TOXICOLOGICAL ASSESSMENT OF THE EFFECT OF MOTORWAY RUNOFF ON STREAM MACROINVERTEBRATE COMMUNITY STRUCTURE AND FUNCTION.

by David Malcolm Forrow

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Abstract.

Road runoff contains a complex mixture of contaminants including metals, anions, and hydrocarbons. This runoff discharges into natural water courses which are often small streams. The concentration of these chemicals in the drainage water and receiving stream depends on a number of site specific characteristics such as traffic volume, area of road drained and size of the stream. It was postulated that these pollutants have a deleterious affect on macroinvertebrate community structure which would result in subsequent effects on macroinvertebrate function (i.e. litter processing). Further, it was hypothesised that impacts would be greatest in small streams, receiving drainage waters from large areas of heavily used motorway and that only a limited number of chemicals would be responsible for any effects.

Field surveys demonstrated that macroinvertebrate community structure and function was impacted at one of the three sites studied, namely Pigeon Bridge Brook. The downstream station at this site received motorway runoff drainage from the largest area of road surface, was the smallest stream and had the highest metal and hydrocarbon concentrations in both stream water and sediments (Maltby *et al.*, 1995a). Macroinvertebrate species richness and diversity were significantly reduced below the discharge. Species generally considered 'sensitive' to pollutants such as stoneflies, gammarids, molluscs and trichopterans were reduced in relative abundance whilst more 'tolerant' opportunistic species such as chironomids and tubificid worms increased in relative abundance downstream of the discharge. An assessment of the trophic composition of the community (i.e. functional feeding groups) indicated that there was a differential loss of functional groups, with significantly lower relative abundances of shredders and scrapers and an increase in collectors downstream of the motorway discharge. The changes in both the structure and trophic biology of the macroinvertebrate community resulted in a significant reduction in macroinvertebrate-mediated leaf processing downstream of the motorway discharge.

Although field surveys indicated macroinvertebrate community structure and function were negatively impacted below the motorway discharge at Pigeon Bridge Brook they cannot establish causal relationships. *In-situ* and laboratory studies were therefore performed to address the mechanistic basis for the impact. *In-situ* and laboratory lethality exposures did not fully explain the field distribution of the species used in toxicological studies; *Gammarus pulex* (L.), *Nemoura cinerea* (Retz.), *Potamopyrgus jenkinsi* (Smith), *Chironomus riparius* (Meigen) and *Tubifex tubifex* (Müller). In acute lethality tests stream water from Pigeon Bridge Brook was not toxic to any of the species. In contrast, *G. pulex* and *N. cinerea* showed slight, but significant mortality when exposed to downstream sediment from this site. Sediment manipulation and sediment solvent and acid extract exposures indicated that the solvent extractable fraction of the sediment was responsible for this toxicity to *G. pulex* but not to *N. cinerea*. These results indicated that aromatic hydrocarbons in the sediment may be responsible for the toxicity and this has subsequently been shown to be the case (Maltby *et al.*, 1995b).

Since lethality studies did not fully explain field distributions of the animals sub-lethal toxicity avoidance behaviour tests were employed using sediment, manipulated sediments and sediment extracts. The sensitivity to downstream field sediment, indicated by avoidance decreased in the order *P. jenkinsi* > *G. pulex* > *C. riparius*> *T. tubifex* = *N. cinerea* and to a solvent extract of this sediment in the order *G. pulex* > *P. jenkinsi* > *C. riparius* > *N. cinerea* > *T. tubifex*. Acid sediment extracts and solvent extracted sediments induced no avoidance responses in these animals.

Gammarus pulex was thought to be the dominant shredding macroinvertebrate at Pigeon Bridge Brook. Reductions in macroinvertebrate-mediated leaf processing could therefore be the result of sub-lethal effects of motorway contamination on the feeding activity of this species. *In-situ* exposures indicated that the consumption of leaf material by *G. pulex* was reduced at the downstream station and laboratory exposures indicated this was principally a result of sediment toxicity. Sediment extract exposures indicated that the solvent extractable fraction was again responsible for the majority of this effect. Accumulation of metals and aromatic hydrocarbons on the leaf material had very little effect on leaf consumption or choice. However, reduced colonisation of leaf material by aquatic hyphomycetes reduced both leaf choice and consumption when the material was conditioned at the downstream station. The major uptake route of aromatic hydrocarbons by *G. pulex* was via aqueous sources and not from food.

In conclusion motorway derived contamination in small streams has both lethal and sub-lethal effects on some macroinvertebrates. This affects macroinvertebrate structural and trophic characteristics which subsequently have a deleterious effect on important ecosystem functions.

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CHAPTER 1.

GENERAL INTRODUCTION.

Road vehicles are a major source of a wide range of potential pollutants including particulate material, metals, anions and organics (including hydrocarbons; Table 1.1). These are derived from a variety of sources including exhaust emissions, tyre wear, brake dust and road wear (Hildemann *et al.*, 1991). Although a proportion of these substances may enter the atmosphere and become associated with atmospheric deposition (Gjessing *et al.*, 1984b; Ball *et al.*, 1991; Adachi and Kobayashi, 1992) the majority (>95%) are deposited on or near the road surface where they become associated with particulate material (Zurcher *et al.*, 1980; Hamilton *et al.*, 1984; Harrison and Johnson, 1985; Post and Beeby, 1993). Consequently, road dust is a complex mixture of vehicle exhaust condensate, lubricating oils, hydraulic fluids, coolants, fuel oils, vehicle load spill, tyre particles, brake and clutch wear particles and deicing chemicals, in addition to atmospheric fallout, road surface particles and soil (Sartor and Boyd, 1972, 1975; Bourcier *et al.*, 1980; Perry and McIntyre, 1986; Takada *et al.*, 1990). The majority of this particulate material accumulates at the road edges and is either washed off the road into gravel filled ditches (french drains, open systems; Wignalt and Kendrick, 1981) or collects at kerbs and runs into gullies via a gully grid (closed system). Both systems connect to pipe networks which then discharge into natural water courses which are often small streams (Sartor and Boyd, 1975; Brunner, 1977). Discharges from closed systems may have concentrations of many pollutants an order of magnitude greater than open systems (Dupois *et al.*, 1985; Stotz, 1987).

The contamination of stream systems by runoff from road surfaces therefore presents a significant source of potential pollutants (Perry and McIntyre, 1986). Some studies have suggested that although roads may occupy only 5-8 % of an urban catchment area, road drainage can contribute as much as 50 % of the total suspended solids, 16 % of the total hydrocarbons and 75 % of the total metal inputs to a receiving stream (Ellis *et al.*, 1987). The pollutant load, in-stream concentrations and the subsequent effects on the stream biota are site specific. Important site-specific characteristics include traffic volume, length of motorway drained, road design and maintenance, drainage system, surrounding land use, accidental spills and the size of receiving water body (Kobriger *et al.*, 1984; Mudre, 1985; Stotz, 1987; Bellamy, 1990). The pollutant load may also exhibit temporal variation. For example many studies have demonstrated that the pollutant load entering a stream in any particular storm event is dependent on traffic

flow during, and length of, the antecendent dry period (Colwill *et al.*, 1984; Jones and Tinker, 1984; Perry and McIntyre, 1986; Hewitt and Rashed, 1992).

Table 1.1. Inorganic and organic contaminants in road runoff and their common sources.

Contaminant	Source	Reference
SUSPENDED SOLIDS	Exhaust condensate, road surfaces, soil, atmospheric fallout, traffic load loss vehicle wear	2, 9, 11, 15
METALS		
Zinc	Tyres, underseal, lubricants	1-8, 10-12, 18-21
Lead	Fuel, tyres, brake linings, underseal, lubricants, bearings	
Cadmium	Plated surfaces, tyres, diesel oil	
Copper	Brake linings, tyres, bearings and bushings, deicing salts	
Chromium	Tyres, brake linings, plated surfaces, steel parts, deicing salts	
Nickel	Tyres, brake linings, underseal, steel parts, diesel oil, deicing salts, road surfaces, bearing bushings	
	oil, deicing salts, road surfaces, bushings	
Iron	Road/bridge structures, steel parts, engine and car body	
Magnesium	Engine wear, brake linings	
Calcium/ Sodium	Deicing salts	
Aluminium	Engine wear	
Manganese	Moving engine parts	
Titanium	Tyres	
Tungsten	Tyres, Steel parts	
Platinum	Steel parts, catalysts	
Vanadium	Steel parts	
Molybdonen	Steel parts	
Barium	Lubricating oil	
ANIONS		
Bromides	Lubricating oil, exhaust condensate	11, 15, 16, 20
Chloride	Lubricating oil, deicing salts	
Sulphates	Lubricating oil, roadway beds, fuel, deicing salts	
Phosphates	Lubricating oil, roadside fertilisers, lorry load loss	
Nitrates	Lubricating oil, roadside fertilisers, lorry load loss	
Ammonia	Roadside fertilisers, lorry load loss	
Asbestos	Clutch and brake linings	
Cyanides	Deicing salts	
ORGANICS		
Polycyclic aromatic hydrocarbons	Combustion products,	1, 11, 13, 14, 17, 20
Aliphatic hydrocarbon	Lubricants	
Alicyclic hydrocarbons	Lubricants	
Polychlorinated biphenyls	Tyres	
Pesticides	Verge weed control	
Fatty acids	Lubricants, road spillages	
Surfactants	Additives	

1. Sartor and Boyd, 1972; 2. Hedley and Lockley, 1975; 3. Shaheen, 1975; 4. Brunner, 1977; 5. Laxen and Harrison, 1977; 6. Christensen and Guinn, 1979; 7. Bourcier *et al.*, 1980; 8. Van Hassel *et al.*, 1980; 9. Zürcher *et al.*, 1980; 10. Cole *et al.*, 1984; 11. Kobriger, 1984; 12. Harrison and Johnson, 1985; 13. Hoffman *et al.*, 1985; 14. Ellis, 1989; 15. Bellamy, 1990; 16. Ohno, 1990; 17. Takada *et al.*, 1990; 18. Scanlon, 1991; 19. Amrhein *et al.*, 1992; 20. Environment Canada, 1994; 21. Wei and Morrison, 1994.

In general, road runoff raises the conductivity, sediment loads, metal and hydrocarbon concentrations in receiving streams (Bellamy, 1990; Baekken, 1994). Most of the contamination is inorganic mineral-like matter although this contains appreciable quantities of organic and inorganic toxicants (Sartor and Boyd, 1972, 1975; Zurcher *et al.*, 1980; Bellamy, 1990). For instance, Cowley (1985) found Zn, Pb and Cu concentrations were elevated in a runoff-impacted stream and Bellamy (1990) concluded that suspended solids, BOD, conductivity, ammonia, nitrate, phosphate, chloride and Pb, were all elevated downstream of a motorway runoff discharge in an oligotrophic upland system. Despite the potential impact of these discharges on the biota of receiving waters relatively few studies have assessed the effect of the road runoff on freshwater communities and none has investigated the implications of changes in community structure for ecosystem functioning.

Shaheen (1975) suggests there are two plausible mechanisms by which urban/ road runoff could threaten the physical, chemical and biological integrity of an aquatic ecosystem. First a shock-loading effect may occur during periods of rainfall or thaw when settled and suspended solids, toxic materials, nutrients and oxygen demanding substances suddenly enter a watercourse. Initial drainage flushes from roads generally bring in the more soluble components of the runoff (e.g. Na, Ca, Mn, Cu and Cd, chlorides and sulphates) with constituents associated with particulate material entering later in the runoff event or during later, more intense, events (Harrison and Wilson, 1985b). Because these episodes of runoff occur repeatedly, more or less permanent changes in an aquatic habitat may appear which in turn produce corresponding alterations in community structure and function. Secondly, there may be a more long-term affect associated with contaminants that settle and accumulate on the stream bed. These contaminated sediments may act as a reservoir of slowly mobilised toxic substances exerting a persistent stress on the system. It is this second mechanism which will be the focus of the current study.

Stressed ecosystems have been shown to exhibit certain predictable changes in community structure (Sheehan *et al.*, 1984; Schindler, 1987) and methods have been developed to characterise these effects (Washington, 1984; Schaeffer *et al.*, 1988; Metcalfe, 1989). Most usually species diversity decreases and dominance increases (Odum, 1985). Changes in community structure often have subsequent important effects on ecosystem function including primary productivity, decomposition and nutrient cycling (Pratt, 1990). For instance, rates of decomposition may be reduced resulting in decreases in the efficiency of resource use with the subsequent loss of nutrients from a system (Odum, 1985).

The two main sources of energy in streams are photosynthesis by aquatic plants within the stream and decomposition of organic matter, mostly leaf fall, from outside the stream. Several studies have suggested that detrital material, particularly leaf litter is the major energy source in small streams (Vannote *et al.*, 1980; Naimo *et al.*, 1988) and may account for up to 99 % of the annual energy budget (Fisher and Likens, 1973). The breakdown of leaf litter in streams is brought about by a combination of chemical leaching, microbial colonisation and decomposition (i.e. conditioning; *sensu* Cummins, 1974), macroinvertebrate feeding and physical abrasion (Kaushik and Hynes, 1971; Petersen and Cummins, 1974; Webster and Benfield, 1986; Maltby, 1992). These processes are not independent. For instance, microbial colonisation (mainly by aquatic hyphomycete fungi) of leaf material makes it more palatable and of higher food quality to macroinvertebrates (e.g. Cummins, 1973).

During the leaching stage, leaves lose about 15 % of their weight as soluble matter leaving behind more refractory structural compounds such as cellulose, hemicelluloses, proteins and lignins (Petersen and Cummins, 1974; Wilson *et al.*, 1986). The biological breakdown of this material requires catabolism by enzymes. Enzymes are generally substrate specific so the more heterogeneous the plant material the greater are the number of enzymes necessary to degrade it (Wilson *et al.*, 1986). Macroinvertebrates have been shown to have few of the enzymes necessary to degrade recalcitrant plant material in aquatic systems (Monk, 1976, 1977; Harris, 1993). This material is therefore generally not directly assimilated by detritivores and must first undergo microbial conditioning to yield more available products (Bärlocher and Kendrick, 1975). Both bacteria and fungi are thought to be important in leaf processing (Lawson *et al.*, 1984; Cargill *et al.*, 1985). However, fungi, principally aquatic hyphomycetes, dominate the early stages of decomposition, whereas bacterial numbers rise during the later stages (Bärlocher and Kendrick, 1974; Webster and Benfield, 1986; Bengtsson, 1992). Although, different fungal species have been shown to have different enzyme activities (Hasija and Singhal, 1991) together the aquatic hyphomycetes produce all the enzymes required to degrade the structural polysaccharides of leaf cell walls (Chamier and Dixon, 1982; Chamier *et al.*, 1984; Chamier, 1985; Zemek *et al.*, 1985; Abdullah and Taj-Aldeen, 1989; Hasija and Singhal, 1991). During conditioning the fungi use the leaf material as a nutrient base but also utilise dilute dissolved nutrients from the water column (Fenchel and Jorgensen, 1977; Wilson *et al.*, 1986). For instance, radiotracer releases have indicated that dissolved ^{32}P and ^{15}N accumulated on CPOM (Lawson *et al.*, 1984; Mulholland *et al.*, 1985b). Microbes use the assimilated nutrients from the leaf material and the surrounding water to produce proteins thus decreasing the C:N

ratio of the leaf material and rendering nitrogen rich detrital material available to detritivorous animals (Mulholland *et al.*, 1985a; Webster and Benfield, 1986).

Macroinvertebrates that feed on conditioned leaf material are known as shredders (Cummins, 1974; Cummins, 1988) and are the most important group of animals in controlling the breakdown of coarse particulate organic matter (CPOM) in streams (Merritt and Cummins, 1984). Although microbes can decompose litter completely in the absence of shredding macroinvertebrates, shredders accelerate litter decomposition by about 20 % (Petersen and Cummins, 1974). Leaf substrates with higher microbial activities and biomass are generally preferred by detritivores. Moreover, preferred foods have been demonstrated to produce greater growth, increase fecundity and result in higher survivorship of detritivores (Kostalos and Seymour, 1976; Willoughby and Sutcliffe, 1976; Sutcliffe *et al.*, 1981; Soderstrom, 1988; Graça *et al.*, 1993). Different hyphomycete fungal species are preferred by and offer different nutritional value to shredding macroinvertebrates. (Suberkropp *et al.*, 1983; Arsuffi and Suberkropp, 1984; 1985; Bermingham, 1993). Consequently the assemblage of fungi on the detrital material affects its food value and attractiveness to shredders (Phillips, 1984; Cargill *et al.*, 1985).

Shredding detritivores have low assimilation efficiencies and much of the material consumed is egested as fine particulate organic matter (FPOM) and dissolved organic matter (DOM) (Petersen and Cummins, 1974; Cuffney *et al.*, 1990). In addition, the feeding activity of shredding macroinvertebrates results in the fragmentation of CPOM to FPOM and DOM (Meyer and O'Hop, 1983). FPOM also becomes colonised by microbes resulting in a high energy food resource for macroinvertebrates known as collectors. Because of their role in converting CPOM to FPOM shredders play a pivotal role in the incorporation of detrital energy in stream food webs (Short and Maslin, 1977; Shepard and Minshall, 1981; Richardson and Neill, 1991). If community composition is altered by changing the system from a shredder-dominated system to a collector/gatherer system, then leaf processing rates are massively decreased reducing the amount of available FPOM to the rest of the community (Cuffney *et al.*, 1984; Shurr, 1989; Wallace *et al.*, 1991). For example, when shredders are excluded, or are at naturally low densities, in a system leaf processing is generally reduced by up to 50 to 70% even if microbial populations are unaffected (Kirby *et al.*, 1983; Cuffney *et al.*, 1984; 1990; Barnes *et al.*, 1986; Webster and Benfield, 1986; Stewart, 1992; Chung *et al.*, 1993; Howe and Suberkropp, 1994). Reductions in shredder-mediated leaf processing will result in increases in the standing crop of CPOM and will have consequences for communities further downstream (Cuffney *et al.*, 1990; Wallace *et*

al., 1991). Streams are linked, open systems and the energy supply at a particular site is dependent, to a greater or lesser degree, on processes occurring upstream (Vannote *et al.*, 1980; Mulholland *et al.*, 1985a; Calow, 1992). Reduced production and entrainment by shredding macroinvertebrates may reduce FPOM transport out of the system and CPOM washout may increase the transport of non-utilisable CPOM to downstream animals, possibly resulting in a loss from the entire system (Cuffney *et al.*, 1990; Wallace *et al.*, 1991).

The temporal and spatial diversity of different macroinvertebrate feeding strategies and life histories increases retention of CPOM in streams (Allan, 1995). When systems are stressed, communities lose structural complexity and energy utilisation becomes increasingly inefficient (Warwick, 1992). Cycling patterns are affected if any of the key processors along a nutrient pathway are compromised resulting in stressed systems generally having low nutrient cycling efficiency with much of the potential energy leaving the system (Sheehan *et al.*, 1984; Odum, 1985; Rapport *et al.*, 1985; Pratt *et al.*, 1990). Functional assessments of toxic stress, such as leaf processing and functional feeding group analysis, provide ecologically relevant information and have been found to be useful tools (McCarthy and Bartell, 1988; Bruns *et al.*, 1992). However, a stressed system may replace sensitive species with functionally similar but less sensitive species maintaining ecosystem function. For instance, Sarranno *et al.* (1993) found that experimental acidification altered the dominance of zooplankton species in lakes but this had no effect on the growth of phytoplankton communities. This was a consequence of the zooplankton species utilising similar resources so nutrient regeneration and the subsequent supply to phytoplankton was maintained. In lotic systems perturbed by organic effluents more 'sensitive' *Gammarus* may be replaced by more 'tolerant' but functionally similar *Asellus* (Whitehurst and Lindsey, 1991). Since function may therefore, not necessarily, be altered it is often important to assess structural properties of the community in addition to functional aspects of the ecosystem (Sheehan *et al.*, 1984).

Pollutants may affect leaf processing either by reducing the diversity and biomass of macroinvertebrates (Webster and Benfield, 1986) or by affecting microbial growth (Forbes and Magnuson, 1980; Fairchild *et al.*, 1984; Frankenhuyzen and Geen, 1986; Bärlocher, 1993). For instance, metals have been shown to have a detrimental effect on microbial and macroinvertebrate communities involved in leaf processing and subsequently on leaf decomposition itself. Reduced microbial colonisation (Giesy, 1978; Gray and Ward, 1983; Maltby and Booth, 1991; Bermingham, 1993; Tattersfield, 1993) and reduced microbial activity have been reported either as a result of direct metal

toxicity or due to floc precipitation of metals on leaf material (Carpenter *et al.*, 1983; Leland and Carter, 1985; Bermingham, 1993). Macroinvertebrate-mediated leaf decomposition may be affected by direct metal toxicity or by altered food quality caused by the pollutant (Bermingham, 1993). The effect of organic pollutants on leaf processing is equivocal. For instance, whereas McKinley *et al.* (1982) reported that hydrocarbon pollution had no effect on microbial activity on detrital material, Werner *et al.* (1984a) reported increased microbial activity. However, both studies reported that hydrocarbons significantly reduced the mineralisation of lignocellulose and detrital material.

Several studies have demonstrated that road runoff reduces macroinvertebrate diversity in the receiving waters (e.g. Bellamy, 1990; Baekken, 1994). However, reductions in abundances or complete extinction of macroinvertebrates at polluted sites are not necessarily attributable to mortality, but may either be due to a reduction in an animal's ability to function successfully and maintain competitive and trophic interactions or may be due to increased emigration and/or increased immigration (Sheehan *et al.*, 1984). Depledge (1989) maintains that ecosystem protection should ensure that the normal repertoire of behavioural and physiological responses of organisms to environmental fluctuations should remain fully intact. The primary reaction of macroinvertebrates to pollutants is often a behavioural response and catastrophic drift has been shown to provide a useful index of disturbance (Hall *et al.*, 1980; Armitage, 1994). For instance Cowley (1985) found that *Gammarus pulex* responded to zinc stress by increasing drift and postulated that this was one possible reason for the reduction in the abundance of this species in a stream impacted by road runoff.

As contaminants in road runoff are associated with the sediments, effects are more likely to be exerted on benthic organisms (Pratt and Coler, 1979). Assessments of the impacts of pollutants using benthic macroinvertebrate community surveys are useful as they incorporate the response of both lethal and sub-lethal responses to contaminants. However, they cannot elucidate causal relationships. Laboratory tests can qualify observations from field surveys and determine the mechanistic basis for these observations. In order to be ecologically meaningful, laboratory bioassays should use species that are widely distributed and play an important role in the functioning of the ecosystem (Sheehan *et al.*, 1984). Population-oriented assessments of impacts may not necessarily reflect changes in functional properties. Furthermore, as Schindler (1987), states '...changes in ecosystem function....cannot be properly interpreted without analogous information on the organisation and structure of the biotic communities

which perform the functions'. It is therefore useful to assess both structural and functional attributes of the system to assess impact (Sheehan *et al.*, 1984).

The overall aim of this thesis is to assess the effect of road-runoff discharges on macroinvertebrate structure and function, concentrating on how the discharge effects those organisms important in leaf processing in streams. The effect of road runoff on aquatic community structure and function will be greatest where contaminants from heavily used roads are discharged into small receiving waters (Willemsen *et al.*, 1990); that is, where motorway runoff enters small streams. In these types of systems the runoff may constitute >50 % of the stream flow below the runoff discharge (Harrison and Wilson, 1985c). In the UK, in 1993, although motorways accounted for 1 % of the total road network length they carried approximately 30 % of all road traffic. Traffic on motorways is increasing more than any other road type having doubled between 1982-1992 whereas motorway length increased by only 17 %. Predictions indicate growth is set to continue with estimates of 65-106 % increases in the numbers of vehicles in the UK between 1992-2025 (Dept. of Transport, 1993). The M1 motorway was opened between 1959-1977 and carries one of the heaviest flows of traffic in the UK (Charlesworth, 1984; Colwill *et al.*, 1984; Perry and McIntyre, 1986). The stretch of motorway used in this study was opened between 1967-1968 (Charlesworth, 1984). Since site-specific characteristics of the streams determine their potential toxic loading, three sites which varied in size and which received runoff from different areas of motorway surface were studied. These sites were considered not to be impacted by other types of contaminant inputs and were examples of 'worse case scenarios'.

1.1. Hypothesis and approach.

The central hypothesis being addressed by this study was that runoff from the M1 motorway would reduce the taxa richness of the benthic macroinvertebrate communities in the receiving waters. In addition, there would be a reduction in detritus processing at sites receiving runoff due to changes in macroinvertebrate and microbial diversity and activity. Although motorway runoff contains vast numbers of potential pollutants it was hypothesised that the effects on the biota of the receiving waters was due to only a small number of chemicals. These hypotheses were addressed using both field and laboratory studies.

Field surveys were conducted to describe the effect of the motorway runoff on the structure and function of both macroinvertebrate and fungal communities (Chapter 2). Field and laboratory studies were then performed to assess the mechanistic basis of the

observed effects. Results from studies of lethal (Chapter 3) and sub-lethal (Chapter 4) effects were used to provide an insight into the mechanisms that may affect the distributions of macroinvertebrates observed in the field. Further, both the broad classes of chemicals responsible for toxicity and the compartments of the ecosystem in which the toxicity resides were identified (Chapters 3 and 4). The sub-lethal effects on the feeding activity of the macroinvertebrate shredder, *G. pulex,* were used to understand *in-situ* patterns of leaf decomposition (Chapter 5). The implications of motorway discharges on macroinvertebrate community structure and function are discussed (Chapter 6).

CHAPTER 2.

COMMUNITY STRUCTURE AND FUNCTION.

2.1. INTRODUCTION.

As organisms have particular environmental requirements, they generally show restricted and variable tolerances to environmental conditions (Hellawell, 1986). Many factors affect community structure in lotic aquatic ecosystems including food quality and quantity, substrate, water velocity, temperature and water chemistry (Hawkins and Sedell, 1981; Hawkins *et al.*, 1982; Townsend *et al.*, 1983, 1987; Armitage, 1994). Roads influence many of these factors. Modification of stream channels and natural drainage systems due to road construction may alter water velocity, temperature, riparian cover and sediment supply and particle size in near-by water courses. Sedimentation affects habitat characteristics and may have a direct physical effect on organism health (Newcombe and MacDonald, 1991). Runoff from operational roads generally contains a complex mixture of contaminants including inorganic and organic toxicants which change the chemistry of receiving waters (Chapter 1). These changes may consequently have an impact on the biota in the streams that receive road drainage discharges (Whitney and Bailey, 1959; Stout and Coburn, 1989; Bellamy, 1990; Baekken, 1994).

Many anthropogenic perturbations may affect communities resulting in instability and altered community structure. Community-based determinations of change, reflect system-wide changes which may not be obvious from single-species population assessments. Biological communities also integrate the effect of multiple stresses and demonstrate cumulative impact (Metcalfe-Smith, 1994). Effects on the biology of streams receiving road-runoff drainage was investigated by describing changes in community structure. Measures of community structure include species richness (i.e. number of species present) and evenness (i.e. distribution of individuals across species). Several diversity indices take account of both richness and evenness, although the way in which they do this may differ (Washington, 1984; Magurran, 1988). The Shannon index is the most commonly used index in freshwater lotic systems (Resh and McElvary, 1993). This index, however, relies on sampling a defined and random sample from a conceptually infinite population and assumes that that all species are represented in the sample; a feat almost impossible to achieve. Moreover the probabilities of occurrence or the proportion of each species in the community can never be known and a precise estimation would require unreasonably large samples, especially for rare species. The result may therefore depend heavily on the sampling design and in general

the Shannon index has been found to be heavily influenced by the abundance of the commonest species and to be fairly insensitive to community change (Southward, 1978; Magurran, 1988). A second commonly used index, the log series alpha index (α, Fisher *et al.*, 1943), reflects species richness and is considered a good descriptor of sample differences (Southward, 1978; Magurran, 1988). It is relatively independent of sample size or of the abundance of rare/common species (Kempton and Taylor, 1974; Taylor, 1978). The index's only disadvantage is that it cannot discriminate between situations where species richness and number of individuals are identical due to the lack of evenness component in its calculation. This is rarely observed, however, in real situations.

Comparisons of communities between sites is achieved using indices of beta diversity. Beta diversity was assessed using the Sorenson similarity index modified by Bray and Curtis (1957). This index has previously been used to successfully detect community changes between reference and impacted sites and has been recommended in comparative indices studies (Perkins, 1983; Magurran, 1988; Pontasch and Brusven, 1988). In order to assess macroinvertebrate community changes as a consequence of species sensitivity to pollution a biotic score was used. Biotic indices assign scores to groups of organisms according to their tolerance or sensitivity to specific types of pollutants. The sum of scores provides a value by which sites can be compared on general pollution status; the lower the score the poorer the water quality. Generally, biotic indices have been based on the sensitivity of macroinvertebrates to organic pollution (i.e. sensitivity to low dissolved oxygen and high ammonia concentrations) although they have been applied more widely (Rosenberg and Resh, 1993). In this study the Biological Monitoring Working Party (BMWP) biotic index was used (ISO, 1979); a qualitative score which operates at the family level. This index has been widely adopted and is recommended due to its simple application and sensitivity in detecting differences between polluted or non-polluted sites (Rico *et al.*, 1992).

Changes in community structure often have subsequent important effects on ecosystem function. A description of community structure which considers the functional properties of the community is that of guilds or functional groups. Functional groups refer to the manner in which organisms obtain their nutrient and energy sources and the major functional feeding groups in streams are shredders (feed on CPOM), collectors (feed on FPOM), scrapers (feed on benthic algae) and predators (feed on live animals; Cummins, 1973). Changes in the relative abundance of functional groupings reflect changes in the trophic status of an ecosystem which may suggest further consequences on ecosystem functioning.

Changes in the functional characteristics of ecosystems (e.g. primary production and nutrient cycling) incorporate the responses of several of the component assemblages as well as the interactions between them. They therefore have the potential to provide a useful indicator of stress. Although there is some dispute over the sensitivity, and therefore usefulness, of functional responses of ecosystems to stress (Schindler, 1987; Pratt, 1990) the application of these measurements has been shown to be useful in a number of cases (Sheehan *et al.*, 1984). In particular, litter decomposition, an element of nutrient cycling, has been shown to be a sensitive ecosystem response to some pollutants. For instance Bruns *et al.* (1992) concluded that litter decay was the best evaluation criteria for assessing the impact of acidic contamination of a stream, whereas a biotic index (biotic condition index) proved least reliable. The methodology used in measuring leaf decomposition in streams usually employs the use of leaf material either enclosed in mesh bags (leaf bags) or tethered by fasteners or lines (leaf packs). Both of these artificial methods have inherent problems, particularly in determining natural decomposition rates, but both can be useful in assessing the relative rates of decomposition between contaminated and non-contaminated sites (Boulton and Boon, 1991).

Most monitoring studies of pollutants in lotic ecosystems concentrate on benthic macroinvertebrates (Hellawell, 1986). The animals are generally sampled by disturbing a defined area of stream bed for a fixed period of time collecting any dislodged animals in a net downstream of the disturbed area (Williams and Feltmate, 1992). Macroinvertebrates have several advantages over other assemblages (Metcalfe-Smith, 1994):

1. they are differentially sensitive and react rapidly, in a graded response to a range of pollutants;
2. they are often abundant, represent many phyla and trophic levels and have a ubiquitous distribution;
3. they are easy to collect and their taxonomy is fairly well established;
4. they are often sedentary and reflect local environmental conditions;
5. they have life-spans which are long enough to provide a record of environmental quality but short enough for all life stages of the population to be exposed to contaminants.

Changes in macroinvertebrate structure and function in pollutant-impacted streams may be the result of two non-mutually exclusive processes: 1. toxic or physical action of pollutants on the macroinvertebrates or 2. indirect pollutant-induced changes in habitat

characteristics and/or food quality or accumulation of pollutants through the food chain. As described in Chapter 1, aquatic hyphomycete fungi, are an important component in the transfer of energy from the detrital food base to macroinvertebrates. It is therefore pertinent to assess the impact of pollutants on this community as any alterations in species composition or activity may have implications for shredders and leaf processing (Chapter 1; Bermingham, 1993).

Many studies have examined the impact of urban runoff on the biotic communities of streams (e.g. Medeiros *et al.*, 1983, Shutes, 1984; Bascombe *et al.*, 1989; Hogg and Norris, 1991). Urban runoff is often a complex mixture of road surface runoff, industrial runoff and sewage. Few studies have looked exclusively at the impact of road runoff discharges. However, previous studies that have been performed suggest that road runoff results in a decrease in the diversity of benthic macroinvertebrates and aquatic macrophytes (e.g. Gjessing, *et al.*, 1984b; Cowley, 1985; Mudre, 1985; Bellamy, 1990; Baekken, 1994). No studies have related changes in community structure to aspects of ecosystem function.

2.1.1. Objectives and approach.

Motorway drainage waters discharge high loads of toxic chemical mixtures into receiving waters. This contamination has the potential to deleteriously effect the structure and function of the biota in the streams into which this water discharges. This chapter describes field-based survey and experimental work performed to assess the impact of motorway runoff on the biota and sediment quality in three small streams (Rockley Dike, Butterthwaite Ditch and Pigeon Bridge Brook) which receive runoff from the M1 motorway. Studies were performed to assess the effect of the discharges on the communities involved in the processing of allochthonous detrital material and on the subsequent decomposition of this material.

The study concentrated on organisms involved in the processing of allochthonous leaf material and the specific objectives were to:

1. describe site-specific habitat and substrate characteristics of the streams upstream and downstream of the motorway discharge;
2. describe macroinvertebrate communities upstream and downstream of motorway discharges;
3. describe assemblages of aquatic hyphomycetes, fungal biomass (ergosterol) and microbial activity (respiration) on leaf material upstream and downstream of motorway discharges;

4. quantify leaf processing upstream and downstream of motorway discharges.

Four seasonal macroinvertebrate surveys were performed at stations upstream and downstream of motorway discharges. These data were used to calculate diversity, biotic and community similarity indices and to assess the relative abundance of functional feeding groups at each station. Surveys of aquatic hyphomycete assemblages associated with leaf material were also conducted during autumnal leaf fall. To assess the subsequent effect of community changes on leaf decomposition both microbial- and macroinvertebrate-mediated processing was quantified using leaf bag methods (Boulton and Boon, 1991). Habitat characteristics which may affect biotic community structure were also measured and recorded at each site.

More intensive studies were performed at the site which received the greatest motorway runoff pollutant load, Pigeon Bridge Brook. Temporal changes in the macroinvertebrate and aquatic hyphomycetes associated with leaf material were determined as were temporal changes in the biomass (ergosterol) and activity (respiration) of microbes on leaf litter. The subsequent impact of the discharge on the rate of microbial- and macroinvertebrate-mediated decomposition of leaf material was assessed.

2.2. MATERIALS AND METHODS.

A. Preliminary surveys.

2.2.1. Site descriptions.

Three streams were selected from an initial preliminary survey of 7 streams all of which received drainage waters from the M1 motorway (Maltby *et al.*, 1995a). There were two sampling stations per stream: an upstream reference station (<400 m upstream of the motorway runoff discharge point) and a downstream station (<100 m downstream of the discharge). The streams selected for the study represented a cross-section of stream size and received motorway drainage from different areas of road surface. Rockley Dike (NGR SE338023, Plate 2.1.a.) was the largest stream studied (3 m wide and 0.14 m deep) and received drainage water from approximately 26,633 m^2 of motorway surface (Sir Owen Williams and Ptnrs., motorway drainage plans). The substrate was generally of large stones interspersed with mud (see section 2.3.2) and the overhanging canopy was of beech (*Fagus sylvatica,* L.), oak (*Quercus robur,* L.) and hawthorn (*Crataegus monogyna,* Jacq.). Butterthwaite Ditch (NGR SK374944, Plate 2.1.b.) was a smaller stream (1 m wide and 0.05 m deep) that received runoff from a motorway surface area of approximately 38,479 m^2. The substrate was of gravel and mud and the stream had a riparian canopy of hawthorn and birch (*Betula pendula,* Roth.) at the upstream station, and beech and sycamore (*Acer pseudoplatanus,* L.) at the downstream station. Pigeon Bridge Brook (NGR SK479852, Plate 2.1.c. and Plate 2.1.d) was also small (1 m wide and 0.02 m deep) but received drainage from approximately 44,389 m^2 of motorway road surface. At the upstream station the substrate consisted of mud and the canopy was of hawthorn. Downstream of the discharge the substrate was of gravel and coarse mud and the canopy was of hawthorn and beech.

At the Rockley Dike and Butterthwaite Ditch sites the drainage system from the motorway surface is closed apart from french drains at the bottom of embankments (Chapter 1). At the Pigeon Bridge Brook site the drainage entering the stream is collected, equally, by both closed and open systems (Sir Owen Williams and Ptnrs., Civil Engineers, pers. comm.).

A comprehensive study of water and sediment chemistry at the three sites was performed over the same time period (A.B.A. Boxall unpublished, Maltby *et al.*, 1995a). Summary results are displayed in Appendix A2.1. Downstream of the motorway discharges stream water had elevated concentrations of several heavy metals

and sediments were contaminated with a range of heavy metals and hydrocarbons. Total oil concentrations were significantly elevated downstream of the motorway discharge at all three sites whereas concentrations of total aromatic hydrocarbons, including several polycyclic aromatic hydrocarbons, were only significantly elevated downstream of the motorway discharge at Pigeon Bridge Brook and Butterthwaite Ditch. Concentrations of sulphate and chloride ions were only significantly elevated at the downstream station at Pigeon Bridge Brook. There were no significant between-station differences in pH, temperature or dissolved oxygen concentration in stream water at any of the three sites although concentrations of sulphate and chloride ions were significantly elevated at the downstream station at Pigeon Bridge Brook.

2.2.2. Sediment particle size.

Sediment cores (diameter: 15 cm, length: 15 cm) were obtained from upstream and downstream stations at each field site using a metal corer. Three replicate samples were taken from each station at Butterthwaite Ditch and Rockley Dike whereas four replicates were taken from each station at Pigeon Bridge Brook. Samples were dried at 60^{o}C and any conglomerates gently broken up with a pestle and mortar. The samples were then passed through a coarse mesh sieve stack consisting of the following sizes: 43 mm, 23 mm, 10 mm, and 6 mm which was shaken by hand. The sub-6 mm fraction was then passed through a standard sieve stack (B.S. 410) consisting of the following mesh sizes: 2 mm, 1.18 mm, 600 µm, 425 µm, 300 µm, 212 µm, 150 µm and 63 µm. The sediment retained by each sieve was weighed, as was the portion passing through the smallest mesh size. The sub-63 µm fraction was characterised further by analysing settling velocities in water (Day; 1965). The sediment passing through the 63 µm mesh sieve was dispersed in 1 litre of water in a measuring cylinder, mixed thoroughly by repeated inversions and allowed to settle at 15^{o}C. Samples of suspended sediment were taken at fixed time periods and depths below the water surface using a pipette (Table 2.1). The contents of the pipette were discharged into a pre-weighed glass boiling tube and the pipette was rinsed out twice with distilled water into the same tube. The majority of the water in the boiling tube was then evaporated off in a Tecam® block digester at 105^{o}C. The remaining water was then evaporated in a drying oven at 60^{o}C for 2 days to leave a dry sediment fraction. The tubes were then re-weighed and the weight of sediment calculated.

Plate 2.1.a: Rockley Dike downstream of the motorway-runoff discharge pipe.

Plate 2.1.b: Butterthwaite Ditch downstream of the motorway-discharge pipe.

Plate 2.1.c: Pigeon Bridge Brook downstream of the motorway-discharge pipes.

Plate 2.3.d: Pigeon Bridge Brook emerging from beneath the motorway showing the motorway-drainage pipes.

Table 2.1.: Sampling intervals and resulting sediment particle sizes for the sub-63 μm sediment. Phi (Ø) = $-\log_2 X$, where X is particle size in mm.

Sampling intervals		Particle diameter	
Time	Depth(cm)	• m	Phi
1 min 45 sec	10	34.73	4.85
6 min 58 sec	10	17.49	5.85
28 min	10	5.89	6.85
1 hr 51 min	10	5.89	7.41
7 hr 24 min	10	2.24	8.80
14 hr 50 min	5	1.12	9.80

The size of particles in each sample extracted from the water column at each sampling interval is dependent on the settling rate of the particles. According to Stokes' equation, the particle size (X •m diameter) at time t is a function of water depth (h), particle density (ρ_S: quartz = 2.65 g/ml), liquid density (ρ_L: water = 0.99913 g/ml at 15°C), liquid viscosity (η: water = 0.0114 poise at 15°C) and acceleration due to gravity (g: 980.7 cm/s) and can be calculated from equation 2.1:

$$X = \sqrt{\frac{18\eta h}{tg(\rho s - \rho L)}} \qquad \text{Eqn. 2.1.}$$

The percentage of the sample in each particle size class as a percentage of all fractions was calculated on a dry weight basis and graphs of cumulative percentage against particle size as ϕ (i.e. $-\log_2 X$, where X is particle size in mm) plotted. The graphs were then used to calculate 5(ϕ 5), 16(ϕ 16), 50(ϕ 50), 84(ϕ 84), and 95 (ϕ 95) percentiles from which mean particle size, standard deviation around the mean and the skewness of the distribution were calculated according to equations 2.2., 2.3., and 2.4. (Brimblecombe *et al.*, 1982):

$$\text{Mean} = \frac{(\phi 16 + \phi 50 + \phi 84)}{3} \qquad \text{Eqn. 2.2.}$$

$$\text{Standard deviation} = \frac{(\phi 84 - \phi 16)}{4} + \frac{(\phi 95 - \phi 5)}{6.6} \qquad \text{Eqn. 2.3.}$$

$$\text{Skewness} = \frac{(\phi 84 - \phi 50)}{(\phi 84 - \phi 16)} - \frac{(\phi 50 - \phi 5)}{(\phi 95 - \phi 5)} \qquad \text{Eqn. 2.4.}$$

2.2.3. Macroinvertebrate community structure.

Macroinvertebrate samples were taken in October 1990, January 1991, April 1991 and July 1991. On each occasion triplicate semi-quantitative two-minute kick samples were taken at each station (section 2.2.1.) using a standard 20 cm x 20 cm, 1 mm-mesh pond net (Furse *et al.*, 1981). Samples were transported live to the laboratory where the macroinvertebrates were sorted and preserved in 70 % ethanol. Animals which may have been damaged by preservation (i.e. triclads and hydracarina) were identified before preservation. Macroinvertebrates were identified using appropriate taxonomic keys (Freshwater Biological Association; Mellanby, 1963; Fitter and Manuel, 1986; Friday, 1988) and counted. Animals were identified to species except for oligochaetes, coleopteran larvae and dipteran larvae which were identified to family level. The data were summarised using both alpha and beta diversity indices and an index of pollution. The log series alpha index (α, Fisher *et al.*, 1943) which reflects species richness was calculated using a computer program according to equation 2.5. (Moore, Unpublished).

$$\alpha = \frac{S}{\log e\left(1+\frac{N}{\alpha}\right)} \qquad \text{Eqn. 2.5.}$$

Where N is the number of individuals and S is the number of species in the community.

Beta diversity was assessed using the Sorenson similarity index modified by Bray and Curtis (1957). Similarities (C_N) were calculated from equation 2.6. (Washington, 1984).

$$C_N = \frac{\sum_{i=1}^{S} |n_{1i} - n_{2i}|}{\sum_{i=1}^{S} (n_{1i} + n_{2i})} \qquad \text{Eqn. 2.6.}$$

Where n_{1i} and n_{2i} represent the number of individuals of species i at sites n_1 and n_2 respectively. The Bray-Curtis index ranges from 0, which are identical communities, to 1 which are theoretically totally dissimilar communities.

In order to assess changes in the community structure with respect to general species sensitivity to pollution the Biological Monitoring Working Party (BMWP) biotic index was used (ISO, 1979). The index uses family presence/absence data to assign scores to

families using pre-established water quality tolerance values for the major taxonomic groups ranging from 1 to 10. The most sensitive groups are given a score of 10, the least sensitive 1, with intermediates in between. The sum of scores of a sample gives the BMWP score which may range from less than 5 (theoretically down to 0) in polluted rivers to more than 200 in clean rivers (theoretically up to ∞). The BMWP score is often standardised for sampling effort by dividing it by the number of taxa that are used in its calculation to give an average score per taxa (ASPT, Armitage *et al.*, 1983). The BMWP-ASPT ranges from 0 (polluted site) to 10 (clean site).

In order to assess changes in the functional characteristics of the community macroinvertebrates were assigned to functional feeding groups based on their method of food acquisition (Merritt and Cummins, 1984). The four functional feeding groups used were:

1. Shredders: feed on coarse particulate organic matter;
2. Collector/gatherers: feed on fine particulate organic matter;
3. Scrapers: utilise the biofilm which develops on submerged surfaces;
4. Predators: feed on other live invertebrates.

The relative abundance of functional feeding groups was compared between stations at each site.

2.2.4. Aquatic hyphomycete community structure.

Aquatic hyphomycete fungi are identified on the basis of the morphological characteristics of their conidia (Plate 2.2). Conidia have been used as a descriptor of both the presence and relative abundance of fungal species (Bärlocher, 1982; Rosset *et al.*, 1982; Findlay and Arsuffi, 1989). In this study alder leaf baits were used to sample aquatic hyphomycetes at each of the sampling stations (Shearer and Lane, 1983).

Alder (*Alnus glutinosa*, (L.)) leaves were collected post-abscission and pre-leaf fall in the autumn (October-December) from a river-side reference site near Calver, Derbyshire (NGR SK246754). Leaves were returned to the laboratory, air-dried and stored at room temperature until use. In order to assess aquatic hyphomycete communities, groups of five large alder leaves were re-hydrated in distilled water for 2 h. and placed in nylon mesh bags (12 cm x 12 cm, mesh size 350 •m; Lockertex®). A fine mesh was used to exclude the majority of macroinvertebrates which may possibly selectively graze fungal species (Bärlocher, 1980; Suberkropp, 1992) and break up the leaf material thus making it more difficult to sample at the end of the deployment period. Leaf bags were attached to fishing line (100 lb. breaking strain) strung across

the streams and tied to trees. Line and bags were held down in the stream by stones to ensure that they remained submerged. Five alder leaf bags were deployed at each sampling station in November 1990.

After 28 days the bags were recovered, returned to the laboratory (individually in sealed polythene bags containing stream water), and the leaves were removed and washed in distilled water. Eight, 10-mm diameter leaf discs were cut from each of five randomly chosen leaves from each station using a cork borer. Each disc was incubated singly for 4 days at 15°C in 20 ml of distilled water. After the 4 day period samples were fixed and stained by adding 2 drops of 10 % lactic acid and lactophenol-cotton blue (Fisons and Gurr respectively). Stained discs were removed with forceps, mounted on a glass slide in a drop of distilled water and viewed at x150 magnification. Conidia were identified using appropriate keys (Nilsson, 1964; Ingold, 1975; Descals and Webster, unpublished) and their presence or absence recorded.

Relative importance values (RIVs) of aquatic hyphomycetes colonising the leaf material were calculated using the methods of Shearer and Lane (1983). The RIV is a summation of two components. Firstly, the relative leaf frequency (A_i) which is an indication of the patchiness or relative occurrence of a species between different leaves. Secondly, the relative disc frequency (B_i) which is a measure of the patchiness or relative abundance of species on individual leaves.

For any particular species (i) the leaf frequency value (LF_i) is calculated from equation 2.7 where m_i is the number of leaves on which species i occurs and M is the total number of leaves examined. The relative leaf frequency (A_i) for any particular species i is calculated from equation 2.8 where s is the number of species.

$$LFi = \frac{mi}{M} \qquad \text{Eqn. 2.7.}$$

$$Ai = \frac{LFi}{\sum_{i=1}^{s} LFi} \qquad \text{Eqn. 2.8.}$$

For each individual leaf j the sum of the number of leaf discs on which species i is recorded is the disc frequency (DF_i) and is given by equation 2.9 where d is the number of discs on which species i occurs per leaf and M is the number of leaves examined. The mean disc frequency (MDF_i) for any particular species i is given by equation 2.10 and

the relative mean disc frequency (B_i) for any particular species i is given by equation 2.11.

$$DFi = \sum_{j=1}^{M} d_{ij} \qquad \text{Eqn. 2.9.}$$

$$MDFi = \frac{DFi}{M} \qquad \text{Eqn. 2.10.}$$

$$Bi = \frac{MDFi}{\sum_{i=1}^{s} MDFi} \qquad \text{Eqn. 2.11.}$$

The relative importance value for the i th species (RIV_i) is given by equation 2.12.

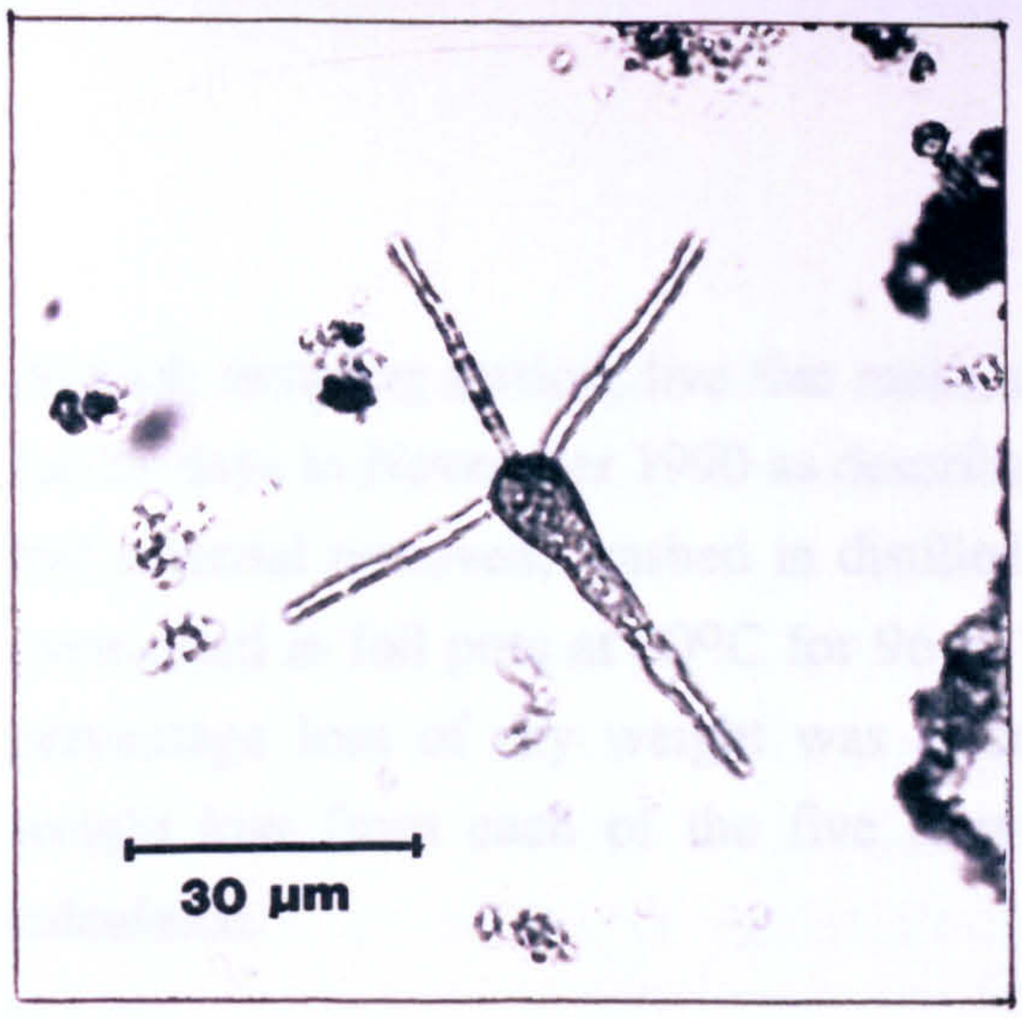
$$RIV_i = A_i + B_i \qquad \text{Eqn. 2.12.}$$

The RIV value for each species, which ranges from 0 (species absent) to 2 (only species present), indicates the importance of that hyphomycete species with regard to the occurrence and abundance of other species.

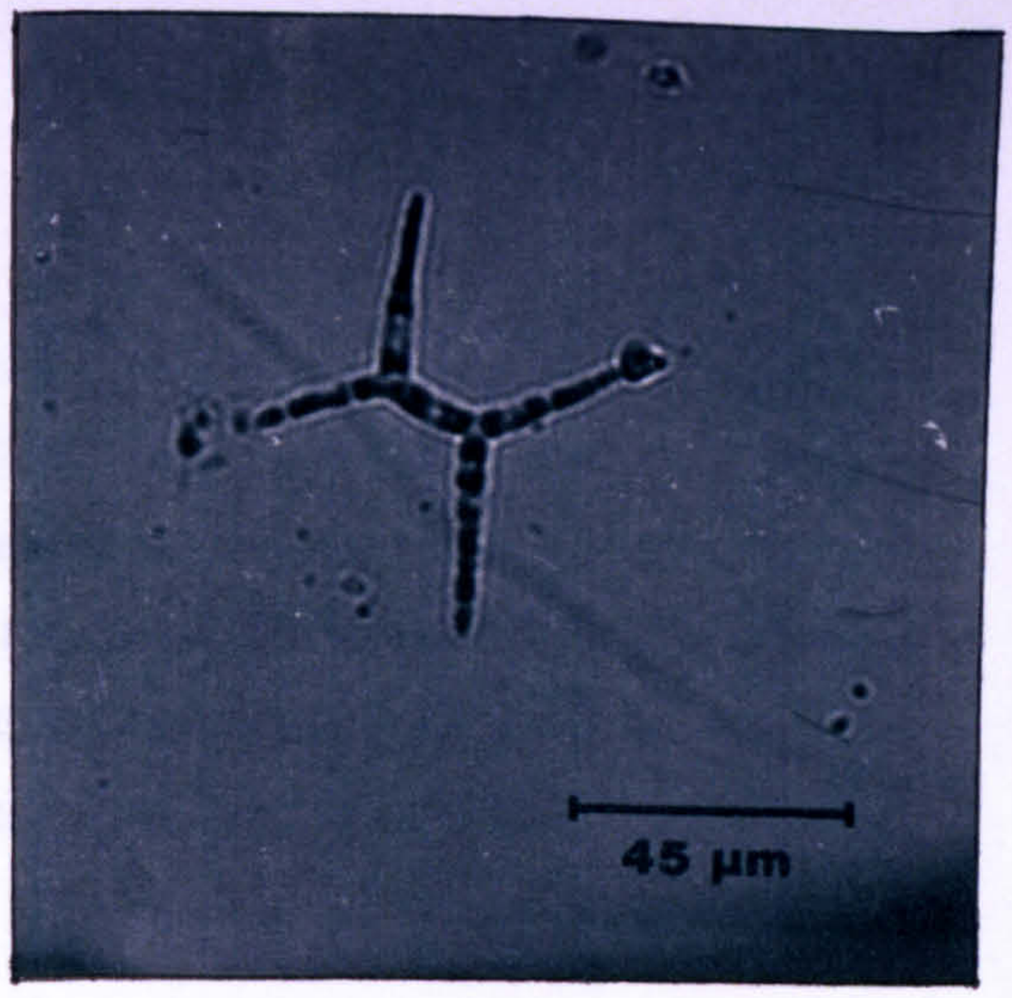
2.2.5. Leaf litter processing.

Weight loss of leaf material in mesh bags was used to assess macroinvertebrate and microbial processing of leaf litter in the stream. Bags of two mesh sizes were used in an attempt to distinguish between microbial and microbial plus macroinvertebrate processing. Breakdown of leaf litter contained within the coarse mesh bags (4-mm mesh size) allowed access to macroinvertebrates whereas fine mesh bags (1-mm) did not. Breakdown of leaf litter within the coarse mesh bags was therefore the result of physical, microbial and macroinvertebrate decomposition whereas breakdown of leaf litter in fine mesh bags was solely due to physical and microbial processes.

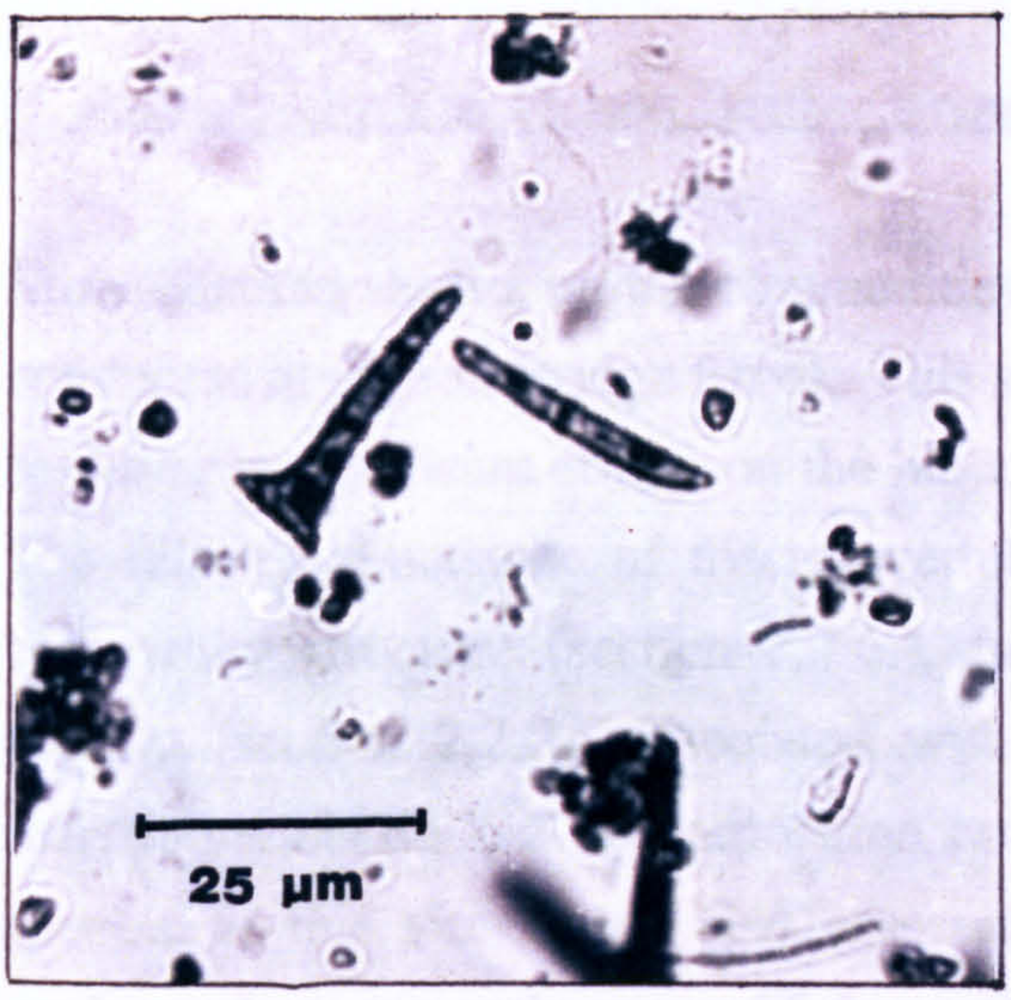
Leaves were collected and stored as described in section 2.2.4. Leaf material was then dried at 60°C for 48 h and cooled in a desiccator. Sixty portions of whole leaf material (approx. 1 g) were weighed on a Sartorius pan balance (model 1712) to determine their dry weight (w_1 to the nearest 0.001 mg), re-hydrated for 2 h in distilled water and placed in leaf bags. Leaf bags were deployed in the streams as described in section 2.2.4.



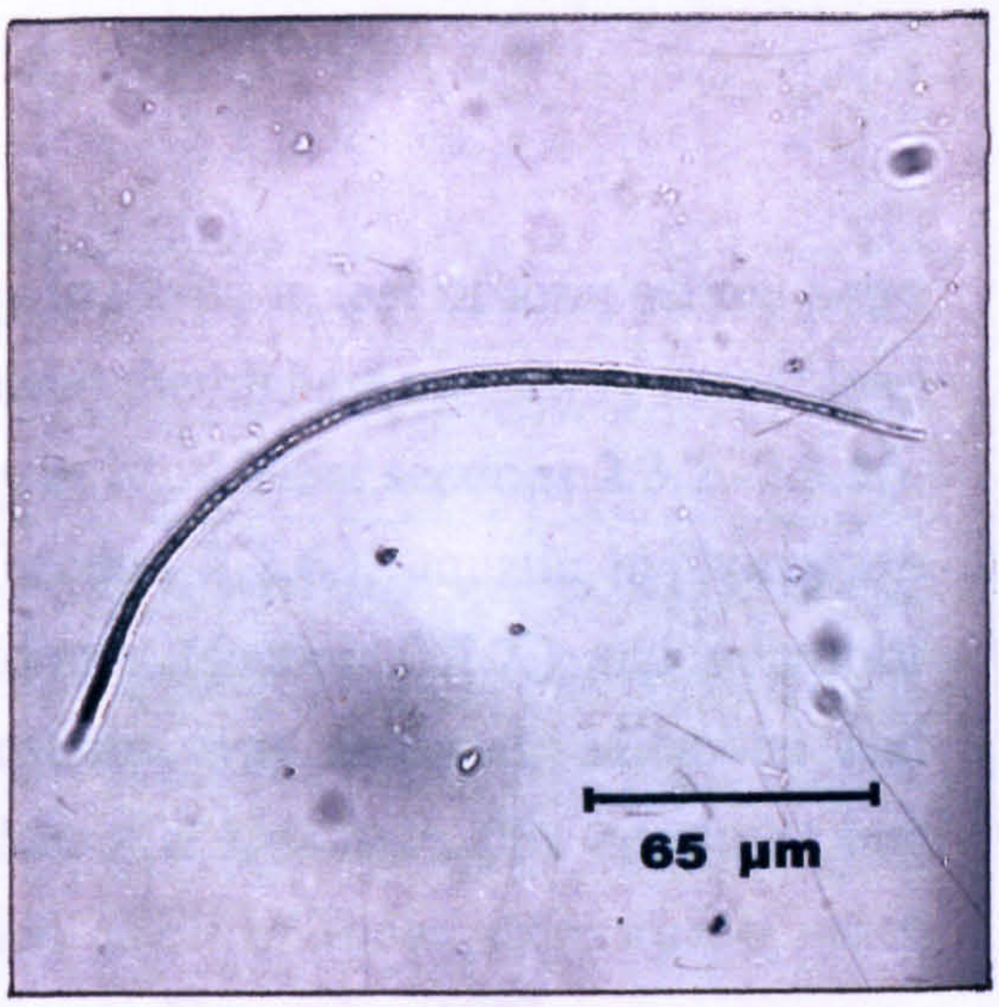
a.) *Clavariopsis aquatica*



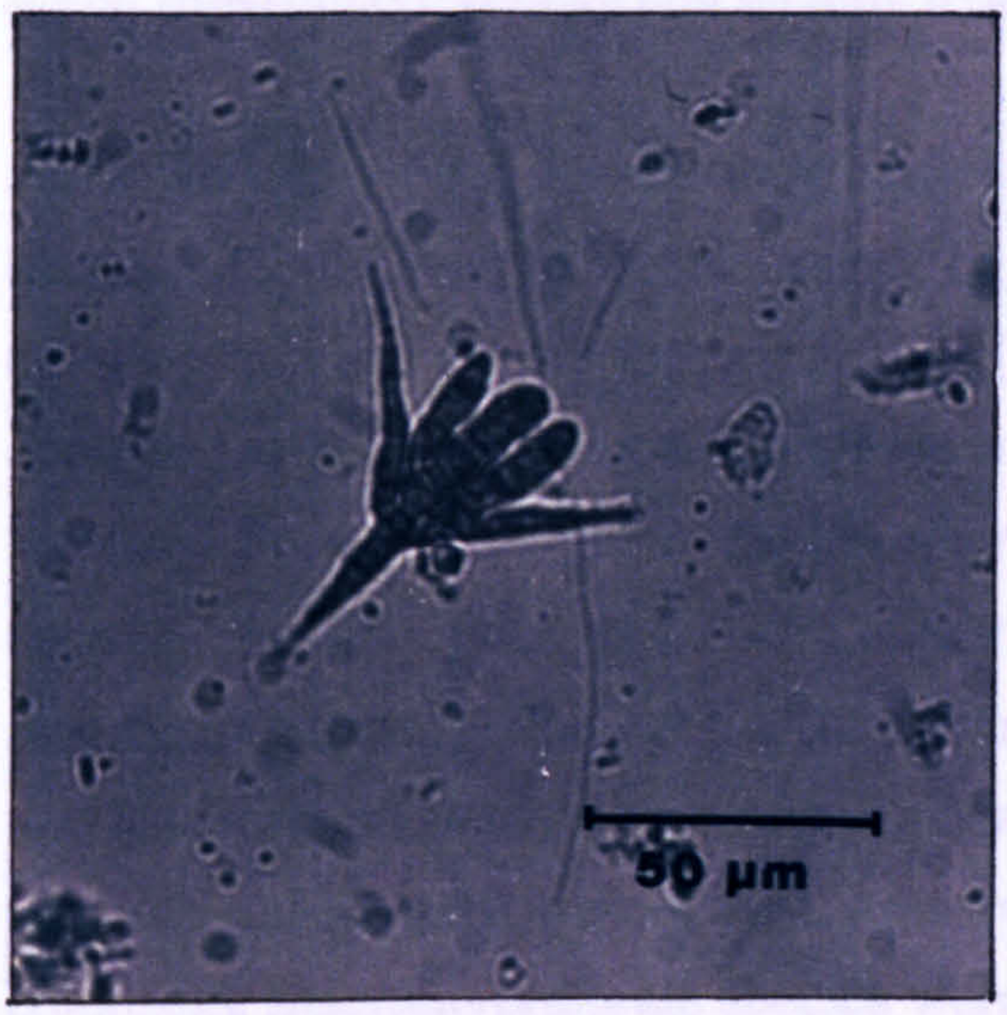
b.) *Tricladium angulatum*



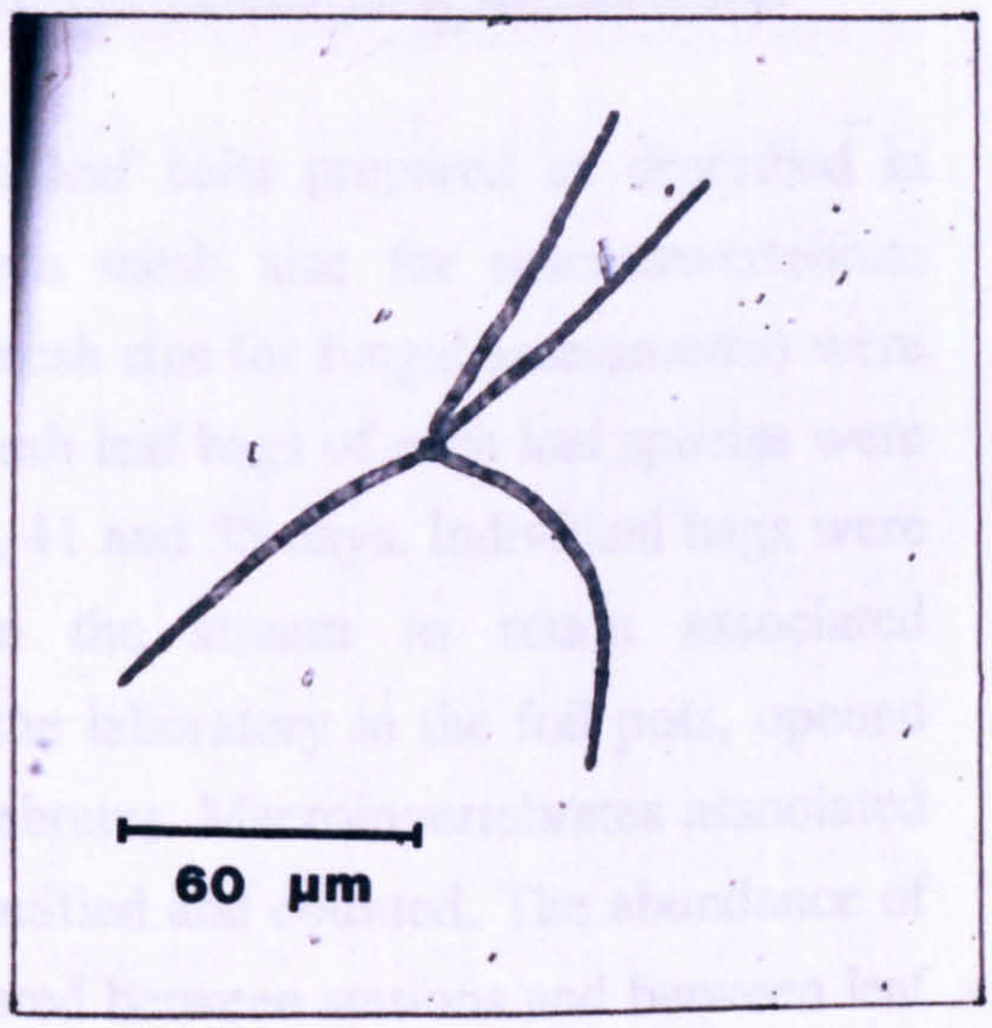
c.) *Heliscus lugdenensis*



d.) *Anguillospora longissima*



e.) *Tetracladium setigerum*



f.) *Tetrachaetum elegans*

Plate 2.2. Examples of conidia used in the identification of aquatic hyphomycete fungi.

At each sampling station, five fine mesh and five coarse mesh leaf bags were deployed for 28 days in November 1990 as described in section 2.2.4. The bags were recovered, leaf material removed, washed in distilled water to remove accumulated sediment and oven-dried in foil pots at 60°C for 96 h. The material was then re-weighed (w_2). The percentage loss of dry weight was calculated using equation 2.13 and the average weight loss from each of the five bags per treatment (site x station x mesh size) calculated.

$$\text{Percentage dry weight loss per leaf bag} = \frac{(w_1 - w_2)}{w_1} \times 100 \qquad \text{Eqn. 2.13.}$$

B. Further work at Pigeon Bridge Brook.

More detailed studies on the communities directly involved in leaf decomposition were conducted at Pigeon Bridge Brook. This site received the highest motorway runoff load resulting in significant effects on the biota (Maltby *et al.*, 1995a; sections 2.3.2.-2.3.5.). The relative abundance of macroinvertebrates (section 2.2.6.), aquatic hyphomycete community structure (section 2.2.6.), fungal biomass (section 2.2.7.) and microbial activity (section 2.2.8.) associated with leaf material was assessed. Microbial and macroinvertebrate leaf decomposition rates of alder and hawthorn (the dominant leaf species at this site) were also assessed (section 2.2.9.). These experiments were performed concurrently over a 55 day period starting in November 1991.

2.2.6. Macroinvertebrates and aquatic hyphomycetes associated with leaf material.

Alder and hawthorn leaves were collected, and leaf baits prepared as described in section 2.2.4. Twenty coarse mesh bags (4-mm mesh size for macroinvertebrate assessments) and twenty fine mesh bags (1-mm mesh size for fungal assessments) were deployed at each sampling station. Five coarse mesh leaf bags of each leaf species were removed from each sampling station after 13, 27, 41 and 55 days. Individual bags were cupped in foil pots prior to removal from the stream to retain associated macroinvertebrates. The bags were returned to the laboratory in the foil pots, opened and leaf material washed to remove macroinvertebrates. Macroinvertebrates associated with the leaf material were sorted, preserved, identified and counted. The abundance of colonising macroinvertebrate species were compared between stations and between leaf species.

In addition, five fine mesh bags of each leaf species were removed from each station after 13, 27, 41, and 55 days deployment and treated as described in section 2.2.4. in

order to study colonisation and successional patterns of hyphomycetes on the leaf material. The number of species and colonisation pattern of fungal species was assessed over the 55 day period. Fungal species diversity was assessed using the Shannon diversity index (*H;* Washington, 1984) which was found to be more independent of low numbers of species in the initial stages of colonisation than the log series alpha index (Equation 2.5.). The index is calculated from equation 2.14 where s is the number of species, n_i is the number of individuals of the i th species and N is the total number of species.

$$H = -\sum_{i=1}^{s} \frac{ni}{N} \ln \frac{ni}{N} \qquad \text{Eqn. 2.14.}$$

2.2.7. Assessment of fungal biomass (ergosterol) on leaf material.

Leaf material deployed at upstream and downstream stations at Pigeon Bridge Brook in fine mesh bags was used to assess fungal biomass using ergosterol analysis (Newell *et al.*, 1988). Leaf baits were prepared as described in section 2.2.4. and 20 bags (1-mm mesh) of each leaf species (alder and hawthorn) and were deployed at the upstream and downstream station for 55 days. Five bags of each species were removed from each station after 13, 27, 41, and 55 days deployment and transported to the laboratory in individual plastic bags containing stream water. Ergosterol was extracted from the leaf discs using the method of Padgett and Posey (1993) as follows: Ten, 10-mm diameter leaf discs were cut from individual leaves of each species from each station and placed in a 100 ml round bottomed flask containing 25 ml of methanol (ALR® grade, Fisons). The sample was refluxed at 80°C on a heating mantle for 2 h after which 1 ml of 4% ethanolic potassium hydroxide (BDH) was added to hydrolyse sterol esters. The sample was refluxed for a further 30 min, cooled and the solvent decanted into a centrifuge tube and centrifuged (MSE Centaur 2®) at 2000 rpm for 5 min to remove suspended particulate material. The supernatant was decanted into a separating funnel to which 10 ml of pentane (ALR®, Fisons) and 10 ml of distilled water were added. The separating funnel was inverted at least 30 times to partition sterols into the pentane. The lower methanol fraction was discharged into a beaker and the upper pentane layer was discharged into a boiling tube. The methanol fraction was re-extracted twice more with 5 ml of pentane and the upper pentane fractions were added to the boiling tube. The pentane fractions were evaporated in a water bath at 30°C under a stream of nitrogen (oxygen free) to dryness and the residue taken up in 1 ml of methanol. The extracts were then filtered through a 0.45 µm nylon filter and stored in foil covered Ebendorf tubes at 4°C prior to analysis. Ergosterol was analysed by high pressure liquid chromatography (HPLC, Philips PU4100® pumping system, PU4110® UV visible

detector). A 20 •L of the sample was injected onto a C18 column (Apex 1, 25 cm long x 4.6 mm internal diameter, 4.6 and 5 mm particle size) using a rheodyne injector. The 100 % methanol (HPLC-grade, Fisons) carrier solvent was pumped at a rate of 2 ml min^{-1} and ergosterol detected at an absorbance of 282 nm. The retention time for ergosterol under these conditions was 4.2 min. An ergosterol/absorbance calibration curve was constructed using 1, 2, 3 and 4 mg/L ergosterol standards. Data were analysed using a Drew® Scientific Chromatographic data analysis package.

2.2.8. Microbial activity on leaf material.

Microbial activity on the leaf material was assessed using methods described in Bermingham (1993). Leaf baits were prepared as described in section 2.2.4. and 20 bags (1-mm mesh) of each leaf species (alder and hawthorn) were deployed at the upstream and downstream station for 55 days. Five bags of each species were removed from each station after 13, 27, 41, and 55 days deployment. Thirty, 5-mm leaf discs of each leaf species were cut from randomly selected leaves from each station with a cork borer and placed in polystyrene petri dishes containing stream water collected from the appropriate station. Randomly chosen samples of 15 discs from each station and each leaf species were treated with antibacterial solution (50 mg/L penicillin + 50 mg/L streptomycin; Sigma) in sterile (autoclaved 121°C, 15 min, 104.3 KPa) water. The remaining fifteen discs were left untreated. Groups of 3 leaf discs from each treatment (station x leaf species x antibiotic treatment/non-treatment) were then placed in 2-ml glass syringes (needle-tipped and sealed with a rubber bung on the needle) which had previously been filled with air-saturated sterile distilled water. Controls consisted of syringes filled with air-saturated distilled water without leaf discs. The syringes were wrapped in silver foil to prevent photosynthesis and incubated at 15°C for 2 h. Five replicates per treatment and four controls were used on each sampling occasion. A Radiometer® oxygen electrode (E5046) linked to a Strathkelvin® oxygen meter (model 781b) was calibrated to 160 torr by injection of ambient air. After 2 h the sample syringes were rotated by hand to ensure thorough mixing of the water, the rubber bung on the needle removed and 1 ml of the sample was injected into the oxygen electrode. The electrode was allowed to stabilise for 2 min and the oxygen concentration, recorded by the meter, noted. Ambient air was then re-injected into the electrode which was reset to 160 torr before the next measurement.

Oxygen consumption (R: $\mu g\ O_2\ ml^{-1}\ h^{-1}$) per sample (3 leaf discs) was calculated using equation 2.15. and the mean oxygen consumption of the 5 replicates per treatment calculated (Bermingham, 1993).

$$R = \frac{(E-C) \times V \times A_1 \times B_1}{t} \qquad \text{Eqn. 2.15.}$$

E is the recorded oxygen concentration in each replicate (torr), C is the recorded oxygen concentration in the control (torr), V is the volume of the water in the syringe (ml), A_1 is the solubility coefficient of oxygen in water at 15°C (2.01 μmol^{-1} $torr^{-1}$), B_1 is the conversion factor for µmols to µg of oxygen (0.032), and t is the incubation period (h.).

2.2.9. Leaf decomposition.

Twenty fine mesh (1-mm) and twenty coarse mesh (4-mm) bags containing pre-weighed portions of either alder or hawthorn (w_1 dry. wt.) were deployed at the upstream and downstream stations at Pigeon Bridge Brook. After 13, 27, 41 and 55 days, five fine and five coarse mesh bags of each leaf species were randomly removed from each station. Leaf material was washed in distilled water to remove sediment, placed in clean foil pots and oven-dried at 60°C for 96 h before being re-weighed (w_2 dry. wt.). The leaf material was then placed in crucibles and ashed in a muffle furnace at 550°C for 12 h. The material was cooled in a desiccator and re-weighed (w_3 dry. wt.).

The percentage loss of ash free dry weight (AFDW) was calculated to adjust for any incomplete removal of accumulated sediment on the leaf material which may have affected the results. Regression equations describing the relationship between ash-weight and dry weight are given in equation 2.16 for alder leaves and equation 2.17 for hawthorn leaves.

$$\text{ash weight} = 0.064\ (\text{dry weight}) + 0.006 \qquad \text{Eqn. 2.16.}$$

$$\text{ash weight} = 0.115\ (\text{dry weight}) - 0.003 \qquad \text{Eqn. 2.17.}$$

The AFDW of the leaf samples in the bags before (w_1) and after (w_2) stream deployment could be calculated from equation 2.18. and equation 2.19. respectively where predicted ash weights of leaf material before deployment were determined using equations 2.16 and 2.17.

$$W_1 = (w_1) - \text{predicted ash-weight} \qquad \text{Eqn. 2.18.}$$

$$W_2 = (w_2) - (w_3) \qquad \text{Eqn. 2.19.}$$

The percentage loss of AFDW (%L) from the bags during the deployment period was then calculated using equation 2.20.

$$\%L = \frac{(W_1 - W_2)}{W_1} \times 100 \qquad \text{Eqn. 2.20.}$$

The relationship between the mean percentage weight remaining for each set of five leaf bags was best described by a single exponential equation of the form $WR = e^{-kt}$ (Webster and Benfield, 1986; WR= percentage dry weight or AFDW remaining at time t, t= number of stream deployment days, k= mass loss rate constant). The mass loss rate constant, k, was given by the slope of the regression line between ln (WR) and deployment time (equation 2.21), where c is a constant.

$$\ln (WR) = -kt + c \qquad \text{Eqn. 2.21.}$$

2.2.10. Statistical analyses.

Percentage data were arcsine transformed prior to analysis and all data were checked for normality using normal probability plots and for homogeneity of variances using Bartletts test. Between-station differences in sediment particle size were assessed using *t*-tests. In the preliminary survey, between-station differences in macroinvertebrate and fungal taxa numbers, macroinvertebrate and fungal diversity, macroinvertebrate BMWP-ASPT scores and leaf decomposition were analysed using analysis of variance (ANOVA), Tukey-multiple comparison tests (*q*-statistic) and *t*-tests. Macroinvertebrate relative abundance and functional feeding group data were analysed using the Friedman test and seasonal changes in functional feeding groups were analysed using the Kruskal-Wallis test. In the detailed study at Pigeon Bridge Brook between-station differences in fungal taxa and activity were analysed using two-way ANOVA and *t*-tests. Macroinvertebrates associated with leaf material, fungal diversity and fungal biomass were analysed using the Friedman and Mann-Whitney test. Between-station differences in leaf decomposition were analysed using analysis of covariance (ANCOVA). The relationship between ergosterol standards and absorbance at 282 nm was analysed using least-squares regression techniques. Bartletts test and the *q*-statistic for Tukey multiple-comparisons were analysed according to Zar (1984) while the remaining analyses were performed using the MINITAB statistical package (Minitab™ Inc., 1991). Significance levels in all cases were $p<0.05$.

2.3. RESULTS.

A. Preliminary surveys.

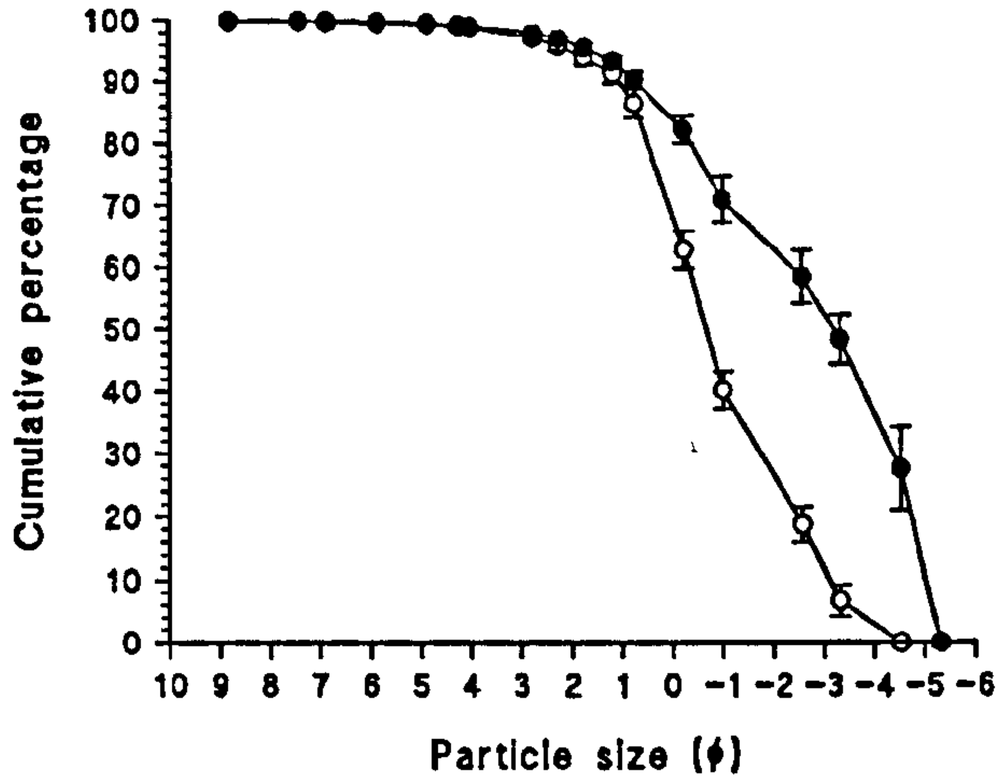
2.3.2. Sediment particle size.

There was significant between-station variation in the mean sediment particle size at both Rockley Dike and Pigeon Bridge Brook with sediments at downstream stations being coarser than the corresponding upstream stations (t>2.54, df=32, Fig. 2.1). In contrast, there was no significant difference in the size of sediment particles at the two Butterthwaite Ditch stations (t=0.35, df=32, Fig. 2.1). Mean particle size and skewness calculated from the particle size distributions are displayed in Table 2.2. Sediments were coarsest at the downstream station at Rockley Dike and finest at the upstream station at Pigeon Bridge Brook. Skewness indicated that Rockley Dike sediments and Pigeon Bridge Brook downstream sediments were generally coarse whereas Pigeon Bridge Brook upstream sediment and Butterthwaite Ditch sediments were generally fine.

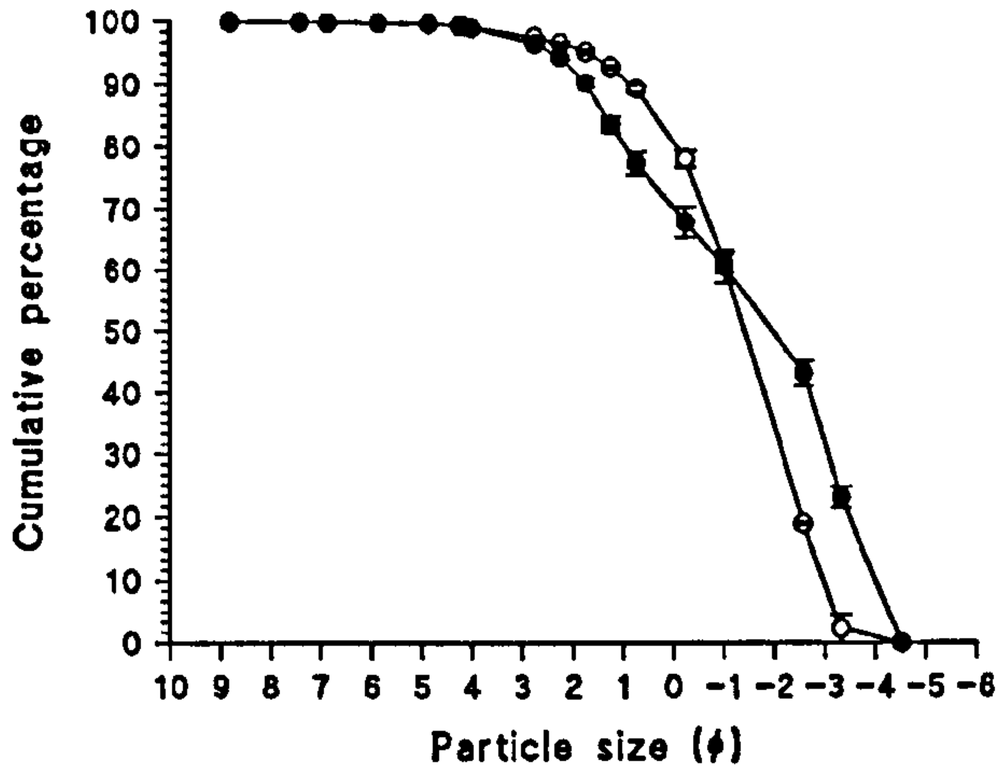
Table 2.2. Mean, standard deviation and skewness of sediment particle size distributions at upstream and downstream stations at the three sites. Phi (ϕ) = $-\log_2 X$, where X is particle size in mm)

Site	Mean (SD) particle size (ϕ)		Skewness	
	Upstream	Downstream	Upstream	Downstream
Rockley Dike	-0.95 (1.68)	-2.68 (2.25)	-0.112	-0.368
Butterthwaite Ditch	-1.28 (1.45)	-1.45 (2.23)	0.250	0.277
Pigeon Bridge Brook	2.25 (1.79)	-0.72 (2.01)	0.035	-0.081

a.)



b.)



c.)

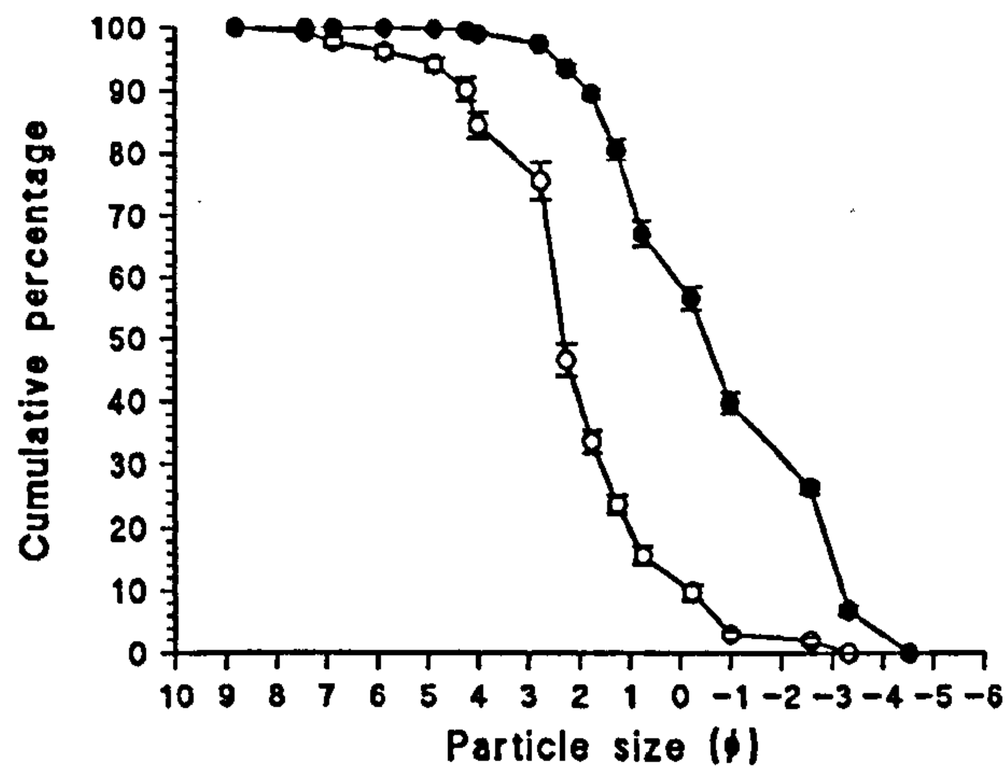


Fig. 2.1. Sediment particle size (where $\phi = -\log_2$ particle size in mm) distributions at a.) Rockley Dike, b.) Butterthwaite Ditch and c.) Pigeon Bridge Brook at upstream (○) and downstream (●) stations. Data presented as means ± 1 S.E..

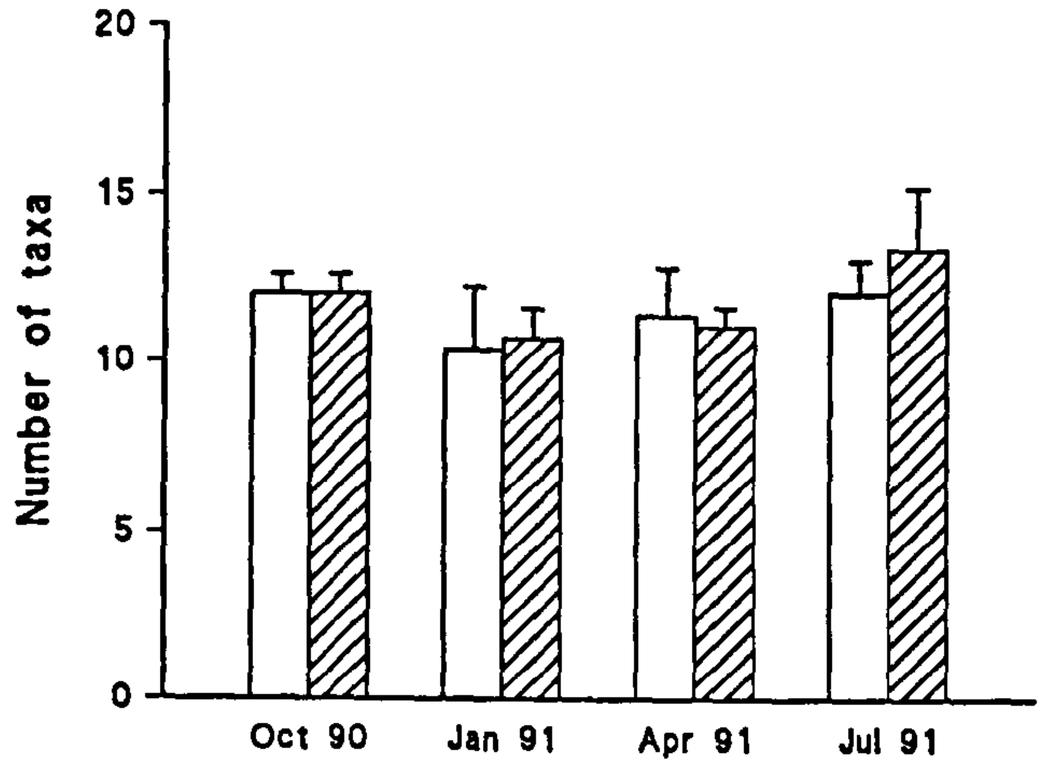
2.3.3. Macroinvertebrate community structure.

Macroinvertebrate community structure was assessed at the three sites (i.e. Rockley Dike, Butterthwaite Ditch and Pigeon Bridge Brook) on four sampling occasions (October, January, April and July) over the period of a year. During the study, a total of 55 different taxa from 35 families was recorded (Appendix A2.2).

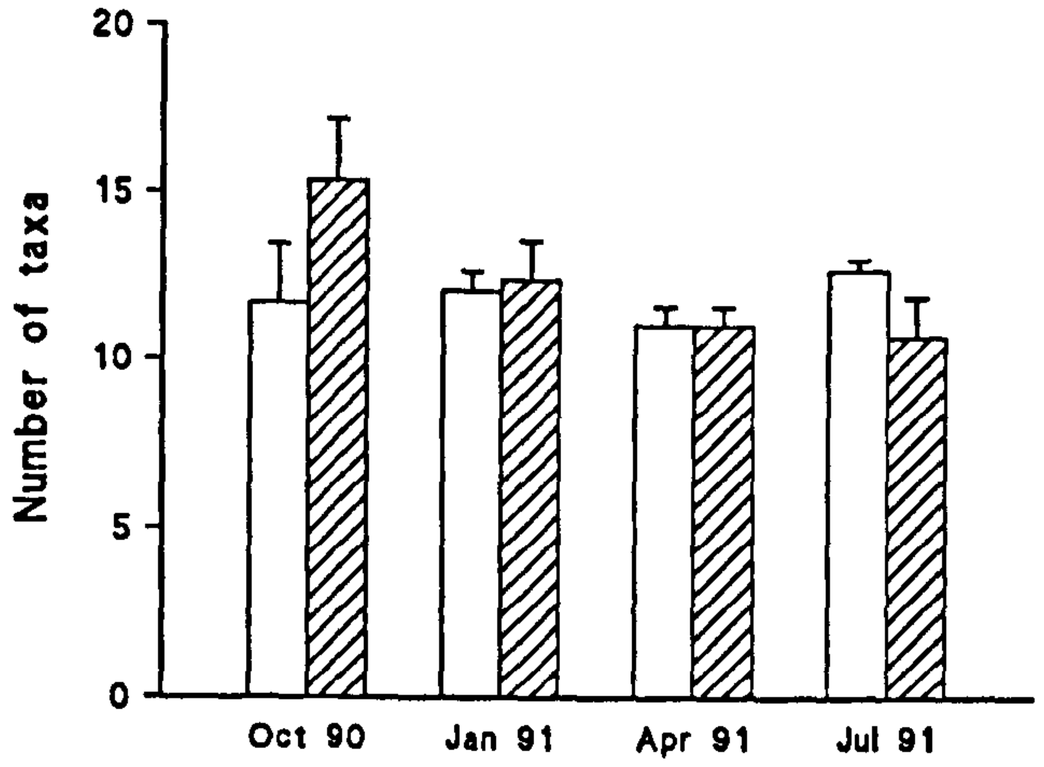
There was no significant between-station or between-sampling occasion differences in the number of taxa at either Rockley Dike or Butterthwaite Ditch (F<1.68, df>7,23, Fig. 2.2.). In contrast, the number of macroinvertebrate taxa was significantly reduced at the downstream station at Pigeon Bridge Brook (F=23.21, df=1,4, Fig. 2.2.). At this site there was a significant reduction in the number of taxa at the downstream station in both January and April (q>5.68, df=14,8) but not in July (q=2.81, df=14,8). Due to low flows at the upstream station at Pigeon Bridge Brook during October only one sample was taken so between-station differences could not be assessed statistically. As with the other two sites there was no seasonal effect on the number of macroinvertebrate taxa present (F<0.89, df>3,6).

Results for diversity (Fishers α) mirrored those for taxon richness. There were no significant between-station or between-sampling occasion differences in diversity at either Rockley Dike or Butterthwaite Ditch (F<0.83, df=5,17, Fig. 2.3.). Scores at Rockley Dike ranged from 3.20 to 3.48 at the upstream station and 2.87 to 3.67 at the downstream station whereas scores at Butterthwaite Ditch ranged from 2.42 to 3.13 at the upstream station and 1.43 to 4.10 at the downstream station. However, macroinvertebrate diversity was significantly reduced downstream of the motorway discharge at Pigeon Bridge Brook (F=18.47, df=1,14, Fig. 2.3.). Scores at the upstream station ranged from 2.3 to 3.4 whereas at the downstream station scores ranged from 1.13 to 1.89. In line with the taxon richness data, there was a significant-between station difference in macroinvertebrate diversity in both January and April (q>5.03, df=14,8) but not in July (q=2.54, df=14,8). Again there was no significant seasonal difference in diversity at this site (F<1.13, df>3,6).

a.)



b.)



c.)

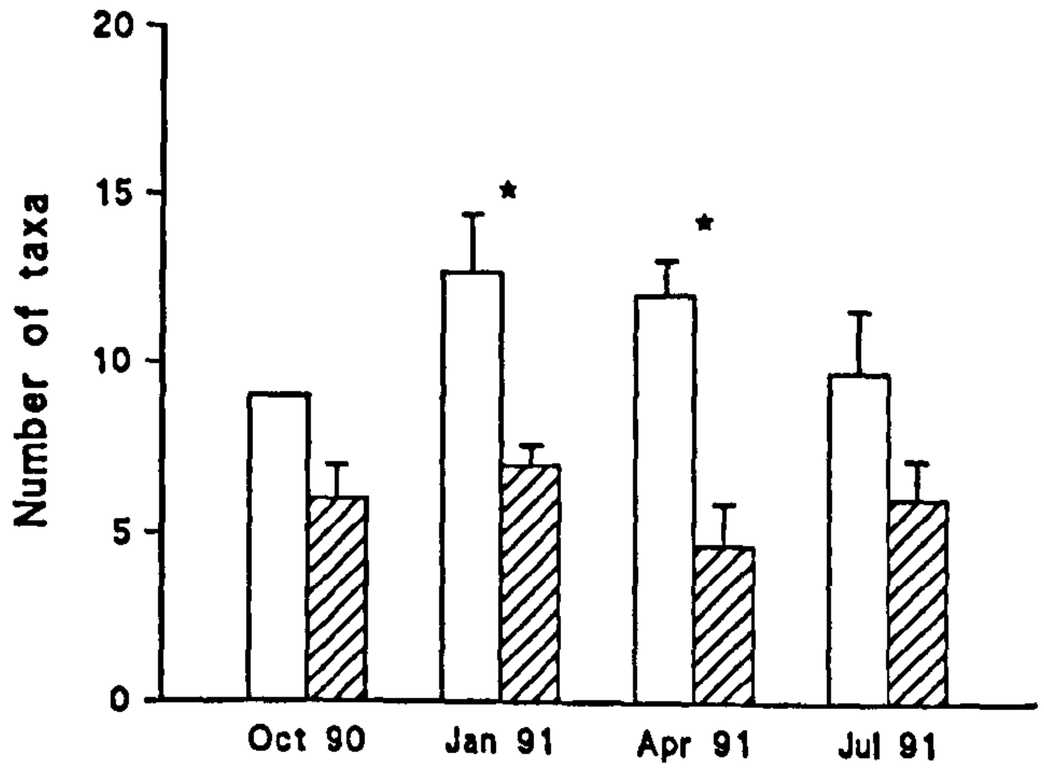
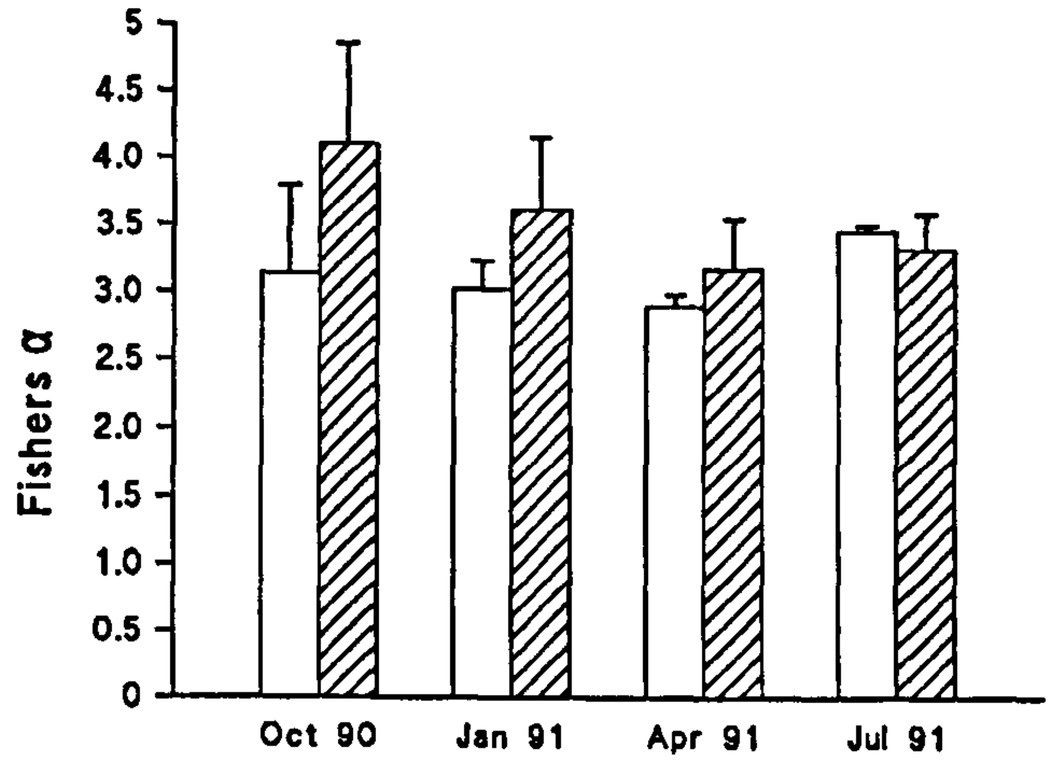


Fig. 2.2. Mean (+1 S.E.) number of macroinvertebrate taxa at a.) Rockley Dike, b.) Butterthwaite Ditch and c.) Pigeon Bridge Brook during the four sampling periods at upstream (☐) and downstream (▨) stations. Asterisk indicates significant between-station differences.

a.)



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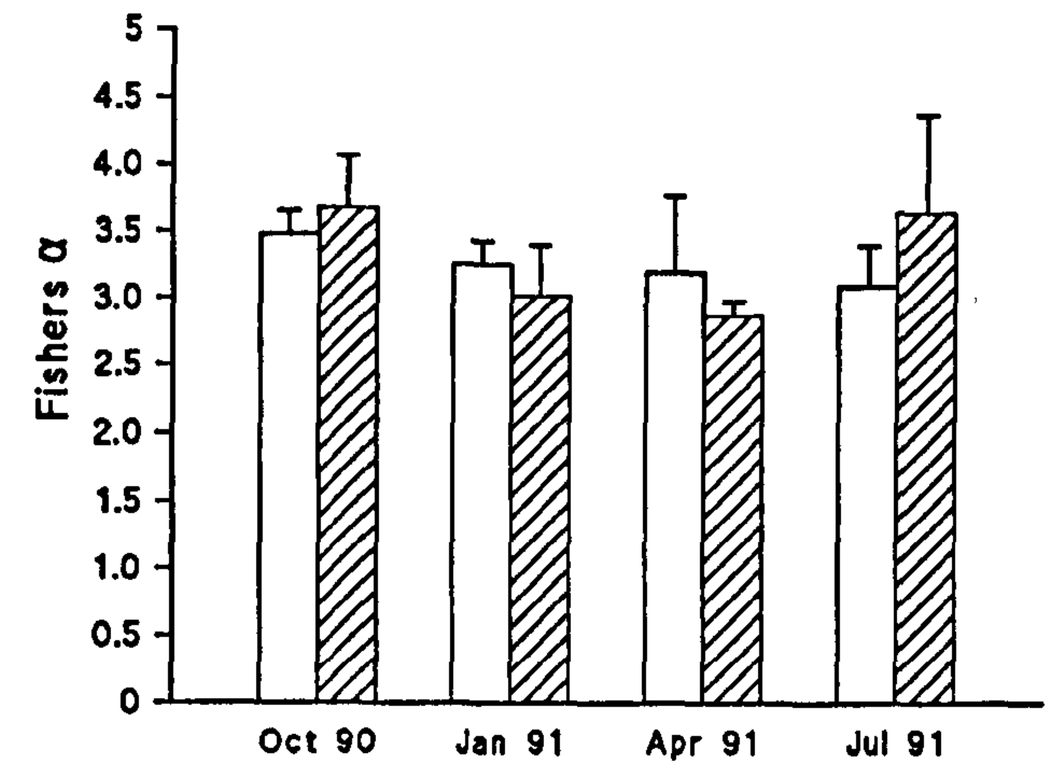

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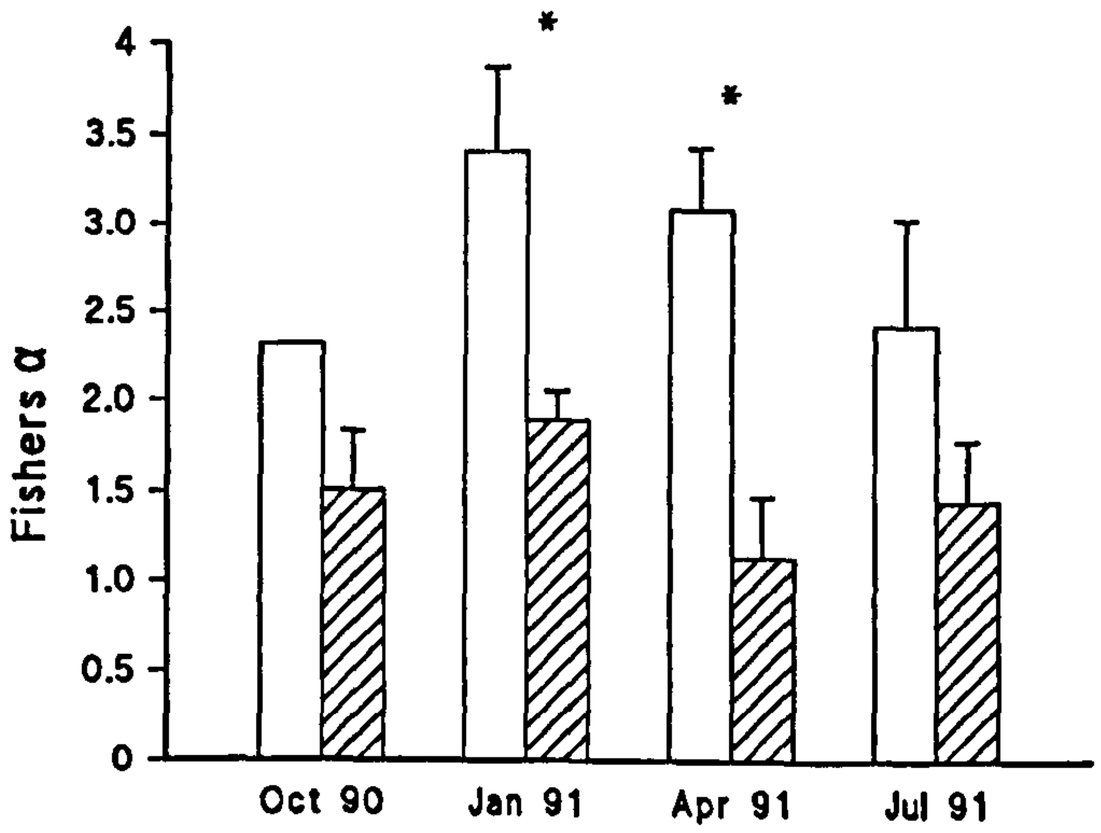


Fig. 2.3. Mean (+1 S.E.) macroinvertebrate diversity (Fishers α) at a.) Rockley Dike, b.) Butterthwaite Ditch and c.) Pigeon Bridge Brook during the four sampling periods at upstream (☐) and downstream (▨) stations. Asterisk denotes significant between-station difference.

Between-station differences in diversity and taxon richness were reflected in the similarity indices. The Bray-Curtis similarity index indicated that between-station differences in community structure were greater at Pigeon Bridge Brook than at either Rockley Dike or Butterthwaite Ditch (Table 2.3.).

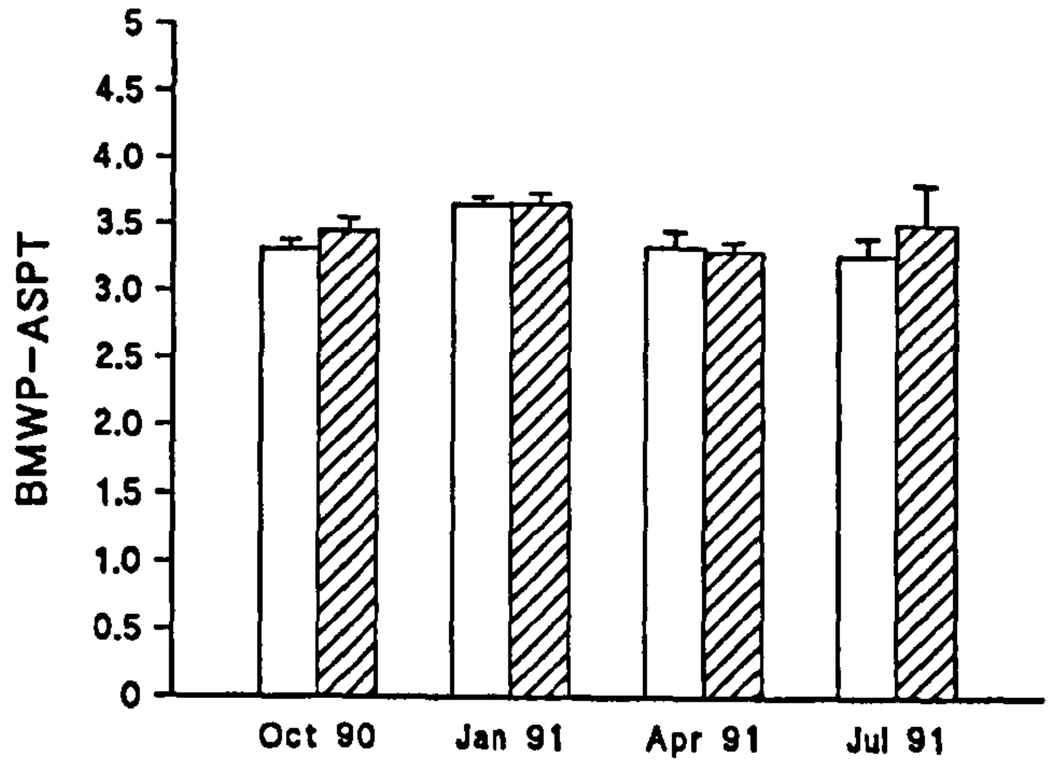
Table 2.3. Between-station similarities in macroinvertebrate community structure (Bray-Curtis similarity index) at the four sampling periods at the three sites. The values range from 0 which are identical communities to 1 which are theoretically totally dissimilar communities.

	October 1990	January 1991	March 1991	July 1991
Rockley Dike	0.37	0.31	0.37	0.39
Butterthwaite Ditch	0.33	0.39	0.34	0.39
Pigeon Bridge Brook	0.86	0.93	0.71	0.81

There were no significant between-station differences in BMWP-ASPT scores (Biological Monitoring Working Party-Average Score Per Taxon) at Rockley Dike or Butterthwaite Ditch (F<2.11 df=7,16, Fig. 2.4.). In contrast, the BMWP-ASPT scores were significantly reduced at the downstream station at Pigeon Bridge Brook compared to the upstream station (F=37.38, df=1,14, Fig. 2.4.). In January and April there were significant (q>5.18, df=14,8) between-station differences in BMWP-ASPT but no significant difference was detected in July (q=3.07, df=14,8). Scores at the upstream station at Pigeon Bridge Brook were generally highest of all stations (upstream stations: Rockley Dike (RD): 3.27 - 3.65, Butterthwaite Ditch (BD): 3.62 - 3.87, Pigeon Bridge Brook (PBB): 4.19 - 4.98), whereas scores at the downstream station at this site were generally the lowest (downstream stations: RD: 3.30 - 3.65, BD: 3.55 - 4.05, PBB: 2.11 - 3.73).

A reduction in the BMWP-ASPT score at the Pigeon Bridge Brook downstream station relative to the upstream station indicates a loss of 'pollution-sensitive' groups downstream of the discharge point. In addition the relative abundance of 'pollution-sensitive' groups present at both stations are reduced below the motorway runoff discharge point (Fig. 2.5.). There was a significant reduction in the relative abundance of stoneflies (S=1, df=1), gammarids (S=4, df=1), ptychopterans (S=4, df=1), caddisflies (S=0.33, df=1) and molluscs (S=4, df=1). In contrast there was significant increase in the relative abundance of 'pollution-tolerant' groups such as chironomids and tubificid worms (S=4, df=1) at the downstream station.

a.)



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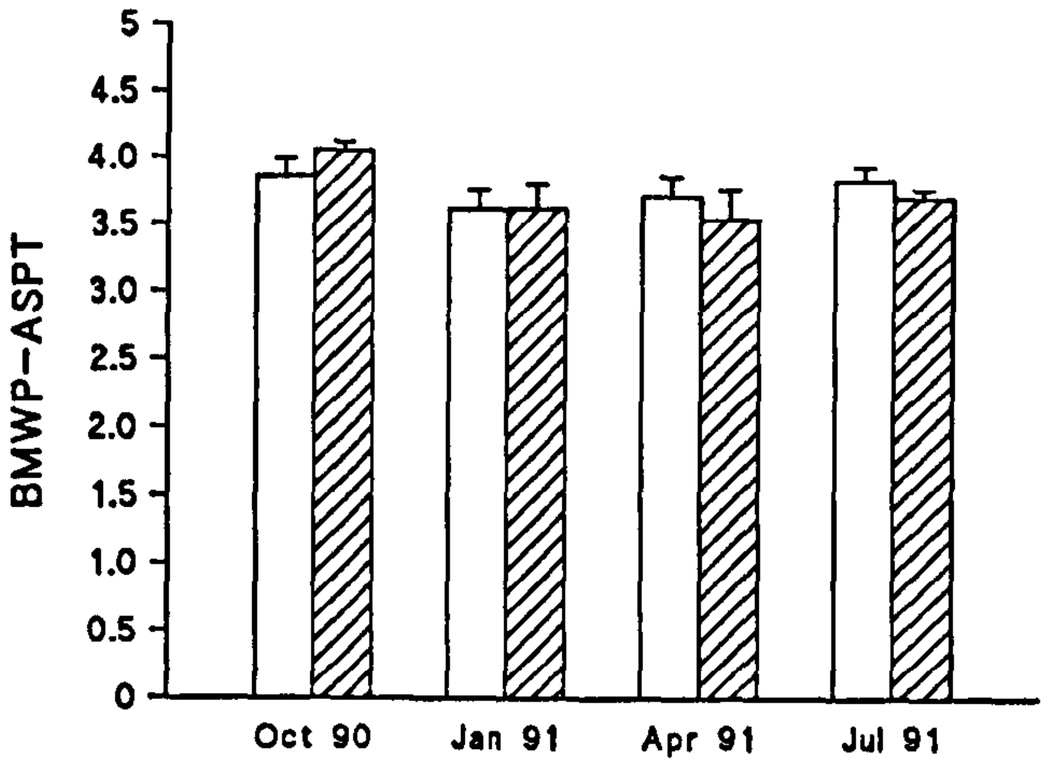

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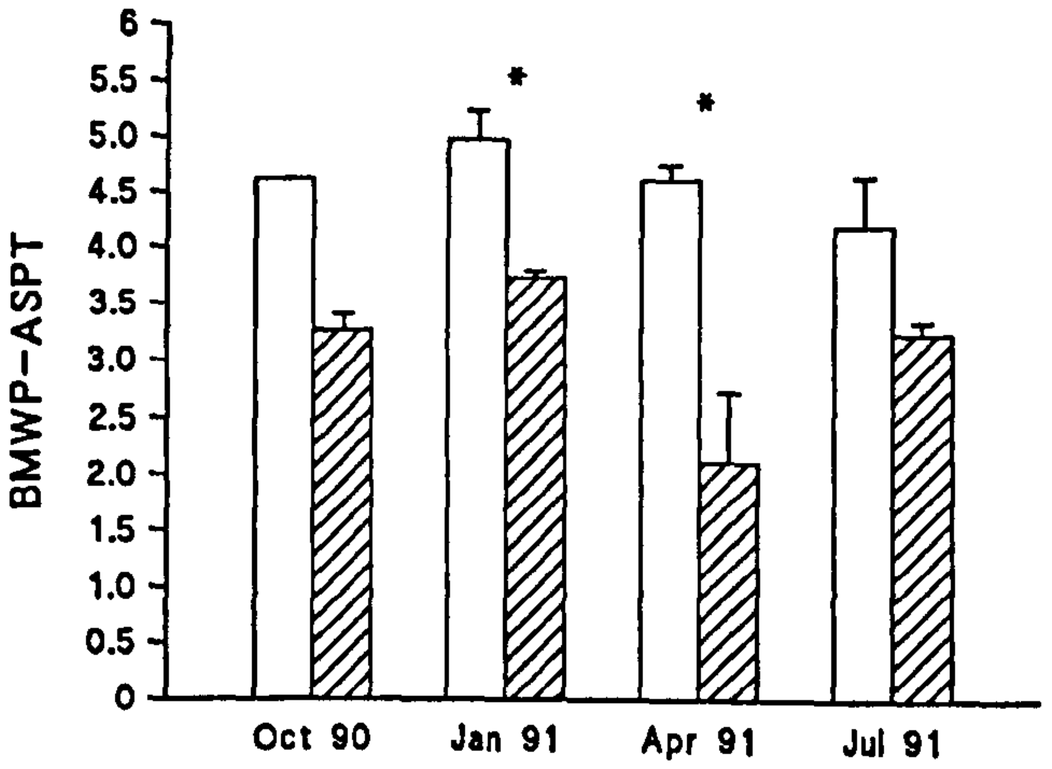


Fig. 2.4. Macroinvertebrate BMWP-ASPT scores (mean and + 1 S.E.) for a.) Rockley Dike, b.) Butterthwaite Ditch and c.) Pigeon Bridge Brook during the four sampling periods at upstream (☐) and downstream (☒) stations. Asterisk denotes significant between-station differences.

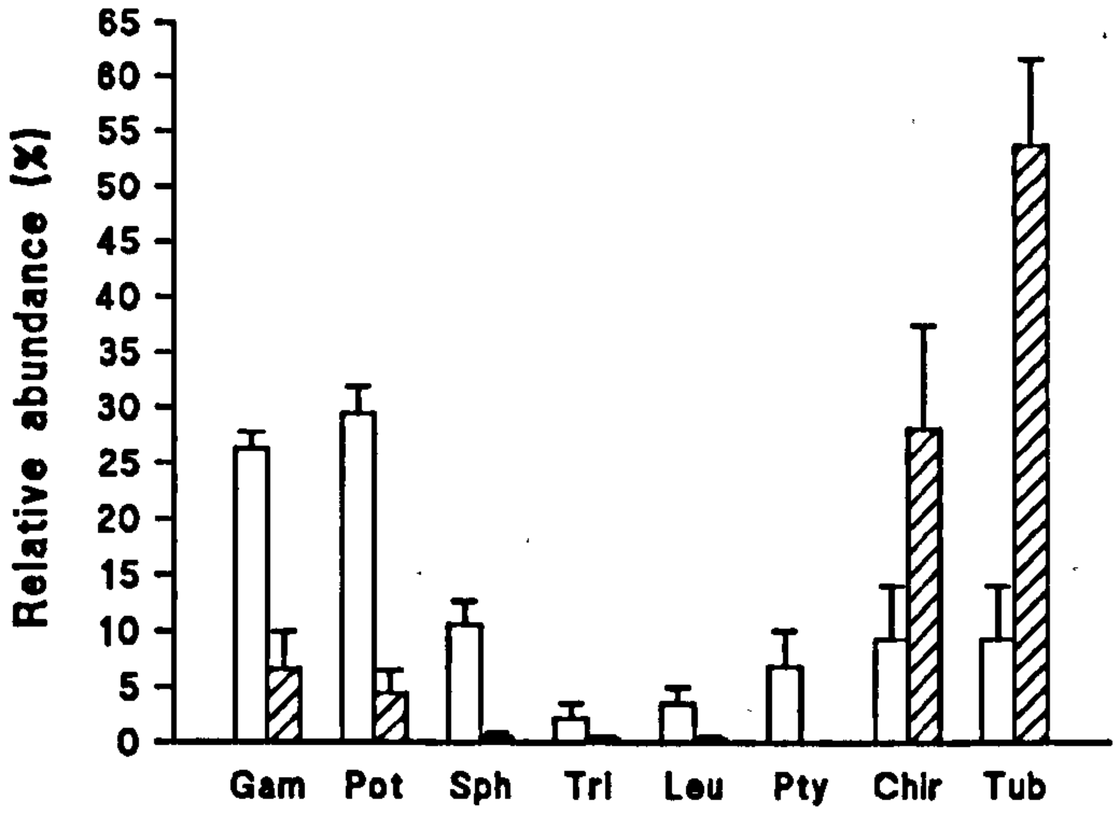


Fig. 2.5. The mean relative abundance (+ 1 S.E.) of selected macroinvertebrates at upstream (□) and downstream (▨) stations at Pigeon Bridge Brook. Gam: *Gammarus pulex* (L.); Pot: *Potamopyrgus jenkinsi* (Smith); Sph: Sphaeriidae; Tri: Trichoptera; Leu: Leutridae; Pty: Ptychoptera; Chir: Chironomidae; Tub: Tubificidae.

Functional feeding group analysis indicated that changes in macroinvertebrate assemblages were associated with changes in functional properties of the community (Fig. 2.6.). At Pigeon Bridge Brook there was a significant decrease in relative abundance of shredder and scraper macroinvertebrates (S=4, df=1) and a significant increase in the relative abundance of collectors (S=4, df=1) downstream of the discharge. There was also a non-significant increase in the relative abundance of predators at the downstream station at this site. At Rockley Dike and Butterthwaite Ditch there were no significant between-station differences in any functional feeding group (S<1, df=1), the only exception being an increase in the relative abundance of predators downstream of the discharge at Butterthwaite Ditch (S=4. df=1).

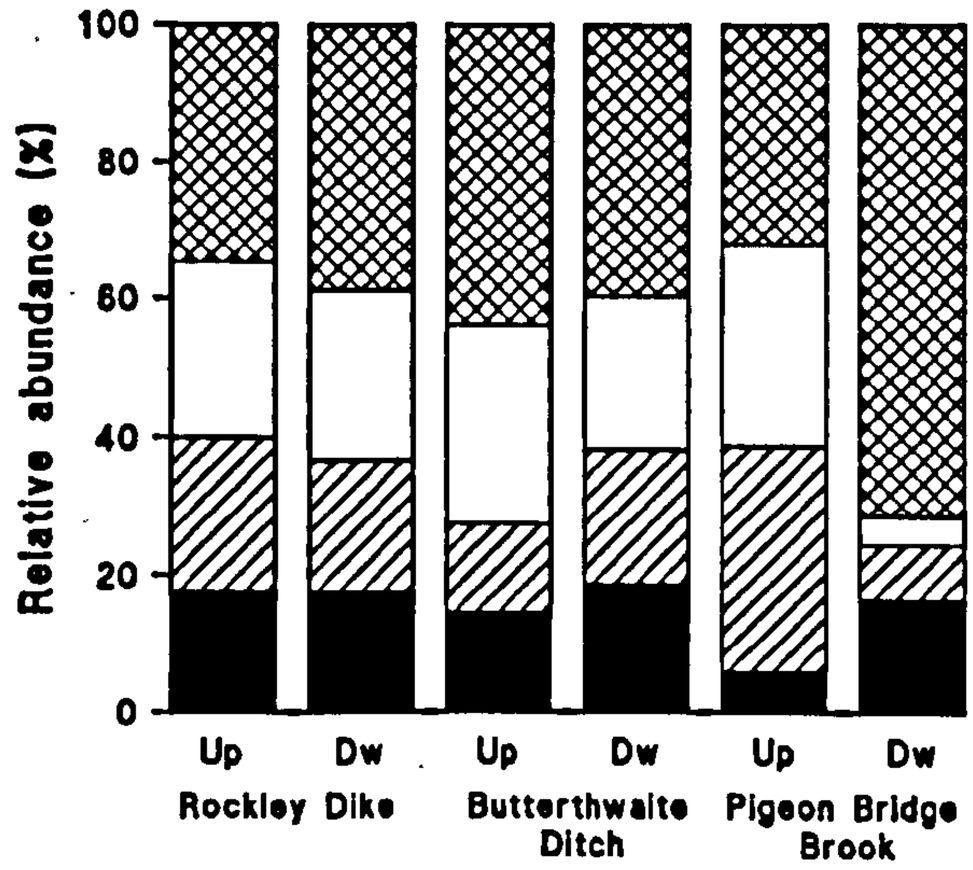


Fig. 2.6. Mean percentage relative abundances of predator (■), shredder (▨), scraper (□) and collector (⊠) functional feeding groups upstream (Up) and downstream (Dw) of the motorway runoff input at the three sites.

Significant seasonal effects were observed on macroinvertebrate functional feeding groups at many of the stations and these are summarised in Table 2.4. The relative abundance of predators generally peaked in July, shredders peaked in October-January, scrapers in October and collectors in April.

Table 2.4. Seasonal changes of macroinvertebrate feeding groups. Asterisk indicates a significant seasonal change (Kruskal-Wallis $H>7.94$, df=3), ns indicates no significant seasonal change and Up=upstream, Dw=downstream.

	Rockley Dike		Butterthwaite Ditch		Pigeon Bridge Brook	
	Up	Dw	Up	Dw	Up	Dw
Predators	*	ns	*	ns	ns	*
Shredders	*	*	*	ns	ns	ns
Scrapers	ns	*	*	ns	ns	ns
Collectors	*	*	*	*	ns	ns

2.3.4. Aquatic hyphomycete community structure.

The fungal communities at the three sites were very diverse with a total of twenty-nine different species of aquatic hyphomycete being identified (Appendix A2.3; Table A2.3.1). No significant between-station or between-site differences in species richness were apparent ($F<0.75$, df>2,24, Fig. 2.7.), however, similarity indices applied to relative importance values (RIVs) indicated that the communities at the Pigeon Bridge Brook stations were less similar ($C_N = 0.37$) than those at either Rockley Dike ($C_N = 0.20$) or Butterthwaite Ditch ($C_N = 0.18$) stations. The lower between-station similarity at Pigeon Bridge Brook can partly be explained by a significant increase in community diversity (Fishers α) below the discharge at this site (t=3.85, df=6; Fig. 2.7). No differences were recorded in species diversity at either Rockley Dike or Butterthwaite Ditch ($t<1.12$, df>7).

RIV scores indicated that *Tetracladium marchalianum* de Wildeman, *Lemonniera terrestris* Tubaki, *Heliscus lugdenensis* Sacc. and Therry and *Alatospora acuminata* Ingold were dominant at all stations (Appendix A3.2, Table A3.2.1). Although no species showed a consistent pattern of upstream or downstream bias in their distribution, *Anguillospora longissima* (de Wildeman) Ingold, *Tricladium angulatum* Ingold and *Articulospora tetracladia* Ingold showed reduced relative abundances at downstream stations at Pigeon Bridge Brook and Rockley Dike. Whereas *Tricladium splendens* Ingold, *Tricelophorus sp.*, *Culicidospora aquatica* Peterson and *Tricladium*

varium Jones and Stewert showed increased relative abundances at the downstream stations at these sites.

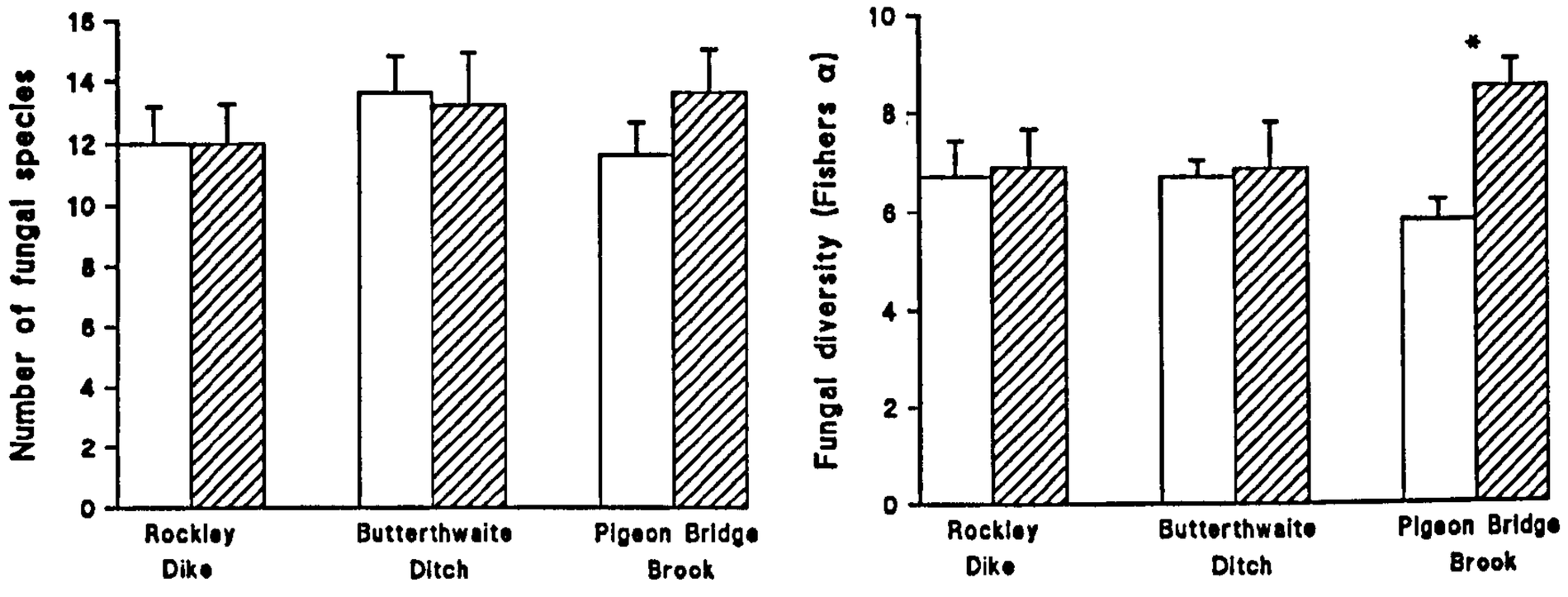
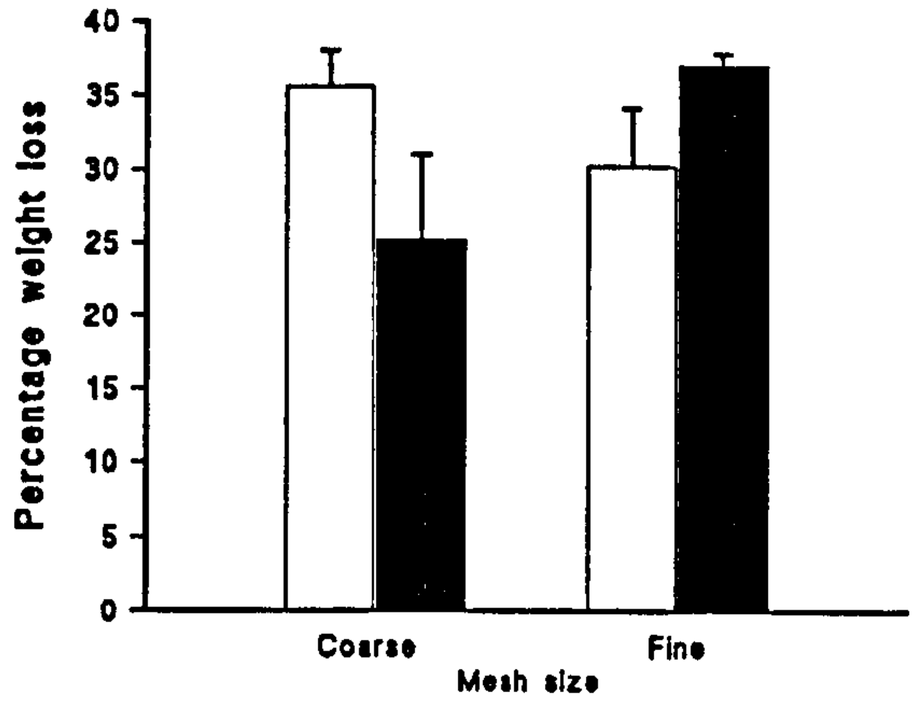


Fig. 2.7. Number of fungal species (a.) and fungal diversity (b.) on leaf material deployed at upstream (☐) and downstream (▨) stations at the three sites. Data displayed as mean +1 S.E.. Asterisk denotes significant between-station difference.

2.3.5. Leaf litter processing.

Leaf material was deployed at upstream and downstream stations at Rockley Dike, Butterthwaite Ditch and Pigeon Bridge Brook for 28 days. Coarse- and fine-mesh leaf bags were used to assess macroinvertebrate- and microbial-mediated leaf decomposition respectively. There were no significant between-station differences in the loss of leaf material from fine mesh bags at any of the three sites (F=0.93, df=1,24, Fig. 2.8) suggesting that motorway runoff did not influence microbial decomposition of CPOM. Neither were there any between-station differences in weight loss from coarse mesh bags at Rockley Dike or Butterthwaite Ditch suggesting macroinvertebrate leaf breakdown was also unaffected by motorway runoff at these sites (t<1.8, df>4, Fig. 2.8). In contrast however, there was a significant reduction in the loss of leaf material from coarse mesh bags deployed at the downstream station at Pigeon Bridge Brook, indicating that motorway runoff inhibited macroinvertebrate-mediated leaf decomposition at this site (t=3.23, df=7, Fig. 2.8).

a.)



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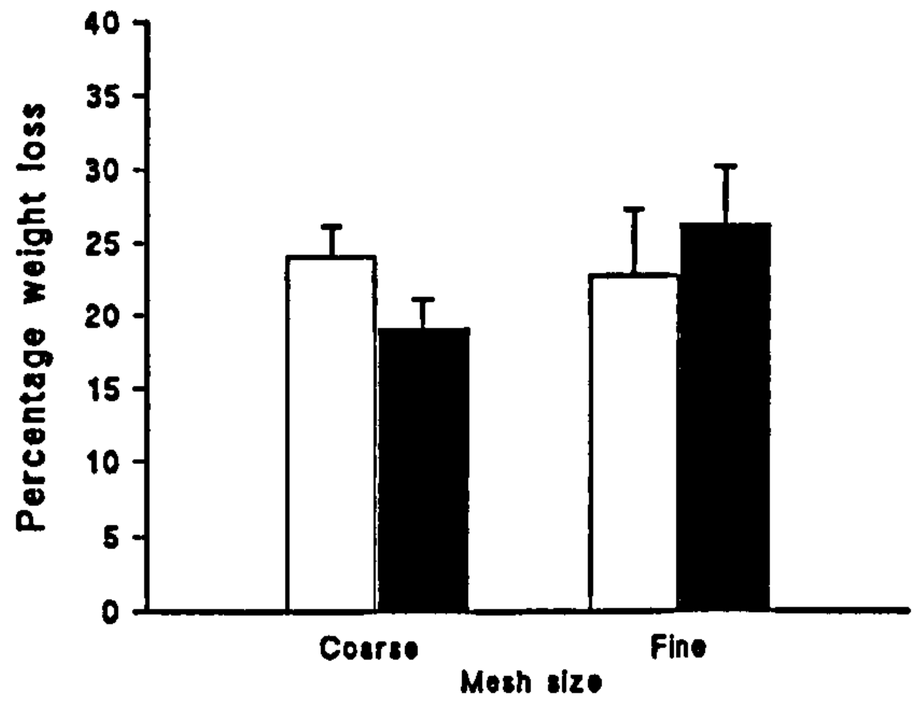

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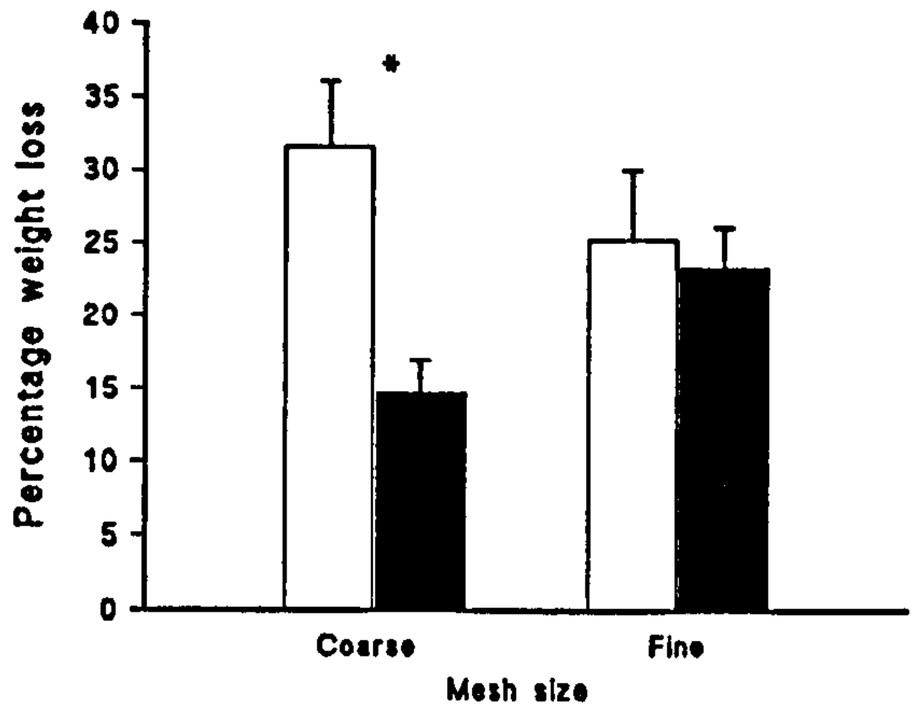


Fig. 2.8. Percentage loss of leaf material from coarse (4-mm) and fine (1-mm) mesh bags at upstream (□) and downstream (■) stations at a.) Rockley Dike, b.) Butterthwaite Ditch and c.) Pigeon Bridge Brook. Data presented as mean (+1 S.E.) and asterisk denotes significant between-station differences.

B. Further work at Pigeon Bridge Brook.

2.3.6. Macroinvertebrates and aquatic hyphomycetes associated with leaf material.

Macroinvertebrates colonising leaf bags deployed at the upstream station were dominated by *Potamopyrgus jenkinsi* and *Gammarus pulex*, whereas leaf bags deployed at the downstream station were dominated by chironomids and oligochaetes (Table 2.5). The abundance of *P. jenkinsi*, *G. pulex* and tipulids in both alder and hawthorn leaf bags were significantly reduced at the downstream station (S=4.0, df=1, Friedman test, station blocked by time). The number of chironomids was significantly elevated in hawthorn leaf bags deployed at the upstream station (S=4 df=1). Tubificidae were only found on upstream alder and downstream hawthorn leaf material although neither of these observations were statistically significant (S<2, df=1). There were no significant

within-station differences between leaf species for any macroinvertebrate species (S<2.25, df=1).

Table 2.5. Mean number (1 S.E.) of selected macroinvertebrate taxa per bag of on alder and hawthorn leaf material deployed at upstream and downstream stations at Pigeon Bridge Brook.

	Alder		Hawthorn	
	Upstream	Downstream	Upstream	Downstream
Gammarus pulex	6.50 (1.63)	0.05 (0.05)	5.55 (2.34)	0.00 (0.00)
Potamopyrgus jenkinsi	12.50 (4.61)	0.15 (0.05)	10.45 (3.97)	0.00 (0.00)
Tipulidae	0.65 (0.17)	0.00 (0.00)	0.30 (0.10)	0.00 (0.00)
Tubificidae	0.05 (0.05)	0.00 (0.00)	0.00 (0.00)	0.20 (0.14)
Chironomidae	0.65 (0.21)	0.15 (0.09)	0.80 (0.21)	0.00 (0.00)

Pythium spp. (Peronosporales, Phycomyceteae) and twenty four species of aquatic hyphomycetes were found on leaf material deployed at Pigeon Bridge Brook during this survey (Appendix A2.3., Table A2.3.2 and Table A2.3.3). There were no significant between-station differences in the number of fungal species colonising alder leaf material over the deployment period (F=0.15, df=1,32). However, there was a significant decrease in the number of species on hawthorn leaves deployed at the downstream station (F=75.62, df=1,32, Fig. 2.9.). At the upstream station significantly more species were recorded on hawthorn compared to alder (F=6.92, df=1,32,) but there was no difference between leaf species at the downstream station (F=0.0, df=1,32).

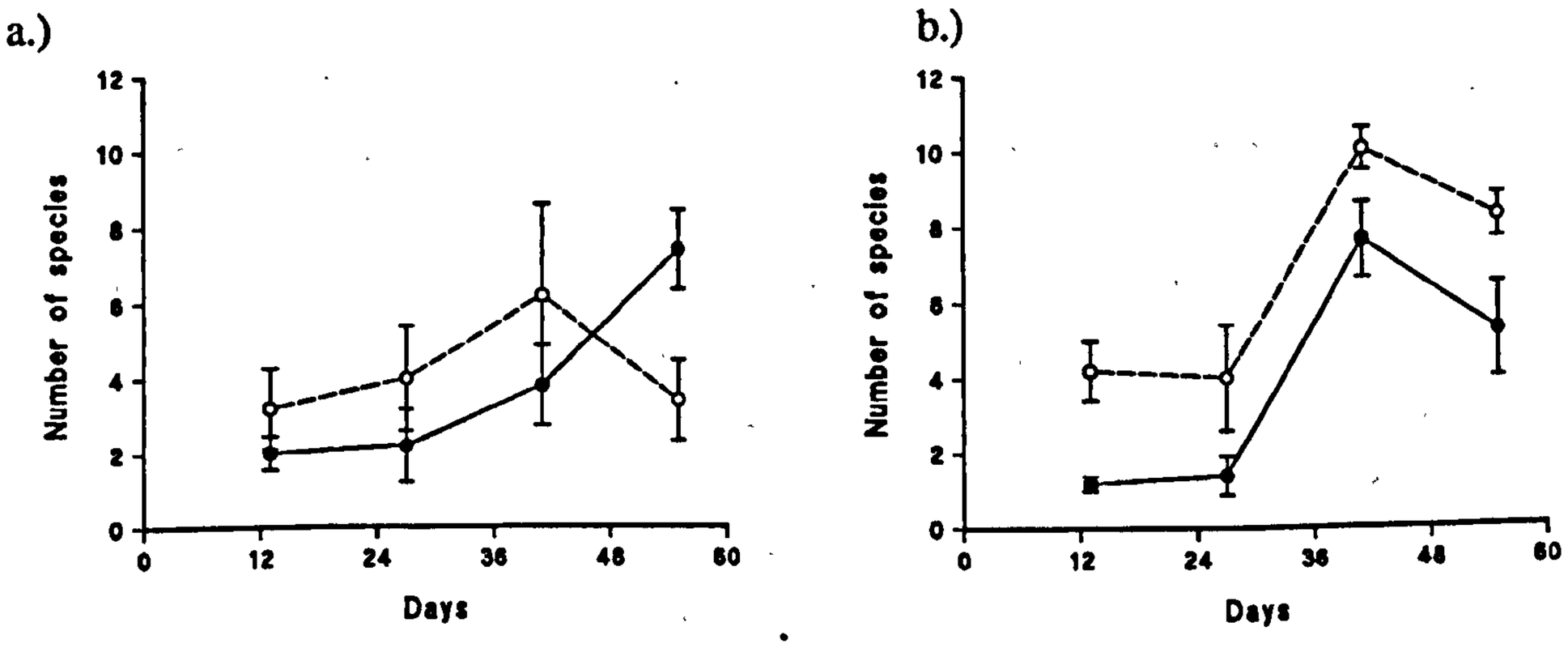


Fig. 2.9. Number of aquatic hyphomycete fungal species on a.) alder and b.) hawthorn leaf material at upstream (O— -O) and downstream (●——●) stations at Pigeon Bridge Brook. Data presented as means ± 1 S.E.

There was no between-station difference in fungal diversity (Shannon index, *H*) on alder leaf material at Pigeon Bridge Brook (*S*=1.00, df=1; Fig. 2.10) over the deployment period. However, there was a significant reduction in diversity on hawthorn leaf material at this site over the same period (*S*=4 df=1). As with species richness, species diversity at the downstream station was low early in the deployment period relative to the upstream station but then either approaches (hawthorn) or exceeds (alder) richness at the upstream station later in the deployment period. There was no significant difference in species diversity between leaf species at either upstream or downstream stations (*S*=1.0, df=1).

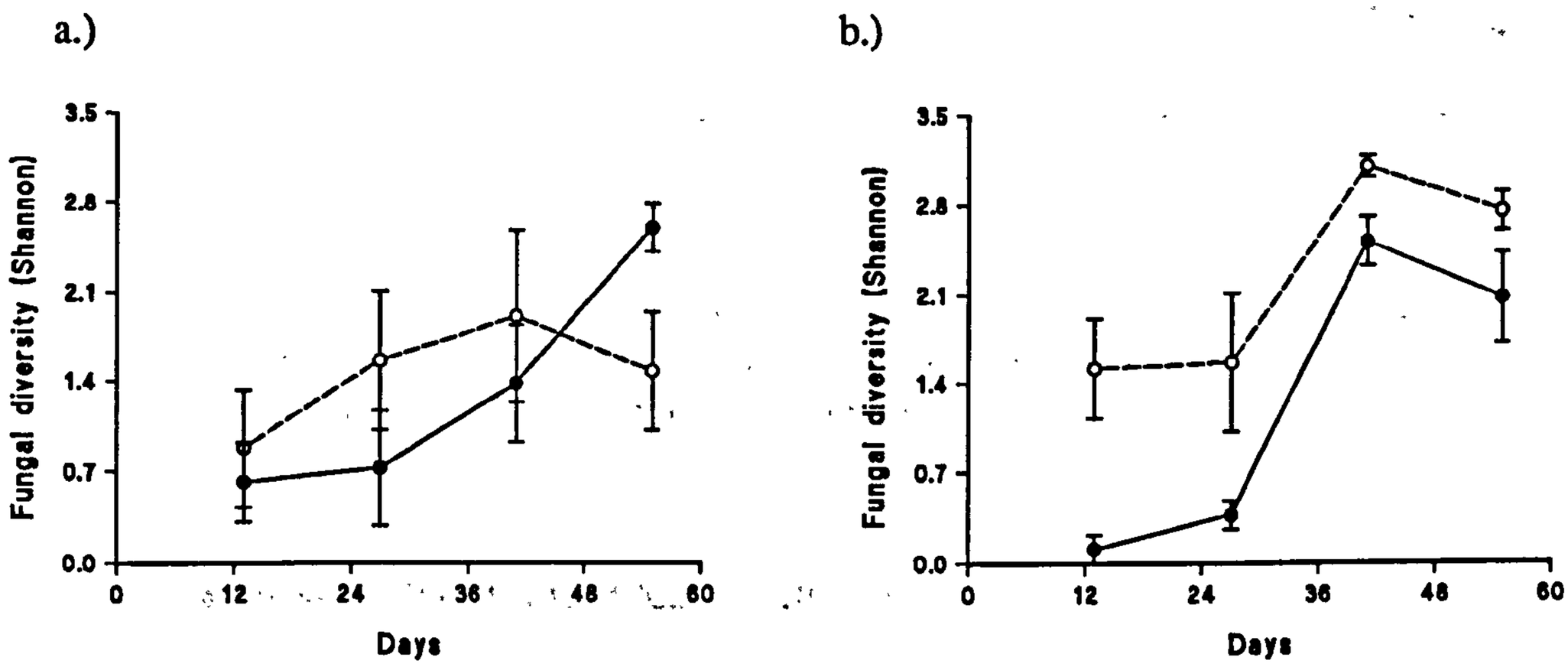


Fig. 2.10. Aquatic hyphomycete fungal species diversity (Shannon index) on a.) alder and b.) hawthorn leaf material at upstream (O— O) and downstream (●—●) stations at Pigeon Bridge Brook. Data presented as means ± 1 S.E..

Generally taxa richness and diversity on the alder and hawthorn leaf material increased over the 55 day experimental deployment period. Relative importance values of fungi on the leaf material indicated that *Pythium* spp. dominated the assemblages on both alder and hawthorn at upstream and downstream stations particularly in the early stages of decomposition (Fig. 2.11). However, at both stations *Pythium* spp. were rapidly replaced by aquatic hyphomycete species such as *A. crassa* and *T. angulatum*, the replacement being more rapid at the upstream station (Fig. 2.11). *Dendrospora sp.* was only found on upstream alder and hawthorn leaf material. This species was also only found upstream at this site in the preliminary survey (section 2.3.4). Although present on alder leaf material downstream of the motorway discharge *Scorpiosporium minutum* Iqbal, *Tetrachaetum elegans* Ingold, and *V. aquatica* were only found on hawthorn at the upstream station. *Clavatospora longibrachiata* (Ingold) Nilsson ex Marvanova and

Nilsson, *Tricelophorus sp.* and two unidentified species were only found on hawthorn leaf material at the upstream station. *Varicosporium* sp., however was only found on hawthorn and only at the downstream station.

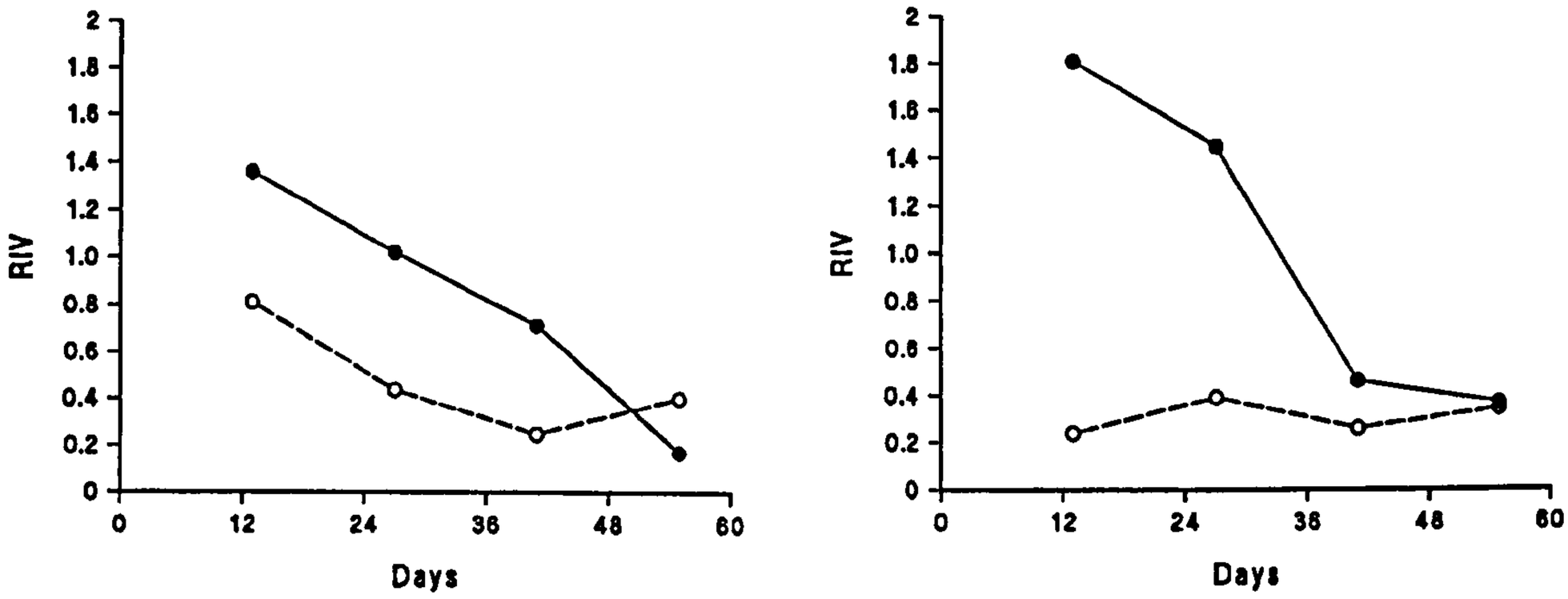


Fig 2.11. Relative importance value of *Pythium spp.* on a.) alder and b.) hawthorn leaf material at upstream (O— -O) and downstream (●——●) stations at Pigeon Bridge Brook. The RIV total for all species on each sampling occasion is 2.

2.3.8. Assessment of fungal biomass (ergosterol) on leaf material.

The calibration curve for ergosterol concentration against absorbance at 282 nm had a correlation coefficient of r=0.996 (df=3). Although the number of sporulating species present on leaf material increased with deployment period for most time periods and treatments (section 2.2.6) there was no significant difference in ergosterol concentration and hence fungal biomass (S<1, df=1, Fig. 2.12.). There was, however, a marked but non-significant increase in ergosterol concentration on alder material deployed at the downstream station on the last sampling occasion (U=3.0, n=2,3). There were no significant between-station differences in fungal biomass (determined by ergosterol concentrations) on the leaf material (S<1, df=1) although ergosterol concentrations on hawthorn were significantly higher than on alder at the upstream station (U=143, n=11,9).

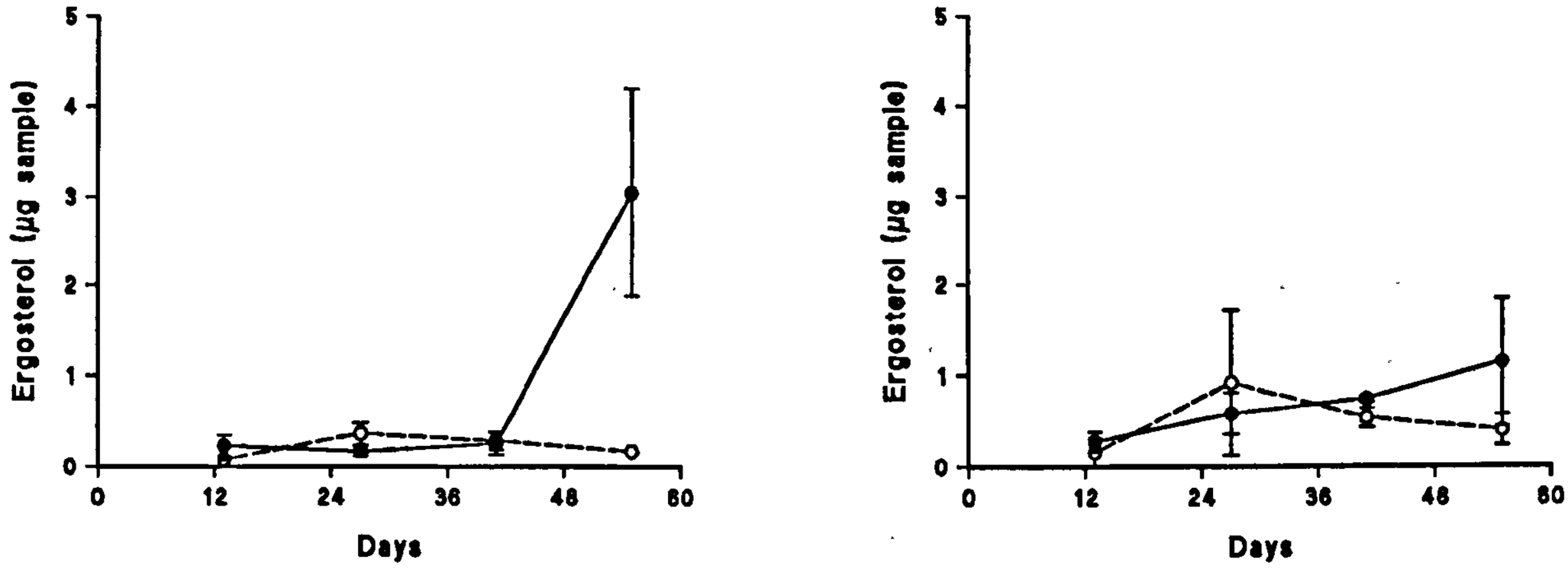


Fig. 2.12. Ergosterol concentrations (mean ±S.E.) on a.) alder and b.) hawthorn leaf material at upstream (○— ○) and downstream (●—●) stations at Pigeon Bridge Brook. Data presented as means ± 1 S.E..

2.3.9. Microbial activity on leaf material.

In all cases respiration rates on downstream incubated material were significantly higher than the corresponding upstream treatment (t>1.91, df>26; Fig 2.13.). In general, between-leaf species differences were not significantly different (t<1.88, df>31) the only exception being significantly higher fungal respiration rates for hawthorn incubated at the downstream station (t=2.27, df=32).

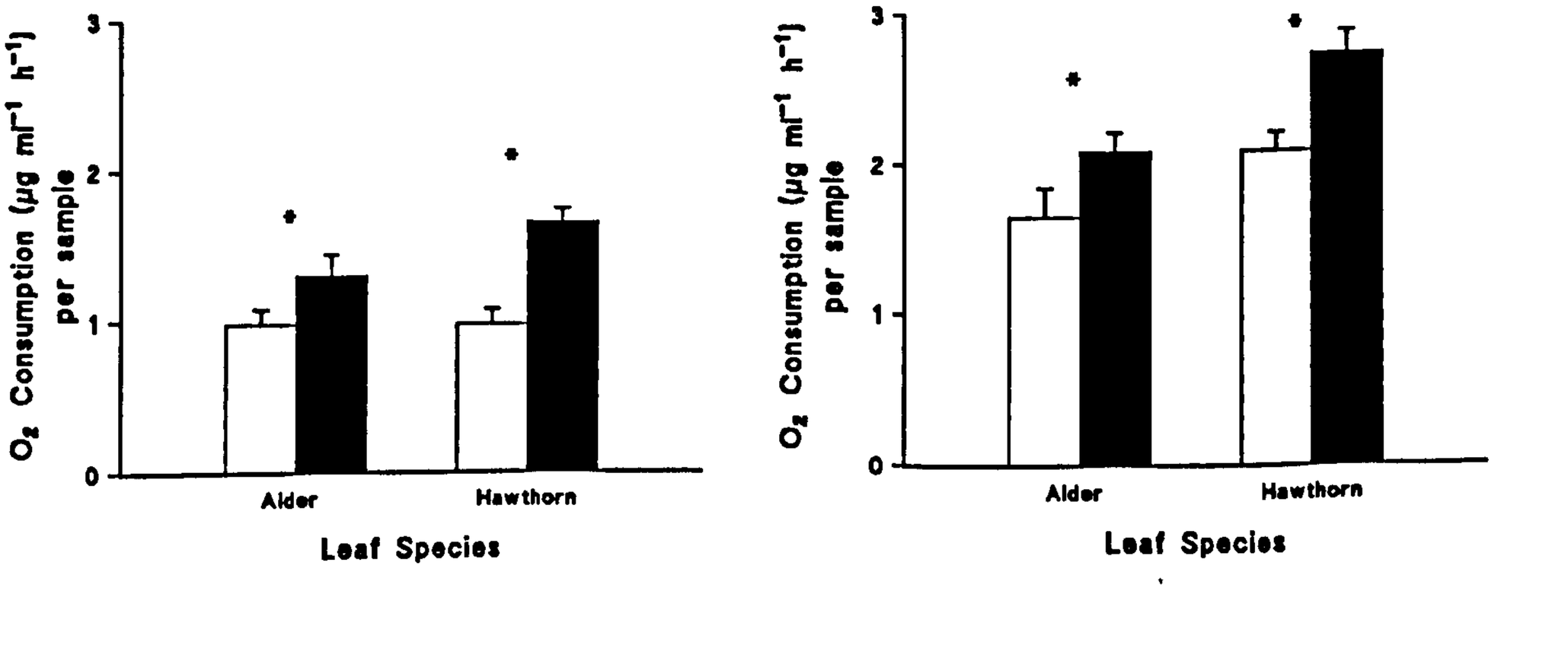


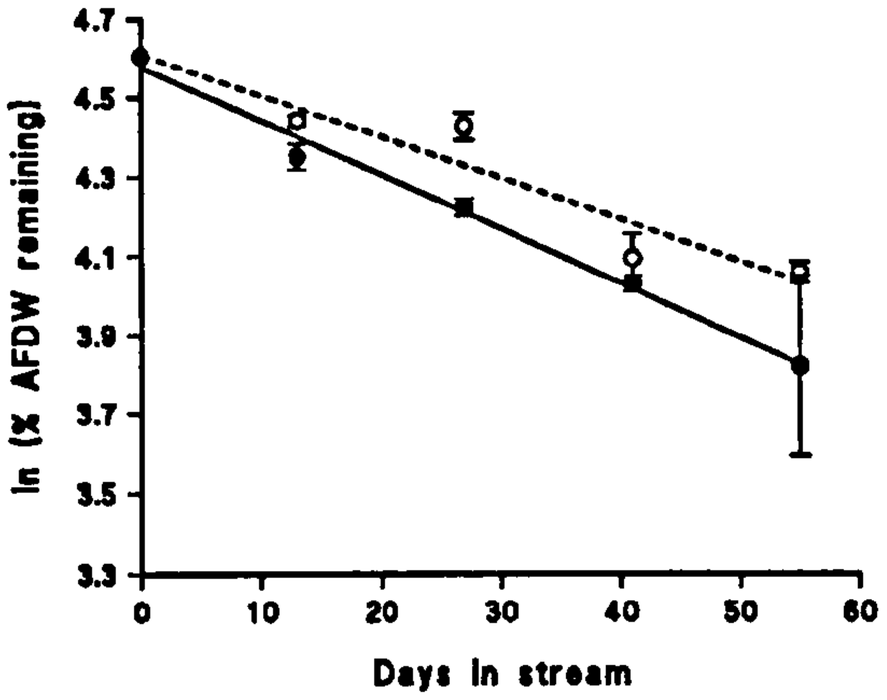
Fig. 2.13. Fungal (a.) and total (b.) respiration on alder and hawthorn leaf material at upstream (□) and downstream (■) stations at Pigeon Bridge Brook. Data presented as means (+ 1 S.E.). Asterisk denotes significant between-station differences.

2.3.10. Leaf decomposition.

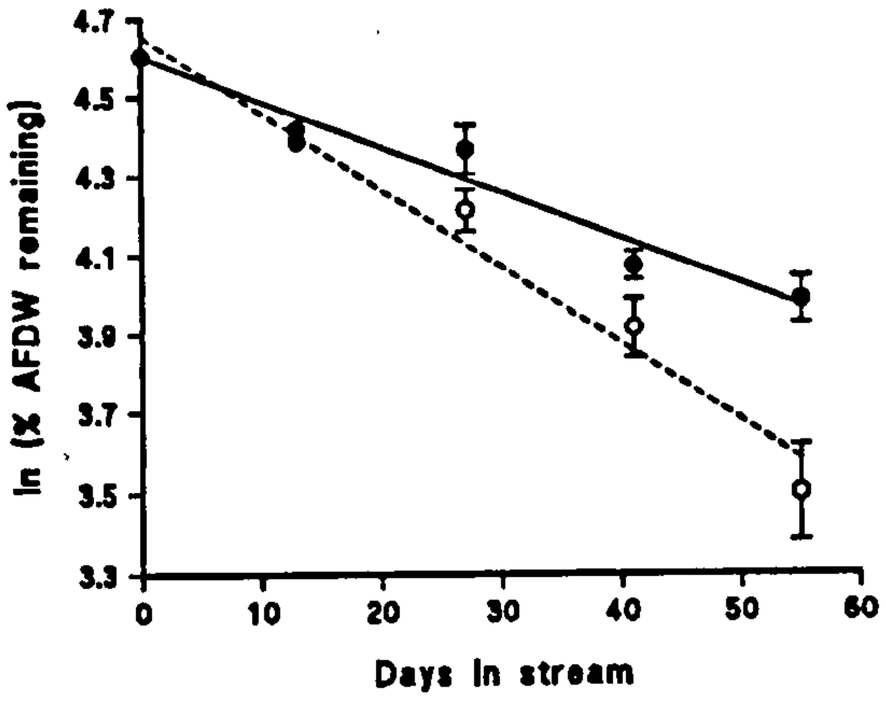
Alder and hawthorn leaf material was deployed at both stations at Pigeon Bridge Brook in either coarse mesh or fine mesh leaf bags. Decomposition rates were determined from the slope of the relationship between time and the natural logarithm of percentage AFDW remaining.

There was a significant correlation between these two variables for leaf material in both the fine and coarse mesh bags at each of the stations (r>0.90, df=3, Fig. 2.14). There was no significant between-station difference in the mass loss of either alder or hawthorn in the fine mesh bags (ANCOVA, F<1.28, df>1,33). Nor was there any significant difference in the mass loss of hawthorn leaf material in coarse mesh bags between stations (ANCOVA, F=2.59 df=1,36). There was, however, a significant between-station difference in mass loss of alder in the coarse mesh bags (ANCOVA, F=11.24 df=1,35) and this was reflected in a significantly reduced decomposition rate at the downstream station in these bags (Fig. 2.15). In all treatments decomposition rates of alder were significantly higher than those of hawthorn (ANCOVA, F>6.3, df>1,34), greatest decomposition rates being for alder deployed at the upstream station in coarse mesh bags (ANCOVA, F>7.4, df>1,36). These data support the results of the preliminary survey (section 2.3.5).

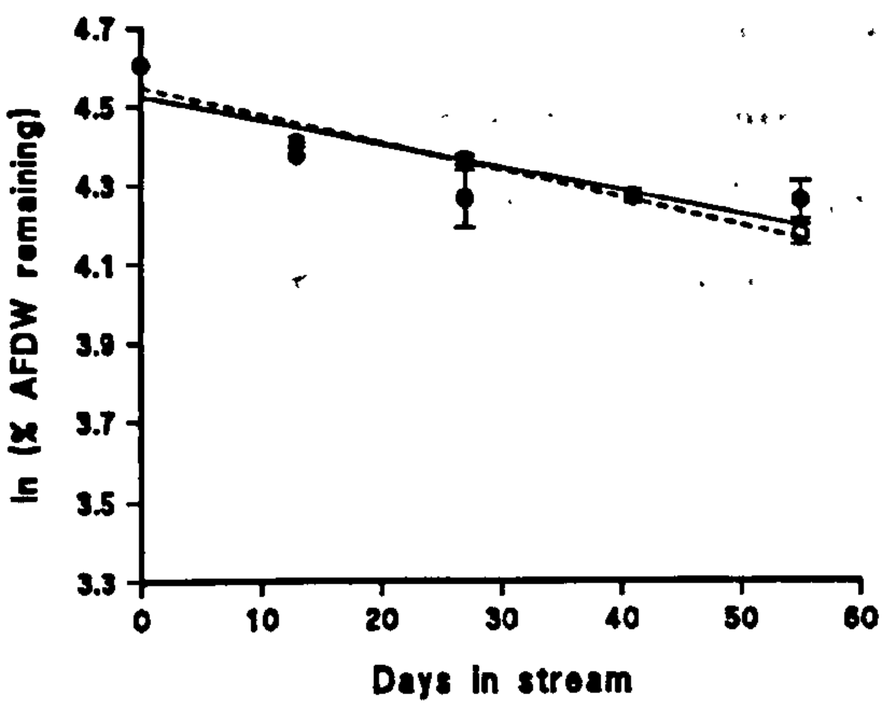
a.i.)



a.ii.)



b.i.)



b.ii.)

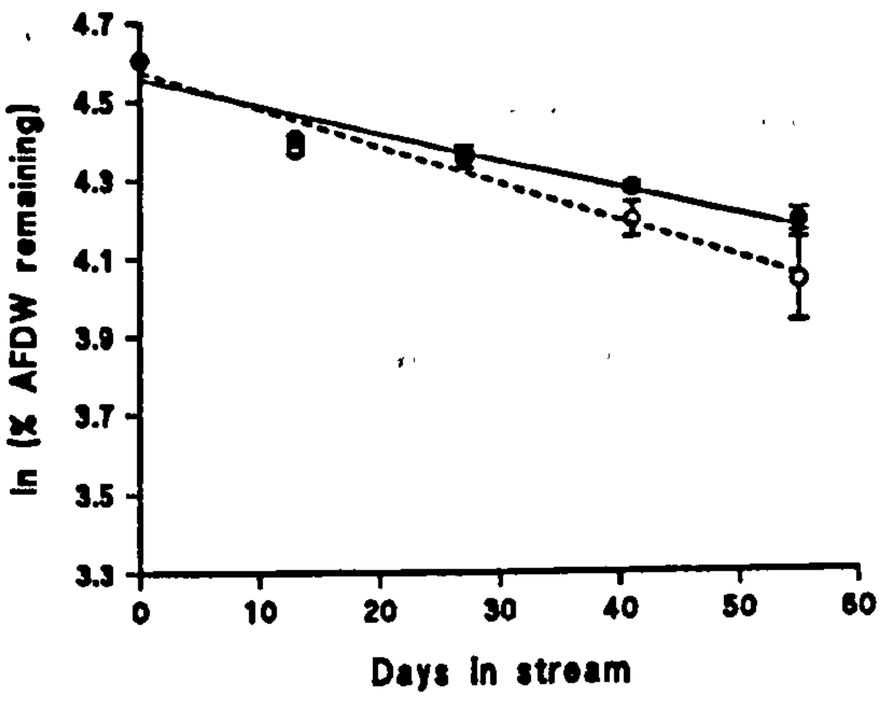


Fig. 2.14. Weight loss of a.) alder and b.) hawthorn leaf material in either i.) fine or ii.) coarse mesh bags incubated at upstream (O— -O) and downstream (●——●) stations at Pigeon Bridge Brook. Data presented as means ± 1 S.E..

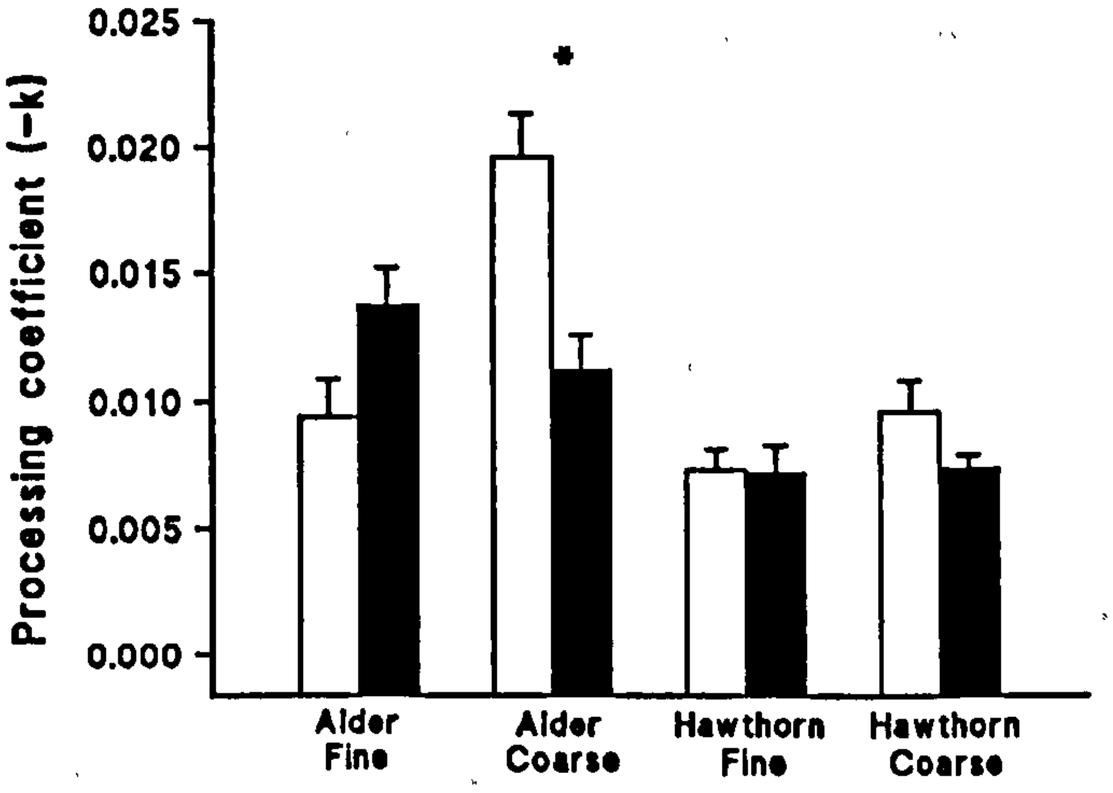


Fig. 2.15. Decomposition coefficients (+ 1 standard deviation) of alder and hawthorn leaf material in fine and coarse mesh bags at the upstream (□) and downstream (■) station at Pigeon Bridge Brook. Asterisk denotes significant between-station difference.

2.4. DISCUSSION.

Previous road runoff studies have suggested that pollutant input and subsequent pollutant loads in streams are dependent on a number of factors including area of road drained, traffic volume and size of receiving water body (Chapter 1). Pigeon Bridge Brook was the smallest stream in the current study (1 m wide x 0.02 m deep) which received runoff from the largest area of motorway (44,389 m^2). Although Butterthwaite Ditch was the same width (but 0.05 m deep) it only drained 38,479 m^2 of motorway whereas Rockley Dike was 3 m wide and 0.14 m deep and drained only 26,633 m^2 of motorway.

All three sites were contaminated with vehicle related pollutants and, as predicted, the greatest between-station differences were observed at Pigeon Bridge Brook (Maltby *et al.*, 1995a). Sediment at the downstream stations had significantly elevated concentrations of aromatic hydrocarbons in general and PAHs in particular. Total aromatic hydrocarbons were significantly elevated in the sediments at downstream stations at all three sites but were below detection levels in the stream waters (Maltby *et al.*, 1995a). The dominant PAHs at Pigeon Bridge Brook were pyrene (10.16 µg/g wet wt.), fluoranthene (3.2 µg/g wet wt.) and phenanthrene (5.62 µg/g wet wt) which is in agreement with the findings of Evans *et al.* (1990). Downstream stations were also heavily contaminated with metals, most of which were associated with the sediment (Maltby *et al.*, 1995a). At Pigeon Bridge Brook there were significantly elevated concentrations of zinc (342.8 µg/g dry wt.), cadmium (2.58 µg/g dry wt.), lead (142.3 µg/g dry wt.) and chromium (98 µg/g dry wt.) in sediments at the downstream station. Although concentrations of metals were generally lower in the stream waters significantly elevated concentrations of calcium (140.94 mg/L), magnesium (77.7 mg/L) and copper (47.7 µg/L) were measured in water downstream of the motorway discharge at Pigeon Bridge Brook.

Sediment at stations below the motorway discharges also differed in terms of total organic carbon (TOC; Maltby *et al.*, 1995a) Concentrations of TOC were elevated at the upstream station at Pigeon Bridge Brook (11.6% SE = 2.9 v 2.3% SE = 0.2 downstream) and at the downstream station at Rockley Dike (8.53% SE = 0.3 v 2.73% upstream). The TOC of sediment at Butterthwaite Ditch were very similar between stations (2.44% SE = 0.5 upstream; 1.93% SE = 0.2 downstream). Sediments at Rockley Dike and Pigeon Bridge Brook were coarser downstream of the discharge relative to the corresponding upstream stations. In contrast there was no between-station difference in sediment particle size at Butterthwaite Ditch. This is contrary to several road runoff studies which have found that road dust inputs in the drainage

discharges generally reduce mean particle sizes downstream of the discharge (e.g. Extence, 1978; Borchardt and Statzner, 1990). Finally, at Pigeon Bridge Brook conductivity and salinity were both increased downstream of the discharge, probably as a result of the significant increase in the concentrations of sulphate and chloride ions at this station.

It is evident that stations downstream of the motorway discharge at the three sites had elevated concentrations of motorway runoff derived pollutants. Therefore there is the potential for these chemicals to have a negative impact on the structure and function of biotic communities. Biological field studies were therefore performed to assess the possible impact of the motorway contamination on the biota of receiving waters concentrating on benthic macroinvertebrates and detritus processing.

The main source of detrital material in the three streams studied were autumn-shed leaves. This material is processed by both microorganisms, in particular, aquatic hyphomycetes, and shredder macroinvertebrates (Chapter 1). The results of preliminary surveys indicated that there were no significant between-station differences in the microbial decomposition of alder leaf material at any of the sites. Neither were there any between-station differences in macroinvertebrate-mediated decomposition at Rockley Dike or Butterthwaite Ditch. There was, however, a significant reduction in macroinvertebrate-mediated decomposition of alder leaf material at the downstream station at Pigeon Bridge Brook. However, the decomposition of hawthorn leaves was not reduced at the downstream station. Hawthorn is amongst a family which have leaves with a thick cuticle and high concentrations of secondary plant metabolites which generally reduces leaf decay rates and provides a poor quality food source to macroinvertebrates (Suberkropp, 1993). This may explain why decomposition rates of hawthorn were consistently lower than those of alder at both upstream and downstream stations and why a significant inhibition of macroinvertebrate-mediated decomposition was not apparent at the downstream station.

The decomposition data were obtained using leaf bags prepared using oven-dried leaf material. The use of dried leaf material for decomposition studies has recently been criticised as higher leaching and hyphomycete colonisation rates have been observed in dried leaf material when compared to fresh material (Bärlocher, 1991; Boulton and Boon, 1991). It has also been suggested that as macroinvertebrate activity may enhance the entry of fungi to leaf litter (Hasija and Singhal, 1991) protecting leaf material in fine mesh bags may reduce fungal decomposition. Moreover, leaf material in the bags may become silted and anaerobic thus inhibiting decomposition. However, detrital material

generally occurs as leaf accumulations and if care is taken when constructing and deploying bags to minimise these factors then the leaf bag method would seem appropriate. The leaf bag method is widely adopted enabling cross-stream comparisons to be made and, as with this study, it is a useful method for investigating relative decomposition rates and to identify the importance of different communities in the decomposition process (Boulton and Boon, 1991).

Given that there was a significant inhibition of macroinvertebrate-mediated decomposition at the downstream station at Pigeon Bridge Brook, what is the mechanism? There are two non-mutually exclusive explanations. Firstly the structure of the macroinvertebrate community was altered below the discharge or secondly the feeding activity of the macroinvertebrates was reduced downstream of the discharge. Changes in the feeding activity of the shredding macroinvertebrates may be a consequence of direct effects of the discharge on feeding and/or an effect on food quality. A reduction in food quality may be a consequence of the accumulation of toxicant on the leaf material and/or an inhibition of the conditioning of leaf material by microbes which makes it less palatable to macroinvertebrates.

Macroinvertebrate surveys were conducted at four periods over a year. Only at one of the three sites studied, Pigeon Bridge Brook, was there a significant difference in the macroinvertebrate communities upstream and downstream of the motorway discharge. At this site, there was a reduction in macroinvertebrate species richness and diversity at the downstream station which was reflected in a high community dissimilarity between the two stations. Biotic scores were also reduced at the downstream station and this was mainly a result of a loss of caddisflies, beetles and dipteran groups (e.g. Tipulidae, Dixidae, Empididae). Species generally considered sensitive to pollutants (Hellawell, 1986) such as the stonefly *Leutra inermis*, the gammarid *Gammarus pulex*, caddisflies and molluscs were reduced in relative abundance below the discharge while more tolerant groups such as chironomids and oligochaetes increased in relative abundance. The trophic structure of the macroinvertebrate community at the Pigeon Bridge Brook also changed between upstream and downstream stations. There was a reduction in the relative abundance of shredders and scrapers below the discharge and an increase in the relative abundance of collectors. Similar effects, in either community structure or trophic characteristics, were not observed at either Rockley Dike or Butterthwaite Ditch.

The changes in community structure observed between upstream and downstream stations at Pigeon Bridge Brook could be due to several factors including changes in the

nature of the substrate as a consequence of sediment inputs from the motorway (Extence, 1978; Hellawell, 1986). Most aquatic organisms have specific preferences for particular sediment particle sizes. For instance, stoneflies and caddisflies prefer coarser sediment particle sizes whereas hydrobiid snails are fairly insensitive to particle size and sphaeriidae, tubificids and chironomids prefer finer sediments (Armitage, 1994). In addition, the abundance and diversity of macroinvertebrate communities in a stream are influenced by the stability and organic detrital content of the sediment (Allan, 1995). Several motorway-runoff studies have reported similar changes in community structure as observed in this study, i.e. reductions in diversity and increases in fine sediment dwellers below the discharge, and have attributed this to finer substrates below the motorway (e.g. Extence, 1978). However, although the substrate was coarser downstream of the discharge at Pigeon Bridge Brook, the relative abundance of fine sediment dwellers, such as oligochaetes and chironomids, increased and animals which generally prefer coarser sediments, such stoneflies and caddisflies, decreased. Previous work on macroinvertebrate substrate preferences (Cummins and Lauff, 1969; Allan, 1995) would suggest that the particle size of the substrate, *per se,* was not responsible for the macroinvertebrate community changes observed at Pigeon Bridge Brook. While TOC concentrations were lower at the downstream station than those at the upstream station the values were similar to that at Butterthwaite Ditch and Rockley Dike (Maltby *et al.*, 1995a). Concentrations of TOC were within the 'normal' range for stream sediments which support diverse macroinvertebrate communities and were not considered to be the determinand for the observed changes in community structure (Burton, 1992a). However, substrate stability and the input of quantities of suspended material which may smother animals and their food sources could have been one of the factors responsible for the observed changes in macroinvertebrate structure and function (Hogg and Norris, 1991).

The structure of macroinvertebrate communities varies both spatially and temporally (Cummins, 1974; Vannote *et al.*, 1980; Hawkins and Sedell, 1981; Bunn *et al.*, 1986). The relative abundance of shredders would be expected to increase during periods of high inputs of allochthonous material in the autumn (leaf fall) and late spring (flowering) and the relative abundance of scrapers will increase in early summer when autotrophic activity is high. These patterns were generally observed in this study. On a spatial scale, the influence of the riparian canopy may affect trophic composition between stations. However, changes in the nature of the canopy cannot explain between-station differences in the relative abundance of shredders at Pigeon Bridge Brook. At this site the canopy at the downstream station was more developed and consisted of more palatable leaf species than the upstream station (Petersen and

Cummins, 1974; Anderson and Sedell, 1979). However, the relative abundance of shredders declined downstream of the motorway discharge. Increased shading downstream would potentially reduce autotrophic growth decreasing the food source and therefore relative abundance of scrapers. However, the significant reduction in the relative abundance of scrapers cannot be attributed to the loss of the primary food source of these organisms, as both algal biomass and species diversity increased at the downstream station (Maltby *et al.*, 1995a). Previous studies have also shown that algal abundance and diversity increase downstream of road runoff discharges (Dussart, 1984), either as a result of reduced grazing pressure (O'Brian and Dixon, 1976; Miller *et al.*, 1979; Dussart, 1984) or direct stimulation of the algae, possibly by oil products in the discharge (Graf and Nowak, 1966; Walker *et al.*, 1975; Gjessing *et al.*, 1984a; Werner *et al.*, 1985a).

Although there were between-station differences in habitat characteristics at Pigeon Bridge Brook these cannot explain macroinvertebrate community differences recorded at this site. Such changes may therefore be the result of toxic contaminants in the discharge. For instance, Medeiros *et al.* (1983) attributed the effects of urban runoff to toxic contamination, mostly originating from the sediments. It was evident that all three streams were contaminated by a complex 'cocktail' of chemicals including metals and hydrocarbons and this was related, in part, to the area of motorway drained and the size of receiving water body. Whereas some authors have suggested road runoff has minor biological effects in receiving waters (Smith and Kaster, 1983) others have demonstrated significant changes in macroinvertebrate community structure (e.g. Cowley, 1985). In a laboratory study lake sediments contaminated with metals, PAHs and polychlorinated biphenyls (PCBs) were acutely toxic to the amphipod *Hyallela azteca* and the chironomid *C. riparius* (Ingersoll and Nelson, 1990). Furthermore, in a wider survey of seven streams receiving motorway drainage waters including the three streams described in the current study there was a negative, though non-significant, correlation between the number of macroinvertebrate families represented at a sampling station and the concentrations of aromatic hydrocarbons in the sediment (r=-0.67, df=12, Maltby *et al.*, 1995a; Appendix A2.4).

The abundance of individual species recorded in the current study was related to hydrocarbon and metal concentrations in the sediment. The mean abundance of *P. jenkinsi* and *G. pulex* at each sampling stations was negatively correlated with sediment aromatic hydrocarbon concentrations (r<-0.79, df=4, Appendix A2.4). The mean number of *G. pulex* was also negatively correlated with sediment lead concentrations (r=-0.79, df=4, Appendix A2.4). In contrast, there was no correlation between sediment

chemistry and the abundance of chironomids but there was a weak positive correlation between the abundance of tubificids and sediment aromatic hydrocarbon concentrations (r=0.61, df=4). There is, therefore, some evidence, albeit equivocal, that contaminants in motorway runoff are toxic to benthic macroinvertebrates and could therefore be responsible for the changes in community structure observed at Pigeon Bridge Brook. This possibility will be investigated in more detail in the following chapters.

The possible effect of motorway runoff on the quality of leaf material as a food for shredders was assessed by investigating the structure, biomass and activity of fungi colonising leaf material. The number of species of aquatic hyphomycetes recorded during this study was high compared to most previous studies (e.g. 29 compared to a usual 4-10; Suberkropp, 1992). Generally hyphomycete surveys have been performed in fast flowing upland streams (Bärlocher, 1993). It is possible that in small lowland streams the influence of stream-side soils, terrestrial plants, more diverse substrates (i.e. crop litter) as well as aquatic plants may provide a potential additional source of hyphomycete fungi (Bandoni, 1981; Park, 1974; Singh and Musa, 1977; Sridhar and Kaveriapa, 1987; Thomas *et al.*, 1992; Sridhar and Bärlocher, 1993).

The initial hyphomycete survey at all sites indicated that there were no significant between-station or between-site differences in aquatic hyphomycete species richness on alder leaf material. However, between-station community similarity was lowest at Pigeon Bridge Brook and this was reflected in higher fungal diversity at the downstream station. In addition the relative importance of some species differed between stations at this site. More detailed surveys at Pigeon Bridge Brook confirmed that there were no significant between-station differences in species richness on alder. However, in contrast to the initial survey, no between-station difference in the diversity of fungi on alder leaves was detected. Moreover, both hyphomycete species richness and diversity were significantly reduced on hawthorn leaves deployed at the downstream station. Colonisation of alder and hawthorn leaf material by hyphomycete fungi at the downstream station appeared to lag behind the upstream station over the 13-41 days deployment. However, after 55 days both hyphomycete species richness and diversity downstream exceeded that at the upstream station on alder but not on hawthorn. At the upstream station significantly more species of fungi were recorded on hawthorn compared to alder, but there were no between-leaf species differences in species number at the downstream station.

Estimates of fungal biomass associated with the leaf material can be obtained by using either direct microscopy (estimates of biovolume followed by biovolume to carbon

conversion), glucosamine or ergosterol assays (Newell, 1992). Using ergosterol as an index of biomass, there were no between-station differences in fungal biomass on either alder or hawthorn leaf material at Pigeon Bridge Brook. Although ergosterol has several advantages over direct microscopy and the glucosamine assay in that it is more specific to living fungal biomass and is less subject to extraction inaccuracies its use as a measure of fungal biomass in multi-species systems has recently been criticised (Paggett and Posey, 1993; Bermingham *et al.*, 1995). Studies on single species isolates have shown that ergosterol levels vary between-species and also within-species where levels depend on the age of cultures and on the nutritional growing conditions of the fungi (Newell *et al.*, 1987; Padgett and Posey, 1993; Bermingham *et al.*, 1995). However, taking these reservations into account, the results in the current study suggest that there was little difference in the structure or biomass of the fungal assemblage colonising alder leaf material at the two sampling stations. Although there were fewer fungal species sporulating on hawthorn deployed at the downstream station, the fungal biomass appears to be similar at both stations. The final aspect that was investigated was microbial activity.

Microbial activity on leaf material at Pigeon Bridge Brook determined by respirometry was similar to previous studies (Groom and Hildrew, 1989; Bermingham, 1993). Total and fungal respiration downstream of the discharge was significantly higher on both alder and hawthorn leaf material indicating elevated activity at this station. The effect of pollutants on microbial biomass and respiration rates has been shown to depend on the nature of the pollutant itself. For instance acid pollution (Allard and Moreau, 1986; Palumbo *et al.*, 1987; Garden and Davies, 1989), metal pollution (Bermingham, 1993), and pentachlorophenol (a wood preservative; Fairchild *et al.*, 1983) were shown to decrease microbial respiration rates on leaf material. Whereas, hydrocarbon pollution has often been found to increase microbial respiration on contaminated leaf material (Werner *et al.*, 1984a); an observation which is consistent with the results presented here. The increase in microbial activity below the discharge was not reflected in an increase in leaf decomposition rates. This was probably because many of the microbes were not utilising the leaf material as an energy source but were using it as a substrate for attachment (Goulder and Baker, 1991). Populations of microbes that mineralise organic pollutants (i.e. oils) often greatly expand in abundance in polluted areas (e.g. Griffiths *et al.*, 1982; Sayler *et al.*, 1982) but little is known of the effect of organic pollutants on the normal resident microbial communities and their functions (Sheehan *et al.*, 1984). It is possible that the hydrocarbons act as an additional nutrient source to the resident leaf decomposing community. It is worth noting that several previous studies have indicated that changes in microbial community structure, biomass and activity in

lake systems heavily contaminated with hydrocarbons have resulted in reduced decomposition of leaf material (McKinley *et al.*, 1982; Werner *et al.*, 1984 a, b; Catallo and Gambrell, 1987).

In summary, therefore, from the results of the field study there is no evidence to suggest that either the structure or functioning of the fungal assemblage on alder leaves was reduced by motorway runoff. It would therefore seem unlikely that the food quality of this material for shredders was impaired.

2.4.1. Conclusions.

At Pigeon Bridge Brook which receives the largest pollutant load from the motorway there was a significant deleterious effect on macroinvertebrate structure and function. The effects on the microbial community on leaf litter may have been more subtle with suggestions of a stimulatory response in microbial structure and function at this site. There was no evidence of any impact of the motorway discharge at either Butterthwaite Ditch or Rockley Dike. The main findings of the field study were:

1. Sediment particle size was significantly larger at downstream stations at Rockley Dike and Pigeon Bridge Brook.
2. Macroinvertebrate community structure was significantly altered downstream of the discharge at Pigeon Bridge Brook, but not at Rockley Dike or Butterthwaite Ditch. Taxon richness, diversity and biotic scores were all reduced at the downstream station at Pigeon Bridge Brook and the trophic structure was altered from a mixed economy, consisting of predators, shredders, scrapers and collectors at the upstream station to an economy dominated by collectors below the discharge. Furthermore, numbers of macroinvertebrates (particularly shredders) associated with leaf material were significantly reduced below the discharge at Pigeon Bridge Brook.
3. The number of aquatic hyphomycete species colonising alder leaves were not affected by the discharge at the three sites. There were no between-station differences in fungal diversity at Rockley Dike or Butterthwaite Ditch. At Pigeon Bridge Brook diversity was lower on hawthorn leaves downstream of the discharge but either higher (initial survey) or not different (second survey) on alder leaf material. There were no between-station differences in fungal biomass at Pigeon Bridge Brook but total and fungal respiration on leaf material was higher downstream of the discharge.

4. Microbial processing of leaf material was not altered by the motorway discharge but macroinvertebrate mediated processing was significantly reduced at the downstream station at Pigeon Bridge Brook.

There are several mechanisms that might be responsible for the reduction of macroinvertebrate abundance, diversity and macroinvertebrate-mediated litter breakdown at Pigeon Bridge Brook. The motorway discharge at this site obviously introduces a high toxicant load into the stream which may reach concentrations in the stream water and sediments that induce lethal toxicity to sensitive species. Lethal toxicological assessments of stream water and sediments are described in Chapter 3. Sub-lethal responses of the animals may also explain their field distributions. One of the initial reactions to toxicants may be the avoidance of the contaminant resulting in drift which can affect community structure and functional properties (Hall *et al.*, 1980). The avoidance reactions of macroinvertebrates to contaminated sediments, where most of the toxicants reside, are described in Chapter 4. The reduction in decomposition may be due to decreases in the activity of detritivores. Sub-lethal effects of the motorway runoff contaminants on the feeding activity of the dominant macroinvertebrate shredder at Pigeon Bridge Brook are described in Chapter 5.

CHAPTER 3.

LETHAL TOXICITY OF RUNOFF-CONTAMINATED WATER AND SEDIMENTS.

3.1. INTRODUCTION.

It was evident from Chapter 2 and from previous studies (see Chapter 1) that motorway runoff contains a complex mixture of potential toxicants that eventually enter receiving waters. Pigeon Bridge Brook received the highest pollutant 'load', of the three streams studied, resulting in the highest levels of metal and hydrocarbon concentrations in the sediments and overlying water downstream of the motorway discharge. These changes in sediment and water quality were associated with changes in the structure and functioning of the benthic macroinvertebrate community. However, to conclude from these observations that the runoff had a direct toxic effect and was responsible for the changes in macroinvertebrate community structure and function would be questionable. Unless all physico-chemical parameters, other than toxicants, and sources of colonising individuals were identical it would be difficult to demonstrate that differences in populations and communities were due to toxic substances in the water or sediments. In addition, short-term chemical and physical stressors such as chlorine, pH or temperature could effect communities but leave no recordable toxic residues (Giesy and Hoke, 1989). Only through controlled laboratory experiments can any toxic effects of these pollutants be ascertained. This chapter describes *in-situ* and laboratory comparative toxicological studies of motorway-runoff contaminated sediments and water using dominant macroinvertebrate species found at the Pigeon Bridge Brook site.

The major contaminants recorded in the stream sediments and water at the downstream station at Pigeon Bridge Brook were metals (primarily zinc, cadmium, chromium and lead), carbonyl compounds and hydrocarbons, in particular PAHs (i.e. fluoranthene, pyrene and phenanthrene: Maltby *et al.*, 1995a). Because of their toxicity, persistence and/or bioaccumulative properties, most of these substances are EEC 'black' or 'grey' list and E.P.A. priority pollutants (Kobriger, 1984; Hellawell, 1988b). In addition to metals and organic contaminants significantly elevated concentrations of sulphate and chlorine ions in stream water at the downstream station at Pigeon Bridge Brook resulted in a non-significant increase in conductivity and salinity. Although previous studies have examined the toxicity of many of the individual pollutants found in motorway runoff (e.g. Cowley, 1985) few have determined the toxicity of the complex 'cocktail' of toxicants in the runoff (Gjessing *et al.*, 1984a; Kobriger, 1984). Further, no

studies have focused on identifying the toxic compartment in the stream ecosystem or the toxicants responsible for any observed toxicity.

At all three sites, the majority of the pollutant load was associated with the stream sediments (Maltby *et al.*, 1995a). For example hydrocarbons were below detection levels in stream water (< 30 µg chrysene equivs. /L), but reached concentrations of >405 µg chrysene equivs. /g (wet wt.) in the sediments. Similarly concentrations of most metals were between 1.3 and 86 times higher in the sediment than in the overlying water. Exceptions were Al, Mg and Ca which were generally at higher concentrations in the water. Pollutants often reach much higher concentrations in the sediments than in water column (Elder and Dresler, 1988). For instance, Woodward and Riley (1983) found that total hydrocarbons in an oil-polluted stream were 55,000 times higher in the sediment than in the overlying stream water and Van Hassel *et al.* (1980) found metals generally accumulated in the sediments of a road-contaminated stream. Sediments are an important component of aquatic ecosystems because of the habitat they provide for benthic organisms. Many species spend a major proportion of their lifecycle living in or on the sediments and direct transfer of chemicals from sediments to organisms is now considered to be a major route of exposure for many species (Adams *et al.*, 1992). Furthermore, sediment detritus may act as a food and pollutant source to benthic macroinvertebrates resulting in these animals accumulating toxicants (Eadie *et al.*, 1985; Landrum *et al.*, 1985; Harkey *et al.*, 1994). It is therefore important to assess the toxicity of contaminated sediments to ecologically relevant species. Species that display apparent sensitivity to toxicants in field studies and which have an important role in the economy of the system are particularly relevant for study.

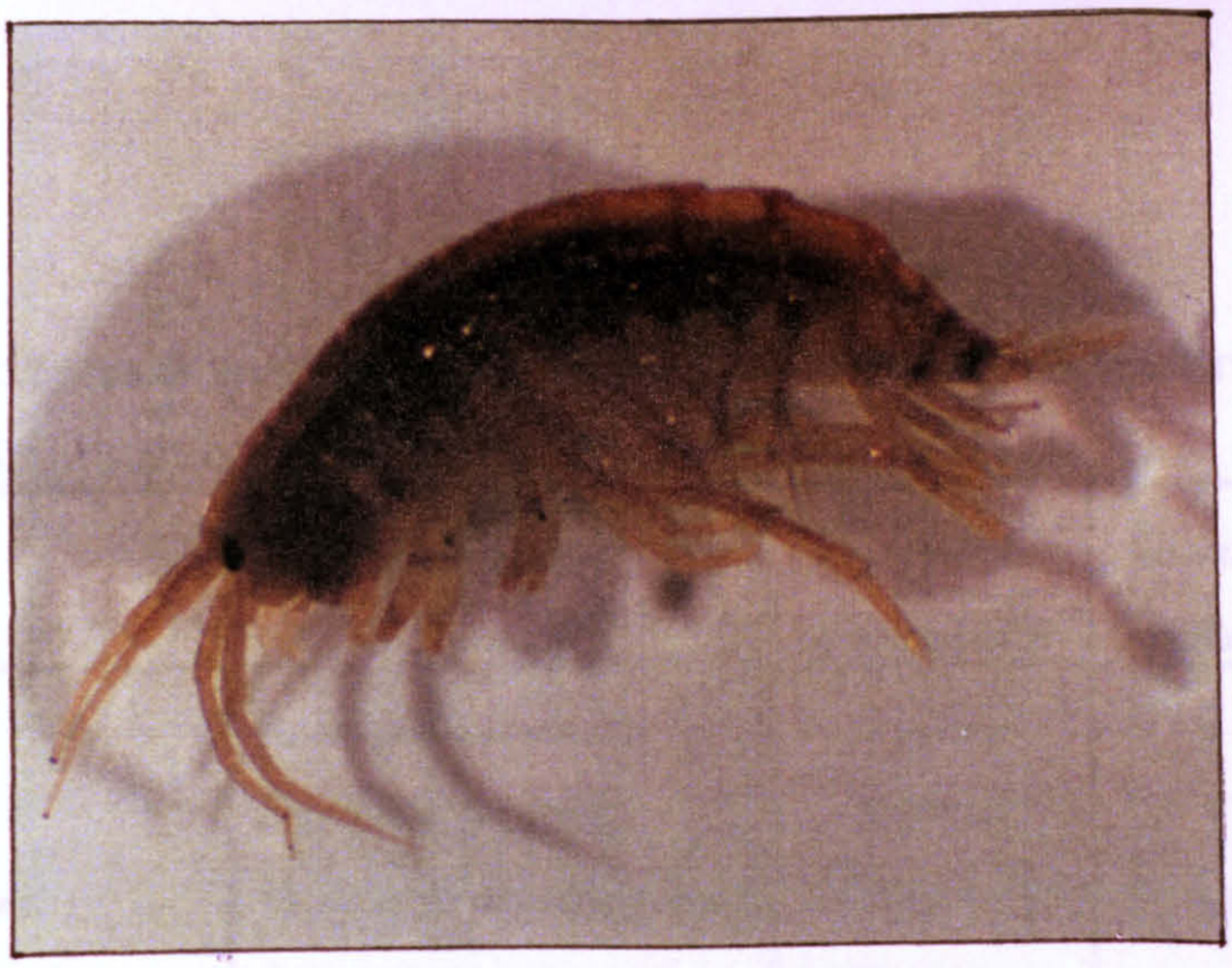
Laboratory studies involving bulk sediments provide a direct measurement and integration of the toxicity of complex mixtures of pollutants but provide no information on which chemicals are responsible for any toxicity observed (Adams *et al.*, 1992). Bishop (1987) stated three basic questions that need to be answered in contaminated sediment situations: 1. what contaminants are present, 2. what is the extent of the contamination and 3. what are the potential effects of these contaminants on the biota. If toxicity identification and evaluation (TIE) procedures are used these methods may provide an insight into causative groups of toxicants. TIE has been described as a "stepwise process that combines toxicity testing and analysis of the physical and chemical characteristics of effluents to identify potentially causative toxicants" (E.P.A., 1985). If the assessment is based principally on toxicity then direct toxicant-toxicity relationships can be established (Burkhard and Ankley, 1989). TIE methods use sequential extraction/ fractionation of particular chemical classes and chemical

manipulations of mixtures to ascertain the toxicity of fractions to standard test species (Doi, 1994). The results of these studies are then used to identify toxic fractions and hence groups of toxicants. The methods generally separate organic and inorganic fractions of the mixture using chemical manipulations or chromatographic techniques. These fractions are tested for toxicity and the toxic fraction may be further separated to identify the toxic components. Additional manipulations, such as acid-base adjustment, aeration and chelation may be used to identify classes of chemicals or specific chemicals causing any toxicity and a comprehensive, parallel programme of chemical analysis is also performed (Doi, 1994). Commonly adopted TIE methodology uses standard test species such as *Ceriodaphnia dubia* with which to test the toxicity of prepared fractions. However, species differ in their response to toxicants (Slooff, 1983; Van der Gaag, 1992) thus undermining inter-species extrapolation. TIE procedures should therefore use a number of test species.

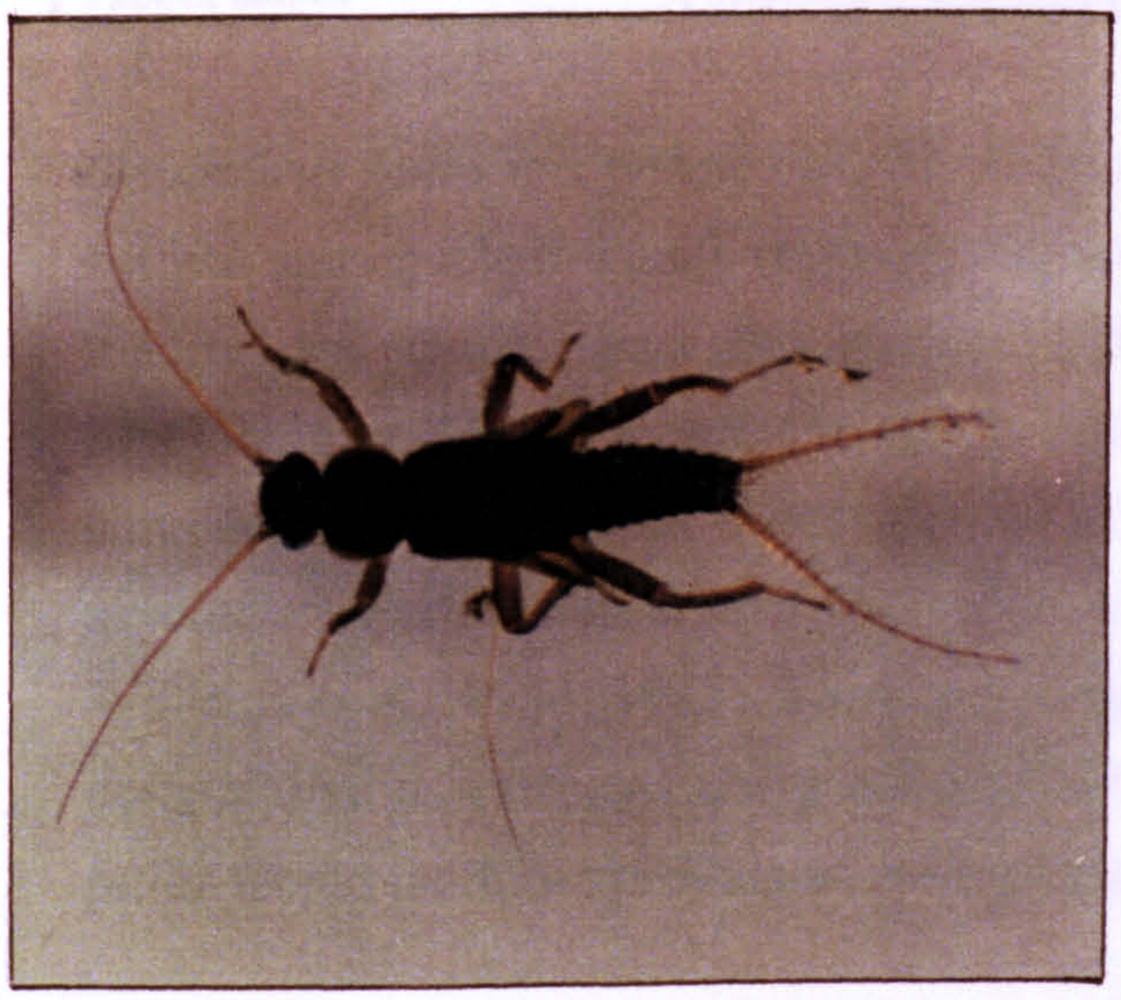
Test species used in this study were selected on the basis of a number of criteria:

1. species relevant to the system studied;
2. species which appeared either sensitive or tolerant to motorway contaminants according to their field distributions at Pigeon Bridge Brook, i.e. either increased or decreased in relative abundance downstream of the motorway discharge and were known to vary in their sensitivity to different pollutant classes;
3. species which were from different phylogenetic groups and had different functional feeding methods. The species chosen were considered to be important in energy and material flow in this system;
4. species which had differing degrees of sediment contact.

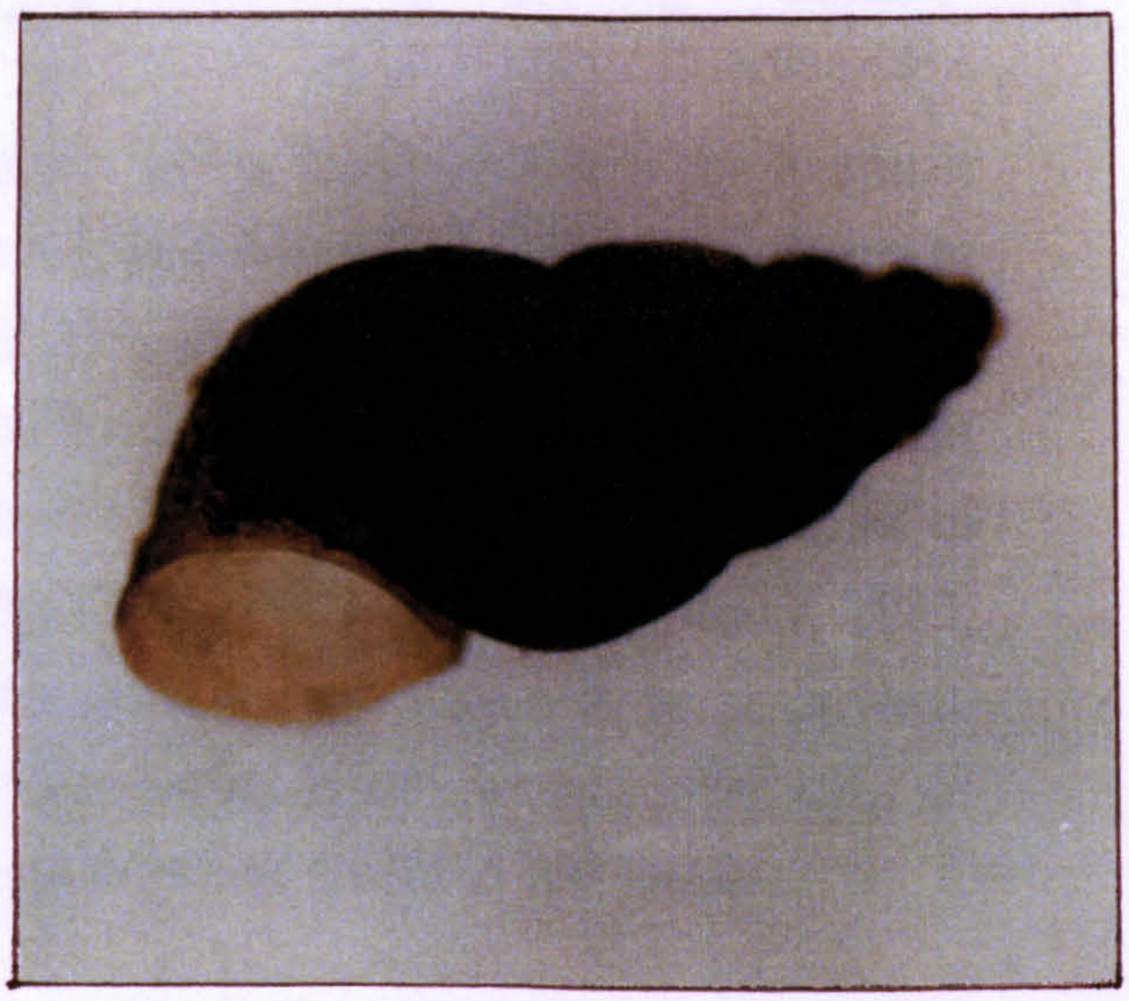
Five species were chosen (Plate 3.1.) and their phylogenetic group, functional feeding group, relative degree of sediment contact and relative abundance at the downstream station compared to the upstream station at Pigeon Bridge Brook are displayed in Table 3.1. Although, at Pigeon Bridge Brook, the stonefly *Leuctra inermis* (Kempny) was more common than *Nemoura cinerea* (Retz.), *N. cinerea* was used as a test species in toxicity tests as no adequate source of *L. inermis* could be found. *N. cinerea* was found at Pigeon Bridge Brook in later surveys with a similar distribution to *L. inermis*.



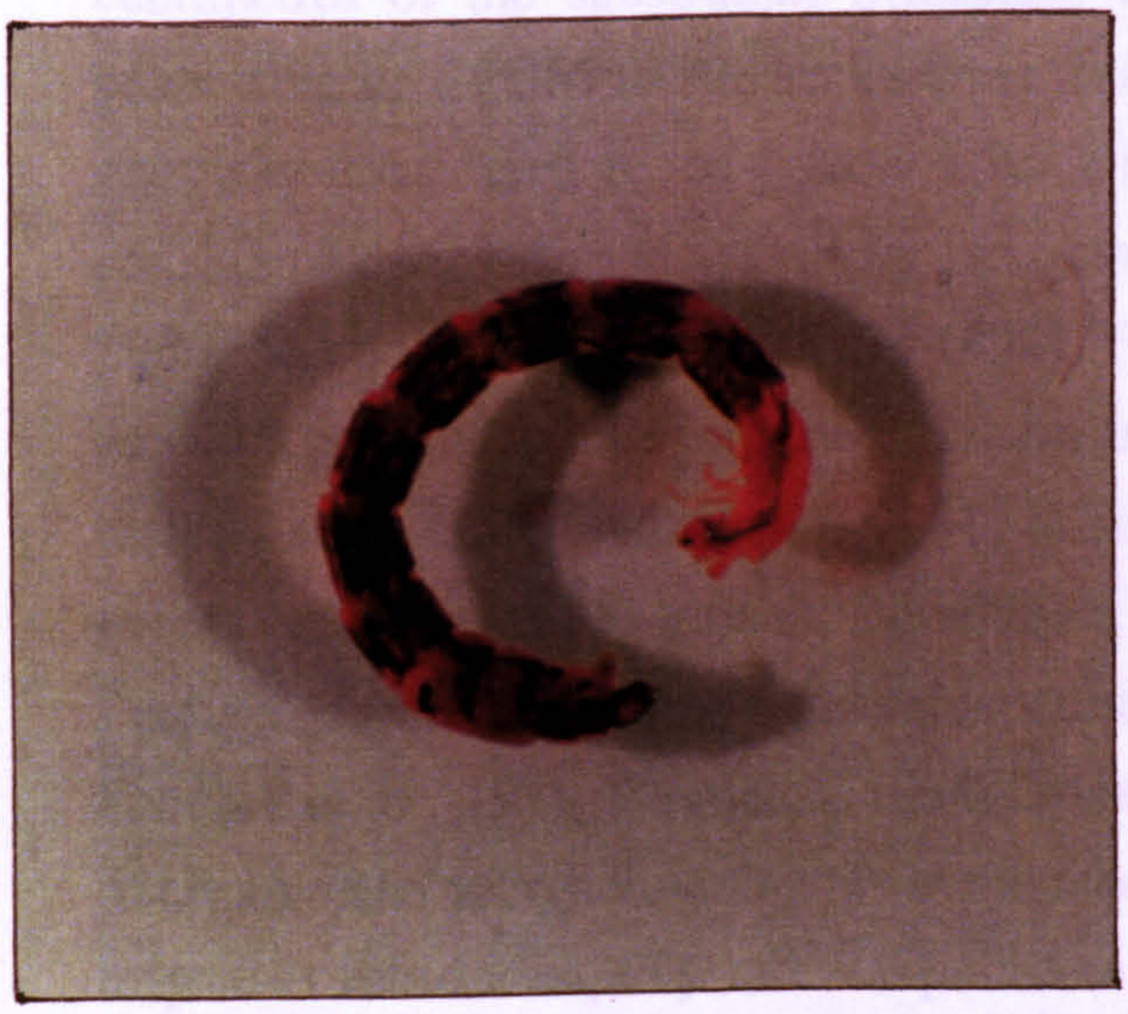
a.) *Gammarus pulex*



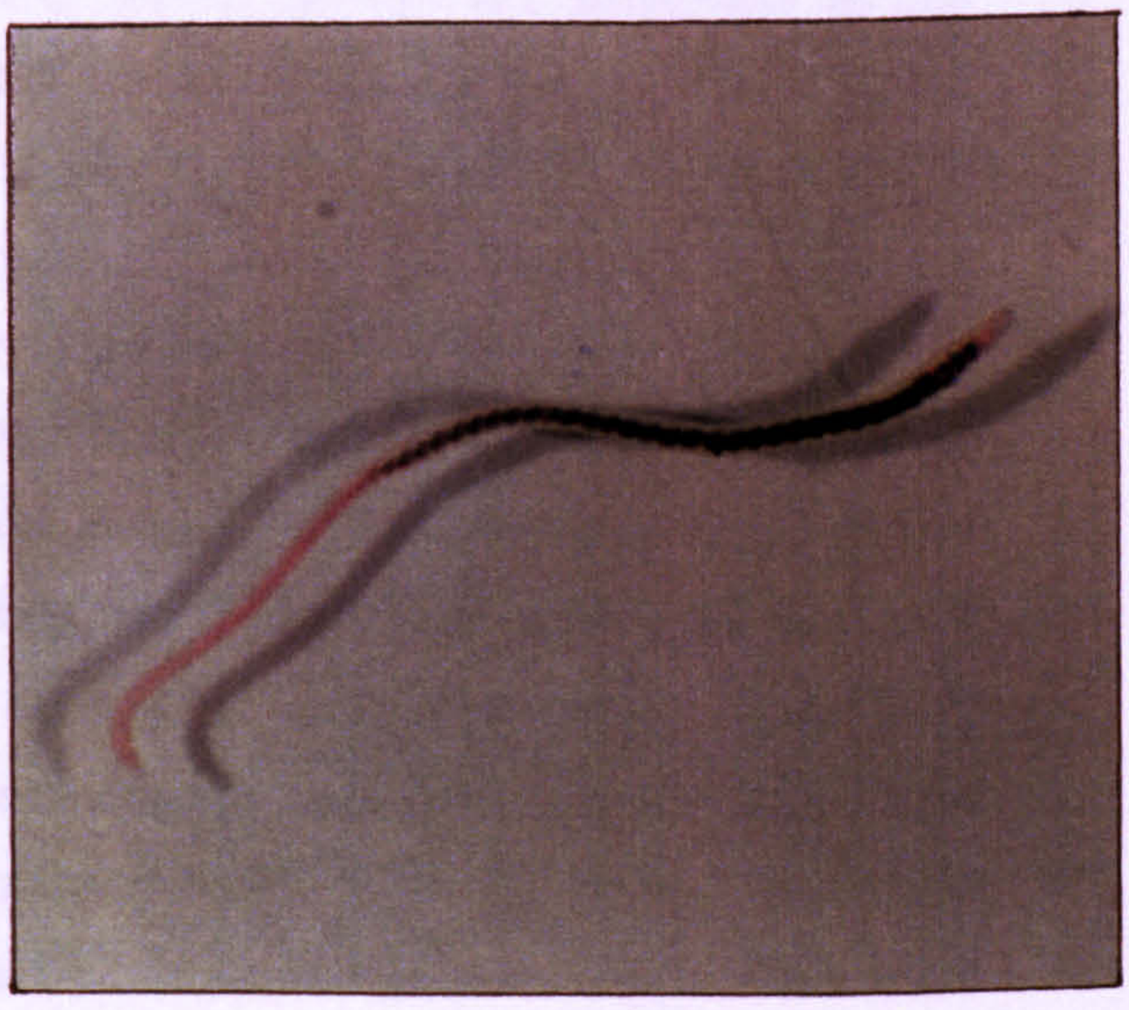
b.) *Nemoura cinerea*



c.) *Potamopyrgus jenkinsi*



d.) *Chironomus riparius*



e.) *Tubifex tubifex*

Plate 3.1. The five macroinvertebrate species used in the toxicity tests.

Table 3.1. Species used in laboratory toxicological studies with motorway-runoff water and sediment. Sediment contact increases from + to +++.

Species	Phylogenetic group	Functional feeding group	Sediment contact	Relative abundance (Downstream)
Gammarus pulex	Amphipoda, Crustacea	Shredder	+	Decreased
Nemoura cinerea	Plecoptera, Uniramia	Shredder	+	Decreased
Potamopyrgus jenkinsi	Mesogastropoda, Mollusca	Scraper	++	Decreased
Chironomus riparius (Meigen)	Diptera, Uniramia	Collector	+++	Increased
Tubifex tubifex	Haplotaxida, Annelida	Collector	+++	Increased

Gammarus pulex is a widely distributed amphipod, which usually inhabits the sediment surface amongst leaf/woody material, although it is free-swimming and is often found in macrophyte stands (Fitter and Manuel, 1986). This species is often important in the processing of CPOM in streams and at Pigeon Bridge Brook was the most abundant shredder (Chapter 2). *G. pulex* has been found to be the most sensitive species in several comparative studies of aqueous toxicity (Rehwoldt *et al.*, 1973; Slooff, 1983; Williams *et al.*, 1984, 1985). Moreover, amphipods have been shown to be sensitive in comparative assessments using contaminated sediments (Ankley *et al.*, 1991 a,b). *G. pulex* would therefore provide an ecologically relevant and sensitive test species.

Stoneflies are generally found on the sediment surface or amongst the upper few centimetres of the substratum (Minshall, 1984). Many species are important in the processing of CPOM in stream systems (Merritt *et al.*, 1984). Although stoneflies are generally considered to be pollution sensitive (Mayer and Ellersieck, 1986), there is considerable variation in sensitivity between families, species and toxicants (Hellawell, 1986; Mance, 1987; Gower *et al.*, 1994). For instance, *Leuctra* spp. were sensitive to zinc in a polluted stream but *Leuctra inermis* were fairly insensitive to cadmium in acute toxicity tests (Abel and Green, 1981; Williams *et al.*, 1985). Whereas stoneflies are generally sensitive to organic enrichment they are tolerant of acid pollution and frequently more tolerant of metals than either mayflies or fish (Warnick and Bell, 1969; Sphehar *et al.*, 1978; Clements, 1994). *Nemoura cinerea* was fairly tolerant to a range of organic and inorganic toxicants in the laboratory (Slooff, 1983). Stonefly populations may also recover rapidly after a pollution event. For example, stoneflies that were immediately absent from a stream after a massive oil spill were found to recolonise before either mayflies or caddisflies (Crunkilton and Duchrow, 1990) and *Nemoura* spp. and *Leuctra* spp. recolonised a metal polluted stream more rapidly than either molluscs or crustaceans (Jones, 1937; Brooker and Morris, 1980).

Potamopyrgus jenkinsi is a sediment surface dwelling, scraping/deposit feeding, gastropod snail with a fairly ubiquitous distribution in lotic freshwater systems (Dorgelo, 1991). Gastropod molluscs have been found to be sensitive to metals in water-only exposures (Nebeker *et al.*, 1986) but fairly insensitive to hydrocarbon pollutants (Green and Trett, 1989). For instance, *P. jenkinsi* was found to be sensitive in comparison to many other macroinvertebrates when exposed to ammonia and copper (Watton and Hawkes, 1984). Again there is great variability in sensitivity between species and between toxicants (Hellawell, 1986; Mance, 1987).

Chironomus spp. are widely distributed, and often highly abundant in freshwater sediments during their larval stage of development where they play an important role in cycling FPOM by feeding and bioturbation (Gerould *et al.*, 1983). Due to their ease of culture and habitat preference, they are routinely used as a standard test organism in sediment assays (Giesy and Hoke, 1990; Burton, 1992). Adult *Chironomus* spp. have often been found to be reasonably tolerant to many pollutants in water and sediment acute toxicity tests, although early instar of some species are sensitive (Williams *et al.*, 1984, 1986; Giesy *et al.*, 1988). Their general tolerance explains their often high abundance in polluted conditions (Crunkilton and Duchrow, 1990; Gower *et al.*, 1994; Canfield *et al.*, 1994).

Oligochaetes are important benthic organisms in the economy of streams and are often considered to be indicators of organic enrichment. Their usual high abundance makes them important prey organisms and as sediment dwellers they are important in bioturbation (Brinkhurst and Jamieson, 1971). In comparative tests, oligochaetes have been found to be fairly insensitive to pollutants (Williams *et al.*, 1984) though they may be sensitive to some metals. For instance, *Tubifex tubifex* are sensitive to some chromium species (Hoekstra *et al.*, 1994). Generally, however, oligochaetes are insensitive to the majority of metals even above the aqueous solubility of the metal salts in laboratory exposures (Schubauer-Berigan *et al.*, 1993). Amongst the oligochaetes, tubificid worms are generally considered very tolerant to pollutants (McCauley, 1966; Bengtsson and Berggren, 1972; Milbrink, 1983; Canfield *et al.*, 1994). Oligochaetes have been shown to be insensitive in comparative assessments of sediments contaminated with metals and complex toxicant mixtures (Ankley *et al.*, 1991 a,b). Although they have been used in standard sediment toxicity assays, endpoints other than mortality are usually assessed (Burton *et al.*, 1992).

The mean concentrations of most metals in the stream water at the downstream station at Pigeon Bridge Brook were below acute lethal concentrations for *Gammarus* sp.,

stoneflies (Pteronarcidae), gastropod molluscs, *Chironomus* sp. and *Tubifex* sp. (Rehwoldt *et al.*, 1973; Hellawell, 1986; Mance, 1987; Table 3.2). Moreover, as the stream water at this station was very hard (672 mg/L calcium carbonate equivalents) those metals that were present would have reduced toxicity (Hellawell, 1986). Data on the sensitivity of stoneflies, oligochaetes, dipterans, molluscs and amphipods to selected PAHs are given in Table 3.3.

Table 3.2. Concentrations of Cd, Cr, Pb, Zn, and Cu in the stream water at the downstream station at Pigeon Bridge Brook expressed as toxic units of the lethal (a= 12 h, b= 24 h, c= 48 h, d= 96 h, e= 7 day and f= 14 day) LC_{50} concentration (Abel, 1989) in water (hardness >40 mg/L calcium carbonate equivalents; Rehwoldt *et al.*, 1973; Hellawell, 1986; Mance, 1987).

	Cd	Cr	Pb	Zn	Cu
Gammarus sp.	1.38×10^{-3} c	2.17×10^{-3} d	0.17 d	0.02 c	1.16 c
Pteronarcidae stoneflies	9.2×10^{-6} d		1.16×10^{-3} f	9.8×10^{-3} e	3.42×10^{-3} f
Molluscs *Physa integra* *Lymnaea emarginata* *Amnicola* sp. *P. jenkinsi*	1.4×10^{-3} e 2.0×10^{-5} d	1.44×10^{-4} c 5.95×10^{-4} d	1.59×10^{-3} c	0.01 c 9.8×10^{-3} d	 0.05 d 0.82 c
Chironomus sp.	6.6×10^{-6} a	4.5×10^{-4} d	4.44×10^{-4} c	2.19×10^{-3} c	0.03 d
Tubifex tubifex	3.6×10^{-7} a	1.1×10^{-3} c	4.94×10^{-5} c	1.05×10^{-3} b	7.43×10^{-4} b

Table 3.3. Sensitivity of aquatic organisms to individual PAHs in aqueous exposures. a= 48 hour (Millemann *et al.*, 1984b), b= 14 day (Maltby *et al.*, 1995b) and c=96 hour LC_{50} (Burton, 1993). Concentrations are in µg/L.

(µg/L)	Naphthalene	Fluoranthene	Phenanthrene	Fluorene	Pyrene
Gammarus sp.	3930[a]	96.8[b]	460[a]		28.9[b]
Peltoperla maria (stonefly)		135			
Chironomus sp.	2810[a]		490[a]	2350[c]	
Physella virgata (snail) *Physa gyrina* (snail)	 5020[a]	>178.5[c]	 $>287 \times 10^{3}$[a]		
Lumbriculus sp. (worm)		>178.5[c]			

3.1.1. Objectives and approach.

Field surveys suggested that benthic macroinvertebrate community structure was impacted by the motorway runoff discharge at Pigeon Bridge Brook. This chapter describes studies performed to assess whether the motorway discharge contaminants were lethal to macroinvertebrates found at this site and whether comparative toxicity could explain the field distributions of the test species. In addition, experiments were designed to identify where the toxicity resides and which major classes of toxicants in the complex mixture were responsible for any toxicity.

The specific objectives were:

1. to assess whether the stream water or sediments from the downstream station at Pigeon Bridge Brook were lethal to the selected macroinvertebrates using *in-situ* and laboratory exposures;
2. to assess which classes of toxicants were responsible for the majority of the toxicity and to quantify the response of sensitive species.

In-situ field deployments were used at Pigeon Bridge Brook to assess the toxicity of stream water and sediments to *G. pulex, N. cinerea and P. jenkinsi*. Laboratory tests were then employed using all five test species (above plus *C. riparius* and *T. tubifex*) to assess stream water and sediment toxicity. Since sediments were usually heterogeneous in physicochemical nature and degree of contamination several field sediments were screened for toxicity against *G. pulex*. This species was considered a sensitive test species and was the dominant shredding macroinvertebrate at this site. The sediment which exhibited the highest toxicity to this species was then used to expose the other species. A stepwise approach based on TIE-type methods were employed, using raw site water/sediment assays, sediment manipulation and preparation of sediment extract fractions to assess the toxicity of ecosystem compartments and to identify broad groups of pollutants responsible for any toxicity to a range of species. Solvent- and acid-extracted sediment fractions were used to evaluate the potential toxicity of sediment-associated contaminants.

3.2. MATERIALS AND METHODS.

3.2.1. Source, collection and maintenance of test animals.

Gammarus pulex and *Potamopyrgus jenkinsi* were collected from Crags Stream, Clowne, Derbyshire (NGR SK497745; water hardness 566.3 mg $CaCO_3$ equivalents /L, pH 7.8). *G. pulex* were collected using a 2-mm mesh Endcott™ laboratory test sieve and *P. jenkinsi* were collected with a 1-mm mesh test sieve. *Nemoura cinerea* were collected from a millrace on the River Rivelin (NGR SK875304; water hardness 37.5 mg $CaCO_3$ equivalents /L, pH 7.2) using a pond net (1-mm mesh size). All three species were maintained in Artificial Pond Water (APW, Appendix A3, Table A3.1) and fed on whole alder leaves (*Alnus glutinosa*) inoculated with *Cladosporium* fungus (Appendix A3, Table A3.2 and Fig. 5.1). Experimental animals were maintained in a 15°C (± 2°C) constant temperature room with a 12 h dark/ 12 h light photoperiod.

Chironomus riparius and *Tubifex tubifex* were obtained from laboratory cultures at Sheffield University. *C. riparius* was cultured in sandwich boxes (12 cm wide x 23 cm long x 7 cm deep) containing 1 kg of sand (BDH™ acid purified 50-150 mesh) and 1 litre of aerated APW. The chironomid culture was kept in a constant temperature room at 15°C (± 2°C) with a photoperiod of 14 h light (of which 1.5 h were dawn and 1.5 h dusk) and 10 h dark. The oligochaete culture was maintained as small colonies in the cells (2 x 2 x 2 cm) of multi-cell trays, over which a continuous flow of de-chlorinated tap water was provided. The culture was kept in an aquarium at ambient temperature and natural lighting conditions. Both *C. riparius* and *T. tubifex* were maintained on a diet of ground Tetramin™ fish food.

A. *In-situ* exposures

3.2.2. *In-situ* exposures.

Animals were placed either individually (*G. pulex* and *N. cinerea*) or in groups of five (*P. jenkinsi*) in cylindrical PVC chambers (diam. 36 mm, 60 mm long) the ends of which were sealed with 1-mm nylon mesh. No food was added to the chambers. Chambers were placed in rectangular cages (13 cm height x 58 cm length x 6 cm width) constructed from PVC garden fencing of 50-mm mesh size and garden crop protection netting of 20-mm mesh size. Each cage held 20 chambers. All three species were deployed at both the upstream and downstream station at Pigeon Bridge Brook. For each species, 80 chambers were deployed at each station, 40 of which were suspended in the water column and the remaining 40 placed in contact with the bed sediments. Animals were deployed *in-situ* for

14 days after which mortality was assessed. *G. pulex* were deployed in November and December 1993 whereas *P. jenkinsi* and *N. cinerea* were only deployed in December 1993.

B. Laboratory toxicity experiments.

All experiments were performed in a constant temperature room at 15°C (± 2°C) with a 12 h dark/ 12 h light photoperiod. Animals were not fed during the experiments.

3.2.3. Stream water.

Survival of *G. pulex*, *N. cinerea*, *P. jenkinsi*, *C. riparius* and *T. tubifex* in APW and stream waters from Pigeon Bridge Brook was assessed. Stream water was collected, mid-depth, from both the upstream and downstream station at Pigeon Bridge Brook in 20-litre PVC drums every four days in October 1993. Water was filtered through Whatman™ No. 1 filters, equilibrated to 15°C and used immediately. Experimental animals were exposed in polystyrene chambers; the details of the experimental design for each species are given in Table 3.4. Test solutions were renewed every 2 d over the 14-d experimental period and analysed both pre- and post-exposure for Ca, Al, Zn, Cu, Cd, Cr, Pb, Fe (triplicate, 15 ml samples) and total aromatic hydrocarbons (triplicate, 250 ml samples) using the methods described in section 3.2.8. At the end of the test period the number of surviving animals was recorded.

Table 3.4. Design of the stream water toxicity tests for the five macroinvertebrate species.

Species	Experimental chamber (mm) diam. x depth	Volume of water (ml)	Replicates per treatment	Chambers per replicate/ animals per chamber
G. pulex	55 x 80	150	5	10 / 1
N. cinerea	55 x 80	150	3	10 / 1
P. jenkinsi	100 x 65	200	6	1 / 20
C. riparius	100 x 65	200	5	1 / 5
T. tubifex	50 x 25	50	8	1 / 5

3.2.4. Field sediments.

As field sediments are spatially and temporally heterogeneous they were collected for toxicity tests on several occasions. The sediments were collected from upstream and downstream stations at Pigeon Bridge Brook in June 1992, July 1992, August 1992 and October 1993. These sediments were initially screened for toxicity using *G. pulex*. The most toxic sediment (collected in October 1993) was then used in studies with *N. cinerea*, *P. jenkinsi*, *C. riparius* and *T. tubifex*. Only results for the exposure of the five test species to this sediment are reported. Sediment samples were collected from the surficial 10 cm of

the stream bed and transported in a cool box. Sediments were passed through a 10-mm mesh sieve to remove large stones and large pieces of vegetation and then thoroughly stirred in PVC buckets to obtain a homogenous test sediment. They were stored at <4°C in the dark for no longer than 2 d before use. Prior to use, the sediments were picked over to remove any live indigenous animals with particular care being taken to remove any remaining chironomids and *Tubifex* spp.. Weighed portions of sediments and sand (control) were allocated to exposure chambers and APW carefully added. Suspended sediment was allowed to settle overnight before the test animals were allocated to the test chambers. Details of the experimental design for each of the five test species are given in Table 3.5. Vessels containing *N. cinerea* were gently aerated for the experimental exposure period. All species were exposed for 14 days after which time the number surviving in each replicate group was recorded. The sediments were sampled at the beginning and end of the experimental period (triplicate samples approx. 3 g) and analysed for Ca, Al, Zn, Cu, Cd, Cr, Pb, Fe, Mg and Ni and total aromatic hydrocarbons (section 3.2.8.).

Table 3.5. Design of the sediment toxicity tests for the five macroinvertebrate species.

Species	Size of experimental chamber (mm) diam. x depth	Wt. of sediment Vol. of water	Replicates	Chambers per replicate /animals per chamber
G. pulex	55 x 80	30 g/ 150 ml	4	20/ 1
N. cinerea	100 x 65	100 g/ 200 ml	6	1/ 5
P. jenkinsi	50 x 25	5 g/ 50 ml	3	10/ 5
C. riparius	50 x 25	5 g/ 50 ml	8	1/ 5
T. tubifex	50 x 25	5 g/ 50 ml	8	1/ 5

3.2.5. Manipulated sediments.

Sediments were manipulated by solvent extraction to remove organic contaminants whilst leaving inorganic contaminants. The toxicity of solvent extracted and untreated downstream sediment was then compared. Sediment was collected from the downstream station at Pigeon Bridge Brook and prepared according to the methods described in section 3.2.4. Four downstream sediments were collected, which corresponded to those described in section 3.2.4, manipulated and screened for toxicity using *G. pulex*. The most toxic sediment was then used in studies with *N. cinerea*, *P. jenkinsi*, *C. riparius* and *T. tubifex*.

A portion of the downstream test sediments was left untreated while a second portion was treated by solvent extraction as follows. Exactly 160 g (wet-weight) of sediment from the downstream station at Pigeon Bridge Brook was weighed into Whatman™ cellulose

thimbles (diam. 40 mm x 120 mm long) and extracted twice in a large soxhlet apparatus with 250 ml of Distol® grade dichloromethane (DCM, Fisons), on a heating mantle at 50°C; each extraction lasting for 24 h. The sediment was then dried at 60°C to remove the solvent and re-hydrated with 50 ml of APW. This sediment was then allocated to experimental chambers. The solvent-extracted downstream sediment was tested for comparative toxicity against the non-extracted downstream sediment. The experimental design for manipulated sediment exposures is displayed in Table 3.5. (section 3.2.4).

N. cinerea test vessels were gently aerated and all the animals were exposed for 14 d after which time the number surviving in each replicate group was recorded. The sediments were sampled (triplicate samples, approx. 3 g) at the end of the experimental period and analysed for Ca, Al, Zn, Cu, Cd, Cr, Pb, Fe, Mn and Ni and total aromatic hydrocarbons (section 3.2.8.).

3.2.6. Dichloromethane ('solvent') sediment extract.

The relative toxicity of solvent extracts of field sediments were assessed using *G. pulex*, *N. cinerea*, *P. jenkinsi*, *C. riparius* and *T. tubifex*. Sediment from upstream and downstream stations at Pigeon Bridge Brook were extracted using dichloromethane (DCM; section 3.2.5.). The extracts were rotary evaporated at 40°C to dryness and the final residue taken up in 80 ml of analytical grade acetone (Fisons). Acetone was used as a carrier solvent as it has minimal toxicity to freshwater organisms (Hoekstra *et al.*, 1994). The extracts were stored at <4°C in a sealed amber glass bottle until use.

Stock solutions were prepared by spiking APW with 0.8 ml extract/ L of either upstream or downstream sediment extract in the acetone carrier. Spiking was performed by injection of the extract into a vortex formed by stirring the APW on a magnetic stirrer. The extract was allowed to slowly dissolve in the APW for 24 h in the dark. This method minimised absorption of the sediment extract to the vessel sides and floatation to the surface and allowed slow solubilisation of the extract. Test solutions were prepared by diluting the stock solutions with APW containing 0.8 ml/ L analytical grade acetone and stirring for 24 h APW spiked with 0.8 ml acetone/ L was used as a carrier solvent control.

Three different sets of upstream/downstream sediment extracts were prepared ('X', 'Y' and 'Z') and used to expose animals at the concentrations displayed in Table 3.6. Details of the experimental design used are given in Table 3.7. Animals were exposed for 14 d during which test solutions were prepared and changed daily. After the experimental exposure period the numbers of animals surviving in each replicate group was recorded. Samples of

all test solutions (3 x 250 ml) were analysed for total aromatic hydrocarbons (section 3.2.8.). and samples (3 x 15 ml) of the 0.4 ml extract/ L test solutions for set 'X' were analysed for Al, Zn, Cu, Cd, Cr, and Pb (section 3.2.8.). *G. pulex* exposed to set 'X' were pooled within replicates and analysed for total aromatic hydrocarbons (section 3.2.8.).

Table 3.6. Exposure concentrations for DCM sediment extracts used in toxicity tests. Letters 'X' - 'Z' refer to sets of extracts. Dashes indicate that the species was not exposed to that extract.

Species	Extract 'X'		Extract 'Y'		Extract 'Z'	
	Upstream ml extract/L	Downstream ml extract/L	Upstream ml extract/L	Downstream ml extract/L	Upstream ml extract/L	Downstream ml extract/L
G. pulex	0.05, 0.1, 0.2, 0.4	0.05, 0.1, 0.2, 0.4	0.4	0.4	---	0.06, 0.12, 0.18, 0.24
N. cinerea	---	---	0.05, 0.1, 0.2, 0.4	0.05, 0.1, 0.2, 0.4	0.4	0.05, 0.1, 0.2, 0.4
P. jenkinsi	0.4	0.05, 0.1, 0.2, 0.4	---	---	---	---
C. riparius	0.05, 0.1, 0.2, 0.4	0.05, 0.1, 0.2, 0.4	---	---	---	---
T. tubifex	---	---	0.05, 0.1, 0.2, 0.4	0.05, 0.1, 0.2, 0.4	0.4	0.05, 0.1, 0.2, 0.4

Table 3.7. Design of toxicity tests using sediment extracts.

Species	Size of experimental chamber (mm) diam. x depth	Vol. of test soln. (ml)	Replicates per treatment	Chambers per replicate /animals per chamber
Gammarus pulex	55 x 80	150	4	15/ 1
Nemoura cinerea	55 x 80	150	3	10/ 1
Potamopyrgus jenkinsi	55 x 80	150	4	1/ 20
Chironomus riparius	55 x 80	150	10	1/10
Tubifex tubifex	50 x 25	50	8	1/ 5

3.2.7. Acetic acid ('acid') sediment extract.

A dilute acid sediment extract was prepared from upstream and downstream field sediments from Pigeon Bridge Brook. A 100 g (wet weight) sample of sediment was stirred with 150 ml of 1 % glacial acetic acid (pH= 1, Fisons) on a rolling sediment stirrer at 30 r/p/m for 48 h at ambient room temperature (21-25^{o}C). The sediment/acid mixture was allowed to settle for 24 h and the overlying dilute acid was then filtered, under vacuum, through Whatman™ No. 1 filters. Triplicate (15 ml) sub-samples of these extracts were analysed for Zn, Pb, Cd, Cr, Cu, Ni, Fe, Al, Mg and Ca (section 3.2.8.). These extracts

were then used to prepare stock solutions by pipetting 50 ml of extract into 5 litres of APW as it was being stirred on a magnetic stirring plate (10 ml extract/ L). The downstream stock solution was used to prepare test solutions of 2, 4, and 6 ml extract /L by dilution with APW containing 0.05 ml acetic acid /L. Animals were exposed to 10 ml upstream extract/ L and 2, 4, 6, and 10 ml downstream extract /L. An acid control was prepared by spiking APW with 3 ml 1% acetic acid/ L to a give a pH similar to that of the sediment extract treatments. Details of the experimental design for each of the test species are given in Table 3.7. Animals were exposed for 14 d and test solutions were exchanged daily. The numbers of animals surviving in each replicate group after 14 d was recorded. Samples of the test solutions (3 x 15 ml) were analysed for metals (Zn, Cu, Cd, Cr, Pb) and total aromatic hydrocarbons (3 x 250 ml; section 3.2.8.) and *G. pulex* were pooled within replicates and also analysed for the same metals (section 3.2.8.).

3.2.8. Chemical analyses.

3.2.8.a. Metals.

Water samples were transferred into 15-ml metal-free polypropylene graduated sample tubes and acidified with 0.1 ml of 30 % Primar® nitric acid (Fisons) before analysis. Sediment or animal tissue samples (approx. 3 g) were dried at 60°C for 48 h in a drying oven. Sub-samples of approximately 1 g (dry weight) of sediment or 0.1 g (dry wt.) of animal tissue were then placed in an acid-washed glass test-tubes containing either 10 ml (sediment) or 5 ml (tissue) of 30 % Primar® nitric acid (Fisons) and acid-digested at 80°C for 2 h in a Tecam® block digester. The resulting solutions were washed into 15-ml metal-free polypropylene centrifuge tubes with 5 ml of distilled water and centrifuged at 5000 rpm for 15 min in a MSE Centaur 2® centrifuge. The supernatant was pipetted from settled undigested material into a 15-ml metal-free polypropylene sample tube and made up to 15 ml volume with distilled water.

Samples for analysis of Al (>0.5 mg/L), Cu (>0.05 mg/L), Cr (>0.1 mg/L), Ni (>0.1 mg/L), Pb (>1.0 mg/L), Zn (> 0.05 mg/L), Fe (>0.1 mg/L), Ca, and Mg were analysed by flame atomic absorption spectrophotometry (AAS, Perkin Elmer M2100® with a AS50® auto-sampler) and samples with concentrations of Al, Cu, Cr, Ni, Pb, Zn, and Fe below those concentrations given in parentheses were analysed by graphite furnace AAS (Perkin Elmer M2100® using a AS90® auto-sampler) according to Perkin Elmer standard techniques (Perkin Elmer, 1989). Minimum detection levels in prepared aqueous/acid samples were Ni = 2, Fe = 5, Zn = 0.5, Cd = 0.1, Cr = 10, Al = 5, Cu = 1 µg/L and Mg = 0.01, Ca = 0.01,

Pb = 0.001 mg/L. The efficiency of the extraction and analytical procedures for assessing metals in sediments was assessed using the standard sediment GBW 07401 (Laboratory of the Government Chemist: Recovery of metals relative to specified concentrations were > 80 % in most cases, the only exception being Cr: 36 %; Maltby *et al.*, 1995a). Efficiency of extraction of tissues was assessed using standard tissue (Commission of the European Communities, Community Bureau of reference CRM 186 Pig Kidney: Cd = 100 %, Cu = 88 %, Fe = 100 %, Mn = 73 %, Zn = 83 %, Pb = 87 %, Ca =100 %, Mg =100 %).

3.2.8.b. Total aromatic hydrocarbons.

Two-hundred and fifty millilitre samples of water were extracted twice with 35 ml of DCM in a glass separating funnel (inverted 30 times). On each extraction the lower DCM layer was allowed to settle before being decanting into a 150-ml glass round-bottomed flask. The DCM extract was then evaporated to approximately 1 ml using a rotary evaporator at 40°C before being transferred to a 3-ml glass sample vial. The flask was rinsed with 1 ml of DCM and the extract made up to a final volume of 2.5 ml. Extracts were then stored in the dark at -20°C until analysis.

Sediment and tissue samples, which had been stored at -20°C, were brought to room temperature and mixed thoroughly. Sub-samples of approximately 5 g (wet weight) of sediment or 0.5 g (wet weight) of tissue were weighed into Whatman™ 10-mm diam. x 50-mm long cellulose extraction thimbles. Hydrocarbons were extracted using a method similar to that described by Brown *et al.* (1985). The cellulose thimbles were placed in microsoxhlet apparatus and the samples saponified by soaking the thimbles in 5 ml of a 58 g/L methanolic potassium hydroxide solution for 15 h. The sample was then extracted with 50 ml of Distol®-grade DCM (Fisons) at 50°C for 4 h on a heating mantle. The resulting extract was added to 20 ml of distilled water in a 250-ml separating funnel and inverted 10 times to partition out the methanolic potassium hydroxide and other water soluble compounds. The aqueous layer was discarded and the DCM extract was rotary evaporated over a water bath (40°C) to approximately 1 ml. The extract was then cleaned-up by loading it onto a column containing 1 g activated alumina (70-230 mesh, activated at 80°C for 24h) over 4 g activated silica (60-120 mesh, activated at 80°C for 24 h) over 0.5 g of sodium sulphate. The column had previously been primed with 15 ml of DCM. The sample was run through the column with DCM until 30 ml of extract was collected from the column. The DCM extract was rotary evaporated over a water bath (40°C) to a volume of approximately 1 ml and made up to a final volume of 2.5 ml. This extract was washed into

a 3-ml sample vial with a further 1 ml of DCM and stored in the dark at -20°C until analysis.

The total aromatic hydrocarbon concentration of water, sediment and tissue extracts were analysed by U.V. absorbance spectroscopy measured at 254 nm on a Pye Unicam SP 8-100® U.V./visible spectrophotometer against a DCM blank (Gachanja, 1993). Results were compared to a chrysene standard curve and expressed in terms of chrysene equivalents. Minimum detection limits were 30 µg chrysene equivs. /L for aqueous samples and 5 µg chrysene equivs. /g for solid samples.

3.2.9. Statistical analyses of data.

Percentage data were arcsine transformed prior to analysis and all data were checked for normality using normal probability plots and for homogeneity of variances using Bartletts test. Chemical and survivorship data from the *in-situ*, stream water, field sediment and manipulated sediment tests were analysed using one-way analysis of variance (ANOVA), Tukey multiple-comparison tests and two-sample *t*-tests. *G. pulex* mortality data in DCM sediment extract set 'Z' concentration-response relationship was Probit transformed. Relationships between survivorship and DCM extract concentration, survivorship and aromatic hydrocarbon concentration, tissue concentration and extract concentration were analysed using least-squares regression techniques. The *q*-statistic for Tukey comparisons and Bartletts test were analysed according to Zar (1984). The remaining analyses were performed using the MINITAB statistical package (Minitab™ Inc., 1991) and significance levels in all cases were $p<0.05$.

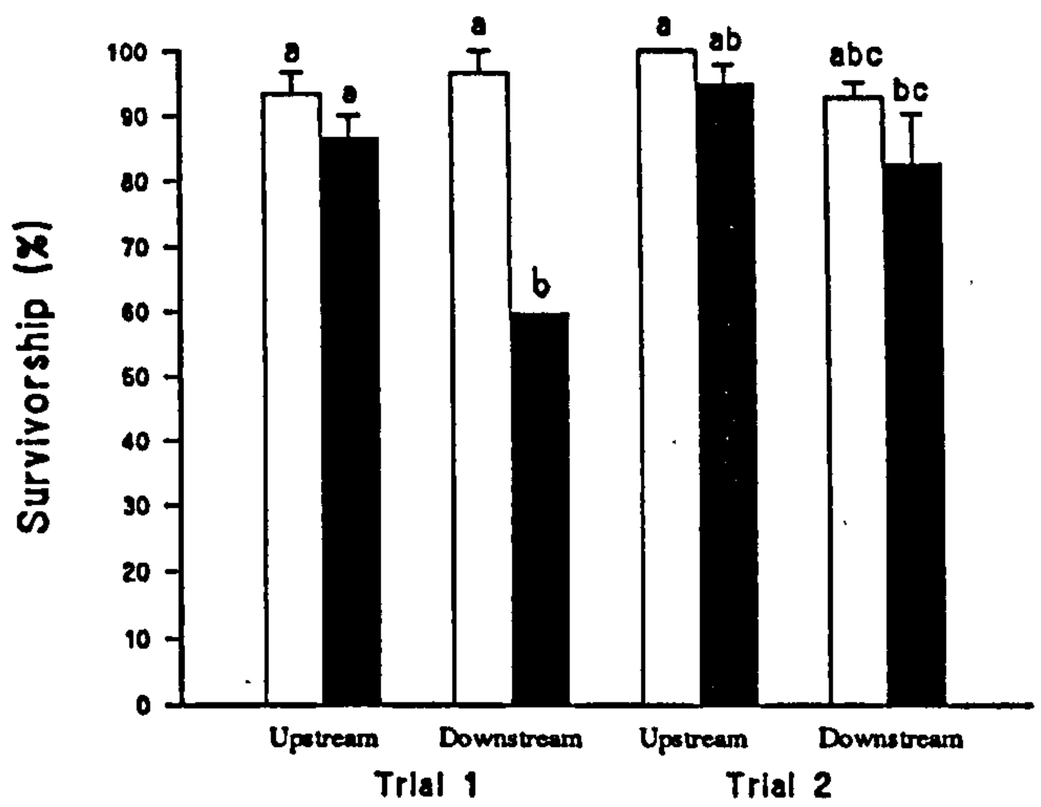
3.3. RESULTS.

A. *In-situ* exposures.

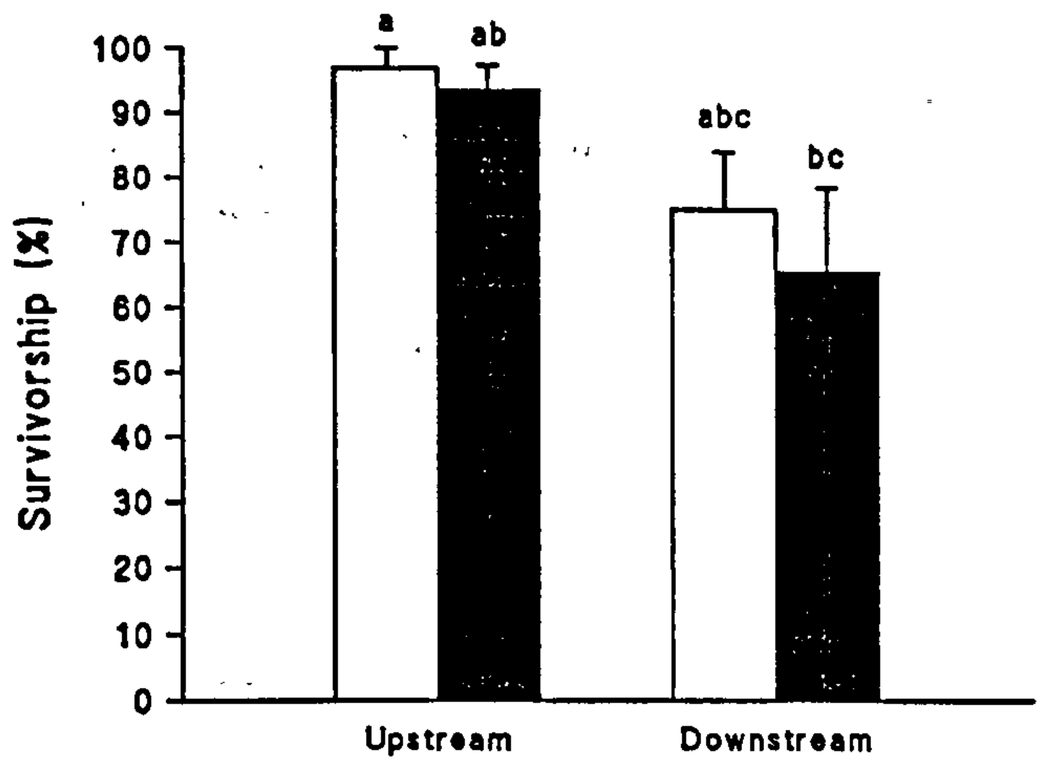
3.3.2. *In-situ* exposures.

In November 1993 (Trial 1), the survival of *G. pulex* exposed in contact with downstream sediment was significantly lower than that of animals exposed to upstream sediment (q=9.23, df=8,4). However, in December 1993 (Trial 2) although the survival of *G. pulex* exposed to downstream sediments was lower than that of animals exposed to upstream sediments this was not statistically significant (q=3.29, df=12,4). There was no significant between-station difference in the survival of *N. cinerea* or *P. jenkinsi* exposed to stream sediments (q<3.81, df=12,4; Fig. 3.1). Moreover, there was no significant between-station difference in the survival of *G. pulex*, *N. cinerea* or *P. jenkinsi* exposed to stream water (q<3.12, df>8,4 Fig. 3.1). Generally, for both *G. pulex* and *N. cinerea*, animals exposed to downstream sediment had the lowest survival whereas those exposed to upstream water had the highest survival (q=4.62, df>8,4, Fig. 3.1). However, within-station differences in survival were only significant for *G. pulex* where animals exposed to downstream sediment in Trial 1 had significantly lower survival than those exposed to downstream water (q=7.27, df=8,4).

a.)



b.)



c.)

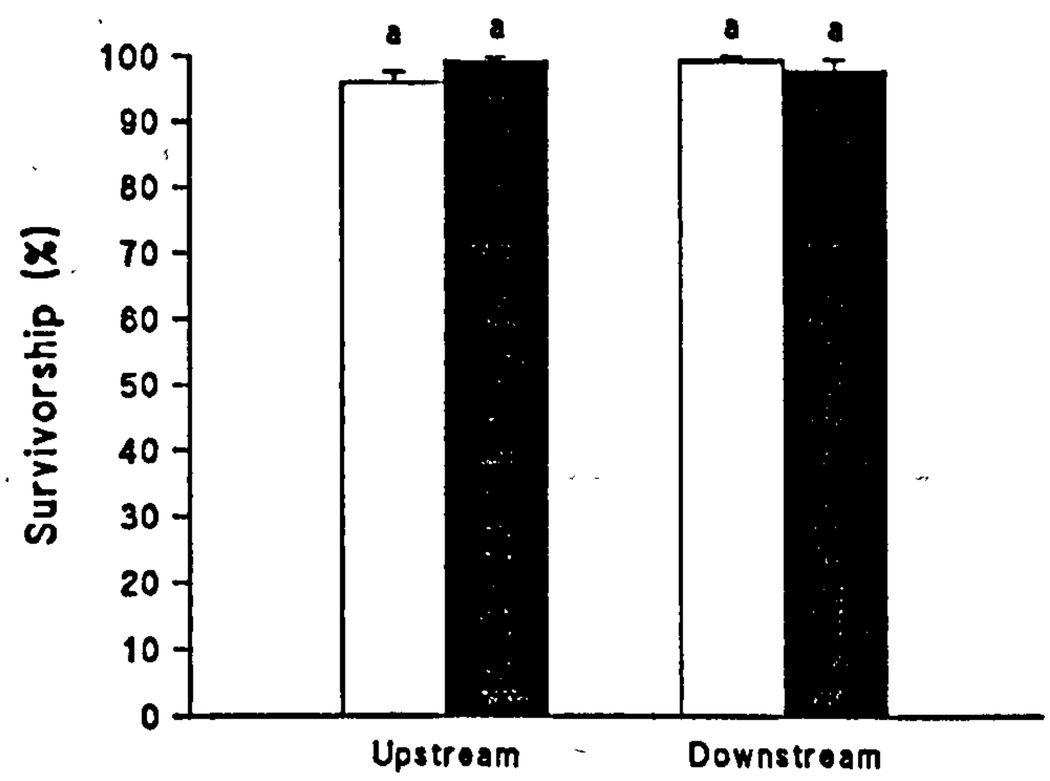


Fig. 3.1. Survivorship of a.) *G. pulex* in two separate trials, b.) *N. cinerea* and c.) *P. jenkinsi* placed *in-situ* at upstream and downstream stations at Pigeon Bridge Brook. Open bars represent animals exposed in the water column and solid bars represent animals exposed at the stream bed sediment surface. Data presented as mean values + 1 S.E.. Bars sharing the same letter code were not significantly different in each trial.

B. Laboratory toxicity tests.

3.3.3. Stream water.

Stream water collected from different stations at Pigeon Bridge Brook differed markedly in water quality (Table 3.8.). Downstream water had significantly elevated concentrations of aromatic hydrocarbons relative to APW and upstream water (q>8.70, df=37,3). Although, concentrations of most metals analysed were elevated in downstream water relative to upstream water and APW, this difference was only significant for zinc and chromium (q>6.14, df>3,3). Concentrations of aromatic hydrocarbons and metals in the downstream water did not alter significantly during exposure (t<6.75, df>21) nor was there any significant within-treatment difference of measured water quality during the 14-d exposure period (t<1.06, df>13).

Table 3.8. Concentrations of metals and total aromatic hydrocarbons (A.H. µg chrysene equivalents/ L) in stream water used in toxicity tests. Concentrations are displayed as means and 1 S.E (in parentheses). ND indicates below detection levels.

	Ca mg/L	Al mg/L	Zn µg/L	Cu µg/L	Cd µg/L	Cr µg/L	Pb mg/L	Fe mg/L	A.H.
APW	61.02 (1.79)	0.106 (0.030)	19.79 (3.61)	15.41 (0.07)	0.39 (0.12)	ND	0.006 (0.002)	0.24 (0.007)	ND
Upstream	74.31 (0.92)	0.029 (0.0003)	9.29 (2.90)	19.76 (1.56)	0.36 (0.10)	0.075 (0.053)	0.009 (0.001)	0.45 (0.01)	ND
Downstream	69.45 (0.09)	0.073 (0.003)	62.77 (5.79)	54.68 (15.00)	1.05 (0.03)	7.600 (1.410)	0.024 (0.013)	1.26 (0.67)	61.82 (7.89)

Despite the differences in water quality between the treatments, there was no significant difference in the survival of the five species tested (F<2.53, df<2,23, Table 3.6.). For all species except *C. riparius*, mean survival was > 80% in all treatments; for *C. riparius* survival ranged from 66-74 % (Table 3.9.).

Table 3.9. Percentage survival of animals after 14-d exposure to either APW or water collected from the upstream or downstream station at Pigeon Bridge Brook.

Species	APW		UPSTREAM		DOWNSTREAM	
	Mean	SE	Mean	SE	Mean	SE
Gammarus pulex	90.00	3.16	86.00	6.00	84.00	6.78
Nemoura cinerea	100	0.00	93.33	5.44	93.33	5.44
Potamopyrgus jenkinsi	98.33	1.67	98.33	1.67	100.00	0.00
Chironomus riparius	74.00	6.78	72.00	8.00	66.00	5.10
Tubifex tubifex	97.50	2.34	100.00	0.00	97.50	2.34

3.3.4. Field sediments.

Sediment collected from the downstream station at Pigeon Bridge Brook had significantly elevated concentrations of Zn, Cr, Cd, Pb, Cu, Mg and Ca relative to the control sand and the upstream sediment (q>4.42, df>6,2, Fig. 3.2.). In contrast, Ni and Fe concentrations were significantly higher in the upstream sediments relative to the downstream sediments and control sand (q>10.69, df>6,2). While there was no significant difference in Al concentrations between upstream and downstream sediment, concentrations were higher in these sediments relative to control sand (q>6.4, df>6,2). Aromatic hydrocarbon concentrations (mean ± 1 S.E µg chrysene equivs. /g wet weight) in downstream sediments (669.35 ± 46.3) were significantly higher than upstream sediment (33.4 ± 4.59) or control sand (2.72 ± 0.19; q>23.69, df>6,3). There was no significant difference in aromatic hydrocarbon concentrations between the control sand and upstream sediment (q=1.14, df=6,3).

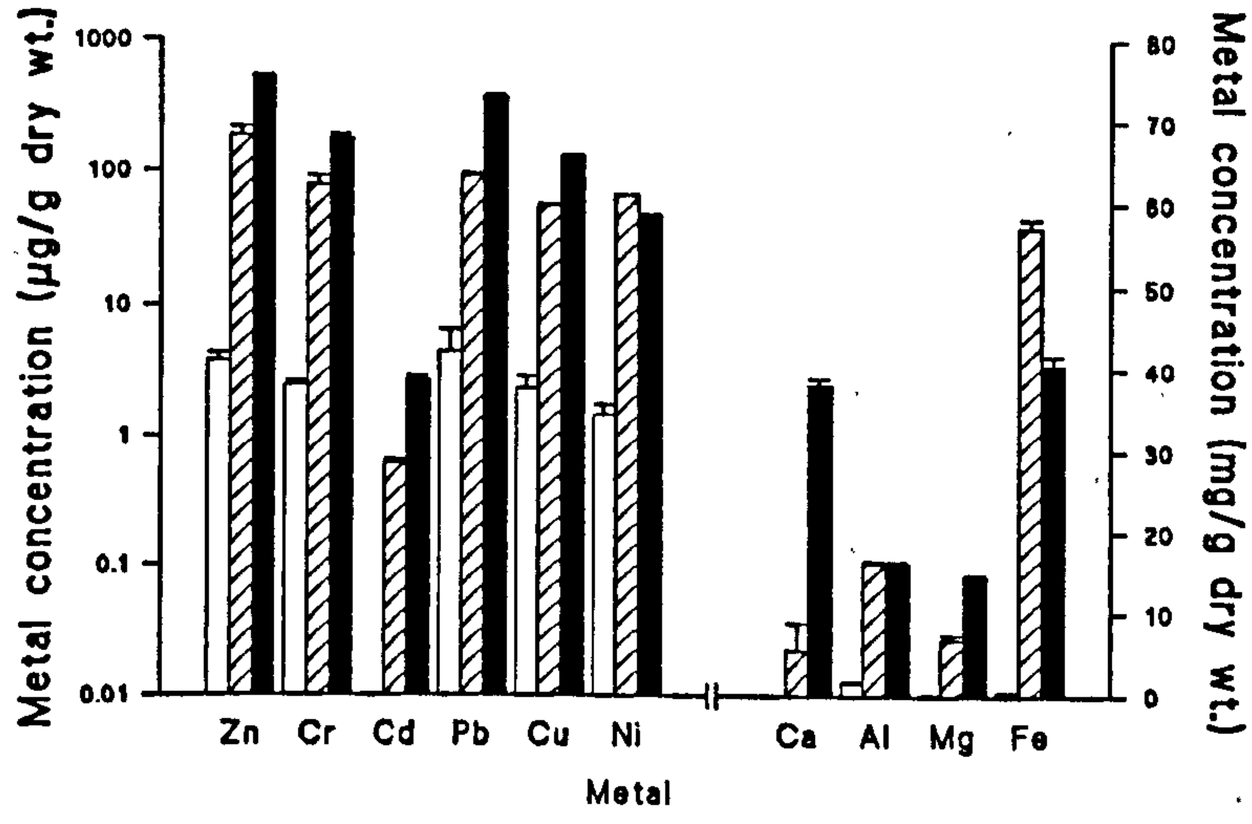


Fig. 3.2. Metal concentrations in sand (open bars), upstream sediment (hatched bars) and downstream sediments (solid bars). Data presented as means + 1 S.E.. See text for significant differences.

When exposed to downstream sediment *G. pulex* displayed a small, but significant, reduction in survivorship (q>3.75, df>9,3, Fig. 3.3.), and *N. cinerea* exhibited a marked reduction in survival (q>4.28, df>15,3, Fig. 3.3). *P. jenkinsi, C. riparius* and *T. tubifex* showed no significant between-treatment differences in mortality when exposed to the sand and field sediments (F<1.99, df>2,21).

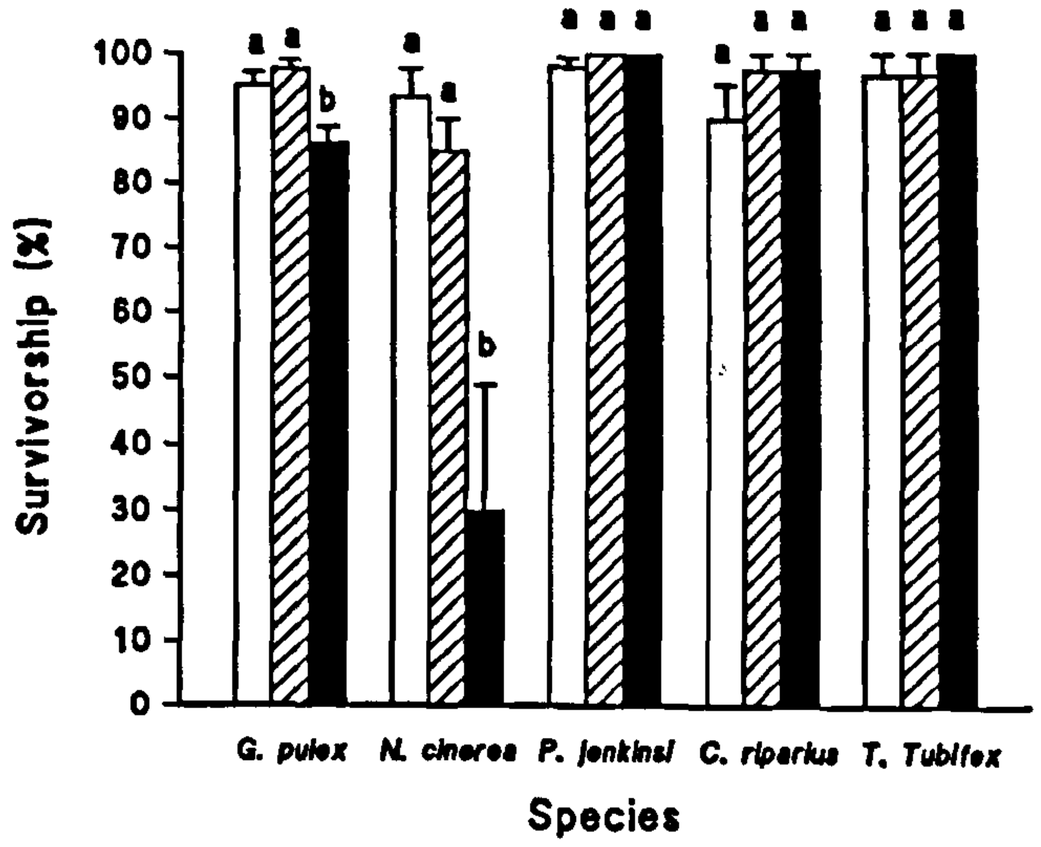


Fig. 3.3. Survivorship of the five macroinvertebrate species in the sediment toxicity tests. Open bars represent sand, hatched bars represent upstream sediment and solid bars represent downstream sediment. Data presented as means and + 1 S.E. Within species treatments which are significantly different are represented by a different letter.

3.3.5. Manipulated sediments.

Extracting downstream sediments with DCM did not generally affect metal concentrations. (Fig. 3.4.). The only exception to this was a significant reduction in Pb concentrations and a significant elevation in Fe concentrations in the extracted sediments (t>4.95, df=2).

Solvent extraction of the downstream sediment successfully removed the majority of aromatic hydrocarbons from the downstream sediment (93.1 %) such that total aromatic hydrocarbon concentrations (mean ± 1 S.E. µg chrysene equivs./ g wet weight) were significantly greater in non-extracted downstream sediment (669.3 ± 46.0) compared to cleaned up downstream sediment (46.44 ± 4.0; t>4.31, df>2). The survival of both *G. pulex* and *N. cinerea* was significantly lower when exposed to downstream sediment rather than solvent-extracted downstream sediment (t=3.51, df=5; Fig. 3.5.). In contrast, *P. jenkinsi*, *C. riparius* and *T. tubifex* showed no between-treatment differences in survivorship (t<1.42, df>7, Fig. 3.5.); survivorship being >86.7 % in all treatments with these species.

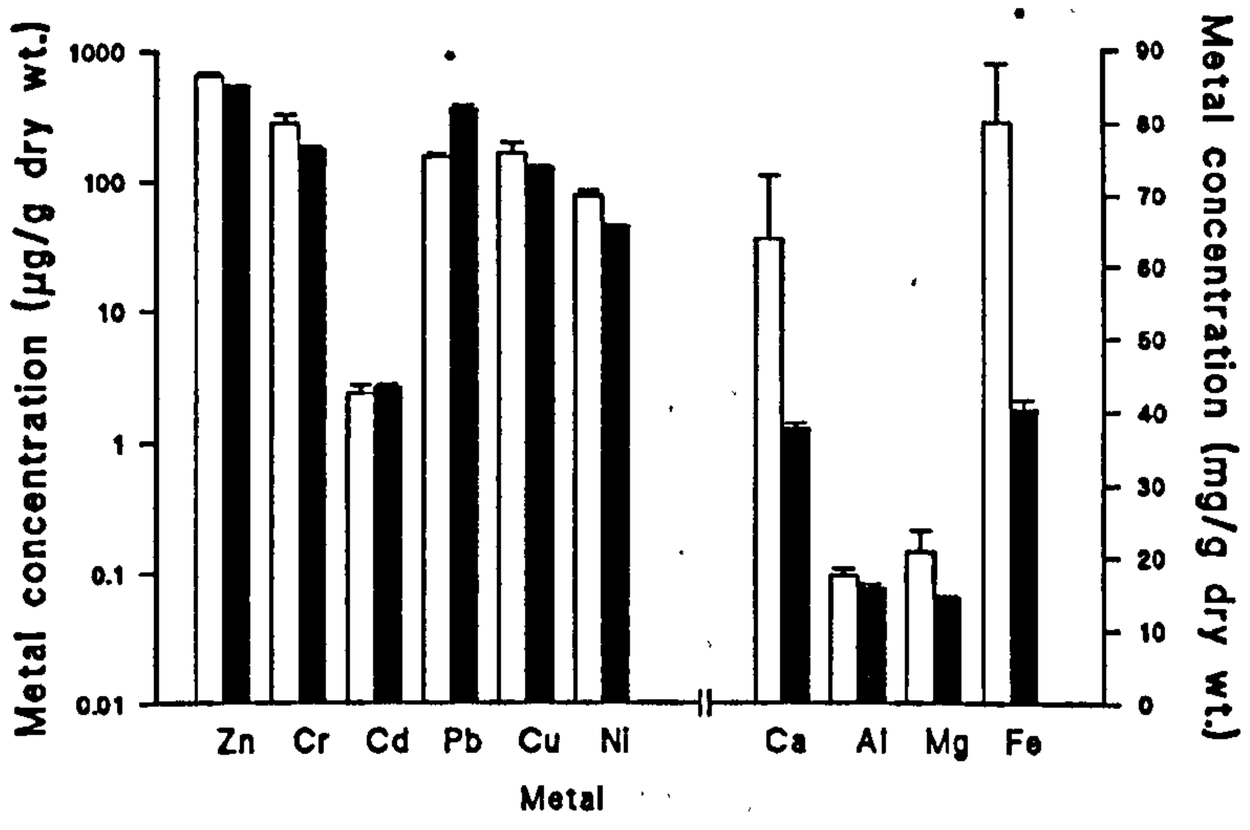


Fig. 3.4. Metal concentrations in solvent extracted downstream sediment (open bars) and non-extracted downstream sediments (solid bars). Data presented as means + 1 S.E.. Asterisks denote significant between-treatment differences.

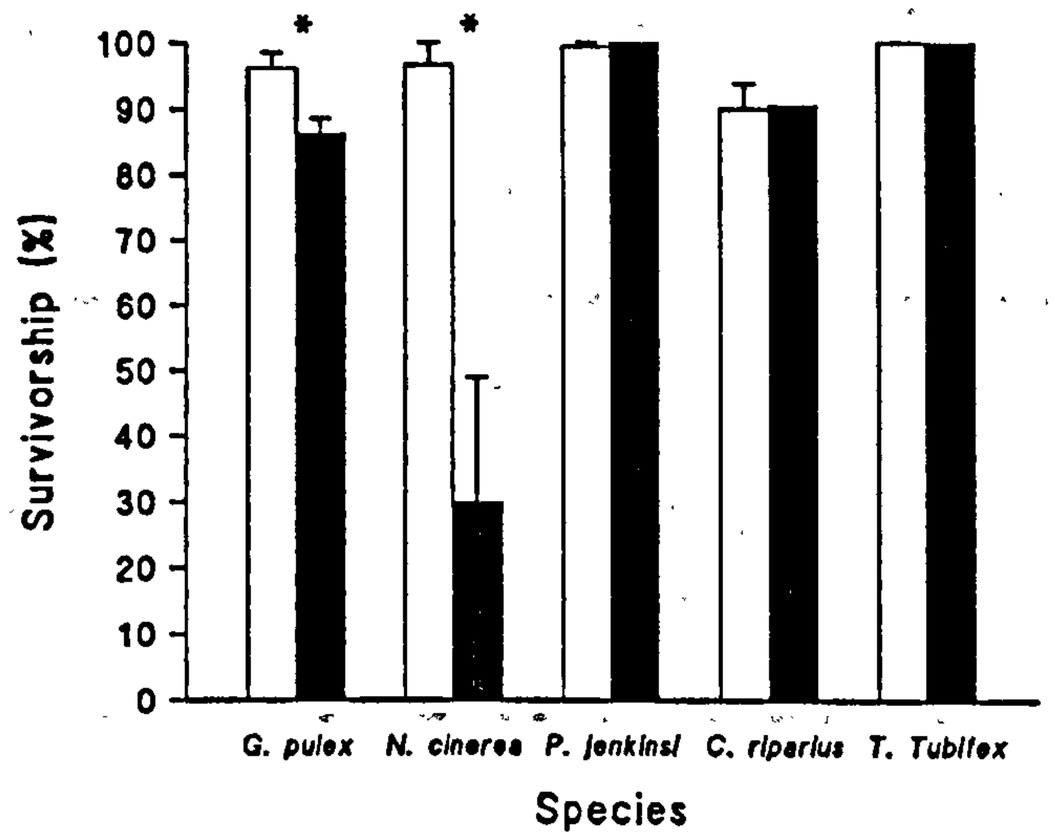


Fig. 3.5. Survivorship of the five macroinvertebrate species in the manipulated sediment toxicity tests. Open bars represent DCM-extracted downstream sediment and solid bars represent non-extracted downstream sediment. Data presented as means and + 1 S.E.. Asterisk denotes significant between-treatment difference.

3.3.6. Dichloromethane ('solvent') sediment extract.

The toxicity of three sets of dichloromethane (DCM) extracts ('X', 'Y' and 'Z') prepared using upstream and downstream field sediments from Pigeon Bridge Brook was assessed. *G. pulex* was exposed to 'X', 'Y' and 'Z', *N. cinerea* and *T. tubifex* to extracts 'Y' and 'Z', and *P. jenkinsi* and *C. riparius* to extract 'X'.

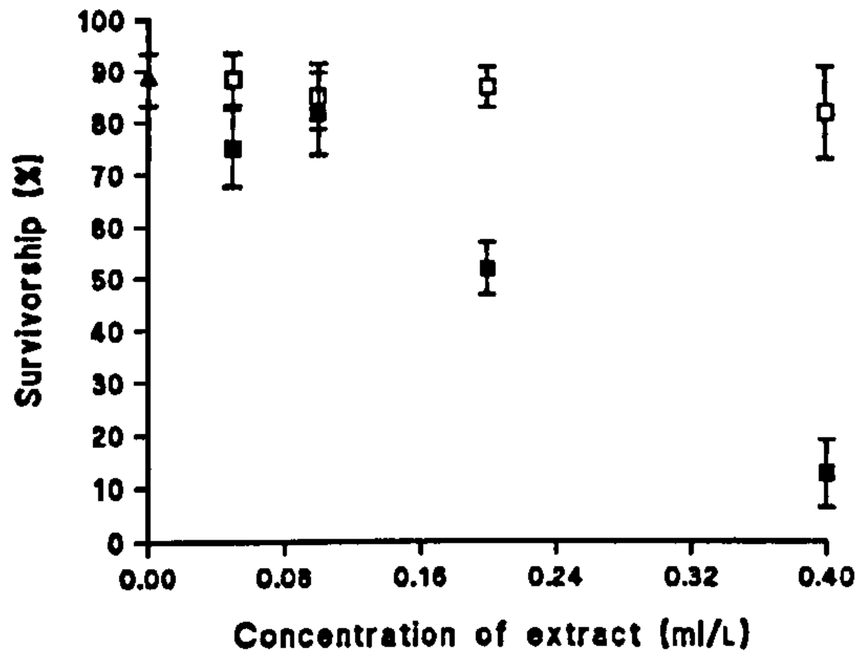
3.3.6.a. Exposure of *G. pulex*.

There was no significant difference in the metal (Al, Cd, Cr, Cu, Pb, and Zn) concentrations of water spiked at 0.4 ml extract/ L with either the upstream or downstream sediment extract in set 'X' (t<1.19, df=4). For two of the three sets of extracts ('X' and 'Z') there was a significant negative correlation between the survival of *G. pulex* and the concentration of downstream sediment extract (r>0.98, df=10, Fig. 3.6.). However, there was no significant difference in the survival of control animals and those exposed to the highest concentration of upstream sediment extract (t<1.43, df>7). Extract 'Y' was used for toxicity comparisons of *G. pulex* with other species and therefore *G. pulex* was only exposed to 0.4 ml extract/ L. When sediment extract concentrations were expressed in terms of total aromatic hydrocarbon concentration, the upstream and downstream data sets merged resulting in common concentration-survivorship relationships (r>0.82, df>13, Fig. 3.6.). The 14-d LC_{50} for *G. pulex* exposed to extracts 'X'-'Z' are displayed in Table 3.10.

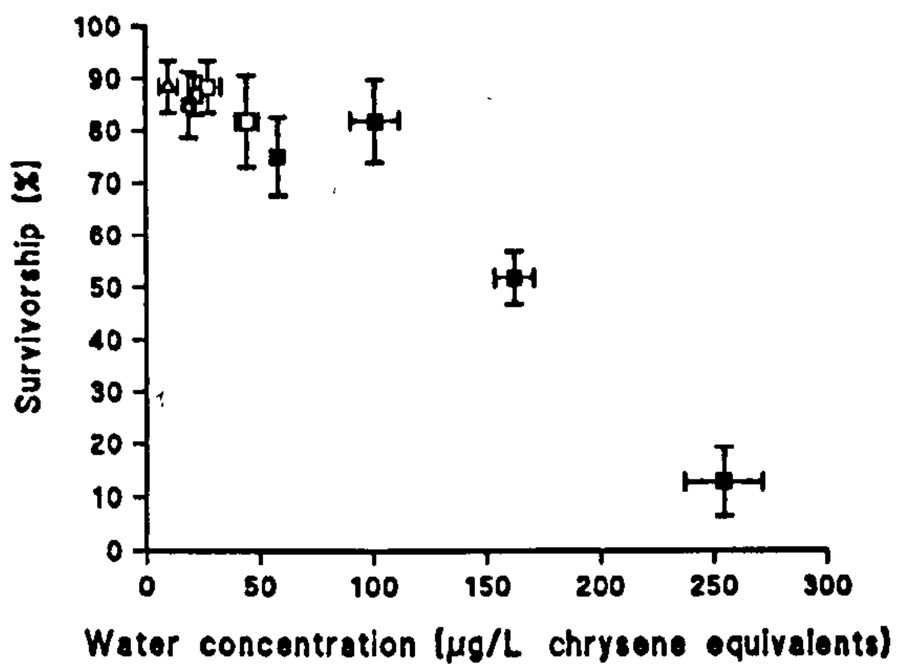
Table 3.10. 14-d LC_{50} values for *G. pulex* survivorship exposed to sediment extract sets 'X'-'Z'

Extract	LC_{50}	95 % Confidence limits	
X	143.2	121.49	164.93
Y	156.4	129.00	183.80
Z	239.4	209.20	269.50

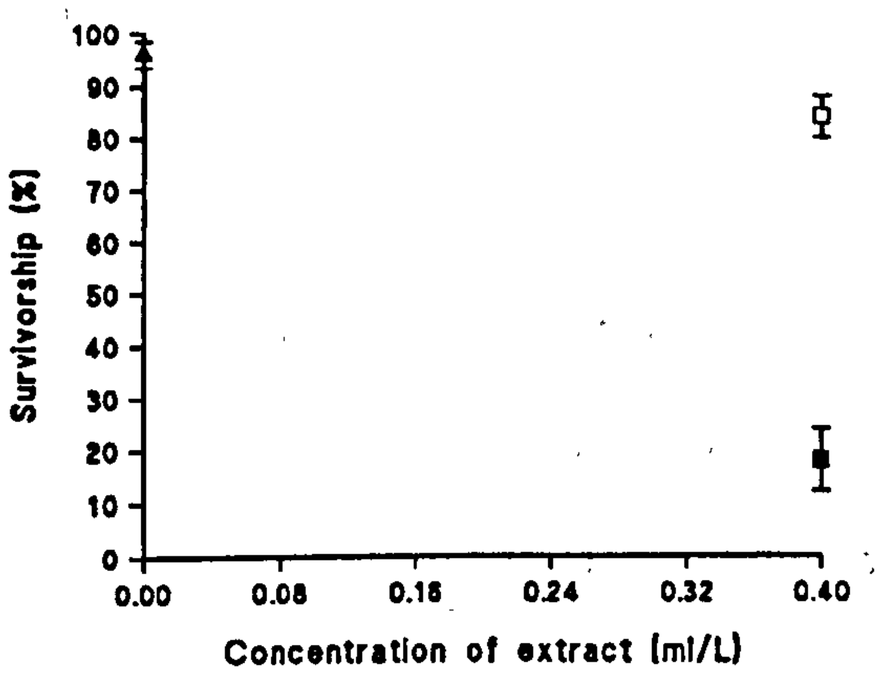
1.) Extract set 'X'



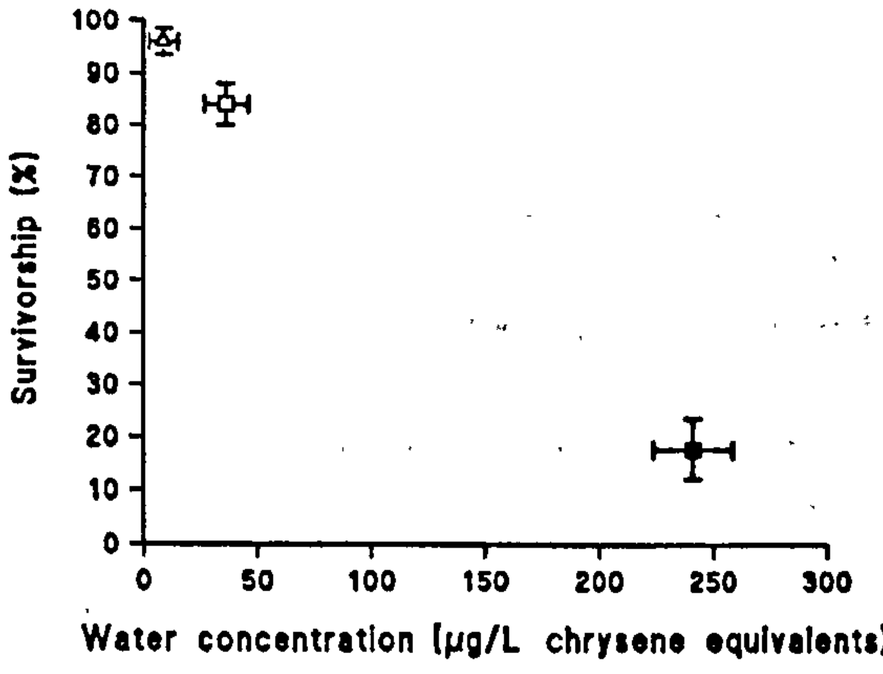
2) Extract set 'X'



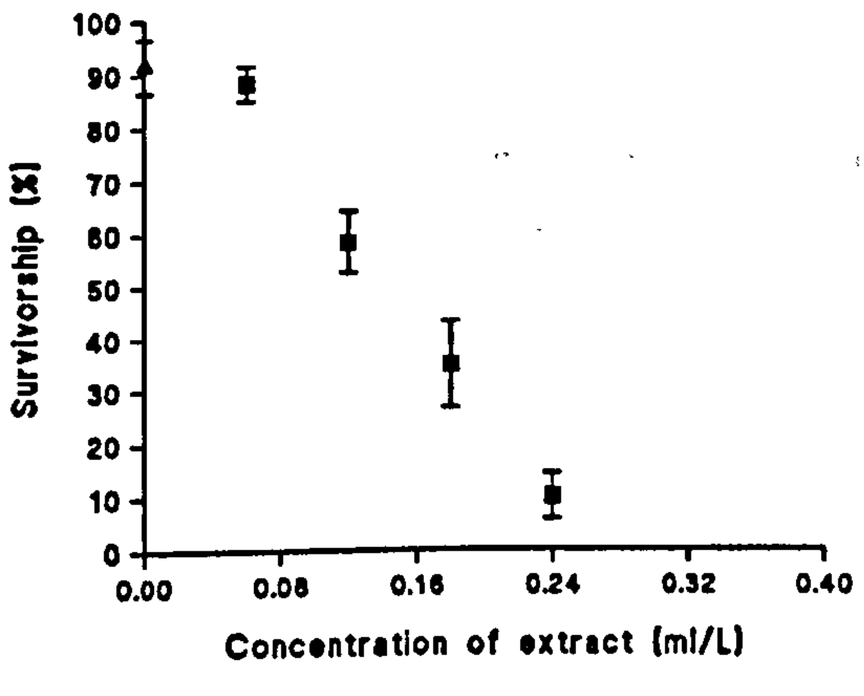
1.) Extract set 'Y'



2.) Extract set 'Y'



1.) Extract set 'Z'



2.) Extract set 'Z'

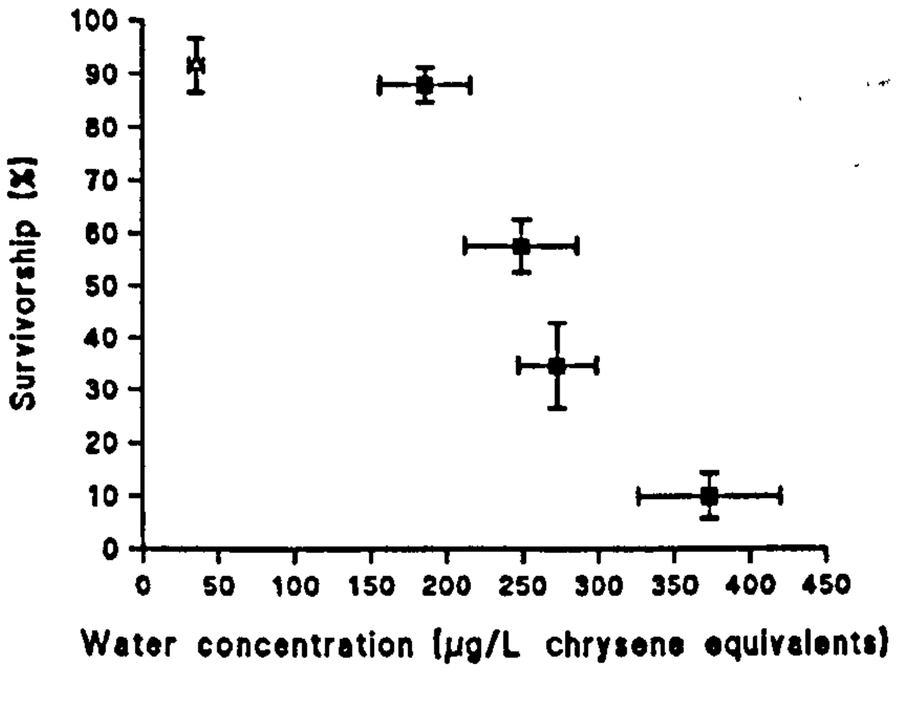


Fig. 3.6. Extract concentration-survivorship relationships 1.) and total aromatic hydrocarbon concentration (µg chrysene equivalents /L)-survivorship relationships 2.) for extract sets 'X'-'Z' in *G. pulex* exposures. Open triangles represent controls, open squares represent upstream sediment extracts and solid squares represent downstream sediment extracts. Data are presented as mean values ± 1 SE..

Analysis of *G. pulex* exposed to extract in set 'X', indicated that aromatic hydrocarbons were accumulated in direct proportion to exposure concentrations (r=0.996, df=7, Fig. 3.7) and there was a significant negative relationship between whole-body hydrocarbon concentrations and percentage survival (r=-0.96, df=7, Fig. 3.7).

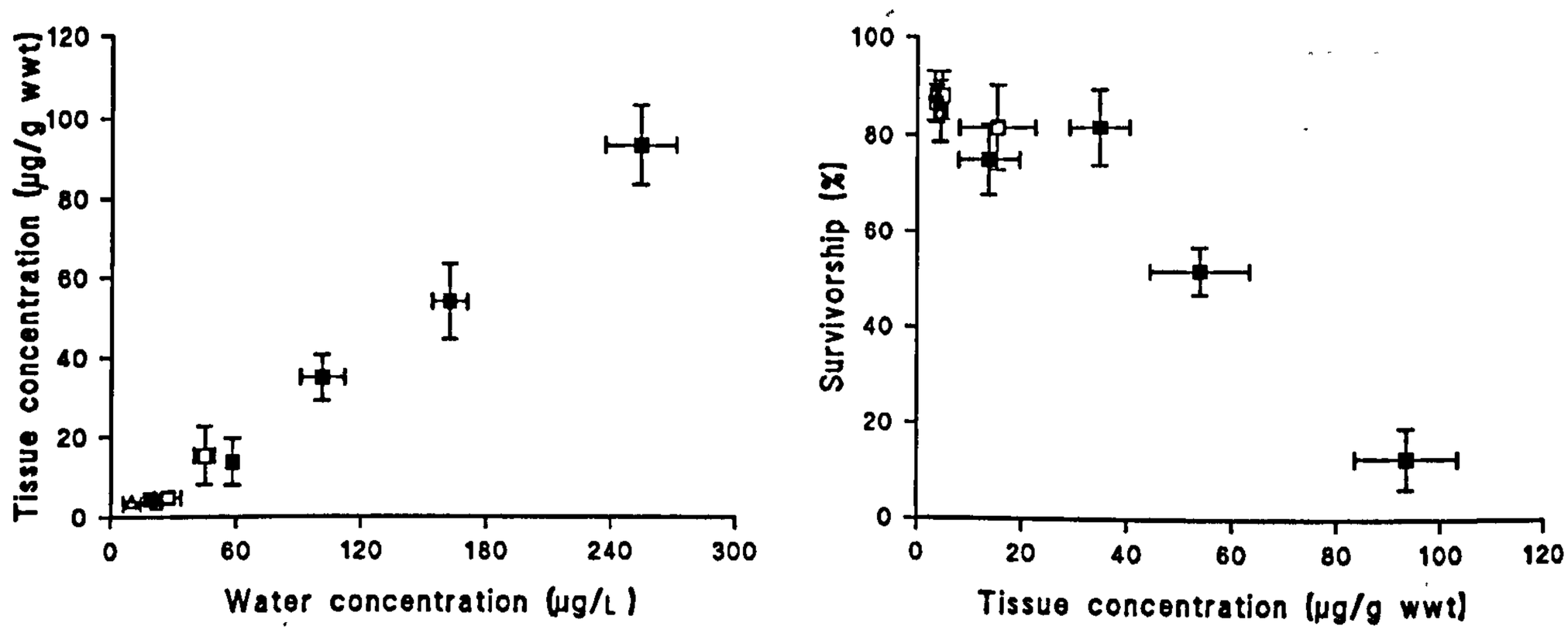
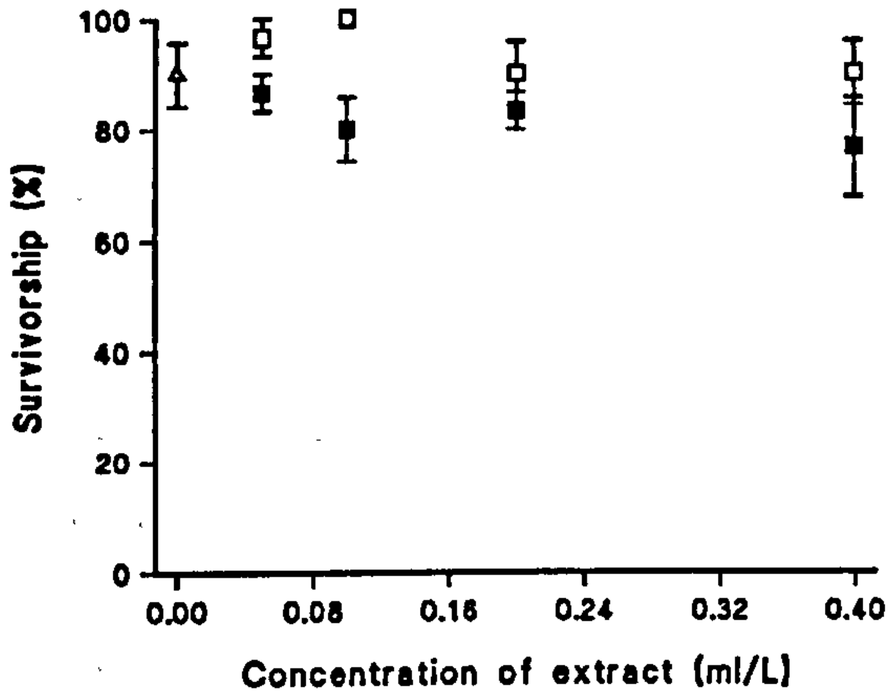


Fig. 3.7. Relationships between the accumulation of aromatic hydrocarbons (µg chrysene equivalents/ g wet wt.) by *G. pulex* and a.) the concentration of hydrocarbons in the water (µg chrysene equivs./ L) b.) survivorship (%) when exposed to either upstream (open squares) or downstream (solid squares) sediment extract 'X'. Data are presented as mean values ± 1 S.E..

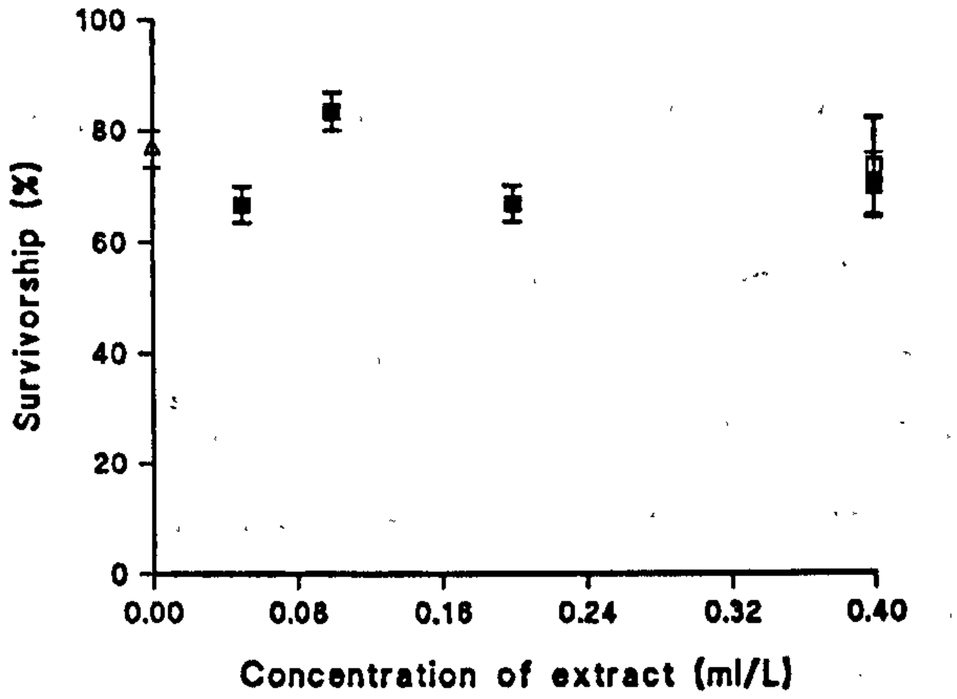
3.3.6.b. Exposure of *N. cinerea, P. jenkinsi, C. riparius* and *T. tubifex.*

In contrast to the results for *G. pulex,* there was no significant relationship between survivorship and sediment extract concentration for either *N. cinerea, P. jenkinsi, C. riparius* or *T. tubifex* (r<0.48, df>10, Fig. 3.8).

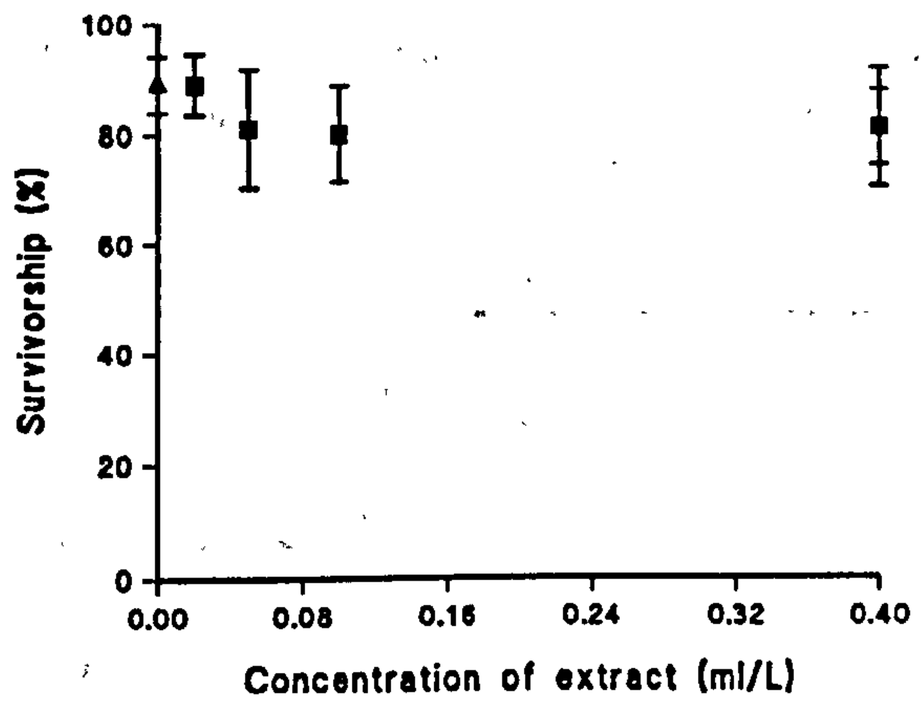
a.) *N. cinerea* extract set 'Y'



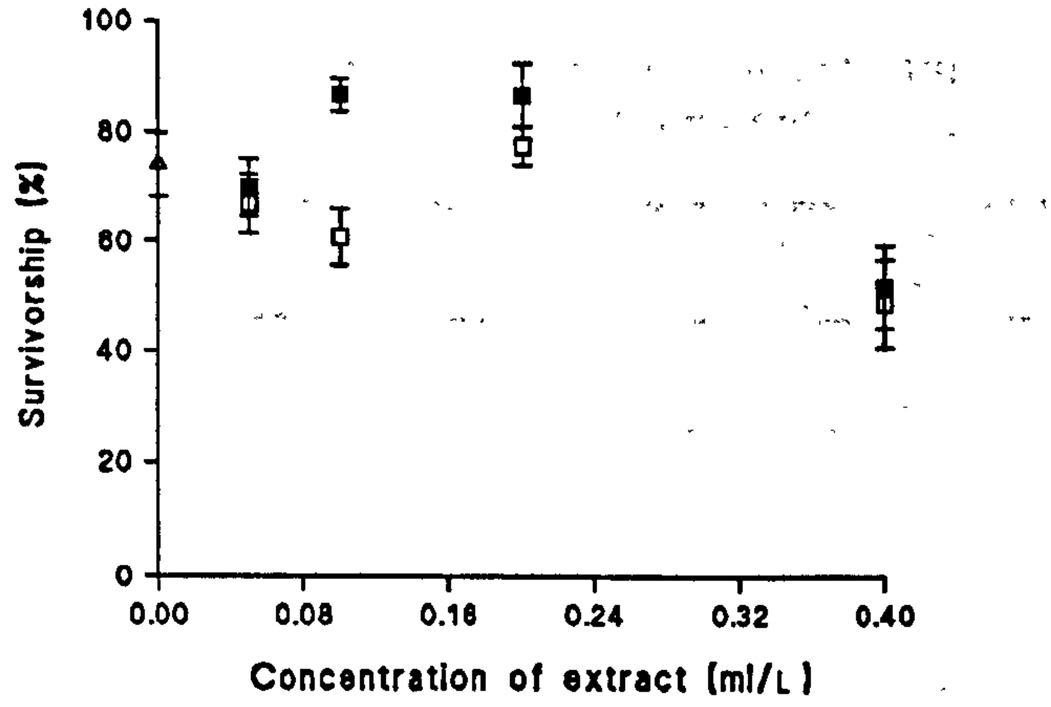
b.) *N. cinerea* extract set 'Z'



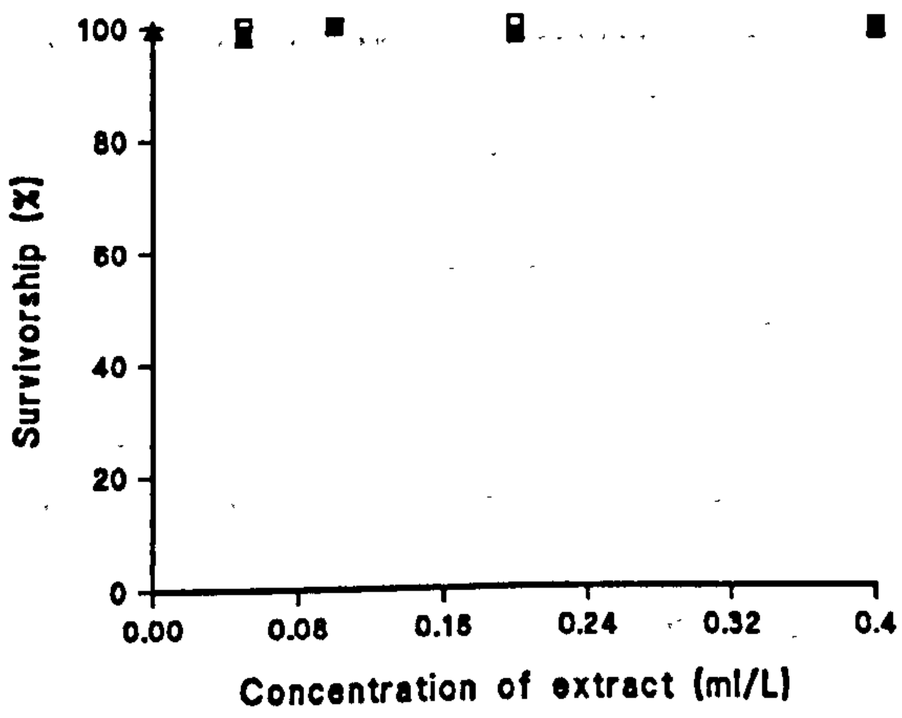
c.) *P. jenkinsi* extract set 'X'



d.) *C. riparius* extract set 'Y'



e.) *T. tubifex* extract set 'Y'



f.) *T. tubifex* extract set 'Z'

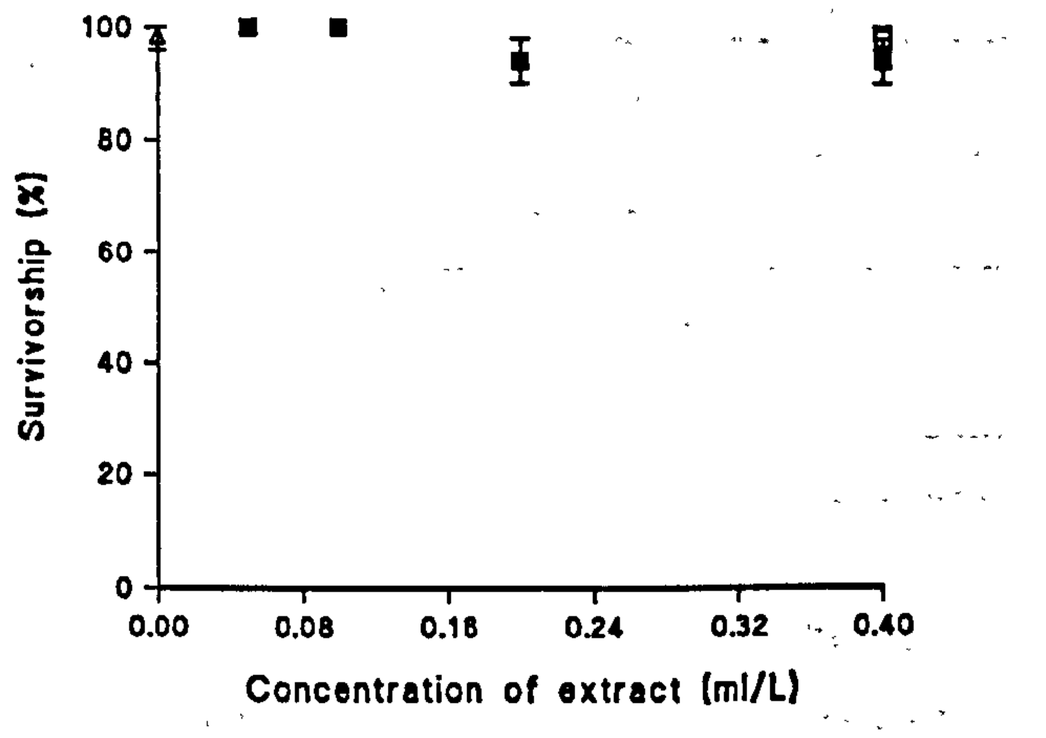


Fig. 3.8. Relationship between the survivorship of *N. cinerea, P. jenkinsi, C. riparius* or *T. tubifex* and extract concentration when exposed to extract sets 'X', 'Y' or 'Z'. Open triangles represents acetone carrier control, open squares represent upstream extract and solid squares represent downstream extract. Data presented as mean values ± 1 S.E.

3.3.7. Acetic acid ('acid') sediment extract.

Acid extracts of sediment from the upstream and downstream stations at Pigeon Bridge Brook were used to spike APW (hardness 192.54 mg $CaCO_3$ equivalents /L). The pH values of the control and highest upstream and downstream extract concentrations (10 ml extract/ L) were 6.45, 6.39 and 7.02 respectively and therefore within the range of pH values for stream water at Pigeon Bridge Brook (Appendix A2.1, Table A2.1.1). The extract prepared from downstream sediment had significantly elevated concentrations of Pb, Cd, Al, Cr, Cu, Fe and Ca relative to the upstream sediment extract (t>3.59, df>2, Table 3.11). Concentrations of Cd in the 10 ml extract/L and Zn in the 10 and 6 ml extract/L downstream sediment extracts were significantly higher than in the control acid and upstream 10 ml extract/L test solutions (q>4.71, df=12,6, Table 3.12). Concentrations of total aromatic hydrocarbons were below detection levels in all the treatments.

Table 3.11. Metal concentrations in acetic acid extracts of upstream or downstream sediments from Pigeon Bridge Brook. Data presented as means and 1 S.E (in parentheses). ND indicates below detection levels.

	Zn mg/L	Pb mg/L	Cd µg/L	Cr mg/L	Cu mg/L	Ni mg/L	Fe mg/L	Al mg/L	Mg mg/L	Ca mg/L
Upstream	6.05 (1.31)	0.85 (0.11)	ND	0.13 (0.02)	0.94 (0.14)	0.52 (0.05)	25.33 (2.90)	8.69 (1.43)	98.11 (4.93)	724.3 (49.9)
Downstream	9.86 (0.99)	1.71 (0.21)	41.30 (7.00)	1.32 (0.13)	4.49 (0.45)	0.45 (0.06)	223.80 (25.50)	33.67 (3.18)	142.96 (13.70)	1663.2 (130.7)

Table 3.12. Metal concentrations of control and test solutions used in toxicity tests using acid sediment extracts. Data presented as means, S.E. given in parentheses. ND indicates below detection levels.

	Zn µg/L	Cu µg/L	Cd µg/L	Cr µg/L	Pb µg/L
Control	19.07 (9.27)	5.97 (0.27)	ND	ND	ND
Upstream extract					
10 ml/L	17.33 (8.59)	4.03 (1.69)	0.09 0.04	ND	ND
Downstream extract					
2 ml/L	28.01 (4.23)	2.67 (0.81)	0.01 0.008	ND	ND
4 ml/L	65.37 (0.26)	2.07 (1.16)	ND	ND	ND
6 ml/L	142.67 (50.7)	4.83 (0.14)	0.02 0.01	ND	ND
10 ml/L	145.00 (7.41)	4.03 (0.75)	0.90 0.41	ND	ND

There was no significant difference in the survival of animals exposed to either the acid carrier control, upstream sediment extract at 10 ml extract/L or any of the downstream sediment extract exposure concentrations (F<1.5, df>5,12, Table 3.13). Although concentrations of Cd, Pb, Cu and Zn in *G. pulex* tissues were higher in the 10 and 6 ml

extract/L downstream sediment treatments compared to the upstream 10 ml extract/L and acid control water, this was only significant for Cd (q>4.95, df=12,3, Table 3.14).

Table 3.13. Percent survival of the five macroinvertebrate species to acid sediment extract. Data presented as means and 1 S.E. (in parentheses).

Species	Acid control	Upstream 10 ml/L	Downstream 2 ml/L	Downstream 4 ml/L	Downstream 6 ml/L	Downstream 10 ml/L
G. pulex	86.70 (13.30)	70.00 (5.77)	93.33 (3.33)	93.33 (3.33)	83.33 (3.33)	93.33 (3.33)
N. cinerea	73.33 (8.82)	76.67 (3.33)	73.33 (3.33)	80.00 (10.00)	66.67 (6.67)	70.00 (5.77)
P. jenkinsi	87.50 (5.26)	92.50 (3.66)	97.50 (2.50)	97.50 (2.50)	92.50 (5.26)	97.50 (2.50)
C. riparius	90.00 (5.35)	87.50 (5.26)	92.50 (5.26)	92.50 (5.26)	92.50 (3.66)	90.00 (5.35)
T. tubifex	87.50 (6.48)	90.00 (3.78)	97.50 (2.50)	95.00 (5.00)	97.50 (2.50)	97.50 (2.50)

Table 3.14. Concentration of metals (µg/g) in *G. pulex* tissue exposed to acid sediment extracts. Data presented as means and 1 S.E. (in parentheses).

	Zn		Cu		Cd		Cr		Pb	
Control	161.92	(15.90)	120.31	(10.28)	6.12	(0.025)	5.25	(3.45)	49.19	(8.63)
Upstream extract										
10 ml/L	191.62	(13.72)	89.96	(8.86)	6.28	(0.56)	2.93	(1.74)	63.82	(8.26)
Downstream extract										
2 ml/L	210.48	(81.40)	111.32	(8.41)	6.06	(0.032)	0.2	(0.2)	52.08	(5.41)
4 ml/L	158.29	(8.81)	98.06	(6.20)	6.09	(0.27)	0.14	(0.14)	60.32	(1.01)
6 ml/L	220.14	(27.41)	128.0	(10.25)	8.58	(0.81)	0.34	(0.34)	89.72	(13.42)
10 ml/L	211.77	(7.87)	137.78	(19.86)	9.68	(0.53)	1.49	(0.89)	71.37	(1.86)

3.4. DISCUSSION.

Results presented in Chapter 2 indicated that downstream of the motorway discharge into Pigeon Bridge Brook there was an increase in the concentrations of heavy metals and hydrocarbons in the sediment, a decrease in the diversity of the benthic macroinvertebrate community and a change in the relative abundance of macroinvertebrate species. The hypothesis that the observed changes in the biota were linked to the observed changes in sediment and water chemistry was addressed by investigating the lethal toxicity of stream water and sediments to selected macroinvertebrates. The macroinvertebrates studied were *G. pulex*, *N. cinerea*, *P. jenkinsi*, *C. riparius* and *T. tubifex* and toxicity was assessed *in-situ* and in the laboratory.

None of the three species deployed *in-situ* at Pigeon Bridge Brook (*G. pulex*, *N. cinerea* and *P. jenkinsi*) showed any significant toxic response to stream water at the station which received motorway runoff. Moreover, neither of these species nor *C. riparius* nor *T. tubifex* were affected by exposure to filtered stream water in the laboratory. It would therefore appear that runoff-contaminated stream water is not toxic to these species. However, one possible limitation of the experimental method used in the laboratory study was the potential loss of toxicants in the stream water due to their retention by filters (Schubauer-Berigan and Ankley, 1991). Zinc and total aromatic hydrocarbons were significantly elevated in the downstream water but the concentrations of zinc in the water used in laboratory exposures (i.e. 0.063 mg/L) was below the reported 96 h LC_{50} values for *Gammarus pulex* (2.1 mg/L), *Leuctra* sp. (37 mg/L), *Tubifex tubifex* (130 mg/L), *Chironomus tendipes* (62.5 mg/L) and the mollusc *Lymnaea emarginata* (4.15 mg/L) (Abel and Green, 1981; Mance, 1987). It is maybe not surprising, therefore, that no lethal toxicity was observed in stream water exposures in the present study. Motorway runoff is generally of an episodic nature (Balades *et al.*, 1984; Bellamy, 1990) and therefore the toxicity of stream water may vary temporally. The results from the current study can only offer a 'snapshot' of possible toxicity. However, previous authors have also found minimal toxicity of road runoff waters (Gjessing *et al.*, 1984a; Kobriger *et al.*, 1984; Dupois *et al.*, 1985). Survival of *C. riparius* in laboratory water exposures was generally poor but still there was no indication of any toxicity. The low survival of *C. riparius* may indicate an additional stress on the animals possibly caused by the lack of a substrate (or food source) in the stream water tests (Lamberson and Swartz, 1988; Giesy and Hoke, 1989; Giesy *et al.*, 1990; Ankley *et al.*, 1994).

Gjessing *et al.* (1984a) assessed the toxicity of complex mixtures of road runoff contaminants to a variety of organisms and concluded that many of the potential organic (and inorganic) pollutants were strongly absorbed to particulate matter. Further, they concluded that the lethal toxicity of runoff water was minimal to bacteria, fungi, protozoa and fish. Kobriger *et al.* (1984) also concluded that road runoff water had minimal effects on *Pimephales promelas* (fathead minnow), *Daphnia magna*, *Asellus intermedius* or *Hexagenia* sp. in flow-through toxicity tests. However, slight toxicity was observed with *Gammarus pseudolimnaeus* when exposed to runoff from a moderately used road but not from a heavily used road. The assays in the study by Kobriger *et al.* (1984) displayed many inherent problems such as high control mortality due to cannibalism. Neither study tested the toxicity of sediments which are the sink for the majority of the runoff pollutants. At Pigeon Bridge Brook concentrations of metals were generally between 1.2 and 22.6 times greater in the sediments than overlying water, exceptions being Al, Mg and Ca which were generally at higher concentrations in the water. And whereas concentrations of total aromatic hydrocarbons were in excess of 405 µg chrysene equivs. /g (wet wt.) in sediments, they were undetectable in stream water (Maltby *et al.*, 1995a; Appendix A2.1, Table A2.1.3).

The survival of both *G. pulex* and *N. cinerea* was reduced when they were placed *in-situ* in contact with contaminated sediment. Although this effect was only statistically significant for the first *G. pulex* deployment, the same pattern of response was observed in both *G. pulex* deployments highlighting the potentially toxic nature of the contaminated sediment. Variability in animal survival within and between *in-situ* trials may be due to a number of reasons including: increased turbidity and dissolved constituents in storm events, spatial variability in toxicant levels, and deployment/ transportation stresses (Cowley, 1985; Elder and Dresler, 1988; Sasson-Brickson and Burton, 1991; Burton, 1992b). In contrast to *G. pulex* and *N. cinerea*, *P. jenkinsi* showed no significant mortality to the stream sediment in the *in-situ* exposures.

Similar results were obtained by Pratt *et al.* (1981) and Medeiros *et al.* (1983) who investigated the effect of urban runoff on a number of benthic macroinvertebrates. They concluded that whereas Plecoptera, Ephemeroptera, Trichoptera and Amphipoda were sensitive to urban runoff Mollusca, Diptera and Oligochaeta were not. The importance of stream sediments as a source of potential toxicants was illustrated in a study by Shutes *et al.* (1992). *G. pulex* and *Asellus aquaticus* were exposed in urban runoff overflows and in the receiving streams. Animals that were caged in the proximity of stream sediments accumulated higher concentrations of lead and copper than those exposed solely to the overflow discharge. Shutes *et al.* (1992) concluded that chronic

exposure resulted in higher uptake of metals, originating from the sediments, than during intermittent storm overflow events. However zinc concentrations in the soluble and suspended phase were high resulting in higher uptake of this metal in discharge-exposed *G. pulex* accounting for a subsequent increase in the mortality of these animals. Medeiros *et al.* (1983) also concluded that toxicity of urban discharges to macroinvertebrates originated from the sediments.

The potential toxicity of Pigeon Bridge Brook contaminated sediments was investigated further in a series of laboratory experiments. Previous studies have suggested that contamination of stream sediments is heterogeneous due to their patchy physicochemical nature (Burton, 1992a). Several field sediments were therefore used to screen for toxicity using *G. pulex*, although only the data for most toxic sediment is reported here. This sediment was also used to expose *N. cinerea, P. jenkinsi, C. riparius* and *T. tubifex*. It was evident that sediment quality differed between treatments. Downstream sediments generally had significantly higher concentrations of those metals analysed (i.e. Zn, Cr, Cd, Pb, Cu, Mg and Ca) compared to other treatments; the exceptions being Ni, Al, and Fe. Despite marked between-station differences in sediment quality only *G. pulex* and *N. cinerea* displayed minor, though statistically significant, increased mortality when exposed to the downstream sediment. *P. jenkinsi, C. riparius* and *T. tubifex* showed no significant mortality to the downstream field sediment with survivorship remaining at > 90 % with all treatments. These laboratory data therefore support the *in-situ* results.

Due to the greatly elevated pollutant concentrations in the sediments from the downstream station at Pigeon Bridge Brook it may be predicted that the sediment would be toxic. However, many factors affect the bioavailability of inorganic and organic pollutants in aquatic sediments to benthic animals. Organic pollutant bioavailability is controlled, to a certain extent, by organic carbon content (Adams, 1987; DiToro *et al.*, 1991; Landrum and Faust, 1991) but other factors such as sediment particle size, DOC, and temperature also play a significant part (Ibanga, 1987; Müller, 1987; Al Mohanna, 1988; Kukkonen and Oikari, 1989; Suedel *et al.*, 1993). Inorganic pollutant bioavailability is partly controlled by organic carbon but also acid volatile sulphides (AVS), particle size, pH, and redox gradients (Di Toro *et al.*, 1990, 1992; Forstner, 1990; Ankley *et al.*, 1994).

Although organic carbon concentrations at the downstream station were lower than at the upstream station at Pigeon Bridge Brook (2.3% v 11.6%, Maltby *et al.*, 1995a) concentrations were still high compared to many sediments (e.g. 0.3-0.43 %; DeWitt *et*

al., 1992) and may go some way to explaining the low toxicity of these sediments. Furthermore, Pigeon Bridge Brook is a small stream with a dense riparian canopy at the downstream station. Large quantities of CPOM in the form of leaves therefore enter the system producing a high pollutant absorptive potential and hence reducing bioavailability (McCarthy and Bartell, 1988). High calcium (and hardness) concentrations in the sediments and water at the downstream station at Pigeon Bridge Brook (a feature of motorway runoff pollution, Kobriger, 1984) may result in reduced bioavailability of metals (Wright, 1980; Amrhein *et al.*, 1992).

It was evident from chemical surveys of the water and sediments at Pigeon Bridge Brook that the sediments were contaminated with a complex 'cocktail' of inorganic and organic contaminants including hydrocarbons and metals (Maltby *et al.*, 1995a). Sediment manipulation experiments were therefore designed to identify broad chemical classes which may be responsible for any toxicity of the downstream sediments sediments. All five test species were used in these experiments to substantiate earlier results. Manipulating downstream field sediments by solvent extraction successfully removed total aromatic hydrocarbons whilst leaving most metals, with the exception of lead. Solvent extraction significantly reduced toxicity in the downstream sediments to *G. pulex* and *N. cinerea*. Neither the extracted nor the non-extracted downstream sediment in this set of experiments were toxic to *P. jenkinsi*, *C. riparius* or *T. tubifex* confirming earlier results.

Based on laboratory and field studies it would appear that the sediment compartment contains contaminants which are lethal to *G. pulex* and *N. cinerea* but not to *P. jenkinsi*, *C. riparius* or *T. tubifex*. Moreover, this toxicity was lost when the aromatic hydrocarbons and lead were removed by solvent extraction. The possibility that contaminants removed by solvent extraction were the major toxicants was investigated further using spiking experiments. APW was spiked with either solvent- or acid-extracts of downstream field sediments. This approach removes the possibility that between-treatment differences in survival were a consequence of differing physical and 'non-toxic' chemical characteristics (i.e. organic content/ food content) of the sediment (Ankley *et al.*, 1994).

Only *G. pulex* showed a significant concentration-survivorship relationship with downstream sediment solvent extract. This response corresponded to increasing concentrations of aromatic hydrocarbons in spiked waters and gave a 14-d LC_{50} between 143.21 and 239.4 µg chrysene equivalents/L for the three extracts tested. The accumulation of aromatic hydrocarbons in the tissues also correlated with increased

mortality of *G. pulex*. The variability in the toxicity of the three sets of extracts to *G. pulex* may have been the result of varying proportions of aromatic hydrocarbons. The extracts were prepared from sediments sampled from the downstream station at Pigeon Bridge Brook on three different occasions and the results reflect the heterogeneous toxic nature of natural sediments. The three extracts also produced different shaped concentration-response relationships which may reflect the complex nature of the extracts and the presence of toxicants with different modes and sites of action.

Water spiked with downstream sediment acid-extracts had no effect on the survivorship of any of the species tested. High calcium levels in the extract and in APW may have reduced the toxicity of metals in the spiked water (Wright, 1980) and tissue analysis indicated that only Cd was significantly elevated in *G. pulex* (exposed to downstream acid-extracts of 6 and 10 ml extract /L). However, the lack of toxicity of this acid fraction is in agreement with the results of the lack of toxicity exhibited by DCM-extracted downstream sediment in the manipulated sediment experiment. DCM extraction removed the hydrocarbon fraction of contaminated sediments whilst leaving the majority of metals.

From the present study it is evident that pollution from motorway runoff reduced the survival of *G. pulex* and *N. cinerea* and that the toxicity originated from the sediments. The pollutant class responsible for the toxicity to *G. pulex* appeared to be the aromatic hydrocarbon fraction, though this fraction did not describe the toxicity of sediments to *N. cinerea*. Mortality of stoneflies in the sediment toxicity assays could possibly have been a result of hypoxia or anoxia (or possibly due to the action of tar product/ insoluble oils on respiratory surfaces (Parker *et al.*, 1976; Simpson, 1980). *N. cinerea*, in fact, has been found to fairly insensitive to a range of organic and inorganic toxicants in comparative tests (Slooff, 1983). Maltby *et al.* (1995b) have subsequently shown that it is the polycyclic aromatic hydrocarbons (PAHs) in the aromatic fraction from the downstream sediments at Pigeon Bridge Brook that are probably responsible for the majority of the toxicity to *G. pulex*.

If, as these and further studies suggest (Maltby *et al.*, 1995b), the aromatic hydrocarbon fraction of the sediment is responsible for the observed mortality then the pattern of sensitivity of the species used in this study are in general agreement with previous studies on organic xenobiotic toxicity. Slooff (1983) assessed the comparative sensitivity of a range freshwater macroinvertebrates to organic and inorganic toxicants and found that it decreased in the order: *Gammarus pulex* > *Chironomus* > sp. > *Lymnaea stagnalis* > *Nemoura cinerea* > Tubificidae. Millemann *et al.* (1984b)

compared the sensitivity of freshwater species to six organic compounds and showed an overall decrease in sensitivity in the order amphipods > midges > snails. More specifically, sensitivity to the PAH phenanthrene decreased in the order: *Gammarus minus* (Amphipoda) > *Chironomus tentans* (midge) > *Daphnia magna* (Cladoceran) > *Physa gyrina* (snail) and to naphthalene in the order *Daphnia magna* > *Chironomus tentans* > *Gammarus minus* > *Physa gyrina* (Millemann *et al.*, 1984b). The results of these, and other studies, therefore suggest that amphipods are sensitive, chironomids are of intermediate sensitivity and mayflies, stoneflies, molluscs and oligochaetes are insensitive to hydrocarbons (Finger *et al.*, 1985; Ramusino and Zanzothera, 1986; Ibanga, 1987; Johnson and Romanenko, 1989; Erben and Pisl, 1993).

Aromatic hydrocarbons, specifically polycyclic aromatic hydrocarbons (PAHs), were the fraction of the motorway runoff considered to be responsible for the mortality of *G. pulex* (Maltby *et al.*, 1995b). Aquatic organisms have varying sensitivity to these PAHs. For example, in a comparative study by Tay *et al.* (1992) the amphipod *Rheopoxynius abronius* was more sensitive than the amphipod *Corophium volutator* when exposed to PAH-contaminated sediments. The relative sensitivity also depends on the specific PAH (e.g. Millemann *et al.*, 1984b). One possibility for the variation in the 14-d LC_{50} values (143.21-239.4 µg chrysene equivs/ L) of DCM sediment extracts to *G. pulex* were that the relative proportions of different PAHs in the extract were probably different affecting the toxicity.

Many organisms can detoxify organic pollutants including PAHs (James, 1989). This is commonly achieved by the conversion of the apolar (lipid soluble) chemicals to more water soluble and readily excreted metabolites by mixed function oxygenase systems (MFOs). Although this transformation means that the chemical can be more readily excreted, many chemicals, including aromatic hydrocarbons, are more toxic when converted to chemically reactive species by MFOs (Payne *et al.*, 1987). There is a great deal of inter- and intra-specific variability in the ability to take up (Müller, 1987; Connell, 1988) and metabolise xenobiotics (Lee, 1976; Landrum, 1988, 1989; James, 1989; Livingstone *et al.*, 1989; Beverley *et al.*, 1991; Tanacredi and Cardenas, 1991 Gobas and McCarquodale, 1992). It is generally assumed for most chemicals that the toxic dose at the target site is similar across species and that differences in toxicity between species are related to physiological and biochemical differences that change the kinetics of absorption, distribution, biotransformation and elimination (James, 1989). For instance, the uptake and retention of these chemicals appears to be directly related to lipid concentrations in organisms. Organisms with a high percentage of lipid to body mass will take up lipophilic organics more readily than organisms with a low

percentage lipid to body mass (Connell, 1988; Barron, 1990). In a study by Oliver and Niimi (1988) fish were shown to have high body lipid levels (4-16 %), the amphipod *Pontoporeia* had intermediate levels (3%), oligochaetes lower levels (1%) and phytoplankton even lower levels (0.5%). Landrum (1988) used this species-specific information together with other information such as water temperature, organic content of the water and sediments, metabolic transformation rates to successfully predict the toxicity and accumulation of organic compounds to a variety of species. Inter-specific differences in the sensitivity of species in the current study may be a consequence of these mechanisms in that crustacea are generally thought to be able to metabolise organic xenobiotics whereas molluscs and annelids generally do not (James, 1989). Moreover, *G. pulex* may have higher lipid to body ratios than some of the other test species increasing the uptake potential of organic xenobiotics (Landrum, 1988).

In the current study *N. cinerea* appeared to be sensitive to runoff-contaminated field sediments but not to sediment extracts. Nemourid stoneflies have been shown to be reasonably tolerant to water soluble fractions (WSFs) of crude oil though they may be may be affected by severe oil pollution events (Ramusino and Zanzothera, 1986). For instance, stoneflies were immediately absent from a stream after a massive oil spill but were soon found to re-appear in the stream after a much shorter period than either mayflies or caddisflies (Crunkilton and Duchrow, 1990). Their eradication from the oil contaminated stream may possibly have been due to respiratory impairment caused by oil droplets rather than direct toxic effects (Simpson, 1980). A similar phenomenon may explain the mortality of *N. cinerea* in field sediment exposures but not in extract solution exposures.

Estimates of the environmental impact of the motorway runoff pollution cannot be based solely on the assessment of mortality to particular life stages. The lethality experiments performed in this part of the study used adult life stages of the test species. Although it was found that toxic effects of sediments on adult male *G. pulex* were fairly minimal the effects on other life stages may be more significant. *Gammarus pulex* juveniles are generally more susceptible to metals than adults (McCahon and Pascoe, 1988b) and first instar *Chironomus riparius* larvae have been shown to be 127 times more sensitive to cadmium than 2nd instars (Williams *et al.*, 1986). Slooff (1983) found that the relative sensitivity of freshwater macroinvertebrates to complex mixtures in the laboratory was not reflected in single organic and inorganic toxicant exposures with inter-species variation in sensitivity (survivorship) to the complex mixtures being lower than that of the single chemicals. Further lethal toxicity did not predict field distributions of organisms. Slooff (1983) suggests that survival data alone are not

sufficient to predict tolerance to chemical pollution and the field distribution of animals in contaminated systems which may be affected by such things as toxicant effects on competition, avoidance responses and downstream drift. In explaining the absence of species from contaminated sites it may be necessary to consider life-history information (Buikema and Benfield, 1979).

Although sediments may be highly contaminated with metals and PAHs they do not necessarily exert lethal toxic effects (Krantzberg and Boyd, 1992). More long-term effects on sub-lethal parameters such as behavioural and feeding responses may affect the population (Linden, 1976). Behavioural changes such as reproductive behaviour, predator avoidance, prey location and habitat selection have been observed as a response to hydrocarbon pollution (Chapter 4). Motorway runoff is obviously a chronic exposure situation (Balades *et al.*, 1984; Baekken *et al.*, 1992) with episodic pollutant input (Balades *et al.*, 1984; Bellamy, 1990). The effect of contaminants in motorway runoff on sub-lethal responses are considered in Chapter 4 and Chapter 5.

3.4.1. Conclusions.

The major findings from this part of the study were:

1. *G. pulex* and *N. cinerea* displayed slight, but significant, mortality when exposed *in-situ* at the downstream station at Pigeon Bridge Brook. The source of toxicants was the sediments. There was no significant mortality of *P. jenkinsi*. Laboratory exposures to Pigeon Bridge Brook downstream sediments resulted in significant mortality of *G. pulex* and *N. cinerea*. The sediments caused no significant mortality of *P. jenkinsi*, *C. riparius* and *T. tubifex*. Further, stream water from the downstream station at Pigeon Bridge Brook caused no significant mortality of any of the test species. Results from the laboratory experiments therefore confirmed the results of *in-situ* exposures.
2. Laboratory sediment manipulations suggested that toxicity to *G. pulex* and *N. cinerea* was removed by DCM extraction of the downstream sediments. Extraction by solvent removed total aromatic hydrocarbons whilst leaving the majority of metals in the sediment. Laboratory sediment extract experiments confirmed that the solvent extract of downstream sediment was toxic to *G. pulex* but not to *N. cinerea*. Sediment acid-extracts caused no mortality of any of the test species. Toxicity of the downstream sediment solvent-extract to *G. pulex* increased with aromatic hydrocarbon concentration. Three separate extracts were tested with LC_{50} values of 143.2, 156.4 and 239.4 µg chrysene equivalents /L.

Mortality was significantly correlated to aromatic hydrocarbon in the tissues of *G. pulex.*

G. pulex was shown to be sensitive to the aromatic hydrocarbons in the sediment possibly, due to this species being able to metabolise PAHs which may produce toxic metabolites within the tissues. Due to the lower bioavailability of PAHs from the sediment, thresholds of internal metabolites of PAHs are probably reached only after chronic exposure or at high aromatic hydrocarbon concentrations in the sediment. This may explain low sensitivity in lethal tests with field sediments. *N. cinerea* did appear to be sensitive to downstream field sediments but the component responsible for any mortality was not clear from these tests. One possible explanation is respiratory 'clogging' by oil/tar 'particulates'. *P. jenkinsi, C. riparius* and *T. tubifex* proved to be insensitive to the motorway runoff pollutants in lethality tests possibly due to low biologically available concentrations to these species and different toxicokinetic characteristics of these animals. In conclusion lethality toxicity tests did not fully explain the distribution of macroinvertebrates from the field surveys (Chapter 2). Sub-lethal responses such as toxicant avoidance may better explain the distribution of these species and are the subject of Chapter 4.

CHAPTER 4.

SUB-LETHAL TOXICITY OF MOTORWAY RUNOFF CONTAMINANTS: I. AVOIDANCE BEHAVIOUR.

4.1. INTRODUCTION.

Results from field distributions of macroinvertebrates at the upstream and downstream station at Pigeon Bridge Brook indicated that the distributions of some species were altered downstream of the motorway discharge (Chapter 2). The absence of particular species could be the result of either lethal or sub-lethal responses to motorway runoff-derived toxicants. Sediment and water from the most contaminated stream, Pigeon Bridge Brook was either not toxic, or showed only slight lethal toxicity to *G. pulex, N. cinerea, P. jenkinsi, C. riparius* and *T. tubifex* (Chapter 3). However the potential toxicity of hydrocarbons in contaminated sediments to *G. pulex* was high (Chapter 3). The fact that pollutant-induced mortality of macroinvertebrates at the downstream station was low, combined with the absence of particular species at this station suggests that long-term and/or sub-lethal effects may be important. Sub-lethal effects have previously been used to explain site-specific field distributions of macroinvertebrates (Abel and Green, 1981; Green, 1984). Behavioural responses are a group of sub-lethal effects influenced by pollutants and one such response, namely toxicant avoidance, is investigated in this chapter.

An organism's habitat range is determined by its tolerance limits to environmental conditions. Generally only a portion of this range is optimal (i.e. allow maximum fitness), with tolerance limits allowing animals to utilise sub-optimal habitat (Sheehan *et al.*, 1984). Animals continually 'sample' their immediate environment and display behavioural characteristics that maintain them in a suitable habitat (Atema *et al.*, 1988). Changes in environmental conditions are detected by chemical, visual, acoustic, electrical and mechanical receptors (Atema *et al.*, 1988) and aquatic organisms have been shown to have a complex array of chemoreceptors capable of detecting aqueous chemicals (Carr and Thompson, 1983; Atema *et al.*, 1988; Read and Williams, 1990). These chemoreceptors have been implicated in a range of activities including: food detection (McLeese, 1970; Thomas, 1986, 1989; Bleckmann, 1988; Smith and Bailey, 1989; Zimmer-Faust, 1989; Graça, 1993), predator avoidance (Mackie, 1973; Dobson *et al.*, 1994), detection of home range and spawning grounds (Atema *et al.*, 1973; Bloom *et al.*, 1978; Mohlenberg and

Kiorboe, 1983; Carr, 1988), mate detection/selection and gregarious behaviour (Blaxter and Hallers-Tjabbes, 1992).

Detection is quantitative and often very sensitive (Zimmer-Faust, 1989); in some cases chemical detection thresholds may approach sub-nanomolar concentrations (Thompson and Ache, 1980). The capacity to respond to chemicals is evident at almost all phylogenetic levels and is a reflection of an organism's necessity to maintain itself in an optimal environment (Olla *et al.*, 1980). It follows that aquatic organisms are also likely to be able to detect certain pollutants. Although this capacity is now well documented, the mechanisms involved are poorly understood (e.g. Olla *et al.*, 1980). Pollutant chemicals in the water column may either disrupt the chemical sensing ability of animals (Olla *et al.*, 1980) and override normal behavioural responses (i.e. aberrant behaviour) or induce additional behavioural responses such as avoidance reactions (i.e. adaptive behaviour, Rand, 1985).

Animals exposed to concentrations of chemicals that exceed their tolerance limits may incur physiological and/or biochemical damage to the sensory organs, nervous system or other tissues resulting in aberrant behavioural patterns. Toxicants have been shown to disrupt normal behaviour such as detection of home range and spawning grounds (Weber *et al.*, 1981; Smith and Bailey, 1990; Benfield and Aldrich, 1994), symbiont detection (Atema *et al.*, 1988), gregarious behaviour (Bloom *et al.*, 1978), self-species identification (Henry and Atchison, 1979), reproductive behaviour (Linden, 1976; Davis, 1978; Little *et al.*, 1985; Borlakoglu and Kickuth, 1990; Poulton and Pascoe, 1990; Pascoe *et al.*, 1994), food detection/avoidance (Jacobson and Boylan, 1973; McLeese, 1975; Percy, 1976; Little *et al.*, 1985; Sandheinrich and Atchison, 1990), competitive behaviour (Little *et al.*, 1985b; Taylor *et al.*, 1994; Vuori, 1994) and predator avoidance (Kania and O'Hara, 1974; Sullivan *et al.*, 1978; Little *et al.*, 1985, 1985b; Taylor *et al.*, 1994). Disturbance of behaviour by disfunctioning chemoreception often happens at concentrations that are lower than those eliciting lethal (Summerfelt and Lewis, 1967; Folmar, 1976) or other sub-lethal effects (Johnson, 1979; Atema *et al.*, 1981). For instance, Finger *et al.* (1985) found that behavioural tests (i.e. swimming impairment, feeding activities, and vulnerability to predation) indicated the bluegill, *Lepomis macrochirus*, was adversely affected by the PAH, fluorene, at concentrations below that predicted by standard long-term toxicity measurements of growth and survival. Because deviations from normal behaviour in response to a specific pollutant are likely to be the result of physiological and/or

biochemical disorder they should provide a sensitive early warning measure of sub-lethal toxicity (Johnson *et al.*, 1993).

Avoidance is part of an animal's normal repertoire of behaviour (i.e. non-aberrant) and is usually one of the first responses of an organism to an environmental stress (Beitinger and Freeman, 1983). It is the primary mechanism for ensuring that animals can evade deleterious or undesirable conditions (Larrick *et al.*, 1978) and examples include valve closure of molluscs, burrowing activity and active drift (Rand, 1985). Assays employing avoidance as a behavioural response can be considered as having high relevance to field responses, although the specific implications are equivocal (Peakall, 1992). For example, avoidance of a contaminated site may reduce exposure to the toxicant but may result in the organism moving into sub-optimal habitat.

The analysis of avoidance/attraction behaviour in response to chemical stimulation is important when estimating the effects of waterborne pollutants on the behaviour of natural populations (Cairns, 1981). The sensory detection of, and attraction/avoidance response to, a pollutant will directly affect the amount of toxic exposure of an organism, or a population, with concominant effects on survival. These responses may prove useful as a hazard assessment tool yielding sensitive estimates of no-effect concentrations (Little, 1990). Avoidance/preference laboratory bioassays have been commonly employed to detect the behavioural responses of animals to low level contamination. Assays have regularly been performed on fish (Folmar, 1976; Hartwell *et al.*, 1988; Smith and Bailey, 1989) and less often on macroinvertebrates (Maciorowski *et al.*, 1977; Folmar, 1978; Smith and Bailey, 1989). The experimental design is generally the determination of avoidance (or preference) of contaminated sediments or the discrimination of animals between two bodies of water across steep pollutant gradients (Smith and Bailey, 1989). Avoidance/preference assays pre-suppose the ability of aquatic animals to determine its own location, detect the chemical, recognise the conditions as unfavourable and act adequately to mitigate any adverse effects (Pearson and Olla, 1980). Avoidance assays can be very sensitive as long as the animal can detect the pollutant. For instance, Keilty *et al.* (1988) found that 96-h EC_{50} responses of two species of oligochaetes to endrin-contaminated sediments determined by burrowing avoidance were 46-150 times more sensitive than 96-h LC_{50} values for the same sediment.

Benthic macroinvertebrates may be considered ideal organisms for behaviour/toxicity assessment because of their close association with the sediments; the sink for many toxic

compounds. In this study, stream sediments were shown to be the major sink of motorway runoff pollutants (Chapters 2 and 3; Maltby *et al.*, 1995a) and many of the pollutants present have the potential to produce avoidance responses (Rand, 1985). Toxicant avoidance may possibly explain the distribution of macroinvertebrates at Pigeon Bridge Brook.

4.1.1. Objectives and approach.

Results from lethality studies (Chapter 3) indicated that lethal toxicity was minimal to the majority of the test species and did not describe the field distributions of the macroinvertebrates assessed from field surveys (Chapter 2). However, the motorway contaminants may be producing sub-lethal responses in these organisms which affect their field distribution. This chapter describes studies to investigate the avoidance behaviour of *G. pulex, N. cinerea, P. jenkinsi, C. riparius* and *T. tubifex* to sediments contaminated with motorway runoff.

The specific objectives were to:

1. determine whether macroinvertebrates avoid sediments contaminated with motorway runoff;
2. assess the degree to which macroinvertebrates can distinguish between contaminated and uncontaminated sediments;
3. determine which groups of chemicals are responsible for eliciting the avoidance behaviour;
4. assess the comparative sensitivity of the test species in their avoidance response to sediment contaminants;
5. assess whether the relative sensitivities of these species to contaminated sediments explains their field distributions.

A comparative assessment of the behavioural preference of animals between field sediments collected from the upstream and downstream stations at Pigeon Bridge Brook was assessed in the laboratory. The experimental design allowed a choice and free movement between the two sediments. Controls were designed to show whether any reaction was due to attraction to, or avoidance of, sediments. An attempt was made to identify broad classes of chemicals that may be responsible for any observed effect and to quantify the response threshold of avoidance activity using sediment manipulation (sediment mixtures) and flow-through choice apparatus with sediment extracts (which exclude sediment effects).

Sediment extracts which induced an avoidance response were used to spike extracted sediments. This experimental method exclude's the possibility of avoidance/attraction due to sediment physical effects since the sediments were essentially identical apart from the addition of sediment extracts to one of the treatments. Avoidance reactions were related back to concentrations of pollutants in the field to extrapolate to station-specific distributions of these animals.

4.2. MATERIALS AND METHODS.

4.2.1. Field and manipulated sediments.

Samples of field sediment were collected from the surficial 5 cm of the stream bed at both upstream and downstream stations at Pigeon Bridge Brook and processed using methods described in section 3.2.4. Sediments samples were also solvent-extracted (DCM) in order to remove hydrocarbons (section 3.2.5).

The test vessels used for experiments with *G. pulex* and *N. cinerea* consisted of PVC trays (Table 4.1) in which the two halves were each spread in an even 1-cm deep layer with each of the two test sediments. With *P. jenkinsi, C. riparius* and *T. tubifex* polystyrene trays (Table 4.1) were used in which two 0.5-cm deep alternating bands of each of the two test sediments were spread (Plate 4.1a). The orientation of the test vessels was randomised between replicates and artificial pond water (APW) was poured carefully into the vessels.

Test animals were distributed evenly between sediment treatments at the start of each experiment, the design of which are given in Table 4.1. All experiments were conducted in a constant temperature room at 15°C (± 2°C), over 24 h. For the *G. pulex*, *N. cinerea* and *P. jenkinsi* tests, dividers were placed between the sediment treatments in each replicate at the end of the experiment and the number of animals removed from each test sediment recorded. In the *C. riparius* and *T. tubifex* tests, the separate sediment treatments were carefully washed into beakers from which the animals were sorted and counted. Triplicate 5 g sub-samples of each sediment treatment were collected at the end of the experimental exposure period and analysed for metals (Ca, Al, Zn, Cu, Cd, Cr, Pb, Ni, Mg and Fe) and total aromatic hydrocarbons (section 3.2.8.).

Table 4.1. Design of avoidance experiments using field and manipulated sediments.

	Test chamber size (cm) length x width x depth	Sediment wet weight (g) per treatment	Vol. of APW ml	No. of animals per rep	Number of replicates
G. pulex	35 x 20 x 5	100	1000	20	6
N. cinerea	35 x 20 x 5	100	1000	12	6
P. jenkinsi	20 x 11 x 2	50	200	20	6
C. riparius	20 x 11 x 2	50	200	12	6
T. tubifex	20 x 11 x 2	50	200	12	6

The combinations of test sediment are displayed in Table 4.2. In an attempt to separate preference for upstream sediment from avoidance of downstream sediment, field

sediments were tested against sand. Solvent-extracted downstream sediment was tested against non-extracted downstream sediment in an attempt to elucidate classes of pollutant which may cause an avoidance response. Finally the solvent-extracted downstream sediment was also tested against upstream sediment.

Table 4.2. Test sediment combinations used in avoidance experiments using field and manipulated sediment.

	Test sediment 1.		Test sediment 2.
I.	Upstream	vs.	Downstream
II.	Upstream	vs.	Downstream solvent-extracted
III.	Sand	vs.	Upstream
IV.	Sand	vs.	Downstream
V.	Downstream solvent-extracted	vs.	Downstream

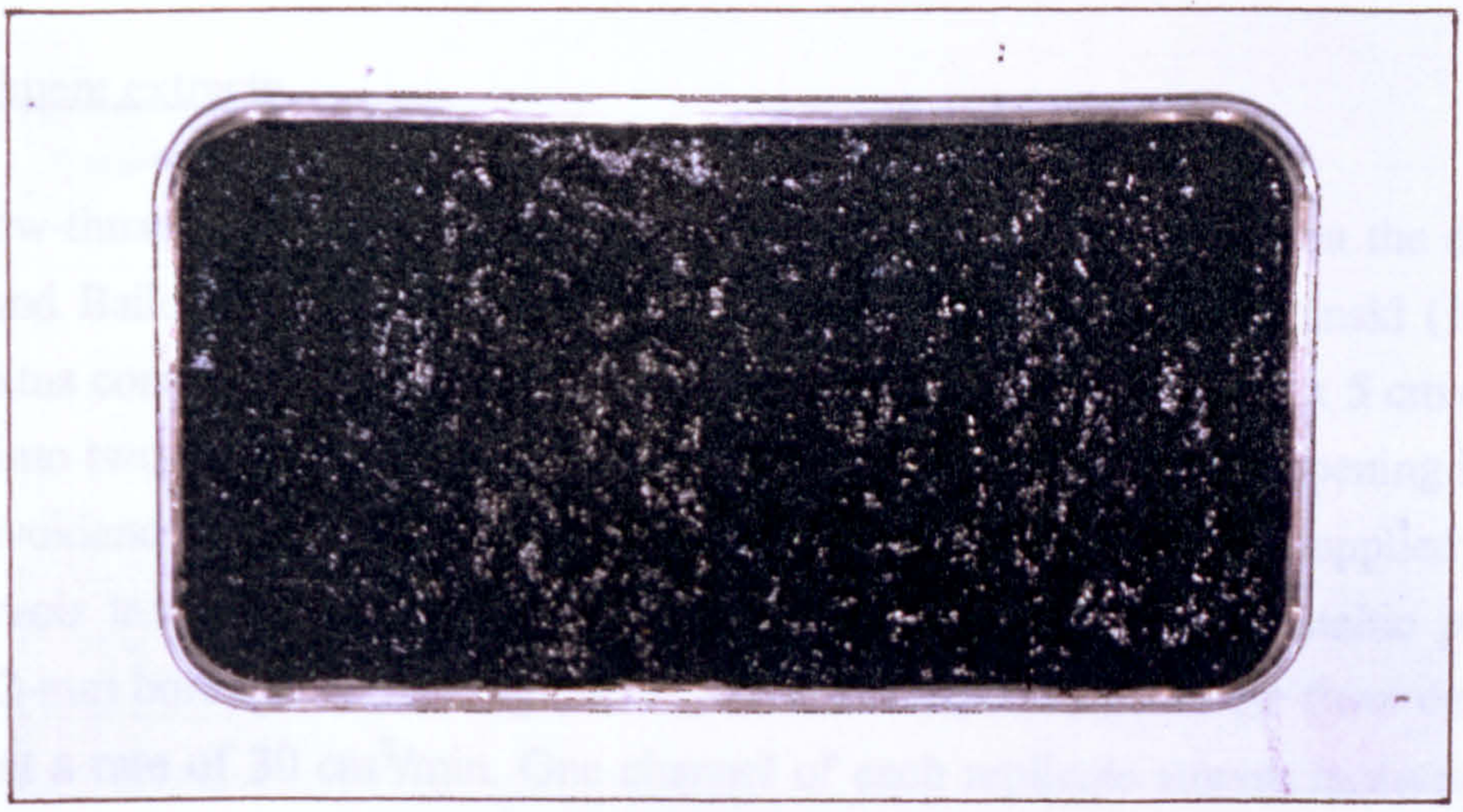
4.2.2. Sediment mixtures.

Sediments were collected from the upstream and downstream stations at Pigeon Bridge Brook and processed using the methods described in section 3.2.4. A sediment-mixture dilution series was prepared by thoroughly mixing upstream sediments with downstream sediments in the following proportions: 2.5, 10, 20, 40, 60, 80, 100 % downstream sediment. These sediments were then used against 100 % upstream sediment to test the avoidance responses of selected macroinvertebrates. The design of avoidance experiments using *G. pulex* and *P. jenkinsi* was as described in section 4.2.1, except that 6 rather than 5 replicates per treatment were used. *C. riparius* and *T. tubifex* were exposed in plastic petri dishes (diam. 9 cm x 1.5 cm depth) containing 5 g (wet weight) of each test sediment, spread evenly on each half of the dish, and 40 ml of APW (Plate 4.1b). As *N. cinerea* demonstrated no response to 100 % downstream sediment in the previous series of experiments (section 4.2.1.) it was not tested with sediment mixtures. The design of the sediment-mixture experiments is given in Table 4.3 and displayed in Plate 4.1c. The exposure period, temperature, treatment of replicates and sediment analysis was the same as described in section 4.2.1.

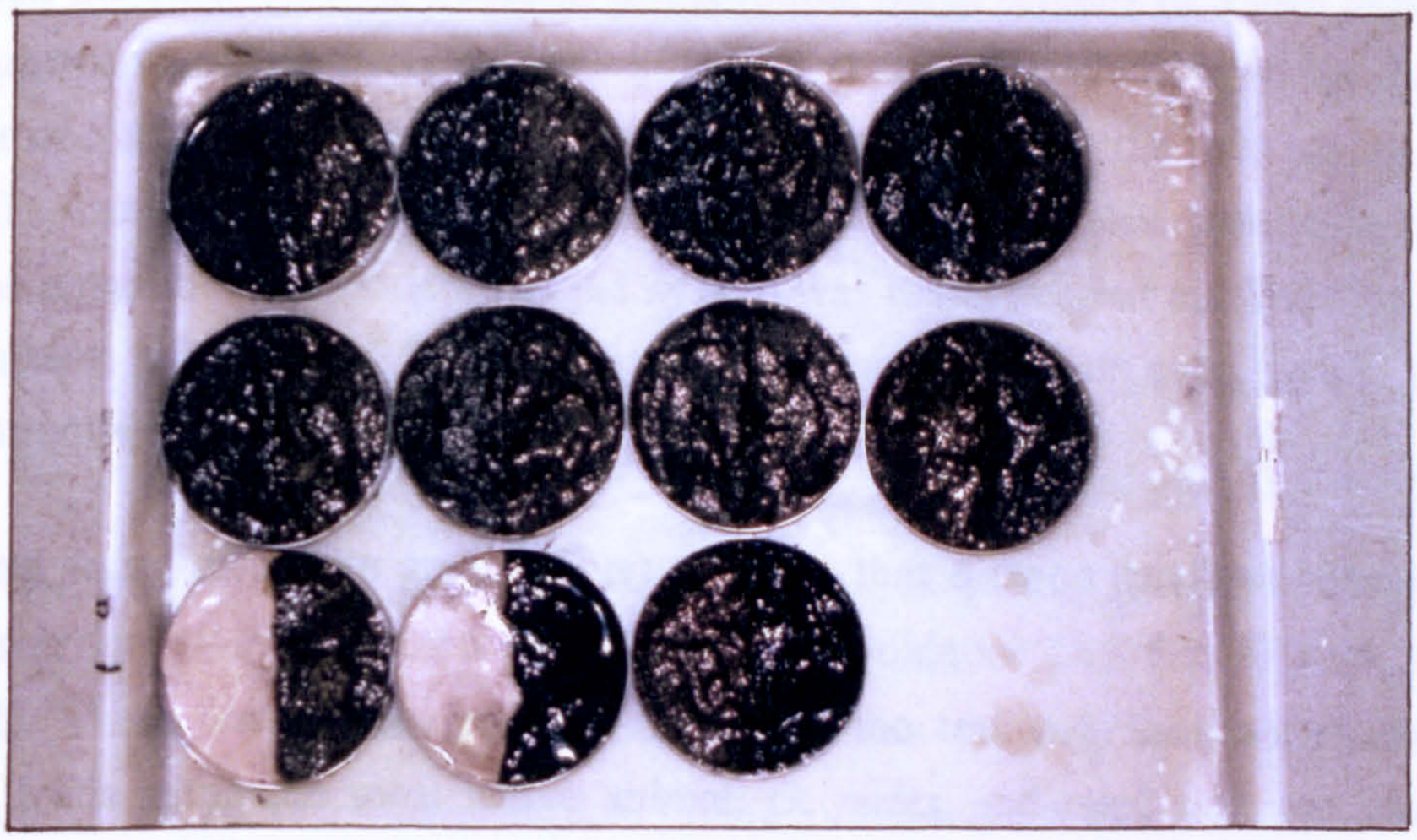
Table 4.3. Design of avoidance experiments using sediment mixtures.

	Test chamber size (cm) length x width x depth or diam. x depth	Sediment wet weight (g) per treatment	Vol. of APW ml	No. of animals per rep	Number of replicates
G. pulex	35 x 20 x 5	100	1000	20	5
P. jenkinsi	35 x 20 x 5	100	1000	20	5
C. riparius	diam. 9 x 1.5	5	40	10	3
T. tubifex	diam. 9 x 1.5	5	40	10	3

a.)



b.)



c.)

Plate 4.1. Trays used in sediment avoidance tests for: a.) *P. jenkinsi, C. riparius* and *T. tubifex* in section 4.2.1, b.) *C. riparius* and *T. tubifex* in section 4.2.2 and c.) *G. pulex* and *P. jenkinsi* in section 4.2.2.

4.2.3. Sediment extracts.

A glass flow-through choice/avoidance apparatus was constructed based on the design of Smith and Bailey (1988) which was modified from Westlake and Lubinski (1976). The apparatus consisted of 5 replicate streams (38 cm long x 11 cm wide x 5 cm deep) each split into two channels for two-thirds of their length; both channels opening into a common avoidance chamber (Fig. 4.1). Control and test solutions were supplied from glass reservoir tanks and pumped using Watson-Marlow 202U/1® peristaltic pumps through 3.2-mm bore silicone tubing (Watson-Marlow TU093®) into the flow-through apparatus at a rate of 30 cm^3/min. One channel of each replicate stream received one test solution the other channel a second test solution. Mesh screens (0.5-mm mesh size) at the upstream end of each channel aided in creating laminar flow by damping turbulence of the incoming waters and prevented animals entering the inlet tubing. Mesh screens (0.5-mm mesh size) at the downstream end of each stream prevented animals leaving the apparatus. Water depth in the system was maintained at ca. 2 cm by means of an adjustable outfall 'weir'. The apparatus was situated under an even light regime in a constant temperature room (15 ± 2°C).

Dye testing, using malachite green (Gurr), showed that a steep gradient between the two test solution was maintained in the common avoidance chamber of each stream (Plate 4.2). There was no evidence of mixing of the test solutions within the two channels. Trials using the most active animal, *G. pulex*, indicated that the gradients between the test solutions were not disrupted by animal activity.

Prior to the addition of the animals, test solutions flowed through the streams for a period of 30 min to establish test solution gradients. For experiments involving *G. pulex*, *N. cinerea*, *C. riparius* and *T. tubifex* there were 12 animals per stream and for experiments with *P. jenkinsi* there were 24 animals per stream. Test animals were initially distributed equally between the two channels and the chamber and their subsequent distribution noted after 5, 15 and 30 min. Only those animals that were recorded in either of the channels after a period of 30 min (i.e. had made a choice) were used in later analyses. Between the experimental runs, the whole apparatus was flushed with APW and test solutions were reversed between channels to avoid possible orientation effects (due to light stimuli etc.). Each species was tested at least twice and a different group of experimental animals was used in each trial run thus avoiding possible acclimation (de-sensitisation) of behavioural responses (Hartwell *et al.*, 1988).

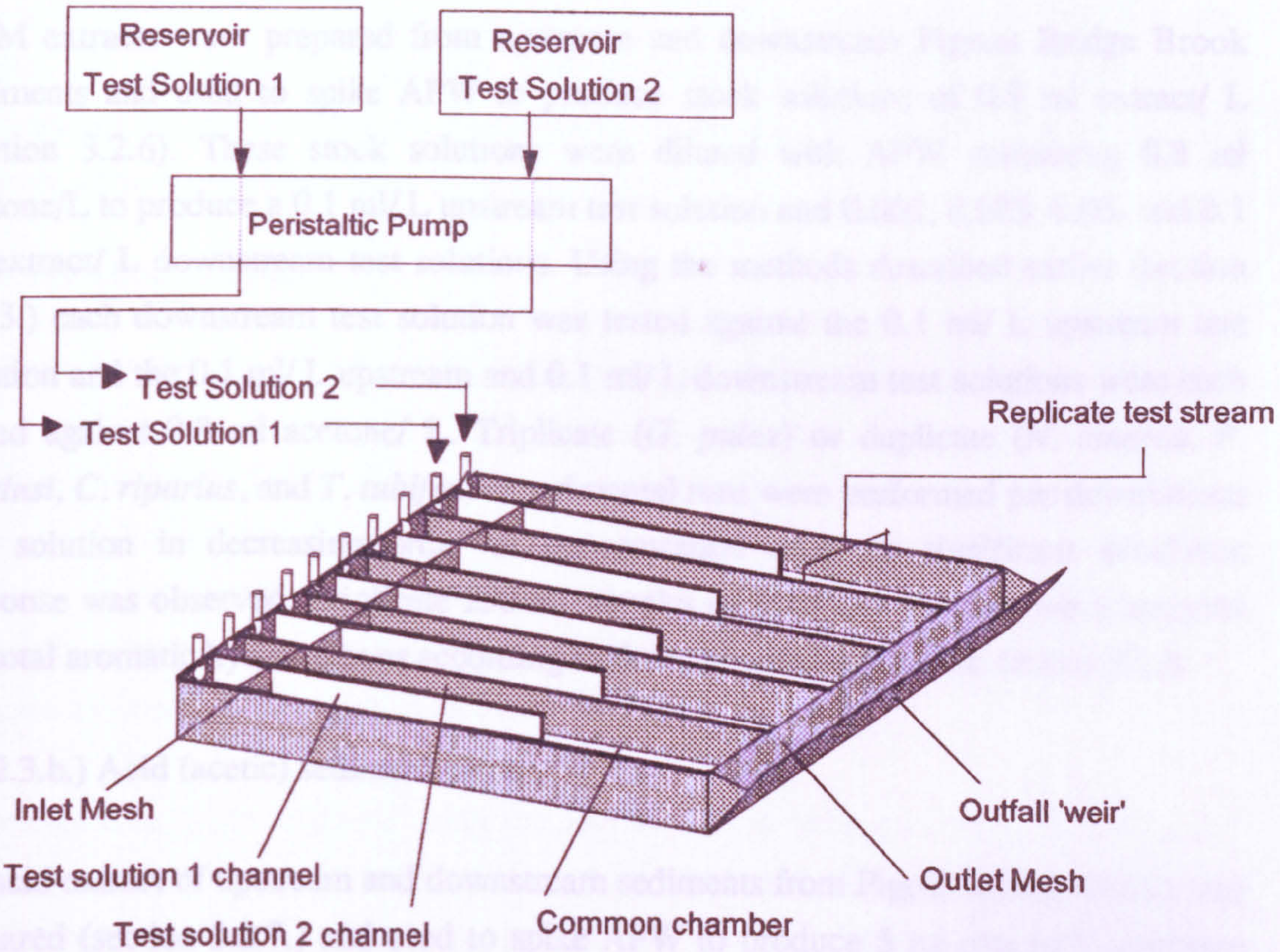


Fig. 4.1. Schematic diagram of avoidance streams.

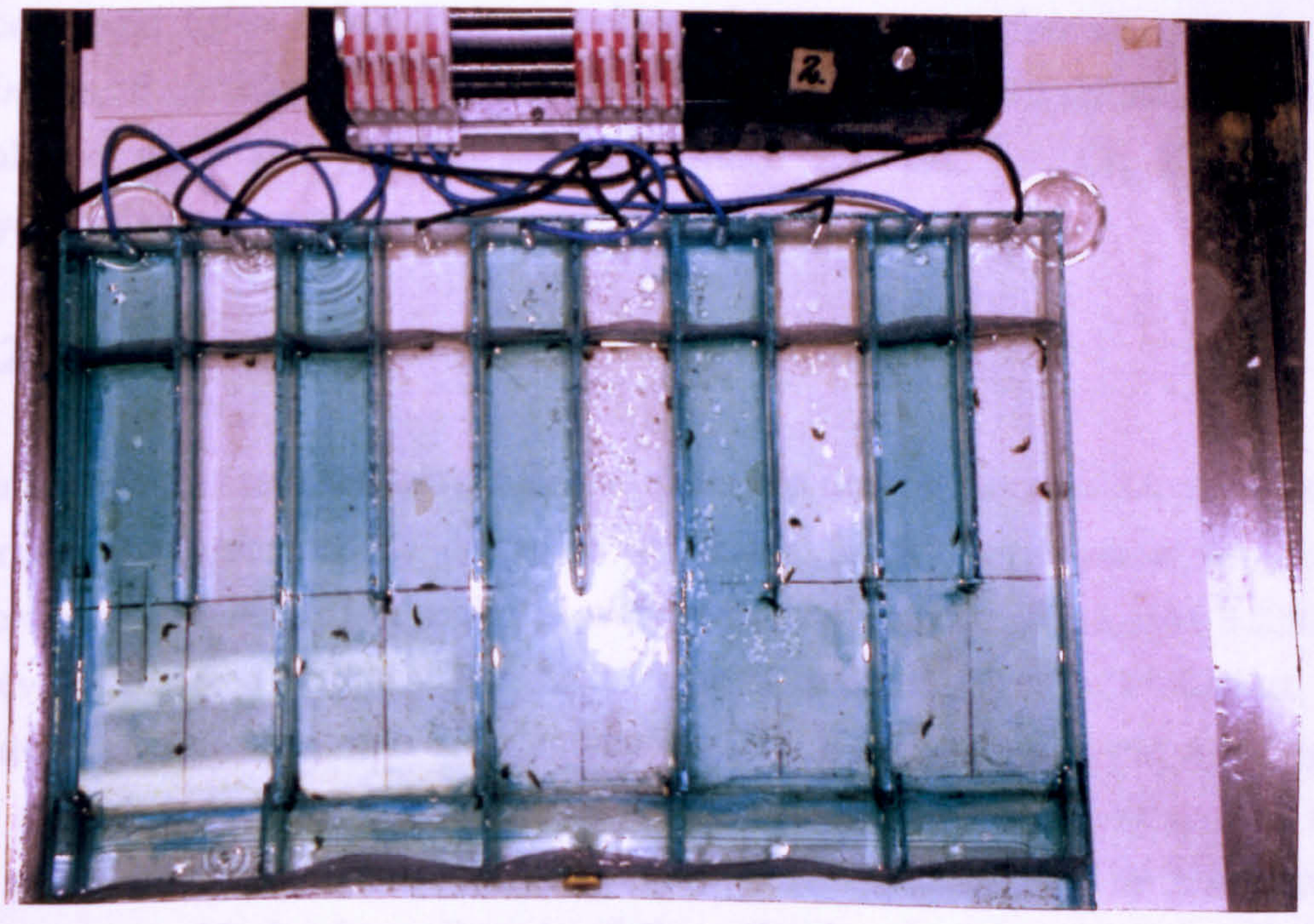


Plate 4.2. Testing avoidance stream 'toxicant' gradients using dye.

4.2.3.a.) Dichloromethane (DCM) sediment extract.

DCM extracts were prepared from upstream and downstream Pigeon Bridge Brook sediments and used to spike APW to produce stock solutions of 0.8 ml extract/ L (section 3.2.6). These stock solutions were diluted with APW containing 0.8 ml acetone/L to produce a 0.1 ml/ L upstream test solution and 0.001, 0.025, 0.05, and 0.1 ml extract/ L downstream test solutions. Using the methods described earlier (section 4.2.3.) each downstream test solution was tested against the 0.1 ml/ L upstream test solution and the 0.1 ml/ L upstream and 0.1 ml/ L downstream test solutions were each tested against 0.8 ml acetone/ L. Triplicate (*G. pulex*) or duplicate (*N. cinerea, P. jenkinsi, C. riparius*, and *T. tubifex*) experimental runs were performed per downstream test solution in decreasing order of concentration until no significant avoidance response was observed. Triplicate 250-ml samples of each test solution were analysed for total aromatic hydrocarbons according to the methods described in section 3.2.8.

4.2.3.b.) Acid (acetic) sediment extract

An acid extract of upstream and downstream sediments from Pigeon Bridge Brook was prepared (section 3.2.7.) and used to spike APW to produce 5 ml extract/L upstream and downstream test solutions. Avoidance behaviour of animals exposed to these solutions was assessed as described in section 4.2.3. The upstream (pH= 6.27) and downstream (pH= 7.15) solutions were also tested against APW spiked with 0.05 ml/L acetic acid (pH= 6.54). Two experimental runs were performed with *G. pulex, N. cinerea, P. jenkinsi, C. riparius* and *T. tubifex*. Triplicate 15 ml samples of each test solution were analysed for selected metals (Zn, Cu, Cd, Cr, Pb, and Fe) and hydrocarbons (section 3.2.8.).

4.2.4. Spiked sediments.

Field sediment samples were collected from the downstream station at Pigeon Bridge Brook and processed as described in section 3.2.4. One-hundred and sixty gram portions (wet weight) of sediment were extracted with DCM (section 3.2.5.) and the solvent-extracted sediment and DCM extract retained. The DCM extract was then reduced and taken up into acetone (section 3.2.6.); 160 g of sediment resulting in 20 ml of acetone sediment extract. Ten millilitres of acetone sediment extract was then used to spike half of the solvent-extracted sediment. This was achieved by thoroughly mixing the extract with the dry sediment and then allowing the acetone to evaporate off in a fume cupboard. The dry sediment was then reconstituted with 50 ml of APW before use. The remaining half of the extracted sediment was spiked with 10 ml of acetone

only and treated in the same manner. These two sediments were then tested against each other as described in section 4.2.2. As in previous experiments, triplicate 5 g sub-samples of each sediment treatment were collected at the end of the experimental exposure period and analysed for metals (Ca, Al, Zn, Cu, Cd, Cr, Pb, Mg, Ni and Fe) and total aromatic hydrocarbons (section 3.2.8.).

4.2.5. Statistical analyses.

All data were checked for normality using normal probability plots and for homogeneity of variances using Bartletts test. All chemical data were analysed using one-way analysis of variance, Tukey multi-comparison tests and two-sample *t*-tests. Correlations between sediment metal or hydrocarbon concentrations and sediment mixtures, sediment extract metal or hydrocarbon concentrations and extract concentration and sediment extract total aromatic hydrocarbon concentration and avoidance were analysed by least-squares regression techniques.

Avoidance data were analysed using the log-likelihood ratio goodness of fit test (*G*-statistic) and techniques for replicated tests of goodness of fit were employed using calculations for data heterogeneity, pooled and total *G* (Sokal and Rohlf, 1981). Statistical tables were used to aid calculation of the *G*-statistic (Table 2 in Rohlf and Sokal, 1981). In all tests, animals were assumed to have a 0.5 probability of being in either treatment (null hypothesis). An avoidance response was only taken as significant when both total and pooled *G* were significant or if both heterogeneity *G* was insignificant and pooled *G* was significant (Navarrete and Castilla, 1988).

The *q*-statistic for Tukey comparisons and Bartletts test were analysed according to Zar (1984). All remaining analyses (except *G*-statistic) were performed using the MINITAB (Minitab™, Inc., 1991) statistical package. Significance levels are $p<0.05$ unless otherwise stated.

4.3. RESULTS.

4.3.1. Field and manipulated sediments.

Sediments used in these experiments markedly differed in their chemical composition. Concentrations of Cd, Cr, Pb, Cu, Zn, Ca and Mg were significantly higher in downstream and downstream solvent-extracted sediments than in either sand or upstream sediment (q>6.07, df=8,4, Table 4.4). Total aromatic hydrocarbon concentrations in the downstream sediment were significantly higher than all other treatments (q>36.2, df=8,2). Extracting downstream sediment with DCM removed over 94 % of the hydrocarbons thus reducing their concentration to a level which was not significantly different to that of sand or upstream sediment (q<2.18, df=8,4). However, DCM extract did not, in general, result in a significant reduction in metal concentrations. The exceptions being Cr and Cu (q>4.86, df=8,4). Concentrations of Mg, Al, Fe and Ni were significantly higher in the upstream sediment than the sand (q>7.70, df=8,4).

Table 4.4. Chemical composition of sediments used in sediment avoidance tests. Concentrations of total aromatic hydrocarbons are expressed as µg chrysene equivalents/g (wet wt) and metal concentrations are given as dry weights. Data are presented as mean values with 1 S.E. in parentheses. For each determinand, treatments which are significantly different are represented by a different letter (q>4.86, df=8,4).

	Sand	Upstream sediment	Downstream sediment	Downstream extracted sediment
Zn (µg/g)	3.76 (0.55) b	142.51 (16.62) b	697.63 (42.12) a	669.88 (50.04) a
Cu (µg/g)	2.27 (0.49) c	34.96 (1.19) c	232.26 (15.21) a	185.20 (11.91) b
Cd (µg/g)	0.00 (0.00) b	0.95 (0.03) b	2.79 (0.41) a	2.81 (0.45) a
Cr (µg/g)	2.42 (0.19) c	55.39 (1.92) c	312.5 (21.95) a	199.50 (11.66) b
Pb (µg/g)	4.31 (1.99) b	96.65 (5.65) b	321.48 (5.99) a	365.28 (53.24) a
Ni (µg/g)	1.43 (0.28) c	63.75 (2.76) a	57.66 (0.11) ab	55.20 (2.06) b
Ca (mg/g)	0.01 (0.01) c	1.17 (0.27) c	30.35 (1.51) b	38.33 (1.45) a
Al (mg/g)	1.61 (0.04) b	12.87 (0.32) a	15.48 (0.13) a	14.80 (1.47) a
Mg (mg/g)	0.11 (0.01) c	6.30 (0.13) b	15.74 (0.35) a	15.76 (1.56) a
Fe (mg/g)	0.48 (0.02) b	57.97 (2.28) a	46.11 (2.54) a	47.48 (5.56) a
Total aromatic hydrocarbons	1.19 (0.04) b	44.08 (15.06) b	1209.10 (57.38) a	79.94 (19.03) b

Differences in sediment chemistry were reflected in the distribution of test animals (Fig. 4.2). During the experiment it was observed that *P. jenkinsi* had a tendency to leave the sediment and crawl up the sides of the exposure trays. However, the proportions leaving the sediment surfaces did not significantly differ between treatments (t=0.9, df=4) therefore statistical analyses of *P. jenkinsi* data included all animals in the experimental trays.

G. pulex, *P. jenkinsi* and *C. riparius* all exhibited a significant movement from downstream sediments onto upstream sediments over a 24 h period (Fig. 4.2; Table 4.5, I). Neither *N. cinerea* nor *T. tubifex* displayed any preference between these two sediments (Fig. 4.2; Table 4.5, Avoidance test I.). There was no significant difference in the distribution of *G. pulex* or *P. jenkinsi* between upstream sediment and sand (Table 4.5, Avoidance test III.) though there was a significant preference for sand over downstream field sediments (Table 4.5, Avoidance test IV.). This suggests that the distribution of these organisms can be explained by avoidance of downstream sediments rather than preference for upstream sediments. *C. riparius* also avoided the downstream sediment to move into sand (Table 4.5, Avoidance test IV.) but in contrast to *G. pulex* and *P. jenkinsi* this species moved from sand into the upstream sediment thus showing a preference for the upstream sediment (Table 4.5, Avoidance test III.). The sediment preferences of *T. tubifex* were markedly different from the other test species in that they demonstrated a preference for both upstream and downstream sediments over sand (Table 4.5, Avoidance tests III. and IV.).

If *G. pulex*, *P. jenkinsi* and *C. riparius* were avoiding downstream sediments because of their hydrocarbon content, solvent-extracted downstream sediments should be more preferable. As illustrated in Fig. 4.2 all three species did prefer solvent-extracted over non-extracted downstream sediment (Table 4.5, Avoidance test V.). Moreover, for two of the three species, *G. pulex* and *C. riparius*, there was no significant difference in the distribution of animals between solvent-extracted downstream sediment and upstream sediment (Table 4.5, Avoidance test II.). *P. jenkinsi* avoided the solvent-extracted downstream sediment to move onto upstream sediment (Table 4.5, Avoidance test II.). *N. cinerea* exhibited no significant choice between solvent-extracted downstream sediment and either upstream or downstream sediment (Table 4.5, Avoidance tests II. and V.). Although *T. tubifex* also exhibited no preference between upstream sediment and solvent-extracted downstream sediments (Table 4.5, Avoidance test II.), they significantly preferred downstream sediment to the solvent-extracted sediment (Table 4.5, Avoidance test V.).

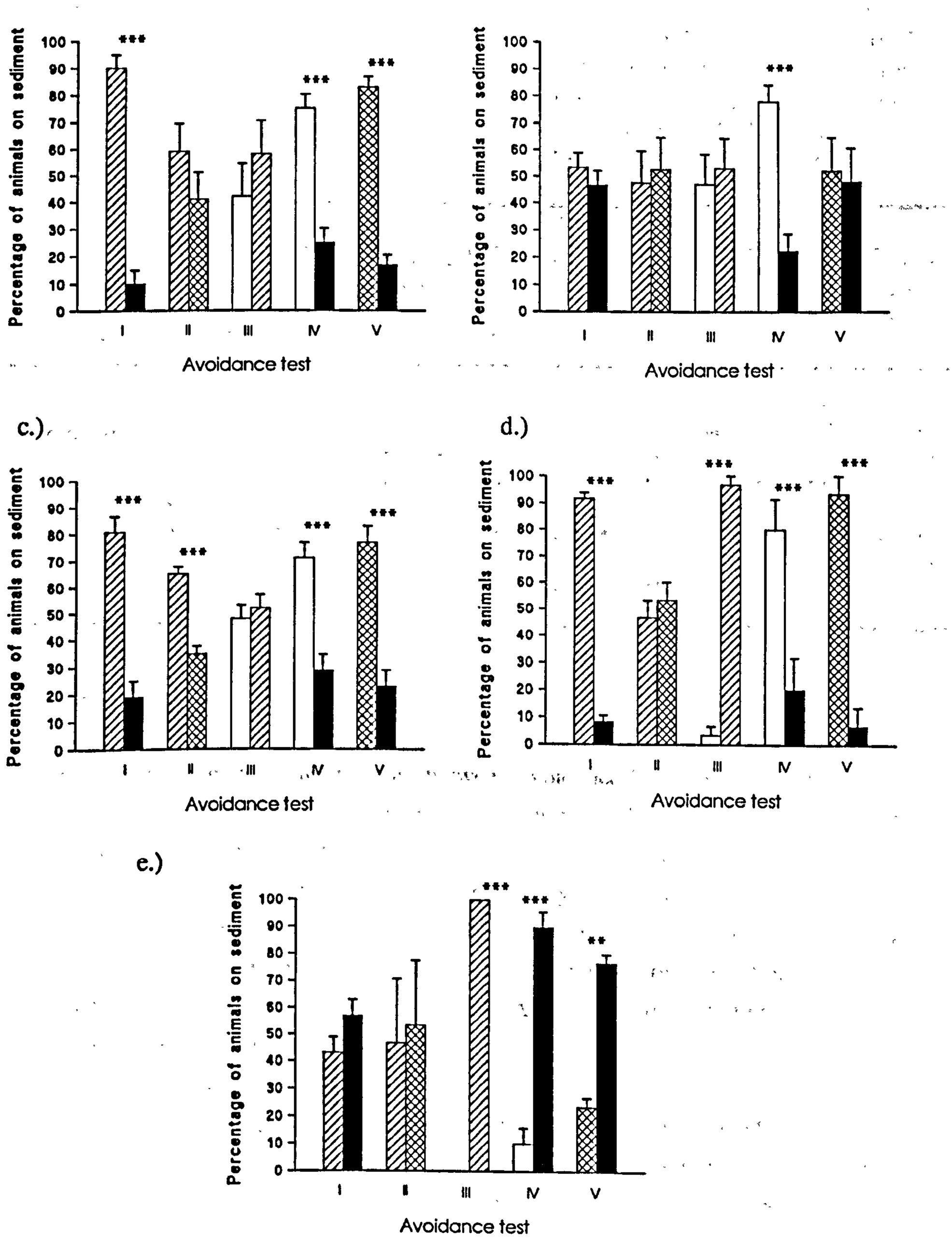


Fig. 4.2. Distribution of a.) *G. pulex*, b.) *N. cinerea*, c.) *P. jenkinsi*, d.) *C. riparius* and e.) *T. tubifex* on different treatments in the five sediment avoidance tests (I-V; see Table 4.2 for details). ☐ = sand; ▨ = upstream sediment; ■ = downstream sediment; ☒ = solvent-extracted downstream sediment. Data presented as means + 1 S.E.. Asterisks denote significant between-treatment differences for pooled *G* ($p<0.05$=*, $p<0.01$=**, $p<0.001$=***).

Table 4.5. Results of statistical analysis of data from sediment avoidance experiments. Asterisks represent significance levels ($p<0.05$=*, $p<0.01$=**, $p<0.001$=***) for pooled (P), heterogeneity (H) and total (T) *G* values. ns indicates no significant difference. Degrees of freedom are: P=1, H=5, T=6 for avoidance test I. for *G. pulex*, *P. jenkinsi*, *C. riparius* and *T. tubifex* and avoidance tests I.-V. for *N. cinerea*; P=1, H=4, T=5 for avoidance tests II.-V. for *G. pulex* and *P. jenkinsi*; P=1, H=2, T=3 for avoidance tests II.-V. for *C. riparius* and *T. tubifex*.

	AVOIDANCE TEST														
	I. Upstream vs. Downstream			II. Upstream vs. Downstream extracted			III. Sand vs. Downstream			IV. Sand vs. Upstream			V. Downstream extracted vs. Downstream		
SPECIES	P	H	T	P	H	T	P	H	T	P	H	T	P	H	T
G. pulex	** *	**	** *	ns	** *	** *	ns	ns	ns	** *	*	** *	** *	ns	** *
N. cinerea	ns	ns	ns	ns	** *	** *	ns	**	**	** *	ns	** *	ns	** *	** *
P. jenkinsi	** *	*	** *	** *	ns	*	ns	ns	ns	** *	ns	** *	** *	ns	** *
C. riparius	** *	ns	** *	ns	ns	ns	** *	ns	** *	** *	*	** *	** *	ns	** *
T. tubifex	ns	ns	ns	ns	** *	** *	** *	ns	** *	** *	ns	** *	**	ns	*

4.3.2. Sediment mixtures.

In order to investigate the relationship between avoidance behaviour and downstream sediment, a dilution series was prepared by mixing downstream and upstream sediments. Concentrations of Zn, Cd, Cr, Pb, Cu, Ca, Mg and total aromatic hydrocarbons in the sediment mixtures were positively correlated with the percentage of downstream sediment in the mixture ($r>0.71$, df=22, Fig. 4.3). In contrast there was a negative correlation between Ni concentration and percent of downstream sediment ($r=-0.64$, df=22) but no relationship between Al and Fe concentration and percent downstream sediment ($r<0.24$, df=22).

For *G. pulex*, *P. jenkinsi* and *C. riparius* the proportion of test animals on upstream sediment increased as the concentration of downstream sediment in sediment mixtures increased (Fig. 4.4.) *P. jenkinsi* avoided sediments with ≥ 20% downstream sediment (Fig. 4.4, Table 4.6), *G. pulex* avoided sediments with ≥ 40% downstream sediment and *C. riparius* avoided sediments with ≥ 60% downstream sediment (Fig. 4.4, Table 4.6). Concentrations of metals and total aromatic hydrocarbons in sediments mixtures corresponding to the LOEC and NOEC for each species are given in Table 4.7. In contrast, *T. tubifex* did not avoid sediment mixtures with high concentrations of

downstream sediment, on the contrary sediments containing downstream sediments were generally preferred (Fig. 4.4, Table 4.6).

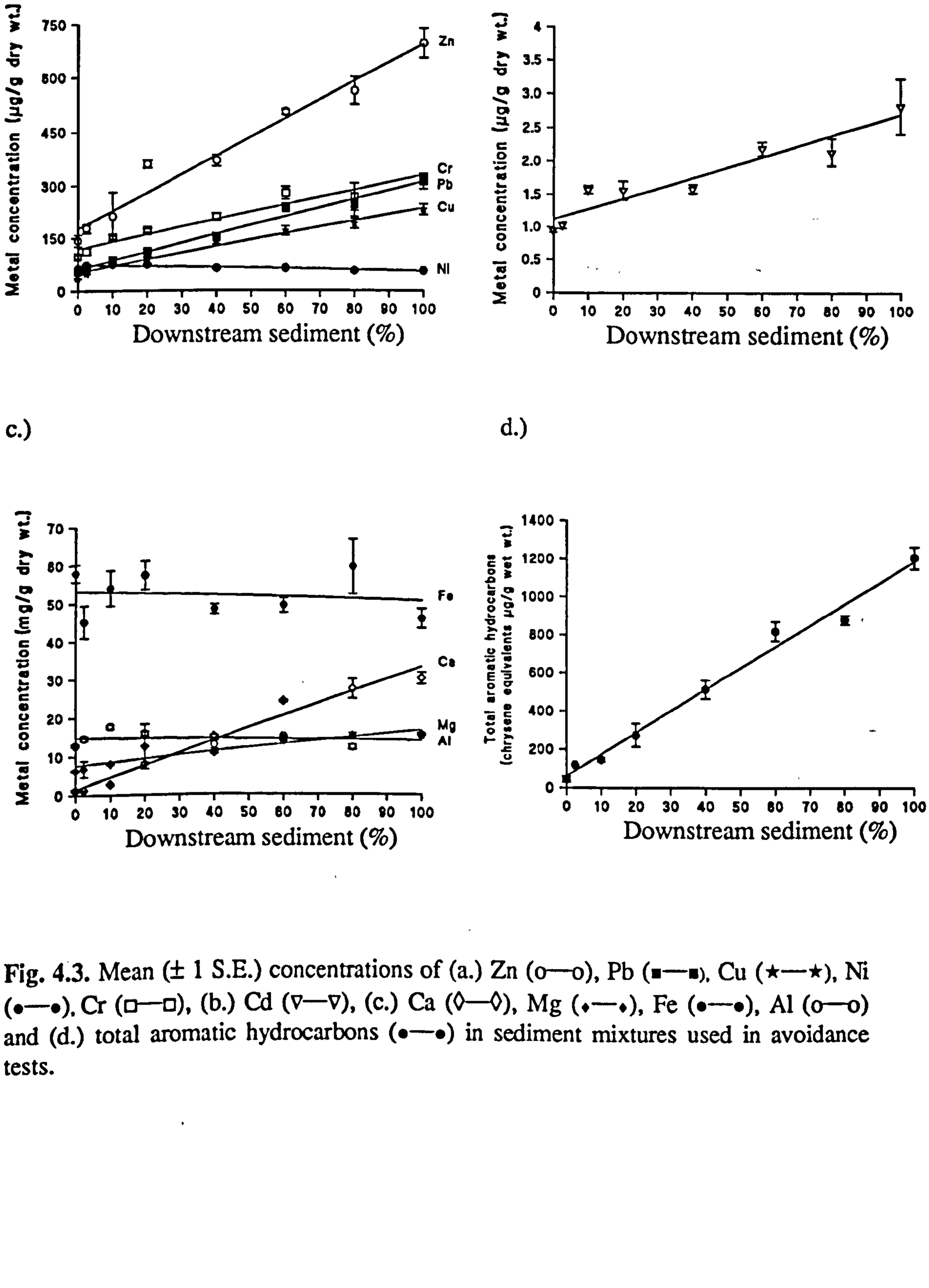


Fig. 4.3. Mean (± 1 S.E.) concentrations of (a.) Zn (o—o), Pb (▪—▪), Cu (★—★), Ni (●—●), Cr (□—□), (b.) Cd (▽—▽), (c.) Ca (◊—◊), Mg (⬧—⬧), Fe (●—●), Al (o—o) and (d.) total aromatic hydrocarbons (●—●) in sediment mixtures used in avoidance tests.

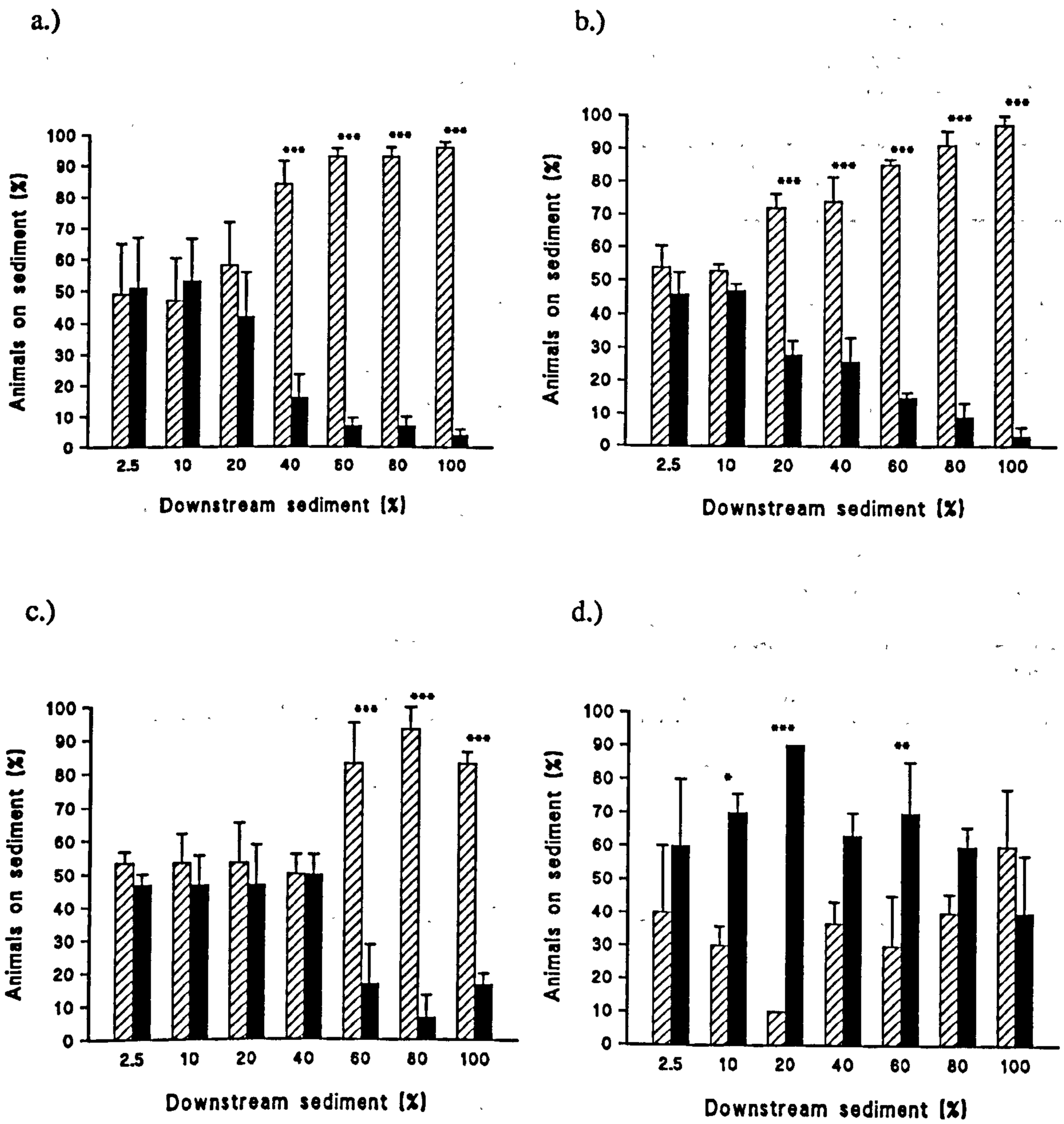


Fig. 4.4. Distribution of a.) *G. pulex*, b.) *P. jenkinsi* c.) *C. riparius*, and d.) *T. tubifex* on 100 % upstream field sediment (▨) and sediment mixtures (■). Mixtures containing between 2.5 and 100 % downstream sediment were tested. Data are presented as mean values + 1 S.E.. Asterisks denote significant differences for pooled *G* ($p<0.05$=*, $p<0.01$=**, $p<0.001$=***).

Table 4.6. Results of statistical analysis of data from sediment mixture experiments. The table displays significance levels ($p<0.05$=*, $p<0.01$=**, $p<0.001$=***) for pooled (P), heterogeneity (H) and total (T) *G* values. ns indicates no significant difference. Degrees of freedom are P=1, H=4, T=5 for *G. pulex* and *P. jenkinsi* and P=1, H=2, T=3 for *C. riparius* and *T. tubifex*.

Downstream sediment (%)	*G. pulex*			*P. jenkinsi*			*C. riparius*			*T. tubifex*		
	P	H	T	P	H	T	P	H	T	P	H	T
2.5	ns	***	***	ns	ns	ns	ns	ns	ns	ns	**	**
10.0	ns	***	***	ns	ns	ns	ns	ns	ns	*	ns	ns
20.0	ns	***	***	***	ns	***	ns	ns	ns	***	ns	***
40.0	***	**	***	***	*	***	ns	ns	ns	ns	ns	ns
60.0	***	ns	***	***	ns	***	***	*	***	*	**	**
80.0	***	ns	***	***	ns	***	***	ns	***	ns	ns	ns
100.0	***	ns	***	***	*	***	***	ns	**	ns	*	*

Table 4.7. The mean concentration of metals (dry weight) and total aromatic hydrocarbon (µg chrysene equivalents /g wet wt.) in sediment mixtures corresponding to the no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) for three macroinvertebrate species.

Species		Zn µg/g	Cd µg/g	Cr µg/g	Pb µg/g	Cu µg/g	Ca mg/g	Mg mg/g	Total A.H.
G. pulex	LOEC	360.70	1.56	111.88	171.99	95.05	8.12	12.56	273.6
	NOEC	370.31	1.58	153.62	209.95	137.40	15.05	10.81	511.8
P. jenkinsi	LOEC	212.77	1.57	86.95	151.76	81.75	2.72	8.08	147.5
	NOEC	360.70	1.56	111.88	171.99	95.05	8.12	12.56	273.6
C. riparius	LOEC	370.31	1.58	153.62	209.95	137.40	15.05	10.81	511.8
	NOEC	505.21	2.18	236.36	278.37	169.63	24.27	13.89	819.9

4.3.3. Sediment extracts.

4.3.3.a.) Dichloromethane (DCM) sediment extract.

There was a significant positive relationship between the concentration of downstream DCM sediment extract and the concentration of total aromatic hydrocarbons in spiked APW (r=0.99, df=11; Fig. 4.5). Mean (± 1 S.E.) treatment concentrations ranged from 106.1 (± 30.4) to 230.6 (± 9.7) µg chrysene equivalents /L. The upstream sediment extract concentration (0.16 ml extract /L) had a total aromatic hydrocarbon concentration of 45.2 (± 10.4) µg chrysene equivalents /L.

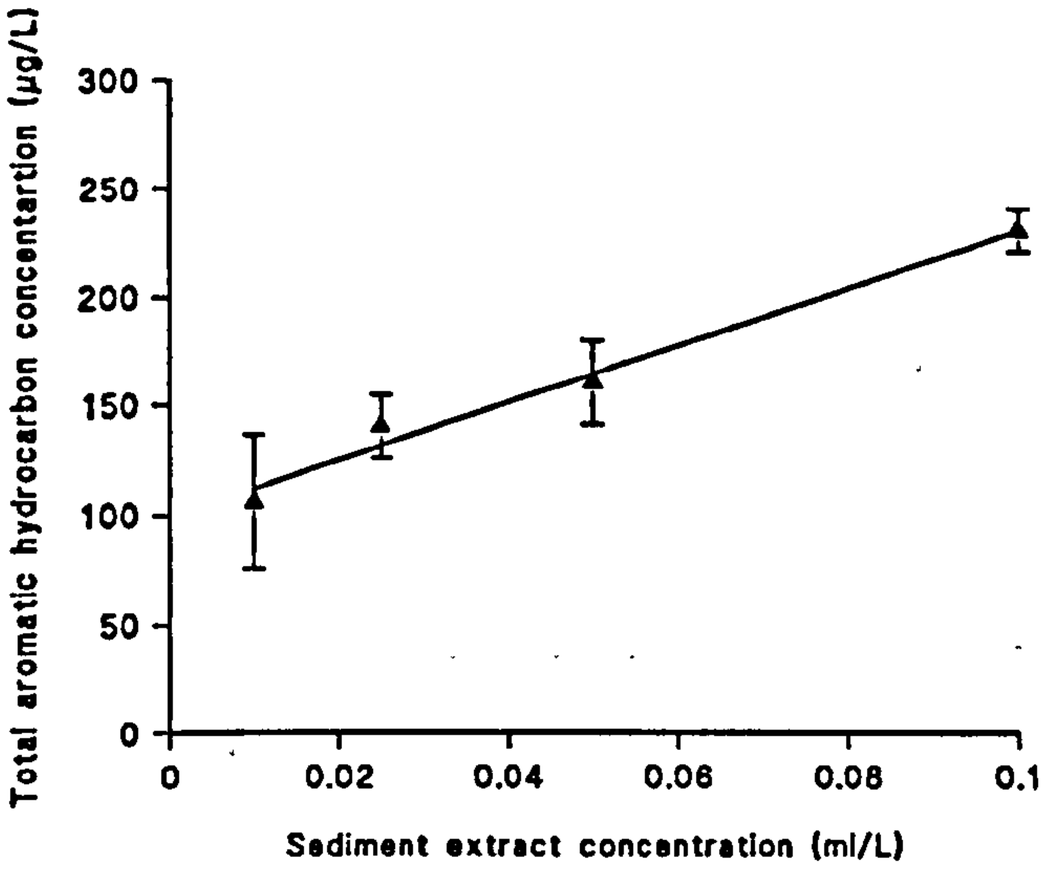


Fig. 4.5. The relationship between downstream sediment extract concentration and total aromatic hydrocarbon (µg chrysene equivalents /L). Data presented as means values ± 1 S.E..

G. pulex, *P. jenkinsi* and *C. riparius* all avoided water spiked with the downstream sediment extract (Fig. 4.6). *G. pulex* demonstrated a significant avoidance behaviour at concentrations ≥ 0.025 ml extract /L, *P. jenkinsi* at ≥ 0.05 ml extract /L, and *C. riparius* at ≥ 0.1 ml extract /L (Table 4.8). The LOEC and NOEC for the three species is given in terms of total aromatic hydrocarbons in Table 4.9. Although *N. cinerea* consistently avoided water spiked with downstream extract the response was only significant at 0.05 ml extract /L (Table 4.8). In contrast to the other species, *T. tubifex* displayed a preference for water spiked with downstream extract and preferred water spiked with upstream extract over control water (Table 4.8).

Neither *G. pulex*, *P. jenkinsi* nor *C. riparius* demonstrated any preference/avoidance between upstream extract (0.1 ml extract /L) or water spiked with acetone (0.1 ml acetone /L, Table 4.8) They did, however, show a significant preference for the acetone spiked water over water spiked with downstream extract (0.1 ml extract /L) (Fig. 4.6, Table 4.8). Taken together with the results from the dilution series, these data suggest that *G. pulex*, *P. jenkinsi* and *C. riparius* show no preference for water spiked with upstream sediment extract but avoid water spiked with downstream extract. *N. cinerea* demonstrated no significant preference for control (acetone spiked) water over water spiked with either upstream or downstream sediment extract (Fig. 4.6, Table 4.8). *T. tubifex*, however, displayed a significant preference for water spiked with either upstream or downstream sediments extracts over the acetone-spiked control water (Fig. 4.6, Table 4.8).

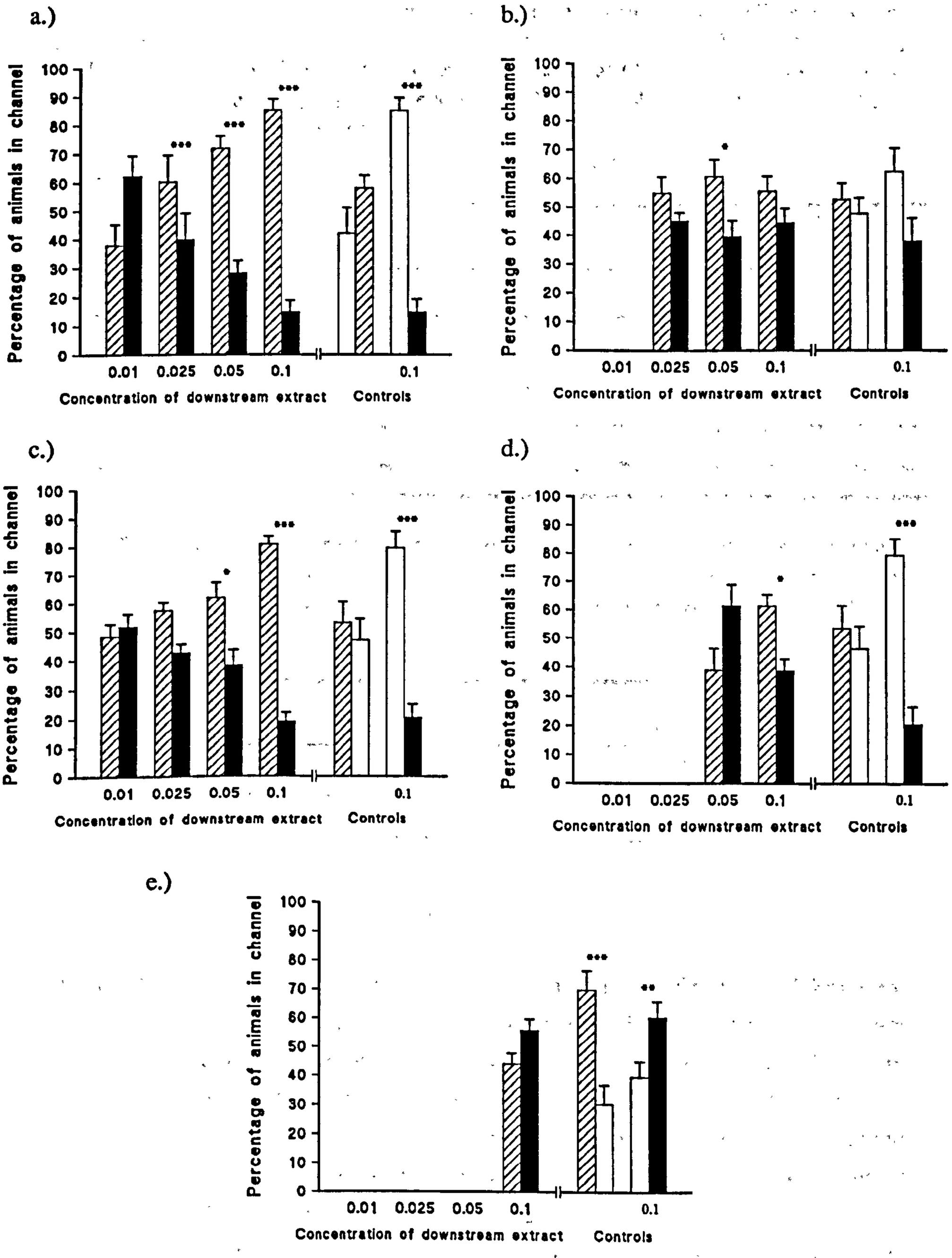


Fig. 4.6. Distribution of a.) *G. pulex*, b.) *N. cinerea*, c.) *P. jenkinsi*, d.) *C. riparius* and e.) *T. tubifex* between channels containing acetone control (0.1 ml extract/L, ☐), upstream sediment extract (0.1 ml extract/L, ▨), or downstream sediment extract (0.01 ml/L - 0.1 ml/L, concentration indicated on x-axis, ■). Data presented as means + 1 S.E.. Asterisks denote significant differences for pooled *G* value ($p<0.05$=*, $p<0.01$=**, $p<0.001$=***).

Table 4.8. Results of statistical analysis of data from DCM sediment extract experiments. The table displays significance levels ($p<0.05$=*, $p<0.01$=**, $p<0.001$=***) for pooled (P), heterogeneity (H) and total (T) *G* value. ns indicates no significant difference. Degrees of freedom are P=1, H=14 (treatment 1 and 2), 9 (treatments 3-6) T=15 (treatment 1 and 2), 10 (treatments 3-6) for *G. pulex* and P=1, H=9, T=10 for *N. cinerea*, *P. jenkinsi*, *C. riparius* and *T. tubifex*.

Treatments	*G. pulex*			*N. cinerea*			*P. jenkinsi*			*C. riparius*			*T. tubifex*		
Concs in ml /L	P	H	T	P	H	T	P	H	T	P	H	T	P	H	T
1. Up 0.1 vs. Dw 0.010	ns	ns	*	–	–	–	ns	ns	ns	–	–	–	–	–	–
2. Up 0.1 vs. Dw 0.025	*	***	***	ns	ns	ns	ns	ns	ns	–	–	–	–	–	–
3. Up 0.1 vs. Dw 0.050	***	ns	***	*	ns	ns	*	ns	*	ns	ns	ns	–	–	–
4. Up 0.1 vs. Dw 0.100	***	ns	***	ns	ns	ns	***	ns	***	*	ns	ns	ns	ns	ns
5. Up 0.1 vs. acetone 0.1	ns	ns	**	ns	ns	ns	ns	***	***	ns	ns	ns	***	*	***
6. Dw 0.1 vs. acetone 0.1	***	***	***	ns	**	**	***	ns	***	***	ns	***	**	ns	ns

Table 4.9. Downstream sediment extract concentration and equivalent total aromatic hydrocarbon concentrations (µg chrysene equivalents /L) for no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) for a significant avoidance response with the various species.

Species	NOEC Total aromatics	LOEC Total aromatics
Gammarus pulex	106.06	139.90
Potamopyrgus jenkinsi	139.90	160.49
Chironomus riparius	160.49	230.63

4.3.3.b.) Acid (acetic) sediment extract.

Although the majority of the metals measured (Zn, Cu, Cd, Cr, Pb, and Fe) were elevated in the acid extract of downstream sediment relative to that of upstream sediment this was only significant for Zn, Cd and Fe ($t>5.26$, df>2, Table 4.10). There was no difference in pH between the two treatments ($t=1.14$, df=2). When given a choice between water spiked with either upstream sediment acid extract (5 ml extract/ L) or downstream sediment acid extract (5 ml extract/ L) none of the five species tested showed any significant difference in distribution between the treatments (Fig. 4.7, Table 4.11). Furthermore, none of the species showed any preference between water spiked with sediment extracts or water spiked with acetic acid (Pooled $G<1.492$, df=1; Heterogeneity $G<8.27$, df>9; Total $G<4.27$, df>10).

Table 4.10. Chemical composition of upstream or downstream acid (acetic) sediment extract at 5 ml extract/ L. Data are presented as mean values and 1 S.E. are given in parentheses. ND indicates not detected. Aromatic hydrocarbons are given in µg chrysene equivs./ L.

	Zn µg/L	Cu µg/L	Cd µg/L	Cr µg/L	Pb µg/L	Fe µg/L	Aromatic hydrocarbons
Upstream extract (5 ml/L)	18.2 (7.3)	3.47 (0.43)	0.057 (0.057)	ND	ND	7.8 (24.0)	ND
Downstream extract (5 ml/L)	123.2 (18.0)	6.47 (0.64)	0.643 (0.096)	ND	0.0003 (0.0003)	687.4 (45.0)	ND

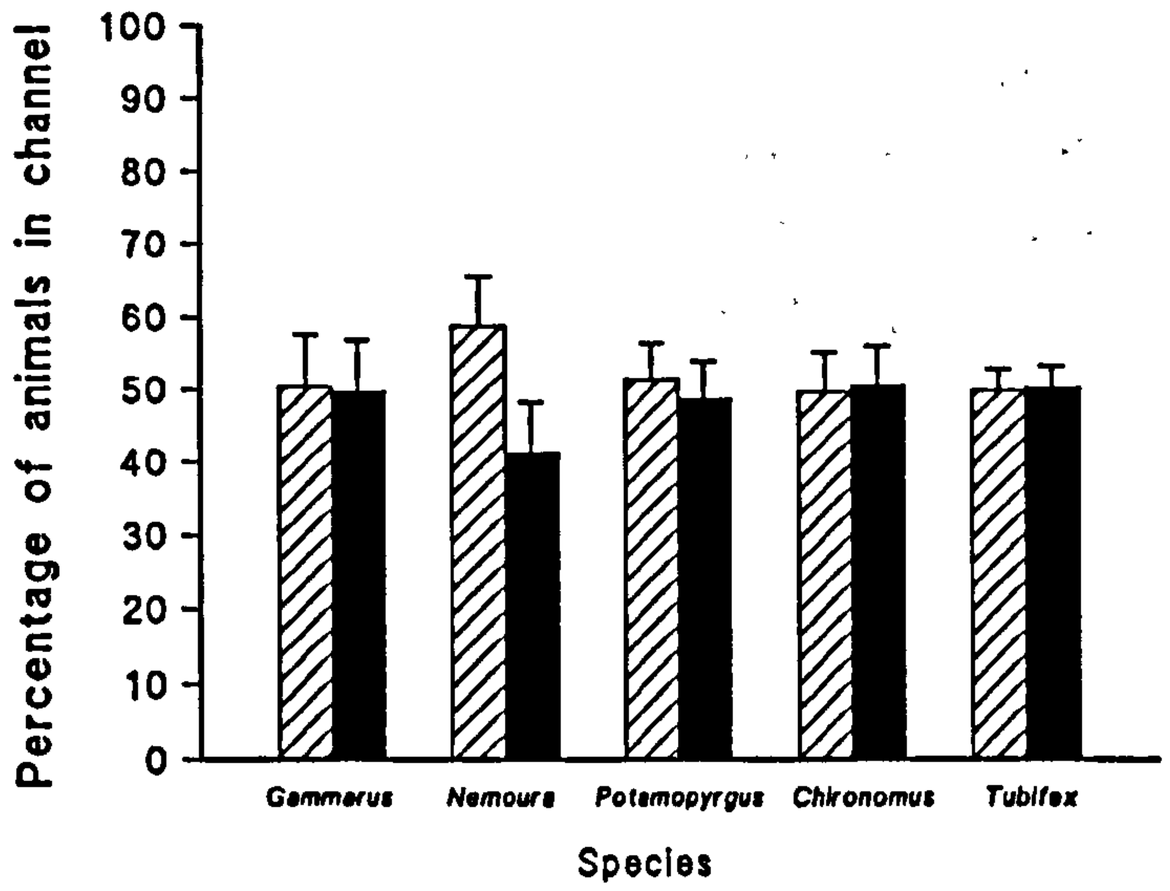


Fig. 4.7. Distribution of *G. pulex*, *N. cinerea*, *P. jenkinsi*, *C. riparius* and *T. tubifex* between test channels receiving water spiked with either upstream acid sediment extract (5 ml extract /L; ▨) or downstream acid sediment extract (5 ml extract /L; ■). Data presented as means + 1 S.E..

Table 4.11. Results from statistical analysis of data from avoidance tests using water spiked with acid sediment extracts. ns indicates no significant difference.

	Pooled *G*		Heterogeneity *G*		Total *G*	
	df	p	df	p	df	p
Gammarus pulex	1	ns	9	ns	10	ns
Nemoura cinerea	1	ns	9	ns	10	ns
Potamopyrgus jenkinsi	1	ns	9	ns	10	ns
Chironomus riparius	1	ns	9	ns	10	ns
Tubifex tubifex	1	ns	9	ns	10	ns

4.3.4. Spiked sediments.

Solvent-extracted downstream sediment that had been spiked with the downstream sediment solvent extract had significantly higher concentrations of total aromatic hydrocarbons (mean = 772.7, S.E.= 41.3) then the same sediment spiked with the

acetone carrier (mean = 79.9, S.E. = 19.0 µg/g chrysene equivalents wet wt.; t=15.22, df=2). The majority of the aromatic hydrocarbons remained associated with the sediments during the course of the experiment and the mean (± 1 S.E.) concentrations of total aromatic hydrocarbons in the overlying water at the end of the experiment was 47.41 (± 6.66) chrysene equivalents /L. Mean (± 1 S.E.) metal concentrations in the solvent-extracted sediment prior to spiking were: Cd 2.11 (±0.09), Cr 311.07 (±24.75), Pb 218.87 (±3.54), Cu 299.94 (±25.02), Zn 903.97 (±77.53), Ni 89.71 (±46.91) µg/g dry wt. and Ca 48.35 (±1.54), Mg 14.58 (±0.59), Al 13.71 (±1.31), Fe 57.32 (±3.00) mg/g dry wt.

Whereas *G. pulex* and *C. riparius* avoided sediment spiked with the downstream sediment extract (Fig. 4.8, Table 4.12), *T. tubifex* showed no significant preference between the two sediments (Fig. 4.8, Table 4.12). When *P. jenkinsi* were placed on the spiked sediment they immediately formed thick mucus trails and died. The effect on this species was obviously lethal rather than sub-lethal and therefore no avoidance results are displayed.

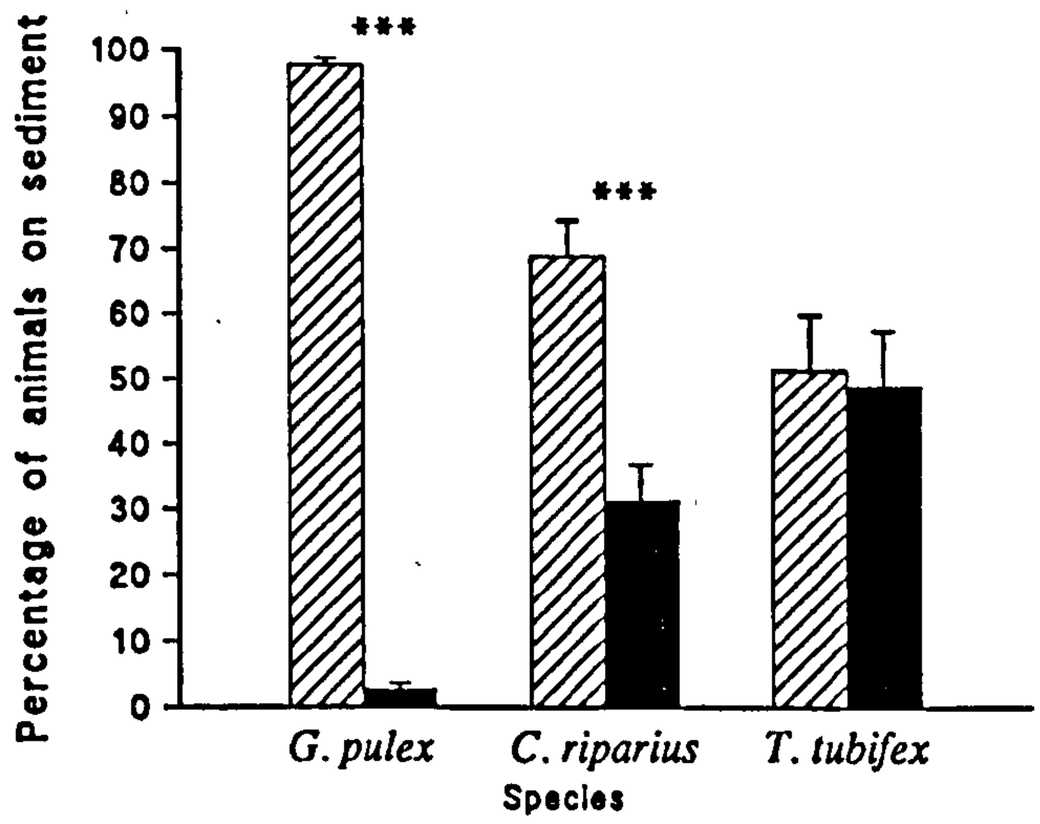


Fig. 4.8. Distribution of *G. pulex*, *C. riparius* and *T. tubifex* between solvent-extracted downstream sediment spiked with either acetone (▨) or downstream sediment extract (■). Data presented as means and 1 S.E.. Asterisks denote significant differences for pooled G ($p<0.05$=*, $p<0.01$=**, $p<0.001$=***).

Table 4.12. Results of statistical analysis of data from DCM extract spiked sediment experiments. ns indicates no significant difference. Asterisks denote significant differences for G values ($p<0.05$=*, $p<0.01$=**, $p<0.001$=***).

	Pooled G		Heterogeneity G		Total G	
	df	p	df	p	df	p
Gammarus pulex	1	***	5	ns	6	***
Chironomus riparius	1	***	7	ns	8	ns
Tubifex tubifex	1	ns	7	**	8	**

4.4. DISCUSSION.

The experiments described in this chapter were designed to investigate whether the selected benthic macroinvertebrates avoid sediments contaminated with motorway runoff. Of the five macroinvertebrates species assayed three species, *G. pulex*, *P. jenkinsi* and *C. riparius*, avoided sediments collected from the downstream station at Pigeon Bridge Brook. Experiments with sediment mixtures indicated that the relative sensitivity of these species decreased in the order *P. jenkinsi* > *G. pulex* > *C. riparius*. In contrast to these species the stonefly *N. cinerea* exhibited no clear pattern of avoidance to any of the field sediments and *T. tubifex* preferred downstream sediments.

Several previous studies have demonstrated avoidance of contaminated sediments, often manifested as changes in burrowing behaviour. For example, Mohlenberg and Kiorboe (1983) found that the burrowing behaviour of the molluscs *Cerastoderma edule*, *Abra alba* and *Macoma baltica*, and the annelids *Nereis diversicolar* and *Scoloplos armiger* was reduced when exposed to pesticide contaminated sediment. In the same study sediment avoidance behaviour was shown by the crustacean *Crangon crangon* and the fish *Solea solea*. Burrowing by the chironomid *Chironomus tentans* was reduced by coal-liquid-spiked sediments (Dauble *et al.*, 1983) and metal contaminated sediments (Wetsel *et al.*, 1977b) and although the oligochaetes *Limnodrilus hoffmeisteri* (Tubificidae) and *Stylodrilus heringianus* (Lumbriculidae) initially burrowed into endrin-contaminated sediment, they soon returned to the surface in numbers proportional to the exposure concentration (Keitly *et al.*, 1988). In the present study, *P. jenkinsi* exposed to contaminated sediments had a tendency to leave the sediment moving to the water/ air interface. This behaviour has commonly been observed with snails attempting to avoid pollutants or low dissolved oxygen (Giddings, 1982).

Although avoiding contaminated sediments reduces exposure to potential toxicants it may not necessarily increase survival probability. For example, Pearson *et al.* (1981) found that sediment contaminated with oil reduced the burrowing behaviour of the littleneck clam, *Protothaca staminea*, resulting in increased rate of predation by the Dungeness crab, *Cancer magister*. Taylor *et al.* (1994) found that in laboratory exposures *G. pulex* avoided copper contaminated sediments in a concentration-related manner spending more time in the water column. Such behaviour would result in *G. pulex* entering the drift and may result in them being predated or swept to a less suitable habitat (Brittain and Eikeland, 1988). Active avoidance will also have an energetic cost thus reducing the amount of energy

available for other metabolic processes (Abel and Green, 1981; Allan, 1995). Whether the benefits of entering the drift to avoid contaminated sediments outweigh the risks will depend on the species and degree of contamination. For example, Wallace *et al.* (1991) concluded that only those animals that drifted survived an insecticide treatment.

Insight into the contaminants in downstream sediments eliciting the avoidance behaviour can be gained by considering the results from experiments using manipulated sediments. DCM-extracted sediments contained significantly lower concentrations of total aromatic hydrocarbons than non-extracted sediment, but similar metal concentrations. In avoidance experiments *G. pulex, P. jenkinsi* and *C. riparius* preferred the extracted sediment to the non-extracted downstream sediment but either showed no preference between the extracted sediment and the upstream field sediment (*G. pulex* and *C. riparius*) or preferred the upstream sediment (*P. jenkinsi*). These results indicate that the contaminants responsible for the avoidance of downstream field sediments by *G. pulex* and *C. riparius* were removed by DCM extraction and were therefore probably organic. However, *P. jenkinsi* may still be avoiding the downstream sediment even when it had been treated by solvent-extraction.

Flow-through avoidance tests provided further evidence that DCM extracts contained compounds which elicited an avoidance response. The use of spiked water also removed any substrate effects which may influence the distribution of benthic organisms (Allan, 1995). The relative sensitivity *G. pulex, P. jenkinsi* and *C. riparius* decreased in the order *G. pulex* > *P. jenkinsi*> *C. riparius*. As with the sediment exposures *N. cinerea* displayed no avoidance behaviour to the downstream DCM extract (≤0.1 ml/ L) and *T. tubifex* preferred downstream extract to control water. None of the five test species displayed any avoidance of the acetic acid downstream sediment extract. Metal concentrations in the spiked water were generally lower than those which have been shown to elicit a response in previous studies (Costa, 1966; Maciorowski *et al.*, 1977; Abel and Green, 1981).

The importance of compounds in the DCM extract in determining the distribution of macroinvertebrates was confirmed using sediment spiking experiments. Downstream sediment which had been DCM extracted (i.e. organics removed) was preferred by *G. pulex* and *C. riparius* over the same sediment which had been spiked with the solvent extract (i.e. organics re-introduced). Whereas *P. jenkinsi* avoided downstream sediment in the sediment avoidance experiment, exposure to spiked downstream sediment resulted in mortality even though the concentration of total aromatic hydrocarbons was lower in the

spiked sediment than in the non-extracted downstream sediments (773 and 1209 µg chrysene equivalents /g wet weight respectively). The high mortality of *P. jenkinsi* exposed to spiked sediments implies that aromatic hydrocarbons were more bioavailable in the spiked sediments.

One of the major groups of chemicals in the DCM extract of downstream sediments were aromatic hydrocarbons. The avoidance of hydrocarbons by aquatic animals has been described by many authors. Percy (1976) reported that marine amphipods were repelled by crude oils and dipteran larvae have been shown to be sensitive to oil spills (Miller *et al.*, 1986). Fractionation of kerosene indicated that polar aromatics (at $<10^{-9}$ ppb) produced an avoidance reaction in lobsters (*Homarus americanus*) and mud snails (*Nassarius obsoletus*; whereas the branched chain cyclic fraction, and to a lesser extent whole kerosene, induced an attraction response in lobsters (Atema *et al.*, 1973). Fractionation of the DCM extract of downstream sediment from Pigeon Bridge Brook into principally aliphatic, PAH and non-PAH aromatic fractions indicated that the fraction containing the PAHs was responsible for the majority of the avoidance response by *G. pulex* (D. M. Forrow and A. B.A: Boxall, unpublished observations). Previous studies have demonstrated that aromatic hydrocarbons do illicit an avoidance response in both fish and macroinvertebrate species. For example, Maynard and Weber (1981) reported that salmon (*Oncorhynchus kisutch*) avoided monocyclic aromatic hydrocarbons and Landrum *et al.* (1991) demonstrated that the amphipod *Diporeia sp.* avoided sediments dosed with PAHs. Although in the present study *T. tubifex* displayed no avoidance of contaminated sediments or sediment extracts, Kukkonen and Landrum (1994) found the oligochaete worm *Lumbriculus variegatus* avoided sediments dosed with the PAH pyrene.

The non-response of *N. cinerea* to contaminated sediments and sediment extracts in this study may be due to less efficient chemoreceptory systems or higher response thresholds. Toxicants may disrupt chemical detection responses by destroying chemoreceptor organs (Bloom *et al.*, 1978; Steele *et al.*, 1987, 1990; Blaxter and Hallers-Tjabbes, 1992) or by increasing the noise to signal ratio of natural chemical cues to a point where they can no longer be sensed (Olla *et al.*, 1980). Kittredge (1973) found that components of oil destroy neuronal dentrites of crustacean chemoreceptor organs and Percy (1978) reported that pre-exposure to an oil emulsion reduced the avoidance response.

The pattern of response of *G. pulex, P. jenkinsi* and *C. riparius* to either increasing proportions of downstream sediments in the sediment mixture experiments or increasing

concentrations of downstream sediment extract in the flow-through experiments was variable. Whereas *P. jenkinsi* appeared to react in direct proportion to concentration, *G. pulex* displayed a linear response to sediment extracts and a threshold response to sediment mixtures, as did *C. riparius*. Variable response curves have previously been reported for sediment dilution series experiments. Nelson *et al.* (1993) found that macroinvertebrate species exhibited sigmoidal or U-shaped lethal concentration-response curves when exposed to contaminated sediments diluted with reference sediments. Differences in response pattern can be attributed, in part, to exposure route, site of action and the sensitivity of individual species.

Differences in the sensitivity of avoidance behaviours of aquatic organisms have been reported between different phyla, similar species, between individuals of the same species and between individuals on different occasions. For example, whereas *Gammarus pulex* avoided copper concentrations ≥ 63 µg/ L (Costa, 1966), *Gammarus lacustris* avoided concentrations of 0.15 and 0.46 mg Cu/ L but were attracted to concentrations of 12.3 and 30.0 mg Cu/ L (Maciorowski *et al.*, 1976). Inter and intra-specific differences in response may be due to extrinsic factors such as temperature and water hardness (Little *et al.*, 1985) or to intrinsic factors such as life stage or physiological state (Conklin and Rao, 1978). The behavioural response of animals to toxicants may be lessened by pre-exposure (Hartwell *et al.*, 1987b) and therefore laboratory assays that use animals from culture or clean field sites may over-estimate their responsiveness to pollutants in the field (Hartwell *et al.*, 1987a). All test species used in the present study were collected from uncontaminated sites or obtained from laboratory cultures.

The results obtained from the present study partly explain the field distribution of the test species (Chapter 2). *G. pulex* and *P. jenkinsi* were less abundant at the downstream station at Pigeon Bridge Brook and avoided downstream sediments in the laboratory. Both species were more sensitive than *C. riparius* in all exposure systems. Although *C. riparius* demonstrated an avoidance of downstream sediments in the laboratory, field surveys indicated that *Chironomus* sp. were abundant at the downstream station (Chapter 2). Chironomids were only identified to generic level in field surveys but *C. riparius* was selected as a representative of this genus in laboratory experiments. It may therefore be possible that *C. riparius* is a sensitive species in this genus or that chironomids at Pigeon Bridge Brook develop a resistance or desensitisation to the chemicals that cause avoidance reactions in the laboratory. *N. cinerea* and *T. tubifex* displayed no avoidance response to the downstream sediments or sediment extracts. The reduced relative abundance of *N.*

cinerea at the downstream station at Pigeon Bridge Brook cannot, therefore, be attributed to avoidance on the evidence from laboratory exposures.

Elimination of some species by avoidance or toxicity may alter the competitive ability between species, releasing some species from competitive pressures and increasing the opportunities for less-sensitive species to increase their abundance (Giddings, 1982; Peterson, 1986; Allan, 1995). It is possible that such factors were partly responsible for the increase in abundance of tubificids at the downstream station at Pigeon Bridge Brook. *T. tubifex* feeds on bacteria which proliferate in organic-rich sediments and in oil contaminated systems (Brinkhurst and Jamieson, 1971; Johnson and Romanenko, 1989) and this may explain why this species avoids the more inorganic sediments (sand and solvent-extracted downstream sediment) and acetone control waters in aqueous exposures.

Although avoidance experiments are instructive they do not necessarily reflect the reactions of organisms *in-situ* (Laughlin *et al.*, 1978). Behavioural responses to toxicants are modified by natural behaviour (Burris *et al.*, 1990) and the bioavailability of toxicants in the field will be altered by the physicochemical characteristics of the system. Moreover, whereas field gradients of chemicals are usually ephemeral, extend for hundreds of meters and undergo short and long term changes, laboratory gradients are steep and confined to the laboratory apparatus (Laughlin *et al.*, 1978; Smith and Bailey, 1989). However bearing these reservations in mind, the results of these experiments support those reported in Chapter 3 which highlight the sensitivity of *G. pulex* to sediments contaminated with motorway runoff. The sensitivity of *G. pulex* is of particular importance since this species is the major shredding macroinvertebrate at Pigeon Bridge Brook. Its sensitivity to contaminants in motorway runoff may result in deleterious effects on macroinvertebrate leaf processing at the downstream station as discussed in Chapter 5.

4.4.1. Conclusions.

The major findings from this part of the study were:

1. *G. pulex, P. jenkinsi* and *C. riparius* avoided downstream sediments to move to upstream sediments, whereas *N. cinerea* showed no preference and *T. tubifex* preferred downstream sediments.
2. Sediment mixture experiments indicated that the avoidance response shown by *G. pulex, P. jenkinsi* and *C. riparius,* was related to the proportion of downstream sediment with more animals avoiding sediments with higher proportions of this

sediment. *G. pulex* avoided mixtures with ≥ 40 %, *P. jenkinsi* ≥ 20 % and *C. riparius* ≥ 60 % downstream sediment. This was related to the increasing concentrations of total aromatic hydrocarbons and most metals in the sediments.

3. Sediment manipulation and sediment extract experiments suggested that the DCM extract rather than the acid extract of sediment was responsible for the avoidance response of *G. pulex, P. jenkinsi* and *C. riparius*. Increasing avoidance was related to increasing aromatic hydrocarbon concentration of the extract. Avoidance of the DCM extract of downstream sediment by *G. pulex* and *C. riparius* was confirmed using sediment spiking experiments. However, the spiked sediment proved to be lethal to *P. jenkinsi* possibly due to increased bioavailability of aromatic hydrocarbons.
4. Sensitivity to downstream sediment solvent-extracts decreased in the order *G. pulex* > *P. jenkinsi* > *C. riparius* > *N. cinerea* > *T. tubifex*. The NOEC concentrations of total aromatic hydrocarbons in spiked water for *G. pulex, P. jenkinsi* and *C. riparius* were 139.9, 160.5 and 230.6 µg chrysene equivs. /L respectively.
5. Avoidance behaviour may partly explain the distribution of selected macroinvertebrates at Pigeon Bridge Brook. *G. pulex, P. jenkinsi* and *C. riparius* all avoided sediment and solvent sediment extracts from the downstream station. Moreover, *G. pulex* and *C. riparius* avoided solvent-extracted downstream sediment spiked with downstream sediment solvent extracts. Spiked sediment was lethal to *P. jenkinsi*. *P. jenkinsi* was more sensitive than *G. pulex* in sediment exposures whereas *G. pulex* was more sensitive than *P. jenkinsi* to aqueous sediment extracts. Both species were more sensitive than *C. riparius* in all exposure systems. Although *C. riparius* demonstrated an avoidance response to downstream sediment, field surveys indicated that *Chironomus* sp.. were not absent at the downstream station. It is possible that *C. riparius* is a sensitive representative of this genus or this group of organisms had become acclimated to the contaminated sediments and benefited from reduced competition. *N. cinerea* and *T. tubifex* displayed no avoidance response to the downstream sediments or sediment extracts. The reduced relative abundance of *N. cinerea* at the downstream station at Pigeon Bridge Brook may, therefore be due to other factors which may, or may not, be related to the motorway runoff. *T. tubifex* do not appear sensitive to contaminants in the sediments but shows a preference for more organic sediments avoiding the sand and solvent-extracted sediments.

CHAPTER 5.

SUB-LETHAL TOXICITY OF MOTORWAY RUNOFF CONTAMINANTS: II. FEEDING.

5.1. INTRODUCTION.

Since the seasonal input of leaf material to streams may be rapidly lost from the system as coarse particulate organic matter (CPOM; Richardson, 1992), the rapid and efficient processing of this material is vital. CPOM loss may be even more significant in systems subjected to modified hydraulics due to drainage flushing or channelization such as motorway drainage streams (Hellawell, 1988a). The primary step in leaf-litter processing by macroinvertebrates is the breakdown of the conditioned whole leaf material by shredders into fine particulate organic matter. This is considered to be a major rate limiting step in the processing of the leaf material in aquatic systems (Cummins *et al.*, 1973). Efficient food processing is vital not only to the system as a whole but also to the viability of the shredder populations themselves. Inefficient processing as a consequence of reduced feeding may result in reduced growth, adult size, fecundity and survivorship of individuals (Bayne *et al.*, 1975; Kostalos and Seymour, 1976; Bayne *et al.*, 1978; Anderson and Cummins, 1979; Lawson *et al.*, 1984; Widdows, 1985; Maltby and Naylor, 1990; Bermingham, 1993). Affects on different populations may eventually be manifested as an effect on the structure of the whole stream community (Sutcliffe and Hildrew, 1989).

It was evident from previous results in this study (section 2.2.8) that, although processing rates of leaf litter in fine mesh bags was unaltered, the processing of leaf litter deployed in coarse mesh bags was reduced at the downstream station at Pigeon Bridge Brook. These results suggest that macroinvertebrate-mediated leaf breakdown was reduced below the motorway discharge. There are two non-mutually exclusive explanations for this. Firstly the abundance of macroinvertebrates involved in leaf-litter breakdown was reduced and secondly the feeding activity of the shredders present was reduced. Results from the macroinvertebrate surveys at Pigeon Bridge Brook indicated that both the relative and absolute abundances of shredders were reduced downstream of the motorway discharge (sections 2.3.3. and 2.3.4.) possibly due to lethal (Chapter 3) or sub-lethal (Chapter 4) toxicity. This chapter will assess the second of these possible explanations for reduced leaf processing rates, namely sub-lethal effects of the motorway discharge on macroinvertebrate feeding.

Impaired feeding efficiency of shredders present may be due to i) direct exposure to, and uptake of, toxicants which affect the physiology of the animal resulting in reduced feeding, or ii) an indirect behavioural response of the animal to contaminated food (Little *et al.*, 1985). Contaminated food may be avoided because the toxicant itself is detected or because the food is of low quality due to effects of the toxicant on leaf conditioning (Swartz and Lee, 1980; Maltby and Booth, 1991; Frankenhuyzen and Geen, 1986; Bermingham, 1993).

The energy budget of an individual animal is an integration of basic physiological processes such as feeding, food absorption, respiration, excretion and production. The most sensitive component of the energy budget of aquatic organisms to toxicants has often been found to be food absorption (Stickle *et al.*, 1984; Widdows *et al.*, 1987a, b; Widdows and Johnson, 1988; Naylor *et al.*, 1989; Maltby *et al.*, 1990a, b) usually as a result of reduced consumption (Widdows *et al.*, 1980-81; Widdows, 1985; McCahon *et al.*, 1988; Maltby and Naylor, 1990; Bermingham, 1993; Tattersfield, 1993). Reduction in food consumption is commonly observed in contaminated systems and may in fact be a more sensitive sub-lethal endpoint than reduced growth (Sandheinrich and Atchison, 1990). It is often the case that measurement of consumption rates may be sufficient to describe the effects of toxicants on animals without measuring the complete suite of energy budget parameters (Maltby, 1994).

The difference between the energy absorbed and that lost via respiration and excretion is the amount of energy available for production and is referred to as the 'Scope for Growth' (SfG, *sensu* Warren and Davis, 1967). SfG has been shown to be a sensitive sub-lethal response parameter to a variety of organic and inorganic toxicants in both laboratory and field studies in marine (Widdows *et al.*, 1980-81) and freshwater systems (Maltby *et al.*, 1990a, b). Although Widdows (1985) states it is important to measure both rates of energy acquisition and expenditure in order to extrapolate to a reduction in growth, many studies have found that some of the energy budget parameters in pollutant stressed animals are less sensitive to pollutants. In the majority of cases respiration is not altered at low toxicant concentrations, whereas energy uptake is. The main exception to this is when animals are exposed to respiratory inhibitors (Widdows *et al.*, 1982; Willows, 1994). It was not the intention in this part of the study to assess the full energy budget of particular species of macroinvertebrate but rather to assess the effect of motorway runoff pollution on the feeding efficiency of the dominant shredder, *G. pulex*, at the most contaminated site, Pigeon Bridge Brook. In addition the effects of food quality on feeding behaviour were investigated.

If animals cannot discriminate between contaminated and clean food then consumed toxicants may have subsequent effects on leaf consumption due to stress caused by toxicant accumulation. Shredder macroinvertebrates can discriminate between food types and selectively feed on fungal patches on leaves (Arsuffi and Suberkropp, 1985). These fungal patches may accumulate contaminants and will therefore increase the animal's exposure (Duddridge and Wainwright, 1980; Karickhoff, 1984; Pinkney *et al.*, 1985; Abel and Bärlocher, 1988). The mechanism of contaminant accumulation by fungi is mainly by passive absorption, however, active uptake does occur and this may vary between fungal species (Abel and Bärlocher, 1988). Leaf material with high microbial activity/biomass is preferred by shredders (Kostalos and Seymour, 1976; Willoughby and Sutcliffe, 1976; Graça *et al.*, 1993). Further, shredders can differentiate between, and show preferences for, different species of fungi on leaf material (Suberkropp *et al.*, 1983; Arsuffi and Suberkropp, 1984, 1985; Bermingham, 1993). Consequently the microbial biomass and fungal community structure will potentially have consequences for toxicant uptake by the leaf material and hence the animals that feed on it. Leaf material inoculated with either a single species (*Cladosporium* sp.) or a mixed microbial assemblage was used as food in this study. The importance of leaf material as a contaminant source was also investigated. Toxicants may affect the assemblage of fungi on the detrital material affecting its food value and attractiveness to shredders which, in turn, may reduce consumption by shredders (Phillips, 1984; Cargill *et al.*, 1985; Bermingham, 1993).

5.1.1. Objectives and approach.

Gammarus pulex was the dominant macroinvertebrate shredder at the upstream station at Pigeon Bridge Brook (Chapter 2). Lethal (Chapter 3) and sub-lethal avoidance (Chapter 4) assessments of toxicity indicated it was also sensitive to motorway-derived toxicants which affect its abundance at the downstream station. Additionally these toxicants may have an impact on the feeding activity of shredders downstream of the motorway discharge which will affect litter processing and further explain reduced detritus processing rates at this station (Chapter 2).

The specific objectives of this chapter were to:

1. assess whether leaf consumption by *G. pulex* was reduced *in-situ* downstream of the discharge;
2. determine whether consumption was reduced as a result of direct sub-lethal toxicity or affects of the toxicants on food quality;

3. determine whether food quality was affected by i.) toxicant accumulation on the leaf material and/or ii) an impact on the microbial assemblage on the leaf material;
4. ascertain which major classes of contaminants were responsible for any reduced consumption, where the toxicity resided and whether the response could be quantitatively related to concentrations of specific chemical classes;
5. determine whether material contaminated with motorway derived toxicants or material conditioned at the downstream station was less preferred than non-contaminated material or material incubated upstream;
6. assess whether leaf material was a major uptake route of motorway-derived toxicants.

The combined impact of direct and indirect effects (leaf quality) of the motorway runoff on the consumption of *Cladosporium*- and naturally-conditioned leaf material was assessed using *in-situ* deployments. Laboratory experiments were employed in an attempt to separate direct and indirect effects on both consumption and absorption of leaf material and to assess which broad classes of toxicant were responsible for the observed effects.

The effect of leaf quality on food choice by *G. pulex* was assessed in order to differentiate between behavioural and physiological effects on reduced consumption. The uptake route of major motorway runoff pollutant classes was assessed in order to address the possible exposure route for accumulated toxicants which may elicit a subsequent physiological response on reduced consumption.

5.2. MATERIALS AND METHODS.

5.2.1.a.) Source and collection of *Gammarus pulex* and alder leaf material.

The sensitivity of animals to toxicants is affected by intrinsic factors such as reproductive condition, body size and parasitism (Buikema and Benfield, 1979; Adcock, 1982; McCahon and Pascoe, 1988a; McCahon *et al.*, 1988). Consequently, adult male *Gammarus pulex* of a standard size (6.5 - 12.0 mg dry wt) were collected from Crags stream, near Clowne, Derbyshire (NGR SK497745) using a 2-mm Endcott™ laboratory test sieve. All experimental animals were checked to ensure that they were free of the acanthocephalan parasite, *Pomphorhynchus laevis* (Müller) which appears as orange areas in the animal's tissue. Animals where returned to the laboratory in stream water, maintained in APW in a constant temperature room (15°C ±2°C; 12 h light; 12 h dark photoperiod) and fed on a diet of whole alder leaves (*Alnus glutinosa*) inoculated with *Cladosporium* sp. fungus.

Alder leaves were collected post-abscission and pre-leaf fall in the autumn (October-December) from a river-side reference site near Calver, Derbyshire (NGR SK246754). Leaves were returned to the laboratory, air-dried and stored at room temperature until use.

5.2.1.b.) Preparation of *Cladosporium* sp. -inoculated leaf discs.

Unconditioned leaf material is a poor quality, less preferred food source to macroinvertebrates than conditioned leaf material (Hanson *et al.*, 1982; Bärlocher, 1990; Graça, 1993). A standard food prepared by inoculating alder leaf material with *Cladosporium* sp. fungus was therefore used. Alder is a 'fast' decomposing leaf species that is generally rapidly colonised by microorganisms in streams and is considered a good quality food source for shredders (Chergui and Pattee, 1990). *Cladosporium,* a hyphomycete fungus is commonly found on aquatic detritus (Graça, 1993), is faster growing than most aquatic hyphomycetes and produces a uniform hyphal mat when grown on nutrient broth in Petri dishes allowing standardisation of fungal inoculation of leaf material. Fungally-inoculated leaf discs were prepared according to the scheme in Fig. 5.1. Leaf discs were generally used in groups of five and dry-weighed on a Mettler™ ME30 micro-balance (accuracy ±0.001 mg).

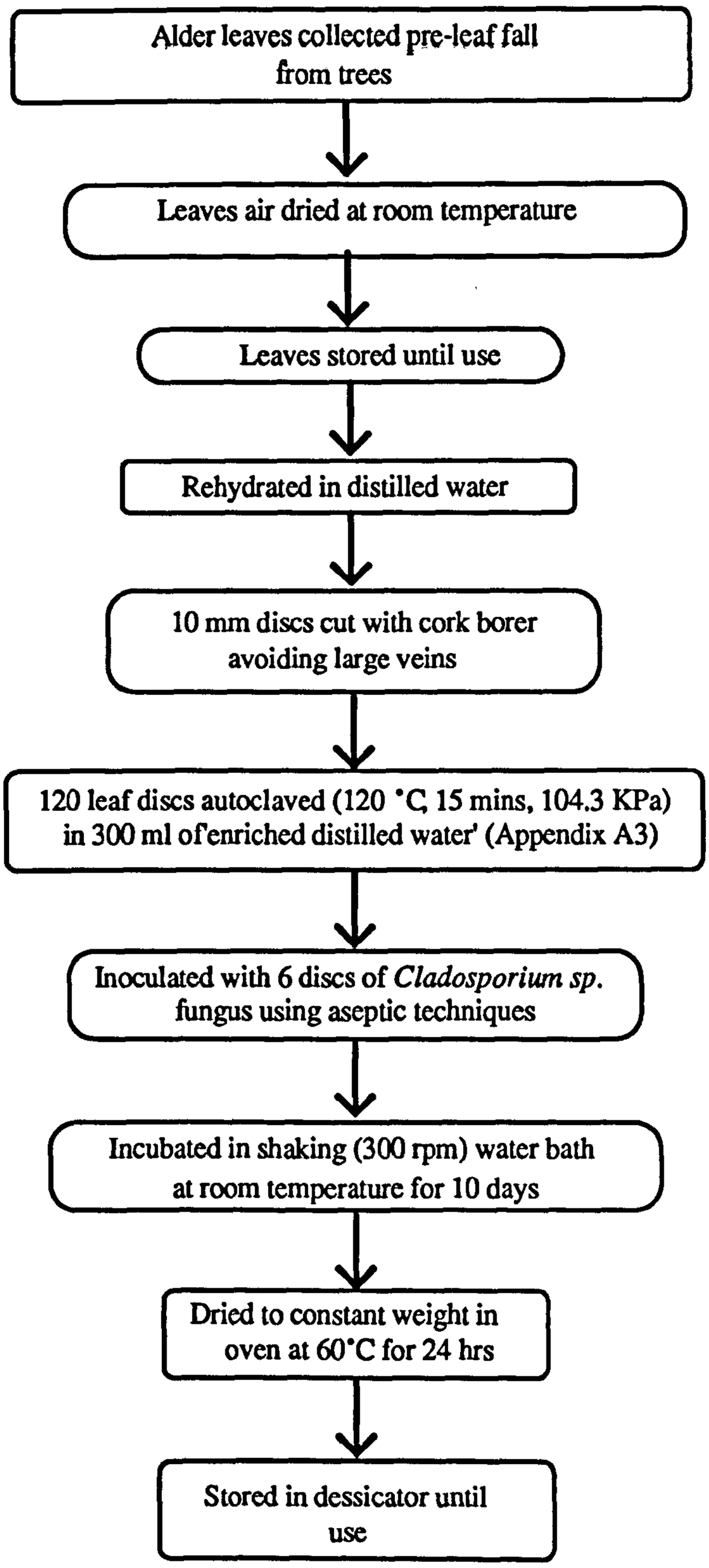


Fig. 5.1. Preparation of *Cladosporium*-inoculated leaf discs.

5.2.1.c.) Preparation of naturally (stream) -inoculated leaf discs.

In order to establish a natural assemblage of aquatic microbes, alder leaves were incubated in the field. Samples of five large alder leaves were re-hydrated in distilled water for 2 h and placed in nylon mesh bags (12 cm x 12 cm, mesh size 350 µm). Leaf bags were deployed at the upstream station at Pigeon Bridge Brook for 21 d according to methods described in section 2.2.4 after which they were washed in distilled water and leaf discs were cut from whole leaves using a 10-mm diameter cork borer. These discs were wet-weighed (excess water removed using absorbent paper) in groups of five and used immediately.

5.2.2. General methods: field.

Individual *G. pulex* were placed together with a ration of five pre-weighed leaf discs (section 5.2.1.) in cylindrical chambers made of PVC piping (diam. 36 mm, 60 mm long) the ends of which were sealed with 1-mm mesh. Control chambers were prepared in the same way, but without the addition of an animal and were used to control for weight loss by physical and microbial processes. Chambers were placed in rectangular cages and deployed at the upstream and downstream station at Pigeon Bridge Brook (section 3.2.2; Plate 5.1.). After 6 d, all cages were recovered from the stream and the animals and leaf material removed, washed and placed individually in numbered cells of cell trays. Naturally-inoculated leaf discs were wet-weighed (section 5.2.1.c.) before being dried at 60°C in a drying oven for 48 h, and re-weighed. For each treatment (i.e. station x leaf type) wet weight to dry weight relationships were calculated for post deployment leaf material and the regression equation was used to convert the initial wet weight to dry weight. *Cladosporium*-inoculated leaf discs were dried and dry-weighed before and after deployment. Consumption of leaf disc material was calculated using equation 5.1 (section 5.2.11.) and individual chambers were treated as separate replicates.

5.2.3. *In-situ* leaf consumption: I. upstream/ downstream deployment.

Consumption of *Cladosporium*- and naturally-inoculated leaf discs at the upstream and downstream station at Pigeon Bridge Brook was assessed by *in-situ* deployment. Adult male *G. pulex* were deployed in individual chambers containing pre-weighed leaf discs (section 5.2.2.). Six additional chambers per treatment containing leaf material only were also deployed and used for chemical analyses. The experimental design is displayed in Table 5.1. Leaf discs from three of the chambers were analysed for total

aromatic hydrocarbons (section 3.2.8.) and leaf discs from the other three chambers were analysed for metals (Zn, Cu, Cd, Cr, and Pb; section 3.2.8.).

Table 5.1. Experimental design for *in-situ* consumption experiment.

Leaf Treatment	Test replicates per treatment	Control replicates per treatment
Cladosporium-inoculated	23	5
Naturally-inoculated	40	5

5.2.4. *In-situ* leaf consumption: II. reciprocal transfer experiments.

In order to assess whether the motorway runoff affected the food quality of leaf material, discs were pre-exposed in the experimental chambers at either the upstream or downstream station for 6 d prior to use in consumption experiments. Ninety chambers each containing five *Cladosporium*-inoculated leaf discs or 50 chambers each containing five naturally-inoculated leaf discs were initially deployed at each station (section 5.2.2). After 6 d, all of the chambers were recovered and a single male *G. pulex* was added to each chamber with the exception of 10 control chambers per leaf type per station. Half of the chambers containing animals (i.e. 40 *Cladosporium*-inoculated; 20 naturally-inoculated) and five control chambers were then transferred to the alternative station whilst the remaining half plus five controls were returned to their original station. Chambers were deployed for a further 6 d and consumption was assessed as described in section 5.2.3. The final treatments were material that had been pre-exposed at either the upstream or downstream station and consumed at the same station (i.e. Up⇒Up and Dw⇒Dw) or material that had been pre-exposed at the upstream or downstream station and transferred to the opposing station (i.e. Up⇒Dw and Dw⇒Up). Six additional chambers per treatment (final station x leaf type) containing leaf material only were also deployed and used for chemical analysis. Leaf discs from three of the chambers were analysed for total aromatic hydrocarbons (section 3.2.8.) and leaf discs from the other three chambers were analysed for metals (Zn, Cu, Cd, Cr, and Pb section 3.2.8.).

5.2.5. General methods: laboratory.

Laboratory experimental exposure pots consisted of two chambers (Plate 5.2). The upper chamber (diam. 7.5 cm x 8 cm deep) which contained an individual adult male *G. pulex* and a ration of pre-weighed leaf material (i.e. 5 leaf discs) was separated from a lower chamber (diam. 5.5 cm x 3 cm deep) by nylon mesh (1-mm mesh size). Faecal pellets produced by the animal in the upper chamber fell through the mesh into the lower chamber preventing re-ingestion and mechanical destruction of the pellets. Pots containing food only were used to control for leaching and microbial decomposition. One-hundred and fifty millilitres of APW were added to each of the two-chambered pots and each pot was treated as an individual replicate in the experiments.

The experiments were conducted over a 6-d period in a constant temperature room at 15°C (± 2°C) with a 12 h dark / 12 h light photoperiod. After 6 d, the animals and remaining leaf material were removed and placed into individual numbered cells of cell trays. Animals were dried at 60°C for 24 h and dry-weighed at the end of the experiment. *Cladosporium*-inoculated food was dry-weighed (dried to a constant weight at 60°C for 24 h) at the beginning and end of each experiment. Naturally-inoculated leaf discs were wet-weighed at the beginning and end of each experiment, dried (dried to constant weight at 60°C for 24 h) and re-weighed at the end of the experiment and wet weight-dry weight relationships used to calculate initial dry weights (section 5.2.2.). Faecal pellets were filtered onto numbered pre-weighed 5.5-cm Whatman™ No. 1 filter papers which were then dried at 60°C for 24 h and weighed. Five control filter papers were processed in the same way, filtering distilled water rather than faeces to control for changes in the weight of filter papers due to the experimental procedure.

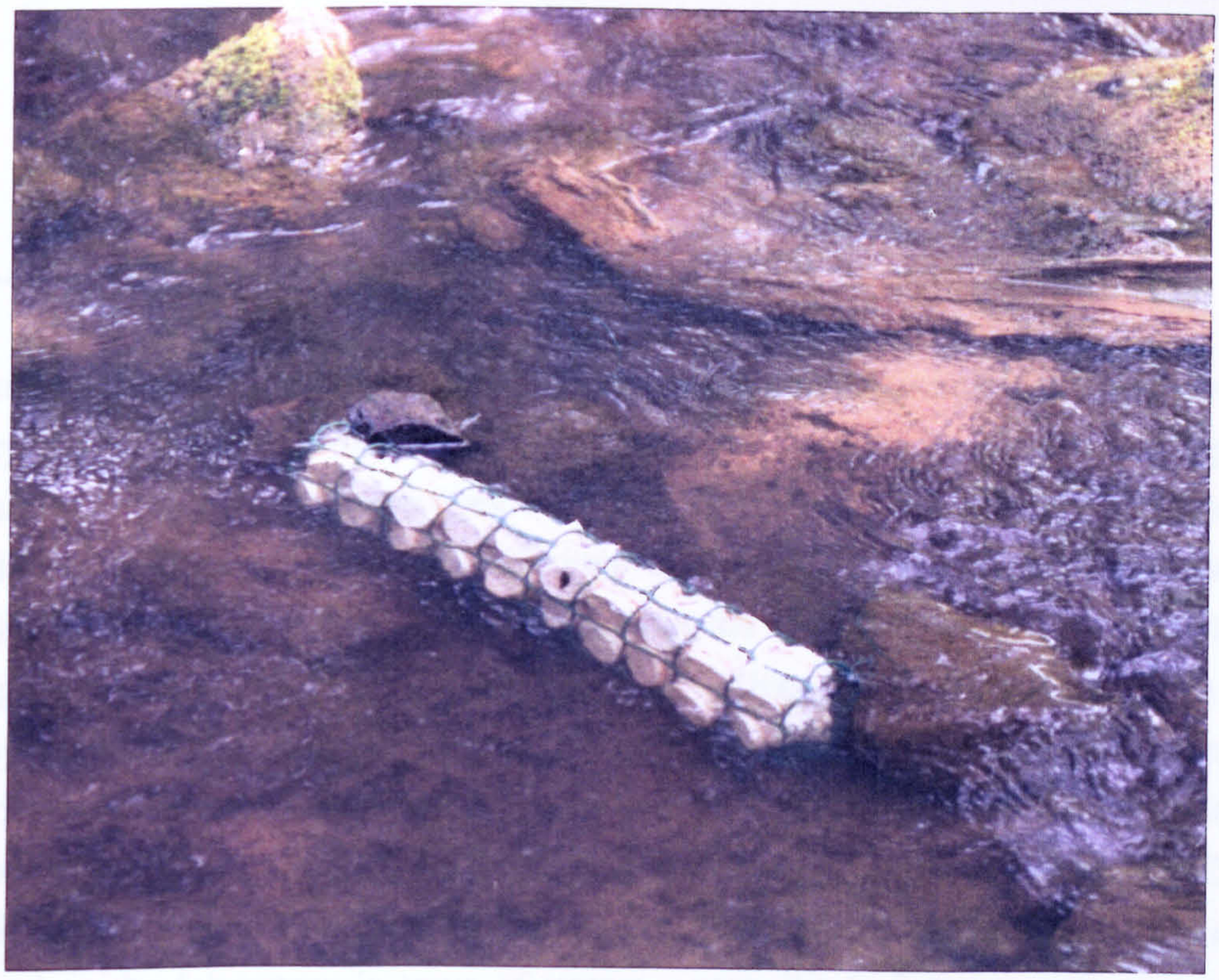


Plate 5.1. Chambers deployed in the field to assess *in-situ* consumption of leaf material by *G. Pulex.*

Plate 5.2. Two-chambered pot used for measuring consumption and faecal production by *G. pulex* feeding on leaf material.

5.2.6. Effects on food quality; I. stream water.

To assess whether stream water contaminated with motorway runoff affected the food quality of *Cladosporium*- or naturally-inoculated leaf material, leaf discs were deployed at upstream and downstream stations, returned to laboratory and consumption assessed. A ration of five pre-weighed *Cladosporium*- or naturally-inoculated leaf discs (section 5.2.1) were placed in chambers and deployed at the upstream and downstream station at Pigeon Bridge Brook (section 5.2.2). Thirty-five chambers per treatment were deployed at each station. In addition, six chambers per treatment containing leaf material only were deployed; three sets of leaf discs were analysed for total aromatic hydrocarbons (section 3.2.8.) and three sets were analysed for metals (Zn, Cu, Cd, Cr, and Pb section 3.2.8.). After 6 d, chambers were recovered from the stream and returned to the laboratory in trays of stream water, the leaf discs were washed with distilled water and placed in their individual rations in numbered cells of cell trays. This material was then used to assess consumption as described in section 5.2.5. Thirty replicates with animals and five control replicates without animals were used for each treatment (station x food type). Consumption, faecal production, absorption and absorption efficiencies were calculated using the equations in section 5.2.11.

5.2.7. Effects on food quality; II. stream sediment.

Since most of the runoff pollutant load resides in the sediments (Chapter 2) experiments were designed to assess whether the proximity of the leaf material to the sediments effects food quality and therefore consumption by *G. pulex* in the laboratory. *Cladosporium*- and naturally-inoculated leaf discs were deployed in a similar manner to that described in section 5.2.6., however the position of deployment differed. Half of the chambers at each station were directly in contact with the stream bed sediments and half were suspended in the water column by attaching polystyrene floats to the cages retaining the chambers. For each leaf type and deployment station, thirty chambers containing pre-weighed leaf discs were deployed on the bed sediments and thirty in the stream water (section 3.2.2). Six additional chambers per treatment were also deployed: three sets of leaf discs were analysed for total aromatic hydrocarbons (section 3.2.8.) and three sets analysed for metals (Zn, Cu, Cd, Cr, and Pb; section 3.2.8.). Chambers were recovered after 6 d and treated as described in section 5.2.6. Consumption, faecal production, absorption and absorption efficiencies were determined using the methods described in sections 5.2.5. and 5.2.11. For each treatment (station x leaf type) there were 25 replicates with animals and 5 control replicates without animals.

5.2.8. Effects on food quality: III. *in-situ* conditioning.

Leaf material was incubated in the stream to assess whether food quality due to changes in conditioning and/or pollutant accumulation had any affect on its consumption by *G. pulex* in the laboratory. Twenty leaf bags were prepared and deployed at the upstream and downstream station at Pigeon Bridge Brook as described in section 2.2.4. Leaf material was removed after 13, 27, 41 and 55 d deployment, washed in distilled water and leaf discs cut with a 10-mm diameter cork borer. However, decomposition rates of leaf material deployed at the upstream station were such that sufficient leaf discs could not be cut from this material after 41 d. Consequently, in subsequent consumption experiments (section 5.2.5), although there were 30 replicates per deployment station for leaves deployed for 13 and 27 d, there were only 10 upstream replicates and 30 downstream replicates for leaves deployed for 41 d. Triplicate samples (approx. 1 g) of leaf material from each station and deployment period (except 55 d) were analysed for metals (Ca, Mg, Fe Al, Cu, Cd, Cr, Pb, Ni and Zn) and total aromatic hydrocarbons (section 3.2.8.).

5.2.9. Effects on consumption: I. stream sediments.

This experiment was designed to assess whether contact with sediments or stream water reduced consumption of leaf material by *G. pulex*. Sediments were collected from the upstream and downstream station at Pigeon Bridge Brook and processed as described in section 3.2.4. Fifty grams of either upstream or downstream sediment were placed in experimental chambers (section 5.2.5) and 150 ml of water from the appropriate field station was poured carefully on top of the sediment. There were three treatments for each sediment type: animals were either in direct contact with the sediment, maintained immediately (1 mm) above or maintained 40 mm above the sediment (Plate 5.3). There were 25 replicates per treatment each containing one adult male *G. pulex* and pre-weighed *Cladosporium*-inoculated leaf discs. In addition there were eleven control pots per treatment containing leaf material only. Leaf material in five control pots was used to control for autogenic changes in the weight of leaf material, three sets of leaf discs were analysed for total aromatic hydrocarbons (section 3.2.8) and three sets were analysed for metals (Zn, Cu, Cd, Cr, and Pb; section 3.2.8). Triplicate samples (approx. 3 g) of each sediment type were also analysed for the same metals and total aromatic hydrocarbons. Consumption of leaf discs was assessed over 6 d, after which time animals and leaf discs were removed and treated according to the methods in section 5.2.5. Consumption of leaf discs was calculated using the equations in section 5.2.11.

5.2.10. Effects on consumption: II. sediment extracts.

These experiments were designed to quantify the feeding response of *G. pulex* when exposed to sediment extracts in an attempt to identify the major pollutant classes causing reduced feeding activity. Solvent (DCM) and acid (acetic acid) sediment extracts were prepared from field sediment collected from the upstream and downstream stations at Pigeon Bridge Brook according to the methods in section 3.2.6. and section 3.2.7. For the DCM extract, stock solutions (0.4 ml extract/L) were used to prepare a 0.16 ml extract/L upstream solution and 0.0001, 0.0005, 0.001, 0.005, 0.01, 0.05, 0.1 and 0.16 ml extract/ L downstream test solutions. An acetone test solution (0.16 ml acetone/ L) was used as a control. For the sediment acid extract exposures, upstream and downstream sediment extract stock solutions (10 ml extract/ L) were used to prepare an upstream 10 ml extract/L and 1, 2, 4, 6, and 10 ml extract/L downstream test solutions. An acid-spiked test solution (0.05 ml/ L) was used as a control. Pre-weighed *Cladosporium*-inoculated leaf discs were spiked with the sediment extracts by soaking them in the highest test concentration (0.16 ml solvent-extract /L or 10 ml acid-extract /L) for 6 d; changing the solution daily. The discs were then soaked for a further 2 d in the respective experimental test concentrations. This procedure was employed to ensure that the leaf material was saturated with contaminants to avoid the effects of differential leaf quality on consumption, and to leach any excess toxicant from the leaf discs before use in the feeding experiment. Consumption of leaf discs was assessed over a 6 d period according to the methods described in section 5.2.5. There were 28 experimental and five control chambers per treatment and test solutions were changed every 2 d. Consumption, faecal production, absorption and absorption efficiencies were calculated using the equations in section 5.2.11. Three replicate samples of each type of leaf material and test solution were analysed for total aromatic hydrocarbons and metals (Zn, Cu, Cd, Cr, and Pb) at the start of the experiment according to the methods in section 3.2.8.

Plate 5.3. Experimental design to assess the effects of the proximity *G. pulex* and food to the sediment on feeding (section 5.2.9).

5.2.11. Calculation of energy budget components.

Consumption (C) is expressed as energy consumed in joules per mg of animal per day and is calculated from equation 5.1.

$$C = \frac{\left[\left((L_b \times C_L) - L_a\right) \times E_L\right]}{W \times T} \qquad \text{Eqn. 5.1.}$$

where L_b and L_a refer to the initial and final dry weights respectively (mg) of the leaf discs fed to animals, T is the duration of the experiment (i.e. 6 d), W is the dry weight (mg) of the animal and E_L is the joule equivalent of alder leaf material determined by bomb calorimetry (21.552 J/mg, Naylor *et al.*, 1989). C_L is the correction factor for weight loss due to leaching of soluble organics from leaf discs and is calculated from equation 5.2.

$$C_L = \frac{\sum_{1}^{N} (Lc_a / Lc_b)}{N} \qquad \text{Eqn. 5.2.}$$

where N is the number of control replicates and Lc_b and Lc_a are the initial and final dry weights (mg) of the control leaf discs respectively.

The amount of energy lost as faeces (F) in joules per mg of animal per day is calculated from equation 5.3.

$$F = \frac{\left[\left(F_a - (F_b \times C_f)\right) \times E_f\right]}{W \times T} \qquad \text{Eqn. 5.3.}$$

where F_b and F_a refer to the initial and final dry weights respectively (mg) of the filter paper and the final dry weight (mg) of the filter paper plus faeces and E_f is the joule equivalent of *G. pulex* faeces determined by bomb calorimetry (18.737 J/mg, Naylor *et al.*, 1989). C_f is the correction factor for change in weight of control filters during the experimental procedure calculated using equation 5.4.

$$C_f = \frac{\sum_{1}^{N} (Fc_a / Fc_b)}{N} \qquad \text{Eqn. 5.4.}$$

where Fc_b and Fc_a are the initial and final dry weights respectively (mg) of the control filters.

The amount of energy absorbed (A) in joules per mg of animal per day is calculated from the difference between C and F and the absorption efficiency AE expressed as a percentage is calculated from equation 5.5.

$$AE = \frac{A}{C} \times 100 \qquad \text{Eqn. 5.5.}$$

5.2.12. General methods: leaf choice.

In order to assess whether exposure of leaf material at the downstream station affects the attractiveness of leaf material as a food source to *G. pulex*, leaf choice experiments were performed in the laboratory.

Pairs (1 pair = 1 replicate) of either pre-weighed *Cladosporium*-inoculated (section 5.2.1.b.) or naturally-inoculated (section 5.2.1.c.) leaf discs were colour coded using coloured map pins and treated as described in the experiments below (sections 5.2.13. - 5.2.15.). The choice experiment consisted of leaf disc pairs from each treatment (i.e. station and/or leaf type and/or spiking solution) being placed in numbered glass choice arenas (diam. 10 cm x 8 cm deep) containing 400 ml of APW. The combination of arena number and pin colour identified particular leaf disc treatment replicates. Groups of five adult male *G. pulex* were placed in half of the arenas, the remaining half being left as controls for non-animal weight loss. Equal numbers of test and control replicates were a requirement of the statistical test method applied to these data (section 5.2.17.). Animals were left to feed on the leaf discs for 24 h and all experiments were conducted in a constant temperature room at 15°C (± 2°C) with a 12 h dark/ 12 h light photoperiod. After the 24-h test period, animals were removed and placed in their groups of five in numbered cell trays. The leaf disc replicates were recovered, map pins removed and leaf discs placed in numbered cell trays. Naturally-inoculated leaf discs were blotted on tissue paper and wet-weighed, dried at 60°C for 48 h and dry-weighed. Wet weight-dry weight relationships for each treatment were obtained by regression and used to calculate dry weights. *Cladosporium*-inoculated material and test animals were dried at 60°C for 48 h and dry-weighed. Consumption (C_c) of each leaf treatment over the 24 h period was calculated using equation 5.6.

$$C_c = \frac{[((L_b \times C_L) - L_a)]}{W_c} \qquad \text{Eqn. 5.6.}$$

where L_b and L_a refer to the initial and final dry weights respectively (mg) of the leaf discs fed to animals, and W_C is the dry weight (mg) of *G. pulex* (sum of 5 animals) in each test arena. C_L is the correction factor for weight loss due to leaching of soluble organics from leaf discs and is calculated from equation 5.2.

Mean (+1 S.E.) data calculated from these equations were used to plot histograms of consumption of the different treatments by *G. pulex*. Statistical analyses of choice data required weight loss of leaf discs (mg dry wt.) in the test arenas (WT_a, equation 5.7) and control arenas (WT_C, equation 5.8) to be calculated (Roa, 1992). Statistical analyses were performed using the methods described in section 5.2.17.

$$WT_a = \frac{(L_b - L_a)}{W} \qquad \text{Eqn. 5.7.}$$

$$WT_c = Lc_b - Lc_a \qquad \text{Eqn. 5.8.}$$

5.2.13. Effect on leaf choice: I. stream water and sediment pre-exposure.

Pairs of pre-weighed *Cladosporium*-inoculated (section 5.2.1.b.) or naturally-inoculated (section 5.2.1.c.) leaf discs were prepared as described in section 5.2.12, placed in numbered cylindrical polythene chambers and deployed at the upstream and downstream station at Pigeon Bridge Brook as described in section 5.2.2. Fifty-two chambers were deployed at each station with half of the chambers in direct contact with the sediments while the other half were kept off the sediments by attaching polystyrene floats to the cages retaining the chambers. After 6 d, all chambers were recovered from the stream and pairs of leaf discs removed, washed in distilled water and placed individually in numbered cells of cell trays. Leaf choice experiments were then conducted as described in section 5.2.12 with each of the ten control and ten test replicate arenas having two leaf replicates of each treatment. This experiment ran in parallel with the experiment described in section 5.2.7 and metal and total aromatic hydrocarbon concentrations for leaf material are displayed in section 5.3.7.

5.2.14. Effect on leaf choice: II. *in-situ* conditioning.

Alder leaves were deployed at the upstream and downstream station at Pigeon Bridge Brook for 13, 27 and 41 d (section 2.2.4). After each deployment period the leaves were washed in distilled water and leaf discs were cut. Leaf discs from each treatment (station x time) were weighed in pairs and identified by coloured map pins. Each of ten control and ten test arenas had two leaf disc pairs per treatment (i.e. deployment station). This experiment ran in parallel with the *in-situ* leaf conditioning experiment described in section 5.2.8 and metal and total aromatic hydrocarbon concentrations for leaf material are displayed in section 5.3.8.

5.2.15. Effect on leaf choice: III. sediment extracts.

Leaf discs were spiked with either solvent (DCM) or acid (acetic) extracts of field sediments (sections 3.2.6 and 3.2.7). *Cladosporium*-inoculated leaf discs were weighed in pairs and spiked with DCM sediment extract at 0.4 ml extract/ L or acid sediment extract at 5 ml extract/ L for 6 d (section 5.2.10). Different treatment leaf disc pairs were identified with coloured map pins and the solvent and acid extracts leaf discs were tested separately. For each choice experiment, there were eight control and eight test arenas each containing two leaf pairs of each treatment (upstream or downstream extract-spiked material). Samples of solvent extract-spiked leaf material were analysed for total aromatic hydrocarbons (section 3.2.8) and acid extract-spiked leaf material was analysed for metals (Zn, Cu, Cd, Cr, and Pb; section 3.2.8).

5.2.16. Uptake route of motorway-runoff pollutants.

Contaminated leaf material is a potential source of pollutants to detritivores such as *G. pulex*. Experiments were therefore designed to assess whether contaminated leaf material or water was the major uptake route for motorway-runoff pollutants. Adult male *G. pulex* were exposed to either control water (APW, Treatments 1-4) or water spiked with a sub-lethal concentration of solvent (DCM; section 3.2.6) or acid (acetic acid; section 3.2.7) downstream sediment extracts (Treatments 5-8). Animals were exposed in groups of 15 in polystyrene containers (diam. 11 cm x 5 cm deep) each containing 250 ml of the test solution and supplied with either uncontaminated food or food that had been spiked with downstream sediment extracts. For both the solvent and acid sediment extract experiments there were eight experimental treatments (Table 5.2).

Table 5.2. The design of the uptake experiments using either solvent or acid sediment extracts. Test concentrations for contaminated water were 0.05 ml extract/L for solvent sediment extract and 5 ml extract/L for acid sediment extract.

Treatment	Water	Food	Food accessibility
1	Control	None	----
2	Control	Control	Accessible
3	Control	Contaminated	Accessible
4	Control	Contaminated	Inaccessible
5	Contaminated	None	----
6	Contaminated	Control	Accessible
7	Contaminated	Contaminated	Accessible
8	Contaminated	Contaminated	Inaccessible

In Treatments 4 and 8, two-chambered pots were used to separate the animals from contaminated food so the contaminated leaf material was essentially inaccessible. The upper chamber contained the animals, the lower chamber contained the leaf material and a mesh screen separated the chambers. This design was to control for the uptake of pollutants that leach from the contaminated leaf material into the test solutions. Leaf material was prepared as follows: *Cladosporium*-inoculated alder leaf material (section 5.2.1.b) was spiked by soaking dry leaf material in a solution containing either 0.4 ml extract /L of solvent or 10 ml extract /L acid downstream sediment extract in APW for 6 d (prepared as in section 3.2.6 and 3.2.7 respectively), changing the solutions daily. Leaf material was then soaked for a further 2 d (changed each day) in either 0.05 ml extract /L solvent sediment extract or 5 ml extract/L acid sediment extract to equilibrate to test conditions and minimise pollutant leaching during the experiment. Control leaf material was prepared in the same way but was soaked in APW spiked with either acetone (0.4 ml acetone /L) or acetic acid (0.05 ml acid /L).

There were six replicates per treatment and test solutions and food were changed every second day. The experiment was run for 14 d, at the end of which animals from each replicate were combined to produce three replicates per treatment, each of 30 animals, for analysis. Three sub-samples of control and contaminated leaf material were analysed as were three samples of control and contaminated exposure solutions. Faeces were collected from Treatments 2 and 3 and also analysed. Animal, faeces, water and leaf material were analysed for either total aromatic hydrocarbons (solvent sediment extract exposed) or heavy metals (Zn, Cu, Cd, Cr, and Pb; acid extract exposed) according to the methods in section 3.2.8.

Tissue total aromatic hydrocarbon and metal concentrations were corrected for non-absorbed hydrocarbons and metals in the material within the gut. Ten animals fed *Cladosporium*-inoculated alder leaf material for 10 d were used to obtain an approximate measure of the percentage weight of the gut contents relative to the whole animal. Ten adult male *G. pulex* were wet-weighed (F_G), their guts were dissected out and their contents squeezed out and wet-weighed (F_g). Total tissue concentrations (C_T) corrected for gut contents (C_F) was defined as C_t and calculated using equation 5.9.

$$C_t = \frac{C_T - C_F\left[\frac{F_g}{F_G}\right]}{\left(1-\left[\frac{F_g}{F_G}\right]\right)} \qquad \text{Eqn. 5.9.}$$

5.2.17. Statistical analyses of data.

Survivorship was high (i.e. > 90%) in all experiments and replicates in consumption experiments in which the animals died during the 6-d experimental period were excluded from analysis. Proportional or percentage data were arcsine transformed prior to analysis and all data were checked for normality using normal probability plots and for homogeneity of variances using Bartletts test. Relationships between wet weight and dry weight of naturally-inoculated leaf material were analysed using least-squares regression techniques. These relationships were tested for homogeneity using ANCOVA or multiple-ANCOVA (coincident regression, Zar, 1984) and combined within an experiment if homogenous to give a common relationship. For *in-situ* and laboratory stream water exposures chemical, consumption, faecal production and absorption data were analysed using one-way analysis of variance and *t*-tests. For the reciprocal transfer, laboratory sediment exposed leaf material, stream-inoculated leaf material and laboratory sediment contact experiments, data were analysed using Kruskal-Wallis, Mann-Whitney and non-parametric Tukey-type multiple comparison tests (Zar, 1984). For the sediment extract tests, data were analysed using ANOVA and Tukey multiple comparison tests. Relationships between consumption and extract concentration, and consumption and total aromatic hydrocarbon concentration were analysed using least-squares regression techniques. Absorption efficiency values of <0 and >100% were treated as 0 and 100% respectively and data were arcsine transformed and analysed by ANOVA, *t*-tests and Tukey multiple comparisons. Choice consumption data were analysed using methods based on Roa (1992) from data derived from randomised pairs of treatment (WT_a) and control (WT_c) weight loss data. Uptake route

data were analysed using one-way analysis of variance and Tukey multiple-comparison tests and two-sample *t*-tests. The *q*-statistic for parametric and non-parametric Tukey comparisons, Bartletts test, and multiple-ANCOVA were calculated according to Zar (1984) and choice data were analysed using a computer-programme (Dr N. Fieller, Dept. of statistics, Sheffield University- pers. comm.) based on the method of Roa (1992). The remaining analyses were performed using the MINITAB statistical package (Minitab™ Inc., 1991) and significance levels in all cases were $p<0.05$.

5.3. RESULTS.

5.3.3. *In-situ* leaf consumption: I. upstream/downstream deployments.

Although the concentrations of most metals were elevated for leaf material deployed at the downstream station (*Cladosporium*-inoculated; Cd, Cu, Zn; naturally-inoculated Cd, Cr, Pb, Cu and Zn), this was only statistically significant for Cd, Pb and Zn concentrations in naturally-inoculated leaf material (t>3.76, df=3, Table 5.3). The concentration of total aromatic hydrocarbons for leaf material deployed at the downstream station at Pigeon Bridge Brook was approximately 3-5 times greater than similar material deployed at the upstream station (Table 5.3). However, between-station differences in the concentration of aromatic hydrocarbons were not significant for either *Cladosporium*-inoculated or naturally-inoculated leaf material (t<2.40, df=2). There was, however, a significant reduction in the consumption of both *Cladosporium*-inoculated and naturally-inoculated alder leaf material deployed at the downstream station at Pigeon Bridge Brook (t>3.70, df>39, Fig. 5.2.). There was a significant relationship when the dry weight of naturally-inoculated leaf discs was plotted against the weight wet of discs deployed at both the upstream and downstream stations (F>264.0, r>0.93, df= 1,43). The initial dry weight of naturally-inoculated leaf material was calculated using the dry weight-wet weight relationship. As these relationships varied across treatments (ANCOVA F>27.3 df=1,176) treatment specific regression equations were used (Appendix A5 Eqns. A5.1-A5.2).

Table 5.3. Metal (µg/g dry wt.) and hydrocarbon (µg/g wet wt. chrysene equivalents) concentrations of leaf material used to assess *in-situ* consumption of *Cladosporium*-inoculated and naturally-inoculated leaf material by *G. pulex* at upstream and downstream stations at Pigeon Bridge Brook. Data presented as mean values and S.E. are given in parentheses.

	Cladosporium-inoculated				Naturally-inoculated			
	Upstream		Downstream		Upstream		Downstream	
Zn	237.59	(9.15)	455.40	(82.19)	260.57	(0.11)	646.84	(77.74)
Cd	1.02	(0.03)	1.19	(0.21)	0.70	(0.11)	2.38	(0.20)
Cr	56.80	(4.18)	8.96	(1.30)	17.44	(4.81)	37.19	(11.58)
Pb	108.80	(5.22)	90.35	(13.44)	99.38	(19.81)	193.39	(15.20)
Cu	62.85	(3.27)	201.93	(116.59)	87.72	(26.27)	134.25	(8.06)
Hydrocarbons	10.48	(2.32)	31.54	(8.91)	10.6	(4.43)	57.85	(19.18)

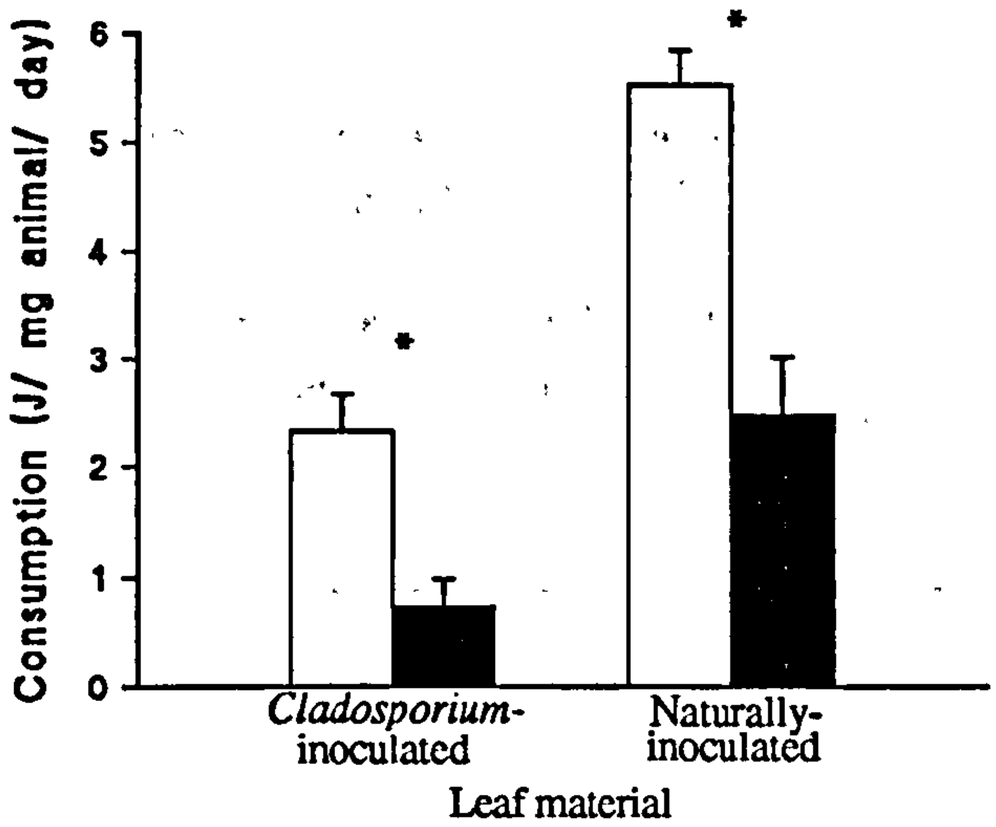


Fig. 5.2. The mean (+ 1 S.E.) consumption of *Cladosporium*-inoculated and naturally-inoculated leaf material deployed at the upstream (□) and downstream (■) station at Pigeon Bridge Brook. Asterisk denote a significant between-station difference.

5.3.4. *In-situ* leaf consumption: II. reciprocal transfer experiments.

Generally, metal concentrations were highest on leaf material that was first pre-exposed at, and then returned to, the downstream station (Table 5.4). There were no significant differences in the aromatic hydrocarbon concentrations of leaf material that had either been deployed at the upstream or downstream station at Pigeon Bridge Brook for 12 d (Up⇒Up and Dw⇒Dw respectively) or deployed at one station for 6 d and then transferred to the alternative station for a further 6 d (Up⇒Dw and Dw⇒Up; $F<3.75$, df=2,8). However, there was a significant reduction in the consumption of both *Cladosporium*-inoculated and naturally-inoculated leaf material at the downstream station irrespective of the station at which the material was incubated for the first 6 d ($q>3.78$, df>∞;4, Fig. 5.3.). In fact, pre-exposure had no significant effect on consumption ($q<0.98$, df>∞;4). The initial dry weight of naturally-inoculated leaf material was calculated using dry weight-wet weight relationships. As these relationships varied across treatments (Multiple ANCOVA, $F=3.68$ df=6,72) treatment specific regression equations were used (Appendix A5 Eqns. A5.3-A5.6).

Table 5.4.: Metal (μg/g dry wt) and total aromatic hydrocarbon (A.H.; μg chrysene equivalents/g wet wt.) concentrations of leaf material used to assess *in-situ* consumption of *Cladosporium*- and naturally-inoculated leaf material by *G. pulex* in reciprocal transfer experiments. The arrow indicates the direction of transfer from the initial site of pre-exposure to the site at which consumption was assessed (Up= upstream; Dw= downstream). Data are presented as mean values and 1 S.E. are given in parentheses. For each chemical determinand, treatments not sharing a common lower case letter were significantly different (q>4.66, df=8,4).

	Zn	Cd	Cr	Pb	Cu	A.H.
Cladosporium						
Up⇒Up	289.72 b (14.18)	0.84 a (0.42)	4.47 c (1.14)	63.92 a (9.44)	72.57 abc (6.29)	16.55 a (2.08)
Dw⇒Up	373.19 ab (23.94)	1.59 a (0.19)	21.28 ab (1.56)	43.97 a (2.36)	58.95 c (9.74)	39.80 a (4.99)
Dw⇒Dw	330.61 b (54.64)	4.79 a (1.80)	33.81 a (6.26)	78.75 a (27.18)	121.67 ab (14.67)	86.14 a (30.56)
Up⇒Dw	528.06 a (51.59)	3.14 a (0.73)	9.5 bc (3.26)	127.07 a (37.76)	131.61 a (19.34)	66.81 a (32.89)
Natural						
Up⇒Up	230.70 b (4.39)	0.87 b (0.12)	2.19 a (0.52)	64.80 b (16.05)	32.36 b (3.23)	21.35 a (8.13)
Dw⇒Up	289.16 ab (33.54)	1.24 b (0.21)	10.39 a (9.79)	61.66 b (10.83)	59.80 ab (6.08)	31.44 a (3.67)
Dw⇒Dw	465.49 a (55.69)	5.88 a (1.07)	68.94 a (61.59)	181.11 a (16.10)	174.70 a (52.18)	147.58 a (55.01)
Up⇒Dw	331.28 ab (57.60)	1.88 b (0.63)	48.60 a (10.14)	70.96 b (21.74)	100.32 ab (7.03)	100.63 a (26.39)

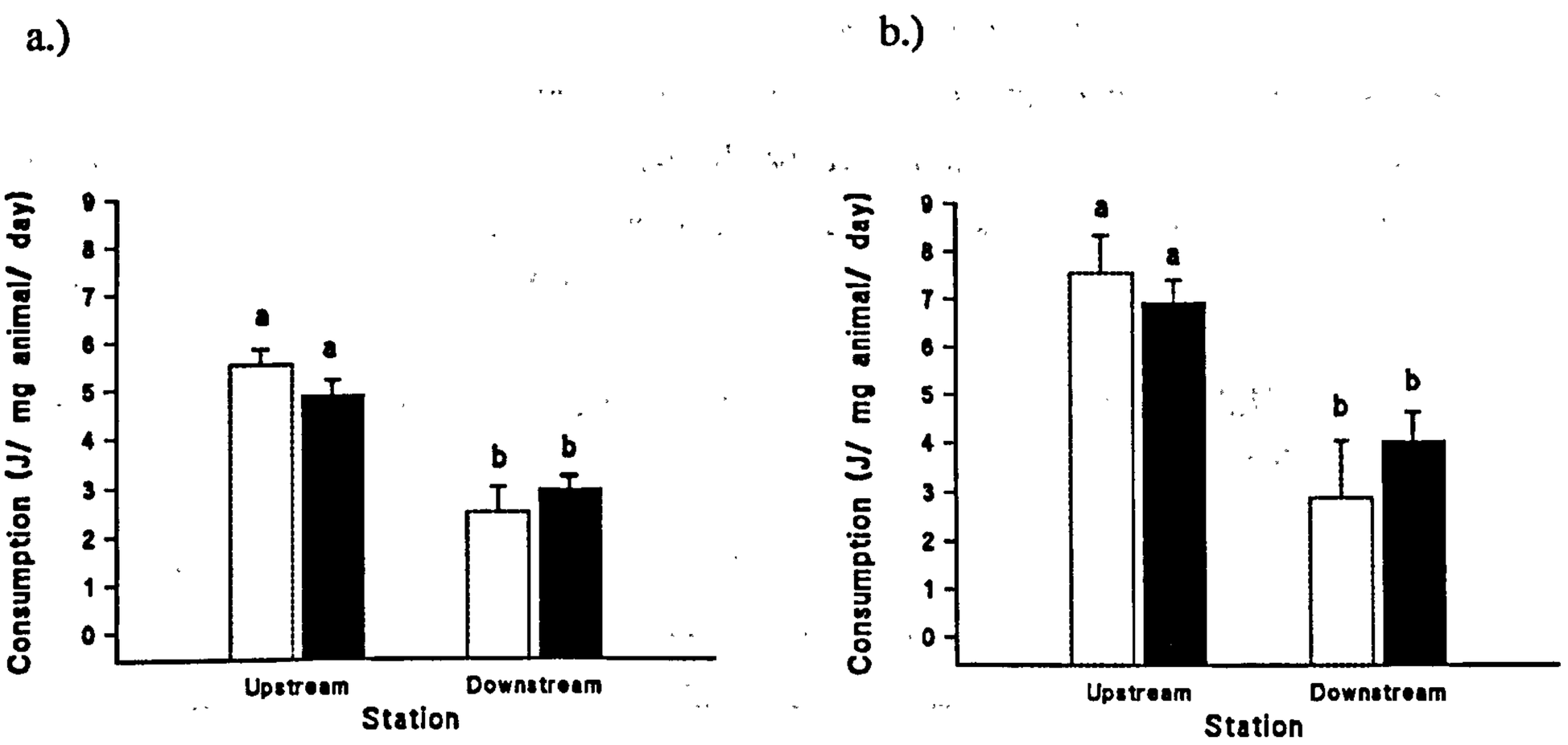


Fig. 5.3. The mean (+1 S.E.) consumption by *G. pulex* of a) *Cladosporium*-inoculated or b) naturally-inoculated leaf material previously deployed for six days at either the upstream (□) or downstream (■) station at Pigeon Bridge Brook. The labels on the X-axis refer to the station at which consumption was assessed. Within a graph, bars sharing the same letter code were not significantly different.

5.3.6. Effects on food quality: I. stream water.

Leaf discs were deployed at the upstream and downstream station at Pigeon Bridge Brook for 6 d before being used in laboratory consumption experiments. All metals on both *Cladosporium*- and naturally-inoculated leaf material were elevated on downstream deployed material compared to the upstream material although this was only significant for Cd on naturally-inoculated leaf material (t=4.83, df=2, Table 5.5). Both *Cladosporium*-inoculated and naturally-inoculated leaf material exposed at the downstream station had significantly higher concentrations of aromatic hydrocarbons than material exposed at the upstream station (t>8.61, df=2, Table 5.5). However, there was no significant difference in the consumption of leaf material deployed at upstream and downstream stations for 6 d and then fed to animals in the laboratory (F<0.86, df>1,58, Fig. 5.4). Moreover, there was no significant effect of deployment station on either the faecal production or amount of energy absorbed by *G. pulex* feeding on *Cladosporium*-inoculated leaf material (F<2.39, df=1,58). In contrast, with naturally-inoculated material, faecal production was significantly lower (F=10.17, df=1,58) for animals fed downstream deployed material resulting in higher absorption (F=4.08, df=1,58). The absorption efficiency of *Cladosporium*-inoculated and naturally-inoculated leaf material deployed at the downstream station was significantly higher than that deployed at the upstream station (t>2.11, df=54, Fig. 5.4.). Further, the absorption efficiency of *Cladosporium*-inoculated leaf material was greater than that of

naturally-inoculated from both upstream and downstream stations (q>1.43, df=116,4). The initial dry weight of naturally-inoculated leaf material was calculated using dry weight-wet weight relationships. As these relationships varied across treatments (ANCOVA F>4.74, df=1,116) treatment specific regression equations were used (Appendix A5: Eqns. A5.7-A5.8).

Table 5.5.: Metal (µg/g dry weight) and total aromatic hydrocarbon (A.H.; µg chrysene equivalents/g wet wt.) concentrations of leaf material used to assess consumption by *G. pulex* of *Cladosporium*- and naturally-inoculated leaf material previously deployed at upstream and downstream stations at Pigeon Bridge Brook. Data presented as mean and 1 S.E. given in parentheses.

Treatment	Zn	Cd	Cr	Pb	Cu	A.H.
Cladosporium						
Upstream	327.69 (10.88)	4.29 (1.00)	6.20 (1.43)	58.91 (16.59)	129.63 (17.21)	2.67 (1.19)
Downstream	473.52 (39.84)	6.67 (2.80)	21.56 (11.15)	22.03 (56.92)	140.36 (29.94)	125.49 (14.22)
Natural						
Upstream	297.05 (38.89)	0.36 (0.03)	3.89 (1.26)	74.08 (15.84)	97.32 (18.07)	9.91 (2.93)
Downstream	627.90 (97.09)	4.31 (0.82)	14.89 (4.73)	178.86 (48.59)	175.24 (50.5)	142.07 (10.94)

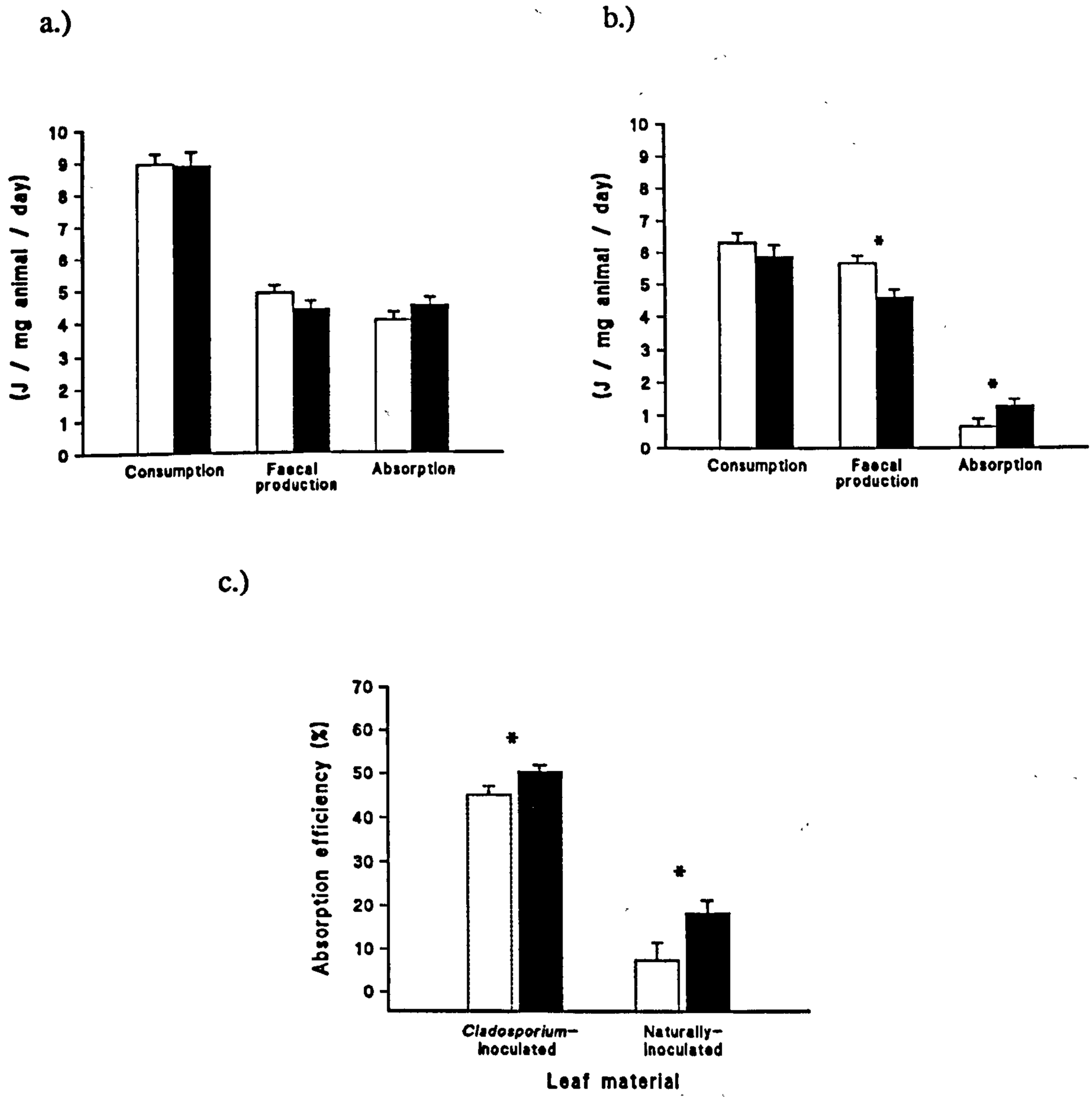


Fig. 5.4. Consumption, faecal production and absorption of leaf material by *G. pulex* feeding on a.) *Cladosporium*- or b.) naturally-inoculated leaf material and c) absorption efficiency of leaf material deployed at the upstream (□) and downstream (■) station at Pigeon Bridge Brook for six days prior to use in laboratory feeding experiments. Data presented as mean + 1 S.E.. Asterisks denote significant between-station differences.

5.3.7. Effects on food quality: II. stream sediment.

In order to assess whether the proximity of leaf material to the sediment had any affect on food quality, leaf material was placed either in direct contact with the sediment or suspended in the water column for 6 d before being used in consumption experiments.

The concentration of Cr in naturally-inoculated leaf material was significantly higher in downstream sediment exposed material than the upstream and downstream water and upstream sediment treatments (q>11.37, df=8,4, Table 5.6). There was no significant between-treatment difference in the concentrations of any of the other metals (F<3.7, df>3,8). *Cladosporium*-inoculated leaf material suspended in the water column at the downstream station had significantly higher concentrations of aromatic hydrocarbons than material exposed to upstream water or upstream sediment (t>4.62, df=2; Table 5.6.). Naturally-inoculated leaf material exposed to downstream sediment had significantly higher concentrations of aromatic hydrocarbons than material exposed to upstream water (t=6.19, df=3; Table 5.6).

There was no significant between or within-station difference in consumption or faecal production by *G. pulex* feeding on *Cladosporium*-inoculated leaf material (H<3.7, df=3, Fig. 5.5.). There was, however, significantly higher absorption of leaf material by *G. pulex* pre-exposed in the water column or sediment at the downstream station compared to pre-exposed upstream material (q>3.65, df=∞,4 Fig. 5.5). Within-station differences in absorption were not significant (q<0.18, df=∞,4). The efficiency with which *Cladosporium*-inoculated leaf material deployed in the water column and on the sediment at the downstream station was absorbed was significantly higher than that of leaf material deployed at the upstream station (q>5.07, df=95,3). There were no within-station differences in absorption efficiency of *Cladosporium*-inoculated leaf material (q<1.65, df=95,3).

Naturally-inoculated material deployed in the water column at the downstream station was consumed more than material deployed in upstream water and upstream sediment treatments but not the downstream sediment treatment (q>4.61, df=∞,4 Fig. 5.5.). There was no significant between or within-station difference in faecal production by *G. pulex* feeding on naturally-inoculated leaf material (H=1.21, df=3). There was, however, significantly higher absorption of material deployed in the water column at the downstream station than all other treatments (q>1.55, df=∞,4, Fig. 5.5.). Further, the absorption efficiency of naturally-inoculated leaf material deployed in the water column at the downstream station was significantly greater than that deployed in the water column at the upstream station (q=1.54, df=∞,4). There was no significant within-station difference in absorption efficiency of naturally-inoculated leaf material by *G. pulex* (q<2.05, df=96,3, Fig. 5.5.).

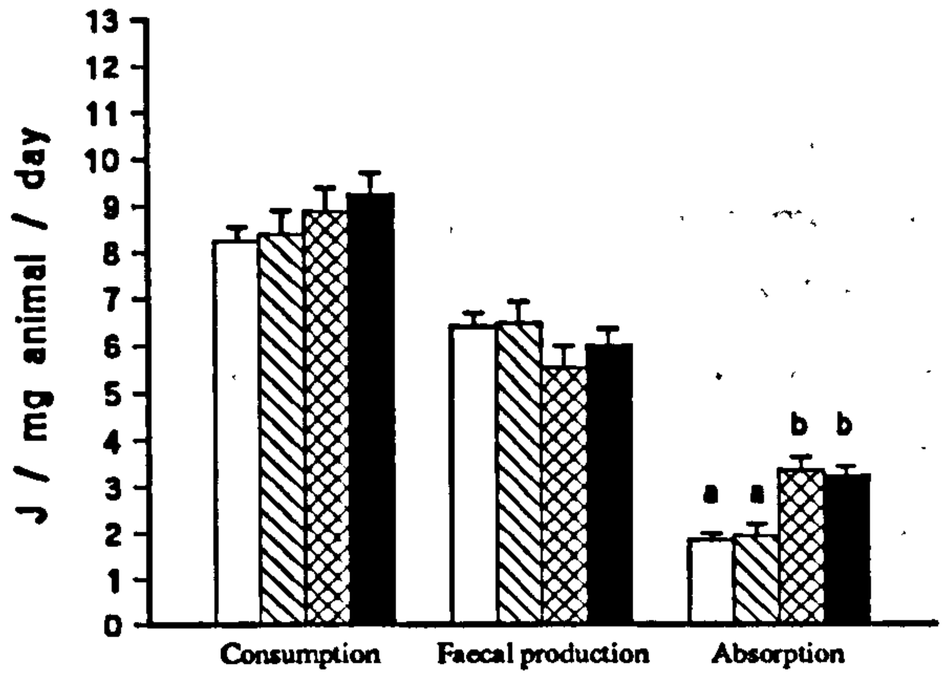
There was a significant relationship between the dry weight and weight wet of the naturally conditioned leaf deployed at both the upstream and downstream station

(r>0.92, df=28). However, these relationships were not homogenous (multiple ANCOVA F=3.359, df=3,112) and therefore treatment specific regressions were used (Appendix A5: Eqns. A5.9-A5.12).

Table 5.6.: Metal (µg/g dry wt.) and total aromatic hydrocarbon (A.H. µg chrysene equivalents/g wet-wt.) concentrations of leaf material used to assess laboratory consumption by *G. pulex* of *Cladosporium*-inoculated and naturally-inoculated leaf material previously exposed to stream water or sediment at the upstream or downstream station at Pigeon Bridge Brook. Data presented as mean and 1 S.E. in parentheses.

	Cladosporium-inoculated				Naturally-inoculated			
	Water exposed		Sediment exposed		Water exposed		Sediment exposed	
	Up	Down	Up	Down	Up	Down	Up	Down
Zn	324.50 (61.6)	353.13 (59.52)	338.44 (15.02)	249.99 (75.51)	255.06 (123.67)	251.42 (72.51)	266.94 (70.08)	525.96 (19.13)
Cd	0.36 (0.06)	1.51 (0.27)	1.04 (0.52)	3.12 (1.34)	1.74 (0.83)	1.82 (0.31)	1.31 (0.17)	2.24 (0.26)
Cr	4.06 (1.13)	15.18 (1.58)	5.44 (1.79)	24.13 (5.28)	4.22 (1.52)	4.97 (2.14)	0.34 (0.09)	30.31 (3.59)
Pb	80.16 (19.79)	74.06 (11.82)	75.14 (12.36)	49.74 (10.13)	55.21 (21.34)	50.37 (22.48)	9.74 (1.16)	62.07 (2.57)
Cu	106.99 (25.99)	71.43 (2.98)	84.26 (2.63)	91.48 (23.95)	54.48 (23.30)	104.42 (35.26)	5.36 (1.26)	83.74 (14.46)
A.H.	10.67 (2.13)	113.33 (15.64)	34.74 (6.71)	159.21 (52.63)	31.98 (11.28)	73.37 (41.29)	91.92 (26.12)	139.61 (13.23)

a.)



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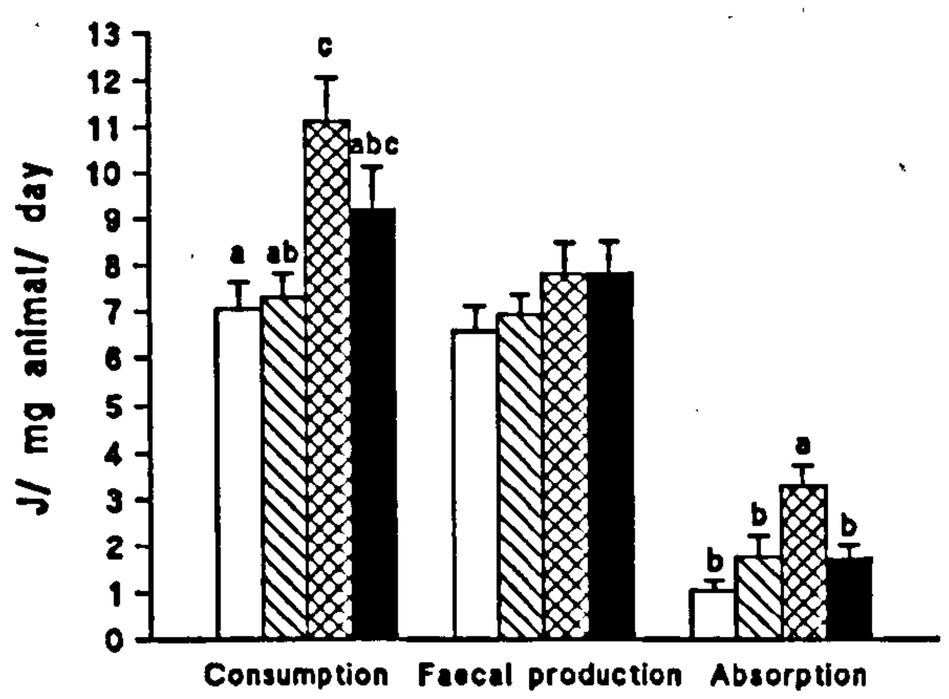

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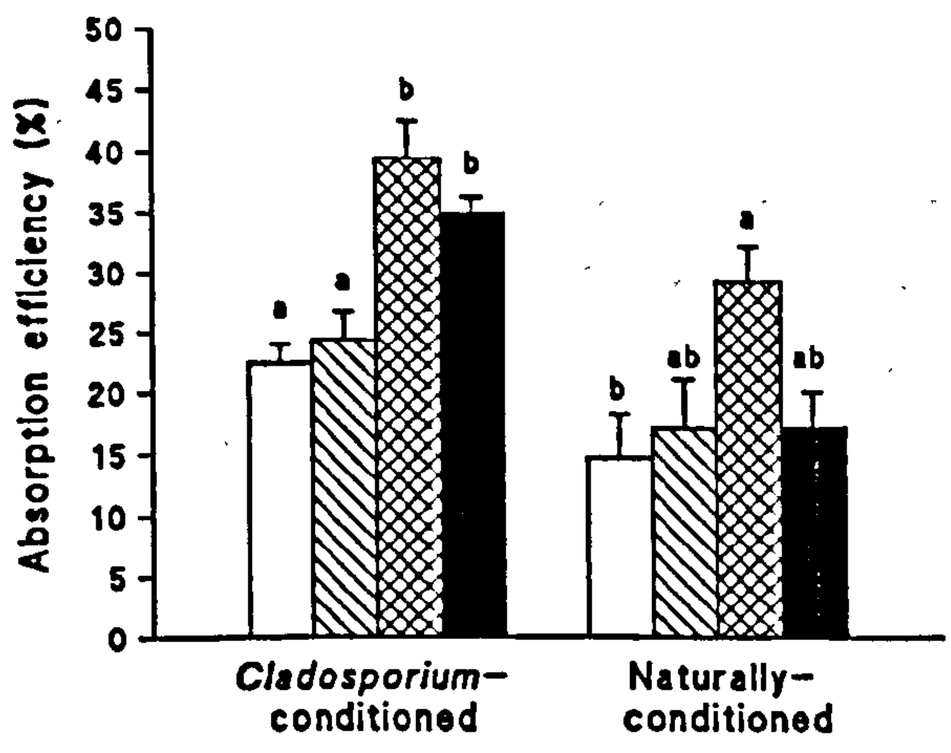


Fig. 5.5. Mean (+1 S.E.) consumption, faecal production and absorption of a.) *Cladosporium*-inoculated and b.) naturally-inoculated leaf material and c.) absorption efficiency of this material previously incubated in upstream water column (☐), upstream sediment (▨), downstream water column (☒) or downstream sediment (■) at Pigeon Bridge Brook. Within each group of four bars, those sharing the same letter code were not significantly different.

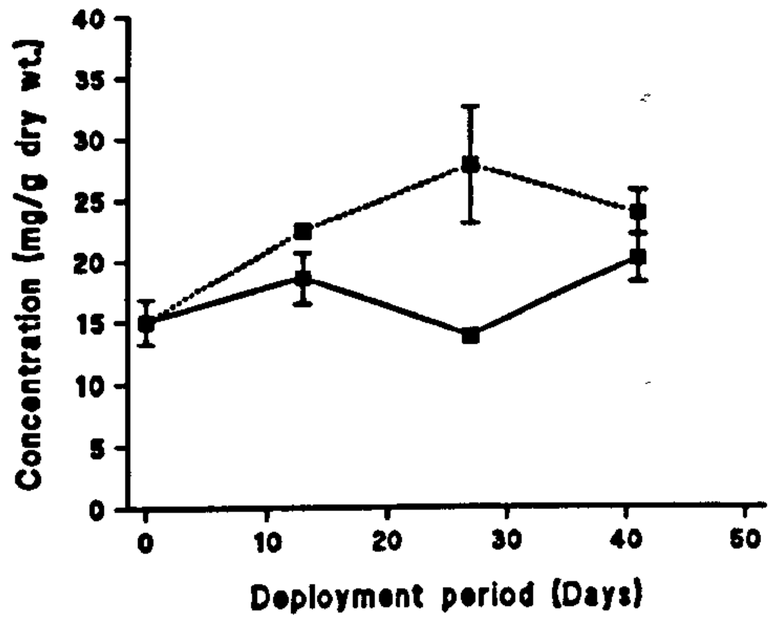
5.3.8. Effects on food quality: III. *in-situ* conditioning.

Leaf material was incubated at the upstream and downstream station at Pigeon Bridge Brook for up to 41 d and consumption, faecal production, absorption and absorption efficiencies determined after 13, 27 and 41 d deployment. After 13 d deployment, only Mg was significantly elevated on the downstream-deployed leaf material (t=3.50, df=3; Fig. 5.6). After 27 d deployment Cu, Cr, Pb and total aromatic hydrocarbons were significantly elevated on the downstream material (t>3.26, df>2; Fig. 5.6) and after 41 d deployment Cu, Cr, Pb and Zn were significantly elevated on the downstream leaf material (t>4.22, df>2; Fig 5.6.). In addition Fe was significantly elevated on the upstream material compared to the downstream material (t=5.56, df=2).

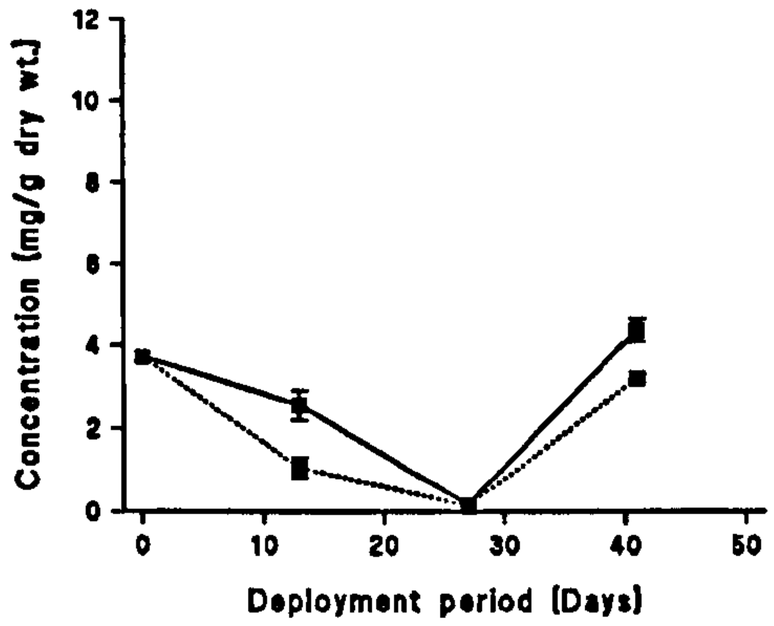
After 13 d deployment in the field, although there was no significant between-station difference in consumption (U=390, n=30,30; Fig. 5.7.), there was a significant reduction in faecal production (U=163, n=30,30) by animals fed downstream material resulting in a higher absorption of this material (U=67, n=30,30). After 27 d deployment consumption, faecal production, and absorption were all significantly reduced when the animals were fed material from the downstream station (U<347, n=30,30) but after 41 d deployment there was no significant between-station difference in consumption, faecal production or absorption (U>1181, n=30,30). Whereas the absorption efficiency of downstream-incubated leaf material was higher than upstream-incubated leaf material after 13 d (t=6.99, df=57), there was no significant between-station difference after 27 or 41 d (t<0.39, df>10).

The initial dry weight of naturally-inoculated leaf material was calculated using dry weight-wet weight relationships. As these relationships varied across treatments after 13 and 41 d (ANCOVA F>6.95, df>1,136) treatment specific regression equations were used. After 27 d, however, the relationships were homogenous (ANCOVA F<4.13, df=1,136) and therefore the data were combined to obtain a common relationship (Appendix A5: Eqns. A5.13-A5.17).

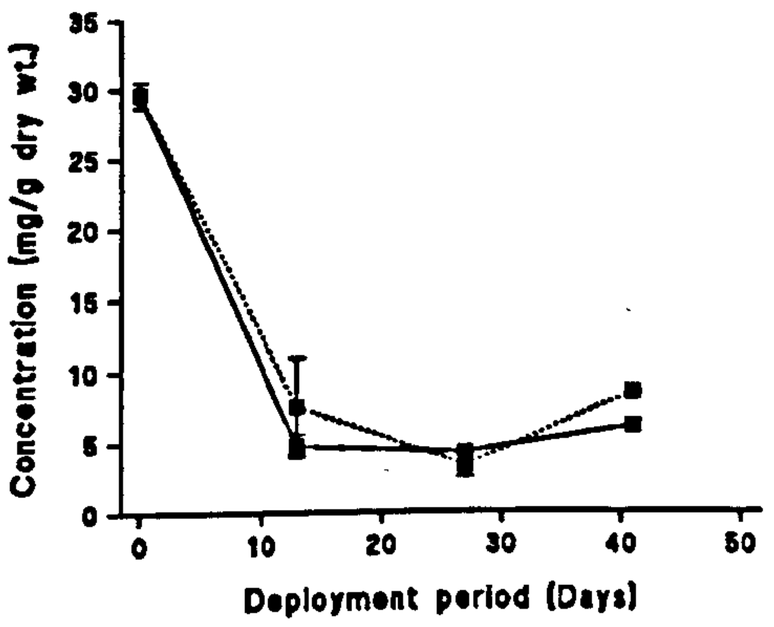
a.) Calcium



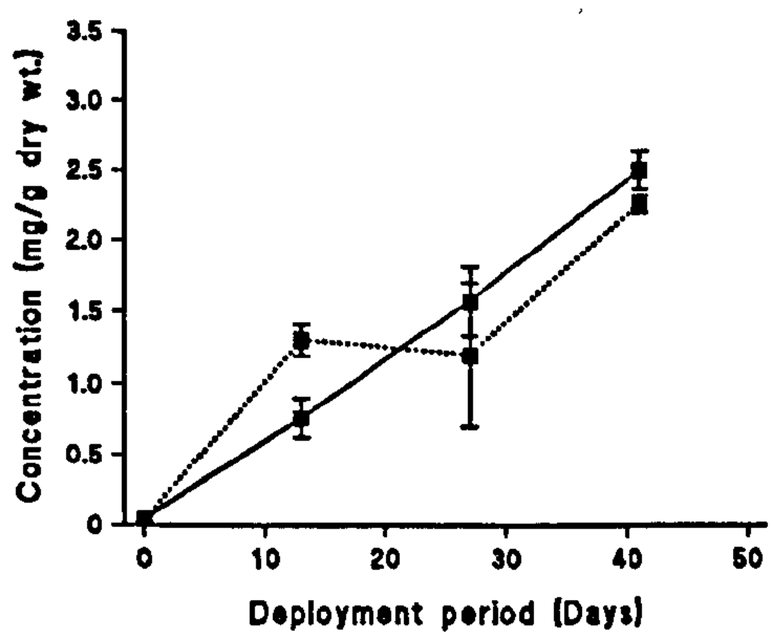
b.) Magnesium



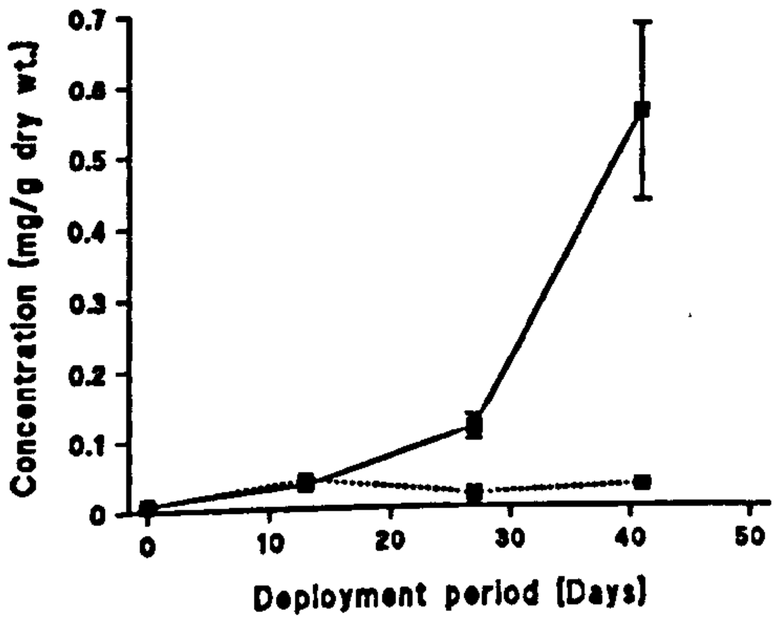
c.) Iron



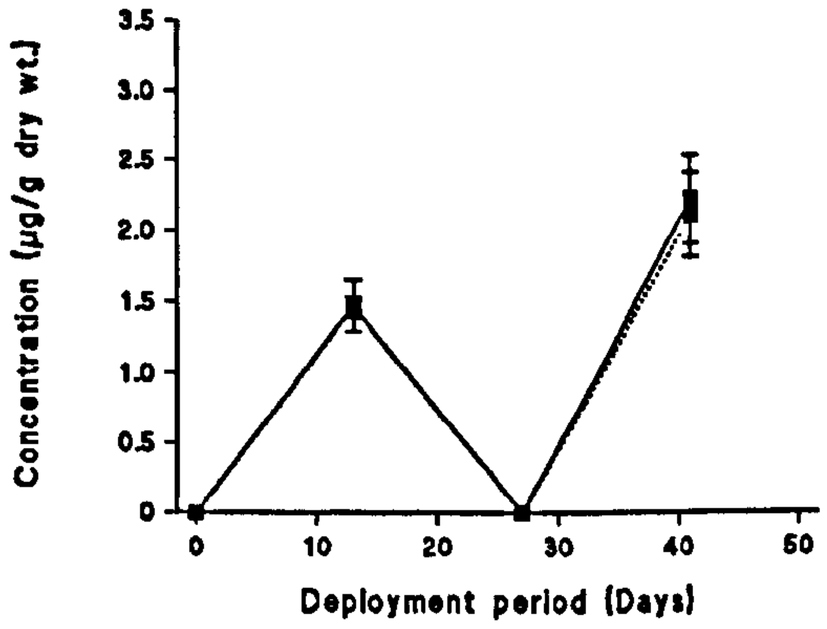
d.) Aluminium



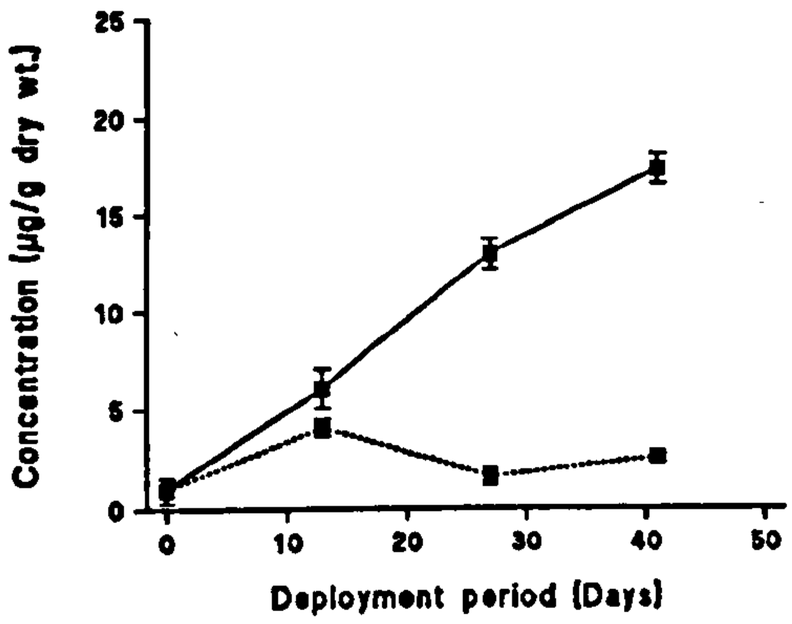
e.) Copper



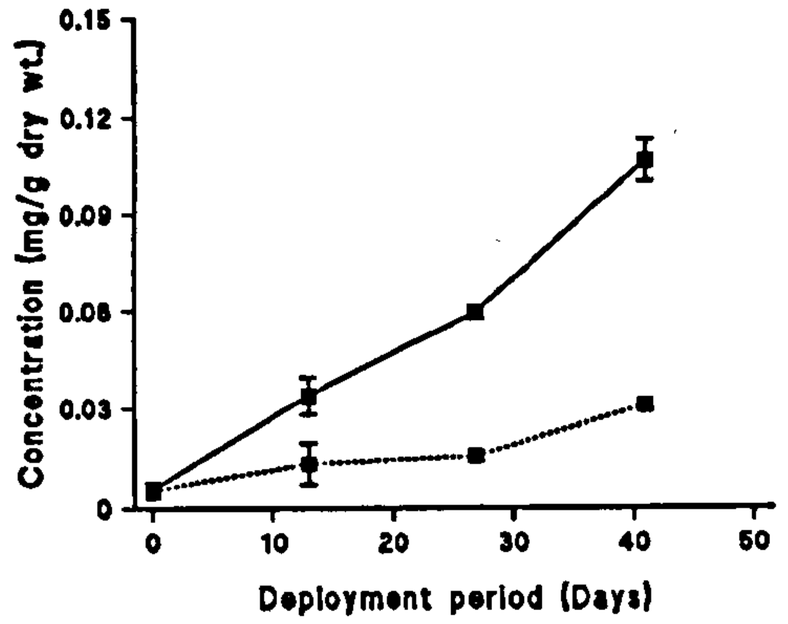
f.) Cadmium



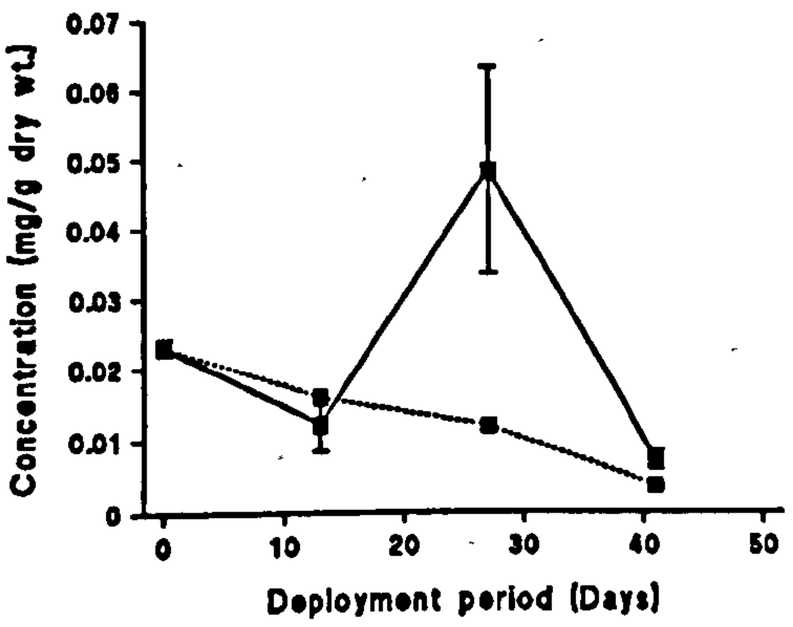
g.) Chromium



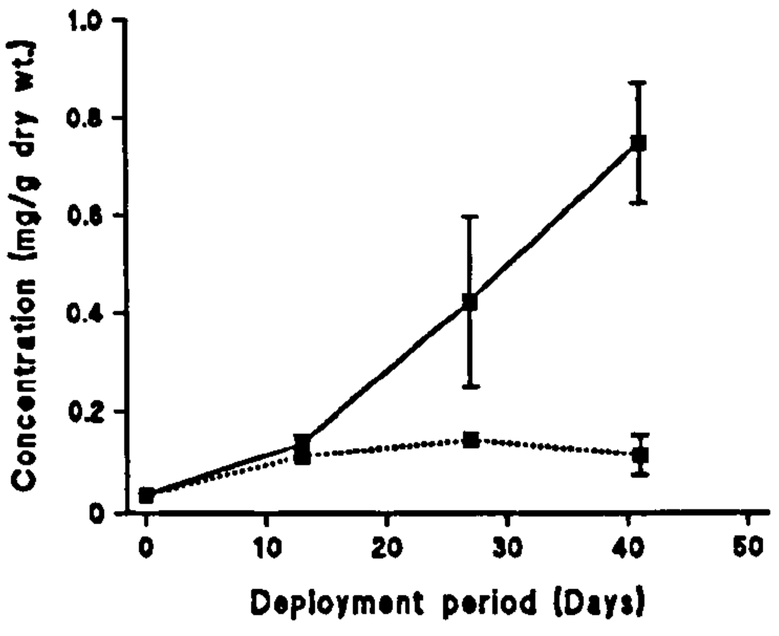
h.) Lead



i.) Nickel



j.) Zinc



k.) Total aromatic hydrocarbons

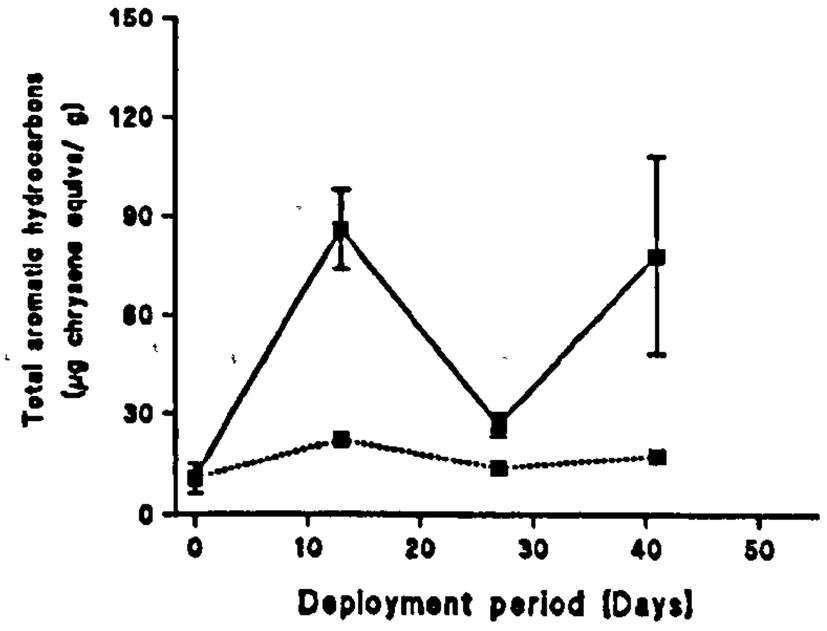


Fig. 5.6. Mean (± 1 S.E.) metal and hydrocarbon concentrations for leaf material deployed at the upstream (■-----■) or downstream (■——■) station at Pigeon Bridge Brook for up to 41 d.

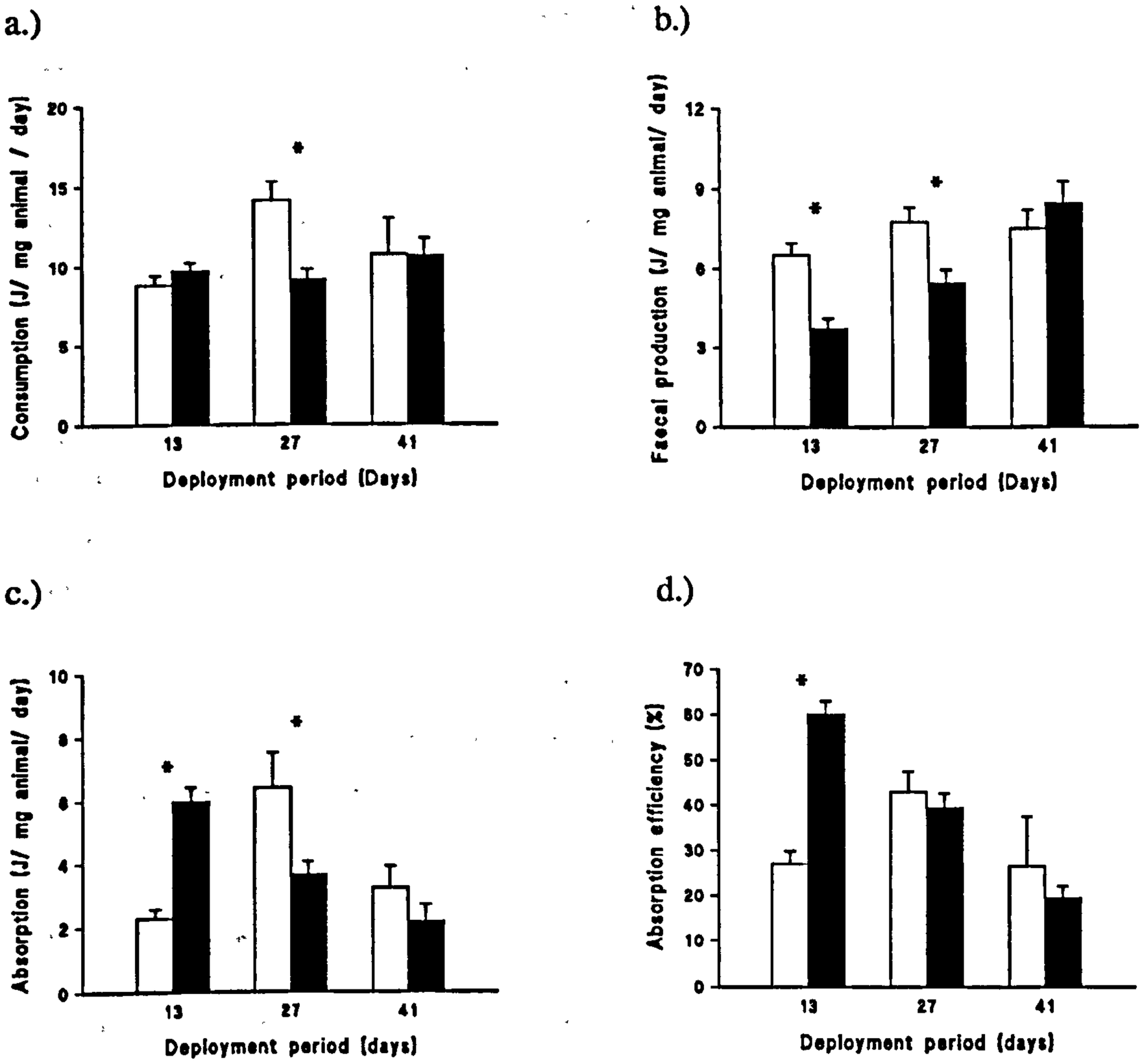


Fig. 5.7. Mean (+ 1 S.E.) a.) Consumption, b.) faecal production, c.) absorption and d.) absorption efficiency of *G. pulex* feeding on leaf material deployed either at the upstream (□) or downstream (■) station at Pigeon Bridge Brook for up to 41 d prior to use in feeding experiments. Asterisks denote significant between-station differences.

5.3.9. Effects on consumption: I. stream sediments.

The aim of this experiment was to assess whether the position of food in relation to sediments had any affect on the consumption of *Cladosporium*-inoculated leaf material by *G. pulex*. Consequently, leaf discs and animals were either in direct contact with sediments, 1 mm above sediments or 40 mm above sediments. Both upstream and downstream sediments were investigated and although total aromatic hydrocarbon concentrations were significantly elevated in the downstream sediments (t=13.68, df=2; Table 5.7.), the only significant difference in the concentration of total aromatic hydrocarbon of leaf material was that concentrations were elevated for material that was in contact with the downstream sediments relative to material isolated from the upstream sediment (t>3.87, df=3; Table 5.7.). All metals analysed (i.e. Cd, Cr, Pb, Zn

and Cu) were significantly elevated in the downstream sediment (t>5.71, df>2) and relative to all other treatments. Concentrations of Cr on leaf material that was in contact with downstream sediments was significantly greater than all other treatments (q>6.2, df=12,6, Table 5.7). Concentrations of Cu were significantly greater on material in contact with downstream sediments than leaf material that was in contact with upstream sediment or 40 mm above downstream sediment (q>5.15, df=12,6).

Animals in direct contact with downstream sediment consumed significantly less leaf material than animals in direct contact with upstream sediment (q=4.41, df=∞,6; Fig. 5.8.). However, this between-station difference was not apparent when animals were separated from the sediments (q<1.88, df=∞,6, Fig. 5.8). The position of food and animal in relation to the upstream sediment had no significant effect on consumption (q<2.01, df=∞,6). In contrast, animals in contact with downstream sediment consumed significantly less than those restrained 40 mm above the sediment (q=5.01, df=∞,6). There was no significant difference in consumption between those in contact with downstream sediment or restrained 1 mm above the sediment or those restrained 40 mm above downstream sediment and those restrained 1 mm above it (q<1.91, df=∞,6).

Table 5.7. Mean (1 S.E.) metal (µg/g dry wt.) and total aromatic hydrocarbon (µg chrysene equivalents/g wet wt.) concentrations of sediment and *Cladosporium*-inoculated leaf material used in consumption experiments. Sediment was either from the upstream station (Up) or downstream station (Down) at Pigeon Bridge Brook and leaf material was either in direct contact with the sediment or separated from it by 1 mm or 40 mm.

	Sediment		Leaf material					
			Direct Contact		1 mm above		40 mm above	
	Up	Down	Up	Down	Up	Down	Up	Down
Zn	81.37 (13.06)	389.98 (8.35)	176.62 (48.35)	319.16 (13.08)	217.67 (104.80)	193.82 (40.05)	187.37 (61.30)	207.70 (9.34)
Cd	0.29 (0.03)	1.97 (0.14)	0.72 (0.51)	1.60 (0.23)	0.64 (0.37)	0.51 (0.21)	0.28 (0.03)	1.58 (0.93)
Cr	36.95 (9.28)	120.47 (3.28)	1.93 (0.49)	22.45 (6.59)	3.23 (0.95)	5.26 (1.49)	5.06 (2.85)	4.41 (1.18)
Pb	41.91 (7.09)	276.75 (0.97)	13.87 (8.08)	64.07 (9.72)	39.46 (15.84)	22.96 (5.07)	29.45 (16.40)	29.81 (9.19)
Cu	25.25 (4.46)	91.77 (5.72)	38.33 (2.44)	97.17 (18.19)	53.13 (9.85)	54.53 (13.21)	24.10 (8.15)	44.32 (10.49)
Aromatic Hydrocarbons	33.40 (3.74)	669.35 (37.77)	82.47 (30.35)	150.92 (16.67)	43.29 (22.24)	125.34 (26.52)	37.85 (16.85)	89.58 (27.65)

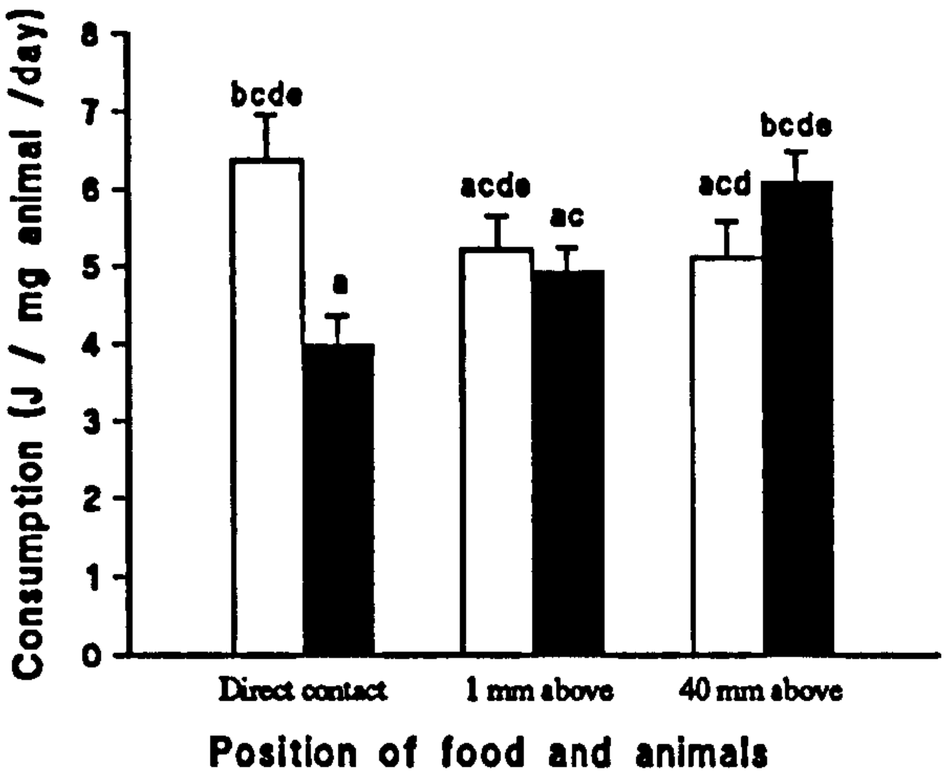


Fig. 5.8. Mean (+ 1 S.E) consumption of *Cladosporium*-inoculated leaf discs by *G. pulex* when in direct contact, 1 mm above or 40 mm above upstream (□) or downstream (■) sediment from Pigeon Bridge Brook. Bars sharing the same letter code are not significantly different.

5.3.10. Effects on consumption: II. sediment extracts.

Consumption of standard *Cladosporium*-inoculated leaf material by *G. pulex* when exposed to water and food spiked with either solvent or acid sediment extracts was assessed. The mean ± 1 S.E. concentration of total aromatic hydrocarbons of the spiked leaf material used in the solvent extract exposure was 94.14 ±11.15 (µg/g wet wt. chrysene equivalents). There was a significant relationship in the extract concentration and concentration of total aromatic hydrocarbons in spiked water (r=0.98, df=2). The mean (±1 S.E.) recorded aromatic hydrocarbon concentrations were 21.44 (by extrapolation: Eqn. 5.10.), 28.29 (±2.96), 36.28 (±4.47), 72.82 (±24.77) and 99.50 (± 10.42) for water spiked with 0.005, 0.01, 0.05, 0.1 and 0.16 ml extract/ L respectively.

Consumption and faecal production were significantly reduced relative to the control for animals exposed to water spiked with downstream sediment solvent extract (q>4.74, df=189,6, Fig. 5.9.). In contrast, only faecal production was significantly reduced for animals exposed to water spiked with upstream solvent sediment extract at 0.16 ml extract/L (q=4.04, df=189,6, Fig. 5.9.). Absorption of the leaf material was significantly reduced at a downstream extract concentration of 0.16 ml extract/L compared to acetone and upstream treatments (q>4.51, df=189,6) whereas absorption was significantly elevated in the upstream treatment relative to the control (q=4.88, df=189,6, Fig. 5.9.). Animals exposed to water spiked with downstream extract at concentrations of 0.1 and 0.16 ml extract/L and the acetone treatment absorbed significantly less leaf material than those exposed to water spiked with upstream extract

(q=4.88, df=189,6). The absorption efficiency of animals exposed to the upstream treatment and 0.005, 0.05, and 0.1 ml downstream extract/L was significantly higher than that of animals exposed to the acetone control (q>4.25, df=189,6). There was no within-downstream extract difference in the absorption efficiencies of leaf material by *G. pulex* (q<3.86, df=189,6), neither were there any differences between upstream extract or any of the downstream extracts (q<4.0, df=189,6).

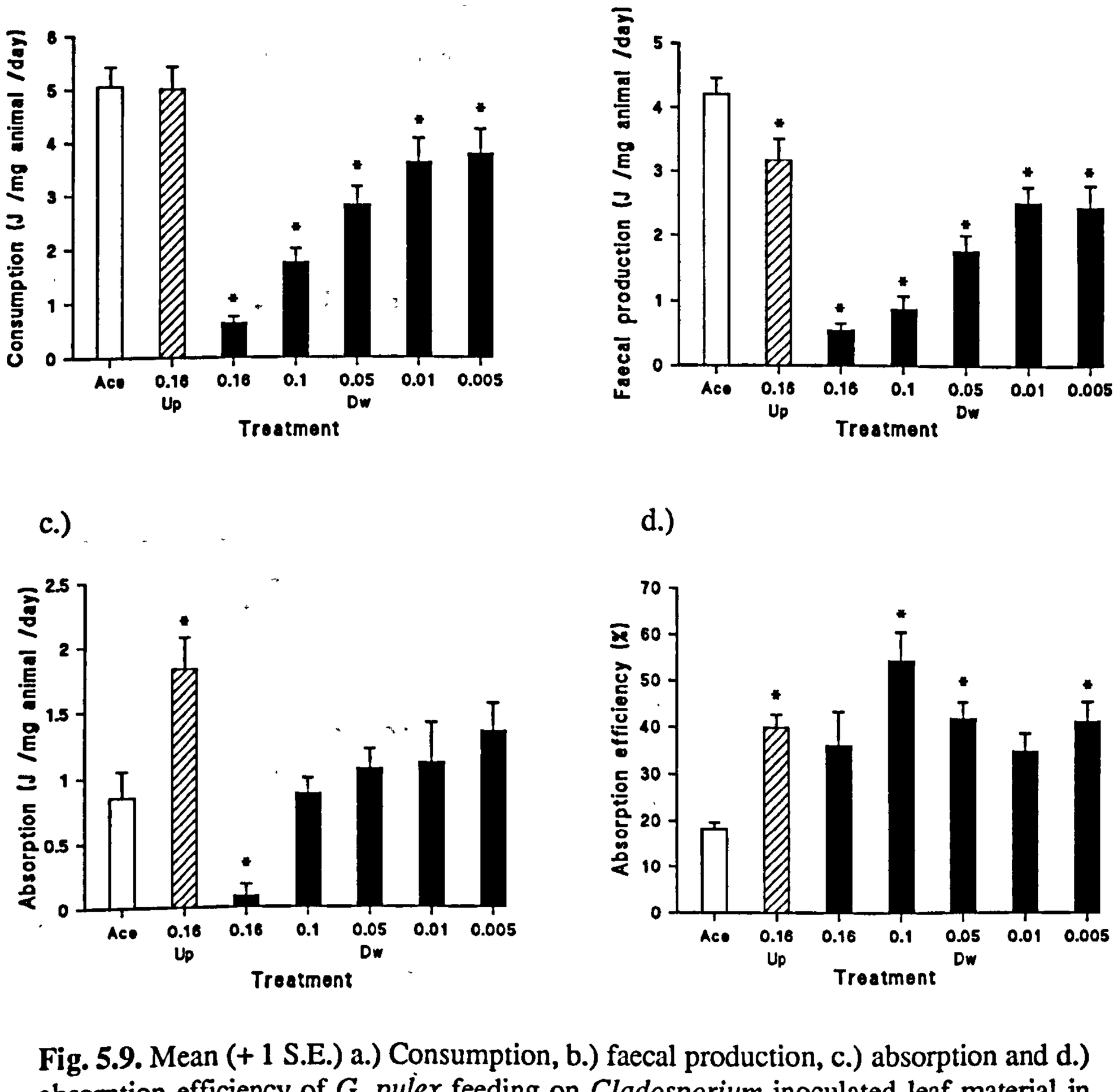


Fig. 5.9. Mean (+ 1 S.E.) a.) Consumption, b.) faecal production, c.) absorption and d.) absorption efficiency of *G. pulex* feeding on *Cladosporium*-inoculated leaf material in water spiked with either acetone carrier solvent control (ace, ☐), upstream solvent (DCM) sediment extract (0.16 ml/L Up, ▨) or a dilution series of downstream solvent sediment extract from 0.005 - 0.16 ml/L (Dw, ■). Asterisks denote a significant difference from control.

This experiment was repeated using water spiked with lower concentrations of downstream extract (i.e. 0.005-0.0001 ml extract/L) again using an acetone treatment (0.16 ml extract/L) as a control. There was no significant between-treatment difference in consumption (Fig. 5.10.), faecal production or absorption (F<0.95, df>4,135) in this second experiment. The lowest observed effect concentration (LOEC) for a significant reduction in consumption relative to the control was therefore 0.005 ml extract/L and the no observed effect concentration (NOEC) was 0.001 ml extract/L. As with the first experiment, animals exposed to water spiked with downstream extract generally had a higher absorption efficiency than control animals, although this was only significant for animals exposed to 0.001 ml extract/L (q=3.66, df=135,4; Fig. 5.10.).

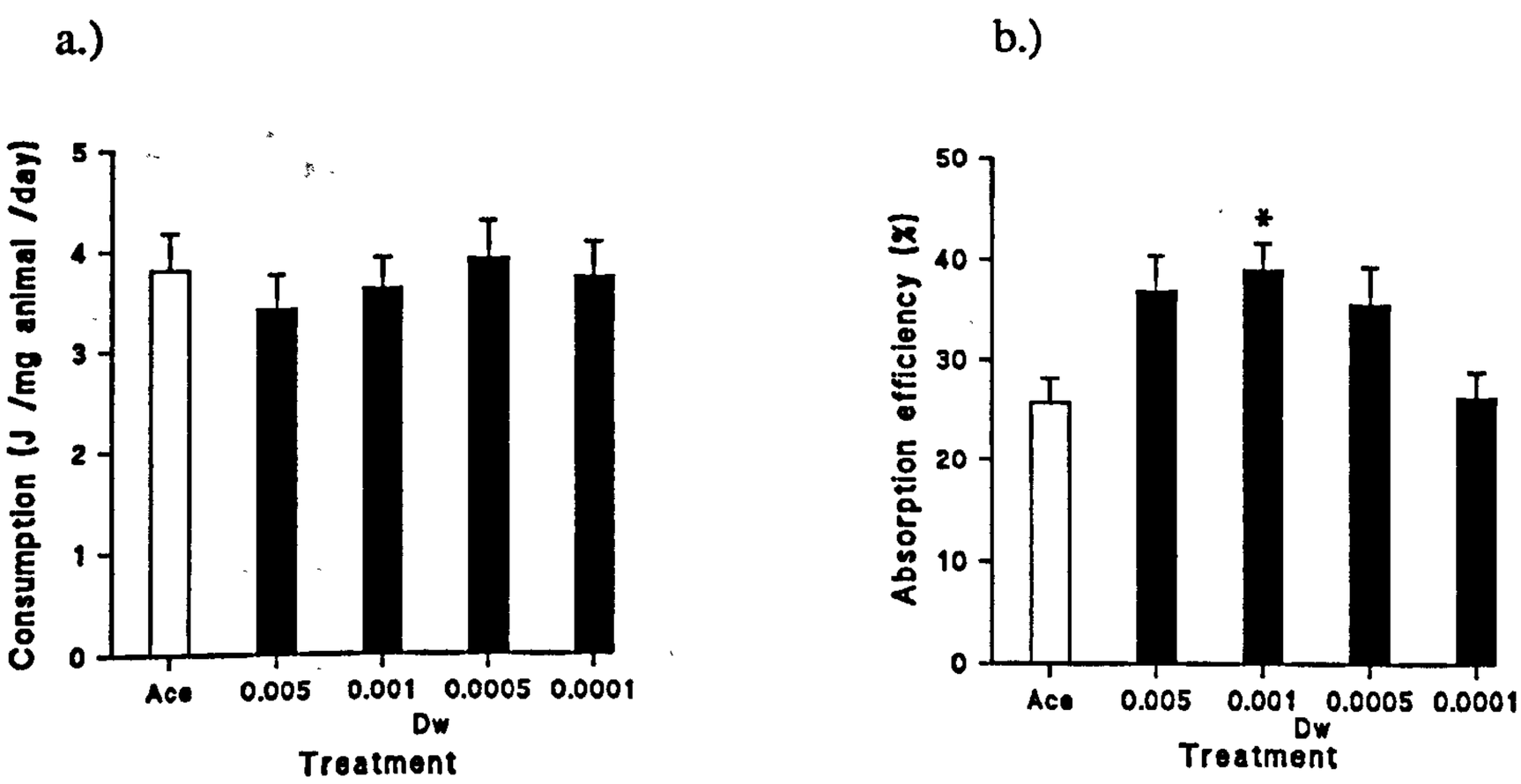


Fig. 5.10. Mean (+1 S.E.) a.) Consumption and b.) absorption efficiency of *G. pulex* feeding on *Cladosporium*-inoculated leaf material when exposed to water spiked with acetone carrier solvent at 0.16 ml/L (ace, ☐) or a dilution series of downstream solvent sediment extract from 0.0001 - 0.005 ml/L (Dw, ■). Asterisk denotes significant difference from control.

The data set from the first and second experiments were combined by plotting the ratio of treatment consumption and acetone control consumption against extract and aromatic hydrocarbon concentrations. Aromatic hydrocarbon concentrations in extract concentrations below 0.01 ml extract/L were obtained by extrapolation from Eqn. 5.10, r=0.82, df=10).

Aromatic hydrocarbon conc. = 503.79 (extract concentration) + 18.92 Eqn. 5.10.

There was a significant negative relationship (Eqn. 5.11.) between downstream extract concentration and the ratio of control (C_a) to test (C_t) consumption (r=-0.92, df=7, Fig. 5.11.) and total aromatic hydrocarbon concentration of the spiked water and relative consumption (r=-0.95, df=8, Fig 5.11.). The LOEC and NOEC equate to 23.96 and 21.44 µg chrysene equivalents /L total aromatic hydrocarbons respectively and the calculated EC_{50} from a deviation from control consumption was 51.72 µg chrysene equivalents /L (95 % C.I. 40.36, 66.28) total aromatic hydrocarbons.

$$C_a/C_t = 0.125 - 0.133 \log_{10} (\text{downstream sediment extract conc.}) \qquad \text{Eqn. 5.11.}$$

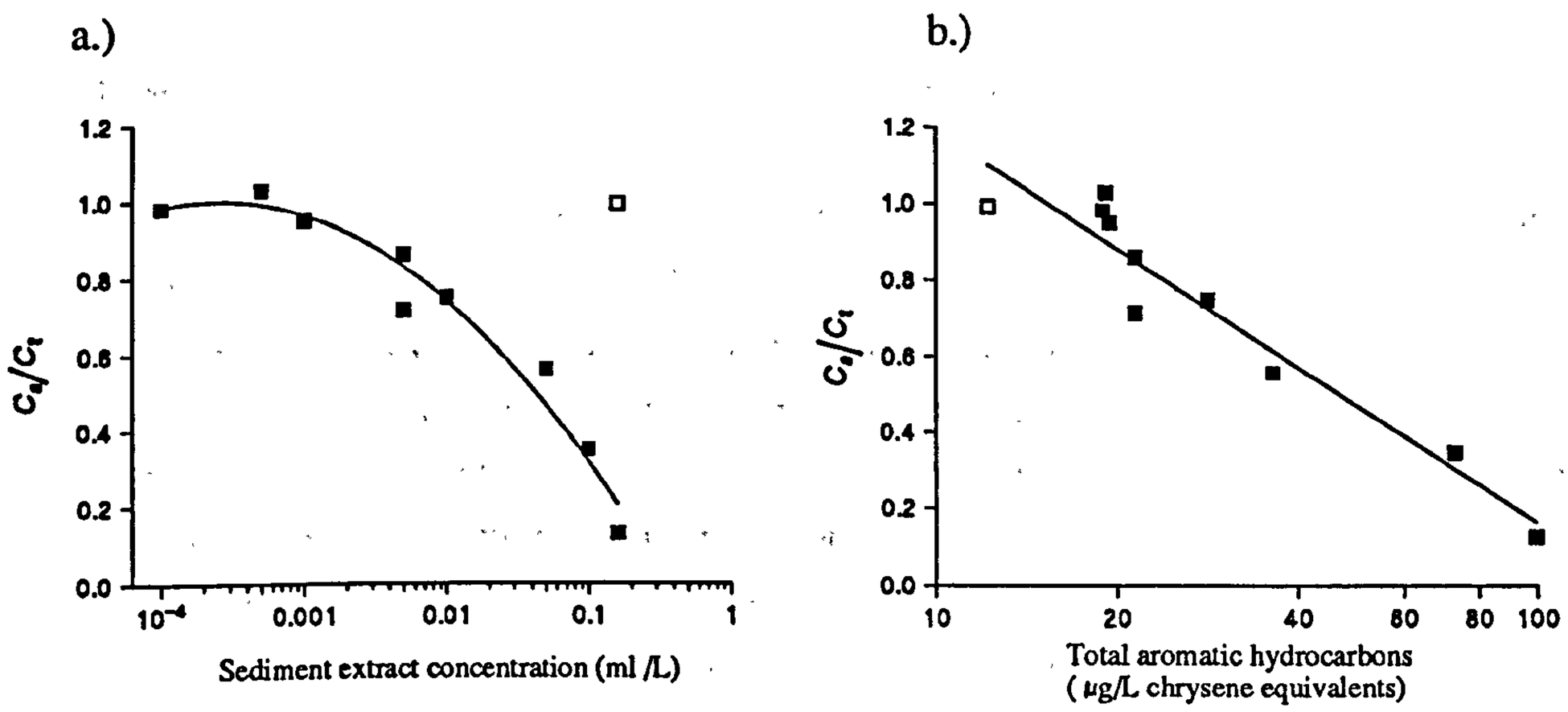


Fig. 5.11. Relationship between the ratio of mean consumption in the treatment (C_t) to mean consumption in the acetone control (C_a) and a.) the solvent sediment extract (ml/L) and b.) concentration of total aromatic hydrocarbons (µg chrysene equivalents /L) in the spiked water. Open symbols represent water spiked with upstream sediment extract and solid symbols represent water spiked with downstream extract.

To assess whether the dilute acid extractable fraction of the sediment had an effect on consumption of leaf material by *G. pulex*, consumption by animals exposed to a series of spiked test solutions was assessed. The leaf material was spiked with acid sediment extract to initially establish an equilibrium between the material and test solutions and standardise the quality of the leaf material between-treatments. Mean (±1 S.E.) metal concentrations (µg/g dry wt.) of spiked leaf material were: Cd, 0.08 (±0.06); Cr, 4.08 (±2.14); Pb, 68.54 (±13.33); Cu, 150.55 (±22.92) and Zn 242.39 (±8.99). Concentrations of Cd at 10 ml downstream extract /L and Zn at 10 and 6 ml downstream extract/L were significantly higher than in the acid control or the 10 ml extract /L test solutions (q>4.71, df=14,7, Table 5.8.). Total aromatic hydrocarbons were not detected in any of the treatments.

Table 5.8. Mean (± 1 S.E.) concentrations of metals (µg/L) in APW spiked with various concentrations of acid sediment extracts. ND indicates not detected.

	Zn		Cu		Cd		Cr		Pb	
Control	19.07	(9.27)	5.97	(0.27)	ND		ND		ND	
Upstream extract										
10 ml/L	17.33	(8.59)	4.03	(1.69)	0.090	(0.04)	ND		ND	
Downstream extract										
10 ml/L	145.00	(7.41)	4.03	(0.75)	0.900	(0.41)	ND		ND	
6 ml/L	142.67	(50.7)	4.83	(0.14)	0.020	(0.01)	ND		0.002	0.001
4 ml/L	65.37	(0.26)	2.07	(1.16)	ND		ND		ND	
2 ml/L	28.01	(4.23)	2.67	(0.81)	0.010	(0.008)	ND		ND	
1 ml/L	21.1	(9.58)	1.00	(0.20)	ND		ND		ND	

There was no significant difference in consumption (F=0.72, df=6,189) or faecal production (F=0.98, df=6,189, Fig. 5.12.) of animals exposed to water spiked with either upstream extract, downstream extracts or the acid control. There was, however, a significantly higher absorption of leaf material by animals exposed to water spiked with downstream sediment acid extract at a concentration of 1 ml/L compared to those exposed to water spiked with 10 ml upstream extract/L (q=4.33, df=189,6, Fig. 5.12.). There was no significant difference in absorption efficiency between animals exposed to the acid control and either the upstream or downstream sediment extract treatments (q<3.24, df=189,6, Fig. 5.12.). However, absorption efficiencies of animals exposed to downstream sediment extract concentrations of 10, 6 and 1 ml extract/L were significantly higher than those of animals exposed to the upstream sediment extract (q>4.2, df=189,6).

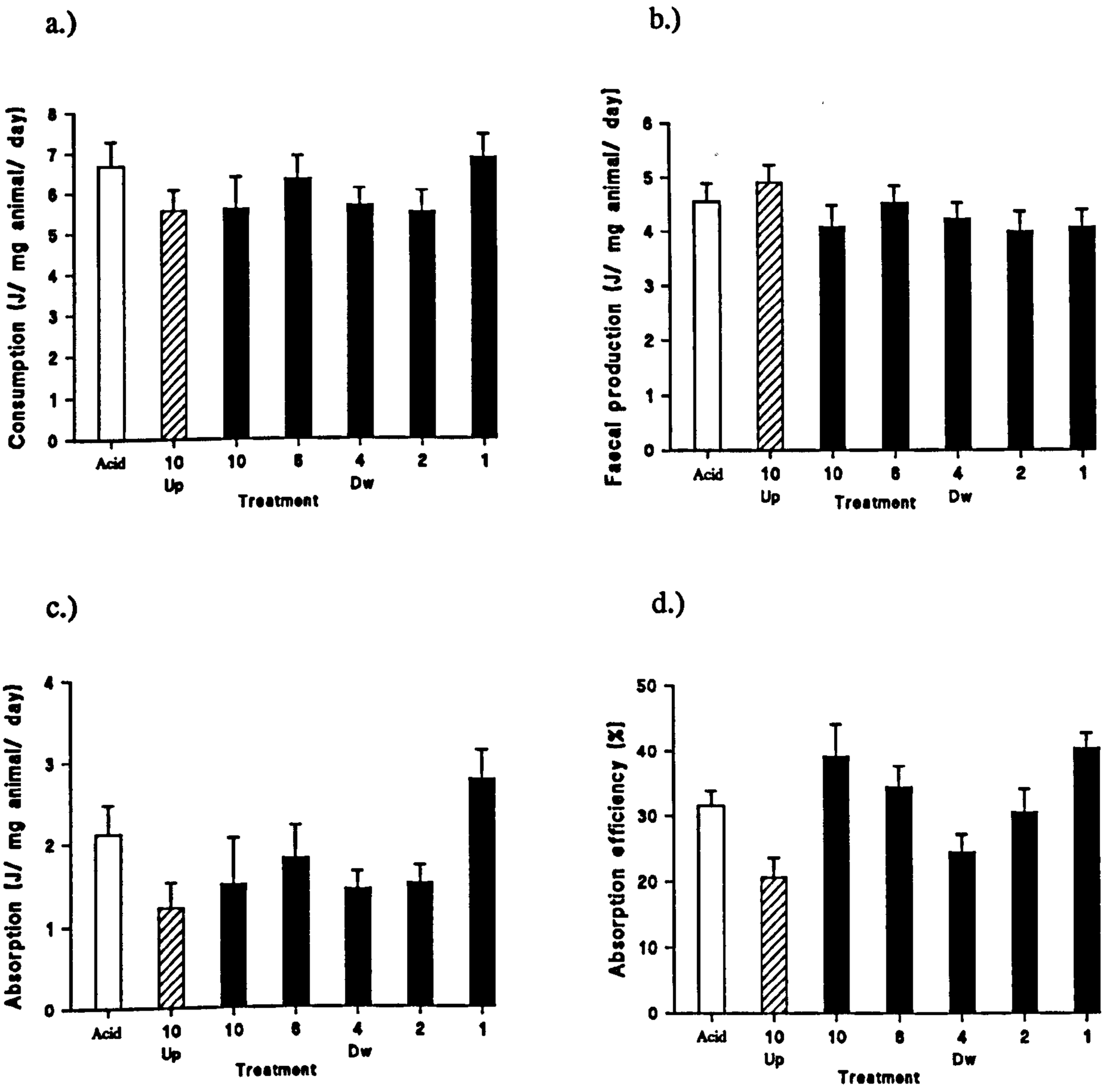


Fig. 5.12. Mean (+1 S.E.) a.) Consumption, b.) faecal production, c.) absorption and d.) absorption efficiency of *G. pulex* feeding on *Cladosporium*-inoculated leaf material in either water spiked with acid (acetic) carrier (acid, ☐), upstream sediment acid extract (10 ml extract/L Up, ▨) or a dilution series of downstream sediment acid extract from 1- 10 ml extract/L (Dw, ■).

5.3.13. Effect on leaf choice: I. stream water and sediment pre-exposure.

The ability of *G. pulex* to distinguish between, and show a preference for, leaf material pre-exposed to either water or sediment at the upstream or downstream station at Pigeon Bridge Brook was assessed. The experiment was performed with both *Cladosporium*-inoculated and naturally-inoculated leaf material; chemical data for which are presented in Table 5.6. (section 5.3.7.).

Pre-exposure resulted in leaf material which varied in metal and hydrocarbon concentrations (Table 5.6., section 5.3.7.). However, *G. pulex* exhibited no significant preference, assessed by leaf disc consumption, of either *Cladosporium*-inoculated or naturally-inoculated leaf discs exposed to stream water or sediments ($F< 4.13$, df=4,6, Fig. 5.13.). The initial dry weight of naturally-inoculated leaf material was calculated using dry weight-wet weight relationships. As these relationships varied across treatments (multiple ANCOVA $F=3.58$ df=6,72) treatment specific regression equations were used (Appendix A5: Eqns. A5.18-A5.21).

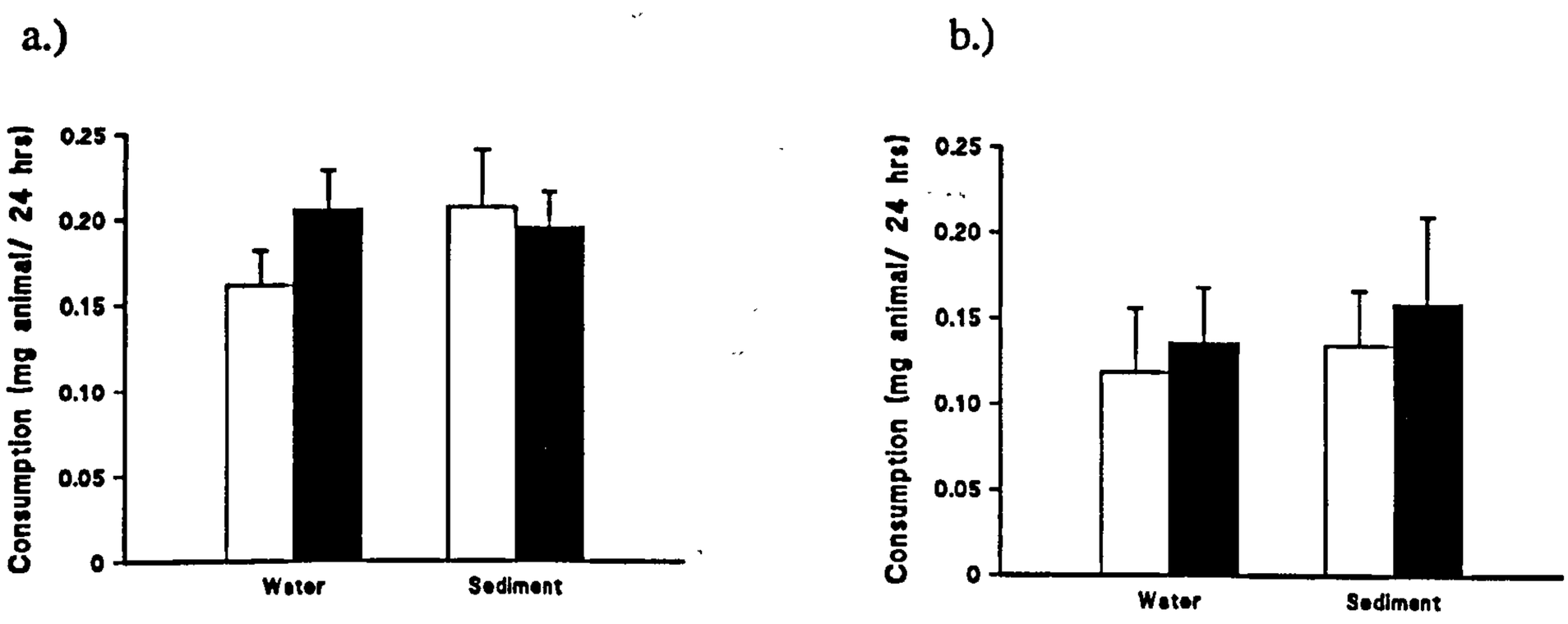


Fig. 5.13. Mean (+ 1 S.E.) consumption of a) *Cladosporium*- and b.) naturally-inoculated discs pre-exposed at either upstream (□) or downstream (■) stations at Pigeon Bridge Brook either suspended in the water column or in contact with the sediments and offered to *G. pulex* in food choice experiments.

5.3.14. Effect on leaf choice: II. *in-situ* conditioning.

Material was deployed at the upstream and downstream station at Pigeon Bridge Brook for up to 41 d to assess whether the discharge affected leaf conditioning and hence food quality as assessed in choice experiments. Chemical data for leaf material used in this experiment is presented in Fig. 5.6. (section 5.3.8). Although the leaf quality varied between treatments (Fig. 5.6., section 5.3.8), after 13 and 41 days deployment there was no significant between-station difference in leaf choice ($F<3.51$, df=2,18; Fig. 5.14.). However, after 27 days deployment animals significantly preferred leaf material deployed at the upstream station ($F=4.54$, df=2,18).

The initial dry weight of naturally-inoculated leaf material was calculated using dry weight-wet weight relationships. As these relationships varied across treatments after 27 and 41 days deployment (ANCOVA $F>5.54$, df=1,156) treatment specific regression equations were used. After 13 days deployment, the relationships were homogenous

(ANCOVA F<0.76, df=1,156) and combined to form a common relationship (Appendix A5: Eqns. A5.22-A5.26).

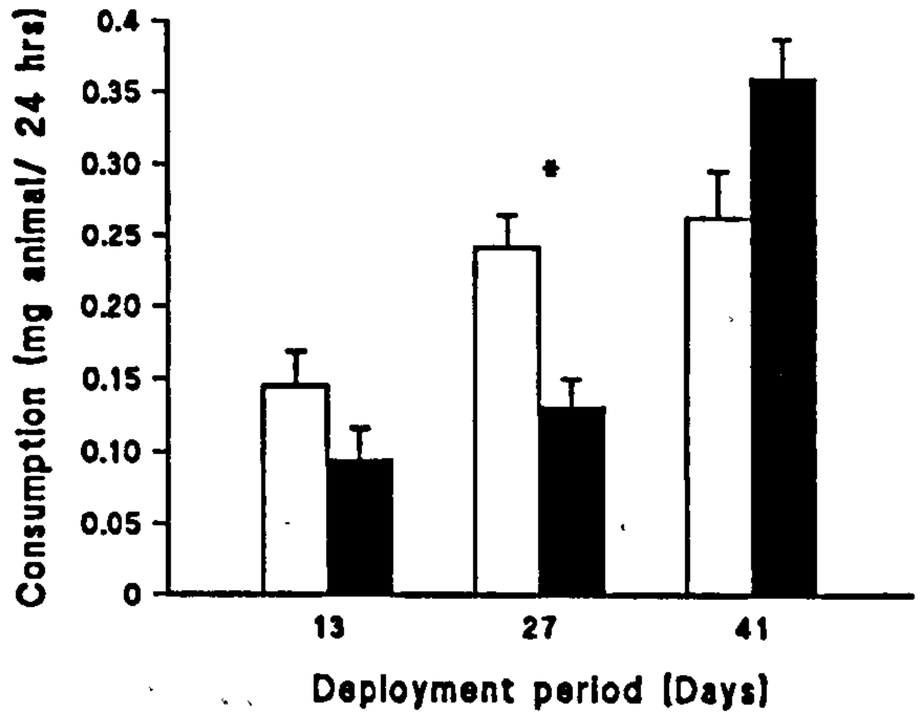


Fig. 5.14. Mean (+1 S.E.) consumption by *G. pulex* of alder leaf material deployed at Pigeon Bridge Brook at the upstream (□) or downstream (■) station for 13, 27 or 41 d and used in food choice experiments. Asterisks denote significant between-station differences.

5.3.15. Effect on leaf choice: III. sediment extracts.

Leaf material was spiked with sediment extracts and the subsequent effect on food choice assessed. Although for all the metals analysed (i.e. Zn, Cd, Cr, Pb and Cu) leaf material spiked with downstream sediment acid extract had higher concentrations than material spiked with upstream sediment acid extract (Table 5.9.) this was only significant for Pb and Cu (t>3.4, df=3). *G. pulex* demonstrated a significant preference for leaf material spiked with upstream sediment acid extract over leaf material spiked with downstream sediment acid extract (F = 19.22, df=2,14, Fig. 5.15.).

Table 5.9. Mean (1 S.E.) metal concentrations (µg/g dry wt.) of leaf material spiked with acid extracts of upstream and downstream sediment from Pigeon Bridge Brook.

	Zn		Cd		Cr		Pb		Cu	
Upstream	168.30	(18.0)	1.61	(0.43)	2.70	(0.35)	50.00	(14.0)	64.00	(15.0)
Downstream	364.00	(19.0)	2.72	(0.47)	23.70	(6.00)	126.94	(7.5)	146.39	(19.1)

Total aromatic hydrocarbon concentrations of leaf material spiked with downstream sediment solvent extract was significantly greater (145 µg chrysene equivalents /g, wet wt. 1 S.E.= 18.0) than leaf material spiked with upstream sediment solvent extract (52.5 µg/g, 1 S.E.=6.0; t=4.91, df=2). *G. pulex* demonstrated a significant preference for leaf material spiked with upstream solvent sediment extract over that spiked with downstream sediment solvent extract (F = 7.70, df=2,14, Fig. 5.15.).

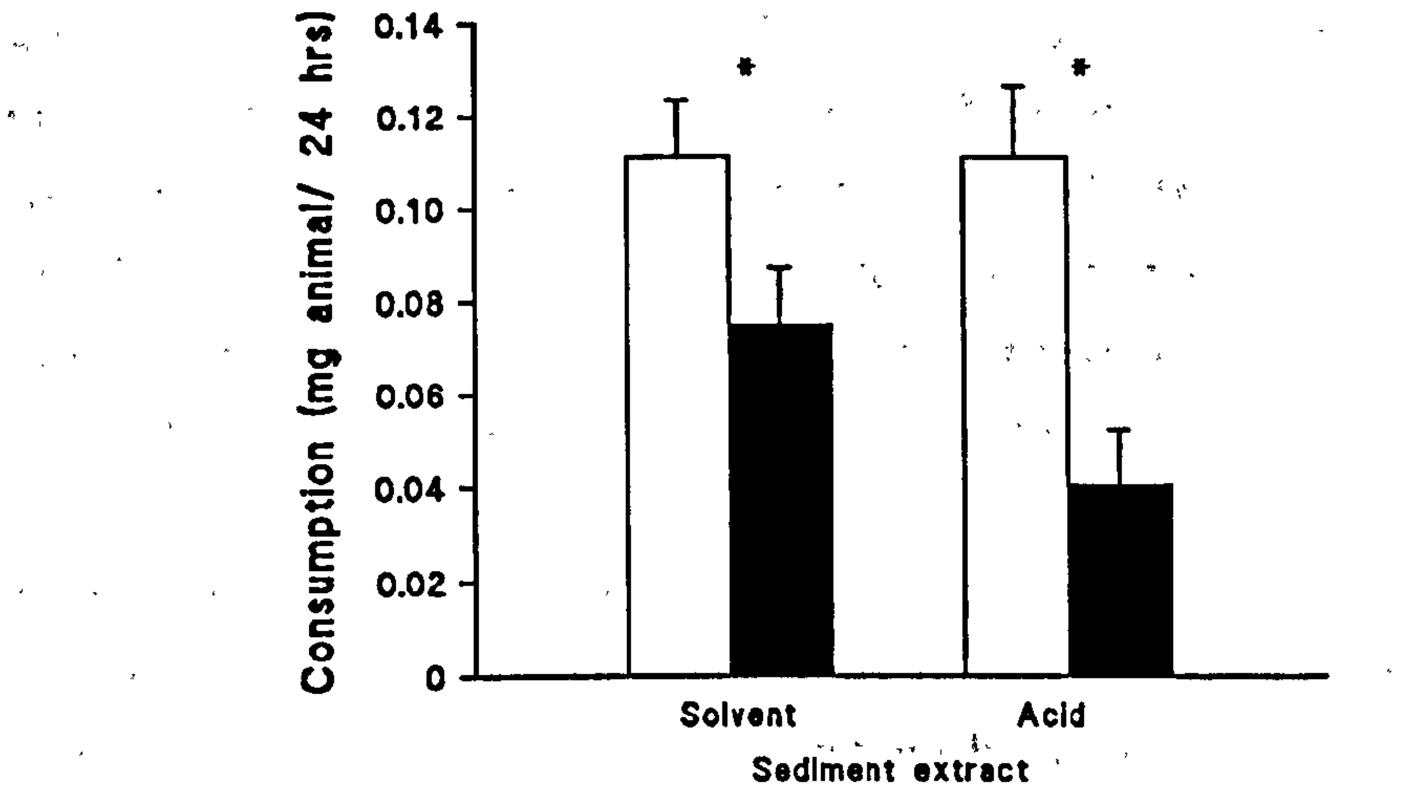


Fig. 5.15. Mean (+ 1 S.E.) consumption by *G. pulex* of *Cladosporium*-inoculated leaf material spiked with either solvent or acid extracts of upstream (□) or downstream (■) sediments from Pigeon Bridge Brook and offered in choice experiments. Asterisks denote significant between-station differences.

5.3.16. Uptake route of motorway runoff pollutants.

In order to assess the possible uptake of toxicants from contaminated food or water animals were exposed to spiked water and contaminated food in combination and separately. The results from the experiment using solvent sediment extracts are given in Fig. 5.16. Animals in Treatment 5-8 were exposed to water spiked with a concentration of 0.05 downstream solvent sediment extract whereas water in Treatments 1-4 was spiked with the acetone carrier. Consequently the concentration of total aromatic hydrocarbons was significantly elevated in Treatments 5-8 (53.92 ±6.40 µg/L chrysene equivalents) compared to Treatments 1-4 (29.65 ±3.23 µg chrysene equivalents/L, t>3.39, df=3). Contaminated food in Treatments 3, 4, 7 and 8 had a significantly higher concentration of total aromatic hydrocarbon concentrations (141.49 µg chrysene equivalents/g wet wt.; 1 S.E.= ±14.81) than the control food in Treatments 2 and 6 (52.5 µg chrysene equivalents /g wet-wt.; 1 S.E.= ±6.0; t=4.71, df=2). Faecal pellets produced by animals consuming control food in acetone-spiked water contained 39.06 µg/g wet-wt. chrysene equivalents 1 S.E. = ±2.18 total aromatic hydrocarbons whereas faecal pellets produced by animals consuming contaminated food under the same conditions had a total aromatic hydrocarbon concentration of 421 µg chrysene equivalents/g wet wt 1 S.E. = ±24.60. The gut contents of *G. pulex* fed on *Cladosporium*-inoculated alder leaves accounted for approximately 2.5% of the animal fresh weight. The total aromatic hydrocarbon concentration of the faeces in the guts of the animals were subtracted from the whole tissue concentrations.

Food in Treatments 4 and 8 was contaminated but inaccessible to the animals. There was no evidence of increased uptake of hydrocarbons leached from this inaccessible material since there was no significant difference in *G. pulex* hydrocarbon tissue concentrations from Treatments 1 and 4 (q=0.06, df=16,8, Fig. 5.16.) or Treatments 5 and 8 (q=1.6, df=16,8). Animals exposed to water spiked with downstream sediment extract (Treatments 5-8) had significantly higher hydrocarbon concentrations than those exposed to water spiked with acetone only (Treatments 1-4; q>5.19, df=16,8).

There was no significant difference in the hydrocarbon concentration of animals exposed to the control water treatments (q=1.20, df=16,8). Generally there was no significant difference in tissue concentrations between animals exposed to water spiked with the downstream solvent extract, the only exception being between the accessible spiked leaf material (Treatment 7) and the inaccessible spiked leaf material (Treatment 8, q=6.19, df=16,8). There was no significant difference in aromatic hydrocarbon concentrations of animals in contaminated water fed clean or contaminated food (Treatments 6 and 7 respectively, q=4.56 df=16,8). These results suggest the major uptake route of aromatic hydrocarbons is via the aqueous phase.

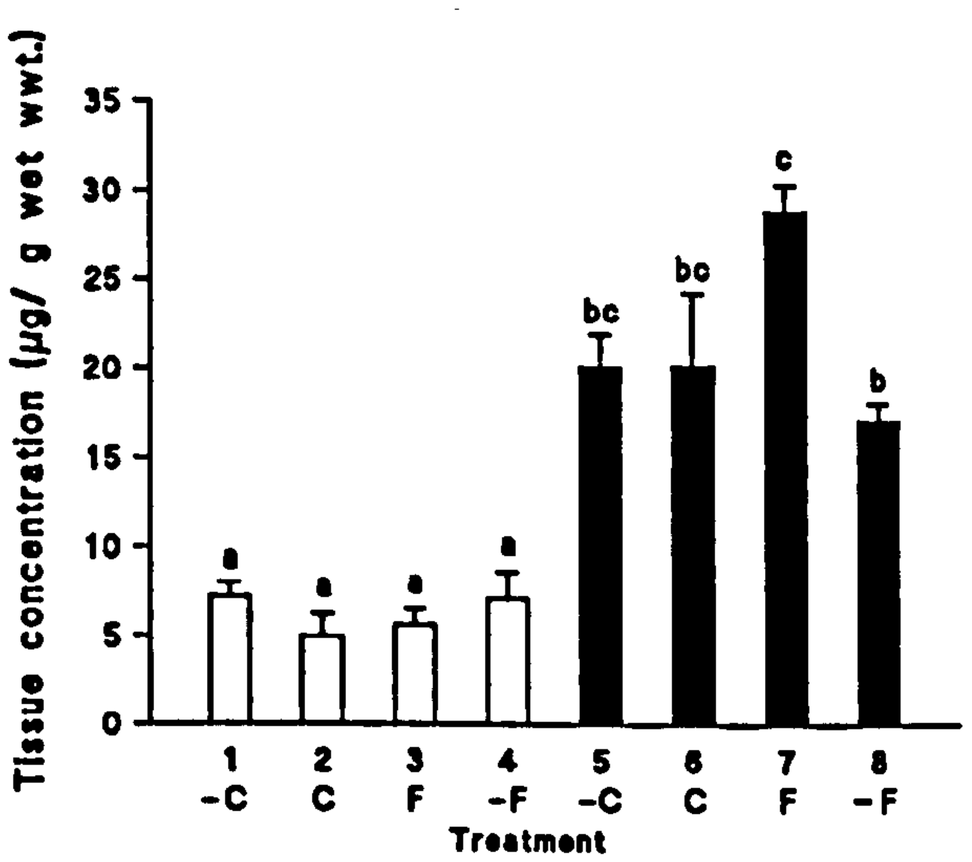


Fig. 5.16. Mean (+ 1 S.E.) concentration of total aromatic hydrocarbons in *G. pulex* (corrected for faeces content) exposed to water spiked with either acetone (□) or downstream sediment solvent extract (■) and provided with no food (Treatments 1,5), acetone-spiked food (C, Treatments 2,6), downstream extract-spiked food which was either accessible (F, Treatments 3,7) or not (-F, Treatments 4,8). Treatments (bars) sharing the same letter code are not significantly different.

The experiment was repeated using acid sediment extracts. Animals in Treatment 5-8 were exposed to water spiked with a concentration of 5 ml downstream sediment acid extract /L whereas those in Treatments 1-4 were exposed to water spiked with 0.05 ml/L acetic acid. Although the majority of the measured metals were elevated in water spiked with the downstream sediment acid extract compared to water spiked with acetic acid this was only significant for Zn and Cd (t>5.26, df>2; Table 5.10.). Treatments 1 and 5 contained no leaf material, Treatments 2 and 6 contained leaf material spiked with acetic acid (0.05 ml/L) whereas Treatments 3, 4, 7 and 8 contained leaf material that had been spiked with downstream sediment acid extract (10 ml extract/L). Although the concentrations of the majority of metals were elevated for the contaminated leaf material, this was only statistically significant for Cu and Pb (t>4.22, df=2; Table 5.10.).

Table 5.10. Mean (1 S.E.) metal and total aromatic hydrocarbon concentrations (A.H. µg chrysene equivs./ L: water or /g: leaf material) of water spiked with either acid or downstream sediment acid extract and leaf material spiked with either acid ('Clean') or downstream sediment acid extract ('Contaminated'). ND indicates not detected.

Water	Zn µg/L	Cu µg/L	Cd µg/L	Cr µg/L	Pb µg/L	A.H.
Acid (5 ml/L)	18.2 (7.3)	3.47 (0.43)	0.057 (0.057)	ND	ND	ND
Downstream extract (5 ml/L)	123.2 (18.0)	6.47 (0.64)	0.643 (0.096)	ND	0.0003 (0.0003)	ND
Leaf Material	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g CE
'Clean' (acid spiked)	176.6 (48.0)	38.33 (2.40)	0.719 (0.510)	1.929 (0.490)	13.90 (8.10)	ND
'Contaminated' (extract spiked)	266.9 (16.0)	109.50 (7.00)	2.045 (0.053)	1.800 (0.210)	49.57 (3.10)	ND

The concentrations of Pb, Cu, and Zn in the faecal pellets of animals feeding on contaminated leaf material in 'clean' water (Treatment 2) were significantly higher than in faecal pellets of animals feeding on clean food in acid spiked water (Treatment 3; t>6.66, df=2; Table 5.11.). *G. pulex* tissue metal concentrations were adjusted for metal gut contents as described in section 5.2.16. After this correction there were no clear patterns of metal accumulation between treatments (Table 5.11.).

Table 5.11. Mean (±1 S.E.) metal concentrations (µg/g dry wt.) in faeces and *G. pulex* tissue (corrected for gut content) when exposed to water spiked with acid (0.05 ml/L, Treatments 1-4) or downstream sediment acid extract (5 ml extract/L, Treatments 5-8) and provided with no food (Treatments 1,5), acid-spiked food (Treatments 2,6) or downstream sediment acid extract spiked food which was either accessible (Treatments 3,7) or not (Treatments 4,8). ND indicates not detected. Treatments not sharing a common lower case letter for each determinand are significantly different (Faeces t>6.66, df=2; Tissue q>4.66, df=16,8).

	Zn		Cd		Cr		Pb		Cu	
FAECES										
Treatment 2	261.54 (6.85)	x	2.71 (0.10)	x	1.05 (0.07)	x	27.75 (0.50)	x	96.42 (2.56)	x
Treatment 3	437.95 (22.54)	y	5.96 (1.15)	x	1.81 (0.22)	x	57.24 (3.52)	y	238.09 (21.13)	y
TISSUE										
Treatment 1	160.44 (4.96)	abc	6.52 (0.68)	abc	1.77 (0.57)	a	45.86 (4.75)	a	158.07 (5.16)	a
Treatment 2	132.68 (4.40)	abc	4.35 (0.19)	c	0.35 (0.28)	a	55.46 (7.86)	a	130.05 (7.14)	abc
Treatment 3	129.11 (2.76)	abc	4.64 (0.28)	bc	ND (ND)	a	43.11 (7.94)	a	135.53 (3.34)	abc
Treatment 4	162.51 (7.84)	ab	5.77 (0.63)	abc	2.80 (1.44)	a	53.82 (2.14)	a	130.99 (5.71)	abc
Treatment 5	186.28 (10.84)	a	7.19 (0.46)	ab	0.83 (0.11)	a	59.43 (18.39)	a	141.34 (8.17)	ab
Treatment 6	118.53 (9.94)	c	4.50 (0.28)	c	1.77 (0.81)	a	36.76 (3.96)	a	116.21 (13.12)	bc
Treatment 7	111.22 (11.48)	c	5.68 (0.92)	abc	0.76 (0.60)	a	57.16 (2.26)	a	99.27 (11.56)	c
Treatment 8	138.28 (7.67)	abc	7.34 (0.42)	a	0.65 (0.39)	a	48.78 (6.40)	a	117.81 (4.27)	bc

5.4. DISCUSSION.

Field assessments of leaf decomposition indicated that macroinvertebrate-mediated leaf processing was reduced below the motorway discharge into Pigeon Bridge Brook. There are two non-mutually exclusive explanations for this: a reduction in the number of macroinvertebrates that breakdown this material and/or a reduction in the feeding activity of these animals. Surveys of the macroinvertebrate community (section 2.3.3.) indicated that the relative abundance of shredders in general and dominant shredders (i.e. *Gammarus pulex*, *Leutra inermis*, and *Ptychoptera* sp.) in particular was reduced at the downstream station. Lethal (Chapter 3) and sub-lethal (avoidance, Chapter 4) responses of *G. pulex* in toxicological assessments went some way in explaining the reduction in the abundance of this species at the downstream station. Reductions in the relative abundance of shredders may also be due to sub-lethal affects on their feeding activity and energy budget which will affect their population dynamics and impede leaf processing. The possibility that contaminants associated with motorway runoff reduce shredder feeding activity was investigated in this chapter using *G. pulex* as the test species.

Resource quality is one factor which affects the breakdown of detrital material (Sheehan *et al.*, 1984). Animals may regulate their feeding according to the quality of the food by pre-ingestive selection or by reducing ingestion to decrease gut passage time (Iglesias *et al.*, 1992; Navarro *et al.*, 1992) and hence increase assimilation efficiency (Schindler, 1971). If the nutritional value of food is poor, animals may be able to 'adjust' gut through-put times in order to increase assimilation rates (Welton *et al.*, 1983). Gut through-put times in *G. pulex* alter according to environmental conditions such as differences in temperature. For instance, Welton *et al.* (1983) determined gut through-put times were 119-160 min at 7°C and 45-59 min at 13.8°C whereas Sutcliffe *et al.* (1981) recorded through-put times of 8-12 h at 15°C. As stated in the introduction to this chapter (section 5.1) there are two non-mutually exclusive mechanisms for reduced food quality to shredder macroinvertebrates; toxicant accumulation and degree of leaf conditioning. Adjusted consumption may generally be a feature of detrital material that differs in food quality due to conditioning rather than toxicant accumulation. Consumption of more highly conditioned leaf material is often greater than less conditioned material (Adcock, 1982).

Initial *in-situ* assessments at Pigeon Bridge Brook identified a significant reduction in the consumption of conditioned leaf material by *G. pulex* at the station receiving motorway runoff compared to the reference station. Subsequent *in-situ* reciprocal transfer experiments indicated that, although material initially deployed at the downstream station accumulated metals (Zn, Cd, Cr, Pb, and Cu) and aromatic hydrocarbons, this had no effect on between-station patterns in consumption. Consumption by *G. pulex* at the downstream station was reduced regardless of the station at which the leaf material was pre-exposed suggesting that it was not dependent on differential leaf quality due to differences in toxicant accumulation.

Laboratory experiments confirmed the findings of the *in-situ* experiments. Conditioned leaf material was deployed at the upstream and downstream station at Pigeon Bridge Brook before being fed to *G. pulex* in the laboratory. While leaf material deployed in the water column and on the bed sediments at the downstream station accumulated metals (Zn, Cd, Cr, Pb, and Cu) and hydrocarbons, there was no effect of deployment position on the consumption of this material by *G. pulex*. There was, however, some evidence of increased absorption of more contaminated material. This is contrary to the results of some other workers using metal contaminated food (Bermingham, 1993; Tattersfield, 1993) but in agreement with research on hydrocarbon contaminated food. For instance, Van Straalen and Verweij (1991) found little effect on consumption, growth, defecation or respiration of benzo(a)pyrene-contaminated food by the isopod *Porcellio scaber* except at very high concentrations (i.e. 125 µg/g BaP). When provided with food contaminated at the highest concentration (i.e. 125 µg/g BaP) male isopods showed significantly higher food assimilation efficiencies but significantly lower growth than animals feeding on food contaminated at lower concentrations (i.e. 1-25 µg/g BaP). These authors suggested that increased assimilation was due to an active mechanism that enhances digestion of food or absorption of digestive products. One possible explanation is that the gut microflora are induced by the toxicant to enhance the degradation of recalcitrant compounds and thereby increase the availability of digestive products (Lawson and Klug, 1989; Harris, 1993). In addition, toxicants may lead to lysis of the cells of the detrital microflora producing enzymes which may aid leaf digestion (Harris, 1993). Hydrocarbons do increase the activity of microbes, particularly heterotrophic bacteria, on leaf material often altering the microbial enzymatic responses (section 2.2.7.; (Sladeckova, 1963; Griffiths *et al.*, 1982; McKinley *et al.*, 1982; Brown *et al.*, 1983; Werner *et al.*, 1984b; Swift *et al.*, 1988; Johnson and Romanenko, 1989; Graça, 1993). Such changes may alter the food quality of this material to shredders resulting in increased assimilation. The role of rapidly-colonising hydrocarbon-utilising

bacteria in the nutrient dynamics of hydrocarbon polluted systems warrants further research.

Changes in food quality due to pollutant-induced changes in microbial assemblages colonising leaf material were also investigated in this study. Microorganisms are important in hydrolysing leaf material making it more palatable to macroinvertebrates and in supplying vital nutrients to these animals (Chapter 1). Results from Chapter 2 suggested that although the motorway discharge had little effect on hyphomycete fungal community structure after 28 and 55 d deployment at Pigeon Bridge Brook (sections 2.2.4 and 2.2.7 respectively), the successional colonisation pattern of these fungi was altered by the discharge (section 2.2.7.).

Leaf material conditioned at the upstream and downstream station at Pigeon Bridge Brook for up to 27 d and fed to *G. pulex* in laboratory trials, differed in 'quality' (i.e. amount consumed). However, material conditioned for longer than 27 d did not. After 13 d conditioning there were significant between-station differences in absorption and absorption efficiency; material deployed at the downstream station being absorbed more efficiently. In contrast, after 27 d deployment consumption, absorption and absorption efficiency of material incubated at the downstream station was lower than that incubated at the upstream station. Field surveys (section 2.3.6) indicated that colonisation of alder leaf material by aquatic fungi at the downstream station was reduced during the early stages of conditioning (i.e. up to 41 d) but 'catches up' later into the conditioning period (55 d). This may explain why consumption and absorption of the leaf material incubated at the downstream station was initially reduced but why after 55 d deployment between-station differences in food quality were no longer apparent. Increases in absorption and absorption efficiency of *G. pulex* feeding on leaf material from the downstream station after 13 d may be a consequence of the action of the toxicants on the leaf microflora as discussed above.

Results from the current and previous studies (Bermingham, 1993; Tattersfield, 1993) suggest toxicant accumulation on leaf material had little effect on leaf consumption by shredding macroinvertebrates. However, the effect of toxicants on leaf conditioning may be important. For instance, Bermingham (1993) concluded that a mine effluent was toxic to aquatic hyphomycete fungi resulting in reduced conditioning of leaf material exposed to the effluent and reduced leaf consumption by *G. pulex*. The current study suggests that effects of toxicants associated with motorway runoff on conditioning by

microbes may also affect the consumption of the leaf material. However, much of the leaf litter that enters the contaminated reach at Pigeon Bridge Brook may be from upstream sections of the stream and will therefore be pre-conditioned. Consequently, toxicant-induced changes in microbial conditioning may not be fully manifested. This leads to the conclusion that direct sub-lethal effects of motorway runoff contaminants in stream waters and sediments on *G. pulex* may be the major mechanism reducing feeding with indirect effects via changes in conditioning of material playing a minor role.

Sediments at the downstream station at Pigeon Bridge Brook were a sink for the majority of the toxicants in the motorway discharge (both metals and hydrocarbons; Maltby *et al.*, 1995a). Previous studies have demonstrated that sediments contaminated with metals, PCBs, and PAHs reduced the feeding activity of the benthic amphipod *Hyalella azteca* and tubificid worms (Winger *et al.*, 1993; Lotufo, 1994). In the present study the proximity of *G. pulex* to the downstream sediments had a significant affect on the consumption of leaf material. Animals in direct contact with downstream sediment consumed less material than either those in direct contact with the upstream sediment or those suspended 40 mm above the downstream sediment (Fig. 5.8, p158). These results suggest that contaminants in the sediment are responsible for the reduction in feeding activity. This was confirmed using sediment extract spiking experiments. Consumption of leaf material by *G. pulex* was significantly reduced by exposure to water spiked with 0.05 ml downstream solvent extract/L. In contrast, exposure to an upstream sediment solvent extract concentration of 0.16 ml/L had no effect on consumption. The solvent sediment extract probably contained many organic chemicals including aromatic hydrocarbons and there was a significant negative relationship between relative consumption and total aromatic hydrocarbon concentrations in the spiked water. The NOEC and LOEC for a significant reduced consumption relative to control were 21.44 and 23.96 µg chrysene equivalents /L respectively and the EC_{50} was 51.72 µg chrysene equivalents /L.

Consumption of leaf material by *G. pulex* was therefore affected at aromatic hydrocarbon concentrations in the range of pore water concentrations (i.e. 19.94 µg chrysene equivalents/L; A.B.A. Boxall, pers. comm.) and below reliable detection limits (i.e. <30 µg chrysene eqivs./L). Fish and invertebrates exposed to chronic oil exposure often show reduced feeding activity, an affect diminished by ageing of the oils suggesting that the more volatile/degradable fractions of the oil are responsible (Engelhardt *et al.*, 1982). The feeding rates of the marine crustaceans *Idotea baltica basteri* and *Gammarus olivii*, for example, were reduced when exposed to an oil

solution at 0.01 ml/L and 0.1 ml/L respectively (Milovidova, 1974). Moreover, a significant negative correlation between the tissue concentration of aromatic hydrocarbons and scope for growth (SfG) in mussels (*Mytilus edulis*) has been reported (Donkin and Widdows, 1986; Widdows *et al.*, 1987). Many authors have studied the SfG of aquatic species (most usually *Mytilus edulis*) in contaminated field situations. In the marine environment metals, organometals, non-hydrocarbon and hydrocarbon contaminants may all be present, although it is often petroleum hydrocarbons that are responsible for the observed reduction in SfG (Widdows *et al.*, 1990). Amongst the petroleum hydrocarbons, the aromatic hydrocarbons are thought to be the main contaminant responsible for reductions in SfG. Aromatic hydrocarbons act through the mechanism of 'non-specific narcosis' depressing SfG over a wide concentration range principally by reducing the activity of the gill cilia and hence consumption rates (Gillfillan *et al.*, 1977; Widdows, 1985; Widdows *et al.*, 1985; Widdows *et al.*, 1987; Widdows and Johnson, 1988; Widdows *et al.*, 1990). One order of magnitude increase in the tissue concentrations of 2- and 3-ringed aromatic hydrocarbons in *M. edulis* resulted in a 50% decrease in SfG. Other studies have reported a decrease in growth, fecundity and survival of aquatic species during chronic exposures to hydrocarbons which may also be a result of reduced feeding activity (Perkins, 1968; Ott *et al.*, 1978; Percy, 1978; Engelhardt *et al.*, 1982).

Reduced consumption may not be the only response of organisms to oil pollution. Chronic oiling at sub-lethal concentrations of crude-oil have been shown to reduce growth in parr of the Atlantic salmon, *Salmo salar*, due to reduced food conversion efficiencies rather than reduced food intake (Vignier *et al.*, 1992). Digestion of food material may be disrupted by chronic oil pollution (Engelhardt *et al.*, 1982) resulting in high defecation rates. In the current study, high rates of faecal production relative to consumption were displayed by *G. pulex* at the highest organic extract concentration (0.16 ml extract/L) resulting in low absorption. One possible reason for this is gut irritation by the toxicants which may either result in an active defecation response or mucus production to facilitate gut clearance resulting in overestimates of faecal production and underestimates of absorption (Bermingham, 1993). Petroleum hydrocarbons are known to produce cellular pathological responses in digestive cells and gut tissues (Berlin and Micks, 1973; Moore, 1985; Lowe *et al.*, 1981; Moore *et al.*, 1986).

In contrast to the spiking experiment using solvent extracts, exposing animals to water spiked with acid sediments extracts from Pigeon Bridge Brook had very little effect on

consumption or absorption of leaf material by *G. pulex*. This is despite the fact that Zn and Cd concentrations were significantly higher in water spiked with the top two downstream sediment acid extract concentrations, at 6 ml and 10 extract/L, than either control or upstream water and were within the range of concentrations recorded in interstitial water (A.B.A. Boxall, pers. comm.). However, concentrations of Cu, Cr and Pb were much lower in these experimental exposures than interstitial water concentrations.

The combined results of laboratory and *in-situ* feeding experiments suggest that consumption by *G. pulex* was reduced by exposure to organic contaminants which accumulate in downstream sediments at Pigeon Bridge Brook. There was no evidence to suggest that the accumulation of toxicants on leaf material influenced consumption when offered as the sole food source. However, given a choice, animals may avoid this material and search for non-contaminated food. Detritivorous aquatic organisms have been shown to be able to discriminate between food of different quality selecting foods which enhance their growth, fecundity and survivorship (Kostalos and Seymour, 1976; Willoughby and Sutcliffe, 1976; Sutcliffe *et al.*, 1981; Soderstrom, 1988; Graça *et al.*, 1993). Such preferences are probably determined by the degree of conditioning and the microbial assemblage on the leaf material (Chapter 1). However, animals may also be able to differentiate between 'clean' and 'contaminated' leaf material (Bermingham, 1993). In natural situations, leaf material contamination would be heterogeneous (Burton, 1992) and avoidance of contaminated material may be one mechanism by which an animal may reduce its exposure to toxicants (Chapter 4).

In laboratory choice experiments *G. pulex* did not apparently distinguish between leaf discs exposed to stream water or sediments at Pigeon Bridge Brook even though the material differed in contaminant concentrations. Neither did the animals make any choice between leaf material that was conditioned at the upstream or downstream station at Pigeon Bridge Brook for 13 or 41 d. They did, however, significantly prefer upstream material to downstream material after 27 d incubation. These results are similar to those discussed earlier (p172) for the consumption of field-incubated test material. As toxicant accumulation has no apparent effect on choice these results suggest that although after 13 d there was little microbial conditioning and hence between-station difference in quality of the leaf material, after 27 d the quality of the downstream material was lower due to reduced conditioning. However, after 41 d the food qualities are again similar. This explanation is in agreement with the pattern of

colonisation of leaf material by aquatic hyphomycetes described in section 2.3.7 and discussed in Chapter 2.

Results of the choice experiments indicate that *G. pulex* chooses leaf material only on the basis of the degree of conditioning. Many studies have shown that low pH and toxicants have an indirect effect on the consumption of leaf material due to alterations in microbial conditioning (e.g. Groom and Hildrew, 1989; Bermingham, 1993). Toxicants may affect the colonisation, community structure and growth of fungi and bacteria on the leaf material and altered community structure of aquatic hyphomycete fungi exposed to metal contaminants and organic pollution have been observed (Abel and Bärlocher, 1984; Bärlocher, 1993; Bermingham, 1993; Tattersfield, 1993). Despite the lack of evidence from field-incubated leaf material that toxicant accumulation influences food choice, when leaf material was spiked with either solvent or acid downstream sediment extract it was rejected by *G. pulex*. *G. pulex* significantly choose leaf material spiked with upstream sediment extract over leaf material spiked with downstream sediment extract. Although total aromatic hydrocarbon concentrations of leaf material spiked with downstream solvent extract was only slightly higher than that of material exposed in the field (145 and 121 µg chrysene equivalents /g respectively) avoidance of the spiked material was much more marked. This may have been due to a higher bioavailability of the hydrocarbons on spiked material.

Different hydrocarbons may result in differing responses of aquatic organisms to contaminated food. For instance, Atema *et al.* (1973) investigated the effect of kerosene fractions and food choice and concluded that whereas whole kerosene oil and branched-chain cyclic fractions were attractive to the lobster *Homarus americanus*, polar aromatics made the food more repulsive, and the straight chain fraction had no effect on feeding behaviour. Water soluble fractions (WSFs; mostly aromatics) of the kerosene also repulsed the mud snail *Nassarius obsoletus*, and the WSF of a crude oil inhibited food choice by the shrimp *Pandalus americanus*, possibly due to chemoreceptor interference (Jacobson and Boylan, 1973; Malins *et al.*, 1982). Hydrocarbons in water have been shown to affect the detection of food sources even at very low concentrations (Atema, 1977, 1979). Takahashi and Kittredge (1973) and Kittredge *et al.* (1974) working with the shore crab *Pachygrapsus crassipes*, demonstrated that monoaromatics produced temporary, reversible affects on food detection whereas PAHs produced more permanent, irreversible effects. The fact that *G. pulex* also consumed less acid-extract spiked food would also suggest that they may avoid the metal-contaminated food. Leaching of metals off the spiked leaf material

(Brown, 1977; Xu and Pascoe, 1994) and the subsequent avoidance of the metal toxicants may possibly explain this result. However, avoidance experiments with acid sediment extracts provided no evidence of avoidance (section 4.3.3.). The reasons for avoidance of food spiked with the downstream sediment acid extract are therefore unclear.

The final question addressed in this part of the study concerned the uptake route of toxicants in the motorway runoff. Reductions in consumption due to physiological stress are often due to uptake of the toxicant into the tissues which may result in cellular damage such as digestive tubule dilation and breakdown (Krishnakumar *et al.*, 1990). Choice experiments indicated that *G. pulex* do not generally discriminate between 'contaminated' and 'clean' food and consumption experiments indicated that they ate as much 'contaminated' as 'clean' leaf material when fed in non-contaminated water. Contaminants on detrital material generally reach concentrations higher than in the water and as high, or possibly higher, than the sediments (Williams and Murdoch, 1969; Xu and Pascoe, 1994). For instance, Odum and Drifmeyer, (1978) found marsh-grass detritus near a road had Pb concentrations of 1415 µg/g and Eadie *et al.* (1988) found that PAH concentrations of detrital material were approximately a factor of 5-10 times higher than in fine (<53µm) sediments. This may be related to the fact that high concentrations of organic carbon in detritus binds pollutants (Karickhoff, 1984). The uptake of toxicants may be due to microbial uptake (adsorption and metabolic absorption), association with detrital and microbial lipids, electrostatic adsorption or the formation of complexes and chelates at active sites on the detritus (Odum and Drifmeyer, 1978; Duddridge and Wainwright, 1980; Pinkney *et al.*, 1985; Abel and Bärlocher, 1988). Animals feeding on contaminated detritus have often been found to accumulate the highest concentrations of contaminants (Parkman and Meili, 1993). This may either be due to uptake from food or a consequence of their close association with contaminated sediments (Porte and Albaieges, 1994). For instance, Van Hassel *et al.* (1980) found that road-derived Pb in macroinvertebrate tissue was not related to diet but to the degree of sediment contact. The relative importance of sediment, food or water as a source of toxicants to aquatic organisms has been a point of controversy among ecologists for many years (Knezovich *et al.*, 1987). Although concentrations of Zn and Cd were significantly elevated in water spiked with acid sediment-extract and Cu and Pb were significantly elevated on spiked leaf material, there was no evidence of the uptake of metals by *G. pulex* from either of these sources.

In the current study there was also little evidence of the uptake of total aromatic hydrocarbons from contaminated food material. Animals exposed to contaminated water did have higher hydrocarbon tissue concentrations when fed contaminated rather than uncontaminated food although this may have been due to inadequate correction for contaminated material in the gut of the animals. This study estimated the gut contents to be responsible for 2.5 % of the total wet weight of the animal. Sutcliffe *et al.* (1981) attributed 6.7-6.8 % of the total weight of *G. pulex* to the full gut contents when fed on leached elm (*Ulmus carpinifolia*). Moreover, tissue hydrocarbon concentrations were not elevated in animals fed contaminated leaf material in the clean water treatment. There was, however, significantly higher concentrations of total aromatic hydrocarbons in animals exposed to water spiked with the downstream sediment solvent extract than those exposed to water spiked with the carrier solvent only. These results indicated that water was the major source of aromatic hydrocarbons to *G. pulex*. Given that the exposure concentration used in these experiments was approximately 2.5 times that of sediment interstitial water concentrations (A.B.A. Boxall, pers. comm.) accumulation by *G. pulex* in the field is possible from sediment interstitial waters (Roesijadi *et al.*, 1978b; Oliver, 1987; McElroy *et al.*, 1990). The majority of previous studies in both the aquatic and terrestrial environments have suggested that the uptake of hydrocarbons via the food is low thus supporting the findings of the current study (Anderson *et al.*, 1977; Rossi, 1977; Oliver, 1987; Van Brummelen *et al.*, 1991). Roesijadi *et al.* (1978a) concluded that the position of feeding rather than the food source itself was the factor that resulted in deposit feeders accumulating hydrocarbons more than suspension feeders since the former were exposed to high interstitial water concentrations.

Interestingly the aromatic hydrocarbon concentrations of faecal material produced by animals consuming leaf material spiked with downstream sediment solvent extract were three times higher than that of the leaf material itself and over ten times greater than that of the faecal material produced by animals consuming uncontaminated leaf material. Similarly Zn, Cu, and Cd concentrations in the faecal pellets of animals feeding on leaf material spiked with downstream sediment acid extract were between 1.5 and 3 times greater than those of the leaf material itself. Lasenby and Van-Duyn (1992) concluded that little Cd or Zn was assimilated from contaminated food by the mysid shrimp *Mysis relicta* and most was egested in the faecal pellets. Similarly Corner *et al* (1973) observed that much of the naphthalene administered on food was lost in the faeces where it accounted for 18.8% of the total dry weight of faeces. The reason for the high toxicant concentrations on the faeces may be due to breakdown and adsorption of the leaf material leaving the ratio of toxicant to remaining undigested material high. It is also possible that the animals may select areas of leaf material that are high in organic

value such as fungal patches rejecting other patches, such as leaf veins (Arsuffi and Suberkropp, 1985; Webster and Benfield, 1986). These patches are likely to have high pollutant concentrations due to the association of the contaminants with fungal lipids and other organic chemicals (Bärlocher, 1993).

Abnormal feeding responses of animals provide an initial indication of physiological stress that may lead to eventual growth retardation (Sheehan *et al.*, 1984). The fact that consumption is reduced in the short term may not, necessarily, be relevant to reduced growth, reproduction and survival if other physiological processes such as respiration and food assimilation are adjusted (Schindler, 1971; Sandheinrich and Atchison, 1990). If the food quality is poor then *G. pulex* may be able to compensate by reducing respiration rates (Graça *et al.*, 1993) or by increasing assimilation efficiencies (Arsuffi and Suberkropp, 1988). For instance, the feeding rate of *Gammarus pulex* was significantly reduced during dosing of a stream with ammonia but this recovered (but not to the original levels) post-exposure (McCahon *et al.*, 1991). However, exposure in the field is generally long-term and negative effects of toxicants on consumption, even in the short term, have been shown to affect growth, fecundity and survivorship. Maltby and Naylor (1990), for instance, demonstrated that Zn stress reduced SfG (mostly feeding) of female *G. pulex* which subsequently had a negative effect on offspring size and resulted in increased abortion rates.

Reduced energy consumption may also have implications on other processes, such as pollutant detoxification mechanisms which require additional energy (Calow, 1991). The combined effects of increased activity due to toxicant avoidance (Chapter 4) and reduced consumption may put an even greater nutritional stress on the animal.

5.4.1. Conclusions.

The major findings of this part of the study were:

1. *In-situ* consumption of leaf material by *G. pulex* was reduced downstream of the discharge at Pigeon Bridge Brook.
2. Direct toxicity of motorway-contaminants to *G. pulex*, as apposed to indirect effects on food quality, was the major mechanism reducing feeding activity by this species.
3. Toxicant accumulation on leaf material after pre-exposure to water and sediment at the downstream station at Pigeon Bridge Brook in *in-situ* and laboratory experiments had no effect on consumption in the laboratory. However, absorption

and the absorption efficiency of this material was increased in some cases. In laboratory assessments leaf material 'conditioned' at the downstream station was consumed and absorbed less than material 'conditioned' at the upstream station after 27 d. This corresponded with lower numbers of fungal species colonising the leaf material (section 2.3.6).

4. Consumption of leaf material by *G. pulex* was reduced when animal and food were in direct contact with downstream sediments in laboratory experiments. Solvent (DCM) extracts of downstream sediment appeared to be responsible for the effects on *G. pulex* feeding. This was shown to be related to total aromatic hydrocarbon concentrations in the sediment with a EC_{50} (for reduced consumption relative to control) of 51.72 µg chrysene equivs. /L. Acid sediments extracts had no effect on the consumption of leaf material by *G. pulex*.
5. *G. pulex* appeared to make no choice between 'contaminated' and 'non-contaminated' leaf material (except when toxicant availability was high) in laboratory choice experiments. They did, however, prefer material that was potentially 'more-conditioned' (higher numbers of fungal species-section 2.3.6).
6. The uptake of aromatic hydrocarbons by *G. pulex* was principally from aqueous sources and not from the food.

The motorway contaminants associated with the sediments appear to have a direct effect on the feeding activity of *G. pulex*. The solvent (DCM) extracted fraction of downstream sediments containing aromatic hydrocarbons was responsible for the majority of the reduced consumption and the main uptake route of hydrocarbons was from the water rather than from the food. In the field, accumulated contaminants probably originate from sediment interstitial waters. Indirect effects of food quality on feeding, due to pollutant accumulation on leaf material were not important. However, food quality and subsequently consumption may be reduced as a consequence of reduced conditioning of leaf material below the discharge during the middle periods of conditioning. Moreover, high toxicant concentrations on leaf material may affect leaf choice, possibly through avoidance of toxicants leaching off the leaf material.

CHAPTER 6.

GENERAL DISCUSSION.

Pollutants entering streams may have considerable impacts on community structure and functioning (Sheehan *et al.*, 1984). One potentially important source of pollutants is runoff from roads which contains a complex mixture of organic and inorganic contaminants (e.g. Van Hassel *et al.*, 1980; Sartor and Boyd, 1975; Harrison and Johnson, 1985; Baekken, 1994). The concentrations of the contaminants in streams are dependent upon a number of site specific factors, including traffic density and area of road drained and are often greatest in small receiving streams.

The central hypothesis of the current study was that the contaminants that accumulate in stream systems below motorway runoff discharges would have a negative impact on community structure with ensuing effects on ecosystem function. As runoff contaminants accumulate in sediments it was predicted that effects were most likely to occur in communities and processes that were intimately associated with stream sediments. In particular, it was postulated that benthic macroinvertebrate diversity, abundance and activity would be reduced below road runoff discharges and that macroinvertebrate-mediated leaf processing would subsequently be impeded. Additionally it was hypothesised that the streams most severely impacted would be small streams receiving drainage waters from large areas of heavily used road (e.g. motorway) and that impacts would be due to a small number of chemicals.

Of the three sites in the current study, Pigeon Bridge Brook, drained the largest area of motorway surface (44,389 m^2) was the smallest stream studied. As predicted it had the highest toxicant load in stream water and sediments below the motorway discharge (Maltby *et al.*, 1995a) and was the only site that exhibited an apparent deleterious effect on macroinvertebrate community structure. Species richness and diversity were reduced at the downstream station and species generally considered more sensitive to pollutants, such as stoneflies and gammarids, showed a reduction in relative abundance whereas more tolerant chironomids and tubificids increased in relative abundance. In addition, biotic scores were reduced at the downstream station as a consequence of the eradication of some caddisfly, beetle and dipteran groups that were present upstream. Several previous studies have demonstrated that macroinvertebrate diversity is reduced below roads, generally with more sensitive groups such as stoneflies, crustaceans, caddisflies, beetles and molluscs being lost from the system and more tolerant groups such as orthocladiinae chironomids and tubificids proliferating (Shutes, 1984, Cowley, 1985; Mudre, 1985; Davis and George, 1987; Bellamy, 1990; Field and Pitt, 1990).

Macroinvertebrate community changes reported in this study may, or may not, result in changes in ecosystem functioning. One process that can occur in contaminated systems is the replacement of sensitive species with functionally similar but less sensitive species (Sheehan *et al.*, 1984; Warwick, 1992). In addition, the fact that species overlap in important ecological functions can mean that some species can be lost from the system without any deleterious consequence on function (i.e. functional redundancy). This preserves overall functional properties of the system such as productivity and nutrient cycling. For example, DeNoyelles *et al.* (1985) found only short-term changes in planktonic chlorophyll *a* and photosynthesis in an atrazine-contaminated system whereas there was a long-term shift in community composition to a more resistant assemblage of algae. However, some communities may have low functional redundancy or the stress may be so great that redundancy cannot compensate for the reductions in ecosystem function (Frost *et al.*, 1995). In such situations shifts in community structure will translate into effects on community functioning.

One measure of community structure that has direct implications for ecosystem function is the assessment of the trophic characteristics of the community. A functional assessment of the macroinvertebrate communities at Pigeon Bridge Brook indicated that the community changed from a mixed community of predators, shredders, scrapers and collector-gatherers at the upstream station to one dominated by collector-gatherers downstream of the discharge. No between-station differences in functional feeding groups (FFG) were observed at either Butterthwaite Ditch or Rockley Dike. No previous studies have assessed the effect of motorway runoff on functional aspects of the macroinvertebrate community. The reduction in shredders at the downstream station at Pigeon Bridge Brook was associated with a reduction in macroinvertebrate-mediated processing of leaf material indicating that community structure was intimately linked to ecosystem function. Tubificid worms are able to process large amounts of coarse particulate organic matter (CPOM; Chauvet *et al.*, 1993), however their high abundance at the downstream station at Pigeon Bridge Brook did not compensate for the loss of shredders. Functional redundancy or functional replacement did not, therefore, occur at this site possibly as a consequence of severe long-term contamination (Sheehan, 1984). In accordance with the lack of between-station differences in macroinvertebrate species number, diversity and FFG at Butterthwaite Ditch and Rockley Dike there were no between-station differences in macroinvertebrate-mediated leaf processing at these sites.

In addition to reduced abundance of shredders, decreases in macroinvertebrate-mediated leaf processing rates could be due to reduced feeding activity by shredders as a consequence of changes in food quality. Food quality can be reduced due to an impact of the motorway contaminants on the microbial communities that partially hydrolyse the leaf material making it more palatable to macroinvertebrate shredders. For example, Bermingham (1993) demonstrated that mine effluents were toxic to some aquatic hyphomycete fungi and changed the fungal assemblage on leaf material. This resulted in reduced consumption rates of this material by shredders in the laboratory. In the current study the assemblage, biomass and activity of fungi on the leaf material in field surveys were not negatively affected by the motorway discharge indicating conditioning had little effect on leaf processing by macroinvertebrates at this site.

Many biotic and abiotic factors, such as substrate and trophic status, can affect macroinvertebrate community structure in streams so the observed affects may not necessarily be due to toxicological impacts. Some studies have suggested that downstream of motorway discharges increases in the abundance of fine sediment dwellers were a result of increases in fine sediment deposition (Extence, 1978; Smith and Kaster, 1983). However, even though substrates were coarser downstream of the discharge at Pigeon Bridge Brook, changes in community structure were similar to that reported in previous studies. It is, therefore, unlikely that substrate preferences were the determinand affecting community changes at this site (see section 2.4). Previous studies also suggest that increased loads of inorganic sediment may result in nutrient poor sediments and hence impoverished benthic communities (Carter and Knisley, 1984; Stout and Coburn, 1989). Although total organic carbon (TOC) content of the sediment was lower at the downstream station at Pigeon Bridge Brook, concentrations were similar to those recorded at Butterthwaite Ditch and Rockley Dike (Maltby *et al.*, 1995a). Moreover, TOC concentrations were within the 'normal' concentration range for stream sediments which support diverse macroinvertebrate communities and are, therefore, not considered to be a major reason for the observed changes in macroinvertebrate communities (Burton, 1993).

It was evident from the field surveys that the motorway discharge had an impact on macroinvertebrate community structure and function although this was only significant at the site which had the highest concentrations of motorway derived contaminants in the stream sediments and water (Maltby *et al.*, 1995a). However, to conclude that these contaminants were responsible for the observed biotic changes is invalid. Only through controlled laboratory investigation can causal relationships be established. To investigate possible causal relationships toxicological assessments of the motorway

contamination were carried out using animals from different trophic groups with differing habitat preferences.

Field and laboratory lethality studies indicated that sediments from the downstream station at Pigeon Bridge Brook were slightly, but significantly, toxic to *G. pulex* and *N. cinerea* but were not toxic to either *P. jenkinsi*, *C. riparius* or *T. tubifex*. Sediment manipulation and sediment-extract exposures indicated that lethal toxicity to *G. pulex* was attributable to the dichloromethane (DCM) extractable fraction of the sediment. In contrast, the other species tested were insensitive to DCM sediment extracts in lethality studies even at concentrations which caused > 85 % mortality of *G. pulex*. Mortality of *G. pulex* was significantly correlated to the concentrations of total aromatic hydrocarbons in the extract and in *G. pulex* tissues. The DCM fraction contained high concentrations of polycyclic aromatic hydrocarbons (PAHs) which have subsequently been shown to be responsible for the majority of the toxicity to *G. pulex* (Maltby *et al.*, 1995b).

It may be surprising that the lethal toxicity of sediments to the test species was low considering the high concentrations of metal and hydrocarbon contaminants recorded downstream of the motorway discharge at Pigeon Bridge Brook (Maltby *et al.*, 1995a). However, many factors control the bioavailability of toxicants in sediments and therefore their toxicity to animals in the field (section 2.4). Although lethal toxicity in the laboratory was low, field surveys indicated that the distribution of some of species was impacted by the discharge. Laboratory tests often only use particular life stages, usually adult animals, and tests are often short-term lethality assessments of toxicity. The toxicants in road runoff may effect more sensitive life stages and/or only elicit toxicity over longer time periods. In fact spiking experiments using DCM extracts indicated that contaminants in sediments were potentially very toxic to *G. pulex*. In performing experiments in controlled laboratory conditions possible causative effects present in the field may be removed. For instance, the impact of suspended sediment or photoinduced toxicity of organic chemicals. Large amounts of inert suspended sediments in the discharge or the re-suspension of stream bed sediments by the turbulent discharge can cause lethal or sub-lethal effects (e.g. drift) in streams (Newcombe and MacDonald, 1991). Photooxidation may be a significant factor in systems polluted by PAHs and the potential for this to occur in the field has recently been demonstrated (Monson *et al.*, 1995). Photooxidation of PAHs by U.V. in sunlight can increase the toxicity of PAHs by several orders of magnitude due to the formation of more toxic metabolites or degradation products (Landrum *et al.*, 1987; Burton, 1993; Brooke *et al.*, 1994). This activation can occur either outside or within the animal

and therefore the life-history/ behaviour of the animal may be important in determining exposure to U.V. radiation (Burton, 1993). The major PAHs that accumulated in sediments at Pigeon Bridge Brook were pyrene and fluoranthene and it is these hydrocarbons (plus anthracene) which seem to be most susceptible to photoactivation (Evans *et al.*, 1990; Takada *et al.*, 1990; Burton, 1993; Maltby *et al.*, 1995a). Initial tests in this laboratory have indicated that the PAH-contaminated sediments collected from the downstream station at Pigeon Bridge Brook show significantly enhanced toxicity to *G. pulex* as a result of photoactivation by artificial U.V. In 10-d lethality tests sediment from the downstream station caused > 80 % mortality in UV light whereas sediments in the dark caused < 5 % mortality to *G. pulex* (Maltby, unpublished results). This is in agreement with PAH-contaminated sediment studies by previous authors and confirms the importance of PAHs as the major toxicants in road-runoff contaminated sediments (Burton, 1993; Ankley *et al.*, 1994a, b; Bell *et al.*, 1994). Previous authors have found that crustaceans are generally sensitive to hydrocarbons (Ibanga, 1987) and that *Gammarus* spp. are sensitive to urban and motorway runoff (Shutes, 1984; Cowley, 1985; Bellamy, 1990; Baekken, 1994).

The downstream sediments at Pigeon Bridge Brook were not only contaminated with hydrocarbons but also with a number of metals (Maltby *et al.*, 1995a). The majority of the metals in the sediments appeared to be non-extractable, even with dilute acid and consequently the acid extract was non-lethal to all of the test species. Extraction of downstream sediments with solvents removed hydrocarbons whilst leaving the majority of metals. However, solvent-extracted sediment did not reduce the survival of *G. pulex* whilst non-extracted sediment did. These results confirm that the bioavailability of the metals in contaminated sediment was low and/or below lethal concentrations.

The laboratory sediment exposures were fairly short-term and sediment extract exposures were of a 'worst-case' type designed to demonstrate the potential for toxicity. Although the difference in sensitivity to sediments and sediment extracts demonstrated a possible explanation for the distribution of *G. pulex, T. tubifex* and *C. riparius* at Pigeon Bridge Brook it doesnt explain the reduction in relative abundance of *N. cinerea* and *P. jenkinsi* at the downstream station. Laboratory ecotoxicological assessments often use lethal endpoints but these are not always useful for extrapolation to field situations (Maltby and Calow, 1989). Environmentally realistic concentrations of contamination over long periods of exposure often initially produce sub-lethal or long-term responses.

One of the first sub-lethal responses of macroinvertebrates to toxicants is avoidance (Beitinger and Freeman, 1983). Experiments were therefore designed to assess the avoidance response of the five test species to contaminated sediments. *G. pulex*, *P. jenkinsi* and *C. riparius* all avoided downstream field sediments whereas *N. cinerea* showed no avoidance and *T. tubifex* showed a slight preference for downstream sediments over upstream sediments. Evidence from solvent-extracted sediments, sediment extract exposures and DCM-extract spiked sediments all suggested that the toxicants responsible for the avoidance were in the DCM sediment extract. Again, this suggests that aromatic hydrocarbons were probably responsible for this reaction. Further studies not reported here have confirmed that a fraction of the DCM sediment extract containing predominantly PAHs was responsible for the observed avoidance shown by *G. pulex* (D.M. Forrow and A.B.A. Boxall, unpublished results). In concordance with lethality studies, acid sediment-extracts and solvent-extracted sediments caused no avoidance response with most of the test species. The only exceptions being *P. jenkinsi* that preferred upstream sediment to DCM-extracted downstream sediment and *T. tubifex* which preferred non-extracted downstream sediment to extracted downstream sediment. Metal concentrations in acid sediment extracts were generally lower than those that have previously been reported as causing avoidance responses (Costa, 1966; Abel and Green, 1981). However, Cowley (1985) did demonstrate that *G. pulex* responded to aqueous Zn in a motorway-runoff contaminated stream by increasing drift and postulated that this was one possible reason for the reduction in the abundance of this species in the stream. Therefore, metals in aqueous phases may possibly be important in causing avoidance responses in some road runoff impacted streams.

Lethal and sub-lethal toxicological assessments went some way in explaining the field distribution of the test species used in the assays. Downstream sediments produced lethal and/or sub-lethal (avoidance) responses with *G. pulex* and *P. jenkinsi* which may explain their reduced abundance at the downstream station at Pigeon Bridge Brook. *T. tubifex* did not respond in either lethal or sub-lethal (avoidance) laboratory exposures which may explain their high abundance at the downstream station. *N. cinerea*, however, was found to be generally insensitive in laboratory exposures but had reduced relative abundance downstream of the discharge, and results in the current study cannot explain the absence of this species. Avoidance tests indicated that chironomids were reasonably sensitive to the organic fraction of the sediment in both whole sediment and DCM sediment extract exposures. This is contrary to their high relative abundance at the downstream station at Pigeon Bridge Brook. One possible explanation for their distribution is that these animals may become desensitised to the toxicants and

opportunistically colonise available habitats (Hartwell *et al.*, 1988). They may also be able to avoid highly-contaminated sediment patches in the stream (Wentsel *et al.*, 1978).

Toxicants not only affect the distributions of animals in streams but they may also affect their activity. If this activity is important in nutrient processing then it may have consequences on the nutrient dynamics of the ecosystem as a whole. One such important process in lotic ecosystems is the processing of detrital material which is controlled, to a large extent, by macroinvertebrate feeding. At Pigeon Bridge Brook the dominant shredding macroinvertebrate was *Gammarus pulex* which was obviously sensitive in lethal and sub-lethal (avoidance) exposures to runoff contaminants. It was hypothesised that the motorway runoff would have sub-lethal effects on the feeding activities of this species resulting in reduced macroinvertebrate-mediated leaf processing downstream of the motorway discharge. Experiments were therefore conducted to assess the direct effects of the motorway runoff on consumption by *G. pulex* and to investigate whether contamination affects food quality and food choice.

Food quality can be affected by the accumulation of toxicants on the leaf material or by toxicant-induced reductions in microbial conditioning. Although leaf material accumulated toxicants when placed in contact with downstream water and sediment, this did not affect choice or consumption of the material by *G. pulex*. However, between-station differences in conditioning (*viz.* number of fungal species) of leaf material at Pigeon Bridge Brook did have an effect in laboratory assessments. Downstream-inoculated (*viz.* conditioned) material was eaten less in choice experiments and consumed less in consumption experiments than upstream-inoculated (*viz.* conditioned) material. It appears that food quality is affected therefore, principally by a retardation in colonisation by conditioning hyphomycetes rather than an accumulation of pollutants. Previous studies have obtained similar results (Giesy, 1978; Gray and Ward, 1983). For instance, Bermingham (1993) found that indirect toxic effects of a mine effluent on food quality as a result of the toxic effects on hyphomycetes fungi reduced consumption of leaf material by *G. pulex*, whereas metal toxicant accumulation on leaf material had no effect on consumption. If food quality is not satisfactory, an animal may expend considerable energy searching for good quality food, increasing energy expenditure and reducing its nutritional state (Buikema and Benfield, 1979).

It is quite likely that in lotic systems material that is conditioned upstream of a discharge will be washed down and be available for macroinvertebrates in the contaminated reach. Consequently, an impact on leaf conditioning in the impacted zone may not limit the

availability of high quality food. Affects on shredder feeding due to direct sub-lethal were therefore considered to be the major mechanism reducing the feeding activity of *G. pulex*. Direct sub-lethal toxicity of motorway-derived pollutants had a negative effect on the consumption of leaf material by *G. pulex* in field exposures. Laboratory exposures indicated that the majority of this toxicity originated from the sediments and was attributed to the DCM-extractable fraction of downstream sediments. Consumption decreased in concentration-related manner with increasing aromatic hydrocarbon concentration with a 6-d EC_{50} (reduced consumption relative to control) of 51.72 µg chrysene equivalents/L. Toxicant-induced reductions in feeding may result in nutritional deficiencies making the animal less fit and more susceptible to toxic chemicals and other stresses (Sheehan, 1984).

Since *G. pulex* neither avoided nor consumed less leaf material contaminated by metals and hydrocarbons there is the potential for bioaccumulation from their food. However, there was no evidence that either metals or total aromatic hydrocarbons were accumulated from the food in contaminated food/water exposures. In contrast, *G. pulex* demonstrated significantly increased uptake of aromatic hydrocarbons from spiked water in contaminated food/water exposures and DCM sediment extract exposures. Further this uptake into tissues was significantly correlated with mortality of *G. pulex*. These results suggest that that *G. pulex* takes up runoff contaminants from the aqueous phase.

Coarse and fine particulate organic matter (CPOM and FPOM) and dissolved organic matter (DOM) absorbs PAHs and metals more than inorganic particles thus reducing their bioavailability to macroinvertebrates (Herbes, 1977; McCarthy and Bartell, 1988; Leversee *et al.*, 1982; Kukkonen and Oikari, 1991). The presence of CPOM, FPOM and DOM also reduce photo-induced toxicity due to shading (Gensemer, 1994). It may therefore be environmentally beneficial to increase the CPOM load of contaminated streams by maintaining a suitable canopy. In addition, the enzyme systems of microbes on leaf material which have evolved to degrade aromatic compounds in the leaf material (particularly ligninases) may also be capable of degrading aromatic xenobiotics such as organohalides and PAHs (Bumpus *et al.*, 1985; Haemmerli *et al.*, 1986; Bumpus and Aust, 1987; Bumpus, 1989). Accumulation of toxicants on leaf material may therefore bring them into contact with organisms capable of their degradation without increasing their availability to shredders.

However, if increased CPOM inputs, as leaf fall, into streams is to be considered as a remediation measure careful consideration of site-specific properties of the system must

be taken into account. If litter is excessive it may accumulate in the stream causing problems of efficient drainage. In addition, although the motorway runoff toxicants may not be generally available in consumed food, transfer to non-shredder trophic groups through FPOM, DOM and faeces must be assessed.

The results from this study demonstrate that motorway contaminants at Pigeon Bridge Brook reduced shredder abundance and feeding activity. Consequently, inefficient processing of leaf material in an ecosystem may result in increased export of CPOM particularly in systems which are subject to drainage flushes (Hellawell, 1988a; Wallace *et al.*, 1991). This may have further ramifications in contaminated systems since the trophic status of a system affects the bioavailability of toxicants (McCarthy *et al.*, 1985; McCarthy and Bartell, 1988). Further, the breakdown of CPOM by shredders may influence microbial colonisation and activity on CPOM and FPOM (Morrison and White, 1980; Smith *et al.*, 1982; Wensen, 1989). As shredders comminute organic matter, the FPOM and faeces produced provide an important food supply for fine-particle feeders such as filter feeders and collector-gatherers (Short and Maslin, 1977; Vannote *et al.*, 1980; Richardson and Neill, 1991). DOM and mineral nutrients produced by these organisms and by the shredders themselves, are used as a nutrient source by primary producers (Woodall and Wallace, 1975; Meyer and O'Hop, 1983; Sheehan, 1984). Therefore, reductions in the feeding activities of shredding macroinvertebrates will have an impact on the entire stream ecosystem.

In conclusion, the main hypothesis of the current study was that road-runoff contamination would have a negative impact on macroinvertebrate community structure and function in streams. It was predicted that this would be most evident in small streams that received drainage from large areas of heavily used roads (e.g. motorways). The results presented indicate that this is indeed the case and offer causative mechanisms for the effects observed at an impacted stream. The major findings of the current study were that motorway runoff had a deleterious effect on macroinvertebrate leaf processing as a result of:

1. decreased abundances of shredding macroinvertebrates as a result of lethal and sub-lethal (avoidance) toxicity originating from the sediments and attributed to aromatic hydrocarbons;
2. decreased feeding activity of the major shredder, *G. pulex* in the system due to both reduced food quality as a result of changes in fungal colonisation and, more importantly, a direct effect of aromatic hydrocarbons on the physiology of the animal.

Although Pigeon Bridge Brook can be regarded as a 'worst case situation' in that it was a very small stream receiving drainage from a large area of heavily used road, this study demonstrates the potential for road runoff-derived impacts on macroinvertebrate community structure and function. Lethal effects of contaminated sediments were small and probably localised, however, sub-lethal experiments and the possibility of photoinduced toxicity indicated the potential for impacts at low concentrations of sediment-derived contaminants. Runoff from roads provide a diffuse, but frequent source of contaminants to freshwaters (Evans *et al.*, 1990). The results of this study demonstrate the potential effects of such discharges, which given the ever increasing traffic densities, should not be ignored (Dept. of Transport, 1993).

Appendix A2.1.: Summary of stream water and sediment chemistry over the period October 1990-July 1991 (With kind permission of A.B.A. Boxall, Unpublished results).

Table A2.1.1: Physicochemical characteristics of stream water upstream (Up) and downstream (Dw) of motorway runoff discharges at the three study sites over the period October 1990-July 1991. Data presented as median values and an asterisk denotes a significant between-station difference.

	ROCKLEY DIKE		BUTTERTHWAITE DITCH		PIGEON BRIDGE BROOK	
	Up	Dw	Up	Dw	Up	Dw
pH	8.19	8.23	7.59	7.69	7.48	7.21
Temp. (^{o}C)	9.25	9.0	8.45	8.3	8.73	9.3
Dissolved O_2(mg/l)	11.7	11.8	10.8	11.2	10.5	9.6
Salinity ($^{o}/_{oo}$)	0.63	0.63	0.38	0.35	0.5	1.0
Conductivity (µmho)	612	567	1600	1350	438	925
PO_4^{3-}(mg/l)	1.4	1.47	1.08	0.77	0.49	0.16
SO_4^{2-}(mg/l)	229.7	247.9	114.4	103.6	60.4	111.6*
NO_3(mg/l)	44.6	44.0	21.8	20.5	66.6	74.2
Cl^-(mg/l)	105.7	112.1	86.2	86.4	65.1	229.1*

Table A2.1.2.: Mean metal concentration in the stream sediment (µg/g dry wt.) and water (µg /L) at stations upstream (Up) and downstream (Dw) of motorway runoff discharges at the three study sites over the period October 1990-July 1991. Asterisks indicate significant between-station differences between either stream sediments or water.

A.) Rockley Dike.

	SEDIMENT		WATER	
	Up	Dw	Up	Dw
Al	17320	10870	87.6	94.5
Fe	135610*	99230	1140	1820
Cr	20.81	26.83	2.42	3.17
Pb	47.3	99.7	6.74	76.69
Ni	99.16	103.37	9.49	8.66
Mg	6370	4360	78500	65500
Cu	36.29	72.29	34.7	14.1
Cd	1.41	1.988*	0.05	0.073
Zn	334.47	374.96	90.7*	36.3
Ca	12600	7590	180000	115000

B.) Butterthwaite Ditch.

	SEDIMENT		WATER	
	Up	Dw	Up	Dw
Al	14920	10480	139	137
Fe	137000	75770	2010*	880
Cr	22.59	73.5*	2.69	1.05
Pb	73.5	164.1*	16.8	38.7
Ni	60.51	66.42	12.5	11.9
Mg	4250	7630	59900	48700
Cu	30.14	74.07	28.1	47.7
Cd	2.44	2.85	0.106	0.328
Zn	377.58	396.82	137.2	62.3
Ca	8140	12740	99000	109900

C.) Pigeon Bridge Brook.

	SEDIMENT		WATER	
	Up	Dw	Up	Dw
Al	11980	33440	103	179
Fe	55260	51870	850	2200
Cr	20.7	98*	0.93	5.06
Pb	97.3	142.3*	7.01	22.18
Ni	62.8*	45.1	7.02	7.34
Mg	3910	12110	63720	7770*
Cu	37.43	57.57	21.8	47.44*
Cd	1.15	2.58*	0.054	0.165
Zn	152.3	342.8*	54.48	137.12
Ca	64600	49120	100120	140940*

(Appendix A2.1. cont.)

Table A2.1.3.: Mean total aromatic hydrocarbons concentrations (µg chrysene equivalents/ g wet-wt.) and specific PAHs (µg /g wet wt.) of sediments at stations upstream (Up) and downstream (Dw) of motorway runoff discharges at the three study sites over the period October 1990-July 1991. Asterisks indicate significant between-station differences.

	ROCKLEY DIKE		BUTTERTHWAITE DITCH		PIGEON BRIDGE BROOK	
	Up	Dw	Up	Dw	Up	Dw
Total aromatic hydrocarbons	203.68	283.72	36.54	279.7*	44.36	405.6*
Naphthalene	2.986	4.521	1.739	3.462	0.01	2.857*
Acenaphthylene	0.083	0.268	0.162	0.076	0.00	0.048
Acenapthene	0.910	0.906	0.387	1.251*	0.206	1.23
Fluorene	0.233	0.186	0.217	0.225	0.00	1.213
Phenanthrene	5.56	5.924	4.729	5.262	2.518	14.478
·Anthracene	1.809	1.782	1.280	1.693	1.103	5.977
Fluoranthene	1.615	2.188	0.860	2.277	0.16	18.488*
Pyrene	4.748	4.427	1.258	4.26	3.396	19.325
Benzanthracene	0.585	1.417	0.280	1.00	0.159	5.482
Chrysene	0.359	1.056	0.244	0.67	1.1	4.877
Benzfluoranthene	1.089	0.669	1.213	1.507	0.505	7.719
Benzpyrene	0.779	1.138	1.069	1.633	0.584	3.657

Appendix A2.2.: Macroinvertebrate taxa present in streams receiving runoff from the M1 motorway and sampled over the period October 1990- July 1991. Up = Upstream and Dw = Downstream sampling stations. * = present, -- = absent.

	Rockley Dike		Butterthwaite Ditch		Pigeon Bridge Brook	
	Up	Dw	Up	Dw	Up	DW
PLATYHELMINTHES						
Polycelis felina	*	*	--	--	--	--
ANNELIDA						
Naididae	*	*	*	--	*	*
Lumbriculidae	--	--	*	*	*	*
Tubificidae	*	*	*	*	*	*
Erpobdella octoculata	*	*	*	*	--	*
Glossiphonia complanata	*	*	--	--	--	--
Helobdella stagnalis	*	*	--	--	--	--
MOLLUSCA						
Potamopyrgus jenkinsi	*	*	*	*	*	*
Lymnaea peregra	*	*	*	*	*	*
Sphaeriidae	*	*	*	*	*	*
CRUSTACEA						
Gammarus pulex	*	*	*	*	*	*
Asellus aquaticus	*	*	--	*	--	--
CHELICERATA						
Hygrobatidae	*	*	--	--	*	--
Limnocharidae	*	--	--	--	--	--
UNIRAMIA						
Sialis lutaria	*	*	*	*	--	--
Baetis rhodani		--	*	*	--	--
Leuctra inermis	--	--	--	--	*	*
Apatania sp.	--	--	--	*	--	--
Halesus radiatus	--	*	--	--	--	--
Limnephilus extricatus	--	--	--	--	*	--
Micropterna lateralis	--	--	--	*	*	--
Plectrocnemia geniculata	--	--	*	*	*	*
Plectrocnemia conspersa		--	*	--	*	--

(Appendix A2.2 cont.)

Stenophylax permistus		--	*	*	--	--
Hesperocorixa sahlbergi		--	--	*	--	--
Sigara concinna		*	--	--	--	--
Velia caprai	--	--	*	*	*	--
Dytiscidae larvae	*	*	*	*	*	*
Elminthidae larvae	--	--	--	--	*	--
Helodidae larvae	--	--	--	--	*	--
Hydrophilidae larvae	--	--	--	--	*	--
Agabus sp.	--	--	*	*	*	*
Anacaena globulus	--	--	*	*	*	--
Hydroporus sp.	--	--	--	*	--	--
Halticinae sp.	--	--	--	*	--	--
Helophorus sp.	--	*	*	*	*	*
Hydrobius fuscipes	--	--	--	--	--	*
Ilybius guttiger	--	--	*	--	--	--
Culicidae	--	--	*	--	--	--
Dixidae	--	--	--	--	*	--
Empididae	--	--	--	--	*	--
Simuliidae	*	*	*	*	--	--
Tipulidae	*	*	*	*	*	--
Chironominae	*	*	*	*	*	*
Orthocladiinea	*	*	*	*	*	*
Prodiamesinae	*	*	*	*	*	*
Tanypodinae	*	*	*	*	*	*
Dicranota sp.	*	*	--	--	*	--
Eristalis sp.	--	--	--	*	--	--
Forcipomyia sp.	*	*	--	--	*	--
Pedicia sp.	*	--	--	--	--	--
Pericoma sp.	*	*	*	*	*	*
Ptychoptera sp.	--	--	--	*	*	--
Lepidoptera larvae	--	*	*	*	*	*

Appendix A2.3. Relative Importance Values (RIV) for hyphomycete fungi colonising leaf material at sites upstream and downstream of the M1 motorway.

Table A2.3.1.: Hyphomycete fungi colonising alder leaves deployed in the three streams in November 1990 (Section 2.3.4) at stations upstream (Up) and downstream (Dw) of the M1 motorway. Classes of RIVs are 1 = RIV<0.05; 2 = 0.05 < RIV < 0.1; 3 = 0.1 < RIV < 0.2; 4 = 0.2< RIV < 0.4; 5 = 0.4 < RIV < 0.8; 6= > 0.8.

	Rockley Dike		Butterthwaite Ditch		Pigeon Bridge Brook	
	Up	Dw	Up	Dw	Up	Dw
Alatospora acuminata	3	3	3	3	4	4
Anguillospora crassa					1	1
Anguillospora curvula					1	1
Anguillospora longissima			1		1	1
Anguillospora rosea	3	2	3	3	2	1
Articulospora tetracladia	1		3	2	1	
Clavariopsis aquatica	1			1		
Clavariopsis longibranchiata	2	2	1	1		
Culicidospora aquatica		1	2	1		3
Dendrospora sp.					1	
Flagellospora curvula	4	3	3	3		
Heliscus lugdenensis	4	3	3	4	2	3
Lemonniera terrestris	4	4	3	3	2	4
Lemonniera cornuta						1
Lemonniera aquatica	2	1	1		2	2
Lemonniera centrosphaera	1	2			3	3
Scorpiosporium minutum	3	3	3	3	3	3
Sympodiocladium frondosum					1	1
Tetrachaetum elegans	1	1			2	1
Tetracladium furcatum	1			1	1	1
Tetracladium marchalianum	4	4	4	3	4	4
Tetracladium setigerum		1	3	3	3	3
Tricelophorus sp.	1	2	1		1	2
Tricladium angulatum	2		1	2	3	1
Tricladium chaetocladium	3	3	2	2	3	1
Tricladium splendens	1	2	3	3		1
Tricladium varium		1				1
Vargamyces aquatica			1		2	1
Varicosporium sp.	2	2	2	2	3	

(Appendix A2.3. cont)

Table A2.3.2.: Hyphomycete fungi colonising alder leaf material deployed at Pigeon Bridge Brook in November 1991 (section 2.3.6) at stations upstream and downstream of the M1 motorway for up to 55 d. Classes are RIVs of 1 = RIV<0.05; 2 = 0.05 < RIV < 0.1; 3 = 0.1 < RIV < 0.2; 4 = 0.2< RIV < 0.4; 5 = 0.4 < RIV < 0.8; 6= > 0.8.

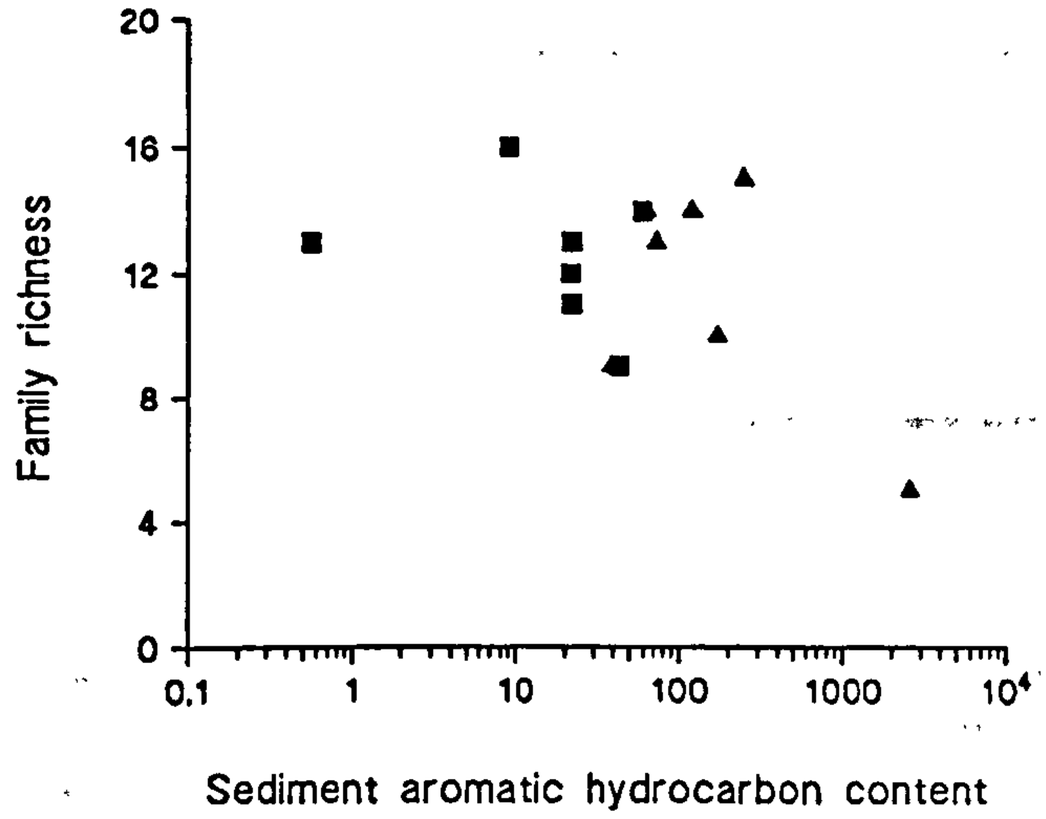
	UPSTREAM				DOWNSTREAM			
	Days				Days			
	13	27	41	55	13	27	41	55
Alatospora acuminta		3	2	2				2
Anguillospora crassa	4	2	3	5		4	3	4
Anguillospora longissima	3	2	4			3	4	4
Cylindrocarpon aquatica			2		3			
Dendrospora sp.	2							
Flagellospora curvula	2		2		3			2
Heliscus lugdenensis			3		3	3	2	
Isthmotricladia sp.								2
Lemonniera aquatica	3	2	4	2			3	2
Piricularia aquatica		2	2	2		4	3	1
Pythium spp.	6	5	4	4	6	6	6	3
Scorpiosporium minutum		4	3					1
Tetracladium marchalianum	2	4	2	4	4	3	5	4
Tetracladium setigerum	3	2	2				2	4
Tricladium angulatum	3	4	4	4			2	3
Tricladium chaetocladius	3	4	2					1
Vargamyces aquatica			3	3				2

Table A2.3.3.: Hyphomycete fungi colonising hawthorn leaf material deployed at Pigeon Bridge Brook in November 1991 (section 2.3.6) at stations upstream and downstream of the M1 motorway for up to 55 d. Classes are RIVs of 1 = RIV<0.05; 2 = 0.05 < RIV < 0.1; 3 = 0.1 < RIV < 0.2; 4 = 0.2< RIV < 0.4; 5 = 0.4 < RIV < 0.8; 6= > 0.8.

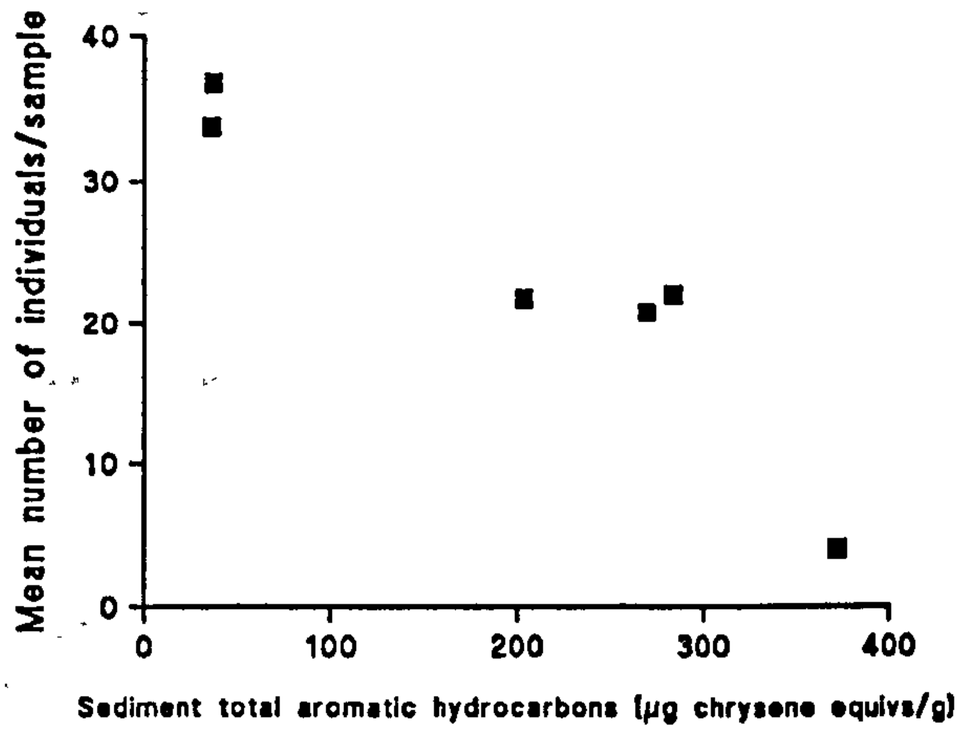
	UPSTREAM				DOWNSTREAM			
	Days				Days			
	13	27	41	55	13	27	41	55
Alatospora acuminata		3	3	4			3	3
Anguillospora crassa		4	4	4		3	5	4
Anguillospora longissima		3	4	4			4	2
Clavariopsis longibranchiata	2							
Cylindrocarpon aquatica	2			1				4
Dendrospora sp.		1						
Flagellospora curvula		3		1			2	2
Heliscus lugdenensis	3		2	1	3		2	4
Isthmotricladia sp.								
Lemonniera aquatica	2	3	4	3			2	
Lemonniera terrestris			1				1	
Piricularia aquatica		1	1	3		3	3	4
Pythium spp.	6	4	1		6	6	3	4
Scorpiosporium minutum	2	2	3	3				
Tetrachaetum elegans			1					
Tetracladium marchalianum			3	1			1	
Tetracladium setigerum		3	3	3			4	
Tricelophorus sp.		1						
Tricladium angulatum	4	4	4	4		3	3	3
Tricladium chaetocladius		2	3	1			1	3
Unknown 1	4							
Unknown 2	2							
Vargamyces aquatica	4		1	3				
Varicosporium sp.								2

Appendix A2.4.: Relationships between the mean number of macroinvertebrate families or abundance of individual species at the sampling stations and concentrations of total aromatic hydrocarbons or lead in the sediments downstream of the motorway discharges.

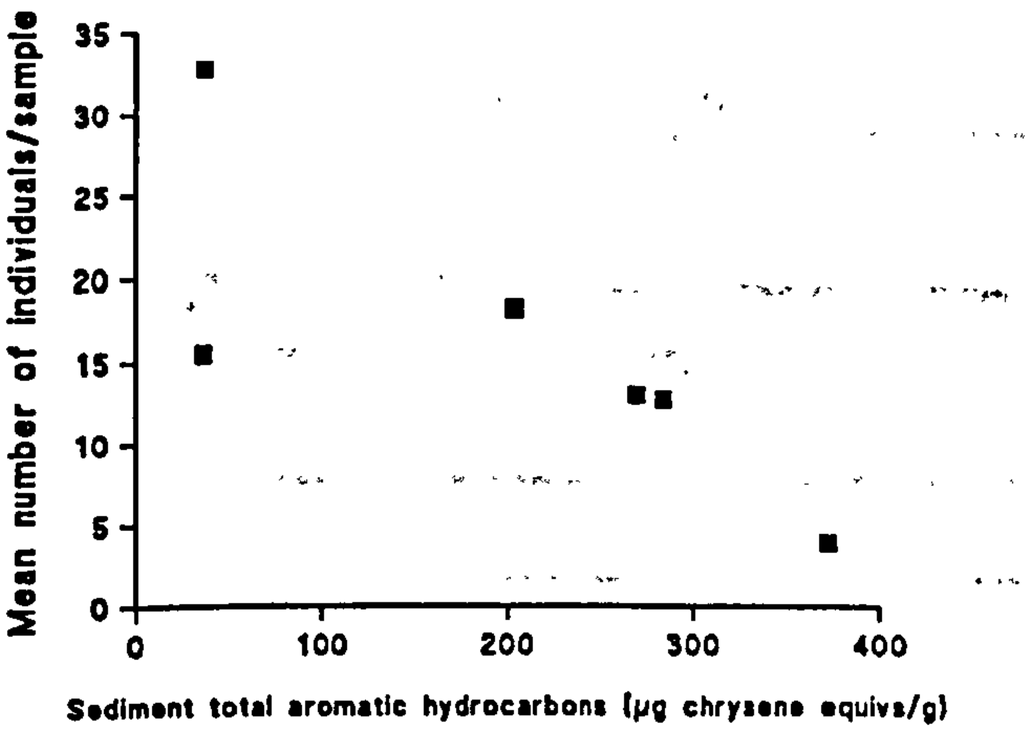
a.)



b.)



c.)



d.)

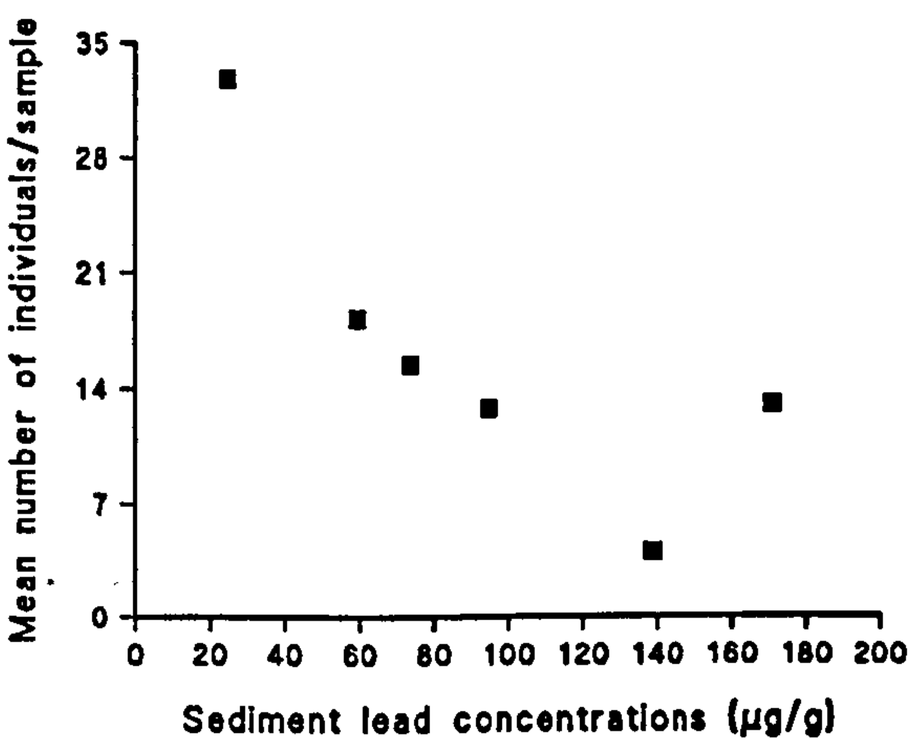


Fig. A2.4.1.: Relationships between a.) the number of macroinvertebrate families and sediment total aromatic hydrocarbon concentrations at seven sites receiving runoff-discharges from the M1 motorway in a wider study (Maltby *et al.*, 1995a). Squares represent upstream stations and triangles represent downstream stations. And relationships between b.) mean number of *P. jenkinsi* per sample and sediment total aromatic hydrocarbon concentrations; c.) mean number of *G. pulex* per sample and sediment total aromatic hydrocarbon concentrations; and d.) mean number of *G. pulex* per sample and sediment lead concentration at Rockley Dike, Butterthwaite Ditch and Pigeon Bridge Brook upstream and downstream stations in the current study.

Appendix A3. Recipes for Artificial Pond Water, Malt Extract Broth and Enriched Distilled Water.

Table A3.1: Artificial pond water (APW) comprised of 50 ml each of stock solutions 1-4 in 10 litres of distilled water.

	Compound	Concentration (g/L)
1.	$CaCl_2.2H_20$	58.80
2.	$MgSO_4.7H_20$	24.65
3.	$NaHCO_3$	12.95
4.	KCl	1.15

Table A3.2.: Plates of *Cladosporium* sp. fungus were grown in petri dishes containing 30 ml of malt extract broth at 15°C for approximately two weeks until the surface of the broth had an even mat of fungus. Discs of fungus were cut from the plates using sterile cork borers according to the methods in the general text.

Product	Quantity (g/L)
Malt extract (Oxoid®)	30
Mycological peptone (Oxoid®)	5

Table A3.3: Enriched Distilled Water comprised of 0.5 ml of Stock 1 and 0.1 ml of Stock 2 per litre of distilled water.

Stock	Compound	Concentration (g/L)
1.	$(NH_4)_2HPO_4$	66.04
	KH_2PO_4	68.05
	K_2HPO_4	87.09
2.	$MgCl_2.6H_20$	25.42
	$CaCl_2.2H_20$	18.38

Appendix A5.: Wet-weight/ dry-weight relationships for leaf material used in *in-situ* and laboratory consumption and food choice experiments (Chapter 5).

Table A5.: Summary details for the equations describing wet-weight/ dry-weight relationships used in seperate feeding and choice experiments in Chapter 5.

	Slope	Intercept	*r*	d.f.	Eqn.
Section 5.3.3.					
Upstream	3.62	5.64	0.93	43	A5.1
Downstream	3.02	21.1	0.95	43	A5.2
Section 5.3.4.					
Upstream⇒Upstream	3.43	13.7	0.84	23	A5.3
Downstream⇒Upstream	4.05	-8.03	0.96	23	A5.4
Upstream⇒Downstream	3.85	-3.60	0.93	23	A5.5
Downstream⇒Downstream	3.50	19.2	0.96	23	A5.6
Section 5.3.6.					
Upstream	3.32	3.30	0.95	33	A5.7
Downstream	3.52	3.91	0.98	33	A5.8
Section 5.3.7					
Upstream water	4.26	-7.50	0.98	28	A5.9
Upstream sediment	3.94	-9.14	0.92	28	A5.10
Downstream water	4.30	-10.0	0.99	28	A5.11
Downstream sediment	3.88	-4.92	0.98	28	A5.12
Section 5.3.8.					
13 d Upstream	3.11	-7.51	0.98	33	A5.13
13 d Downstream	1.91	22.0	0.77	33	A5.14
27 d	3.11	2.67	0.97	67	A5.15
41 d Upstream	3.77	-1.85	0.99	8	A5.16
41 d Downstream	2.41	-1.66	0.96	19	A5.17
Section 5.3.13.					
Upstream water	3.45	-2.35	0.96	18	A5.18
Upstream sediment	3.34	-1.38	0.97	18	A5.19
Downstream water	3.81	-3.66	0.99	18	A5.20
Downstream sediment	4.26	-7.50	0.98	18	A5.21
Section 5.3.14					
13 d	2.84	-0.04	0.92	75	A5.22
27 d Upstream	2.90	0.27	0.97	37	A5.23
27 d Downstream	2.84	0.18	0.95	37	A5.24
41 d Upstream	2.17	3.71	0.79	37	A5.25
41 d Downstream	1.66	14.0	0.50	37	A5.26

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THE EFFECTS OF MOTORWAY RUNOFF ON FRESHWATER ECOSYSTEMS: 1. FIELD STUDY

LORRAINE MALTBY,*† DAVID M. FORROW,† ALISTAIR B.A. BOXALL,†
PETER CALOW† and CLIFFORD I. BETTON‡
†Department of Animal and Plant Sciences, The University of Sheffield, P.O. Box 601, Sheffield S10 2UQ, U.K.
‡Castrol International, Burmah Castrol House, Pipers Way, Swindon SN3 1RE, U.K.

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Abstract—The effects of motorway runoff on the water quality, sediment quality, and biota of small streams were investigated over a 12-month period. Downstream of motorway runoff discharges there was an increase in the sediment concentrations of total hydrocarbons, aromatic hydrocarbons, and heavy metals and an increase in the water concentrations of heavy metals and selected anions. Hydrocarbon contamination of sediments was positively correlated with potential contaminant loading (i.e., length of road drained/stream size). The greatest effect was observed at Pigeon Bridge Brook, a small stream receiving drainage from a 1,500-m stretch of the M1 motorway. The dominant PAHs in contaminated sediment at this site were phenanthrene, pyrene, and fluoranthene, whereas the dominant metals were zinc, cadmium, chromium, and lead. Differences between the station upstream and downstream of discharges in the diversity and composition of the macroinvertebrate assemblages were detected in four out of the seven streams surveyed. However, there was no evidence of an effect on either the diversity or abundance of epilithic algae. The diversity of the aquatic hyphomycete assemblage was only affected at the most impacted site. Reductions in macroinvertebrate diversity were associated with reductions in the processing of leaf litter and a change from an assemblage based on benthic algae and coarse particulate organic matter to one dependent upon fine particulate organic matter.

Keywords—Road runoff Sediment contamination Benthic communities Hydrocarbons

INTRODUCTION

Road-vehicle-related activities produce a number of potentially toxic substances including oil and tar products, dioxins, oxygenated compounds, halogenated phenols, metals, deicing salts, and asbestos [1–9]. These contaminants are derived from a wide range of sources. For example, hydrocarbons are present in lubricating oils and fuels, whereas metals are present in fuel (e.g., lead), brake linings (e.g., copper), vehicle tyres (e.g., zinc and cadmium), and road deicing salts (e.g., copper and chromium) [10,11]. Contaminants from roads can enter river systems via runoff or atmospheric deposition, the relative importance of these two routes being dependent on the particular contaminant in question. Whereas low-molecular-weight polycyclic aromatic hydrocarbons (PAHs) are emitted mainly in the gas phase and are therefore dispersed in the atmosphere, higher-molecular-weight compounds are emitted in particulate form and are deposited on or near the road [12]. Other contaminants may be associated with crankcase oil and leak directly onto the road surface where they become associated with particulate material [13].

Road runoff therefore contains a complex mixture of potential toxicants that are discharged, untreated, into receiving waters. Although several studies have described the chemical composition of road runoff [5–7,10,14] relatively few detailed studies have been performed on the fate of these contaminants in receiving waters [15–17]. The potential impact of road runoff on receiving water quality will be dependent on several factors, including volume of traffic, rainfall, and size of receiving water. In Britain, there are presently over 380,000 km of road and more than 24 million vehicles [18]. Although major trunk roads and motorways only account for approx. 4% of the road network, they carry approx. 30% of road traffic [18]. Motorways in Britain are drained using a system of pipes or channels [19], which, whenever possible, drain into the nearest watercourse from point sources. Soakaways, pits into which runoff is diverted before percolating through the adjacent soil [19], are only installed where there are no suitable watercourses. Consequently, if road runoff does have a detrimental effect on receiving-water quality, this should be most apparent where motorway runoff directly enters small watercourses.

Despite the potential impact that road runoff may have on the biota of receiving waters [20,21], relatively few studies have assessed the effect of road runoff on freshwater communities [22,23] and none have investigated the implications of changes in community structure for ecosystem functioning. Many of the contaminants in road runoff are associated with particulate material and accumulate in the sediments of receiving waters where they may reach concentrations orders of magnitude greater than those present in the overlying water [24,25]. The organisms most at risk, therefore, will be members of the benthic community as they are exposed to both dissolved and deposited contaminants. Although several different groups of benthic organisms may be used to assess water and sediment quality [26,27], most studies have concentrated on macroinvertebrates [28]. These play an im-

*To whom correspondence may be addressed.

portant role in energy flow and nutrient processing in freshwaters, as well as providing prey for vertebrates such as fish and birds [29,30].

The major energy sources in small streams are benthic algae and detritus, coarse particulate organic matter (CPOM) (e.g., leaf litter) being the dominant energy input in wooded streams [31]. The breakdown of CPOM is brought about by a combination of microbial decomposition, macroinvertebrate feeding, chemical leaching, and physical abrasion [32]. Previous studies have shown that conditioning of leaf material by fungi increases its palatability to macroinvertebrate shredders [33-36] and that aquatic hyphomycetes (Fungi Imperfecti), in particular, play an important role in the microbial decomposition of leaf material [37]. The processing of CPOM by microorganisms and shredders produces fine particulate organic matter (FPOM), which is consumed by filter feeders and collector-gatherers. The latter are in turn consumed by invertebrate and vertebrate predators [38]. Hence, efficient decomposition is key to the energy budget (and therefore the integrity) of many stream ecosystems. A major rate-limiting step in the incorporation of CPOM into the freshwater food web is the conversion of detrital material into fungal and macroinvertebrate biomass.

Here we report on a study investigating the effects of motorway runoff on several small streams in northern England. The study consisted of an initial survey of several sites along the M1 motorway, followed by a more detailed investigation of three sites. The section of motorway studied was opened in 1967/68 and is drained by a combination of French drains and gullies that discharge directly into local streams [19,39]. The study concentrated on point sources and was designed to examine "worst-case" scenarios. Consequently, sampling stations were immediately downstream of discharges into small watercourses. The specific objectives were to investigate the effects of runoff on (a) water and sediment quality, in particular heavy metal and hydrocarbon concentrations; (b) algal, fungal, and macroinvertebrate assemblages; and (c) macroinvertebrate and microbial processing of CPOM.

MATERIALS AND METHODS

Sampling sites

Seven streams receiving drainage from the M1 motorway were sampled as part of the initial survey (Table 1). There were two sampling stations per site, one less than 400 m upstream of the point at which motorway runoff entered the stream and one less than 100 m downstream of this point. All were wooded, the main tree species being hawthorn (*Crataegus monogyna*), beech (*Fagus sylvatica*), birch (*Betula pendula*), sycamore (*Acer pseudoplatanus*), alder (*Alnus glutinosa*), and oak (*Quercus robur*). Rainfall data for the main sampling period (i.e., October 1990–July 1991) are given in Figure 1.

Three streams, Pigeon Bridge Brook, Butterthwaite Ditch, and Rockley Dike, were investigated in more detail. Mean substrate particle size was smallest at the upstream station at Pigeon Bridge Brook (i.e., upstream = 2.25 ϕ; downstream = −0.72 ϕ) and greatest at the downstream station at Rockley Dike (i.e., downstream = −2.68 ϕ; upstream = −0.95 ϕ). There was little difference in the size of substrate particles at the two Butterthwaite Ditch stations (i.e., upstream = −1.28 ϕ; downstream = −1.45 ϕ). The combination of stream size and area of road drained meant that the ratio of drainage input to dilution by stream water increased in the order Rockley Dike < Butterthwaite Ditch < Pigeon Bridge Brook.

Sample collection and preparation

Samples of sediment were collected from all 14 sampling stations during July and August 1990. Sediment, stream water, and outfall water samples were obtained from Pigeon Bridge Brook, Rockley Dike, and Butterthwaite Ditch at three monthly intervals between October 1990 and July 1991. Additional sediment samples were obtained from these three sites in April 1993.

Three 2-L samples of stream water and outfall water were collected in precleaned polypropylene containers and stored at 4°C until analysis. One 2-L sample was analysed for anions and metals, and two 2-L samples were analysed for hydrocarbons. For each station, three 15-ml subsamples of water were used for anion analysis, and a further three 15-ml subsamples were acidified with 1 ml of concentrated nitric acid prior to metal analysis. One-litre subsamples of stream and outfall water were prepared for hydrocarbon analysis by extracting twice with 250 ml Distol-grade dichloromethane (DCM) in a 2-L solvent-rinsed glass separating funnel [40]. The two extracts were then combined for each subsample and evaporated to approximately 10 ml using a rotary evaporator at 40°C. Extracts were then further evaporated to exactly 3 ml in a graduated centrifuge tube under a stream of oxygen-free nitrogen.

Table 1. Details of sites sampled along the M1 motorway

Stream (sample abbrev.)	Grid reference upstream/downstream	Minimum stream size (m) (width × depth)	Length of road drained (m)
River Doe Lea (DLa)	SK 453630/SK 453631	1.02 × 0.15	498
River Doe Lea (DLb)	SK 453653/SK 455653	0.71 × 0.42	1,046
Pigeon Bridge Brook (PBB)	SK 479852/SK 476851	0.9 × 0.02	1,500
Ulley Brook (UB)	SK 488896/SK 476886	1.42 × 0.08	2,000
Butterthwaite Ditch (BD)	SK 374944/SK 374937	0.7 × 0.05	1,300
Rockley Dike (RD)	SK 338023/SK 343024	2.6 × 0.14	900
House Carr Dike (HCD)	SE 335036/SE 337036	3.7 × 0.14	300

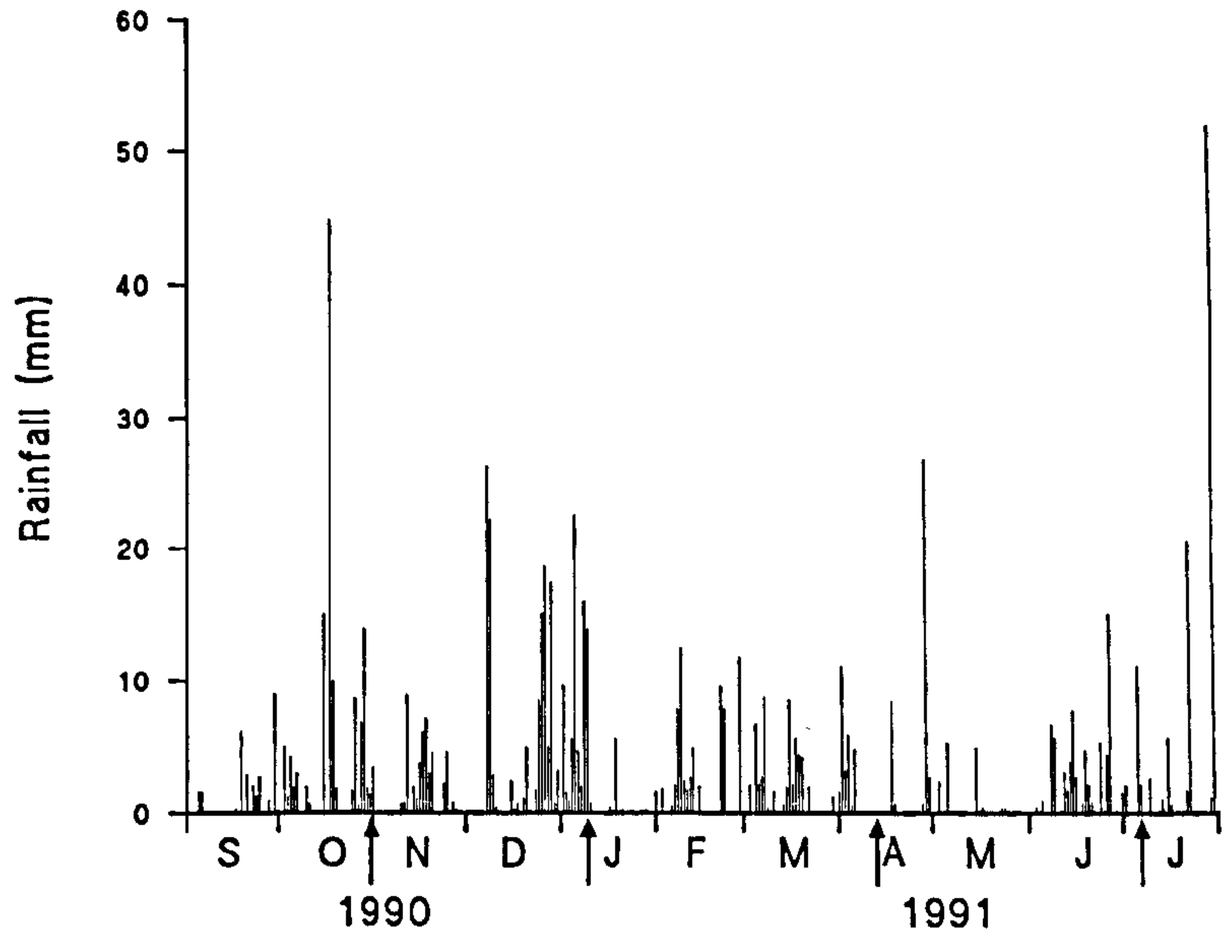

Fig. 1. Rainfall data for the period October 1990 to July 1991. Arrows indicate sampling dates. Data provided by Weston Park Meteorological Station, Sheffield, UK.

Triplicate sediment samples (approx. 20 g) were randomly collected from the upper 2 cm of sediment using a trowel and stored separately in DCM-rinsed glass vials at −17°C. Before preparation, sediment samples were brought to room temperature and mixed thoroughly. A weighed subsample (approx. 4 g) was taken from each sample for hydrocarbon analysis and placed in a Whatman 10 × 50 mm cellulose extraction thimble. The remaining sediment was dried at 80°C for 48 h before being processed for metal analysis (see below).

The method used to extract hydrocarbons from sediments was similar to that described by Brown, Pierce, and Rice [40]. The thimbles were placed in micro-Soxhlet wells and soaked overnight in 5 ml of a 58 g/L potassium hydroxide in methanol solution. The sediment was then micro-Soxhlet-extracted into 50 ml of Distol-grade DCM for 4 h. The resulting extract was shaken with 20 ml of distilled water in a 250-ml separating funnel to partition out the methanolic potassium hydroxide. The aqueous layer was then discarded and the organic extract was rotary-evaporated to approximately 10 ml before being evaporated to approximately 1 ml under a stream of oxygen-free nitrogen. The sediment extract was loaded on a column containing 1 g activated alumina (70 to 230 mesh, activated at 80°C for 24 h) on 4 g activated silica (60 to 120 mesh, activated at 80°C for 24 h) on 0.5 g sodium sulphate. The column had previously been primed using 15 ml of DCM before loading 25 μl of a standard mixture of deuterated PAHs (1 mg/ml of pyrene-d_{10}, phenanthrene-d_{10}, and naphthalene-d_8 in DCM; MSD isotopes) on the top of the column to act as an internal standard [41]. Distol-grade pentane was run through the column until 15 ml had been collected; this was designated the F_1 fraction, and it contained essentially saturated hydrocarbons. Dichloromethane was then run through the column until 30 ml had been collected; this was designated the F_2 fraction, and it contained unsaturated hydrocarbons including PAHs. Each fraction was then evaporated to exactly 3 ml under a stream of oxygen-free nitrogen before being analysed for hydrocarbons. Concentrations presented in the Results section are the sum of the F_1 and F_2 fractions.

Sediment was prepared for metal analysis by placing weighed subsamples (approx. 0.2 g) of dried sediment into acid-washed glass test tubes and adding 15 ml of 30% Primar nitric acid. The sediment was then digested at 80°C for 2 h in a Tecam DG1 block digester. The resulting solutions were transferred to acid-washed graduated tubes and made up to exactly 15 ml with distilled water.

Analytical procedures

Water samples were analysed for metals, aromatic hydrocarbons, anions, pH, dissolved oxygen, and temperature. Sediment samples were analysed for metals, aromatic hydrocarbons, and a range of selected PAHs [42]. In addition, sediment samples collected in 1993 were analysed for total hydrocarbons and total organic carbon (TOC).

The TOC content of sediments was determined using a modified version of the Kalembasa and Jenkinson [43] method [44], and a Dionex 2000i ion chromatography module was used to measure concentrations of phosphate, nitrate, sulphate, and chloride in water samples [45]. Hand-held meters were used to measure pH (Bibby, model SMP 1) and dissolved oxygen and temperature (Jenway, model 9070) in the field.

Metals were analysed using a Perkin-Elmer 2100 atomic absorption spectrophotometer with an AS50 autosampler according to Perkin-Elmer standard flame techniques [46]. The efficiency of the extraction and analytical procedures for assessing metals in sediments was assessed using the standard sediment GBW 07401 (Laboratory of the Government Chemist).

Aromatic hydrocarbon content of samples from the initial survey was determined by measuring fluorescence at 360 nm with 310 nm excitation [41]. Subsequent samples were analysed by UV absorbance measured at 254 nm using a Pye Unicam SP 8-100 UV/vis spectrophotometer [47]. In all cases, results were compared to a chrysene standard curve and expressed in terms of chrysene equivalents.

The concentration of total hydrocarbons was measured using infrared (IR) spectrophotometry [48]. Samples of sediment extract (2 ml) were evaporated to dryness under a stream of oxygen-free nitrogen and dissolved in 2 ml of carbon tetrachloride. Samples were then placed in 0.1-mm-path cells, and a transmittance scan between 4,000 and 1,700 wave numbers was obtained using a Perkin-Elmer 684 IR spectrophotometer. The height of the 2,932 cm^{-1} peak, which corresponds to the C—H bond stretch, was used as a measure of total hydrocarbons. A calibration curve was produced using a standard base oil (SN150, Castrol Research Laboratory), and results were expressed in terms of SN150 equivalents. Sediment samples from Pigeon Bridge Brook were also analysed using a Perkin-Elmer FTIR 1720X spectrophotometer equipped with KBr beam plates and a deuterated triglycine sulphate (DTGS) detector. The sample was transferred to a KBr disc and evaporated to dryness under an IR lamp. The disc was then scanned from 4,000 to 400 cm^{-1} ten times at 4 cm^{-1} resolution. Scans were then cross-referenced with known spectra using computer software.

Sediment extracts were analysed for 12 selected PAHs: naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[*a*]anthracene, chrysene, benzo[*b* and *k*]fluoranthene and benzo[*a*]pyrene [42]. The PAH analysis was carried out using a Perkin-Elmer GC8300 gas chromatograph fitted with an ion trap detector and a 20 m × 0.25 mm DB5 column (J & W Scientific). Samples (0.5 ml) were introduced using a Perkin-Elmer 8100 autosampler, and operating parameters were as follows: a 1-μl shot was injected and split 10:1; the injection port temperature was 195°C; the column was kept at 80°C for 5 min, after which the temperature was increased at 6°C/min up to 295°C; the column was then kept at 295°C for 15 min. Quantification was by peak area comparison with known standards (Alltech Associates). The accuracy of the procedures used to measure PAHs in sediments was assessed using the standard sediment SES-1 (National Research Council Canada).

Macroinvertebrate community structure

Macroinvertebrate samples were obtained from all 14 sampling stations during July and August 1990. In addition, samples were obtained from Pigeon Bridge Brook, Butterthwaite Ditch, and Rockley Dike every 3 months between October 1990 and July 1991. On each occasion, triplicate 2-min kick samples were obtained from each station using a pond net (net size 20 × 20 cm; mesh size 1 mm) [49]. Samples were integrative of all microhabitats present. Macroinvertebrates were sorted and either identified immediately (i.e., flatworms and mites) or preserved in 70% ethanol. Most individuals were identified to species using standard Freshwater Biological Association (FBA) keys. However, oligochaetes, coleopteran larvae, and dipteran larvae were identified to family level only. The abundance of each taxon was recorded, and the data were used to calculate diversity, biotic, and similarity indices. Published information [50,51] was used to assign macroinvertebrates to functional feeding groups (FFG) and the relative abundance of each FFG at each sampling station was determined.

Hyphomycete community structure

Aquatic hyphomycetes were sampled using alder leaf baits [52] placed in similar microhabitats at both upstream and downstream stations. Alder leaves were collected postabscission but preleaf fall (i.e., Oct.–Dec. 1990) and stored dry until use. Five mesh bags (11 × 11 cm; mesh size 1 mm), each containing five large alder leaves, were deployed at each of the sampling stations in February 1991. The bags were retrieved after 2 weeks and the leaves removed and washed in distilled water. For each station, eight 10-mm leaf discs were cut from each of five randomly chosen leaves and incubated individually in 20 ml of distilled water for 4 d at 15°C. After this period the discs were stained with lactophenol-cotton blue in 10% lactic acid, scanned under 100× magnification, and the conidia of aquatic hyphomycetes identified [53,54] and counted. Relative importance values (RIVs) were determined for each species using the methods of Shearer and Lane [52]. Diversity and similarity indices were calculated using the methods outlined below.

Algal community structure

Triplicate random algal samples were obtained in October 1990 using standard techniques [55]. For each sample, a 2-cm^2 area of epilithon was removed with a razor blade and placed into glass vials containing 2 ml of water. Subsamples were mounted on glass slides and observed under 400× magnification. At least 200 algal cells per sample were examined and identified to genera [56–58]. Chlorophyll-*a* concentrations were used as a measure of algal biomass [59]. Three stones were collected from each station at random. Algae were removed from a 2-cm^2 area on each stone and washed onto filter papers. Chlorophyll-*a* was then extracted using 90% acetone and analysed by UV spectroscopy [60].

Leaf litter processing

A measure of leaf litter processing was obtained by measuring the weight loss of leaves in coarse (4-mm mesh) and fine (1-mm mesh) mesh bags (11 × 11 cm) [61]. Five coarse-mesh bags and five fine-mesh bags, each containing approximately 1 g of preweighed dried alder leaves, were deployed at each station for 2 weeks in February 1991. The bags were then retrieved and the leaves washed to remove inorganic particulate material before being oven-dried (60°C) and reweighed. Processing was expressed in terms of percentage weight loss.

Indices

The diversity of the macroinvertebrate, algal, and fungal assemblages was determined by calculating the log series index (α) [62], and a modified Sorenson index (C_N) was used to compare assemblages [63,64]. The C_N ranges from 0 (i.e.,

totally dissimilar assemblages) to 1 (i.e., identical assemblages) and α ranges from 0 (i.e., no individuals present) to infinity (i.e., same number of individuals as species).

The macroinvertebrate data were also used to calculate Biological Monitoring Working Party (BMWP) scores for each sampling station [65]. The BMWP score ranges from 0 (i.e., no macroinvertebrates present) to infinity (i.e., large number of pollution-sensitive taxa present); the higher the score the "cleaner" the water [66]. The BMWP score was standardised by calculating an average score per taxon (ASPT), which ranges from 0 to 10 [67].

Statistical analyses

All data were tested for normality using normal probability plots. Abundance, diversity, ASPT, leaf processing data (arcsine transformed), and total hydrocarbon concentrations were analysed by two-sample *t* tests or fixed-effects two-way analysis of variance, the factors being station (two levels) and time (four levels). Between-station differences in sediment quality, water quality, FFGs, and the relative abundance of specific macroinvertebrate taxa were assessed using the Friedman test [68]. As the Friedman test is based on one observation per cell, data were averaged for each time interval. Correlations between IR and UV data and sediment contamination and loading were assessed using least-squares regression techniques. Least-squares regression techniques were also used to evaluate the relationship between the abundance of macroinvertebrates and sediment quality. All analyses were performed using the MINITAB statistical package [69]. Significance levels were $p < 0.05$.

RESULTS

Efficiency of sediment preparation and analytical procedures

On average, 98% of the PAHs applied as internal standards were extracted during the 4-h micro-Soxhlet procedure, and recovery values for PAHs in the standard sediment ranged from 73 to 102%: anthracene, 73%; fluoranthene, 94%; pyrene, 73%; chrysene, 102%; and benzo[*b* and *k*]fluoranthene, 90%. Recovery of metals from standard sediment was more variable, but generally greater than 80%: cadmium, 93%; chromium, 36%; copper, 95%; lead, 101%; manganese, 80%; nickel, 102%; zinc, 100%.

Sediment quality

Results of the preliminary survey indicated that the concentration of aromatic hydrocarbons (μg chrysene equivalents/g wet wt.) in sediments was elevated at all sites (see Table 1) receiving motorway drainage (i.e., DLa: 22 upstream, 39 downstream; DLb: 21.8 upstream, 120 downstream; PBB: 43 upstream, 2,881 downstream; UB: 22 upstream, 250 downstream; BD: 0.6 upstream, 171 downstream; RD: 9.1 upstream, 73 downstream; HCD: 59.5 upstream, 64 downstream). The magnitude of the between-station differences varied greatly between sites and was greatest for Pigeon Bridge Brook. Sites differed in terms of the size and length of road from which they received runoff (Table 1). Consequently, considerable between-site variation existed in potential loading (PL), defined as the ratio of the length of road drained to minimum stream size (width × depth). A significant positive correlation was seen between the downstream increase in the concentration of aromatic hydrocarbons in the sediment (ΔAH_S) and PL (Fig. 2):

$$\log \Delta AH_S = 1.61(\log PL) - 4.88 \quad (r = 0.88)$$

Results from the initial survey were confirmed in a detailed study of three of the sites over a 12-month period. Statistically significant elevations in median aromatic hydrocarbon concentrations (μg chrysene equivalents/g wet wt.) were measured for sediments collected from downstream stations at Pigeon Bridge Brook (upstream = 55.4, downstream = 383.5) and Butterthwaite Ditch (upstream = 50.9, downstream = 295.2) ($S = 4$, $d.f. = 1$), and mean concentrations of total hydrocarbons (μg SN150 equivalents/g wet wt.) were significantly elevated at all three downstream stations (PBB: upstream = 129, downstream = 1,806; BD: upstream = 131, downstream = 1,777; RD: upstream = 172, downstream = 1,014) ($t > 4.9$, $d.f. = 5$).

The IR spectra of extracts of sediments from Pigeon Bridge Brook indicated that, in addition to aromatic hydrocarbons, carbonyl compounds were also present in contaminated sediments but were absent from uncontaminated sediments (Fig. 3).

Gas chromatograms for downstream sediment extracts had an unresolved region that was characteristic of oil contamination (Fig. 4) [70]. Although the median concentrations (μg/g wet wt.) of most of the PAHs measured were elevated in runoff-contaminated sediments, between-station differences were only statistically significant for naphthalene (upstream = 0.017, downstream = 0.52) and fluoranthene (upstream = 0.27, downstream = 3.2) at Pigeon Bridge Brook and for acenaphthene (upstream = 0.24, downstream = 1.35) at Butterthwaite Ditch ($S = 4$, $d.f. = 1$). The dominant PAHs at the most contaminated site (i.e., Pigeon Bridge Brook) were pyrene (10.16 μg/g wet wt.), fluoranthene (3.2 μg/g wet wt.), and phenanthrene (5.62 μg/g wet wt.). In addition,

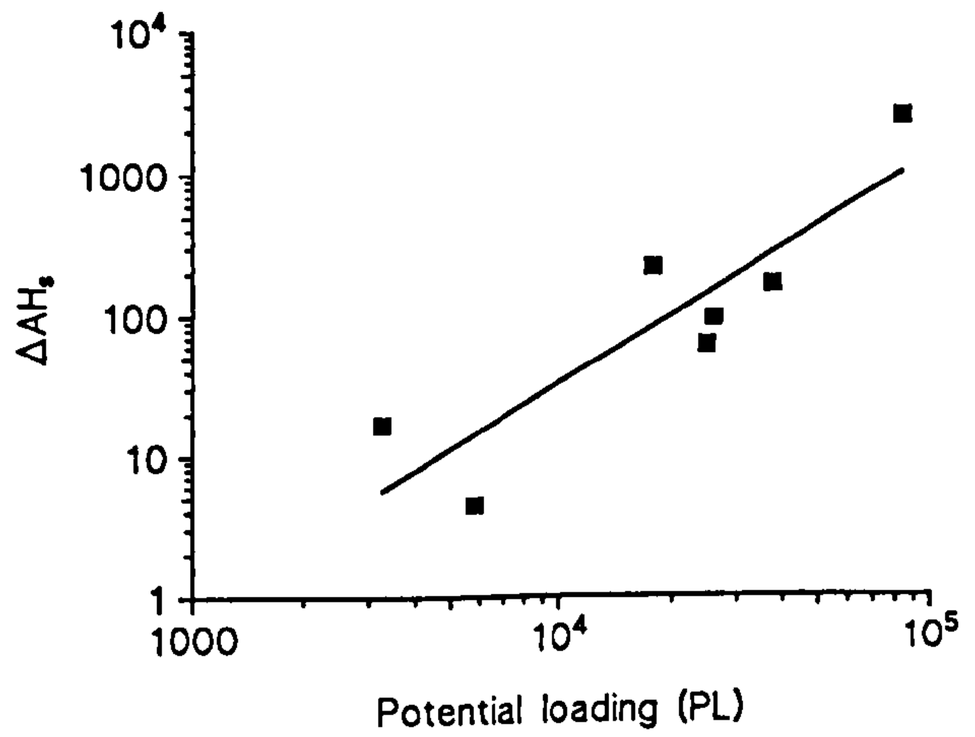

Fig. 2. Relationship between downstream elevation in sediment aromatic hydrocarbon concentration (ΔAH_s, μg chrysene equivalents/g wet wt.), and potential loading (PL, see text for definition).

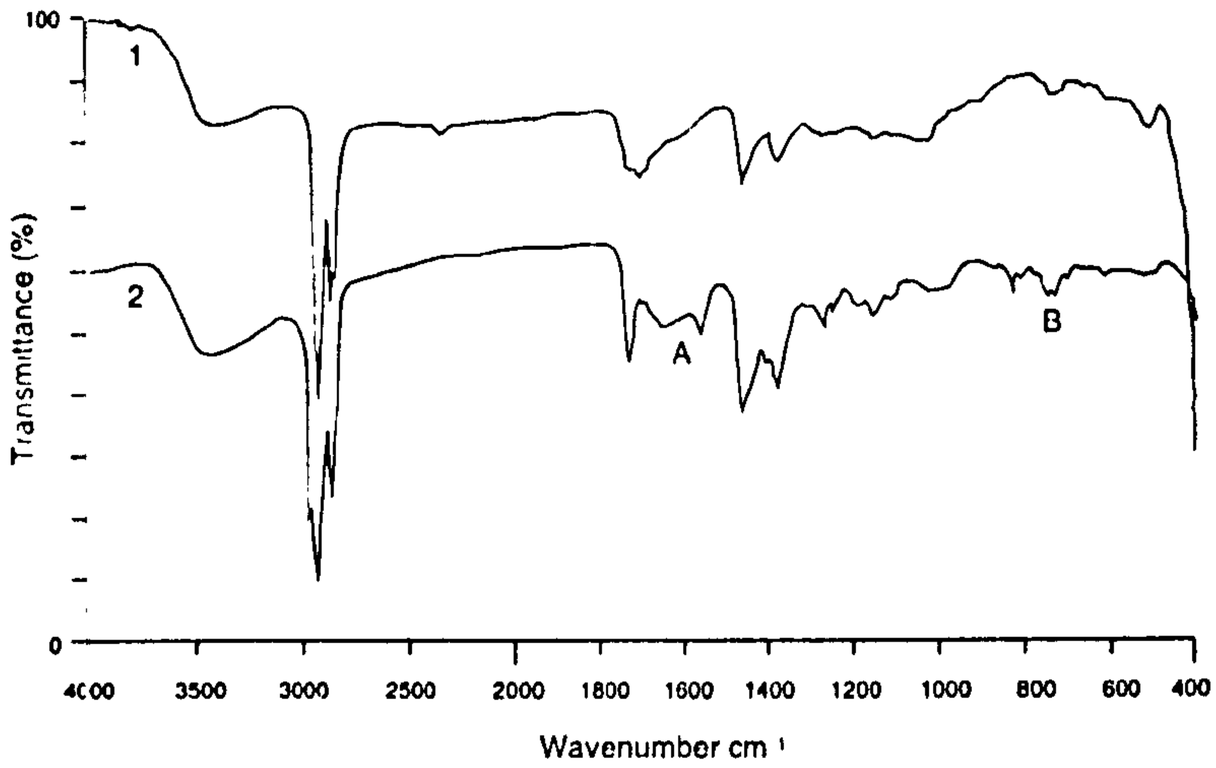

Fig. 3. Infrared spectra for extract of sediments collected upstream (1) or downstream (2) of the point at which motorway runoff is discharged into Pigeon Bridge Brook. The peaks in region A are indicative of carbonyl compounds. The peaks in region B indicate aromatic compounds.

benzo[*b* and *k*]fluoranthene and benzo[*a*]pyrene were elevated in contaminated sediments at Butterthwaite Ditch (i.e., 2.45 and 4.63 μg/g wet wt., respectively), and naphthalene was abundant in contaminated sediments at both Butterthwaite Ditch (2.28 μg/g wet wt.) and Rockley Dike (2.89 μg/g wet wt.).

Sediments at stations receiving motorway runoff were also contaminated with a number of heavy metals and differed in TOC concentration. Downstream sediments at Pigeon Bridge Brook contained significantly elevated median concentrations of zinc (upstream = 137 μg/g dry wt.; downstream = 338 μg/g dry wt.), cadmium (upstream = 0.93 μg/g dry wt.; downstream = 2.28 μg/g dry wt.), lead (upstream = 86 μg/g dry wt.; downstream = 133 μg/g dry wt.), and chromium (upstream = 21 μg/g dry wt.; downstream = 76 μg/g dry wt.), and significantly reduced concentrations of nickel (upstream = 66 μg/g dry wt.; downstream = 46 μg/g dry wt.) ($S = 4$, $d.f. = 1$). Downstream sediments at Butterthwaite Ditch contained significantly elevated median concentrations of chromium (upstream = 28 μg/g dry wt.; downstream = 84 μg/g dry wt.) and lead (upstream = 69 μg/g dry wt.; downstream = 84 μg/g dry wt.), whereas downstream sediments at Rockley Dike contained significantly reduced median concentrations of iron (upstream = 129 mg/g dry wt.; downstream = 107 mg/g dry wt.) and significantly elevated median concentrations of cadmium (upstream = 0.91 μg/g dry wt.; downstream = 1.39 μg/g dry wt.) ($S = 4$, $d.f. = 1$). The TOC concentrations were elevated at the upstream station at Pigeon Bridge Brook—11.6% (SE = 2.9) vs. 2.3% (SE = 0.2)—and the downstream station at Rockley Dike—2.73% (SE = 0.23) vs. 8.53% (SE = 0.3). However, concentrations of TOC in sediments collected from the two sampling stations at Butterthwaite Ditch were very similar: upstream = 2.44% (SE = 0.5), downstream = 1.93% (SE = 0.2).

Water quality

There were no significant between-station differences in pH, temperature, or dissolved oxygen (Table 2) although concentrations of sulphate and chloride ions were significantly

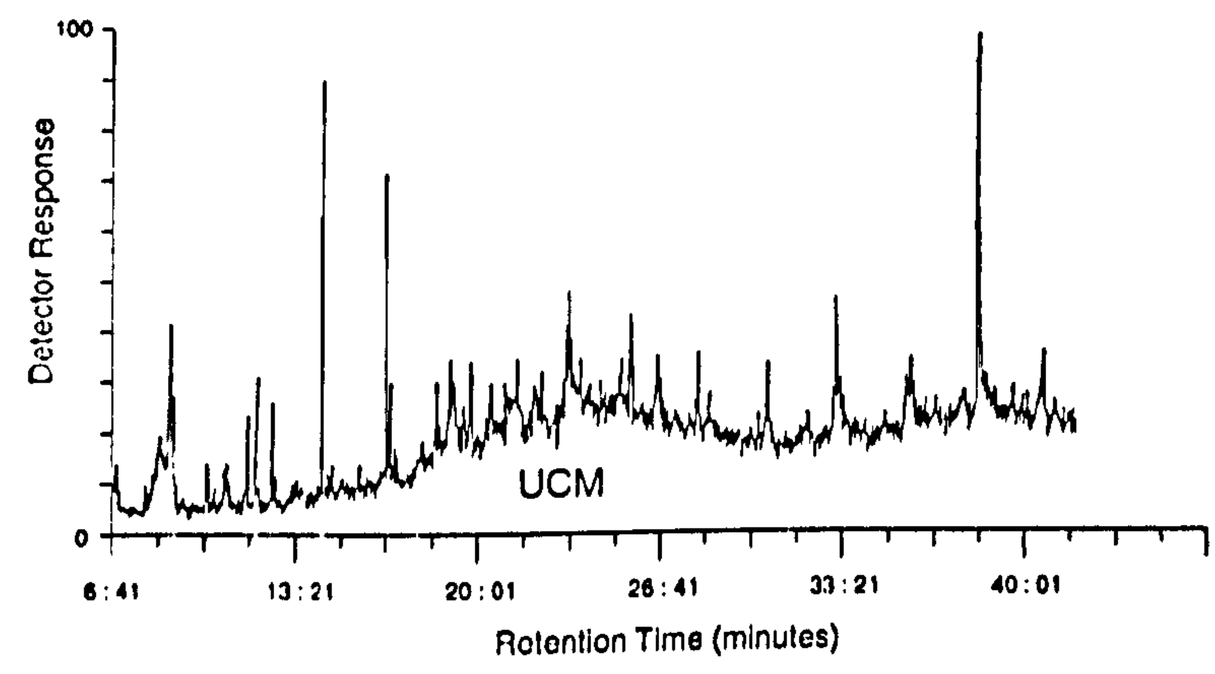

Fig. 4. GC-MS chromatogram for sediment contaminated with motorway runoff. The unresolved complex mixture (UCM) is indicative of oil pollution.

Table 2. Physicochemical properties of stream water upstream (us) and downstream (ds) of motorway runoff discharges into the three study sites

	Pigeon Bridge Brook		Butterthwaite Ditch		Rockley Dike	
Determinand	us	ds	us	ds	us	ds
pH	7.48	7.21	7.59	7.69	8.19	8.23
Temperature (°C)	8.73	9.3	8.45	8.3	9.25	9.0
Dissolved O_2 (mg/L)	10.5	9.6	10.8	11.2	11.7	11.8
PO_4^{3-} (mg/L)	0.49	0.16	1.08	0.77	1.40	1.47
SO_4^{2-} (mg/L)	60.4	111.6*	114.4	103.6	229.7	247.9
NO_3^- (mg/L)	66.6	74.2	21.8	20.5	44.6	44.0
Cl^- (mg/L)	65.1	229.1*	86.2	86.4	105.7	112.1

Data are presented as median values, and an asterisk denotes significant between-station differences.

elevated at the downstream station at Pigeon Bridge Brook ($S = 4$, $d.f. = 1$). Even though motorway runoff draining into the three main study sites was contaminated with aromatic hydrocarbons (all >30 μg chrysene equivalents/L), dilution by the receiving waters was such that concentrations in stream water were below detection levels. However, several metals were detected in both motorway runoff (Table 3) and stream water collected upstream and downstream of the discharges. Significant elevations in the median concentrations of calcium (upstream = 83 mg/L, downstream = 116 mg/L), magnesium (upstream = 43.7 mg/L, downstream = 46.8 mg/L), and copper (upstream = 26 μg/L, downstream = 47.2 μg/L) were detected in stream water obtained from the downstream station at Pigeon Bridge Brook ($S = 4$, $d.f. = 1$). Calcium concentrations were significantly elevated (upstream = 82.6 mg/L, downstream = 89.4 mg/L), and iron concentrations were significantly reduced (upstream = 1.98 mg/L, downstream = 0.74 mg/L) in stream water collected from the downstream station at Butterthwaite Ditch, whereas zinc concentrations were significantly reduced in water samples obtained from the downstream station at Rockley Dike (upstream = 76 μg/L, downstream = 25.1 μg/L) ($S = 4$, $d.f. = 1$).

Macroinvertebrates

The diversity (α) of the macroinvertebrate assemblage was reduced at four out of the seven stations receiving runoff. Reductions in diversity were associated with reduced biotic scores, indicating that contaminated stations had fewer pollution-sensitive macroinvertebrates than did noncontaminated stations (Table 4).

Three of the most impacted sites, Pigeon Bridge Brook, Butterthwaite Ditch, and Rockley Dike, were monitored over a 12-month period during which representatives of more than 50 taxa were identified (see Appendix). The number of macroinvertebrate taxa sampled from these streams did not differ significantly with time ($F < 1.7$, $d.f. < 3, 16$) nor were there any significant between-station differences in the number of macroinvertebrate taxa present at Butterthwaite Ditch and Rockley Dike ($F < 0.38$, $d.f. = 1, 16$). Throughout the year the mean number of taxa ranged from 10.3 to 15.3. There was, however, a significant reduction in the number of macroinvertebrate taxa present below the motorway runoff discharge into Pigeon Bridge Brook (minimum decrease of 38%, range 4.7 to 6 taxa; $F = 23.21$, $d.f. = 1, 14$).

Values of α for the macroinvertebrate assemblages at upstream stations ranged between 2.3 and 3.7, the least diverse assemblage being that at Pigeon Bridge Brook (i.e., Pigeon Bridge Brook, $\alpha = 2.3$ to 3.4; Butterthwaite Ditch, $\alpha = 2.89$ to 3.1; Rockley Dike, $\alpha = 3.2$ to 3.48). Although there was no significant downstream reduction in macroinvertebrate diversity at either Butterthwaite Ditch or Rockley Dike ($F < 1.86$, $d.f. = 1, 16$), values of α were significantly reduced at the downstream station at Pigeon Bridge Brook ($\alpha = 1.13$ to

Table 3. Average concentrations of metals in motorway runoff discharging into three sites along the M1 motorway

Metal	Pigeon Bridge Brook	Butterthwaite Ditch	Rockley Dike	Minimum detection levels
Aluminium (μg/L)	131.5	112.9	<80	80
Iron (μg/L)	590	3,850	470	9
Chromium (μg/L)	4.0	3.6	3.1	3
Lead (μg/L)	42.1	<30	<30	30
Nickel (μg/L)	<9	15.5	11.8	9
Magnesium (mg/L)	60.4	57.0	55.8	7
Copper (μg/L)	37.9	128.6	29.2	14
Cadmium (μg/L)	<1	<1	<1	1
Zinc (μg/L)	74.7	489.1	126.4	2
Calcium (mg/L)	175.46	131	147.1	0.4

Table 4. Characteristics of the benthic macroinvertebrate assemblages present in streams receiving drainage from the M1 motorway

Sampling station[a]	No. of families	Diversity index (α)	Biotic index (ASPT)
DLa: upstream/downstream	13/9	4.7/2.6	4.5/3.3
DLb: upstream/downstream	12/14	3.0/5.0	4.2/3.7
PBB: upstream/downstream	9/5	2.2/1.4	4.2/3.0
UB: upstream/downstream	11/18	4.5/6.4	3.7/3.9
BD: upstream/downstream	13/10	5.0/3.0	3.9/3.8
RD: upstream/downstream	16/13	4.9/4.6	4.2/3.4
HCD: upstream/downstream	14/14	3.5/5.1	3.7/3.8

[a]See Table 1 for site codes.

1.89; $F = 18.47$, $d.f. = 1, 14$). These between-station differences in diversity were reflected in the similarity indices, which were greater than 0.6 for the Butterthwaite Ditch (i.e., 0.61 to 0.67) and Rockley Dike (i.e., 0.61 to 0.69) assemblages but less than 0.3 for Pigeon Bridge Brook assemblages (i.e., 0.07 to 0.29).

The reduction in macroinvertebrate diversity at the downstream station at Pigeon Bridge Brook was associated with a significant reduction in the average score per taxon (ASPT) ($F = 37.38$, $d.f. = 1, 14$). Whereas the upstream station at Pigeon Bridge Brook had the highest ASPT (i.e., Pigeon Bridge Brook, 4.19 to 4.98; Butterthwaite Ditch, 3.62 to 3.87; Rockley Dike, 3.27 to 3.65), the downstream station had the lowest ASPT (i.e., Pigeon Bridge Brook, 2.11 to 3.73; Butterthwaite Ditch, 3.55 to 4.05; Rockley Dike, 3.30 to 3.65), indicating that there was a loss of pollution-sensitive taxa below the motorway discharge. Moreover, there was a shift in the relative abundance of particular taxa at the two stations, with stoneflies (Plecoptera), gammarids (Amphipoda), caddisflies (Trichoptera), and snails (Mollusca) being more abundant upstream of the discharge, and chironomid larvae (Diptera) and tubificid worms (Oligochaeta) being more abundant downstream of it (Fig. 5).

Changes in the structure of the macroinvertebrate assemblages were associated with changes in the relative abundance of functional feeding groups (Fig. 6). Downstream of the discharge into Pigeon Bridge Brook, there was a significant decrease in the relative abundance of scrapers and shredders ($S = 4$, $d.f. = 1$) and a significant increase in the relative abundance of collectors ($S = 4$, $d.f. = 1$). The only other significant change was at Butterthwaite Ditch where there was a significant increase in the relative abundance of predators at the downstream station ($S = 4$, $d.f. = 1$).

Aquatic hyphomycetes

Twenty-nine different species of aquatic hyphomycetes were identified from samples collected from Pigeon Bridge Brook, Butterthwaite Ditch, and Rockley Dike in February 1991 (Table 5). The dominant species at all sampling stations were *Tetracladium marchalianum*, *Lemonniera terrestris*, *Heliscus lugdunensis*, and *Alatospora acuminata*. *Articulospora tetracladia* was the only species to exhibit a consistent pattern of upstream bias in its distribution. However, *An-*

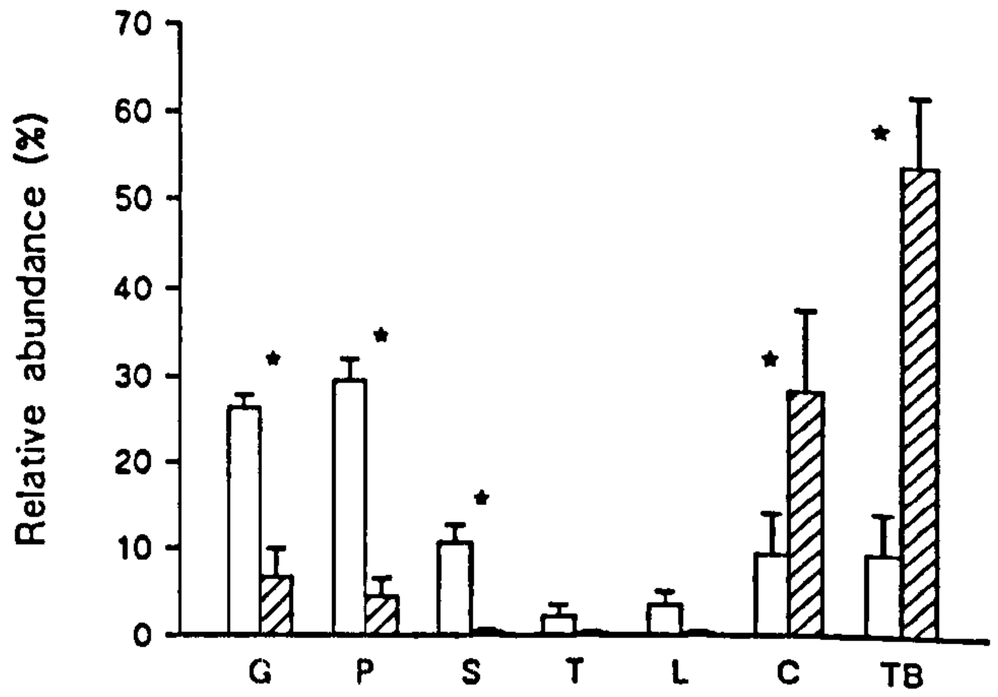

Fig. 5. The relative abundance of selected macroinvertebrates at stations upstream (open bars) and downstream (hatched bars) of the motorway discharge at Pigeon Bridge Brook. G: *Gammarus pulex* (Amphipoda); P: *Potamopyrgus jenkinsi* (Mollusca); S: Sphaeriidae (Mollusca); T: Trichoptera; L: *Leutra inermis* (Plecoptera); C: Chironomidae (Diptera); TB: Tubificidae (Oligochaeta). Data are presented as mean values + 1 SE, and asterisk denotes significant between-station differences.

guillospora longissima and *Tricladium angulatum* showed reduced relative abundances at downstream stations at Rockley Dike and Pigeon Bridge Brook, whereas *Tricladium splendens*, *Triscelophorus* sp., *Culicidospora aquatica*, and *Tricladium varium* showed increased relative abundances at these stations.

Although there were no significant between- or within-site differences in species richness (mean values ranged from 11.6 to 13.6, $F < 0.75$, $d.f. < 2, 24$), in terms of RIVs, the Pigeon

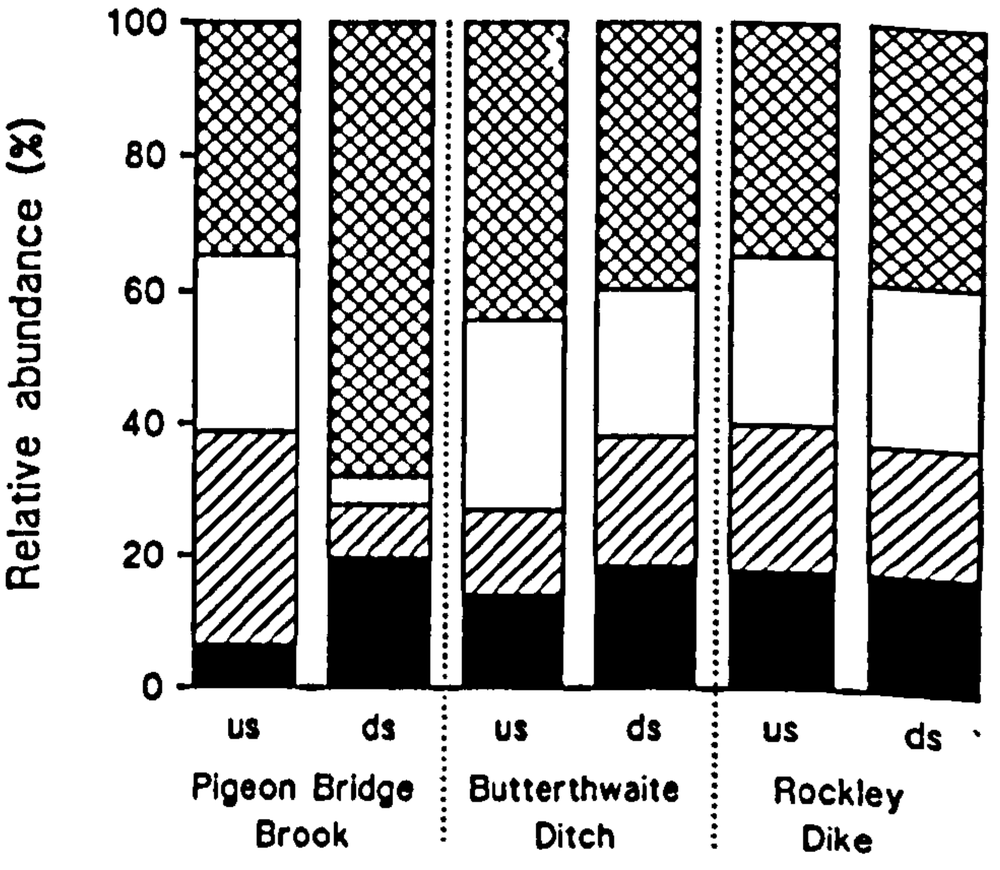

Fig. 6. Mean relative abundance of functional feeding groups upstream (us) and downstream (ds) of the input of motorway runoff. Solid bars represent predators, hatched bars represent shredders, open bars represent scrapers, and cross-hatched bars represent collectors.

Table 5. Relative importance values (RIVs) for hyphomycete fungi colonizing alder leaves deployed in three streams receiving runoff from the M1 motorway

Taxon	Pigeon Bridge Brook	Butterthwaite Ditch	Rockley Dike
Alatospora acuminata	4/4	3/3	3/3
Anguillospora rosea	1/1	0/0	0/0
Anguillospora crassa	1/1	0/0	0/0
Anguillospora curvula	1/1	1/0	0/0
Anguillospora longissima	2/1	3/3	3/2
Articulospora tetracladia	1/0	3/2	1/0
Clavariopsis aquatica	0/0	0/1	1/0
Clavariopsis longibrachiata	0/0	1/1	2/2
Culicidospora aquatica	0/3	2/1	0/1
Dendrospora sp.	1/0	0/0	0/0
Flagellospora curvula	0/0	3/3	4/3
Heliscus lugdunensis	2/3	3/4	4/3
Lemonniera terrestris	2/4	3/3	4/4
Lemonniera cornuta	0/1	0/0	0/0
Lemonniera aquatica	2/2	1/0	2/1
Lemonniera centrosphaera	3/3	0/0	1/2
Scorpiosporium minutum	3/3	3/3	3/3
Sympodiocladium frondosum	1/1	0/0	0/0
Tetrachaetum elegans	2/1	0/0	1/1
Tetracladium furcatum	1/1	0/1	1/0
Tetracladium marchalianum	4/4	4/3	4/4
Tetracladium setigerum	3/3	3/3	0/1
Triscelophorus sp.	1/2	1/0	1/2
Tricladium angulatum	3/1	1/2	2/0
Tricladium chaetocladium	3/1	2/2	3/3
Tricladium splendens	0/1	3/3	1/2
Tricladium varium	0/1	0/0	0/1
Vargamyces aquatica	2/1	1/0	0/0
Varicosporium sp.	3/0	2/2	2/2

Data are presented for upstream/downstream sampling stations and expressed as RIVs. Classes were: 0 = species absent; 1 = RIV < 0.05; 2 = 0.05 < RIV < 0.1; 3 = 0.1 < RIV < 0.2; 4 = RIV > 0.2.

Bridge Brook assemblages were less similar ($C_N = 0.63$) than either the Butterthwaite Ditch ($C_N = 0.82$) or the Rockley Dike assemblages ($C_N = 0.80$). Moreover, the diversity of the hyphomycete assemblage was significantly increased downstream of the discharge into Pigeon Bridge Brook (upstream = 3.81 (SE = 0.87), downstream = 8.52 (SE = 0.55), $t = 3.85$, $d.f. = 6$).

Epilithic algae

Representatives of 20 algal genera were recorded from the three sites during October 1991 (Table 6). Although algal assemblages at downstream stations were more diverse (upstream α = 1.5 to 2.1; downstream α = 1.9 to 2.6) and contained, on average, either a similar number or more genera than assemblages at upstream stations (upstream, 7 to 9.4 genera; downstream 7 to 11 genera), none of these differences were statistically significant ($F < 2.35$, $d.f. = 1, 12$). There were also no significant between-station differences in chlorophyll-*a* concentrations ($F = 0.01$, $d.f. = 1, 12$). Mean chlorophyll-*a* concentrations ranged from 12 $\mu g/cm^2$ (SE = 2.26) at the upstream station at Pigeon Bridge Brook to 28.8 $\mu g/cm^2$ (SE = 0.9) at the upstream station at Butterthwaite Ditch.

Table 6. Epilithic algal genera sampled from streams receiving runoff from the M1 motorway

Taxon	Pigeon Bridge Brook	Butterthwaite Ditch	Rockley Dike
Cyanophyceae			
Oscillatoria	*/*	–/*	–/*
Chlorophyceae			
Cladophora	–/*	–/*	*/–
Closterium	–/*	–/*	–/–
Bacillariophyceae			
Achnanthes	–/–	–/–	*/*
Amphora	–/–	*/–	*/*
Ceratoneis	–/–	–/–	–/*
Cyclotella	–/–	–/–	*/–
Cymatopleura	–/–	*/–	–/*
Diatoma	*/–	–/*	–/*
Entomoneis	–/*	–/–	*/*
Gomphonema	–/*	–/*	*/*
Gyrosigma	–/–	*/–	–/*
Melosira	–/*	–/*	*/*
Meridion	–/*	*/*	*/*
Navicula	*/–	*/*	*/*
Nitzschia	*/*	*/*	*/*
Rhoicosphenia	*/*	*/*	*/*
Sellaphora	*/*	–/*	*/*
Surirella	*/*	*/*	*/*
Euglenophyceae			
Euglena	–/*	–/–	–/*

Data presented for upstream/downstream sampling station, * = present, – = absent.

Leaf litter processing

The two mesh sizes used in leaf litter decomposition studies either prevented (i.e., fine mesh) or allowed (i.e., coarse mesh) macroinvertebrates access to the leaf material, thus enabling microbial and macroinvertebrate decomposition to be separated. There were no significant between-station differences in the loss of leaf material from fine-mesh bags at any of the three sites (Fig. 7; $F = 0.93$, $d.f. = 1, 24$). However, there was a significant reduction in the loss of leaf material from coarse-mesh bags deployed at the downstream station at Pigeon Bridge Brook ($t = 3.23$, $d.f. = 7$), suggesting that motorway runoff inhibited macroinvertebrate-mediated leaf decomposition at this station. Similar effects were not observed for leaves deployed in coarse-mesh bags at either Butterthwaite Ditch or Rockley Dike ($t < 1.8$, $d.f. > 4$).

DISCUSSION

Road runoff is an important source of potentially toxic contaminants in fresh waters [71]. The most frequently detected contaminants in a nationwide survey of urban runoff in the Untied States included the metals copper, lead, zinc, chromium, cadmium, and nickel, and the PAHs phenanthrene, naphthalene, pyrene, and fluoranthene [10]. The concentration of aromatic hydrocarbons in drainage waters sampled during the present study ranged from 20 to 64 μg chrysene equivalents/L, which was slightly lower than the concentration range of 40 to 70 μg/L reported by MacKenzie and Hunter [13] but well within the range of 1 to 50 μg/L reported by Stenstrom et al. [72]. The runoff from the M1

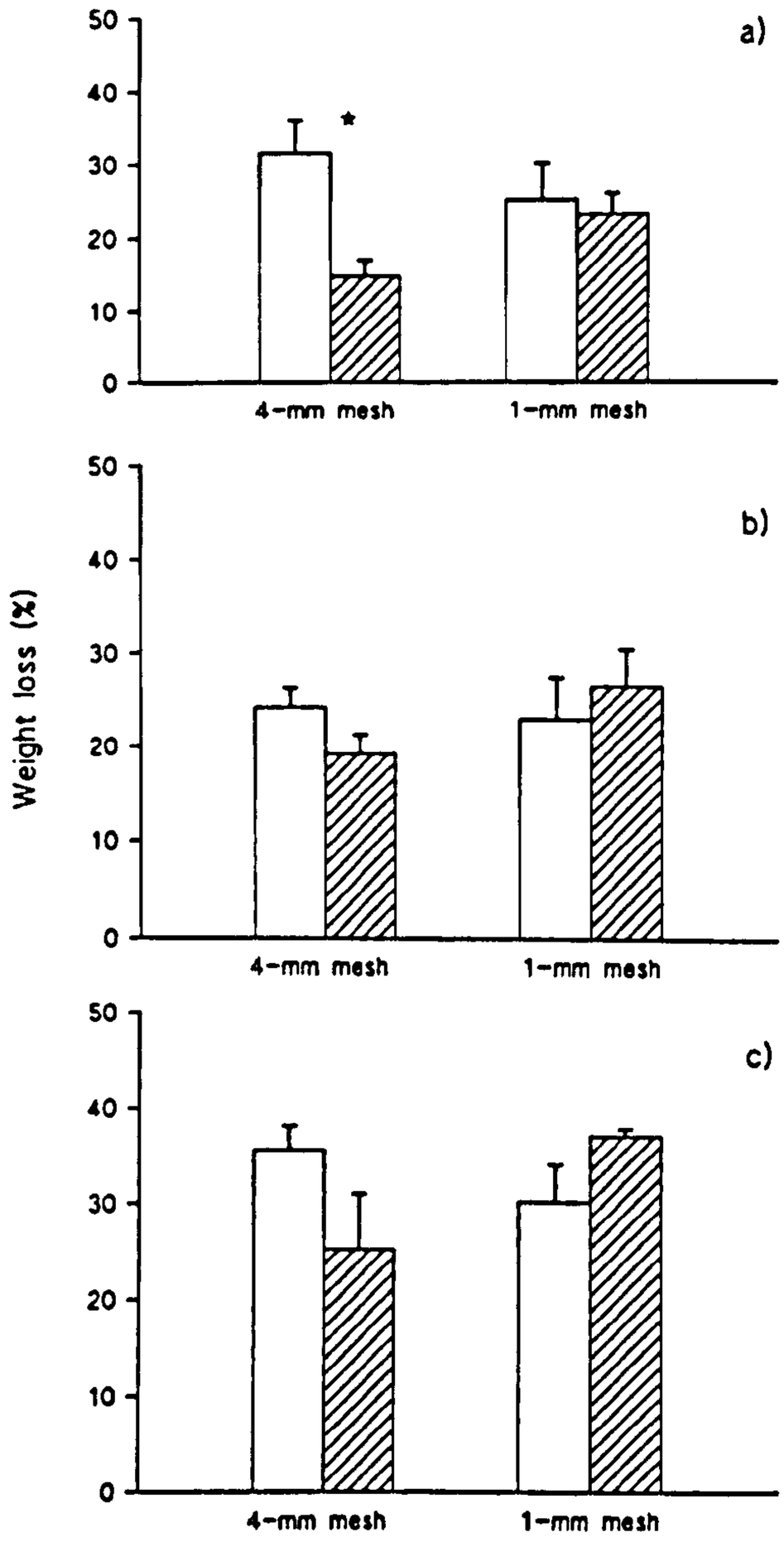

Fig. 7. Loss of leaf material from coarse (4 mm) and fine (1 mm) mesh bags deployed upstream (open bars) and downstream (hatched bars) of the motorway runoff into (a) Pigeon Bridge Brook, (b) Butterthwaite Ditch, and (c) Rockley Dike. Data are presented as mean values + 1 SE, and the asterisk denotes significant between-station differences.

motorway was also contaminated with a number of heavy metals, the concentrations of which were generally similar to those reported in previous studies [2,5,10,73,74].

Numerous studies have demonstrated that the majority of pollutants in road runoff are associated with particulate material [4–6,13,74,75]. It is not surprising, therefore, that contaminants in road runoff accumulate in the sediments of receiving waters. Sediments collected from sites receiving drainage from the M1 had elevated concentrations of both metals and hydrocarbons.

Contaminated sediments contained between 1,014 and 1,806 μg total hydrocarbons/g wet wt. Sediments were analysed for 12 PAHs, all of which were elevated in sediments collected from the contaminated station at Pigeon Bridge Brook and Butterthwaite Ditch, and the majority were elevated in sediments collected from the contaminated station at Rockley Dike. Sediment concentrations were greatest at Pigeon Bridge Brook, where the most abundant PAHs were pyrene, fluoranthene, and phenanthrene. Two of these compounds, fluoranthene and pyrene, have been shown to be important components of crankcase oil and account for 74% of the PAHs present [76].

A significant correlation existed between the increase in the concentration of aromatic hydrocarbons in sediments collected from the downstream station and the potential loading of road-related pollutants. Hydrocarbons in road runoff may be derived from lubricating oils, fuel, exhaust emissions, and road wear [71]. Leakage of crankcase oil is a major contributor to the hydrocarbon content of urban runoff and may account for up to 88% of the total loading [77]. Moreover, chromatograms of road runoff closely resemble those of used crankcase oil [40,76,78]. Although fresh oil contains low concentrations of PAHs, their abundance increases with increased oil use [79]. The PAHs either dissolve directly from the fuel into the oil or are produced by incomplete fuel combustion. Fuel combustion also produces carbonyl compounds such as aldehydes and ketones [80], which have been previously recorded in road runoff [81] and which were detected in runoff-contaminated sediments collected from Pigeon Bridge Brook.

Concentrations of several metals were significantly elevated in runoff-contaminated sediments at Pigeon Bridge Brook (i.e., zinc, cadmium, lead, and chromium) and to a lesser degree at Butterthwaite Ditch (i.e., lead and chromium). The distribution of metals in contaminated sediments was similar to that reported previously [14,82] and generally mirrored the abundance of metals in stream water. These metals were probably derived from a number of sources, including brake linings, fuel additives, tyres, and corrosion products [1,10,11].

There was also evidence of a change in the diversity and composition of the macroinvertebrate assemblages at four of the seven stations receiving motorway drainage, although this was only statistically significant at Pigeon Bridge Brook. A negative, though nonsignificant, correlation existed between the number of macroinvertebrate families present at a sampling station and the concentration of aromatic hydrocarbons in the sediment (Fig. 8; $r = -0.67$, $d.f. = 12$).

Changes in diversity were associated with reductions in biotic indices, indicating that there was a loss of pollution-sensitive species at stations receiving motorway runoff. There was also a shift in the relative abundance of taxa common to both stations. For example, whereas the macroinvertebrate assemblage at the upstream station at Pigeon Bridge Brook was dominated by *Gammarus pulex* and *Potamopyrgus jenkinski*, the assemblage at the downstream station was dominated by chironomid larvae and tubificid worms. Similar changes in the structure of macroinvertebrate assemblages affected by road runoff have been reported in previous studies [22,83], although some data are equivocal [84].

Many factors, both biotic and abiotic, affect the distribution of macroinvertebrates in streams [85]. Consequently,

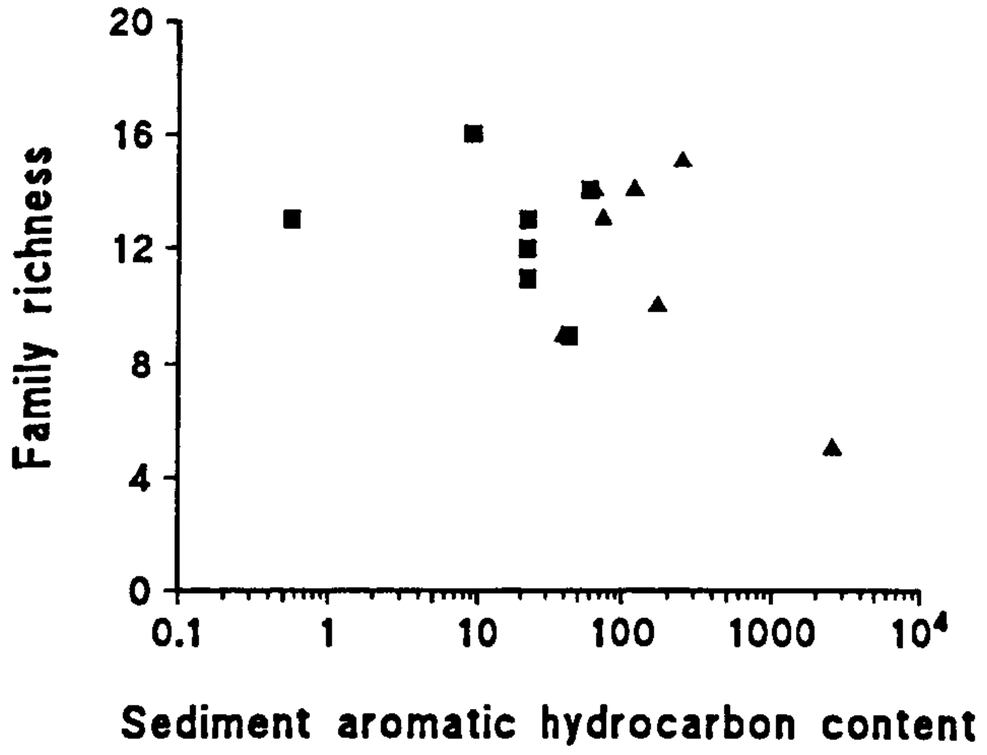

Fig. 8. Correlation between the number of macroinvertebrate families present at a station and the concentration of aromatic hydrocarbons in the sediment. Squares represent upstream stations, and triangles represent downstream stations.

differences in macroinvertebrate assemblages observed in this study may not be related to direct toxicity at all. For example, particulates washed off road surfaces may alter the structure of the stream bed, and bridge and drainage structures may alter flow conditions. Furthermore, the design used in this study (i.e., upstream/downstream comparison) means that between-station differences in environmental factors unconnected with motorway runoff cannot be separated from those that are. Although the data collected during a study such as the one described here cannot be used to establish cause–effect relationships, they can be used to rule out certain factors and identify plausible causal factors that can be assessed further by experimental analysis.

Previous studies have demonstrated that particulate material from roads alters substrate characteristics affecting the distribution of burrowing animals such as chironomid larvae and tubificid worms. For example, Extence [86] noted that the abundance of burrowing macroinvertebrates was reduced below a motorway discharge and attributed this effect to a reduction in the stability and organic content of the sediment. Alternately, fine particulate material in motorway runoff may be deposited on the stream bed, thus favoring burrowing organisms. In the present study, chironomids and oligochaetes were more abundant at the downstream station at Pigeon Bridge Brook than at the upstream station. However, this increased abundance was not associated with either a reduction in particle size or an increase in the organic content of the sediment. Sediment particle size was smallest and TOC concentration was greatest at the upstream station.

Assessing the relative importance of altered flow conditions in determining species distributions at the study sites is more problematic. Detailed hydrological analyses of the sampling stations were not conducted as part of this study, although rainfall data were available (Fig. 1). Despite the fact that samples were obtained throughout the year and under different hydrographic conditions, statistically significant temporal changes in macroinvertebrate assemblages were not detected.

There is little evidence, therefore, that changes in the physical structure of the habitat were responsible for the observed changes in macroinvertebrate distributions. Another possibility is that runoff-induced changes in food quality or quantity were responsible [87–89]. The main food sources for nonpredatory macroinvertebrates in small streams are benthic algae and detritus. Hydrocarbons have been shown to increase algal abundance either by reducing grazing pressure [90–92] or by direct stimulation [93–95]. In the present study there was a significant reduction in the relative abundance of algal feeders (i.e., scrapers) at the contaminated station at Pigeon Bridge Brook. However, there was no evidence that runoff from the M1 motorway had a significant effect on either the abundance or distribution of benthic algae.

Autumn-shed leaves were the main source of detrital material in the study streams and are the main food of macroinvertebrate shredders. Leaves were colonised by a range of aquatic hyphomycete species, the most diverse assemblage being recorded for the downstream station at Pigeon Bridge Brook. Aquatic hyphomycetes are important both as decomposers of leaf material and as modifiers of leaf material for macroinvertebrate consumption [38,96–98], and as such they have the potential to influence both the breakdown of CPOM and the distribution of shredders in streams. Oil, a major component of road runoff, has been shown to reduce the decomposition of leaf litter in lakes [99–101], and metals are known to inhibit the growth and sporulation of aquatic hyphomycetes [102].

In the present study, microbial decomposition of leaf material was not affected by motorway runoff, but macroinvertebrate-mediated breakdown was significantly reduced at Pigeon Bridge Brook. As the canopy at Pigeon Bridge Brook was more dense at the downstream station, it can plausibly be predicted that shredders would be most abundant at this station and hence macroinvertebrate-mediated breakdown would be increased. However, the opposite was observed. The abundance of shredders was significantly reduced downstream of the discharge. Moreover, those that were present (e.g., *Gammarus pulex*) had reduced feeding rates (D. Forrow, unpublished data).

In summary, therefore, the results of this study indicated that the quality of both receiving water and sediment was altered at stations a short distance (<100 m) from point-source inputs of motorway runoff. There are reasonable grounds for assuming causality, especially because there was a good fit to a "mechanistic" model linking downstream concentrations of aromatic hydrocarbons in the sediment and an index of potential loading. The main contaminants were hydrocarbons, in particular PAHs (i.e., fluoranthene, pyrene, and phenanthrene), carbonyl compounds, and metals (i.e., zinc, cadmium, chromium, and lead). In addition, there was also an elevation in the concentrations of chloride and sulphate ions in stream water. Although there was evidence of contamination at all the sites surveyed, it was most severe where drainage entered small streams, the most impacted site being Pigeon Bridge Brook.

For 57% of the streams surveyed, the macroinvertebrate assemblages at the station receiving motorway runoff was less diverse and contained fewer pollution-sensitive taxa than the assemblage at the uncontaminated station. At the most im-

pacted site, Pigeon Bridge Brook, the change in the structure of the macroinvertebrate assemblage was associated with a significant reduction in macroinvertebrate-mediated leaf breakdown and a shift from a mixed-economy to one dependent upon FPOM and dominated by collectors. Changes in macroinvertebrate distributions were possibly due to direct toxic effects as there were no significant between-station differences in either the abundance of epilithic algae or detritus and associated fungi. Furthermore, the major changes observed (i.e., increased abundance of chironomids and oligochaetes, decreased abundance of *G. pulex*) could not be explained on the basis of changes in substrate particle size or TOC.

Although the effects observed in this study were limited and localized, they were significant and should therefore be taken into consideration when assessing the potential risk of road runoff to stream biota. The toxicity of runoff-contaminated sediment to *G. pulex*, the dominant macroinvertebrate at the upstream station at Pigeon Bridge Brook, is discussed elsewhere [103].

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APPENDIX

Macroinvertebrate taxa present in streams receiving runoff from the M1 motorway. Data are presented for upstream/downstream sampling stations. * = present; – = absent.

Taxon	Pigeon Bridge Brook	Butterthwaite Ditch	Rockley Dike
Platyhelminthes			
Polycelis felina	–/–	–/–	*/*
Annelida			
Naididae	*/*	*/–	*/*
Lumbriculidae	*/*	*/*	–/–
Tubificidae	*/*	*/*	*/*
Erpobdella octoculata	–/*	*/*	*/*
Glossiphonia complanata	–/–	–/–	*/*
Helobdella stagnalis	–/–	–/–	*/*
Mollusca			
Potamopyrgus jenkinsi	*/*	*/*	*/*
Lymnaea peregra	*/*	*/*	*/*
Sphaeriidae	*/*	*/*	*/*
Crustacea			
Gammarus pulex	*/*	*/*	*/*
Asellus aquaticus	–/–	–/*	*/*
Chelicerata			
Hygrobatidae	*/–	–/–	*/*
Limnocharidae	–/–	–/–	*/–
Uniramia			
Sialis lutaria	–/–	*/*	*/*
Baetis rhodani	–/–	*/*	–/–
Leuctra inermis	*/*	–/–	–/–
Apatania sp.	–/–	–/*	–/–
Halesus radiatus	–/–	–/–	–/*
Limnephilus extricatus	*/–	–/–	–/–
Micropterna lateralis	*/–	–/*	–/–
Plectrocnemia geniculata	*/*	*/*	–/–
Plectrocnemia conspersa	*/–	*/–	–/–
Stenophylax permistus	–/–	*/*	–/–
Hesperocorixa sahlbergi	–/–	–/*	–/–
Uniramia, continued			
Sigara concinna	–/–	–/–	–/*
Velia caprai	*/–	*/*	–/–
Dytiscidae larvae	*/*	*/*	*/*
Elminthidae larvae	*/–	–/–	–/–
Helodidae larvae	*/–	–/–	–/–
Hydrophilidae larvae	*/–	–/–	–/–
Agabus sp.	*/*	*/*	–/–
Anacaena globulus	*/–	*/*	–/–
Hydroporus sp.	–/–	–/*	–/–
Halticinae sp.	–/–	–/*	–/–
Helophorus sp.	*/*	*/*	–/*
Hydrobius fuscipes	–/*	–/–	–/–
Ilybius guttiger	–/–	*/–	–/–
Culicidae	–/–	*/–	–/–
Dixidae	*/–	–/–	–/–
Empididae	*/–	–/–	–/–
Simuliidae	–/–	*/*	*/*
Tipulidae	*/–	*/*	*/*
Chironominae	*/*	*/*	*/*
Orthocladiinea	*/*	*/*	*/*
Prodiamesinae	*/*	*/*	*/*
Tanypodinae	*/*	*/*	*/*
Dicranota sp.	*/–	–/–	*/*
Eristalis sp.	–/–	–/*	–/–
Forcipomyia sp.	*/–	–/–	*/*
Pedicia sp.	–/–	–/–	*/–
Pericoma sp.	*/*	*/*	*/*
Ptychoptera sp.	*/–	–/*	–/–
Lepidoptera larvae	*/*	*/*	–/*
Mean no. individuals	135/82	140/115	110/119

Pergamon

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THE EFFECTS OF MOTORWAY RUNOFF ON FRESHWATER ECOSYSTEMS: 2. IDENTIFYING MAJOR TOXICANTS

LORRAINE MALTBY,*† ALISTAIR B.A. BOXALL,† DAVID M. FORROW,†
PETER CALOW† and CLIFFORD I. BETTON‡
†Department of Animal and Plant Sciences, The University of Sheffield, P.O. Box 601, Sheffield, S10 2UQ, U.K.
‡Castrol International, Burmah Castrol House, Pipers Way, Swindon SN3 1RE, U.K.

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Abstract—Previous studies have provided prima facie evidence that runoff from the M1 motorway, UK, affects both the quality of the receiving water and the biota living there, in sites short distances from point sources—i.e., possible worst-case situations. Because discharges contain a wide variety of contaminants, both the identification of toxicants and the establishment of causal relationships between observed changes in water/sediment quality and biology are often difficult. In this particular case, the problem was addressed by conducting a series of toxicity tests using the benthic amphipod *Gammarus pulex*. The abundance of this species was greatly reduced downstream of the point where motorway runoff entered the stream. Stream water contaminated with motorway runoff was not toxic to *G. pulex*. However, exposure to contaminated sediments resulted in a slight reduction in survival over 14 d, and sediment manipulation experiments identified hydrocarbons, copper, and zinc as potential toxicants. Spiking experiments confirmed the importance of hydrocarbons, and fractionation studies indicated that most of the observed toxicity was due to the fraction containing polycyclic aromatic hydrocarbons. Animals exposed to contaminated sediments and water spiked with sediment extract accumulated aromatic hydrocarbons in direct proportion to exposure concentrations.

Keywords—Motorway runoff *Gammarus pulex* Sediment toxicity Hydrocarbons TIE

INTRODUCTION

A recent detailed study into the impact of discharges from a British motorway on the quality and biology of receiving waters concluded that small streams receiving motorway runoff had elevated concentrations of metals and hydrocarbons in both the sediments and overlying water [1]. Moreover, these changes in water and sediment quality were associated with localized changes in the structure and functioning of the benthic community at some sites. Runoff from roads may contain hundreds of different compounds making identification of potential toxicants far from straightforward. One approach is to use toxicity identification evaluation (TIE) procedures [2–4]. The TIE procedure has been defined as "a stepwise process that combines toxicity testing and analysis of the physical and chemical characteristics of effluents to identify potentially causative toxicants" [5]. Various standard procedures have been designed to characterise, identify, and confirm toxicants in acutely toxic effluents [6,7], sediment pore waters [8,9], waste leachates [10], and ambient waters [11]. This has proven useful in identifying toxicity caused by a range of compounds including ammonia, nonpolar organic compounds, and metals [12].

A stepwise approach was used in this study to assess the toxicity of sediments and water contaminated with motorway runoff and to identify the group(s) of compounds responsible for the observed toxicity. The experiments concentrated on the benthic amphipod *Gammarus pulex* and on one particular study site, Pigeon Bridge Brook. This was the most contaminated of the seven sites in the original survey [1]. At this site, concentrations of hydrocarbons in sediments downstream of the motorway discharge averaged 368 μg chrysene equivalents/mg wet wt. (SE = 63) compared to 50 μg chrysene equivalents/mg wet wt. (SE = 10) upstream of the discharge. In addition, metal concentrations were elevated in both the sediment and the overlying water. *Gammarus pulex* was the most abundant benthic macroinvertebrate upstream of the discharge. However, downstream of the discharge the macroinvertebrate assemblage was dominated by chironomid larvae and oligochaetes, and the abundance of *G. pulex* was greatly reduced.

MATERIALS AND METHODS

Source of test material and organisms

Adult male *Gammarus pulex* were collected from Crags Stream, Derbyshire (National Grid Reference SK497745). They were maintained under experimental conditions (15°C, 12-h light:12-h dark) and fed a diet of alder leaves until use. Test sediments and water were obtained from stations upstream (National Grid Reference SK476851) and downstream (National Grid Reference SK479852) of the point where runoff from the M1 motorway enters Pigeon Bridge Brook. Sediment and water were stored in polypropylene containers in the dark at 4°C and used within 4 d of collection.

Toxicity of stream water

Animals were exposed to either an artificial pond water (APW) [13] or stream water collected from the two sampling

*To whom correspondence may be addressed.

stations. Stream water was collected every 4 d and filtered through Whatman No. 1 filters before use. For each replicate, 10 adult male *G. pulex* were each placed in individual plastic experimental chambers (5.5 cm diameter) containing 150 ml of test solution. There were five replicates per treatment, and animals were exposed for 15 d, after which the number surviving was recorded. Animals were not fed during exposure. Test solutions were changed every other day, at which time duplicate independent samples of "old" test solutions and duplicate subsamples of "new" stream water were taken and analysed for aromatic hydrocarbons and heavy metals.

Toxicity of field sediments

Sediment samples, collected from the surficial 10 cm, were thoroughly mixed before being allocated to plastic experimental chambers (5.5 cm diameter). Each chamber contained 30 g of sediment, 150 ml of APW, and one animal. No food was added. There were 50 chambers per replicate and four replicates per treatment. Animals were exposed for 14 d, after which the number surviving in each chamber was recorded. Samples of sediment were taken at the beginning and end of the experiment and analysed for aromatic hydrocarbons. For each treatment, six subsamples were taken at the beginning and six independent samples taken at the end of the experiment. Metal concentrations of sediments at the beginning of the experiment were also determined as was the concentration of aromatic hydrocarbons in surviving animals.

Sediment manipulation

Contaminated sediment (160 g) was Soxhlet-extracted twice with 250 ml of Distol-grade dichloromethane (DCM), each extraction lasting for 24 h. The sediment was then dried to evaporate off the solvent before being rehydrated with APW and allocated to experimental chambers. The experimental design was the same as that used in the previous experiment: the two treatments being extracted and nonextracted downstream sediment.

Toxicity of sediment extracts

Samples (640 g) of upstream and downstream sediment were Soxhlet-extracted using the methods described above. The resulting extracts were rotary-evaporated to dryness before being taken up in 80 ml of analytical-grade acetone. Stock solutions (0.8 ml/L) were prepared for each extract by pipetting 8 ml of extract into 10 L of APW and stirring for 24 h. Test solutions of 0.05, 0.1, 0.2, and 0.4 ml/L were prepared by diluting the stock solutions with APW containing 0.8 ml/L acetone. This diluting solution also served as the control treatment. Animals were exposed individually to 150 ml of test solution for 17 d. There were four replicates per test solution, each consisting of 15 individually exposed animals. Test solutions were prepared daily and analysed for aromatic hydrocarbons. Spiked water was analysed for heavy metals, and surviving animals were analysed for aromatic hydrocarbons.

Toxicity of extract fractions

An 800-g sample of sediment was collected from the downstream station at Pigeon Bridge Brook and Soxhlet-extracted into DCM to produce five 20-ml extracts, each derived from 160 g of sediment. Each extract was mixed with 10 g of activated alumina (70 to 230 mesh) before being transferred to a glass column (100 cm × 1 cm i.d.) packed with 20 g of silica (60 to 120 mesh). The column was then eluted sequentially with 100 ml of each of the following solvents: 100% *n*-pentane, 10% DCM in *n*-pentane, 20% DCM in *n*-pentane, 30% DCM in *n*-pentane, 100% DCM. This procedure was repeated for each of the five sediment extracts. The eluates for each solvent were then pooled to give a total volume of 500 ml per solvent. The 10 and 20% DCM eluates were combined (F2a fraction) as were the 30 and 100% DCM eluates (F2b fraction). The *n*-pentane eluates are subsequently referred to as the F1 fraction. The three resulting fractions (i.e., F1, F2a, F2b) were evaporated to dryness and dissolved in 100 ml of acetone. Test solutions were prepared by spiking 5 L of APW with 1.5 ml of either acetone (control), F1 fraction, F2a fraction, or F2b fraction. Mixtures were prepared by adding together 1.5 ml of the F2a and F2b fractions (i.e., F2a + F2b) or 1.5 ml of each of the three fractions (i.e., F1 + F2a + F2b), evaporating to dryness, and dissolving in 1.5 ml of acetone. The mixture was then used to spike 5 L of APW.

There were four replicates per test solution, each consisting of 15 animals housed separately in individual plastic experimental chambers (5.5 cm diameter) containing 100 ml of test solution. Fresh test solutions were prepared daily and analysed for total hydrocarbons, aromatic hydrocarbons, and selected PAHs. Mortality was recorded daily for 14 d, after which surviving animals were analysed for aromatic hydrocarbons and selected PAHs.

Chemical analyses

Sample preparation and chemical analyses followed procedures described in Maltby et al. [1]. Water and sediment samples were analysed for aluminium, cadmium, copper, chromium, lead, and zinc using atomic absorption spectrophotometry (Perkin-Elmer 2100 AAS with an AS50 autosampler). Prior to analysis, preweighed samples of sediment (~0.2 g) were digested for 4 h at 80°C in 15 ml of 30% Primar nitric acid.

Sediment (ca. 4 g) and animal (ca. 1.5 g) samples for hydrocarbon analysis were saponified for 12 h in 5 ml of methanolic KOH (56 g/L) before being Soxhlet-extracted into 50 ml of Distol-grade DCM for 4 h. Water samples (200 ml) were prepared for hydrocarbon analysis by extracting twice, each with 35 ml of DCM. The DCM extracts were rotary-evaporated to approximately 3 ml before being loaded onto an alumina (70 to 230 mesh) on silica column (60 to 120 mesh) and eluted with 30 ml of DCM. The eluate was then evaporated to 5 ml before analysis.

Aromatic hydrocarbon concentrations were determined using UV spectrophotometry; total hydrocarbon concentrations were measured by IR spectrophotometry; and selected PAHs (i.e., anthracene, phenanthrene, fluoranthene, pyrene, and chrysene) were quantified by HPLC. Also, GC–MS was used to identify specific hydrocarbons, including PAHs and alkylated phenols.

The infrared analysis was performed using a Perkin-Elmer 684 IR spectrophotometer, the size of the 2,932 cm^{-1}

peak being used as a measure of total hydrocarbons. Results are expressed in terms of standard base oil (SN150) equivalents. Ultraviolet absorbance was measured at 254 nm using a Pye Unicam SP 8-100 UV/visible spectrophotometer, and data are expressed as chrysene equivalents. Individual PAHs were quantified using a Phillips PU4100 liquid chromatograph with a Phillips PU5110 UV/vis detector. Ten-microliter samples were eluted using a 15%:85% methanol:water mix at a flow rate of 1,000 μl/min, and absorbance was measured at 254 nm. Samples were also analysed for specific hydrocarbons using a Perkin-Elmer GC8300 gas chromatograph fitted with an ion trap detector.

Statistical methods

Data were tested for normality using normal probability plots. Between-treatment differences were assessed using either one-way analysis of variance or Student's *t* test, and regression analyses were performed using least-squares regression techniques. All analyses were performed using the MINITAB statistical package [14], and significance levels were $p < 0.05$.

RESULTS

Toxicity of stream water

Test solutions differed markedly in water quality (Fig. 1). Water collected from the downstream station contained significantly more zinc and aromatic hydrocarbons than either APW or water collected from the upstream station ($t > 6$; $d.f. > 19$). However, within a treatment, there was no significant difference in water quality during exposure ($t < 1.06$, $d.f. > 13$). There was no significant difference in the mean survival of animals exposed to either APW or stream water ($F = 0.03$, $d.f. = 2, 14$): APW = 90% (SE = 3.16), upstream water = 86% (SE = 6), downstream water = 84% (SE = 6.8).

Whole sediment toxicity

Sediments collected from the two field stations were markedly different in their aromatic hydrocarbon content,

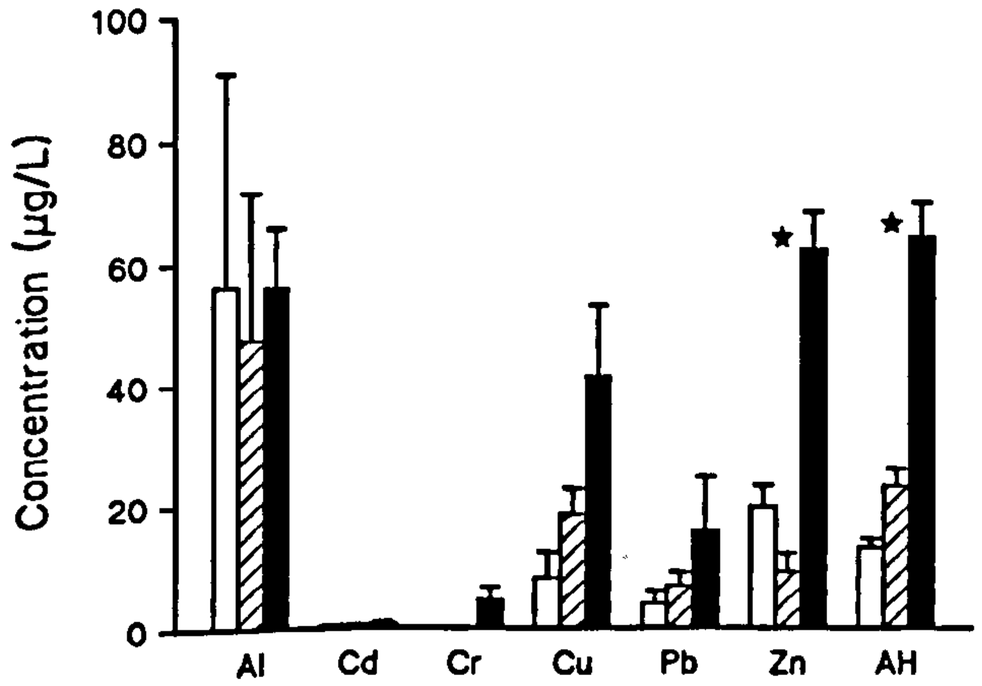

Fig. 1. Concentrations of metals and aromatic hydrocarbons (AH) in artificial pond water (APW) (open bars), upstream water (hatched bars), and downstream water (solid bars). Data are presented as mean values + 1 SE, and asterisk denotes significant differences between downstream water and the other treatments.

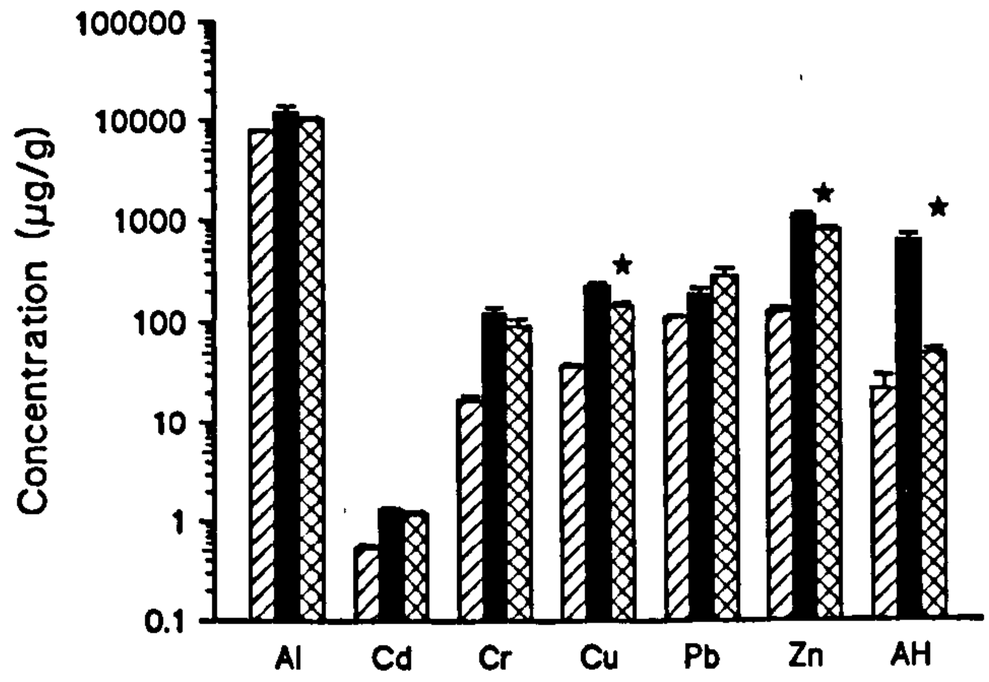

Fig. 2. Concentrations of metals and aromatic hydrocarbons (AH) in upstream sediment (hatched bars), downstream sediment (solid bars), and downstream sediment that has been extracted with dichloromethane (DCM) (cross-hatched bars). Data are presented as mean values + 1 SE. Asterisk denotes significant difference between untreated and treated downstream sediment.

upstream sediment having less than one-tenth the aromatic hydrocarbon content of downstream sediment. Downstream sediments also had significantly elevated concentrations of all the metals analysed (Fig. 2; $t > 2.8$, $d.f. > 5$). The aromatic hydrocarbon content of the sediment did not vary significantly over the course of the experiment ($t < 1.7$, $d.f. = 3$), and exposure to contaminated sediments resulted in a small, but statistically significant, reduction in survival ($t = 3$, $d.f. = 6$). The mean survival of animals exposed to upstream sediment was 96% (SE = 1.4) compared to 90% (SE = 1.4) for animals exposed to downstream sediment. Moreover, animals exposed to contaminated sediment had elevated whole-body concentrations of aromatic hydrocarbons (Fig. 3).

Sediment manipulation

Extracting contaminated sediment with DCM resulted in a significant reduction in the concentrations of aromatic hy-

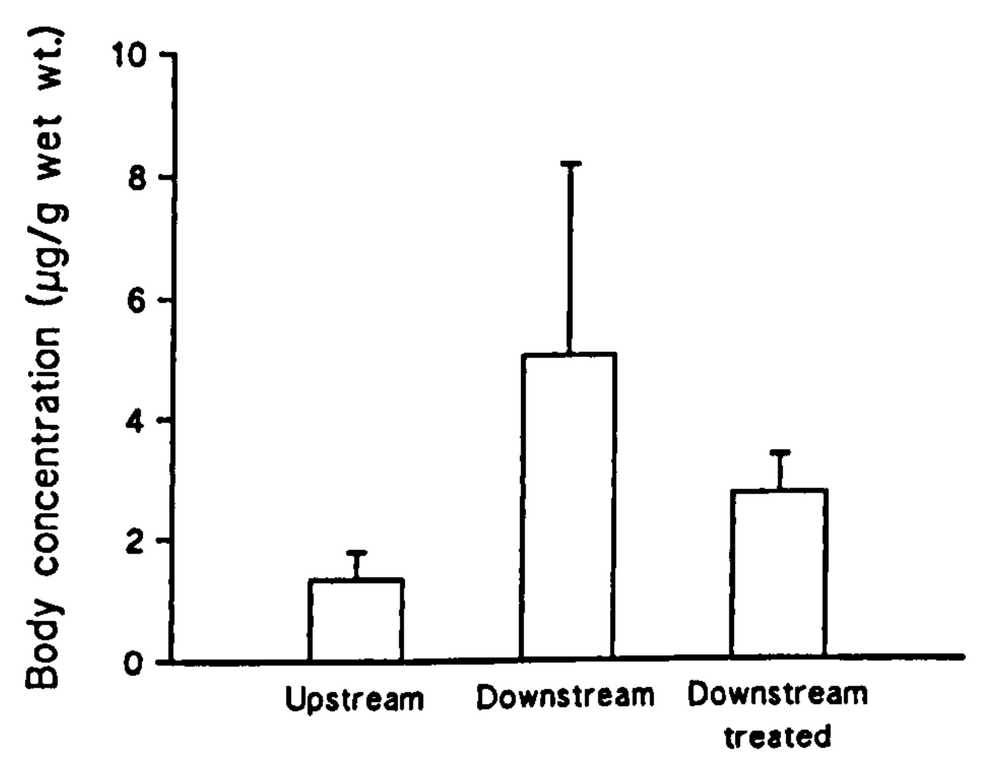

Fig. 3. Mean concentration (+1 SE) of aromatic hydrocarbons in *Gammarus pulex* exposed to upstream, downstream, or extracted downstream sediment.

drocarbons, copper, and zinc (Fig. 2; $t > 4.8$, $d.f. = 9$). There was no significant difference between the concentrations of aromatic hydrocarbons in upstream and treated downstream sediment ($t = 2.6$, $d.f. = 3$) although concentrations of copper and zinc were significantly lower in upstream sediment ($t > 9$, $d.f. > 5$). These changes in the quality of downstream sediment were associated with a statistically significant reduction in mortality ($t = 3.7$, $d.f. = 4$) to a level identical to that for animals exposed to upstream sediment, i.e., 4%. Animals exposed to manipulated sediments also accumulated hydrocarbons less than animals exposed to untreated sediments (Fig. 3).

Spiking with sediment extract

A strong negative correlation existed between the concentration of downstream sediment extract in spiked water and the survival of *Gammarus pulex* ($r^2 = 96\%$). However, no such relationship was apparent for animals exposed to water spiked with upstream sediment extract (Fig. 4a). When the data were expressed in terms of aromatic hydrocarbon concentrations, the two data sets merged (Fig. 4b), resulting in a common LC50 value of 154 μg chrysene equivalents/L.

Animals exposed to sediment extracts accumulated aromatic hydrocarbons in direct proportion to exposure concentrations (Fig. 5a; $r^2 = 99\%$); consequently, a significant negative relationship existed between whole-body hydrocarbon concentration and percentage survival (Fig. 5b; $r^2 = 92\%$). There was no significant difference in the metal concentrations of water spiked with the two extracts (Fig. 6).

Toxicity of extract fractions

The chemical composition of the fractions was markedly different. Whereas the F1 fraction had the highest concentration of total hydrocarbons, it had the lowest concentra-

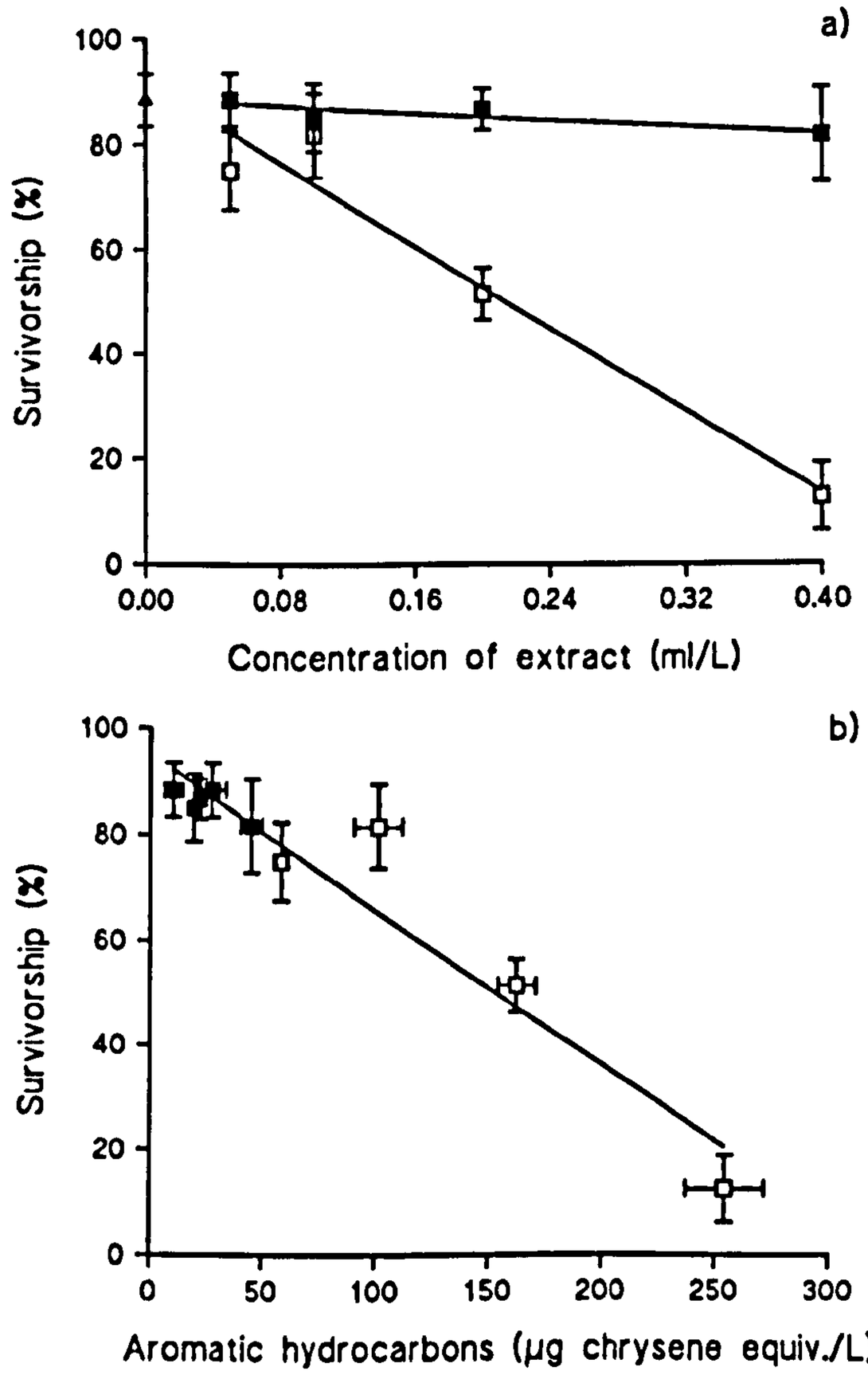

Fig. 4. Mean survival (± 1 SE) of *Gammarus pulex* exposed to water spiked with either upstream (solid squares) or downstream (open squares) sediment extract. The triangle represents control animals. Data are presented as concentration of (a) sediment extract and (b) aromatic hydrocarbons (mean ± 1 SE). Lines fitted by regression analysis.

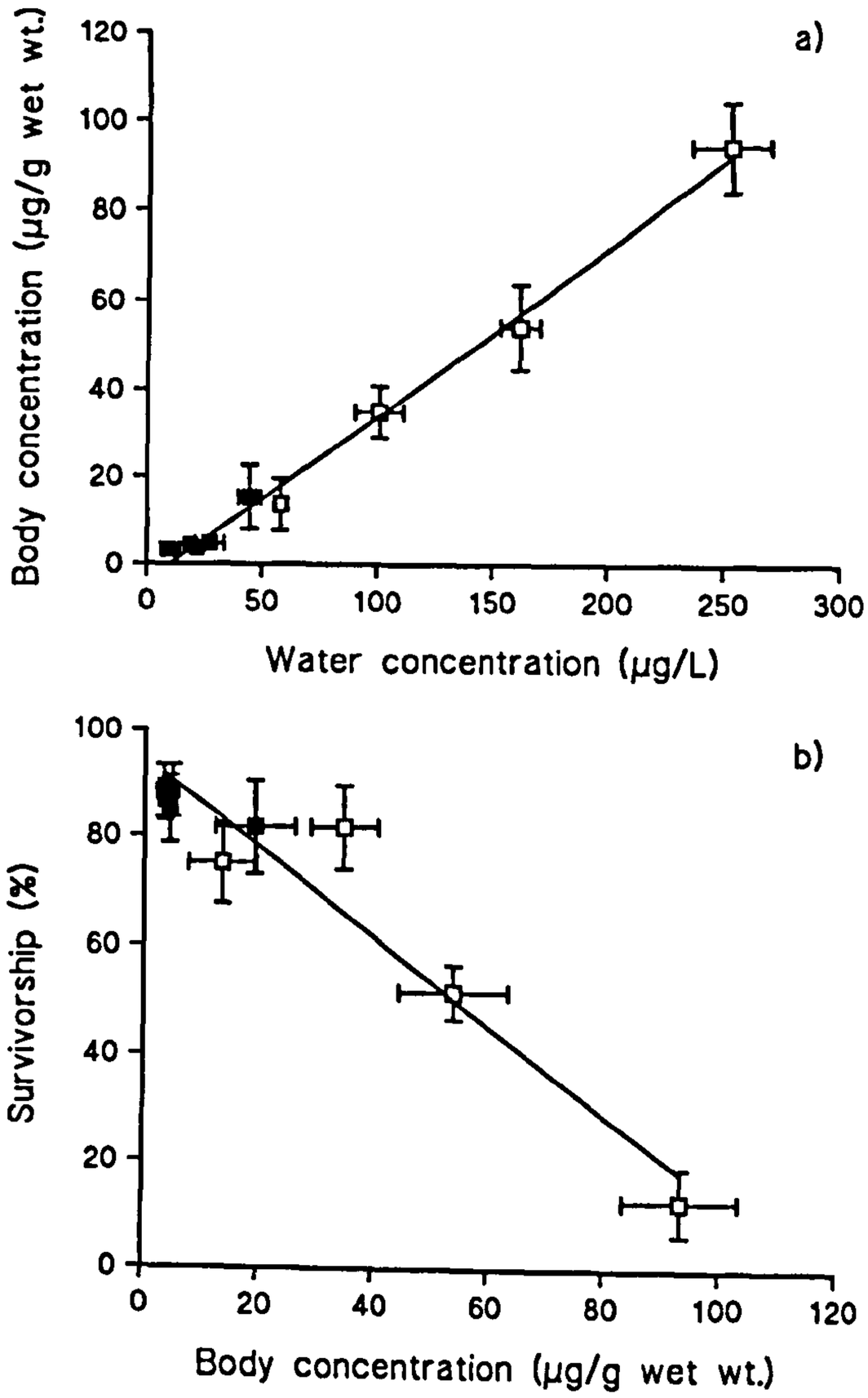

Fig. 5. Relationship between (a) the concentration of aromatic hydrocarbons in the test solution and their accumulation by *G. pulex* and (b) survival and whole-body aromatic hydrocarbon concentration. Open symbols represent animals exposed to downstream sediment extract; solid symbols represent animals exposed to upstream sediment extract. Error bars are 1 SE. Lines fitted by regression analysis.

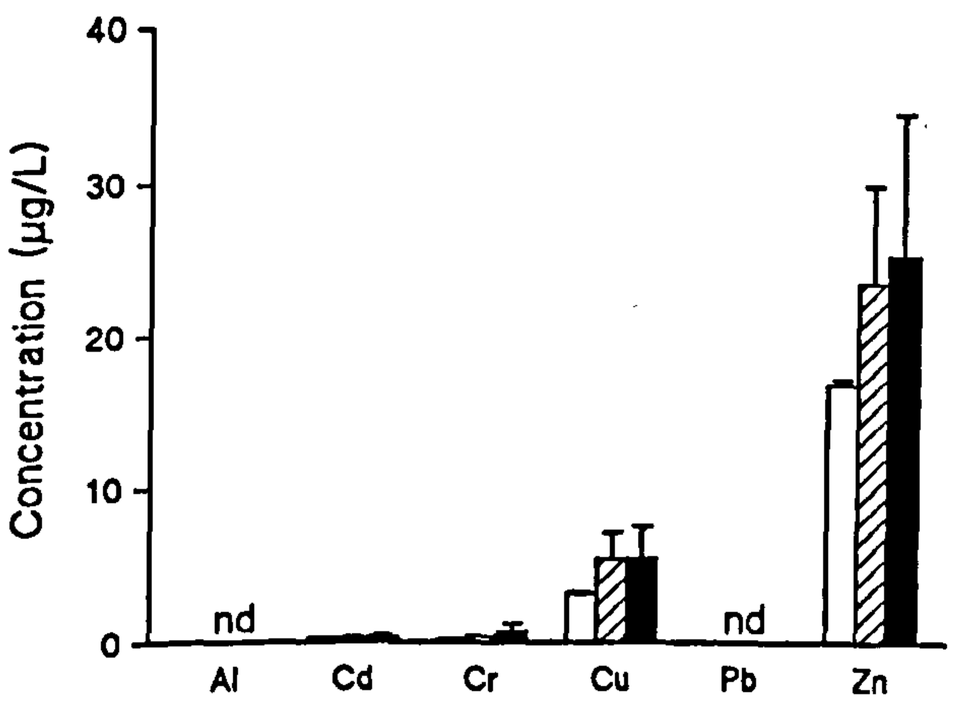

Fig. 6. Mean concentration (+1 SE) of metals in APW (open bars) and APW spiked with either upstream (hatched bars), or downstream (solid bars) sediment extract (nd = not detected).

tion of aromatic hydrocarbons (Fig. 7). The F1 fraction was comprised principally of aliphatic hydrocarbons, the F2a fraction contained PAHs, and the F2b fractions contained non-PAH aromatics including alkylated phenols (Fig. 8).

No significant difference existed in the mortality of animals exposed to water spiked with either acetone, F1, or F2b fractions (Fig. 9; $t < 1.4$, $d.f. = 3$). However, a large and significant increase occurred in the mortality of animals exposed to water spiked with either the F2a fraction or fraction mixtures (Fig. 9; $t > 12.7$, $d.f. = 5$). There was no significant difference in the LT50 values for animals exposed to water spiked with the F2a fraction alone or mixtures containing the F2a fraction. The LT50 values ranged from 9.78 (F2a + F2b) to 10.62 d (F1 + F2a + F2b).

There was a significant linear relationship between the concentration of aromatic hydrocarbons in the test solution (µg/L) and their accumulation by *G. pulex* (µg/g wet wt.) ($r^2 = 0.91$, $d.f. = 5$). However, specific PAHs, with the ex-

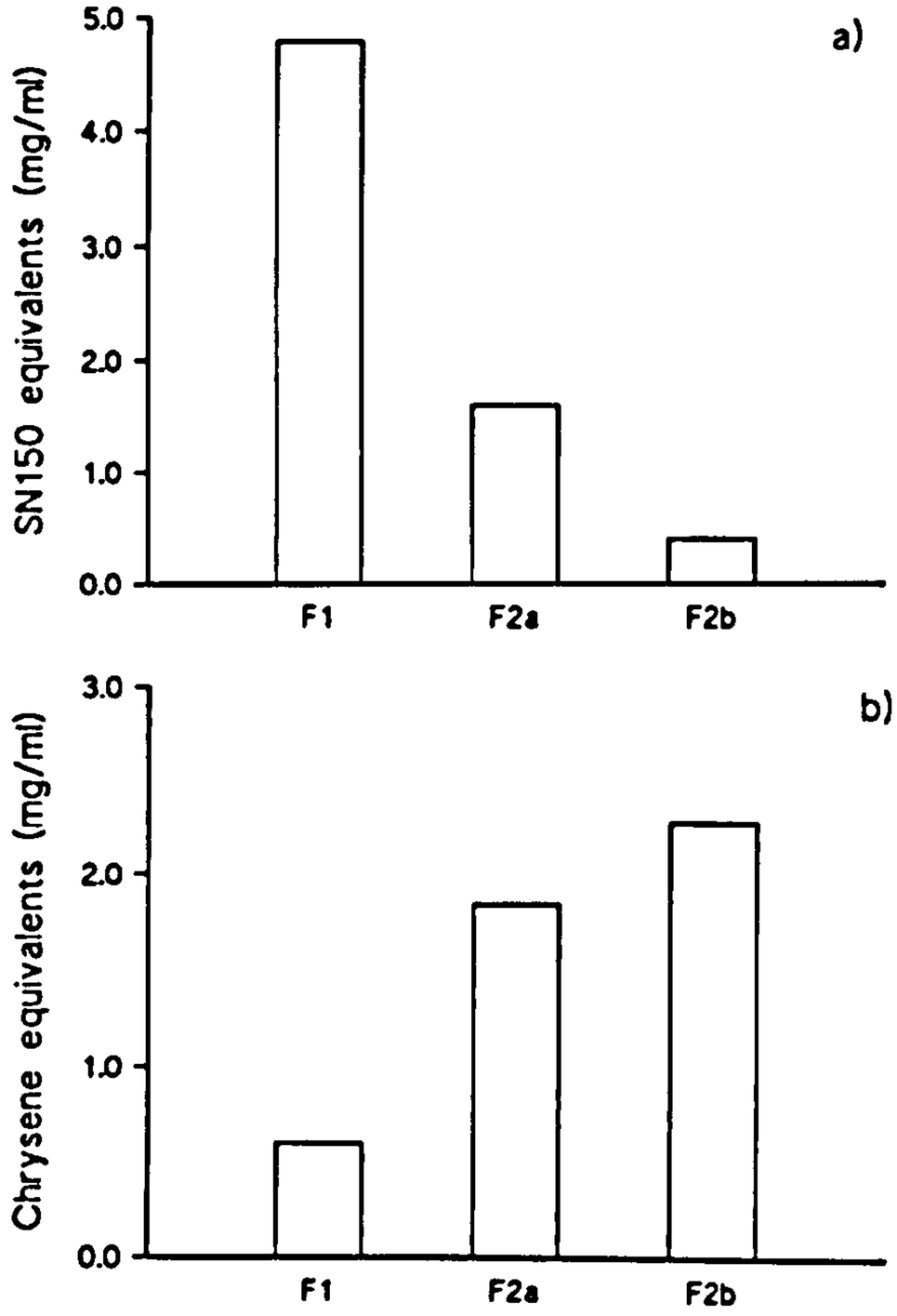

Fig. 7. Concentration of (a) total hydrocarbons and (b) aromatic hydrocarbons in fractions of a downstream sediment extract.

ception of pyrene, did not follow this relationship (Fig. 10). Fluoranthene was accumulated to a greater extent than predicted, whereas chrysene was accumulated to a lesser extent. The greatest discrepancy between observed and predicted accumulation was for anthracene and phenanthrene.

DISCUSSION

Pigeon Bridge Brook was a small stream which received runoff from a busy British motorway. Downstream of the discharge, concentrations of hydrocarbons and metals were elevated in both the sediments and the water column, and there was a change in the structure and functioning of the benthic macroinvertebrate community [1]. However, correlations of effects such as these are not necessarily evidence of causal relationships. The aim of the work described here was to assess whether there was any evidence that contaminated sediments and/or water were toxic to a dominant macroinvertebrate upstream of the discharge (i.e., *G. pulex*) and, if so, to identify the major toxicants.

There was no evidence that contaminated stream water was acutely toxic to *G. pulex*. However, exposure to contaminated sediments did result in a small but statistically significant reduction in survival. Although this effect was slight (i.e., 10% reduction in survival) it was consistent. This experiment has been repeated three times, and on all occasions the mortality of animals exposed to upstream sediment was ≤5% (x=4.3%) compared to an average mortality of 10.3% for animals exposed to downstream sediment. The biological significance of such a relatively small decrease in survival is problematic. However, lethality is an extreme response that is often preceded by a range of sublethal responses, some of which may have been exhibited by animals exposed to contaminated sediments. The fact that there was a consistent reduction in the survival of animals exposed to sediment contaminated with road runoff indicated that there was a potential risk to benthic organisms.

Downstream sediments were contaminated with a variety of potential toxicants including both heavy metals and hydrocarbons [1]. Consequently, various experiments were designed to identify more precisely the possible toxicants in a stepwise fashion. Extracting contaminated sediments with DCM resulted in a reduction in mortality to a level identical to that experienced by animals exposed to upstream sediments. Manipulating sediment in this way resulted in changes in both physical and chemical properties of the sediment. Treated downstream sediment had significantly reduced concentrations of aromatic hydrocarbons, copper, and zinc, thus indicating that they may have been responsible for the observed toxicity. This was investigated by spiking APW with DCM extracts of upstream and downstream sediment. Whereas water spiked with downstream sediment extract was highly toxic, water spiked with upstream sediment extract was not. Although spiked water differed significantly in terms of aromatic hydrocarbon concentration, there was no significant difference in the concentrations of either copper or zinc. Results of these two experiments therefore suggest that sediment-associated toxicants are removed by DCM extraction and that they are hydrocarbons rather than metals. Analysis of exposed animals confirmed that *G. pulex* accumulated aromatic hydrocarbons, and that this accumulation was proportional to the concentration of aromatic hydrocarbons in the test solution.

Runoff-contaminated sediments contain a large number of hydrocarbons, both aliphatic and aromatic [15]. Downstream sediment extract was therefore fractionated using differential solvent extraction in an attempt to identify which group or groups of hydrocarbons were responsible for the observed toxicity. Three fractions were produced, one containing principally aliphatic hydrocarbons (F1) and two containing aromatic hydrocarbons (F2a, F2b). The F2a fraction was characterised by the presence of PAHs, whereas the F2b fraction contained alkylated phenols. Water spiked with the F2a fraction was highly toxic to *G. pulex*, resulting in >90% mortality over 14 d. In contrast, animals exposed to either APW or the other two fractions experienced <12% mortality over the same period. Therefore, it would appear that the toxic components were in the F2a fraction and that they were probably PAHs.

Although animals exposed to spiked water accumulated aromatic hydrocarbons in direct proportion to the exposure concentration, accumulation varied between individual PAHs. Whereas the accumulation of fluoranthene, pyrene, and chrysene was similar to that predicted on the basis of total aromatics, the accumulation of phenanthrene and anthra-

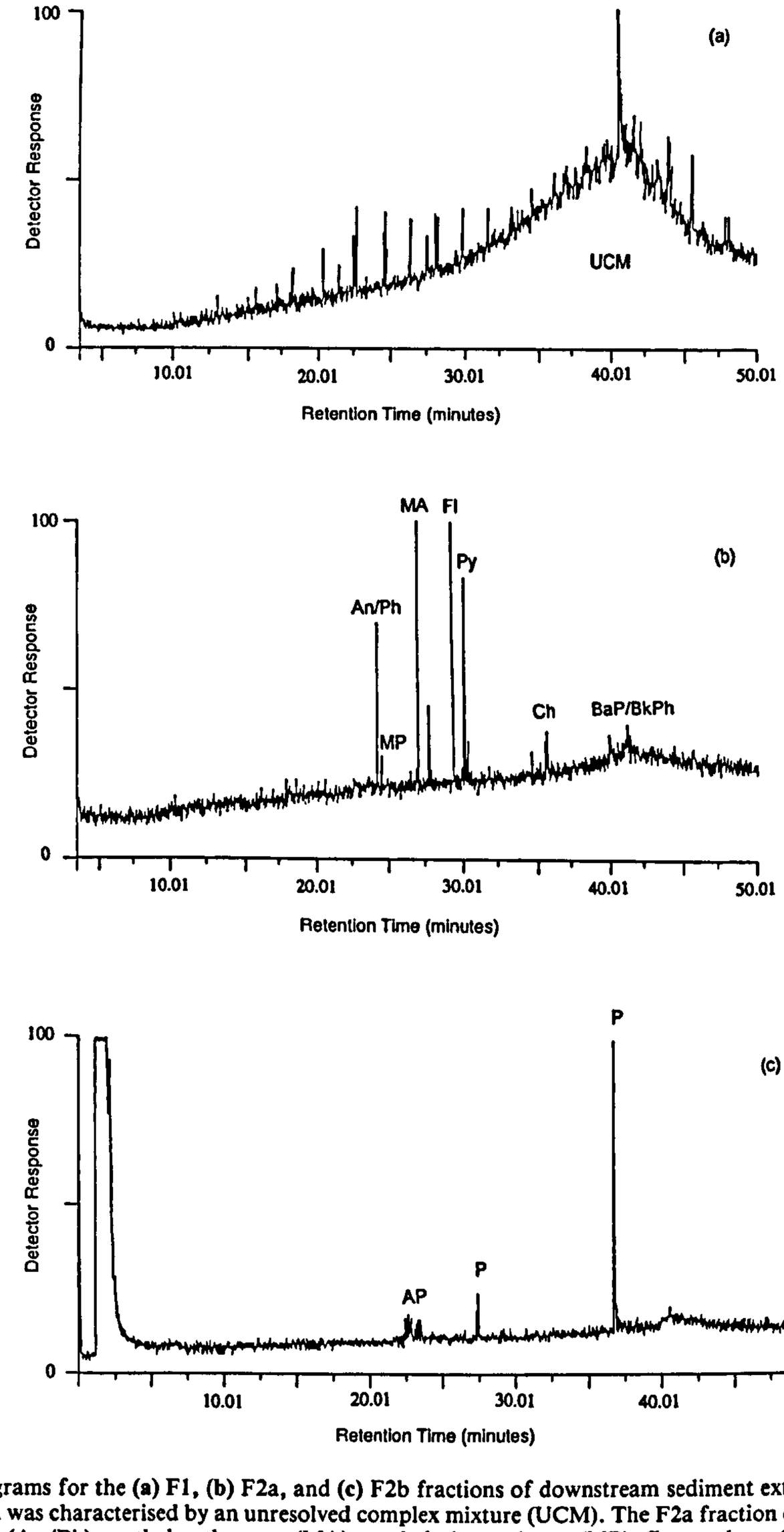

Fig. 8. GC–MS chromatograms for the (a) F1, (b) F2a, and (c) F2b fractions of downstream sediment extract. The F1 fraction contained aliphatic hydrocarbons and was characterised by an unresolved complex mixture (UCM). The F2a fraction contained PAHs indcluding: anthracene and phenanthrene (An/Ph), methyl anthracene (MA), methyl phenanthrene (MP), fluoranthene (Fl), pyrene (Py), chrysene (Ch), and benzo[*a*]pyrene and benzo[*k*]phenanthrene (BaP/BkPh). The F2b fraction contained plasticisers (P) and alkylated phenols (AP).

cene was much less than predicted. Possible reasons for this include differences in bioavailability, biotransformation, and excretion. The accumulation of nonpolar hydrocarbons such as PAHs is proportional to their K_{ow} [16,17]. Although anthracene and phenanthrene have the lowest log K_{ow} values (4.54 and 4.57, respectively) and were accumulated the least, chrysene (log $K_{ow} = 5.79$) was not accumulated to a greater extent than fluoranthene or pyrene, both of which have lower log K_{ow} values (i.e., 5.2) [18]. The differences in accumulation cannot, therefore, be explained in terms of K_{ow} alone. Amphipods can metabolise some PAHs, including anthracene, which may account for their reduced net accumulation

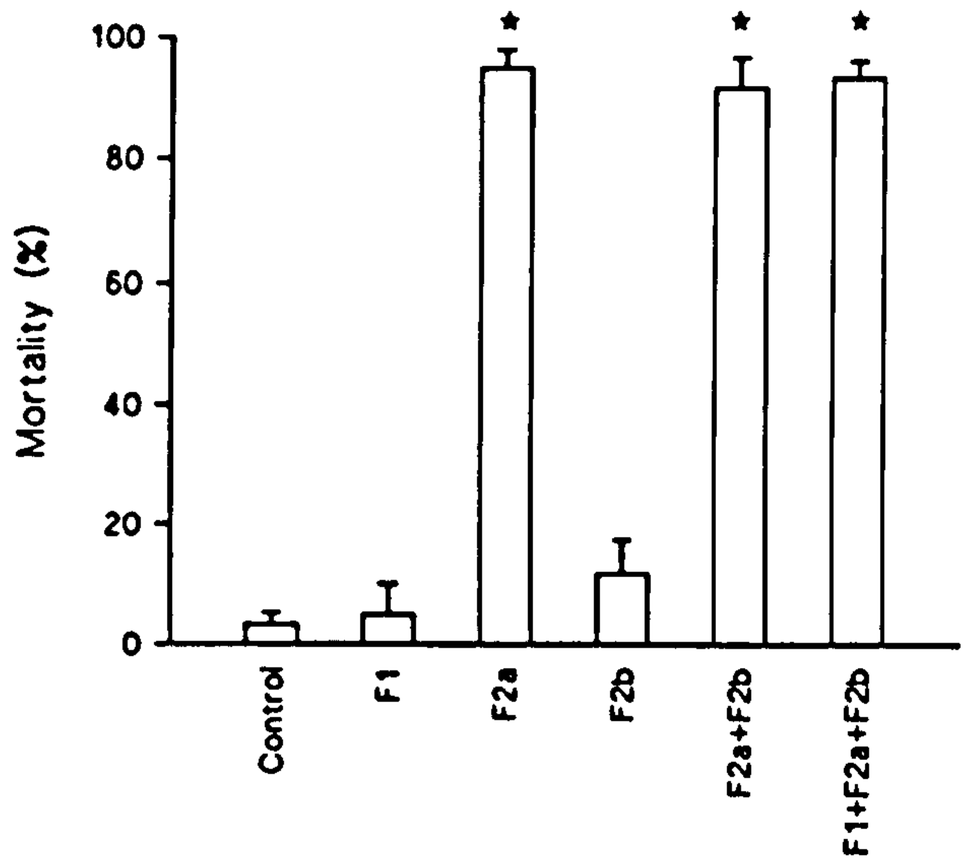

Fig. 9. Mean mortality (+1 SE) of *G. pulex* after 14-d exposure to APW or APW spiked with fractions of downstream sediment extract.

[19]. However, not all amphipods metabolise PAHs to the same extent [20], and the ability of *G. pulex* to metabolize PAHs is unknown.

In summary, the results from this study indicate that sediments contaminated with motorway runoff were slightly toxic to *Gammarus pulex*, but contaminated stream water was not. Most of the sediment toxicity (in terms of lethal effects) could be attributed by sediment manipulation and fractionation studies to hydrocarbons rather than to heavy metals, and of these PAHs were probably most important.

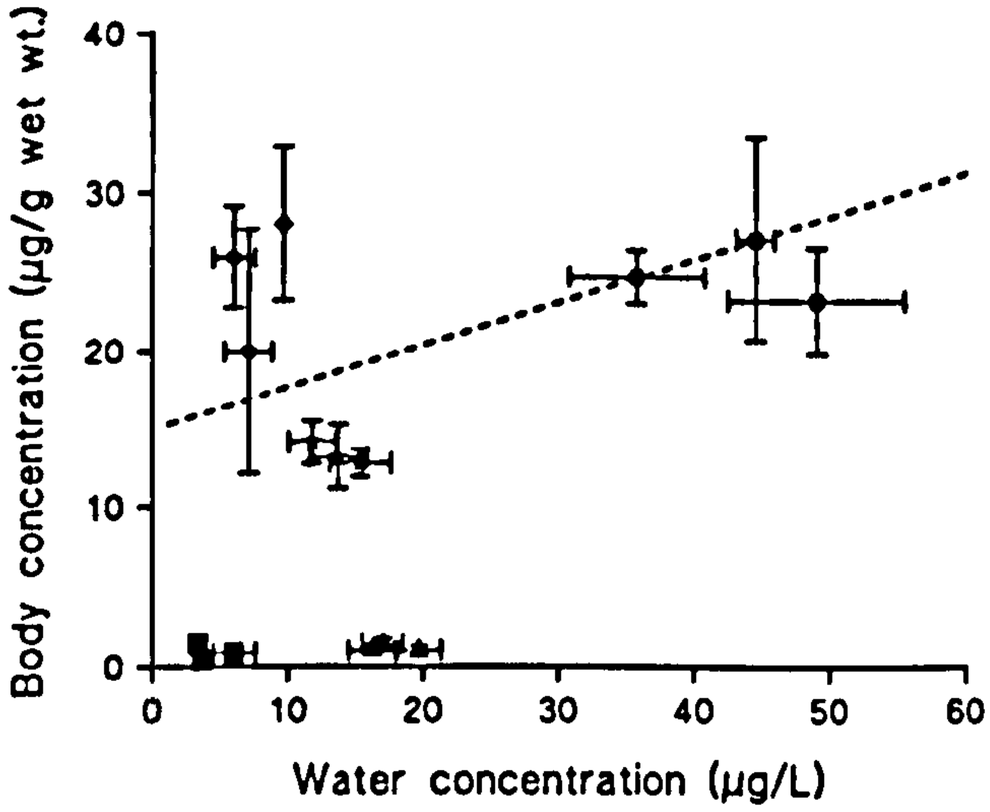

Fig. 10. Accumulation of phenanthrene (squares), anthracene (triangles), chrysene (stars), fluoranthene (diamonds)s, and pyrene (hexagons) by *G. pulex*. Data are presented as mean values + 1 SE. The relationship between water concentration and total aromatic hydrocarbon accumulation is represented by the dotted line and is described by the equation: accumulation = 0.27 (water concentration) + 15.11.

Several previous studies have demonstrated that sediment-associated PAHs are both bioavailable and toxic to amphipods [19–24], bioavailability being determined in part by sediment particle size and organic content [25,26]. Sediments collected from Pigeon Bridge Brook differed in both particle size and organic content; sediments collected from the upstream station had a smaller mean particle size and a higher total organic carbon content [1]. Therefore, not only were contaminants more concentrated in sediment from the downstream station but they were also potentially more bioavailable. The linking of observations from acute toxicity experiments to the observed differences in upstream/downstream populations of *G. pulex* and more general community composition is obviously problematic. However, taken together the field observations and laboratory toxicity experiments suggest that the implication of hydrocarbons, and especially PAHs, in ecological impacts deserves further attention.

The final phase of a TIE scheme is confirmation, where the toxicity of the suspected toxicants (individually or in combination) is compared to that of the complex mixture [2]. Studies investigating the toxicity of individual PAHs and mixtures are currently in progress.

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