The effect of species composition on community responses to toxicants: A comparison of Species Sensitivity Distribution curves and mesocosm studies.

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Thesis submitted to the University of Sheffield for the Degree of Philosophy in the Department of Animal and Plant Sciences.

September 2001

Acknowledgements

My sincerest thanks go to my supervisor Dr. Lorraine Maltby for her supervision and support. Thanks are also extended to my supervisor at Unilever Research, Dr. Stuart Marshall.

This work was funded by a case studentship funded by the Natural Environment Research Council and Unilever Research. The work was carried out at tthe Depatrment of Animal and Plant Sciences, for which I thank Professor John Lee, and at Unilver Research, Port Sunlight, for which I thank John Solbé.

Many people at the Department of Animal and Plant Sciences and Unilever Research have provided help and advice throughout this project. Special thanks go to Suzanne, Beryl, Martin, Hazel, Darren, Andy, Bob and Rob.

Moral support and encouragement offered by staff and postgraduates in the Department of Animal and Plant Sciences has been much appreciated. Special thanks must go to Don for his endurance during fieldwork, to Cheryl for her moral support and friendship, and to Sophie, Louise, Katrien, Lucy, Paul and Jo for their support and encouragement.

Finally, my thanks go to Jamie and my family for their love, support and tolerance throughout my time as a PhD student.

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Summary

Different species are affected at different concentrations of a toxicant. As communities differ in their species composition, they are also likely to be affected at different exposure concentrations. The concentration at which a community will be affected may be extrapolated from single-species tests or determined from multispecies exposures. This work compares the ability of two methods to detect the effect of species composition on communities' responses to toxicant exposure.

The sensitivity of 15 indigenous freshwater macroinvertebrate taxa to a surfactant were experimentally determined in single-species toxicity tests and supplemented with published data. The sensitivity of indigenous macroinverterate taxa to the surfactant varied by four orders of magnitude. Half of the indigenous taxa tested were more sensitive than the standard test species *Daphnia magna*.

The single-species toxicity data were then used to generate species sensitivity distribution (SSD) curves. SSD curves are used to estimate the concentration of a toxicant that is hazardous to 5% of species (HC5), based on the assumption that communities will be able to compensate for this small level of effect. SSD curves were generated for 60 naturally occuring macroinvertebrate communities, all from low order, circumneutral streams. The SSD curves were found to be sensitive to changes in species composition, with community HC5 values differing by more than an order of magnitude. An HC5 value was also calculated using the complete dataset. This value was lower than the HC5 values derived for 92% of natural communities.

To test the validity of these HC5 values, the responses of structurally distinct communities to surfactant exposure were determined under identical test conditions. Prior to running this experiment it was necessary to establish whether structurally distinct communities would remain distinct over time in the stream mesocosms.

Three structurally distinct macroinvertebrate communities were used to colonise a stream mesocosm, and changes in community composition and structure were monitored over ten weeks. At the start of the study, there was considerable overlap in the species composition of the communities, providing the potential for the three communities to become more similar over time, converging to a 'generic mesocosm community'. In fact, the commuities remained remarkably distinct from one another for the duration of the study.

Two structurally distinct communities were then exposed to the surfactant in the stream mesocosms for 28 days. The community dominated by sensitive taxa was found to be more sensitive, as predicted, with changes in species richness and community diversity occurring at different exposure concentrations in the two communities. The two communities exposed to linear alkylbenzene sulphonate (LAS) in the experimental stream study were structurally distinct, but their species composition was very similar. As these curves are generated from presence absence data they were unable to predict the differences in sensitivity of the two communties. However, the HC5 values determined from the SSD curves were found to be protective or overprotective of these communities, based on the responses observed in the mesocosm study.

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1 Introduction

The aim of this thesis was to examine the effect of species composition on community responses to toxicants. This was achieved by determining the sensitivity of structurally distinct communities to a toxicant using species sensitivity distribution (SSD) curves and stream mesocosm studies. This introduction provides an overview of the advantages and limitations of the single-species and multispecies assessment methods currently employed in environmental risk assessments, and considers the use of SSD curves, an alternative method for determining community sensitivity. Section 1.5 identifies the main aims and objectives of the work, and outlines the approaches taken to address these objectives.

1.1 An overview of environmental risk assessments

Environmental Risk Assessments (ERA) are performed to determine the risk a compound poses to the natural environment during the course of its use and disposal (Shaw & Chadwick, 1998). Risk is a function of exposure and hazard. Exposure to a compound is determined by the pathways, rates of movement, transformation and degradation of the chemical in the environment (i.e. the fate and behaviour of the compound in the environment). This information is used to calculate the predicted environmental concentration (PEC) (Van Leeuwen & Hermens, 1996). The hazard associated with a compound is the frequency and severity of adverse effects occurring at a given concentration (i.e. the effect of the compound on exposed organisms). This information is used to determine the predicted no-effect concentration (PNEC) for a compound (i.e. the concentration at which no adverse effect is expected to occur) (Van Leeuwen & Hermens, 1996). Assessment of the risk associated with a given compound is based on interpretation of the PEC, PNEC and resulting margin of safety (i.e. the difference between the PEC and PNEC), by legislative and regulatory authorities and associated industries (Urban, 1994; Shaw & Chadwick, 1998). This thesis focuses on effects assessment of compounds in aquatic systems.

It is not possible to test all species that may come into contact with a compound, under all possible environmental conditions and measure all possible effects. Therefore, uncertainties exist in the extrapolation of the effects of a compound measured on a few species, under a limited set of conditions, to effects on natural communities and ecosystems (Suter, 1995). Under the 7th amendment (92/32/EEC) to the Dangerous Substances Directive (67/548 EEC) a tiered structure exists in effects assessments (Crossland, 1992; Shaw & Chadwick, 1998).

Tier I effects assessment is a screening procedure designed to determine the acute toxicity of a chemical (normally measured as lethality or immobility), consisting of short-term single-species laboratory tests (Schudoma, 1994; Cooney, 1995). Uncertainties in the extrapolation of Tier I studies to natural communities include those associated with extrapolating between species, and across exposure times, endpoints, and environments (i.e. laboratory to field) (Stuhlbacher *et al.*, 1993; Chapman *et al.*, 1998).

Tier II assessments focus on chronic exposures and sublethal endpoints, thus reducing some of the uncertainties associated with extrapolating across endpoints and exposure times (Cooney, 1995; Shaw & Chadwick, 1998). Tier III of an effects assessment is normally only required for high volume chemicals, and involves an investigation of the effect of a compound on species assemblages in microcosms, mesocosms or field trials (e.g. Fairchild *et al.*, 1992). These tests attempt to reduce uncertainties associated with exposure conditions and extrapolations to natural communities, as well as considering the importance of species interactions (Suter *et al.*, 1983; Solomon *et al.*, 1996; Belanger, 1997).

1.2 Single-species toxicity tests

Single-species toxicity tests for aquatic organisms are usually conducted using a few standard species of algae, cladocerans, or fish (Crossland, 1992; Cooney, 1995). These standard test species have been chosen because they are easy to culture, can be kept stress-free under controlled laboratory conditions, and have been found to be relatively sensitive to a wide range of compounds (Thurston *et al.*, 1985; Cooney, 1995; Rand, 1995). The use of a standard test species also allows direct comparisons

of the relative toxicity of different compounds to be determined for each taxon tested (Thurston *et al.*, 1985).

However, problems exist when trying to extrapolate the responses of standard species exposed in controlled, single-species tests to the responses of indigenous communities exposed under natural conditions (Cairns, 1986; Gillespie *et al.*, 1998). Where the test species used is not indigenous to the exposed communities, uncertainties associated with how representative these standard species are of exposed communities are increased (Slooff *et al.*, 1983). For example, *Daphnia* spp., a lentic genus, are used in the risk assessment of compounds discharged into lotic environments (Richardson & Kiffney, 2000). It has previously been shown that species used in single-species tests are not always representative of responses of indigenous species, and may either over- or under-predict the responses of communities to a toxicant (Slooff *et al.*, 1983; Lewis, 1986; Pontasch & Cairns, 1991).

Knowledge of indigenous species responses to a compound would remove some of the uncertainties associated with the use of standard species tests by providing information on the range of sensitivities within species assemblages (Greve *et al.*, 1998, 1999; Stuijfzand *et al.*, 2000; Van der Geest *et al.*, 2000). These data could then be used to determine how representative the standard test species are of species sensitivities within a given community (e.g. Slooff *et al.*, 1983). This thesis will determine the sensitivities of indigenous species from lotic freshwater environments to a reference compound, and compare these data with the sensitivity of the standard invertebrate test species *Daphnia magna*.

1.3 Multispecies studies

Multispecies studies (e.g. mesocosms and field trials) are used in the final tier of environmental risk assessments in order to address areas of uncertainty not addressed in previous tiers of testing (Suter *et al.*, 1983; Belanger, 1997). One such area of uncertainty that cannot be addressed in single-species tests is the effect of exposure on species interactions, and their influence on species sensitivities (Shaw & Kennedy, 1996). Species interactions may be affected by exposure in multispecies

studies due to the direct effect of a compound on one or more species resulting in changes in the viability of another species (e.g. shifts in predator prey interactions). For example, Stephenson & Mackie (1986) found that after treatment with 2,4-D, invertebrate diversity decreased in treated pond mesocosms relative to the control. This was attributed to a reduction in habitat complexity due to the loss of several macrophyte species, which resulted in an increase in fish predation on invertebrates. Belanger *et al.* (1995) observed an increase in periphyton biomass in stream mesocosms exposed to high concentrations of surfactant (224 - 1586 μg surfactant/l), resulting in increased densities of gastropods and oligochaetes. Dorn *et al.* (1997) attributed the increase in periphyton biomass in stream mesocosms exposed to a surfactant to a decrease in grazing pressures at higher treatment concentrations, an effect that was also observed in an indoor microcosm system exposed to carbendazim (Van den Brink *et al.*, 2000b).

However, there are several constraints to determining species sensitivities from multispecies studies. First, if natural communities have been used to stock the mesocosms, then exposed individuals will differ in age and condition within and between species (Stuijfzand *et al.*, 2000). Second, some species will be present at low densities, therefore although a range of species may be present in the test system, it may only be possible to determine the sensitivity of a few dominant taxa (Van Wijngaarden *et al.*, 1996). Third, variability in stocking densities between test units introduces uncertainty as to whether relative changes in species abundance after exposure to a compound are due to the effect of the compound, or differences in stocking densities (e.g. Gillespie *et al.*, 1996; Van den Brink *et al.*, 1996).

If species interactions and exposure conditions are important in determining species sensitivities, then it may be hypothesised that the ranked sensitivity of species in multispecies studies will differ from the ranked sensitivity of the same species in single-species tests (Van Wijngaarden *et al.*, 1996). Comparisons between species sensitivities in single-species and multispecies tests have been made previously (Pontasch *et al.*, 1989; Gillespie *et al.*, 1996; Giddings *et al.*, 2001). However, with a few exceptions (e.g. Van Wijngaarden *et al.*, 1996; Crane *et al.*, 1999), most of these comparisons compared the response of standard species in single-species toxicity tests with the response of indigenous species in multispecies exposures

(e.g. Pontasch *et al.*, 1989; Fairchild *et al.*, 1992; Dorn *et al.*, 1997; Girling *et al.*, 2000). This work will compare the responses of indigenous species and communities exposed in stream mesocosms with their responses extrapolated from single-species toxicity tests and SSD curves to determine if sensitivities determined in single-species and multispecies exposures are significantly different.

1.4 Species sensitivity distribution curves

Previous comparisons between single-species laboratory exposures and mesocosms have indicated that short-term single-species laboratory tests may be protective of natural communities for at least some compounds (Van Wijngaarden *et al.*, 1996). If laboratory tests are representative of a species responses under natural conditions, short-term single-species toxicity tests on indigenous species may be of value in assessing the sensitivity of a community or ecosystem to a compound by examining the distribution of species sensitivity (Van der Geest *et al.*, 2000). Single-species test data may be fitted to a frequency distribution curve, which is then used to extrapolate the proportion of all species that would be affected at a given concentration (e.g. Kooijman, 1987). These curves are traditionally used to determine the concentration hazardous to 5% of species (i.e. HC5; Van Straalen & Denneman, 1989), and the most frequently used distribution models are log-logistic (Aldenberg & Slob, 1993) and log-normal (Wagner & Løkke, 1991). Although these methods are not currently included into environmental risk assessment legislation, the potential value of such methods is receiving much attention (e.g. Solomon *et al.*, 1996; Versteeg *et al.*, 1999; Aldenberg & Jaworska, 2000; Campbell *et al.*, 2000; Newman *et al.*, 2000; Van den Brink *et al.*, 2000a).

Species sensitivity distribution (SSD) curves potentially allow the sensitivity of communities to be determined from single-species toxicity data. However, a fundamental assumption of these methods is that the tested species are representative of the range and distribution of sensitivities that would be determined if all species were tested (OECD, 1992). It has been argued that this assumption can only be met if the tested species were drawn randomly from the pool of all species (Versteeg *et al.*, 1999). A review of single-species toxicity data for surfactants showed that even

for compounds that have been tested extensively, the majority of tests were carried out using standard laboratory test species, and limited data were available for non-standard species (BKH Consulting Engineers, 1992b). This bias in available data means the assumption that species are randomly selected is not met, and therefore estimates of community sensitivities may be inaccurate (e.g. McDaniel & Snell, 1999). If information on the sensitivities of a wider range of species could be collected, then the distribution of species sensitivities in natural communities may be determined more accurately. This project will use the sensitivities of a range of indigenous lotic macroinvertebrate species to explore the effect of species selection on HC5 values generated from SSD curves.

1.5 Overview of thesis

1.5.1 Aim and objectives

The overall aim of this work was to examine the effect of species composition on community responses to toxicants using species sensitivity distribution (SSD) curves and stream mesocosm studies. In particular, the thesis addressed the following objectives;

1. To determine how the sensitivity of standard test species compares to that of non-standard indigenous taxa.
2. To generate SSD curves for natural communities and to assess variability in predicted HC5 values.
3. To establish whether structurally distinct communities remain distinct in a mesocosm study over ten weeks.
4. To experimentally determine whether structurally distinct communities differ in their responses to toxicant exposure in a stream mesocosm.
5. To compare the responses of species and communities to a toxicant, as determined by SSD curves and stream mesocosm studies.

1.5.2 Approach

The toxicant used in this study was the surfactant linear alkylbenzene sulphonate (LAS). LAS is the most extensively used anionic surfactant product world-wide, with annual usage in 1998 estimated at 2.8 million tonnes (Verge *et al.*, 2001). It was developed to replace the less biodegradable surfactant alkylbenzene sulphonate 35 years ago (Rapaport & Eckhoff, 1990). LAS is normally disposed of via the domestic drains system, to be discharged into lotic systems either directly or via sewage treatment works (Kimerle, 1989). Microbial degradation in sewage treatment works removes 99% of LAS from sewage treatment influent, with effluent concentrations ranging from 19 to 71 μg LAS/l (Matthijs *et al.*, 1999). Due to the very high volume usage, extensive tests on the fate and effect of LAS have been conducted (Kimerle & Swisher, 1977; Maki & Bishop, 1979; Holt & Mitchell, 1994; Jorgensen & Christoffersen, 2000). LAS was selected as a test compound due to the extensive exposure dataset available (BKH Consulting Engineers, 1992a).

Objective 1 was addressed by collating acute toxicity data from the literature for LAS, and by conducting novel single-species toxicity tests on fifteen indigenous stream macroinvertebrate taxa. These data were then used to compare the sensitivity of standard and non-standard species to LAS (Chapter 2).

Objective 2 was addressed using toxicity data generated for Objective 1 and community data from low-order streams sampled in Yorkshire and Derbyshire. The toxicity and community data were combined to produce species sensitivity distribution (SSD) curves using all available data, and community-specific subsets of the toxicity data. These curves were then used to determine the proportion of community-specific HC5 values that were lower than the HC5 value generated from a species sensitivity distribution curve using all data (Chapter 3).

In order to test whether structurally distinct communities differ in their response to toxicants (Objective 4) it is necessary to establish that distinct communities can be maintained in the experimental systems for the duration of the proposed study (i.e. Objective 3). Three structurally distinct natural communities were sampled and used to stock a stream mesocosm facility. The mesocosms were run for ten weeks and changes in community structure assessed (Chapter 4).

Objective 4 was addressed by performing a 28 day mesocosm study in which two structurally distinct communities were simultaneously exposed to LAS. The results of this study were used to test the hypothesis that community structure influences sensitivity to a toxicant, and that a community composed of taxa found to be sensitive in short-term single-species toxicity tests would be more sensitive than a community composed of more tolerant taxa (Chapter 5).

Objective 5 was addressed by comparing data from single-species tests and mesocosm studies to determine whether the ranked sensitivities of indigenous species were significantly different in the two test systems. Also, the HC5 values for each community, derived using single-species toxicity data, were compared to community responses observed in the mesocosm study (Chapter 6).

2 Sensitivity of indigenous lotic macroinvertebrates to LAS: Comparisons with the sensitivity of *Daphnia* spp.

2.1 Introduction

Single-species toxicity tests are conducted to determine the effect of a compound on species from different taxonomic groups, typically represented by a crustacean, an alga and a fish (Cooney, 1995). The crustacean selected for these tests is normally a Daphniidae species; *Daphnia magna, D. pulex* or *Ceriodaphnia dubia* (e.g. Lewis & Weber, 1985; McDonald *et al.*, 1996; Bailey *et al.*, 1997). The use of *Daphnia* spp. in single-species toxicity tests is favoured as they are common in many lentic freshwater bodies; are easy to culture in the laboratory; and are sensitive to a wide range of compounds (Rand, 1995). Short-term single-species toxicity tests use juvenile *Daphnia* sp. (≤ 24 hours old) to minimise variability in sensitivity between individuals due to differences in the age or lifestage of tested individuals (Rand, 1995).

Although such tests provide a useful screening tool, and are mandatory under the Dangerous Substances Directive (67/548/EEC) (Shaw & Chadwick, 1998), a number of problems have been identified with the use of standard species in determining the effect of a compound on natural communities. A major criticism is the assumption that there will be one species that is among the most sensitive to all compounds. Slooff (1985) reported *Daphnia*, algae and fish standard species to be less sensitive than other aquatic species by an order of magnitude for 25 to 30 % of test chemicals reviewed, and Slooff *et al.* (1983) found six of the 15 chemicals tested to be less toxic to *Daphnia* spp. than to other invertebrates. Thurston *et al.* (1985) found *Tanytarsus* (Chironomidae) to be more sensitive than *Daphnia* sp. for half of the 10 compounds tested. As relative susceptibility has been shown to be compound specific, the results from one species cannot be used to accurately predict the response of another, untested species (Lewis, 1986; Maltby & Calow, 1989; Pontasch & Cairns, 1991).

Standard toxicity tests are further criticised for not representing the diversity, and hence range of sensitivities, of natural systems (Kiffney & Clements, 1996; Van de Plassche *et al.*, 1999). For example, a review of single-species data for the surfactant linear alkylbenzene sulphonate (LAS) found that although 25.4 % of taxa in Dutch rivers were Insecta, they only accounted for 3.2 % of species for which toxicity data were available. Therefore, although large toxicity datasets may exist for some compounds, such as LAS, some taxonomic groups are under-represented (Blok & Balk, 1993). Hence, it has been proposed that toxicity tests should incorporate species that are representative of the communities exposed to the compound in question (Lewis, 1991; Van der Geest *et al.*, 2000).

The use of indigenous non-standard species may overcome many of these problems, although new problems and considerations are introduced. Large interspecific differences in sensitivity exist, even within the same family, therefore organisms should be selected because they are both sensitive and representative of the exposed community (Cairns, 1986; Leeuwangh *et al.*, 1994; Van der Geest *et al.*, 2000). For example, Slooff (1983) found variability within species groups was as large as variability between groups in tests using *Erpobdella* sp., *Asellus* sp., *Gammarus* sp., *Corixa* sp., *Cloeon* sp., *Nemoura* sp. and *Ischnura* sp..

Interpopulation differences may affect species sensitivities to a compound. For example, Ephemeroptera populations from high altitude streams have been found to be more sensitive to metal exposure than populations from low altitude streams (Kiffney & Clements, 1994, 1996). Previous exposure to a toxicant may also affect a population's sensitivity. For example, animals from populations that have been previously exposed to a toxicant (i.e. zinc, iron) have been shown to display lower sensitivity when compared to animals that were not previously exposed (Maltby *et al.*, 1987; Naylor *et al.*, 1990).

Intrapopulation differences in sensitivity may occur due to variation in the age and health of the constituent individuals (Slooff, 1985; Van der Hoeven & Gerritsen, 1997). For instance, early instars of *Agapetus fuscipes* (Trichoptera) and juvenile *Asellus aquaticus* (Crustacea) have been found to be more sensitive to cadmium than older individuals (Green *et al.*, 1986; McCahon *et al.*, 1989). This variability due to age does not just apply to non-standard species (Van der Hoeven & Gerritsen, 1997),

but it is harder to ascertain the age and condition of individuals collected from the field than it is for laboratory reared species. Methods have recently been developed to allow the rearing of some indigenous species in the laboratory (Greve *et al.*, 1998; Greve *et al.*, 1999; Van der Geest *et al.*, 2000), but are currently only available for a small number of taxa. Despite these difficulties, determining the sensitivities of a number of non-standard indigenous species to a compound would provide information on the range of sensitivities within natural communities (Slooff, 1983), and allow single-species toxicity tests with standard species to be directly compared to the responses of indigenous species in single-species tests.

The aim of this Chapter is to determine the sensitivity of indigenous lotic macroinvertebrate species to the surfactant linear alkylbenzene sulphonate (LAS). A literature search for data on the sensitivity of indigenous species to the LAS was followed by experimentally determining the sensitivity of indigenous lotic macroinvertebrate species to the compound. These data were compared to the sensitivity of *Daphnia* spp. to determine whether the standard test species sensitivities are representative of the sensitivity of indigenous lotic species. LAS was selected as a test compound because its mode of use and disposal (i.e. via sewage treatment works into river systems) means that lotic species are more likely to be exposed than lentic species (e.g. Scott & Jones, 2000).

2.2 Methods

2.2.1 Literature review

Recent reviews have compiled much of the published data on the toxicity of LAS to aquatic organism (i.e. Painter & Zabel, 1988; BKH Consulting Engineers, 1992a, 1992b; Van de Plassche *et al.*, 1999). Data from these sources were supplemented with data from the US EPA ecotoxicology database, ECOTOX (http://epa.gov/ecotox/), and an electronic literature search using Web of Science and Biological Abstracts was carried out (dates searched, 1981-1997). The selection criteria for data to be included are shown in Table 2.1.

Table 2.1: Selection criteria for macroinvertebrate L(E)C50 data for LAS sourced from existing reviews and literature searches (see text for references)

Taxa:	non-Daphniidae macroinvertebrates
Habitat:	freshwater
Test design:	single-species exposures
Test duration:	≤ 96 hours
Compound:	single- or mixed-chain length linear alkyl benzene sulphonate (LAS)
Endpoint:	lethality or immobility (i.e. L(E)C50)

2.2.2 Toxicity tests

Test species

Having established the availability of published data, lotic macroinvertebrate species were identified for testing (Table 2.2). Macroinvertebrates were collected from five sites either by kick-net sampling or stone washing (Mason, 1993). Animals were held in 2-litre tanks filled with aerated artificial pond water (APW, Appendix 2.1) with a small amount of natural substrate at densities of 50 - 150 individuals per tank. Animals were kept in a constant temperature room (15 ± 2 °C) with a 16:8 light:dark photoperiod for between one and four days prior to exposure.

Table 2.2: Source and collection date for macroinvertebrate species used in single-species toxicity tests. The range of nominal test concentrations used is also given for each species

Species	Source (NGR)	Collection date	Nominal test concentrations (mg/l)
Polycelis felina	Crags Stream (SK497745)	March 1999	0 – 20
Lumbriculus variegatus	lab-reared	March 1999	0 – 20
Erpobdella octoculata	River Don (SK315925)	July 1998	0 – 25
Asellus aquaticus	River Don (SK315925)	July 1998	0 – 25
Gammarus pulex	Crags Stream (SK497745)	July 1998	0 – 25
Baetis rhodani	Porter Brook (SK318855)	March 1999	0 – 20
Ecdyonurus dispar	Porter Brook (SK318855)	July 1998	0 – 25
Ephemerella ignita	River Don tributary (SK05933)	July 1998	0 – 25
Rhithrogena semicolorata	Porter Brook (SK318855)	March 1999	0 – 20
Leuctra sp.	Porter Brook (SK318855)	July 1998	0 – 25
Elmis aenea	Barlow Brook (SK345755)	February 2000	0 – 100
Hydropsyche angustipennis	River Don (SK315925)	October 1999	0 – 100
Agapetus fuscipes	Crags Stream (SK497745)	March 1999	0 – 20
Rhyacophila dorsalis	Peakshole Water (SK170834)	July 1998	0 – 25
Chironomus riparius	lab-reared	July 1998	0 – 25

Test set up

Animals were exposed to LAS individually in 60-ml glass jars (5 cm diameter) containing 50 ml APW, for 96 hours. Aeration was provided to each jar through a hypodermic syringe connected to an airline. Each vessel contained one piece of pea gravel (< 1.0 cm) to provide an anchorage point for those animals that required it. The test setup was the same for all species to avoid variability in exposure conditions, and no food was provided during the test. At the start of the test, solutions were added to vessels and animals assigned to each concentration using an

ordered assignation (one individual per treatment in turn) to ensure that the likelihood of fitness differences between treatments was minimised. Sixteen to twenty individuals were randomly assigned to each of five concentrations or control vessels.

The test compound used in these experiments was LAS, provided by Unilever (Port Sunlight, UK) as NANSA HC80/S (manufactured by Albright & Wilson, average chain length C11.8). All solutions were replaced and animals checked every 24 hours and immobile individuals were removed and preserved in 70% industrial methylated spirits (IMS) to allow species identity to be confirmed at a later date. Animals were defined as immobile if there was no movement in response to disturbance (pushing with glass rod) and any individuals still mobile at 96 hours were preserved in 70% IMS to allow later confirmation of species identity.

Chemical analysis

Samples (30 ml) of LAS test solutions were taken at the beginning and end of each 24 hours, preserved with the addition of 3 ml of formalin and stored at < 4 °C (HMSO, 1982). Samples were analysed using a modified micro-methylene blue active substances (micro-MBAS) method (Appendix 2.2). The test for MBAS detects compounds other than LAS, including partially degraded LAS and other inorganic compounds (Kimerle & Swisher, 1977), therefore a calibration curve for known concentrations of LAS in APW was constructed and used to determine the concentration of LAS in test solutions.

Ninety-six hour L(E)C50 values were calculated for each species. Exposure concentrations were calculated using the mean LAS concentrations over the 96 hour exposure (Stephan, 1977; Kooijman, 1987), and they may not have conformed to a geometric series if biodegradation rates vary between test concentrations. The use of non-standard species increases the probability of unequal numbers of individuals being exposed at different concentrations (e.g. due to emergence). The modified Spearman-Karber method is a non-parametric method that does not require concentrations to be a geometric series or an equal number of individuals in each treatment (Stephan, 1977) and was therefore used to analyse these data using the Trimmed Spearman-Karber (TSK) Program, version 1.5 (Newman, 1995; US EPA).

2.3 Results

2.3.1 Literature review

The L(E)C50 values retrieved from the BKH database (BKH Consulting Engineers, 1992b), ECOTOX (ECOTOX; http://epa.gov/ecotox/) and an electronic literature search are listed in Appendix 2.3. Wherever possible original records referenced in reviews were consulted to allow verification of the data. The ECOSOL dataset (i.e. BKH Consulting Engineers, 1992a, b) included 586 effects data values for 93 species. A total of 219 L(E)C50 values were available for 32 invertebrate taxa; 63 % of these values were for *Daphnia magna*. BKH Consulting Engineers (1992b) reported 138 L(E)C50 values for *Daphnia magna* ranging from 0.013 mg LAS/l to 55 mg LAS/l. The geometric mean for all *D. magna* data for tests of less than 96 hours duration with lethality or immobility as an endpoint was 4.9 mg LAS/l.

A total of 53 values for 22 non-Daphniidae species met the selection criteria set out in Section 2.2.1; two Turbellaria, five Oligochaeta, three Gastropoda, two Bivalvia, three Malacostraca, and seven Insecta. The L(E)C50 (<96 hours) values for non-Daphniidae taxa ranged from 0.06 mg LAS/l for *Lymnaea vulgaris* and *Tubifex rivulorum* (Lal *et al.*, 1983) up to 270 mg LAS/l for *Asellus* sp. (Lewis & Suprenant, 1983). Four of the ten references available determined actual LAS concentrations in the test systems using either the MBAS method, ^{14}C labelling, or HPLC (Arthur, 1970; Bressan *et al.*, 1989; Casellato & Negrisolo, 1989; Pittinger *et al.*, 1989). The remaining cited references (Appendix 2.3) did not state a method of determining exposure concentrations, or stated that the L(E)C50 data were calculated from nominal values.

2.3.2 Toxicity tests

The sensitivity (L(E)C50) of indigenous taxa determined in novel toxicity tests ranged from 1.8 mg LAS/l (*Polycelis felina*) to >70.2 mg LAS/l (*Elmis aenea*) (Table 2.3). Five of the 15 species tested exceeded 10% control mortality (Table 2.3) indicating that test conditions may be stressful, particularly for the mayfly larvae, *Ephemerella ignita* and *Baetis rhodani*.

Table 2.3: Ninety-six hour L(E)C50 values for lotic macroinvertebrate species exposed to LAS. Upper and lower 95% confidence limits are given in parentheses and asterisk denotes tests in which reliable 95% confidence limits could not be calculated.

Species	96 hr L(E)C50 (mg/l)	Control mortality >10% (%)
Erpobdella octoculata	7.8 (6.7 – 9.2)	
Lumbriculus variegatus	1.9 (*)	
Asellus aquaticus	24.5 (*)	11
Gammarus pulex	10.4 (8.2 – 13.1)	
Elmis aenea	> 70.2	
Chironomus riparius	15.1 (8.2 – 27.7)	
Ecdyonurus dispar	3.9 (2.5 – 6.2)	
Rhithrogena semicolorata	4.3 (3.9 – 4.9)	
Ephemerella ignita	4.9 (3.7 – 6.4)	21
Baetis rhodani	4.1 (2.7 – 6.2)	25
Leuctra sp.	2.8 (1.8 – 4.2)	
Hydropsyche angustipennis	> 62.2	
Agapetus fuscipes	> 14.4	11
Rhyacophila dorsalis	13.5 (10.5 – 17.4)	13
Polycelis felina	1.8 (*)	

2.3.3 Combined data

The novel toxicity data were combined with the literature values (Appendix 2.4, Figure 2.1) and where more than one value was available for a species, the geometric mean was calculated (French & Lindley, 2000). If data were reported at both genus and species level the data were combined and reported as genera specific values. In total L(E)C50 values were available for 35 taxa, with values ranging from 0.06 mg LAS/l for *Tubifex rivulorum* and *Lymnaea vulgaris* (Lal *et al.*, 1983) to 200 mg LAS/kg sediment for *Anodonta cygnea* (Bressan *et al.*, 1989). Sixteen of the 35 non-Daphniidae taxa have L(E)C50 values lower than *Daphnia magna* (Figure 2.1).

The mean L(E)C50 value all L(E)C50 data available for freshwater indigenous macroinvertebrate taxa was 23.2 mg LAS/l (SE = 7.9 mg LAS/l) and the median L(E)C50 was 4.9 mg LAS/l. Skew and kurtosis values are 3.0 and 9.0 respectively, indicating that the data are concentrated around the mean, with a strong positive skew (i.e. a few very high L(E)C50 values). This strong skew in the data is largely driven by the two bivalve species (Bressan *et al.*, 1989) having reported sensitivities at least double those of any other taxa. These very high values may not truly reflect the sensitivity of the organisms, as at concentrations $\geq$ 10 mg LAS/kg sediment

Bressan *et al.*, (1989) reported that both bivalve taxa closed their valves and increased mucus production, thus limiting their exposure to the toxicant.

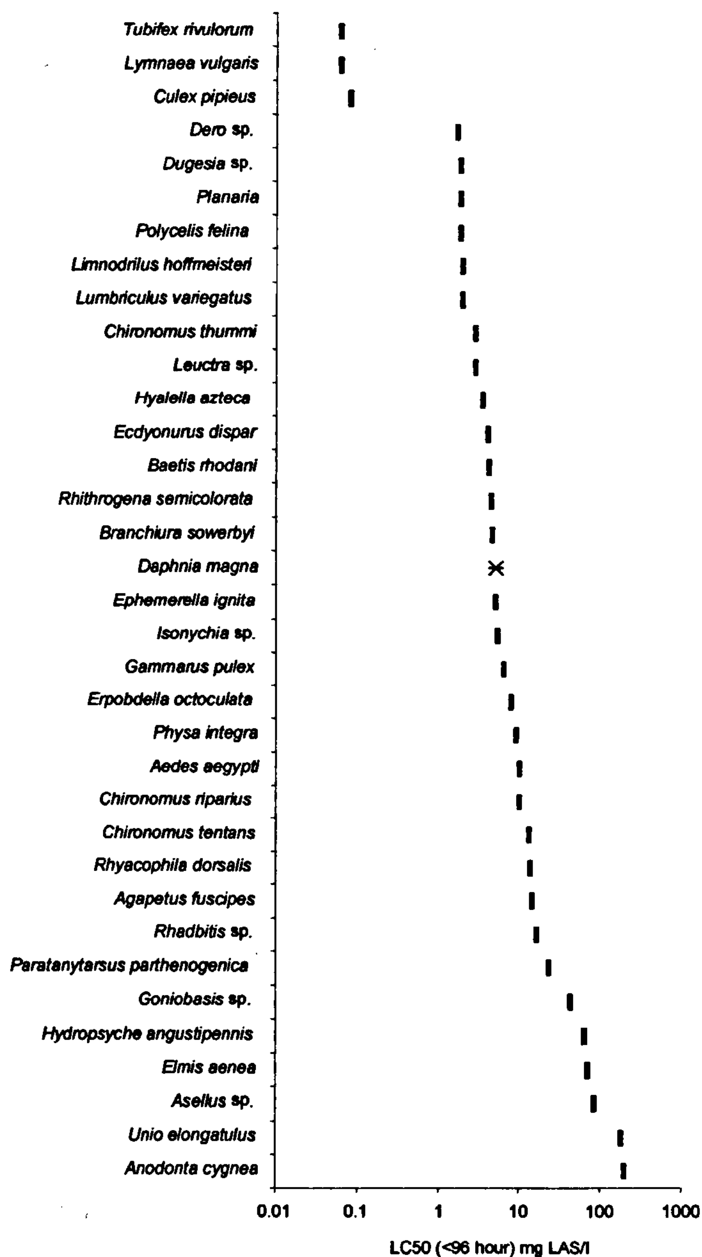


Figure 2.1: L(E)C50 values for freshwater macroinvertebrate species. Where more than one value was available the geometric mean has been calculated. (See Appendices 2.3 and 2.4 for source data). Asterisk represents the standard species *Daphnia magna*.

2.4 Discussion

A large number of single-species toxicity tests have been conducted to determine the toxicity of LAS to freshwater organisms (e.g. BKH Consulting Engineers, 1992a, b), but species have not been selected randomly, resulting in a few standard species, which may not be representative of the sensitivity of lotic macroinvertebrate taxa, being over-represented. Blok & Balk (1993) observed that the standard species *Daphnia magna*, *Pimephales promelas* and *Lepomis machrochirus*, accounted for 55 % of the test data available and did not differ significantly in their responses to LAS. It has been suggested that compounds with a non-specific mode of action, such as LAS, show little variation in toxicity between species (Thurston *et al.*, 1985). This contention could only be verified if data were available for a wide range of taxa. The aim of this Chapter was to determine the sensitivity of a range of indigenous macroinvertebrate species that may occur in receiving waters (i.e. lotic systems).

Concentrations of LAS in UK rivers have been recorded to be as high as 0.25 mg/l (Waters & Garrigan, 1983), although as LAS is readily biodegraded in sewage treatment works, concentrations normally range from 0.03 to 0.04 mg/l (Painter & Zabel, 1988). The upper concentration in water used in hazard assessments is 0.3 mg LAS/l (Kimerle, 1989). Commercial mixtures of LAS are composed of homologues of chain lengths C_{10}-C_{13} (Blok & Balk, 1993; Feijtel *et al.*, 1999) with toxicity increasing with increasing chain length, therefore the toxicity of partially degraded LAS is less than the toxicity of intact LAS (Dolan & Hendricks, 1976). Maki & Bishop (1979) demonstrated the effect of chain length in tests with *Daphnia* sp. using single chain length LAS (C_{14} to C_{10}), and found 48-hour L(E)C50 values ranged from 0.68 to 27.6 mg LAS/l. The novel data presented in this chapter were generated using a commercial mix of LAS (average chain length $C_{11.8}$).

The geometric mean for all *Daphnia magna* data for tests of less than 96 hours duration with lethality or immobility as an endpoint listed in BKH Consulting Engineers (1992b) was 4.9 mg LAS/l, and the median was 6.0 mg LAS/l. This is very similar to the median value for indigenous species (Section 2.3.3). *Daphnia magna* was less sensitive than 16 of the 35 non-Daphniidae species for which data were available (Appendix 2.4). If an application factor of 10 is applied to the

geometric mean L(E)C50 for *D. magna* value to account for uncertainties associated with interspecific extrapolations (e.g. Chapman *et al.*, 1998) then the resulting value would be lower than 32 of the 35 non-Daphniidae taxa. The L(E)50 values for three taxa (*Tubifex rivulorum, Lymnaea vulgaris, Culex pipiens*) would still be an order of magnitude lower than *D. magna* data after the use of an application factor. These values were all generated by Lal *et al.* (1983). This paper has been questioned previously (Painter & Zabel, 1988) as the values quoted are at least an order of magnitude lower than any other values reported. As these data met the data selection criteria set out in Section 3.2.1, they were included in this comparison. The setting of more stringent data selection criteria (e.g. actual test concentrations determined) would have resulted in most of the data available for non-Daphniidae species being excluded. The most tolerant species in the distribution were both bivalves (*Unio elongatulus, Anodonata cygnea*). These values may not be representative of the true sensitivity of these taxa, as Bressan *et al.*, (1989) reported that both species closed their valves at concentrations of ≥10 mg LAS/kg sediment.

The narrower range of values in novel tests (1.8 to 70.2 mg LAS/l for *Polycelis felina* and *Elmis aenea* respectively) may be due to consistency in exposure conditions, chemical analysis, statistical analysis, and the use of a standard LAS mixture. Within the BKH data base, (Blok & Balk, 1993) found that 52 % of the variability in sensitivity to LAS was interspecific and 48 % was intraspecific. The high intraspecific variation can be explained by a combination of factors including differences in chain length toxicity (e.g. Maki & Bishop, 1979); use of nominal or actual concentrations; differences in methodology and statistical analyses (Kimerle, 1989); and the method of chemical analysis used. This last factor is important as the less toxic, partially degraded homologues of LAS may still be included in some measurement techniques such as MBAS analysis (Dolan & Hendricks, 1976; Van de Plassche *et al.*, 1999).

The novel toxicity data presented here were determined using indigenous macroinvertebrates collected from the field. A higher level of intraspecific variability would be expected between field-collected individuals than between individuals of laboratory cultured species (e.g. *Daphnia magna*), which may result in greater variability in the responses of individuals to a toxicant due to variability in

the age, sex and general health of the individuals (Van der Hoeven & Gerritsen, 1997). Control mortality gives a measure of variability in the general health, and ability to survive under test conditions for each species, and control mortalities of up to 10 - 20 % have been proposed for tests using standard species (e.g. *Daphnia* sp.) (Stephan, 1977; Lewis & Weber, 1985; Gelber *et al.*, 1995; Newman, 1995). Thirteen of the fifteen species tested here had control mortalities of less than 13%. *Baetis rhodani* and *Ephemerella ignita* had higher control mortalities (25% and 21% respectively), suggesting that these species were not stress free within the laboratory environment, and factors other than the toxicity of LAS were also affecting their survival (Newman, 1995). However, as these species were not among the most sensitive of those tested the reported range of sensitivities is still valid. The development of standardised short-term tests for non-standard organisms (Greve *et al.*, 1998, 1999), may go some way to reducing these high control mortalities, although while Van der Geest *et al.* (2000) reported control mortality of only 2 % for *Ephoron virgo* (Ephemeroptera) control mortality for *Cyrnus trimaculatus* (Trichoptera) was 22 %.

While the use of non-standard species addresses many of the criticisms levelled at the use of standard species in the determination of a compounds effect on natural communities, several limitations and assumptions remain. The test organisms may directly represent a sensitive species from a natural community exposed to the compound (e.g. Stuijfzand *et al.*, 1999), but greater intraspecific variation in sensitivity exists due to population, age and health differences between individuals (Naylor *et al.*, 1990; Stuijfzand *et al.*, 2000). Furthermore, sensitivities may be overestimated due to lethal or sublethal stresses caused by the test conditions. The order of ranking for these data may therefore change if tests were repeated under different test conditions, or with individuals from a different population, or at a different age (Van der Geest *et al.*, 2000). However, the inability of laboratory tests, to predict precisely the response of every population likely to be exposed to the test compound, even when indigenous species are used, does not invalidate the use of such tests. The use of a range of indigenous non-standard species would increase the probability of protecting the most sensitive, locally important species without resorting to overprotective application factors (Lewis & Suprenant, 1983). A suite of non-standard tests may be more expensive, time consuming and less standardised

than traditional low tier assessments, but consideration should be given to their value in higher tier assessments, providing information on where the greatest effects are likely to occur within a community. One method of utilising the range of sensitivities among tested species to extrapolate the responses of whole communities is explored in Chapter 3.

3 Species sensitivity distributions: Applying SSD's to natural macroinvertebrate communities.

3.1 Introduction

Application factors are applied to single-species toxicity data in Tier I risk assessments to account for the uncertainty in extrapolating from short-term single-species laboratory toxicity tests to natural communities (e.g. Roman & Isnard, 1999). However, these methods do not make use of information on the range or distribution of single-species toxicity data. If single-species data are available for several species, a frequency distribution of species sensitivities may be determined. If the assumption that the sensitivity of tested species are representative of the sensitivity of species in exposed communities is met, then this distribution may be used to extrapolate the proportion of all taxa affected at any given toxicant concentration. This is the rationale behind the use of a species sensitivity distributions (SSD) to estimate hazardous concentrations.

Kooijman (1987) defined the hazardous concentration (HCS) for sensitive species as the "lower bound for concentrations that can be expected to be harmful for a given community" and presented methods for determining this hazard concentration based on the loglogistic distribution of LC50 data. In order to determine the concentration that may be hazardous to the most sensitive species, it would be necessary to extrapolate the concentration at which no effect would be expected in a community from the sensitivity of species tested. Van Straalen & Denneman (1989) proposed no observed effect concentration (NOEC) rather than LC50 values should be used in species sensitivity distribution curves as they incorporate the most sensitive measured endpoint, and may therefore be a better estimate of species sensitivities in the field. A practical limitation of the use of NOEC data to generate SSDs is the limited amount of data available, especially for indigenous species (Hoekstra *et al.*, 1994). As the aim of this Chapter is to make comparisons between distributions, rather than define 'safe' concentrations, LC50 data have been used.

Determining an acceptable hazardous concentration (HC), that will affect a certain proportion of a community (HC*p*) is a political and societal decision, based on

scientific interpretation of available data (Emans *et al.*, 1993). Van Straalen & Denneman (1989) suggested a concentration hazardous to 5 % of species (HC5) may be appropriate, although any centile may be used. For example, Solomon *et al.* (2001) use the 10th centile (i.e. HC10), based on their analysis of community responses in mesocosms. The analysis presented here will calculate hazard concentrations at 5 % (i.e. HC5).

Van Straalen & Denneman (1989) proposed that it was not necessary to set the hazardous concentration at a level that would have no effect on even the most sensitive species for communities to be protected as communities would be able to compensate for low levels of disturbance. The ability of a community to tolerate some loss of species without affecting ecosystem functioning conforms to the redundancy hypothesis (Naeem, 1998; Ruess *et al.*, 2001). Evidence from freshwater mesocosm studies appear to suggest that this assumption may be valid. For example, in a stream mesocosm study with atrazine, sensitive taxa were lost in treated mesocosms, resulting in a change in structure, but functional endpoints were not affected, suggesting that the more tolerant taxa were able to compensate for the loss of sensitive species (Solomon *et al.*, 1996). In a ditch mesocosm study, Van den Brink *et al.* (1996) reported that community structure and function were impacted by exposure to chlorpyrifos immediately after exposure, but that there were no significant measured endpoints between treated and control mesocosms after 24 weeks.

Aldenberg & Slob (1993) modified the methods of Van Straalen & Denneman (1989) to reflect the much greater uncertainties associated with small sample sizes used to generate SSDs. The method of Aldenberg & Slob (1993) determines a median HC5 (50 %), and a 'safe' HC5 (95 %). The HC5 (95 %) is the 95% confidence interval of the 5th percentile, and provides an indication of the level of uncertainty in the calculation (Schudoma, 1994). Wagner & Løkke (1991) developed an alternative method, based on a lognormal distribution, which was found to be in good agreement with the results from loglogistic models (Emans *et al.*, 1993; Roman & Isnard, 1999).

Criticisms of the use of SSD curves to determine 'safe' exposure concentrations include; the fit of the data to the selected distribution model, the ability of the

selected HC*p* to protect communities structure and function, and the non-random selection of species used to generate SSD curves (Versteeg *et al.*, 1999). Newman *et al.* (2000) examined the fit of the data to distribution models by comparing HC values generated from bootstrapping (making no assumptions about distribution) and HC values generated from a lognormal distribution. They found that a strong correlation existed between the two methods, regardless of whether the data were well described by the fitted distribution, suggesting that the model selected is not critical. However, Newman *et al.* (2000) argued that use of a model that doesn't fit the data is hard to defend. The second criticism, that the calculated HC*p* value will protect ecosystem structure and function has been addressed in part by Emans *et al.* (1993), Okkerman *et al.* (1993) and Van Wijngaarden *et al.* (1996), all of whom found extrapolation from single-species data to be a protective indicator of multispecies NOEC values where sufficient data were available.

The third criticism, that species used to generate SSD curves are a non-random selection of species, with a strong bias towards standard species used in Tier I effects assessments is a function of the available data, as a strong bias certainly exists in the available data for a range of compounds. The assessment of species data for linear alkylbenzene sulphonate (LAS) by Blok & Balk (1993) found 55% of tests were carried out using the standard taxa *Daphnia* sp., *Pimephales* sp. or *Lepomis* sp.. Crommentuijn *et al.* (2000) were able to determine maximum permissible concentrations (MPCs) using statistical extrapolation methods for only 11 out of 70 pesticides due to the limited number of taxa tested.

As the majority of single-species toxicity data are generated for standard species, indigenous taxa are under-represented. This chapter aims, with the use of indigenous single-species toxicity data, to determine the effect of species composition on estimates of HC5 calculated from SSD curves. These values will then be compared to the HC5 value calculated using all the indigenous macroinvertebrate data, to determine whether the use of all the data would be protective of natural communities. As LAS is discharged via sewage works into rivers, this would require novel data for lotic taxa.

Toxicity data from Chapter 2 were combined with community data from low order streams sampled in Yorkshire and Derbyshire (UK) to produce SSD curves for each

community. An SSD curve using all available data was also generated. The hazard concentration values (HCp values) for the SSD curve incorporating all data (i.e. HCp_{TOT}) was compared to the HC values from the community SSD curves (i.e. HCp_{COM}). The $HC5_{TOT}$ extrapolates the concentration at which 95% of all species are protected from the measured effect (e.g. LC50, EC50, NOEC). Natural communities will be composed of a subset of this pool of species. This chapter tests whether the $HC5_{TOT}$ was lower than the $HC5_{COM}$ for 95 % of natural communities. Recent studies indicate that determining discrete SSD curves for different taxonomic groups (e.g. algae, invertebrates, fish) will determine more accurate HC5 values, especially for compounds with specific modes of action such as pesticides (Solomon *et al.*, 1996; Brix *et al.*, 2001; Giddings *et al.*, 2001). Versteeg *et al.* (1999) suggested that there may be an increased likelihood of non-unimodal distributions where taxa from different groups are pooled, therefore this analysis was limited to freshwater macroinvertebrates.

3.2 Methods

3.2.1 Acute toxicity data

The results of short-term single-species laboratory toxicity tests (i.e. L(E)C50 data) for linear alkylbenzene sulphonate were taken from Section 2.3.3 (Appendix 2.4). The L(E)C50 data were grouped by genera, except in the case of Oligochaeta, Chironomidae and Tricladida. These taxonomic classifications were used as they correspond to the taxonomic resolutions in the community datasets. Where more than one value was available, the geometric mean of the values was calculated (e.g. Hoekstra *et al.*, 1994). LC50 (μg/l) values for LAS were log-transformed (French & Lindley, 2000).

3.2.2 Community data

One hundred and eighteen sets of community data from 64 sites were collated from three surveys of freshwater macroinvertebrate communities in Derbyshire and Yorkshire (UK), carried out at the University of Sheffield (Grant, 1996; Clayton,

2000; Whittle, 2000). Each macroinvertebrate community dataset consisted of either 10 one-minute kick-samples or 10 Hess samples (30 cm diameter) (Mason, 1993).

Uncertainty still exists in determining the number of data required to generate an SSD curve (e.g. Newman *et al.*, 2000). OECD (1992) suggest that eight or more data should be used to reliably fit a species sensitivity distribution curve; Van Straalen & Denneman (1989) determined a species sensitivity distribution curve from seven data; Roman & Isnard (1999) proposed that SSD curves could be generated where more than 5 data values were available; Versteeg *et al.* (1999) fitted curves with six values; Emans *et al.* (1993) applied the method of Aldenberg & Slob (1993) where at least four data values were available; and Slooff (1992) require at least four NOEC values from different taxonomic groups to determine a maximum tolerable concentration (MTC) from a species sensitivity distribution curve. In this work, the minimum number of data required to generate an SSD curve for a community was set at six L(E)C50 values.

For the calculated $HC5_{COM}$ value to be representative of a community's sensitivity to a toxicant a representative and significant proportion of the community should be represented in the SSD curve. The selection criteria for using a community dataset to determine an SSD curve are given in Table 3.2.

Table 3.1: Selection criteria for community datasets

Criteria	Limit
Minimum proportion of individuals with L(E)C50 data	>30%
Minimum number of taxa with L(E)C50 data	>6
Communities found under similar physiochemical conditions	pH >6 and <8

These criteria were set to ensure that the extrapolated HC5 values would be representative of the sensitivity of a significant proportion of individuals and taxa within the community. The criteria for pH was because a small number of surveyed sites from one source (Grant, 1996) were extremely acidic (i.e. pH <5).

3.2.3 Calculation of the SSD curves

A loglogistic species sensitivity distribution curve was fitted to the full (normalised) dataset, together with 95 % upper and lower confidence limits. Concentrations

hazardous to 5 % of taxa (HC5 values) were then generated using all the available data (i.e. $HC5_{TOT}$), and for each of the subsets of L(E)C50 data as determined by the species composition of selected communities (i.e. $HC5_{COM}$). The method of Aldenberg & Slob (1993) was applied using the following equation;

$$L = \bar{x}_m - k_L \cdot s_m \qquad \text{Eqn. 2.1}$$

where L is the 95 % protection level (i.e. HC5); $\bar{x}_m$ and s_m are the mean and standard deviation of the log test data for a sample size of m; and k_L is the extrapolation constant for sample size m for either the 50 % or 95 % left confidence limit of the HC5 value as defined by Aldenberg & Slob (1993). The 50 % left confidence limit may be taken as the median HC5 value, and the 95% left confidence limit used to provide an indication of the uncertainty associated with the median value (Schudoma, 1994).

3.2.4 Statistical analyses

In order to determine whether the $HC5_{TOT}$ was protective of 95 % of natural communities a χ^2 goodness-of-fit test was used. The expected values were that $HC5_{COM}$ values would be greater than the $HC5_{TOT}$ 95% of the time and lower than the expected values 5% of the time.

3.3 Results

3.3.1 SSD curve using all available data

The geometric means of L(E)C50 data generated in Section 2.6.3 (Appendix 2.4) were log-transformed and used to determine a species sensitivity distribution (SSD) curve using the log-logistic model of Aldenberg & Slob (1993). The resulting 25 L(E)C50 values were fitted to a loglogistic distribution curve (Figure 3.1). The median HC5 value (i.e.$HC5_{TOT}$) was 0.29 mg LAS/l, the confidence limit (i.e. 95% left confidence limit of the HC5 extrapolation) was 0.06 mg LAS/l. Figure 3.1 shows two taxa (*Culex pipiens* and *Lymnaea vulgaris*) with L(E)C50 values that

were more than one order of magnitude lower than any other taxa. These values were both sourced from the literature search (Appendix 2.3).

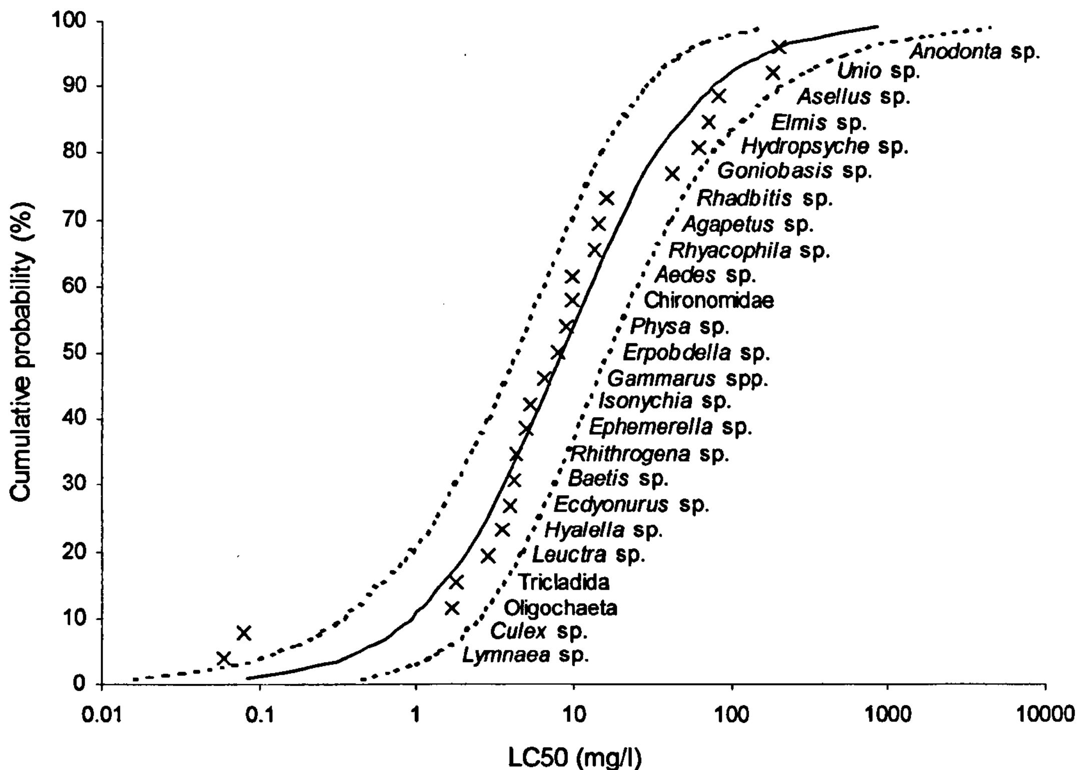


Figure 3.1: Cumulative probability plot of single-species toxicity data with log-logistic model (—) and upper and lower 95 % confidence intervals (- - -) (after Aldenberg & Slob, 1993).

3.3.2 Community selection

Of the original 118 sets of community data from 64 sites, 60 sets of data from 40 sites met the site selection criteria set out in Section 3.2.2. The total community data set included 133 genera, 113 of these genera were present in the communities selected for use in this analysis. The selected communities contained between eight and thirty-seven genera (mean = 17), and L(E)C50 data were available for between six and fourteen genera within each dataset (mean = 8), representing 32 % to 75 % of genera present (mean 50 %). The percentage of individuals in a community dataset for which L(E)C50 data were available ranged from 33 % to 99 % with a mean of 75 %.

3.3.3 Effect of species selection on SSD curves using community data

The SSD curves for each of the 60 community datasets were determined and $HC5_{COM}$ values calculated (Appendix 3.1). Median $HC5_{COM}$ values (i.e. $HC5_{COM}$ 50%) ranged from 0.15 to 1.3 mg LAS/l (mean = 0.8 mg LAS/l), $HC5_{COM}$ confidence limit values (i.e. $HC5_{COM}$ 95%) ranged from 0.01 to 0.4 mg LAS/l (mean = 0.1 mg LAS/l). The distribution of $HC5_{COM}$ values are plotted in Figure 3.2.

The $HC5_{COM}$ values were calculated to allow the relevance of species sensitivity distribution curves to natural communities to be explored by determining the proportion of communities with higher $HC5_{COM}$ values than the $HC5_{TOT}$. The median $HC5_{TOT}$ was lower than 55 of the 60 median $HC5_{COM}$ values. The five community datasets with lower $HC5_{COM}$ values than the $HC5_{TOT}$ were the only communities to include *Lymnaea* sp., which was one of the two most sensitive genera in the sensitivity distribution (Figure 3.1). The $HC5_{TOT}$ (95 %) was lower than 39 of the 60 community $HC5_{COM}$ (95%) values.

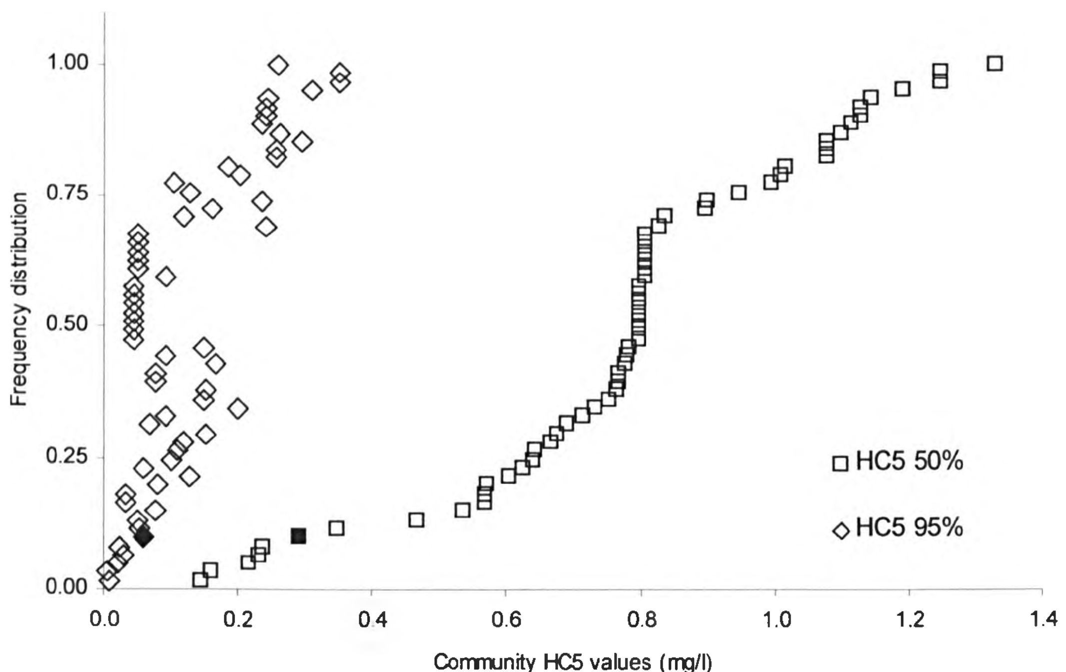


Figure 3.2: Cumulative frequency distributions of median HC5 values (HC5 50%), and associated HC5 confidence limits (HC5 95%) extrapolated from 60 community datasets (shaded points represent the position of the HC5 values calculated using all available data).

The proportion of communities with $HC5_{COM}$ values greater than the $HC5_{TOT}$ was analysed using χ^2 goodness of fit. If the $HC5_{TOT}$ was lower than the $HC5_{COM}$ for 95 % of natural communities, three of the 60 data would be lower than the $HC5_{TOT}$. The proportion of median $HC5_{COM}$ values (i.e. $HC5_{COM}$ 50%) that were lower than the median $HC5_{TOT}$ was not significantly different from the expected value (i.e. 3) ($\chi^2 = 1.42$, df = 1, $p > 0.05$). The proportion of $HC5_{COM}$ (95%) values that were lower than the $HC5_{TOT}$ was significantly different from the expected value (i.e. 3) ($\chi^2 = 178.2$, df = 1, $p < 0.001$).

Two communities were sampled repeatedly over one year. These data allowed the variation in community sensitivity due to species composition to be explored. Table 3.2 shows the species composition of the two communities through time and the generated $HC5_{COM}$ values. At Site 1, the $HC5_{COM}$ (median) varied through time, ranging from 0.56 mg to 0.94 mg LAS/l. Only 25 % of species within this community were present on all sampling dates, and 76 % of species were present in less than half of the 10 samples meeting the selection criteria. Five of the eight L(E)C50 genera were present on all sample dates. At Site 2 a total of 15 species were sampled over the year, with 47% of species present in all samples. L(E)C50 data were available for the same 6 taxa on every sample date.

Table 3.2: The sensitivity of two macroinvertebrate communities collected between May 1993 and April 1994 (Grant, 1996)

	Site 1									
	May	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr
Set	37	38	39	40	41	42	43	44	45	46
Asellus aquaticus								✓		
Baetis rhodani	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Chironomidae	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Gammarus pulex	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Hydropsyche angustipennis	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Leuctra sp.					✓	✓	✓	✓		✓
Oligochaeta	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Rhyacophila dorsalis	✓	✓	✓	✓	✓	✓		✓	✓	
HC5 50%	**0.80**	**0.80**	**0.80**	**0.80**	**0.76**	**0.76**	**0.56**	**0.94**	**0.80**	**0.56**
HC5 95%	**0.05**	**0.05**	**0.05**	**0.05**	**0.07**	**0.07**	**0.03**	**0.12**	**0.05**	**0.03**

	Site 2						
	May	**June**	**Aug**	**Sept**	**Oct**	**Mar**	**Apr**
Set	**50**	**51**	**52**	**53**	**54**	**55**	**56**
Asellus aquaticus	✓	✓	✓	✓	✓	✓	✓
Baetis rhodani	✓	✓	✓	✓	✓	✓	✓
Chironomidae	✓	✓	✓	✓	✓	✓	✓
Gammarus pulex	✓	✓	✓	✓	✓	✓	✓
Hydropsyche angustipennis	✓	✓	✓	✓	✓	✓	✓
Leuctra sp.	✓	✓	✓	✓	✓	✓	✓
Oligochaeta							
Rhyacophila dorsalis							
HC5 50%	**0.8**	**0.8**	**0.8**	**0.8**	**0.8**	**0.8**	**0.8**
HC5 95%	**0.04**	**0.04**	**0.04**	**0.04**	**0.04**	**0.04**	**0.04**

3.4 Discussion

As HC5 values are extrapolated from the left hand tail of a distribution, the fit of the curve in this region is important if hazard concentrations are to be determined accurately (Van Straalen & Denneman, 1989). When all the available data were used, the SSD curve closely followed the plotted loglogistic distribution, with the exception of two values (*Lymnaea* sp. and *Culex* sp.) falling outside the 95%

confidence intervals of the curve (Figure 3.1). These two values were identified in Chapter 2 as being potentially unreliable. However, the descriptions of test methodologies is frequently very limited and there was no evidence that these tests were not carried out in a similar way to other literature data, and therefore there was no justification for removing them. In the context of a risk assessment further testing with these taxa would be required.

Many macroinvertebrate species spend part of their lifecycle out of the water and hence may not be detected when the community is sampled (Elliott *et al.*, 1988). Within the community datasets used here, two sites were sampled every month for a year. At site 1 only 25% of species were present on all sampling dates, and 76 % of species were present in less than half of the ten samples meeting the selection criteria. This resulted in different $HC5_{COM}$ values being calculated for different sampling dates (Table 3.2). The insects *Leuctra* sp. and *Rhyacophila* sp. were not detected on some sampling dates. These taxa both have aerial life-stages and would therefore not be present in the community on some sampling dates (Hynes, 1977; Edington & Hildrew, 1995). *Asellus aquaticus* was also only present on a few sample dates. Although isopods complete their lifecycle in the water (Gledhill *et al.*, 1993), *Asellus aquaticus* were locally scarce, represented by two individuals on only one occasion. This repeated sampling of a community illustrates that the sensitivity of a community to toxicant is dynamic and will change with changes in species composition through time. At site 2 a total of 15 species were sampled over the year, with 47% of species present in all samples. Of the species present in this community *Asellus* sp., *Gammarus* sp., Oligochaeta were present throughout the year as their whole lifecycle occurs within the water (Gledhill *et al.*, 1993). *Baetis* sp., *Hydropsyche* sp. and Chironomidae all have aerial life stages, but are either multivoltine (Elliott *et al.*, 1988) or the aerial stages of different species within the taxa are staggered, resulting in some individuals present as larvae on all sampling dates (Nilsson, 1997; Edington & Hildrew, 1995).

The median HC5 value generated using all available data (i.e. $HC5_{TOT}$) was 0.29 mg LAS/l. This is in good agreement with the Maximum Permissible Concentration calculated by Feijtel *et al.* (1999) of 0.32 mg LAS/l, determined from laboratory generated NOEC data. Similarity in these values will partly be due to the similarity

in the data used, as the extreme values within the distribution (Figure 3.1) are all sourced from the literature. The community HC5 values (i.e. $HC5_{COM}$) are predominantly generated from the novel tests on lotic species carried out in Chapter 2 (Table 2.3). Median $HC5_{COM}$ values ranged from 0.15 to 1.3 mg LAS/l, with a mean value of 0.8 mg LAS/l (Figure 3.2). These values based on novel data are still very similar to the MPC calculated by Feijtel *et al.* (1999), differing by less than a factor of five. This suggests that SSD curves and associated HC5 values using all the data available within a single taxonomic group (i.e. invertebrates) may be representative of the range of sensitivities of indigenous species tested under similar exposure conditions (i.e. short term single-species tests). This hypothesis was tested using χ^2 goodness of fit, and the median $HC5_{TOT}$ was found to be lower than 95% of $HC5_{COM}$ values.

The $HC5_{TOT}$ estimates the concentration that will not affect 5 % of taxa. An assumption inherent within this method is that the distribution of responses observed in the taxa tested will be representative of the distribution of responses of taxa in natural communities (Versteeg *et al.*, 1999). However, this has not always been found to hold true (e.g. McDaniel & Snell, 1999). Freshwater macroinvertebrate communities tend to be dominated by a few abundant taxa, with a large number of taxa present in very low abundances (Clarke & Warwick, 1994). Novel test species may be selected because they are, or have been, a common species in potentially exposed habitats (e.g. Greve *et al.*, 1998). This means that less common species, present in low abundances will not be included within these distributions. As there is no evidence that the range of sensitivities of the tested taxa are representative of the range of sensitivities for taxa present in low abundances in natural streams, considerable uncertainties remain as to whether these SSD curves are in any way representative of the response of natural communities. Objective 5 addresses this problem by exposing structurally distinct communities to LAS in a stream mesocosm and determining the effect of LAS on the whole community. Prior to exposing distinct communities in a stream mesocosm, it was first necessary to establish whether such communities could be maintained within the test system (Chapter 4).

4 Changes in macroinvertebrate communities structure in outdoor stream mesocosms.

4.1 Introduction

If the risk of damage to receiving environments is not disproved with sufficient certainty after Tier I and Tier II single-species testing, higher tier assessments may be conducted. These include multispecies tests in mesocosms or field trials (Graney *et al.*, 1989; Crossland, 1994; Urban, 1994). By exposing multispecies assemblages to a compound under more realistic exposure conditions, mesocosm studies aim to reduce the uncertainty in extrapolating effects assessments to natural systems. Natural communities differ in their species composition (e.g. Vannote *et al.*, 1980), with the structure of a community determined by the presence and relative abundance the composite species (Dewey & DeNoyelles, 1994). Colonisation of stream mesocosms is normally achieved by either allowing natural colonisation of macroinvertebrates over time (e.g. Belanger, 1997) or artificially seeding the mesocosms with natural communities (e.g. Girling *et al.*, 2000). Previous studies have demonstrated mesocosms are able to support diverse communities, closely resembling natural stream communities (Warren & Davis, 1971; Crossland *et al.*, 1991), although they are unable to replicate exactly the conditions experienced in natural systems (Robinson & Minshall, 1986; Mackay, 1992).

Natural streams are a dynamic habitat with many factors affecting the distribution of invertebrate species including current velocity, food availability, substrate type, temperature, dissolved oxygen and water chemistry (Robinson & Minshall, 1986; Ladle & Ladle, 1992; Mackay, 1992; Degani *et al.*, 1993). As relatively little is known about the niche requirements of particular species or assemblages, it is unclear how habitat differences between natural and artificial streams will affect the responses of a species assemblage to a toxicant (e.g. Joern & Hoagland, 1996). Variation due to immigration or emigration results in changes in assemblage composition that may affect responses to a test chemical. Problems with artificial streams include maintaining sensitive taxa, escape of emergent adults and interpreting results from

life-cycle stages that may not be the species most sensitive (Pontasch & Cairns, 1991). Also, spatial and temporal interactions may mask experimental interactions (Perry & Troelstrup, 1988). The extent to which the physiochemical requirements (e.g. habitat structure, food availability and water chemistry) of individual species and communities are met by a stream mesocosm will determine which species colonise the system, or remain in the system after seeding (Swift *et al.*, 1993). Previous studies have shown that artificial streams normally only contain a subset of the species present in the natural source community resulting in a change in the community structure (Arthur *et al.*, 1982; Peckarsky & Penton, 1990).

Prior to testing the effect of species composition on community responses in stream mesocosms (Objective 5), it was first necessary to determine whether communities that were structurally distinct in natural systems could be maintained in a stream mesocosm for the duration of a study. Belanger (1997) reported the median duration of mesocosm studies to be 28 days (SE = 14.46). The study presented in this chapter was run for 10 weeks to allow time for any patterns that may not be significant over shorter timescales to emerge. If structurally distinct natural communities were to lose those species that differentiated them from one another, their species compositions would become more similar, and the communities may ultimately converge to a 'generic mesocosm community', composed of species that were both present in natural communities and were able to persist in the mesocosm system.

Alternatively, if different streams stocked from the same community lose different species, the community structures of the replicate streams will diverge. This may result in communities becoming indistinct from one another due to the high level of variability between replicate channels stocked with the same community. The aim of this chapter is to determine first, whether streams stocked from three structurally distinct freshwater macroinvertebrate communities remain distinct over ten weeks; and second, whether streams stocked from the same natural community remain similar to each other for the duration of the study.

4.2 Methods

4.2.1 Experimental streams

This experiment was conducted in eight experimental streams at Unilever Research (Port Sunlight, UK) from October 1998 to January 1999. Each stream was 6.0 m long x 0.5 m wide x 0.3 m deep and was constructed from marine plywood and coated in water resistant paint. The stream bed consisted of gravel and small pebbles (5-20 cm diameter) to a depth of 0.1 m and the streams were shaded with netting (mesh size, 2 mm), attached approximately 0.7 m above the streams to reduce the amount of direct light reaching the streams. Drift nets (mesh size, 500 μm) were in place at the end of each channel, allowing drift to be sampled or returned to the stream as required.

Water from the River Dibbin (NGR SJ343835) was passed through a high-rate sand filter (particle size, 20-25 μm), and then held in a 1500-m^3 pond from where it was pumped to each stream via a header tank (12000 l). The streams operated on a once through basis at approximately 55 l/min with a flow rate of approximately 25 cm/sec. Leaf packs (35 x 10 cm; mesh size, 6 mm; Collins Nets, Dorset), containing alder and chestnut leaves and preconditioned in pond water for 7-10 days to allow fungal colonisation, were provided in excess as a food source.

Each stream was divided into three longitudinal sections (top, middle and bottom) for the purposes of macroinvertebrate colonisation and sampling to reduce the possibility of unequal macroinvertebrate distributions (animals and water could move freely between sections). Equal number of samples were removed from each section on each sampling day to allow a blocked design to be used if significant longitudinal differences in macroinvertebrate densities or diversities existed within the streams.

Prior to colonisation with macroinvertebrates, 72 stainless steel cylinders (100 mm diameter x 97 mm high, 9.5 mm^2 perforations; Industrial Filtration Services, Birkenhead), were embedded in the substrate of each experimental stream as retainers for the sediment samplers. Sediment samplers (10 cm diameter x 18 cm high) were constructed from coarse-mesh (10 cm diameter x 8 cm; mesh size, 6-10 mm), stitched to a fine-mesh bag (10 cm diameter x 10 cm; mesh size, 0.5-1.0 mm).

Macroinvertebrates could move freely through the coarse mesh during deployment, but were retained by the fine mesh bag when the samplers were removed from the stream. Samplers were removed on at least fortnightly intervals from 26 October 1998 and preserved in 70 - 80 % industrial methylated sprits (IMS). At each sample date four samples were randomly selected from the top, middle and bottom section of each stream. Drift nets were cleared fortnightly and all invertebrates collected preserved in 70 % IMS for later identification.

4.2.2 Invertebrate stocking

Three streams each with structurally distinct macroinvertebrate communities were selected as source communities. The River Wheeler (Denbighshire, NGR SJ102713) was dominated by Ephemeroptera, Plecoptera and Trichoptera. The River Tame (Greater Manchester, NGR SD986042) was dominated by *Asellus aquaticus* and *Erpobdella octoculata,* and was exposed to organic pollution from an upstream sewage treatment works during periods of high flow. The River Wye (Derbyshire, NGR SK096727) was a *Gammarus* dominated community. These communities were used to stock three, three and two artificial streams respectively (randomly assigned prior to stocking). The *Gammarus* dominated community was selected to stock only two experimental streams as it has been shown in previous studies to be relatively stable in experimental channels over time (e.g. Crossland *et al.*, 1991).

Macroinvertebrates were collected from each field site on two occasions between 14th and 23rd October 1998. On each occasion, kick-net samples (Mason, 1993) sufficient to stock each channel with animals from an equivalent area to each experimental stream (i.e. 3 m^2 per channel), were collected. On each stocking day the material collected for each stream was divided into three aliquots and added at 0 m, 2 m and 4 m from the top of each stream. Macroinvertebrates captured in the drift nets during the stocking phase were returned to the top of the riffle section daily.

4.2.3 Water quality analysis

Temperature, conductivity, dissolved oxygen and pH were measured at the head of each stream daily using a YSI DM600 Sonde (HydroData Services Ltd, London). Flow rates from each stream outlet were monitored daily for the first two weeks of the study and then three times a week using a bucket (5 litre) and a stopwatch. Total hardness was determined for one stream twice weekly (HMSO, 1982). Dissolved organic carbon, total organic carbon and suspended solids concentrations were determined twice weekly from samples taken from the source water (i.e. pond water) (HMSO, 1995, 1984).

4.2.4 Invertebrate sampling

Twelve sediment samplers were removed from each stream 0, 14, 28, 42, 56 and 70 days after the stocking phase was completed. Sediment samples were preserved in 50 to 70 % ethanol and returned to the laboratory for macroinvertebrate identification and counting. Macroinvertebrates were separated from the sediment by floating off animals in water and then picking through the sediment to ensure all animals were removed. Identification was performed to species level where possible, except for Chironomidae, Oligochaeta, Tricladida and Hydracarina, using available identification keys (Holland, 1972; Hynes, 1977; Macan, 1977; Reynoldson, 1978; Elliot & Mann, 1979; Elliott *et al.*, 1988; Friday, 1988; Wallace *et al.*, 1990; Gledhill *et al.*, 1993; Edington & Hildrew, 1995; Elliott, 1996; Nilsson, 1997).

4.2.5 Statistical analyses

Macroinvertebrate species counts from the 12 sediment samplers taken from each stream on each sample date were pooled, providing a single sample for each combination (8 streams x 6 times = 48 samples). Data were square root transformed and Bray-Curtis similarity matrices generated. The similarity matrix generated was used to plot a non-metric multidimensional scaling ordination (non-metric MDS) to allow visual interpretation of whether the three communities remained distinct for the duration of the study (Clarke & Warwick, 1994). Data points that were close together

on the ordination were relatively similar (within the variation explained by the plotted axes), while data points that were further apart were less similar (Clarke & Warwick, 1994). A one-way ANOSIM of the Bray-Curtis similarity matrix generated from the square root transformed community data was used to determine whether communities stocked from different communities remained different for the duration of the study. A two-way crossed ANOSIM was used to determine whether communities stocked from the same community were similar to one another.

4.3 Results

4.3.1 Water Quality

There were no significant differences between water quality parameters measured in all streams (Table 4.1). Total hardness (measured as $CaCO_3$ mg/l), total organic carbon , dissolved organic carbon , nitrate, and suspended solids were only recorded in one stream, therefore no statistical comparisons were made between streams.

Table 4.1: Mean values (± 1 SE) for all measured water quality variables

Water quality measurement	Mean (±1SE)
pH	7.7 (0.01)
Temperature (°C)	7.4 (0.10)
Dissolved oxygen (mg/l)	11.4 (0.04)
Conductivity (µs/cm)	619 (4.23)
Stream flow (l/min)	58.2 (1.7)
Total hardness (mg/l $CaCO_3$)	219.25 (11.56)
Total organic carbon (mg/l)	16.0 (0.29)
Dissolved organic carbon (mg/l)	15.1 (0.29)
Nitrate (mg/l)	22.4 (0.27)
Suspended solids (mg/l)	14.4 (2.26)

4.3.2 Macroinvertebrate assemblages

All taxa making up > 0.5 % of a sample were identified to at least genus, with the exception of Chironomidae, 'unidentified Diptera', Oligochaeta, Simuliidae and 'unidentified Tricladida'. Of these taxa, only Chironomidae and Oligochaeta made up more than 2.5% of any sample at any time. Forty-nine macroinvertebrate taxa were identified in total (Table 4.2). Streams stocked from the River Tame, River Wheeler

and River Wye contained 31, 33 and 22 invertebrate taxa respectively. At Day 0, River Tame samples were dominated by *Erpobdella octoculata* (39%), *Asellus aquaticus* (29%) and Oligochaeta (18%), River Wheeler samples were dominated by Oligochaeta (50%), *Gammarus pulex* (14%) and *Rhithrogena semicolorata* (13%), and River Wye samples were dominated by *Gammarus pulex* (79%), Oligochaeta (11%) and *Polycelis* sp. (8%) (Table 4.2).

Table 4.2: Total macroinvertebrate abundances (mean for replicate streams) Species are pooled to genus (>100 individuals = **, >10 = * in any replicate = *).

Macroinvertebrate taxa	Tame	Wheeler	Wye
Chironomidae	**	**	**
OLIGOCHAETA	**	**	**
Asellus aquaticus	**		
Erpobdella octoculata	**		
Gammarus pulex	*	**	**
Hydropsyche spp.	*	*	
Rhyacophila spp.	*		*
Ceratopogonidae		**	
Ancylus fluviatilis		*	
Dicranota spp.		*	
Elmis aenea		*	
Ecdyonurus dispar		*	
Polycelis sp.			**
Lumbricidae			*
Rhithrogena semicolorata		**	
Isoperla grammatica		*	
Limnius volckmari		*	
Silo sp.		*	
Agapetus fuscipes			*

4.3.3 Comparisons between source communities

At the start of the study the three communities were structurally distinct. However, 30% of the taxa identified were present in all three communities, and between 40% and 50% of taxa were present in two of the three communities (Table 4.2). These overlaps in taxonomic composition between the three communities provide the potential for the communities to collapse to a 'generic mesocosm assemblage' over the ten week duration of the study. An alternative scenario would be that the replicate streams stocked with the same community diverge through time until all mesocosm communities are equally distinct from one another. The following analysis focuses on these questions by determining whether communities from different source

communities remain distinct for the duration of the study, and whether communities from the same source community remain similar for the duration of the study.

The MDS ordination of the data, generated from Bray-Curtis similarity matrices is shown in Figure 4.1a. Hierarchical clustering of the similarity matrix (Figure 4.1b) shows that the three tight clusters observed in the ordination represent a complete separation of samples from the three source communities. This separation of the source communities was found to be highly significant in a one-way ANOSIM test ($R = 1.0$, $p < 0.1$ %).

The relative abundances of all taxa in each stream on each sample date are given in Appendix 4.1. Chironomidae increased in abundance in all streams during the study. Oligochaeta numbers increased in streams stocked from the River Tame and the River Wye, but decreased slightly in streams stocked from the River Wheeler. *Baetis rhodani* (Ephemeroptera) were absent from all streams from Day 14 onwards, and *Rhyacophila* sp. (Trichoptera) abundances were severely reduced. In streams stocked from the River Tame, there was an increase in abundance of *Asellus aquaticus* (Crustacea), while in streams stocked from the River Wheeler, *Rhithrogena semicolorata* (Ephemeroptera) abundances were severely reduced. In streams stocked from the River Wye, *Gammarus pulex* declined in relative abundance to Day 42, then increase slightly to the end of the study. *Polycelis* sp. decreased in abundance by Day 70. *Glossiphonia complanata* (Hirudinea) were present at Day 0, but not at Day 70, and *Ancylus fluviatilis* (Mollusca) and *Agapetus fuscipes* (Tricladida) both decreased in abundance, although all three species were only present in low numbers at Day 0.

Despite these shifts in taxa abundances, the mean number of taxa in the stream mesocosms remained similar through time in each community (Figure 4.2 a, c, e). If the three communities were to collapse to a 'generic mesocosm community' large shifts in taxonomic abundances would need to occur. The ordination (Figure 4.1), and associated analysis of similarity (ANOSIM) clearly show that the three communities remained distinct for the duration of the ten week study, despite temporal changes in the relative abundance of some taxa.

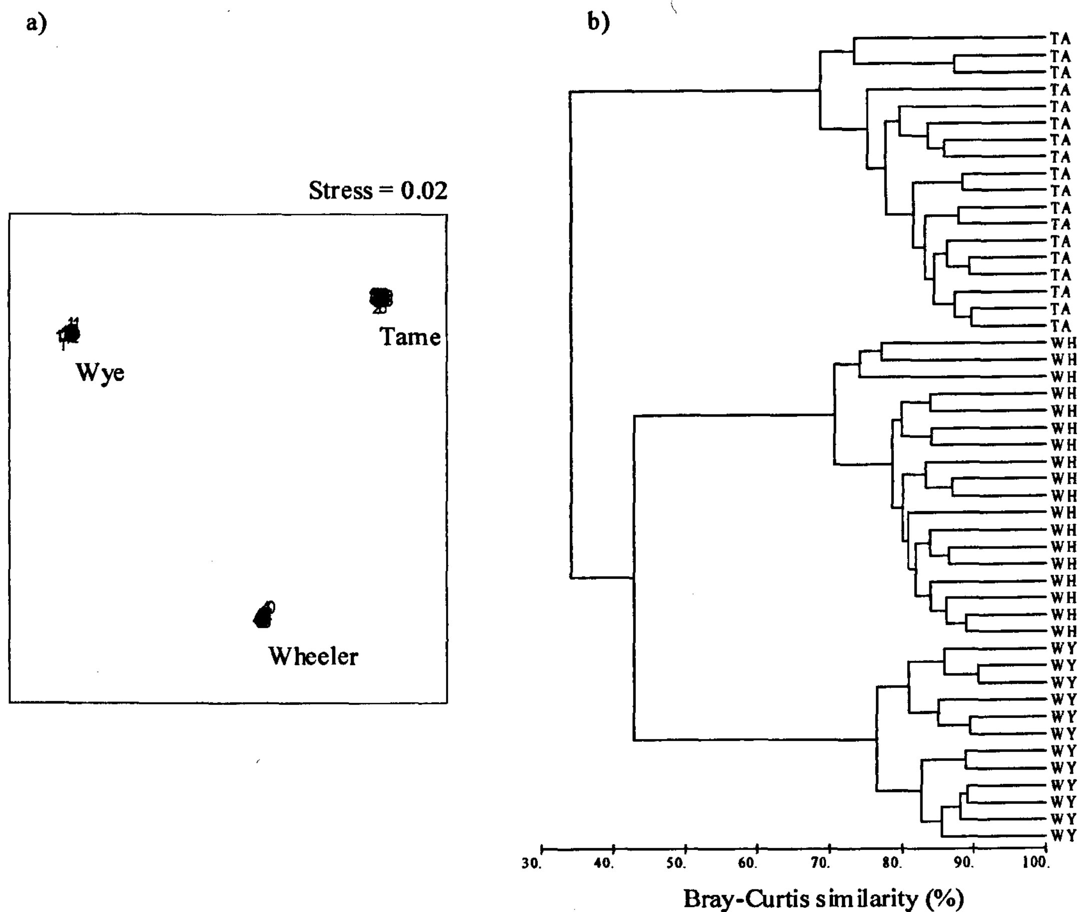


Figure 4.1: a) Ordination (non-metric MDS) of the total number of individuals sampled fortnightly over 10 weeks (8 streams, 6 times). b) Clusters formed are shown in the Bray-Curtis similarity plot for the River Tame (TA), River Wheeler (WH) and River Wye (WY).

4.3.4 Comparisons within source communities

Differences between streams

The number of individuals remained constant in the streams stocked from the River Wheeler and the River Wye, although variability between streams is larger for the River Wye (Figure 4.2). The number of individuals in streams stocked from the River Tame increased through time in all streams. Statistical comparisons between streams stocked from the same community showed no significant differences between streams for either the River Wheeler or the River Tame streams (2-way ANOSIM: River Wheeler, $R = 0.052$, $p > 0.05$; River Tame, $R = 0.066$, $p > 0.05$) (Figure 4.2). River Wye streams did differ significantly (2-way ANOSIM: $R = 0.543$, $p < 0.05$). This

may be due to large differences in initial stocking densities (Figure 4.2f) rather than divergence in species composition through time.

Differences through time

The effect of sample date was significant for all three communities, indicating some shift in community structure through time (2-way ANOSIM: River Wheeler, $R = 0.125$, $p < 0.05$; River Tame, $R = 0.147$, $p < 0.05$, River Wye, $R = 0.244$, $p < 0.05$. Pairwise comparisons showed that all three communities underwent an initial shift in community structure after which no significant differences were detected. In the River Wheeler community Day 0 was significantly different to all other dates except Day 14; Day 0 was significantly different to all other sample days in streams stocked from the River Tame; in the River Wye community Day 0 significantly differed from days 42 to 70, and Day 14 differed from days 56 and 70.

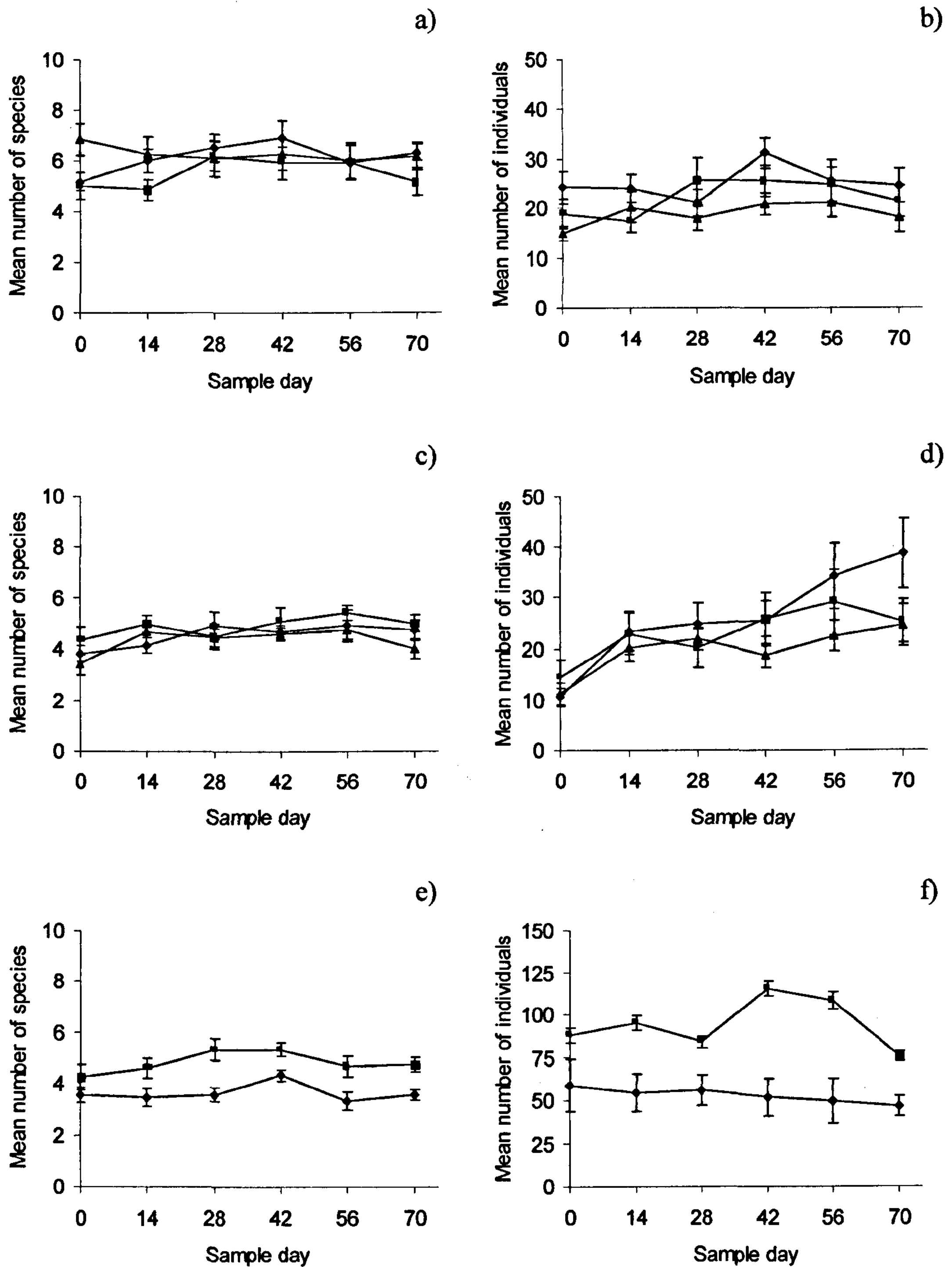


Fig 4.2: Mean number of species (a, c, e) and individuals (b, d, f) per stream at each sample date for streams stocked from River Wheeler (a, b), River Tame (c, d) and River Wye (e, f) (error bars ± 1 S.E.).

4.4 Discussion

4.4.1 Comparisons between communities

There was considerable overlap in the composition of the three communities, with the River Wye community being largely composed of a subset of the River Wheeler community. This presented the potential for the three communities to converge to a 'generic mesocosm community' over the duration of the study, if taxa that were unique to each community were lost. Indeed, several taxa were either lost from the communities or were severely reduced in abundances, including mayflies (*Baetis rhodani, Rhyacophila* sp.), caddis-flies (*Rhithrogena semicolorata, Agapetus fuscipes*) and a snail (*Ancylus fluviatilis*). Other taxa increased in abundance, most notably Chironomidae, which increased by up to two orders of magnitude. However, despite the initial overlap between communities and change in species composition over time the three communities remained distinct from one another for the duration of the ten week study, with less than 45 % similarity between communities.

4.4.2 Temporal changes within communities

The changes occurring within the stream mesocosm were not sufficient to result in distinct communities becoming structurally similar. However, they may have been sufficient to result in similar communities becoming structurally distinct over the duration of the study. Significant changes in community structure were observed in all three communities over time, with different rates of change in different communities. Streams stocked from the River Tame and River Wheeler both underwent a shift in community structure at the beginning of the study, but no significant differences were detected between sample dates after Day 0 and Day 14 respectively. The initial shift in these communities was predominantly due to the increase in Chironomidae abundances in all streams. The River Wye community underwent a more gradual shift in community structure, with significant changes up to Day 28. These changes were due to both an increase in Chironomidae abundances, and the decrease in the high numbers of *Gammarus* over the duration of the study. The effect of these species will be greater in the River Wye than in other communities

due to the River Wye community having the lowest species richness, and highest *Gammarus pulex* relative abundance (79%) at the start of the study. Previous stream studies carried out in the UK have frequently used *Gammarus*-dominated communities as their stocking community (e.g. Tattersfield *et al.*, 1995; Girling *et al.*, 2000). These results suggest that the use of more diverse communities which are not dominated by *G. pulex* may result in reduced levels of between-stream variability, thus improving the ability of the system to determine the effect of toxicants on exposed communities.

4.4.3 Divergence between replicate streams

Replicate streams stocked with either the River Wheeler or the River Tame communities did not diverge from each other over the duration of the study. As toxicity studies in stream mesocosms normally have low, or no replication between communities (Tattersfield *et al.*, 1996), this consistency between streams is important. If the replicate communities are not diverging, then changes between treatments can be attributed to the effect of the toxicant rather than the effect of stochastic variation.

The presence of one highly dominant species within the River Wye community meant that any multivariate analysis of this community would largely be driven by changes in the *Gammarus pulex* population. More extreme data-transformations prior to analysis (i.e. fourth root transformation or presence absence data) could be used to reduce the effect of the *G. pulex* (Clarke & Warwick, 1994). However the aim of this Chapter was to make comparisons between the three communities, therefore the same transformation was used for all data (i.e. square root transformed). The two replicate streams stocked with the River Wye community were significantly different to each other, due to large differences in initial stocking densities in these streams. This resulted in within-stream samples being more similar to each other than between-stream samples. However, it should be noted that the Bray Curtis similarities show that the level of similarity between all the River Wye samples was greater than for either the River Wheeler and River Tame communities.

4.4.4 Conclusions

In conclusion, the three communities remained structurally distinct over the duration of the ten week study. There were significant shifts in community structure over time in all three streams, but only streams from the River Wye showed significant differences between replicate streams. The River Wheeler and River Tame communities were therefore selected to address Objective 4. Chapter 5 uses these two communities to determine whether two structurally distinct communities significantly differ in their sensitivity to LAS.

5 The responses of two distinct macroinvertebrate communities to LAS exposure in experimental streams.

5.1 Introduction

Chapter 4 established that structurally distinct communities could be maintained in an experimental stream system for up to ten weeks. In this chapter Objective 4 will be addressed by determining whether two structurally distinct macroinvertebrate communities differ in their response to LAS exposure. Species differ in their sensitivity to toxicants (e.g. Hoekstra *et al.*, 1994; Stuijfzand *et al.*, 2000), resulting in a range of species sensitivities being present within a community. If community responses are determined by the direct effect of a toxicant on the species present, then it may be hypothesised that structurally distinct communities would differ in their responses to the toxicant (Kiffney & Clements, 1996).

Communities may also differ in their sensitivity to a toxicant due to indirect effects. An example of direct effects on one taxonomic group resulting in indirect effects in other groups was reported by Boyle *et al.* (1996), in a mesocosm study with diflubenzuron. The direct effect of diflubenzuron on macroinvertebrate grazers resulted in indirect effects on both the algal community and the fish population. As grazing pressure from invertebrate grazers decreased, the algal biomass increased. At the same time, there was a significant reduction in *Lepomis macrochirus* reproduction and survival due to a decrease in prey organisms (i.e. grazing invertebrates). Other indirect effects that have been observed involve the presence of one species modifying another species response to a toxicant. For example, Schulz & Liess (2001) observed that the emergence of caddisfly (*Limnephilus lanatus*) was reduced by 20 to 25 % when exposure to the pesticide fenvalerate occurred in multispecies exposures relative to their response in single-species exposures. While Schulz & Liess (2001) were not able to determine whether this change in sensitivity was due to a specific interaction, they speculated that disturbance by *Gammarus pulex* which were present in high abundances may be a contributing factor.

Mesocosms are used in an attempt to determine species or community sensitivity under exposure conditions that are more similar to natural conditions than traditional laboratory tests (Pontasch & Cairns, 1991). A range of test systems are employed, ranging from single-species laboratory tests, through laboratory microcosms containing simple multispecies assemblages, to large scale outdoor mesocosms in which complex communities may be exposed under more environmentally realistic conditions (Brock *et al.*, 1992; Van den Brink *et al.*, 1996).

However, as there is no standardised test design, and no standard test community, it is difficult to make direct comparisons between studies (Brock *et al.*, 1995). Different communities may be selected in different studies (Belanger *et al.*, 2000); the selected communities may be stocked by seeding or natural colonisation (Belanger, 1997; Girling *et al.*, 2000a); the systems may be operated on a flow through or recirculating basis (see Guckert, 1993); and the study duration, which may be as short as six days or as long as 59 weeks (Van den Brink *et al.*, 1996; Richardson & Kiffney, 2000). As it is not possible to make direct comparisons between studies, it is difficult to determine whether the differences between community responses are due to differences in community structure, or differences in exposure conditions (Slooff, 1985). In order to determine whether differences in the response of mesocosm communities are due to species composition *per se*, it is necessary to simultaneously expose different communities to a toxicant in the same test system, thus minimising confounding variables such as differences in environmental conditions, exposure conditions, water quality and season. This was the approach adopted in this study.

5.1.1 Objectives

In Chapter 4 it was shown that it is possible to maintain three structurally distinct macroinvertebrate communities in the experimental stream facility at Unilever, Port Sunlight, over at least ten weeks. These communities were collected from natural streams and transplanted to the experimental stream facility, thus retaining, as far as possible, the natural community structure. The aim of this chapter was to investigate the hypothesis that structurally distinct communities respond differently to toxicant exposure and that community sensitivity is dependent upon the relative sensitivities

of the constituent species. Two distinct communities were simultaneously exposed to LAS for 28 days to test the prediction that the effect of LAS would be greater in the community dominated by more sensitive species and less extreme in the community dominated by more tolerant species.

5.2 Methods

5.2.1 Experimental streams

This experiment was conducted in eight experimental streams at Unilever Research (Port Sunlight, UK) from May 1999 to June 1999. The eight experimental streams were as described in Section 4.2.1, with the following modifications. An additional filtration system had been installed between the header tank and the stream outflow (SpinClean Filter; particle size 20 μm); sediment samplers were placed directly into the stream substrate (i.e. without the stainless steel mesh cylinders); the leaf packs contained alder leaves, not alder and chestnut leaves, and were not conditioned prior to being added to the streams at the beginning of the colonisation phase.

Samplers were removed at fortnightly intervals from 6th May 1999 to 3rd June 1999 and preserved in 70 – 80 % IMS. At each sample date, eight samples were randomly selected from the top, middle and bottom sections of each stream.

5.2.2 Invertebrate stocking

The two macroinvertebrate communities selected were the River Wheeler (Denbighshire, NGR SJ102713), dominated by taxa found to be sensitive to LAS (e.g. *Rhithrogena semicolorata.*, *Baetis rhodani*, Plecoptera), and the River Tame (Greater Manchester, NGR SD986042), dominated by more tolerant species (e.g. *Asellus aquaticus*, *Erpobdella octoculata*). Each community was used to stock four experimental streams.

Macroinvertebrates were collected from each field site between 19th and 27th April 1999. On the first visit, kick-net samples (Mason, 1993) sufficient to stock each channel with animals from twice the substrate area of the experimental streams (i.e. 6 m^2 per channel), were collected. On the two subsequent visits, half this volume of

material was collected (i.e. 3 m^2 per channel). On each stocking day, the collected material was combined and then split into five aliquots. Four aliquots were used for stocking the experimental streams, while the fifth was preserved with 50 to 70 % ethanol. This preserved sample was used to characterise the source macroinvertebrate community. Each aliquot was further divided into three and added in equal volumes at the head of each of the three longitudinal sections. Macroinvertebrates captured in the drift nets during the stocking phase were returned to the top of the riffle section daily.

5.2.3 LAS dosing and analysis

After the two week stocking phase, the experimental streams were dosed continuously with LAS for 28 days using peristaltic pumps (Watson Marlow, Model 202U). There were four LAS treatments, control, 0.8 mg LAS/l, 2.5 mg LAS/l and 8.0 mg LAS/l, and each community was exposed to each LAS treatment. There was no replication of community x LAS treatment.

Separate stocks were prepared for each stream every seven days for the duration of the study (Table 5.1). The stock solutions were mixed with the stream water after the stream water had been filtered, but prior to it entering the experimental streams. Stocks were dosed at a rate of 10 ml/min, which was measured weekly for each stream.

Table 5.1: Stock calculations, based on a stream flow rate of 55 l/min and a dosing stock flow rate of 10 ml/min

LAS added to stock (kg)	Volume of demineralized water in stock	Nominal stock concentration (g/l)	Nominal concentration in stream (mg/l)
0	110	0	0
0.61	110	4.4	0.8
1.90	110	13.8	2.5
6.05	110	44.0	8.0

Water samples (up to 50 ml, see Appendix 5.3) were taken from each stream according to the sampling regime shown in Table 5.2, preserved with 3 % formaldehyde and stored at less than 5°C until analysis. All LAS analysis was carried out by the SEAC Analytical Unit at Unilever using HPLC fluorescence as described in Appendix 5.1.

Table 5.2: Sampling regime for water samples taken for LAS analysis. All streams were sampled on each occasion and one sample was taken per sampling location.

Day	Sampling location	Total no. of samples
0	Top of riffle	8
3	Top and bottom of riffle	16
6	Top of riffle	8
10	Top of riffle	8
13	Top of riffle	8
17	Top and bottom of riffle	16
20	Top of riffle	8
24	Top of riffle	8
27	Top and bottom of riffle	16

5.2.4 Water quality analysis

Temperature, conductivity, dissolved oxygen and pH were measured at the head of each stream daily. Flow rates were measured from each stream outlet at least three times a week. Total hardness was determined for one stream three times a week from water samples taken immediately below the riffle section. Dissolved organic carbon and total organic carbon were determined weekly from water samples taken immediately below the riffle section of each stream. Suspended solid concentrations were measured for one stream once a week from water samples taken immediately below the riffle section. All methods used for all water quality analyses are the same as those referenced in Section 4.2.3.

5.2.5 Invertebrate sampling

Twenty-four sediment samplers were removed from each stream one day prior to dosing (Day –1), and 13 and 27 days after dosing and preserved in 50 – 70 % ethanol. Macroinvertebrates were separated from the sediment, identified and counted according to the methods in Section 4.2.4. During dosing the drift nets were cleared at least once a week and the collected material preserved with 50 to 70 % ethanol.

5.2.6 Water quality measurements and LAS data analysis

Water quality measurements taken from all streams were analysed using one-way ANOVA's to determine if significant differences existed between streams. Where water quality measurements were only taken from one stream, the mean and the standard error were calculated. The mean, maximum and minimum concentrations of LAS measured in each stream were determined and a one-way ANOVA calculated to determine if different nominal dosing concentrations were significantly different (Zar, 1996).

5.2.7 Macroinvertebrate communities – univariate statistics

Species identities, and their relative abundances in the source communities were determined from samples taken on each stocking day. Dominant and abundant taxa (i.e. > 5 % and > 1 % relative abundance respectively) were identified from species counts. The total number of individuals, species richness, and Shannon's index of diversity (H') were determined for each of the 24 sediment samplers removed from each experimental stream on each sampling day. Taking each community in turn, a two-way crossed ANOVA was used to determine if the total numbers of individuals (ln transformed), species richness, or diversity were significantly affected by LAS concentration, or over time, and whether there was any interaction between these terms (Zar, 1996). Where significant differences were identified, Dunnett's test was used to determine which treatments differed significantly from the control stream. The lowest concentration at which there was no significant difference from the control stream was identified as the NOEC value.

As each community x concentration combination was only present in one stream, the 24 sediment samplers removed from each stream on each sample day were treated as replicates. It is recognised that these are not replicates, but pseudoreplicates (Hurlbert, 1984). This was unavoidable in this instance due to the low number of stream mesocosms, however it should be noted that all effects attributed to treatment cannot be separated from between-stream variation. This assumption has been accepted in the following analyses based on the extremely low between stream variation in previous studies in these streams with communities sourced from the same sites (Section 4.3.3).

In order to compare the responses of two structurally distinct communities to a toxicant it was necessary to determine proportional changes in numbers of individuals, species, and diversity indices. This was achieved by determining the proportional change in each sediment sampler removed on Day 13 and 27 with the mean value for the same stream on Day –1 using the following equation;

$$P = 1 - \left(\frac{\bar{x}_{it0} - i_{t1}}{\bar{x}_{it0}} \right) \qquad \text{Eqn. 4.1}$$

where P is the proportional change in endpoint i since Day –1; $\bar{x}_{it0}$ is the mean endpoint value on Day –1; and i_{t1} is the endpoint measured in a sediment sampler. If there was no change, P would equal one, values less than one indicate that the endpoint has declined, and values greater than one indicate the endpoint has increased.

A three-way crossed ANOVA (community x treatment x sample day) was used to determine whether there were significant differences in the responses of the two communities at any treatment concentration or on any sample day, and whether the interaction between the three terms was significant. Where significant differences were found, Tukey's multiple comparison test was used to determine which streams were distinct, by determining pairwise comparisons for all terms. The only terms considered in this analysis were those that compared communities that were exposed to the same treatment on the same day. For example, significant differences between

the two communities on Day 13 at 2.5 mg LAS/l would be included, but differences between the River Wheeler community on Day 13 at 0.8 mg LAS/l and the River Tame community on Day 27 at 8.0 mg LAS/l would not be reported as the comparison was not relevant.

5.2.8 Macroinvertebrate communities – multivariate statistics

Macroinvertebrate species counts from the 24 sediment samplers taken from each stream on each sample date were pooled, and analysed using non-metric multidimensional scaling and cluster analysis (see Section 4.2.5). A one-way ANOSIM was carried out to determine whether the two communities remained distinct for the duration of the study.

The aim of this study was to determine the effect of a toxicant (LAS) on each community relative to the control stream, and separate this treatment effect from the residual effect of natural shifts in community composition. Principal Response Curves (PRC), are a modification of Redundancy Analysis developed by Van den Brink & Ter Braak (1998). PRC constrain the analysis to differences between treatments and the control on each sample day by using 'dummy' covariables (i.e. 'treatment x sample day') (Van den Brink & Ter Braak, 1997, 1998). The resulting ordination plots time intervals along the x-axis and constrains the control data to $y = 0$. The treatment data for each time interval are then plotted relative to the control. Treatments positioned close to the control data on a sampling day are more similar to the control than treatments positioned further away (Ter Braak & Smilauer, 1998). Species scores, generated at the same time as the PRC, indicate the extent to which each species may contribute to the PRC. Large species scores indicate large changes in species abundance relative to the control. Species with a score close to zero were either similar in abundance to the control stream, or the effect of the treatment on that species was not captured by the ordination (Van den Brink & Ter Braak, 1999).

In order to determine the effect of LAS on the structure of each macroinvertebrate community over time a PRC was generated in CANOCO (Ter Braak & Smilauer, 1998). This analysis used macroinvertebrate counts (ln transformed) from sediment samplers taken from streams stocked from either the River Wheeler or the River

Tame. As each species x treatment combination was only present in one stream it was not possible to determine the statistical significance of any observed effects, therefore a weight of evidence approach (incorporating univariate and multivariate analyses) was used.

5.3 Results

5.3.1 LAS analysis

There were significant differences in LAS concentrations between streams (ANOVA, $F = 497.78$, $p < 0.001$, $df = 7, 144$). Streams dosed at different nominal concentrations were significantly different, but streams dosed at the same nominal concentrations were not significantly different from each other (Table 5.3).

Table 5.3: Mean concentrations of LAS (mg/l) measured in water samples taken from the eight mesocosms.

Nominal LAS concentration (mg/l)	Source community	Mean LAS concentration (mg/l) (± 1 standard error)*
0.0	Wheeler	0.06 ± 0.04^{a}
0.0	Tame	0.02 ± 0.00^{a}
0.8	Wheeler	0.64 ± 0.02^{b}
0.8	Tame	0.57 ± 0.02^{b}
2.5	Wheeler	1.79 ± 0.04^{c}
2.5	Tame	1.85 ± 0.06^{c}
8.0	Wheeler	5.17 ± 0.20^{d}
8.0	Tame	5.77 ± 0.22^{d}

* Numbers with the same superscript letter are not significantly different (Tukey's multiple comparison test, $p<0.05$).

5.3.2 Water quality

Mean values for water quality measurements are presented in Table 5.4. Total hardness and suspended solids were only measured in one stream, therefore no statistical comparisons were made between streams. With the exception of flow rate (ANOVA, $F = 12.27$, $p < 0.001$, $df = 7, 120$) there were no significant between-stream differences in water quality variables (ANOVAs: $F = < 1.79$, $p > 0.05$, $df = 7, > 39$). The mean flow rate in the stream stocked with the River

Wheeler community and dosed at 8.0 mg/l was significantly faster (63.1 l/min) than in any other stream (57.5 - 59.7 l/min) (Tukey's multiple comparison test, $p<0.05$).

Table 5.4: Mean (± 1 SE) water quality measurements taken for all streams on all sampling occasions.

Water quality measurement	Mean
pH	8.3 ± 0.04
Temperature (°C)	15.3 ± 0.06
Dissolved oxygen (mg/l)	10.7 ± 0.10
Conductivity (µS/min)	420 ± 6.8
Stream flow (l/min)	58.7 ± 0.2
Total hardness (mg/l $CaCO_3$)	192 ± 8.1
Total organic carbon (mg/l)	14.9 ± 0.2
Dissolved organic carbon (mg/l)	13.7 ± 0.2
Suspended solids (mg/l)	15.8 ± 3.3

5.3.3 Source communities

The benthic sample taken from the River Wheeler contained 37 macroinvertebrate taxa, with a total of 5,523 individuals (Appendix 5.2). Nine taxa were present at abundances greater than 1 % of the total number of individuals, and the community was dominated (greater than 5 %) by four taxa (Table 5.5). The sample from the River Tame contained 26 taxa, with a total of 7,155 individuals. Six taxa were present at abundances of greater than 1 % of the total number of individuals, and the community was dominated (greater than 5 %) by three taxa (Table 5.5). The oligochaete worms had fragmented, preventing identification to a higher resolution and potentially resulting in the number of individuals being over-estimated.

Table 5.5: Dominant (greater than 5 % relative abundance) and abundant (greater than 1 % relative abundance) taxa in macroinvertebrate samples collected from the River Wheeler and the River Tame during stocking.

Taxa	River Wheeler	River Tame
Rhithrogena semicolorata	37 %	
Oligochaeta	26 %	63 %
Baetis rhodani	8 %	17 %
Gammarus pulex	8 %	
Limnius volckmari	4.6 %	
Hydropsyche instabilis	4.0 %	
Chironomidae	3.3 %	11.3 %
Elmis aenea	1.5 %	
Isoperla grammatica	1.5 %	
Erpobdella octoculata		2.8 %
Asellus aquaticus		1.6 %
Ceratopogonidae		1.3 %

5.3.4 Communities in experimental streams prior to dosing

Prior to dosing (day –1) twenty-four sediment samplers were removed from each stream, in effect sampling 9 % of the total volume of each channel. In streams stocked from the River Wheeler, 220 to 322 % of the total number of Chironomidae added to the stream during stocking were found in these sediment samples (Appendix 5.3). In the streams stocked from the River Tame, 50 to 60 % of the total number of Chironomidae added during stocking were found in these sediment samples (Appendix 5.4). During the study, Chironomidae larvae were observed passing through the filtration system and into the artificial streams (S. Marshall, 1999, pers. com.). Chironomidae samples from all sample days and sites were identified to tribe in an attempt to distinguish between Chironomidae from the source communities and those colonising the experimental streams through the water supply (Figure 5.1, Appendix 5.5).

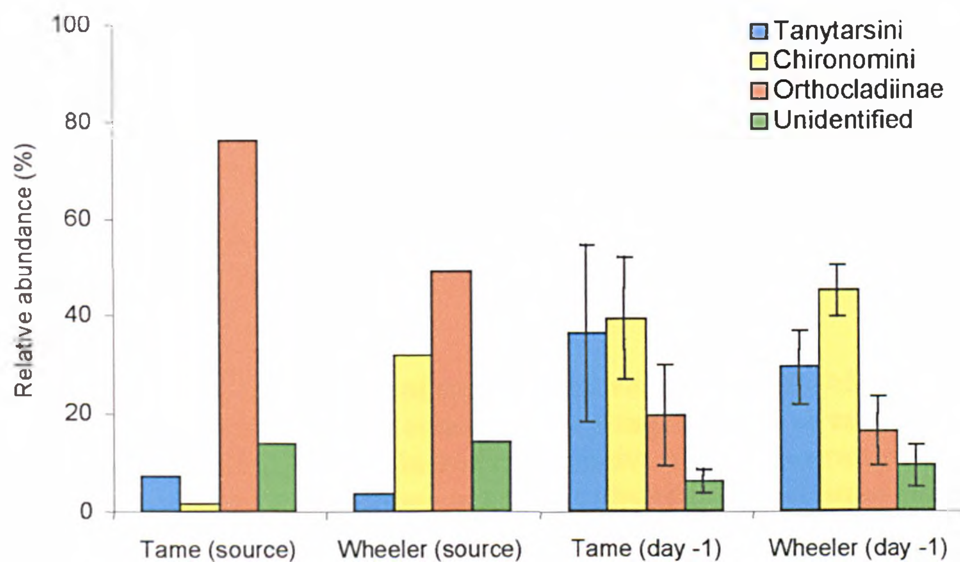


Figure 5.1: Relative abundance of Chironomidae in the source streams, and the artificial streams prior to dosing (error bars ±1 SE).

The Chironomidae tribes found in the two source communities were different, with the River Tame Chironomidae community dominated by Orthocladiinae, and the River Wheeler Chironomidae community composed predominantly of Orthocladiinae and Chironomini. On day –1, all sediment samples removed from the artificial streams were dominated by Tanytarsini and Chironomini. The large increases in numbers of individuals, and the change in the Chironomidae community structure after stocking both indicate additional Chironomidae have entered the artificial streams during the stocking phase. The streams are operated on a flow through basis, therefore if Chironomidae were entering the system through the water supply, new individuals would be introduced throughout the study. As there is no way to determine how long individuals were exposed to the LAS, the Chironomidae abundances may not reflect the effect of LAS exposure, therefore all Chironomidae data were excluded from subsequent analysis.

The mean relative abundance of species across all stream mesocosms stocked from the same source community prior to dosing were determined (Table 5.6). In streams stocked from the River Wheeler, fourteen taxa were present at relative abundances greater than 1 % and the stream communities were dominated (greater than 5%) by *Limnius volckmari* (24.8 %), Oligochaeta (15.1 %), *Elmis aenea* (13 %),

Hydropsyche siltalai (12.6 %), *Rhithrogena semicolorata* (12.4 %) and *Gammarus pulex* (7.8 %). In streams stocked from the River Tame, ten taxa were present at abundances greater than 1 % and the communities were dominated by *Erpobdella octoculata* (31.9%), *Asellus aquaticus* (30 %), Oligochaeta (11.6 %) and Simuliidae (7.1 %).

Table 5.6: Relative abundance of species that were dominant (>5 %) or abundant (>1 %) in the source communities, sampled from stream mesocosms prior to dosing with LAS. (↑ = increase in relative abundance compared to stocking community, ↓ = decrease in relative abundance compared to stocking community).

Taxa	River Wheeler		River Tame	
Limnius volckmari	24.8 %	(↑)		
Oligochaeta	15.1 %	(↓)	11.6 %	(↓)
Elmis aenea	13.0 %	(↑)		
Hydropsyche instabilis	12.6 %	(↑)		
Rhithrogena semicolorata	12.4 %	(↓)		
Gammarus pulex	7.8 %	(↓)		
Potamopyrgus jenkinsi	2.5 %	(↑)		
Ephemerella ignita	2.0 %	(↑)		
Simuliidae	1.5 %	(↑)	7.1 %	(↑)
Empididae	1.2 %	(↑)	4.3 %	(↑)
Sericostoma personatum	1.1 %	(↑)		
Baetis rhodani	(0.3 %)	(↓)	2.1 %	(↓)
Isoperla grammatica	(0.3 %)	(↓)		
Erpobdella octoculata			31.9 %	(↑)
Asellus aquaticus			30.0 %	(↑)
Hydropsyche siltalai			2.9 %	(↑)
Ceratopogonidae			2.2 %	(↑)
Glossiphonia complanata			1.5 %	(↑)
Amphinemura sulcicollis			1.5 %	(↑)

5.3.5 Response to LAS of the River Wheeler community

5.3.5a Multivariate analysis

In order to determine how different concentrations of LAS affected the community structure over time, and which species were driving those changes, a principal response curve (PRC) was generated for macroinvertebrate species counts (Figure 5.2). At day –1 the data from all four stream channels are relatively close together,

but do not all lie on the same point. This is due to a small variation in the relative abundances of species in the streams after the stocking phase was completed. At Days 13 and 27, the position of the streams dosed with 0.8 mg/l and 2.5 mg LAS/l relative to the control were similar to that observed at day –1. Therefore, if these concentrations had any effect on the community structure, it was not sufficient to produce a level of change detectable in this analysis.

At Day 13 the position of the stream dosed with 8.0 mg LAS/l relative to the control was different to that observed at day –1. There was a clear effect of LAS dosing on this stream resulting in the community structure differing from that in the control stream. This difference persisted in samples taken on Day 27. Of the total amount of variance in the data set used to generate the PRC, 5 % is explained by the horizontal axis and can therefore be attributed to time, and 12.3 % is due to the treatment effect (Van den Brink & Ter Braak, 1999). The two axes account for 17.3 % of the total variance which appears very low, but this is in part due to the analysis being constrained by the explanatory variables (i.e. treatment and time). However, the PRC is based on Redundancy Analysis, therefore the axis attempt to fit the data such that the maximum amount of variability due to the treatment is explained. Fifty-six percent of the variance attributed to treatment effects in this analysis is captured by the vertical axis of the PRC. Section 4.3.3 demonstrated that the communities changed significantly through time, with most of this change occurring within the first 28 days. Species driving changes in community structure that are unrelated to LAS concentrations would not be captured in this analysis.

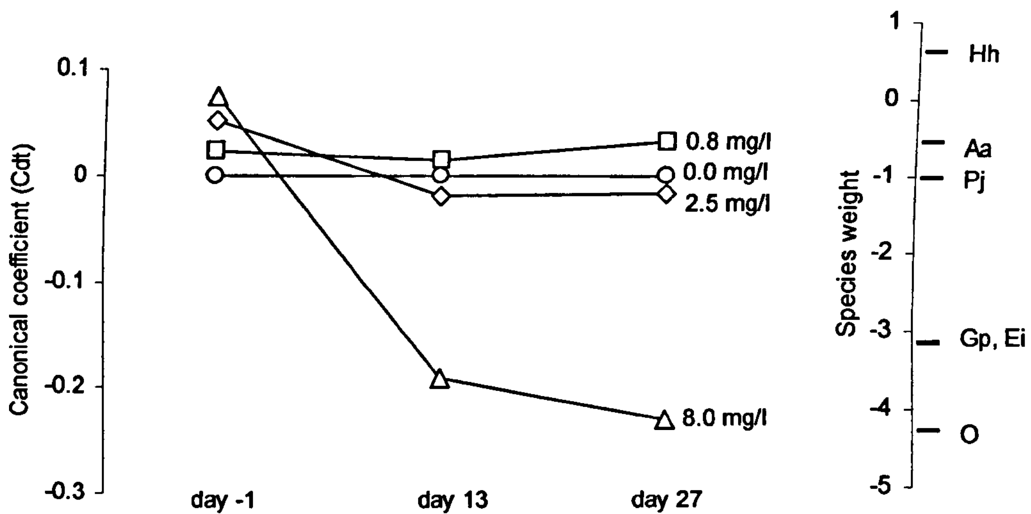


Figure 5.2: Principal Response Curve with species scores (> |0.5|) for community data from streams stocked from the River Wheeler and treated with LAS (Hh *Hydropsyche instabilis*; Aa *Asellus aquaticus*; Pj *Potamopyrgus jenkinsi*; Gp *Gammarus pulex*; Ei *Ephemerella ignita*; O Oligochaeta).

Six taxa had species scores greater than ±0.5 and were therefore important in driving the changes in community structure detected by the PRC (Figure 5.2). The importance of taxa in driving changes in community structure was not a function of their relative abundance as only three of the six dominant taxa had species scores greater than |0.5|. Oligochaeta, which had the greatest influence, comprised 15.1 % of the community on day –1 (Table 5.6). They increased in abundance up to Day 13 at 0.0 mg LAS/l, and up to Day 27 at 0.8 mg LAS/l. At 2.5 mg/l, Oligochaeta decreased in abundance to Day 13, then increased in abundance to Day 27, and at 8.0 mg/l Oligochaeta decreased in abundance by Day 13 and did not increase in abundance by Day 27 (Figure 5.3a). Although Oligochaeta were more abundant in the dosed streams than the control stream at day –1, by Day 13 Oligochaeta abundances at 8.0 mg LAS/l were lower than in the control stream (Figure 5.3b).

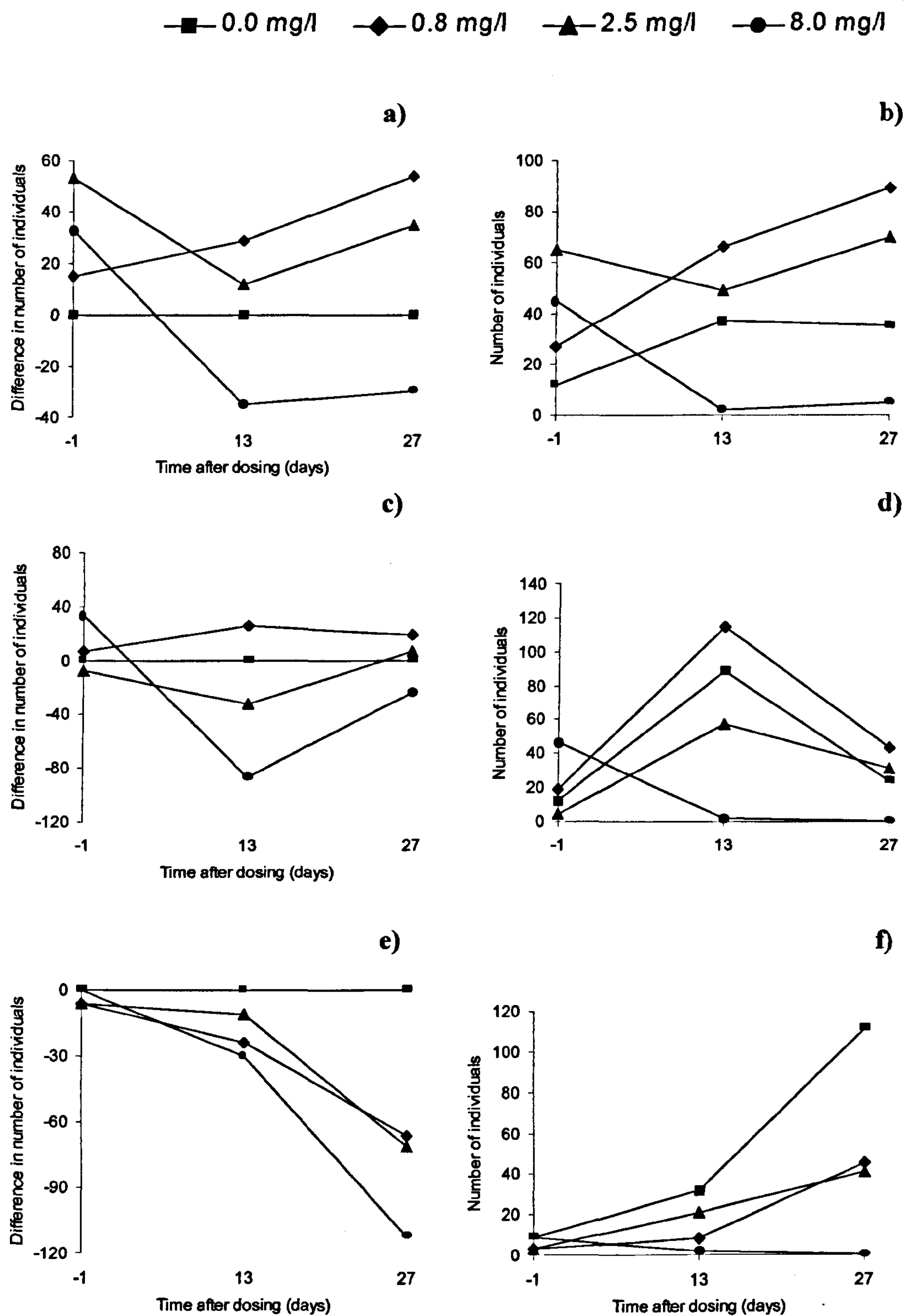


Figure 5.3: Absolute abundance (a, c, e) and abundance relative to the control stream (b, d, f) of Oligochaeta (a, b), *Gammarus pulex* (c, d) and *Ephemerella ignita* (e, f) in the four streams stocked with the River Wheeler community.

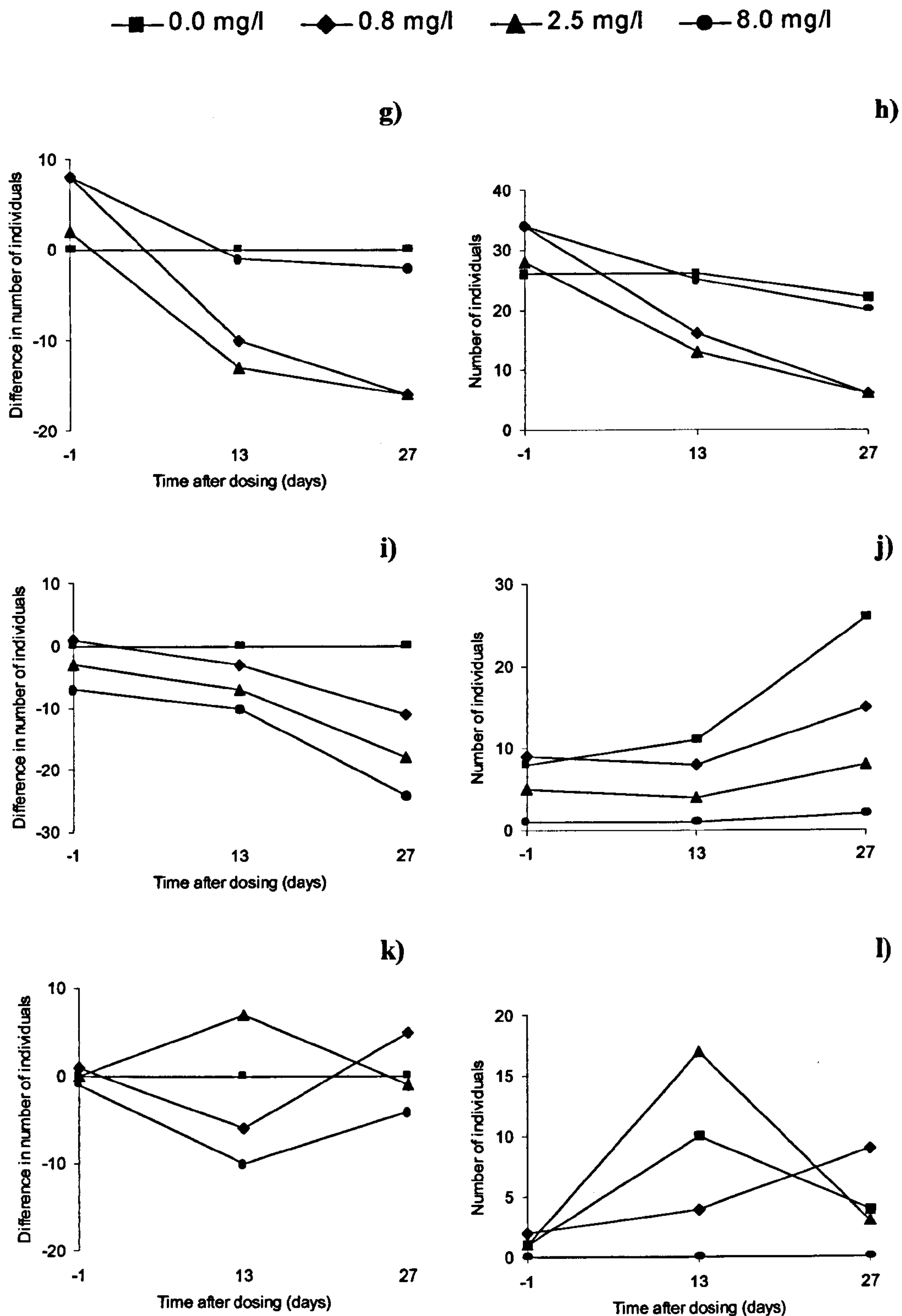


Figure 5.3 continued: Absolute abundance (g, i, k) and abundance relative to the control stream (i, j, l) of *Hydropsyche instabilis* (g, h), *Potamopyrgus jenkinsi* (i, j) and *Asellus aquaticus* (k, l) in the four streams stocked with the River Wheeler community.

Gammarus pulex comprised 7.8 % of the community on day –1 and showed a concentration response with an increase in abundance in all streams except 8.0 mg LAS/l up to Day 13, decreasing again by Day 27. At 8.0 mg LAS/l the number of *G. pulex* decreased on Day 13 and none were present on Day 27 (Figure 5.3c). By the end of the exposure period, *G. pulex* abundance was only less than control in the 8.0 mg/l treatment (Figure 5.3d). On Day 13, *G. pulex* abundances were much higher than on day –1. Examination of the data showed the increase in densities on Day 13 was caused by a sampling effect. On Day 13 the two samplers at the head of the stream were removed, and these two samplers (out of 24 samplers removed) contained 68 - 77 % of all *G pulex* collected on Day 13 (Table 5.7).

Table 5.7: Abundance of *G. pulex* and *A. aquaticus* in samplers removed on Day 13 (Samplers 1 and 2 were the most upstream samplers in each stream).

Nominal concentration (mg LAS/l)	Number of *G. pulex*		Number of *A aquaticus*	
	Samplers 1 and 2	All samplers (n = 24)	Samplers 1 and 2	All samplers (n = 24)
0.0	61	89	9	10
0.8	89	115	1	4
2.5	44	57	1	17
8.0	1	2	0	0

Asellus aquaticus comprised < 1 % of the community on Day –1, and increased in abundance to Day 13 in all streams except 8.0 mg LAS/l, where the increase in abundance continued to Day 27 (Figure 5.3k, l). Examination of the distribution of *A. aquaticus* between samplers shows the same pattern as observed in *G. pulex*, with individuals congregating at the head of the stream (Table 5.7). *Ephemerella ignita* comprised 2 % of the community on Day –1 and increased in all streams except 8.0 mg LAS/l to Day 27 with a clear concentration response (Figure 5.3e, f). *Hydropsyche instabilis* comprised 12.6 % of the community on Day –1 and decreased in abundance in all dosed streams from Day –1, and in the control stream from Day 13 (Figure 5.3g, h). *Potamopyrgus jenkinsi* abundances at Day –1 were variable (Figure 5.3i). There appeared to be a clear dose-response to LAS (Figure 5.3j) although this may be a function of the variable stocking densities (Appendix 5.3).

5.3.5b Univariate analysis

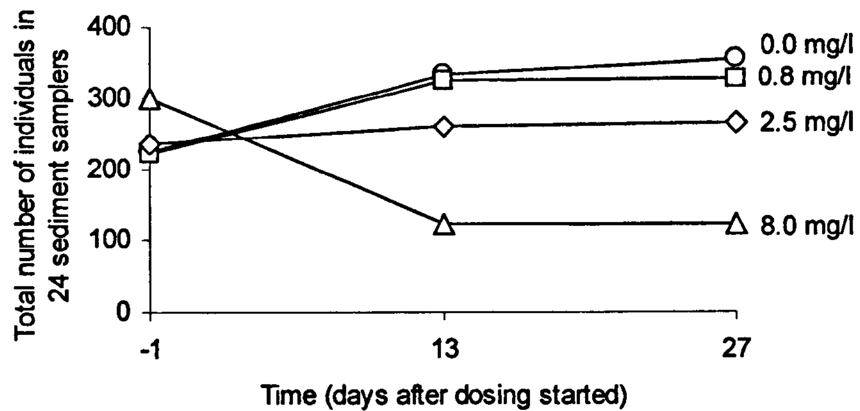
In streams stocked from the River Wheeler, a total of forty-one taxa were recorded across all sample dates and treatments. On any single sample day between thirteen and twenty-three taxa were recorded in any one stream (mean = 19) (Figure 5.4a).

The number of individuals in streams stocked from the River Wheeler increased over time (ANOVA: $F = 14.66$, $p < 0.001$, $df = 3, 275$), with significantly fewer individuals in streams dosed at 8.0 mg/l than in the control stream (Dunnett's test, $p<0.05$). There was no significant change between sample days grouped across streams (ANOVA: $F = 1.06$, $p>0.05$, $df = 2, 275$), but the interaction between LAS concentration and sample date was significant (ANOVA: $F = 14.66$, $p < 0.001$, $df = 3, 275$), with fewer individuals in streams dosed at 8.0 mg/l than in control streams on sample Days 13 and 27. The no observed effect concentration (NOEC) for change in number of individuals due to LAS exposure was 2.5 mg LAS/l (Figure 5.4a).

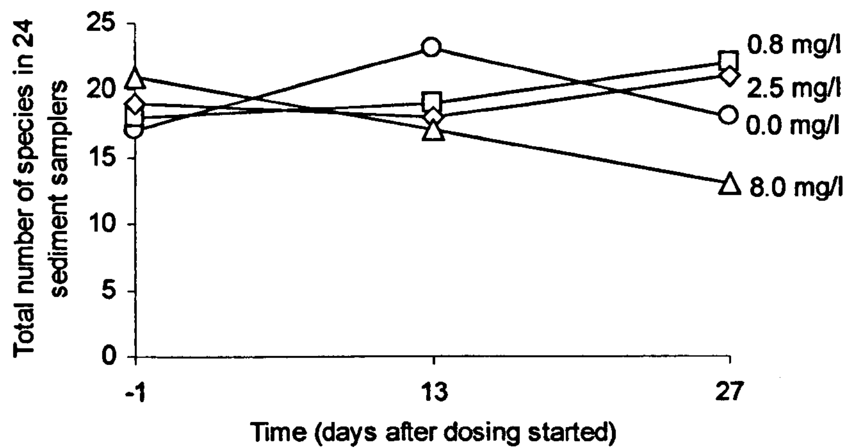
The number of species per sampler in streams stocked from the River Wheeler was also found to differ significantly with concentration (ANOVA: $F = 15.79$, $p < 0.001$, $df = 3, 275$), with streams dosed at 8.0 mg/l having significantly fewer species per sampler than the control streams (Dunnett's test, $p < 0.05$). Sample day was not significant (ANOVA: $F = 0.10$, $p > 0.05$, $df = 2, 275$), but the interaction between sample date and LAS concentration was significant (ANOVA: $F = 4.96$, $p < 0.001$, $df = 6, 275$), with significantly fewer species per sampler in streams dosed at 2.5 and 8.0 mg/l on Day 13, and 8.0 mg/l on Day 27 than in the control stream (Dunnett's, $p < 0.05$). The NOEC for change in species richness due to LAS exposure was 0.8 mg LAS/l (Figure 5.4b).

Community diversity (H') was significantly affected by LAS concentration (ANOVA: $F = 10.7$, $p < 0.001$, $df = 3, 191$) with macroinvertebrate diversity in the stream dosed at 8.0 mg/l being significantly lower than in the control stream (Dunnett's test, $p < 0.05$). Diversity was not significantly affected by sample day (ANOVA: $F = 0.38$, $p > 0.05$, $df = 2, 191$) or the interaction of sample day and LAS concentration (ANOVA: $F = 1.55$, $p > 0.05$, $df = 6, 191$). The NOEC for change in diversity due to LAS exposure was 2.5 mg LAS/l (Figure 5.4c).

a)



b)



c)

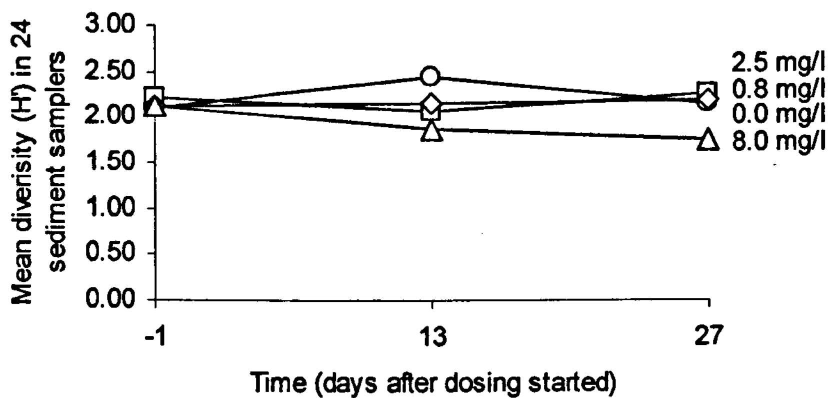


Figure 5.4: Total number of a) individuals, b) species, and c) mean diversity in 24 sediment samplers taken from streams stocked from the River Wheeler and treated with LAS (excluding Chironomidae data).

5.3.6 Response to LAS of the River Tame community

5.3.6a Multivariate analysis

A principal response curve (PRC) was generated for macroinvertebrate species counts for the River Tame community (Figure 5.5). At Day –1 the four streams stocked from the River Tame were more distinct from each other than was observed streams stocked from the River Wheeler (Figure 5.2). At Day 13 the position of streams dosed with 0.8 and 2.5 mg LAS/l relative to the control are similar to that observed on Day –1, indicating that if these concentrations affected community structure it was not sufficient to be observed in this analysis.

At Day 13 the position of the stream dosed with 8.0 mg LAS/l relative to the control was different to that observed on Day –1, producing a clear concentration effect that persisted to Day 27. At Day 27 the position of the streams dosed at 0.8 and 2.5 mg LAS/l were different to those observed on Day –1 or Day 13 (Figure 5.5), indicating a clear effect of LAS exposure. Of the total amount of variance in the data set used to generate the PRC, 15.3 % is explained by the horizontal axis and can therefore be attributed to time, and 15.5 % is due to the treatment effect (of which 79 % is captured by the ordination).

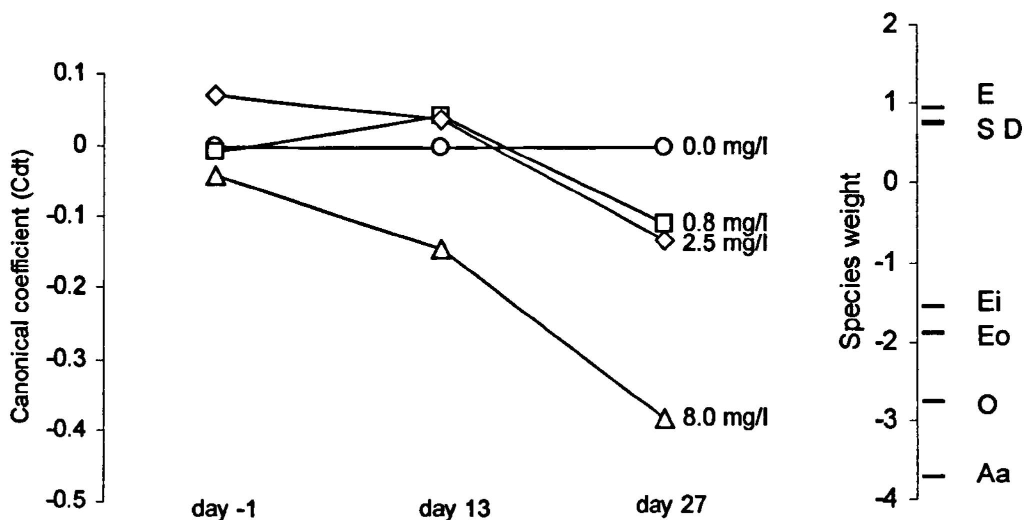


Figure 5.5: Principal Response Curve with species scores (> |0.5|) for community data from streams stocked from the River Tame and treated with LAS (E Empididae; S Simuliidae; D Diptera; Ei *Ephemerella ignita*; Eo *Erpobdella octoculata*; O Oligochaeta; Aa *Asellus aquaticus*).

Seven taxa had species scores greater than |0.5| and were therefore important in driving changes in community structure detected by the PRC (Figure 5.5). Five of these species were classified as dominant taxa prior to dosing (Table 5.6). *Asellus aquaticus*, which had the greatest influence, comprised 30 % of the community on Day –1 (Table 5.6), and increased in abundance up to Day 27 (Figure 5.6g). A clear concentration response was evident . By Day 27 the number of *Asellus aquaticus* in the stream dosed at 8.0 mg/l treatment was approximately 25 % that observed in the control stream (Figure 5.6h).

Erpobdella octoculata comprised 32 % of the community on Day –1 and was therefore the most abundant taxa overall (Figure 5.6c). A clear concentration response was evident; control abundances increased, abundances in streams dosed at 0.8 and 2.5 mg LAS/l decreased slightly, and abundances at 8.0 mg LAS/l decreased by over 30 % relative to control by Day 27 (Figure 5.6d). Oligochaeta comprised 12 % of the community at Day –1 and showed a concentration response with densities increasing at 0.0 and 0.8 mg LAS/l, and decreasing at 2.5 or 8.0 mg LAS/l (Figure 5.6e, f).

Ephemerella ignita were not observed in any streams stocked from the River Tame on Day –1 (Appendix 5.4), but on Day 13 they were recorded in low abundances in all streams except 8.0 mg LAS/l (Figure 5.6a). On Day 27 low numbers were present at 8.0 mg LAS/l, and abundances in all other streams had increased (Figure 5.6b). Empididae comprised <1 % of the community on Day –1, but showed a clear concentration response, increasing in abundance in all streams, with the rate of increase affected by the concentration of LAS (Figure 5.6i,j). Simuliidae abundances were very variable, both between streams and over time, with zero counts on all sample days in the stream dosed at 2.5 mg LAS/l. At Day 27 abundances in the control stream were higher than in any other stream (Figure 5.6k,l). Diptera were present in very low abundances in all streams (n = 0 or 1) except for the stream dosed at 8.0 mg/l on Day 27 (n = 13, Appendix 5.4). Changes in Diptera abundances were therefore not examined further.

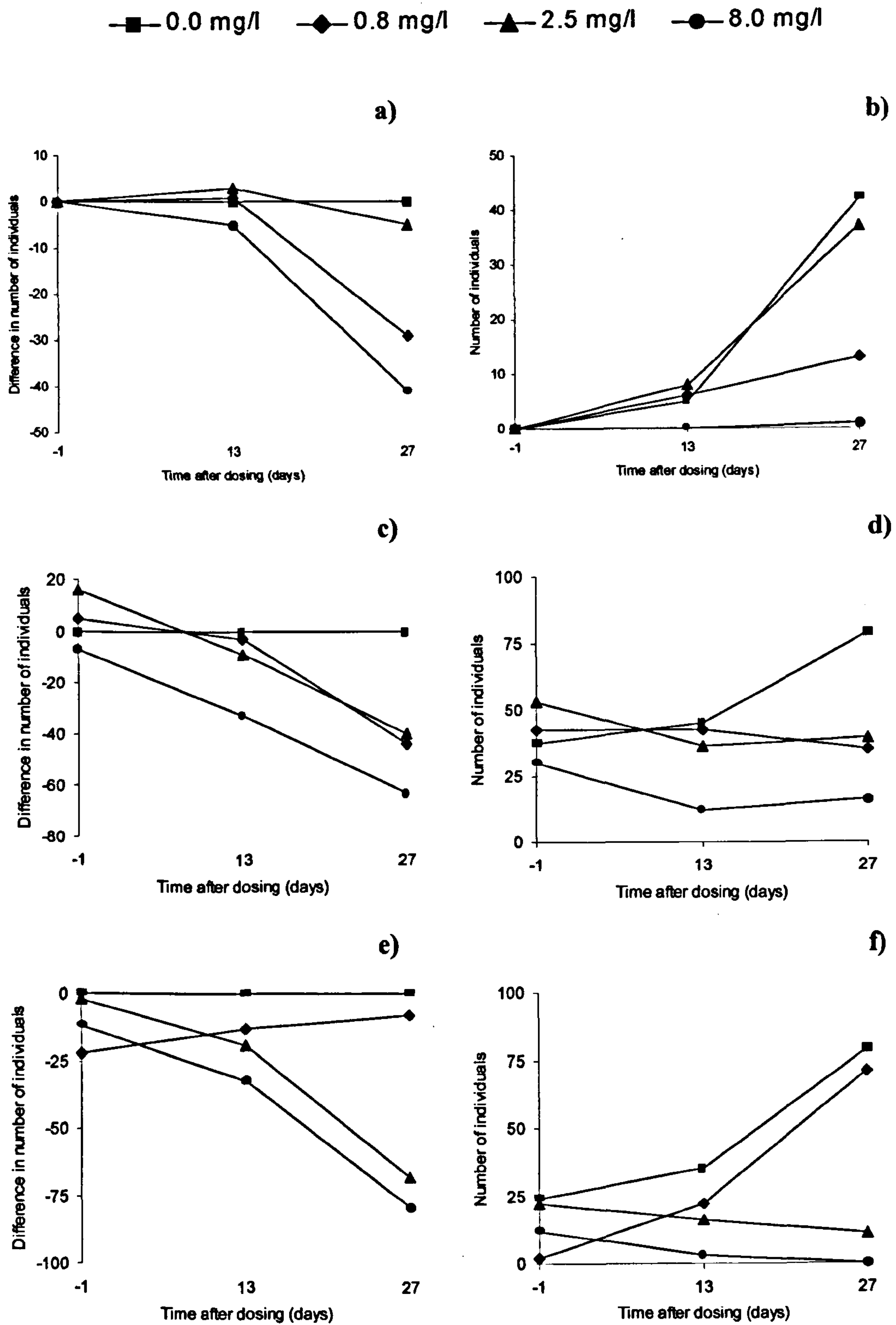


Figure 5.6: Absolute abundance (a, c, e) and abundance relative to the control stream (b, d, f) of *Ephemerella ignita* (a, b), *Erpobdella octoculata* (c, d) and Oligochaeta (e, f) in the four streams stocked with the River Tame community. (Unidentified Diptera data are not shown).

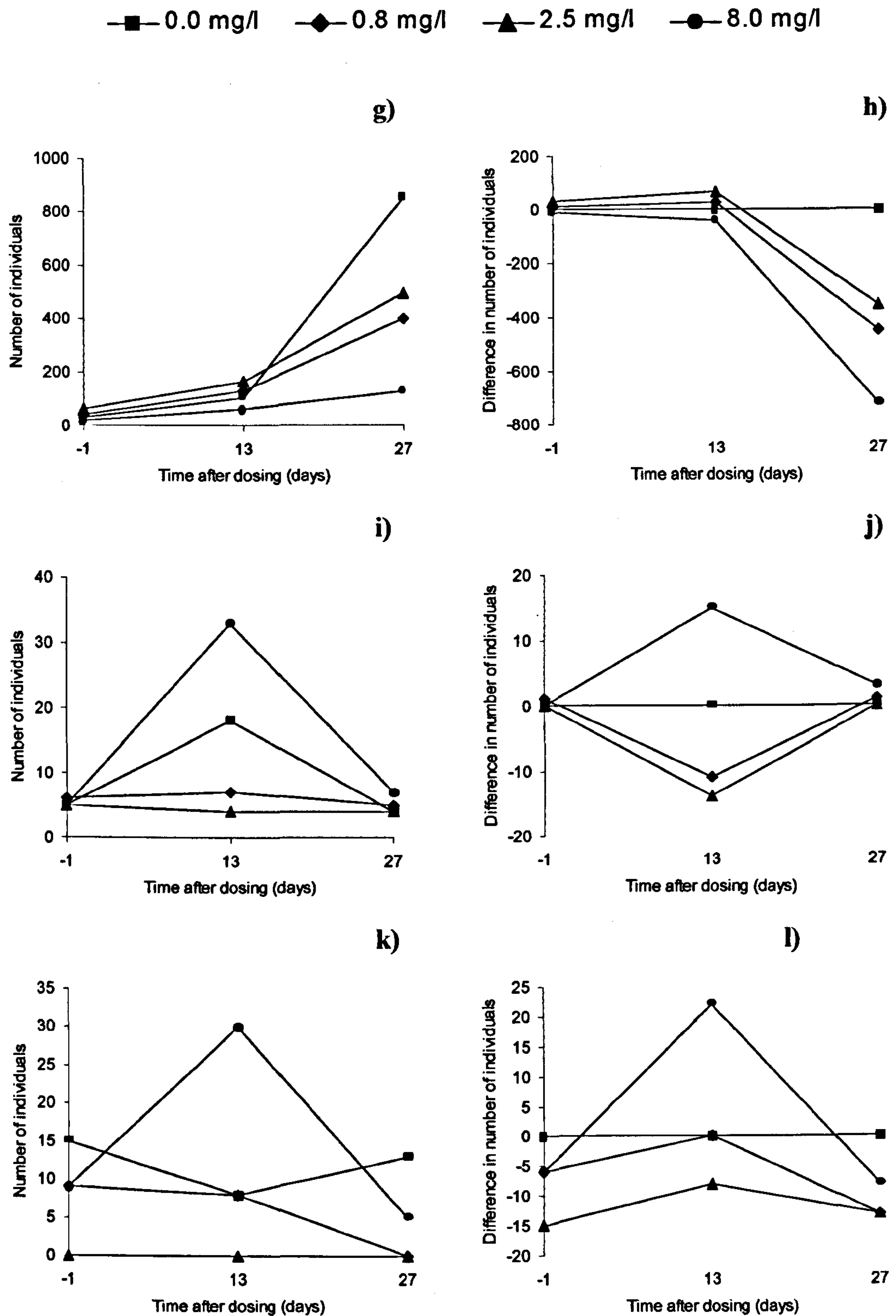


Figure 5.6 continued: Absolute abundance (g, i, k) and abundance relative to the control stream (h, j, l) of *Asellus aquaticus* (g, h), Empididae (i, j) and Simuliidae (k, l) in the four streams stocked with the River Tame community. (Unidentified Diptera data are not shown).

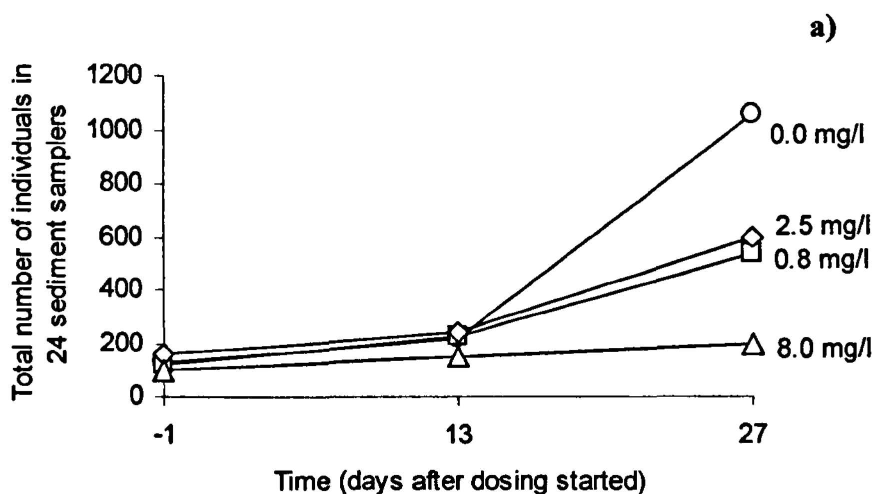
5.3.6b Univariate analysis

In streams stocked from the River Tame, a total of thirty taxa were recorded across all sample dates and treatments. On any single sample day between eleven and fourteen taxa were recorded in any one stream (mean = 12) (Figure 5.7a). The number of individuals (ln(x+1)) in streams stocked from the River Tame decreased with increasing LAS concentrations, and increased over time (Figure 5.7a). In a two-way crossed ANOVA, significant differences were identified due to LAS concentration (ANOVA: $F = 12.14$, $p < 0.001$, $df = 3, 275$), with streams dosed at 8.0 mg/l having significantly lower numbers of individuals than the control stream (Dunnett's test, $p < 0.05$). Comparisons between sample dates also detected significant differences (ANOVA: $F = 49.52$, $p < 0.001$, $df = 2, 275$), with streams sampled on Day 13 and Day 27 both having significantly higher numbers of individuals than streams sampled on Day –1. The interaction between LAS concentration and sample day was significant (ANOVA: $F = 3.52$, $p < 0.001$, $df = 6, 275$). Dunnett's test ($p < 0.05$) showed the stream dosed at 8.0 mg LAS/l to have significantly lower numbers of individuals than the control stream on Day 27 ($p < 0.001$). The no observed effect concentration (NOEC) for change in number of individuals due to LAS exposure was 2.5 mg LAS/l (Figure 5.7a).

The number of species per sampler in streams stocked from the River Tame decreased with increasing LAS concentrations, and increased from Day –1 to Day 13, but then decreased to Day 27 (Figure 5.7b). In a two-way crossed ANOVA significant differences were identified due to LAS concentration (ANOVA: $F = 3.12$, $p < 0.05$, $df = 3, 275$), with significantly fewer species per sampler in the stream dosed at 8.0 mg/l than in the control stream (Dunnett's test, $p < 005$). The effect of sampling day was also found to be significant (ANOVA: $F = 10.59$, $p < 0.001$, $df = 2, 275$), with significantly more species in sediment samplers removed on Day 27 than on Day -1 (Dunnett's test, $p < 0.05$). The interaction between LAS concentration and sample day was not found to be significant (ANOVA: $F = 1.73$, $p > 0.05$, $df = 6, 275$). The NOEC for change in species richness due to LAS exposure was 2.5 mg LAS/l (Figure 5.7b).

No significant changes in diversity were evident due to either LAS concentration or time were evident in streams stocked from the River Tame (Figure 5.7c). In a two-

way crossed ANOVA no significant differences were identified between streams due to LAS concentration (ANOVA: $F = 2.11$, $p > 0.05$, $df = 3, 203$), sample day (ANOVA: $F = 0.77$, $p > 0.05$, $df = 2, 203$) or due to the interaction between the two (ANOVA: $F = 1.63$, $p > 0.05$, $df = 6, 203$).



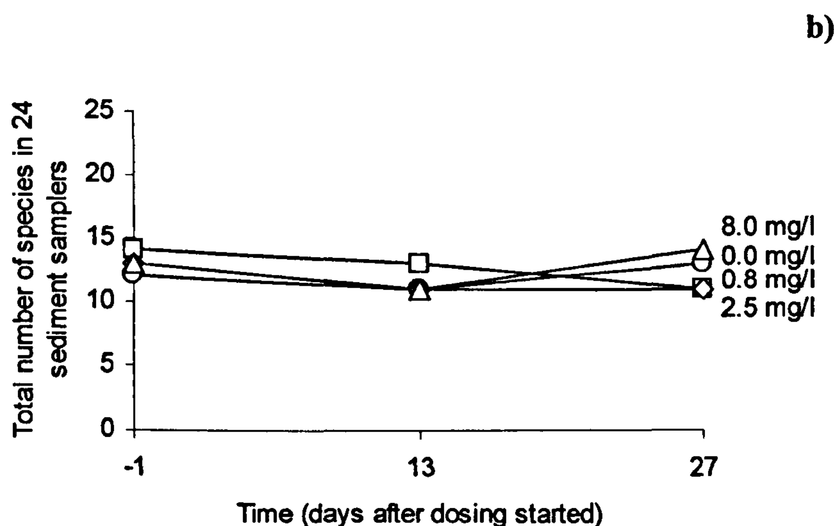


Figure 5.7 continued: Total number of a) individuals, and b) species in 24 sediment samplers taken from streams stocked from the River Tame and treated with LAS (excluding Chironomidae data).

c)

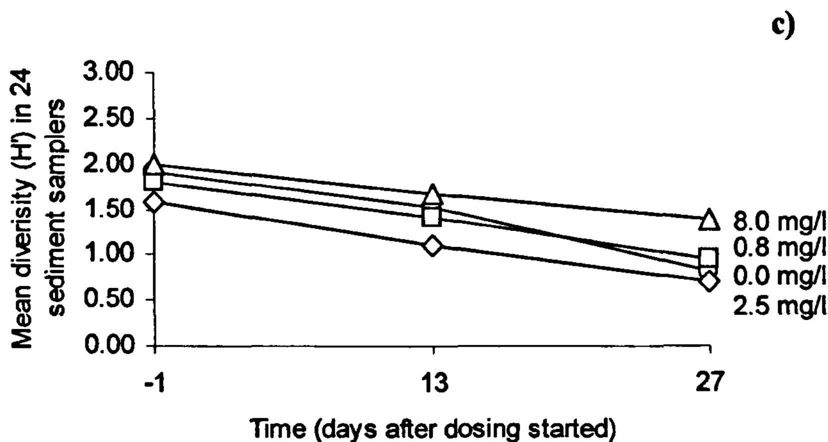


Figure 5.7 continued: Mean diversity in 24 sediment samplers taken from streams stocked from the River Tame and treated with LAS (excluding Chironomidae data).

5.3.7 Comparisons between macroinvertebrate communities

5.3.7a Multivariate analysis

In the ordination of the two communities in the experimental streams (Figure 5.8), each point represents the twenty-four sediment samplers taken from one stream on a single sampling date. Two clusters are formed on the left and right hand side of the plot (Figure 5.8 dashed lines). These clusters represent the two communities used for stocking the streams, and suggest that the two communities have remained distinct from one another for the duration of the study. A one-way ANOSIM was carried out which showed separation of the source communities to be highly significant ($R = 0.998$, 0.1 %). As the clusters are separated due to different values along the horizontal axis, the variance between the two communities is largely explained by this first axis. Within each cluster, the samples from the beginning of the study (Figure 5.8, blue) are at the bottom of the plot, and samples from the end of the study (Figure 5.8, yellow) are at the top of the plot. Changes in the communities through time is therefore largely explained by factors incorporated in the vertical axis. The stress of the ordination (0.07) indicates the plot provided a good interpretation of the data (Clarke & Warwick, 1994).

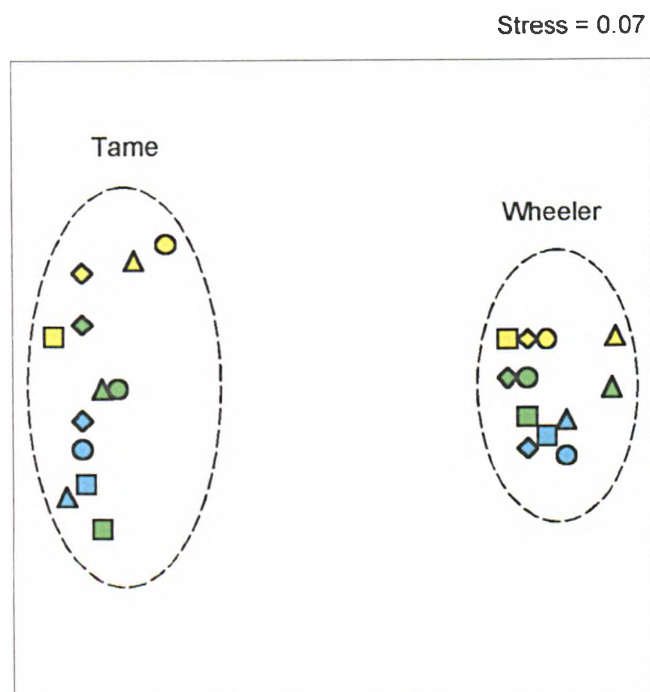


Figure 5.8: Ordination (non-metric MDS) of the total number of individuals sampled on days –1 (blue), 13 (green) and 27 (yellow) for streams stocked from the River Tame or the River Wheeler and dosed at 0.0 mg/l (○); 0.8 mg/l (□); 2.5 mg/l (◇); 8.0 mg/l (△).

5.3.7b Univariate analysis

A three-way ANOVA was carried out to determine whether LAS concentration, sampling day, or an interaction between the two resulted in significant differences in the responses of the two communities. The endpoints measured were numbers of individuals (ln (x+1)), species richness, or community diversity (H'). These analyses were performed using data corrected for mean values per stream on Day –1 (i.e. proportional change in endpoint relative to Day –1; Eqn. 4.1) to allow a direct comparison to be made between the communities.

The number of individuals in both communities increased to Day 27, except in streams dosed at 8.0 mg LAS/l, where there was an overall decrease in numbers of individuals. The rate of increase in the two communities was greater in the streams stocked from the River Tame community than in those stocked from the River Wheeler community. This difference was found to be significant on Day 27 (Table 5.8, Figures 5.4a, 5.7a).

Species richness in the River Wheeler streams increased over the duration of the study, whereas in the River Tame species richness decreased. There was a significant difference in the response of the two communities at all concentrations except 8.0 mg LAS/l, with the greatest relative change in species richness occurring in the River Tame community (Figures 5.4b, 5.7b). However, the analysis of variance for species richness refers to mean species richness between sediment samplers within a stream. Therefore, decreases in species richness may be a function of decreases in overall abundances.

Community diversity decreased in both communities at all concentrations for the duration of the study. There was a significant difference between the two communities, with diversity in streams stocked from the River Tame decreasing at a greater rate than streams stocked from the River Wheeler. There was also a significant effect due to LAS concentrations, with diversity in streams dosed at 2.5 – 8.0 mg LAS/l decreasing significantly more than the control streams. The decrease in diversity relative to the control greater on day 27 than day 13. The interaction between community and LAS concentration was found to be significant. In streams dosed at 0.0 – 2.5 mg LAS/l the decrease in diversity in the River Tame community was significantly greater than the decrease in the River Wheeler community.

The changes in number of individuals, species richness and diversity relative to Day –1 in the two communities were therefore found to be significantly different. The increase in number of individuals was greater in the River Tame stream than in the River Wheeler stream. Species richness decreased in the River Tame and increased in the River Wheeler, and these differences were significant at all concentration except 8.0 mg LAS/l. Diversity decreased in both communities, but the rate of decrease was greater in the River Tame community in streams dosed at 0.0 – 2.5 mg LAS/l.

Table 5.8: Results from a three-way crossed ANOVA comparing relative changes in numbers of individuals (ln(x+1), species richness, or diversity (H') between streams stocked from the River Wheeler or the River Tame due to LAS concentration, sample day, or an interaction of the two. Tukey's pairwise comparisons made for 'relevant' comparisons (See Section 5.2.6) (p<0.05).

	F	p	df	Direction of change	Significant differences (p<0.05)
Change in number of individuals relative to Day -1					
community	6.21	< 0.05	1, 368	both increase	Tame > Wheeler
concentration	26.85	< 0.001	3, 368	0.0-2.5 mg/l increase 8.0mg/l decrease	8.0 mg/l > 0.0 to 25 mg/l
day	27.54	< 0.001	1, 368	d13 decrease Day 27 increase	Day 27 > Day 13
community x concentration	0.43	> 0.05	3, 368		
community x day	15.38	< 0.001	1, 368	decrease Day 13 increase Day 27	Tame > Wheeler, Day 27
concentration x day	2.93	< 0.05	3, 368		
community x concentration x day	1.50	> 0.05	3, 368		
Change in number of species relative to Day –1					
community	57.0	< 0.001	1, 368	Wheeler increase Tame decrease	Tame > Wheeler
concentration	22.0	< 0.001	3, 368	0.0 mg/l increase 0.8-8.0 mg/l decrease	2.5-8.0 mg /l > 0.0 mg/l
day	1.56	> 0.05	1, 368		
community x concentration	11.18	< 0.001	3, 368	Wheeler increase Tame decrease	Tame > Wheeler, 0.0 - 2.5 mg/l
community x day	3.10	> 0.05	1, 368		
concentration x day	0.28	> 0.05	3, 368		
community x concentration x day	2.02	> 0.05	3, 368		
Change in diversity relative to Day –1					
community	98.1	< 0.001	1, 256	both decrease	Tame > Wheeler
concentration	5.54	< 0.001	3, 256	all decrease	2.5-8.0 mg/l > 0.0 mg/l
day	5.2	< 0.05	1, 256	both decrease	Day 27> Day 13
community x concentration	11.92	< 0.001	3, 256	Wheeler 0.0 - 0.8 mg/l increase all others decrease	Tame > Wheeler, 0.0 - 2.5 mg/l
community x day	0.05	> 0.05	1, 256		
concentration x day	0.20	> 0.05	3, 256		
community x concentration x day	1.41	> 0.05	3, 256		

5.4 Discussion

The aim of this experiment was to determine the effect of LAS on two distinct macroinvertebrate communities, and to address the hypothesis that the responses of these communities to LAS exposure was a function of the sensitivity of the composite species and effects on these species interactions.

At the end of the stocking phase the control stream for the River Wheeler community was more diverse than the River Tame community and was dominated by mayflies (*Ephemerella ignita*, 31.5 %), beetles (*Limnius volckmari, Elmis aenea*), Oligochaeta, snails (*Potamopyrgus jenkinsi*), amphipods (*Gammarus pulex*), and caddis larvae (*Hydropsyche instabilis*). In contrast, the control stream for the River Tame community was dominated by isopods (*Asellus aquaticus*, 80.5 %), Oligochaeta, and leeches (*Erpobdella octoculata*). The loss of large numbers of *Rhithrogena semicolorata* and *Isoperla grammatica* during colonisation meant that both communities included dominant taxa that were found to be tolerant to LAS in single-species tests (i.e. *Elmis aenea, Hydropsyche* sp., *Asellus aquaticus*).

5.4.1 Response of the River Wheeler community to LAS exposure

The number of individuals, species richness, and community diversity in streams stocked with the River Wheeler community all decreased with increasing concentrations of LAS, with some endpoints significantly affected at all concentrations. The most sensitive endpoint measured was species richness, with a NOEC of 0.8 mg LAS/l. However, it should be noted that the use of pseudoreplication in the univariate analyses was unavoidable due to each 'community x concentration' only occurring in a single stream (Hurlbert, 1984). As treatments ('community x concentration') were not replicated, the effect of LAS cannot be separated from the variability in community structure between streams. However, it has been demonstrated that replicate streams stocked with these communities remained structurally similar for at least ten weeks (Section 4.3.4).

The principal response curve (PRC) for the River Wheeler community identified *Hydropsyche instabilis, Ephemerella ignita, Gammarus pulex, Asellus aquaticus, Potamopyrgus jenkinsi* and Oligochaeta as species contributing to the observed shifts

in community structure (Figure 5.2) (Van den Brink & Ter Braak, 1999). Two of the taxa identified as having large species scores in the PRC were dominant species at the start of the study (i.e. *Gammarus pulex* and Oligochaeta), and a third was abundant (*Hydropsyche instabilis*). *Ephemerella ignita* was detected at low abundances prior to dosing, but increased throughout the duration of the experiment, thus explaining it's inclusion in the PRC. The patterns of response observed in *Potamopyrgus jenkinsi* and *Asellus aquaticus* are similar to those observed in *Ephemerella ignita* and *Gammarus pulex* respectively, which would account for their inclusion in the PRC as species with relatively large scores (Figure 5.3).

5.4.2 Response of the River Tame community to LAS exposure

Over the duration of the study, the number of individuals decreased with increasing LAS concentrations, and increased over time; species richness decreased with increasing LAS concentrations; and there was no significant effect of LAS on diversity (H') in any stream. The most sensitive endpoints were number of individuals and species richness, with NOECs of 2.5 mg LAS/l.

The PRC identified *Ephemerella ignita, Erpobdella octoculata,* Oligochaeta, *Asellus aquaticus,* Empididae, Simuliidae and 'unidentified Diptera' as the main contributors to the observed shifts in community structure. Four of these taxa were dominant taxa on Day –1 (i.e. *Ephemerella ignita, Erpobdella octoculata, Asellus aquaticus* and Oligochaeta) and increased in abundance in the control streams over the duration of the study. There was a marked decrease in the total number individuals at high concentrations, without any change in the total number of species. This suggests that while a clear effect was identified at the highest concentration, most species were still present in all streams at Day 27.

The PRC showed a response at all concentrations of LAS by Day 27, with the most extreme response at 8.0 mg LAS/l. Changes in the abundance of the dominant taxa appear to be driving the observed change in community structure. Empididae and Simuliidae responded differently to LAS exposure, increasing in abundance in the highest concentration on Day 13 and then decreasing back to pre-dosing abundances on Day 27. No explanation can be provided for these data, and there is no evidence to suggest that these taxa would preferentially colonise mesocosms dosed with very

high concentrations of LAS. As both taxa were only recorded in low abundances, these apparent changes in abundance may be due to a sampling affect.

5.4.3 Comparing the responses of two communities to LAS

The River Wheeler was dominated by more sensitive species on Day –1 (Appendix 2.4; i.e. *Ephemerella ignita,* Oligochaeta and *Gammarus pulex*), whereas the River Tame was dominated by more tolerant taxa on Day –1 (*Asellus aquaticus, Erpobdella octoculata*). It was predicted that the community dominated by more sensitive taxa would respond to LAS exposure at lower concentrations. The prediction was verified in this study, with the River Wheeler responding at lower concentrations of LAS than the River Tame. The lowest NOEC measured for the River Wheeler was 0.8 mg LAS/l. This was determined for the effect of LAS on the total number of individuals. The lowest NOEC for the River Tame was 2.5 mg LAS/l, determined for the total number of individuals and species richness. The NOEC for the effect of LAS on community diversity was also lower in the River Wheeler community (2.5 mg LAS/l) than the River Tame community (>8.0 mg LAS/l).

The NOEC values determined in this study were considerably higher than effect concentrations determined in previous studies. For example, Feijtel *et al.* (1999) determined a maximum permissible concentration (i.e. HC5) of 0.35 mg LAS/l, and Tattersfield *et al.* (1995) determined the lowest NOEC in a 28-day stream mesocosm to be 0.12 mg LAS/l. This difference in measured sensitivity may be due to the loss of a number of species that were sensitive to LAS exposure in single-species tests prior to dosing.

One of the key advantages of using mesocosms to determine the effect of a toxicant on a community is that data on both direct and indirect effects for a diverse range of species may be determined, and longer term endpoints may be determined for indigenous species that cannot be maintained for long periods in the laboratory (Boyle *et al.*, 1996; Guckert, 1996; Greve *et al.*, 1999). Also, as exposure occurs under more realistic conditions, a more realistic assessment of the effect of exposure on natural communities may result (Holt & Mitchell, 1994; Versteeg *et al.*, 1999).

As the results of this study demonstrate, the species composition of the community used in multi-species tests may significantly affect the resulting estimates of 'safe'

concentrations. It was possible to predict which of the tested communities would be more sensitive to LAS exposure from single-species data for indigenous species. These results have implications on the use of mesocosms in effects assessments, as consideration must be given to how representative the responses of the exposed community may be, relative to natural communities.

The ability of some stream mesocosms to represent the sensitivity of natural systems have been theoretically determined. Dyer & Belanger (1999) used an 'insect only community index of sensitivity'(ICS) to determine whether a naturally colonised outdoor mesocosm facility in Ohio might be able to represent the sensitivity of natural communities to toxicant exposure. Based on this index, the mesocosms were more sensitive than 70% of field communities in Ohio 75% of the time. This suggests that while the mesocosm community may be representative of natural communities, a significant proportion (i.e. 30%) of natural stream communities are more sensitive and may potentially be under-protected.

When designing mesocosm studies, consideration should be given to whether such complex systems are needed, or whether smaller, more controlled, laboratory microcosm studies can be used (Shaw & Kennedy, 1996). Alternatively, if the sensitivity of a range of indigenous species were known, SSD curves could be generated for each community and the resulting HC5 values compared (i.e. Section 3.3.3). Chapter 6 will address the final objective of this work by comparing the sensitivity of species and communities exposed to LAS in stream mesocosms to single-species data and HC5 values generated for these communities.

6 Comparison of species and community sensitivities determined from single-species and multispecies exposures.

6.1 Introduction

Short-term single-species toxicity tests provide information on the interspecific sensitivity of a few species to toxicants under controlled laboratory conditions (e.g. Slooff *et al.*, 1983). These tests provide a useful method for screening large numbers of compounds, and identifying those compounds that may be hazardous in natural systems at predicted environmental concentrations (ECETOC, 1993). However, a species' response to a toxicant under controlled laboratory conditions may not represent its response in natural environments, over potentially extended time periods, under variable environmental conditions and in the presence of other species (Gower *et al.*, 1994; Girling *et al.*, 2000). Experimental streams can be used to expose indigenous benthic macroinvertebrate communities to toxicants under more natural conditions and over longer periods of time than would be possible in laboratory studies (ECETOC, 1997). This method potentially allows both direct and indirect effects (e.g. predator prey interactions) to be determined (Fairchild *et al.*, 1992).

Previous comparisons of single-species and multispecies exposures indicate that single-species tests are either representative or over-protective of taxa responses in multispecies test systems such as mesocosms. ECETOC (1997) determined the ratio between single-species and multispecies exposures for 34 compounds by comparing between one and three chronic single-species NOEC values for standard test species with aquatic mesocosm NOECs. Overall, the single-species:multispecies ratios appear to conform to a lognormal distribution with a median value of 1.45. The range of ratios covered 3 orders of magnitude for pesticides, and two orders of magnitude for non-pesticide compounds. Pascoe *et al.* (2000) compared the most sensitive laboratory derived NOEC from tests with standard and non-standard species with the most sensitive field derived NOEC values for copper, lindane, atrazine and 3,4-dichloroaniline, and they found the laboratory NOEC to be

protective of single-species and community endpoints measured in the mesocosm studies. Giddings *et al.* (2001) reviewed single-species toxicity tests and mesocosm studies for pyrethroids in the UK and North America and reported that single-species LC50 tests were generally able to predict the relative sensitivities of species from the same family, class or phyla (e.g. mayflies, snails, fish) in mesocosm studies. However, these comparisons were made between the most sensitive endpoint available for single-species and multispecies exposures, and could therefore relate to comparisons for very broad taxonomic groups. The aim of this study is to compare the sensitivity of the same species or genus in single-species toxicity tests and stream mesocosms.

Previous comparisons between the responses of the same taxa in single-species toxicity tests and mesocosm studies have been restricted by the limited number of single-species data available for indigenous macroinvertebrate species, and the low abundances of many taxa recorded in mesocosm studies (Van den Brink *et al.*, 1996). For example, Emans *et al.* (1993) compared the sensitivity of 'similar or related species on a genus level' exposed in single-species and multispecies tests to 19 organic compounds and 10 metals. After removal of unreliable data, and data reported as greater than or less than values, a total of 17 comparisons were made (across all compounds), and a significant correlation was found. For twelve of the 17 comparisons of species sensitivity in single-species and multispecies tests differed by a factor of > 0.2 and < 5.0. Van Wijngaarden *et al.* (1996) found that laboratory 96-hour EC50 data were within a factor of two of species EC50 values generated from exposure to Dursban in mesocosms, but was only able to make these comparisons for four of the 120 species found in the mesocosms due to low abundances and high temporal and spatial variability between mesocosms. These results indicated that there was no significant difference in species sensitivity in single-species and multispecies exposures. If this is the case, then single-species tests may be used to determine the sensitivity of communities in mesocosms.

Indirect effects between species affect the overall sensitivity of a species in a number of ways. Woin (1998) examined the effect of fenvalerate exposure on an invertebrate pond community and found a >10-fold increase in Oligochaeta abundances in treated ponds. This increase was an indirect effect, resulting from the

decrease in predation by the more sensitive arthropod species, and a subsequent increase in food availability. This effect resulted in changes in community structure that could not have been determined from single-species data. Weis *et al.* (2000) determined that *Fundulus heteroclitus* exposed to natural sediment contaminated with a range of pollutants including mercury displayed a reduced ability to capture live prey (*Palaemonetes pugio*). *Fundulus heteroclitus* collected from the source of the contaminated sediment were found to be smaller than individuals from clean sites, with a much lower proportion of their gut contents consisting of live food. The prey species, *Palaemonetes pugio*, were less affected by the contaminated sediment, and were significantly larger than individuals from clean sites due to the reduced level of predation.

Roast *et al.* (2000) tested the effect of sublethal concentrations of chlorpyrifos on the swimming behaviour of *Neomysis integer* and found an increase in swimming activity and movement away from the substrate into the water column in treated flumes relative to the control flumes. They hypothesised that these responses to chlorpyrifos exposure would result in increased predation due to the increased visibility of *Neomysis integer*.

The similarity in species sensitivity in single-species and multispecies tests suggests that indirect effects are either not important in determining the sensitivity of taxa, and thus communities; or that the multispecies test systems are unable to detect these indirect effects. However, some mesocosm studies have been able to determine indirect effects in mesocosm studies. Mitchell *et al.* (1993), Boyle *et al.* (1996) and Dorn *et al.* (1997) all reported an increase in algal productivity due to decreased grazing by macroinvertebrates after exposure to the insecticides lindane, diflubenzuron and the surfactant, linear alcohol ethoxylate, respectively. Conversely, Belanger *et al.*, (2000) and Giddings *et al.* (2001) reported no evidence of significant indirect effects in mesocosm studies testing the effects of surfactants and pyrethroids.

6.1.1 Objectives

In Chapter 3 the effect of a toxicant (LAS) on a selection of indigenous macroinvertebrate species was extrapolated to macroinvertebrate communities found in natural systems by determining HC5 values from SSD curves. Stream mesocosms

were employed in Chapter 5 to directly determine the effect of LAS on two structurally distinct macroinvertebrate communities. The aim of this chapter is to directly compare these two methods of determining community sensitivities to toxicants. If mesocosm studies are able to capture both direct effects on populations (such as reproduction) and indirect effects (such as changes in predation), then the sensitivities of taxa in single-species toxicity tests and mesocosms may differ. The hypothesis for this chapter was that the ranked sensitivities of species exposed in short-term single-species toxicity tests and longer term mesocosms would be significantly different due to interactions between species within the stream mesocosm study.

6.2 Methods

6.2.1 Data collation

Single-species data for indigenous macroinvertebrate species were collated from Section 2.3.3, and ranked according to their sensitivity to LAS. Where the same genera, but different species were present in single-species data and mesocosm data, the data for that genus were compared. The geometric means of single-species data for Oligochaeta and Tricladida were used in this comparison, as the experimental stream taxa were only identified to this resolution.

Indigenous species with single-species test data were listed in Appendix 2.4, and taxa found in the mesocosm prior to dosing were given in Appendix 5.3. Information in these two appendices were collated to generate a list of taxa for which both single-species and multispecies toxicity data were available.

6.2.2 Comparison of species sensitivities

The single-species data for all indigenous macroinvertebrate species were transformed to natural logarithms and a t-test carried out to determine if the sensitivity of taxa used in the comparison with the mesocosm study were significantly different from the sensitivity of excluded taxa (i.e. taxa absent from

either the control or treated streams on Day –1, or absent from the control streams on Day 27).

The sensitivity of taxa in single-species and multispecies were ranked, and the ranks for the two systems compared using Spearmans' rank correlation to determine whether the ranked sensitivity of taxa to LAS was significantly different in the two test systems (Zar, 1996).

Taxa were excluded from the correlation if there were no individuals found in either the control stream or the stream dosed at 8.0 mg LAS/l on day –1 or in the control streams on day 27. The ranked sensitivities of taxa in the stream mesocosms were determined from the change in total number of individuals from one day prior to dosing (i.e. Day –1) to 27 days after dosing (i.e. Day 27), relative to the change in the total number of individuals in the control stream (Eqn. 6.1).

$$\text{Survival relative to control (\%)} = \left(\frac{(n_{dt2})/(n_{dt1})}{(n_{ct2})/(n_{ct1})} \right) \times 100 \qquad \text{Eqn. 6.1}$$

Where n is the total number of individuals in the taxon of interest, d is the streams dosed at 8.0 mg/l LAS, c is the control streams, $t1$ is one day prior to dosing, and $t2$ is after 27 days of dosing. Only data from the control streams and the streams dosed at 8.0 mg LAS/l were used in this comparison.

6.2.3 Comparison of community sensitivities

Single-species data were collated from Appendix 2.4 for all taxa that were present in the River Wheeler or the River Tame community on day –1 (Appendix 5.1). Species sensitivity distribution curves were generated for each community, and the concentration hazardous to 5% of species (HC5) was extrapolated using the method of (Aldenberg & Slob, 1993). These values were then compared to the lowest NOEC values generated for each community in Section 5.3.7.

6.3 Results

6.3.1 Species comparisons

Single-species toxicity data were available for a total of seven taxa that met the selection criteria for inclusion. Eight taxa were excluded from the comparison between taxa sensitivity in single-species and multispecies tests due to absence in the experimental streams on day –1 (i.e. *Baetis rhodani, Rhyacophila dorsalis, Leuctra* sp., Tricladida) or loss of all individuals in the control streams by day 27 (i.e. *Rhithrogena semicolorata*). The single-species L(E)C50 data used in the comparison with the stream mesocosm were not significantly different from the L(E)C50 data that were excluded ($t = 1.69$, $df = 8$, $p > 0.05$), indicating that the selected taxa were a representative subset of all the L(E)C50) data available.

Table 6.1: The sensitivity of indigenous macroinvertebrate species to LAS in short-term single-species toxicity tests that were either included or excluded from the comparison with mesocosm data.

Selected taxa		Excluded taxa	
Taxa	**LC50 (mg/l)**	**Taxa**	**LC50 (mg/l)**
Oligochaeta	2.3	Tricladida	1.8
Ephemerella ignita	4.9	*Leuctra* sp.	2.8
Gammarus pulex	6.7	*Ecdyonurus dispar*	3.9
Erpobdella octoculata	7.8	*Baetis rhodani*	4.1
Hydropsyche spp.	>62.2	*Rhithrogena semicolorata*	4.3
Elmis aenea	>70.2	Chironomidae	9.6
Asellus sp.	81.3	*Rhyacophila dorsalis*	13.5
		Agapetus fuscipes	14.4

Of the seven taxa used in the comparison between taxa sensitivity in single-species and multispecies tests, five taxa were present in mesocosms stocked from the River Wheeler and four from mesocosms stocked from the River Tame (Table 6.2). Two taxa occurred in both stream mesocosm communities (Oligochaeta and *Hydropsyche* sp.). The ranked sensitivity of these taxa was similar in both streams, therefore the taxa from the two communities were reported as a single ranked value to avoid a large number of tied ranks (Table 6.2). The correlation between the ranked sensitivities of taxa in the single-species and multispecies tests was found to be not significant ($r_s = 0.67$, $p > 0.05$; Figure 6.1).

Table 6.2: Comparison of the sensitivity of species to LAS, tested in both single-species tests and experimental streams (TA = stream stocked from the River Tame; WH = stream stocked from the River Wheeler).

	single-species		experimental streams		
Taxa	**96 hr LC50 (mg/l)**	**ranked sensitivity**	**Survival over 27 days at 8.0 mg/l relative to control (%)**	**ranked sensitivity**	**Source stream**
Oligochaeta	2.30	1	3.81	3	TA/WH
Ephemerella ignita	4.90	2	0.00	1.5	WH
Gammarus pulex	6.66	3	0.00	1.5	WH
Erpobdella octoculata	7.80	4	40.27	5	TA
Hydropsyche spp.	>62.20	5	44.44	6	TA/WH
Elmis aenea	>70.20	6	91.49	7	WH
Asellus aquaticus	81.33	7	22.18	4	TA

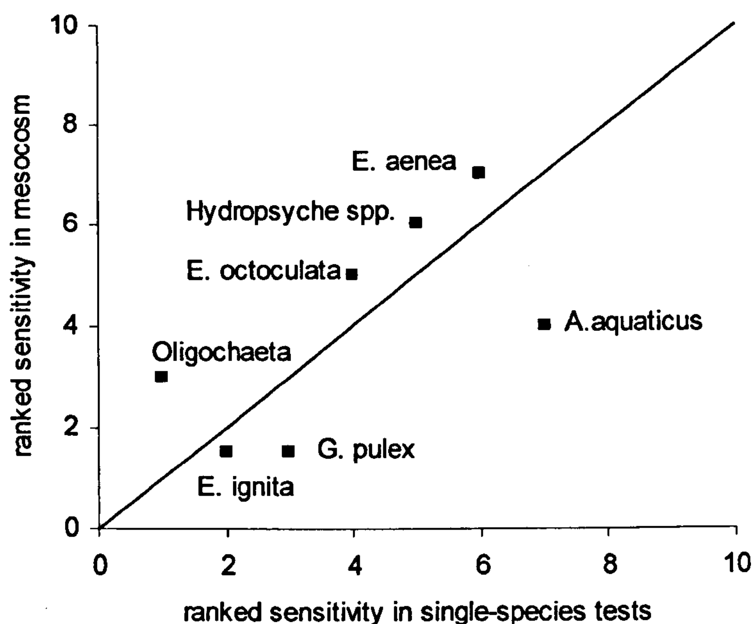


Figure 6.1: Plot of species ranked sensitivity to LAS in single-species toxicity tests and stream mesocosms ($r_s = 0.67$, $p > 0.05$; fitted line = 1:1).

6.3.2 Community comparisons

The single-species sensitivity of taxa present in the mesocosm communities on day – 1 are listed in Table 6.3. HC5 values were calculated from the single-species toxicity data for all species present in each community. The extrapolated HC5 values for the River Wheeler and River Tame communities were very similar (0.85 and 0.73 mg LAS/l respectively). This similarity is largely due to nine of the thirteen species with single-species data identified from the stream mesocosm study being present at both sites.

Table 6.3: Total number of individuals found in all streams stocked from either the River Wheeler or the River Tame prior to LAS exposure, and single-species values determined for each taxa in single-species tests. HC5 values were calculated for each community after the method of (Aldenberg & Slob, 1993).

	River Wheeler	River Tame	96 hour L(E)C50 data (mg/l)
Asellus aquaticus	4	157	>81.3
Baetis rhodani	3	10	4.1
Ecdyonurus sp.	0	1	3.9
Elmis aenea	125	1	>70.2
Ephemerella ignita	24	0	4.9
Erpobdella octoculata	8	162	7.8
Gammarus pulex	81	0	6.3
Hydropsyche spp.	122	15	>62.2
Leuctra sp.	1	1	2.8
Oligochaeta	149	60	2.3
Rhithrogena semicolorata	125	0	4.3
Rhyacophila sp.	4	2	13.5
TRICLADIDA	3	5	1.8
HC5 calculation			
count	12	10	
mean	0.9	1.0	
sd	0.6	0.6	
HC5 95%	0.18	0.10	
HC5 50%	0.85	0.73	

The species sensitivity distribution (SSD) curves, from which the HC5 values are derived, are plotted in Figure 6.2 together with the NOEC values from the stream mesocosms. The mesocosm NOEC for the River Wheeler community was 0.80 mg LAS/l which is in very good agreement with the HC5 value of 0.85 mg LAS/l generated for that community (Section 5.3.5). The mesocosm NOEC for the River Tame was 2.5 mg LAS/l (Section 5.3.6) which was 3.4 times higher than HC5 value

of 0.73 mg LAS/l. The HC5 was therefore found to be either protective, or overprotective of the effects measured in the stream mesocosm study.

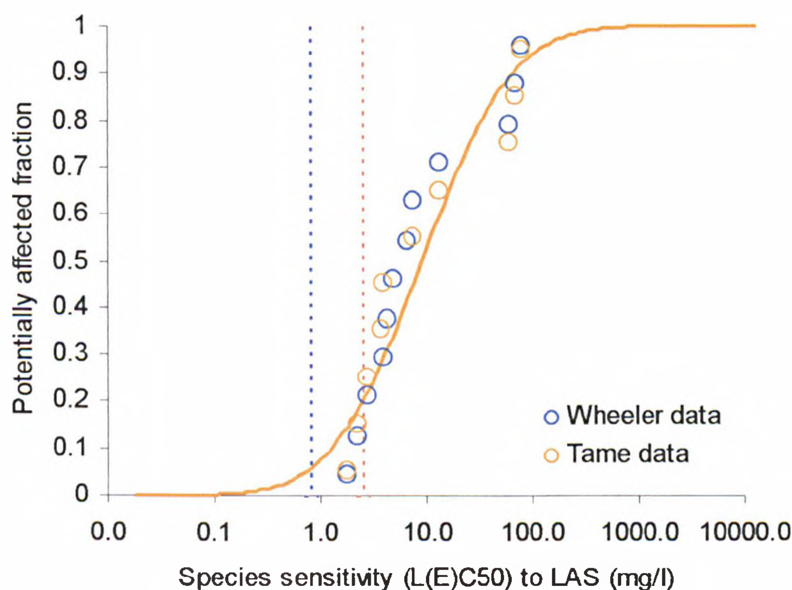


Figure 6.2: Species sensitivity distribution curves for the River Wheeler community and River Tame community. Vertical dashed lines show the community NOEC values determined from mesocosm studies (Chapter 5).

6.4 Discussion

For taxa to be included in the comparison of the two approaches, both short-term single-species toxicity data, and sensitivity data from the mesocosm study were required. Several taxa were present in the mesocosms, but were excluded from the comparison with single-species data as they were absent from the experimental streams prior to dosing, or absent from the control streams by day 27. These excluded taxa included several Insecta species which were found to be sensitive to LAS in the short-term single-species tests (e.g. *Baetis rhodani*). However, there was no statistically significant difference between the L(E)C50 values for taxa that were used in the comparison, and those that were not. The single-species L(E)C50 data

used for this comparison are therefore a representative subset of the available single-species data for indigenous species.

6.4.1 Comparison of single-species sensitivities

Comparing short-term single-species tests (<96 hr LC50) with longer-term mesocosm studies is not comparing like with like (Giddings *et al.*, 2001). However, by comparing the ranked sensitivities of species in the two systems it is possible to determine whether exposure in complex multispecies communities under more realistic exposure conditions than single-species laboratory tests, affects the relative sensitivity of taxa (i.e. is there a change in the rank order of species sensitivities?). If the ranked sensitivity of taxa in single-species tests and mesocosm studies were different, then the sensitivity of species in natural streams may not be accurately determined from single-species data (Cairns, 1986).

In this comparison, no significant correlation was found between the ranked sensitivities of the seven indigenous macroinvertebrate taxa exposed to LAS in single-species tests and in the mesocosms, indicating that there were significant differences in the ranked sensitivities of taxa in the two systems. For instance, *Gammarus pulex* and *Asellus aquaticus* were ranked as more sensitive in the mesocosm study than in the single-species tests. *Gammarus pulex* was the third most sensitive species in the short-term single-species tests but was the most sensitive taxa in the mesocosm study. *Asellus aquaticus* was the most tolerant taxa in the single-species exposures, but was more sensitive than *Erpobdella octoculata, Hydropsyche* spp. and *Elmis aenea* in the stream mesocosm study. In order to ascertain which if any of these factors are affecting *G. pulex* sensitivity further studies would be required. However, this increase in sensitivity of *G. pulex* to LAS in the stream mesocosms is in agreement with the effect of LAS on *G. pulex* observed by Tattersfield *et al.* (1995) who reported an EC50 of 0.24 mg/l LAS after exposure for 28 days in a mesocosm study.

Four taxa were less sensitive in the mesocosm study than in the single-species test. For three of these taxa (*Erpobdella octoculata*, *Hydropsyche* sp. and *Elmis aenea*) this change in ranked sensitivity was partly due to the increase in ranked sensitivity of *Asellus aquaticus* (Table 6.2). Also, the single-species LC50 data for

Hydropsyche spp. and *Elmis aenea* were based on 'greater than' values, and would therefore be an overestimation of the sensitivity of these taxa.

The fourth taxon to decrease in ranked sensitivity in the mesocosm study was Oligochaeta, which was the most sensitive taxa in single-species LC50 tests but was ranked as less sensitive than *Ephemerella ignita* and *Gammarus pulex* in the stream mesocosm study. Due to the low level of taxonomic resolution in the mesocosm data, it is not possible to determine whether the decrease in sensitivity in the mesocosms is simply due to interspecific differences in sensitivity to LAS. However, the change in sensitivity may be due to interactions between species affecting the sensitivity of Oligochaeta in the mesocosm exposures. Chapman *et al.* (1982) reported both *Limnodrilus hoffmeisteri* and *Tubifex tubifex* were less sensitive to cadmium, mercury and pentachlorophenol in multispecies tests than in single-species tests. Another factor that would affect the ranked sensitivity of Oligochaeta in the mesocosms is the increase in ranked sensitivity of *G. pulex*.

6.4.2 Comparison of community sensitivities

The HC5 values extrapolated from species sensitivity distribution curves determined for the River Wheeler and River Tame communities were 0.85 and 0.73 mg LAS/l respectively, and the lowest NOEC determined for each community was 0.8 and 2.5 mg LAS/l respectively. The two estimates of a 'safe concentration' for the River Wheeler community are remarkably similar and suggest that the single-species data are able to determine the sensitivity of communities exposed in stream mesocosms. The difference between the two estimates of a 'safe concentration for the River Tame community are also very similar, differing by less than a factor of four, with the HC5 value being lower than the stream mesocosm NOEC. Therefore the SSD method may be taken as being at least as sensitive as the stream mesocosms for these communities (Versteeg *et al.*, 1999).

The sensitivity of the mesocosm community will partly be determined by the sensitivity of its composite taxa (Larsen *et al.*, 1986). In the stream mesocosm study, NOECs determined for the two communities were different, whereas the HC5 values were very similar. Examination of changes in the structure of the two communities exposed in the mesocosm found significant differences in their responses to exposure

(Section 5.3.7). For example the reduction in community diversity was significantly greater in the River Wheeler community than in the River Tame community. These differences were detected because the relative abundances of taxa are utilised in these analyses, and effects determined by shifts in taxa relative abundances. In comparison, the SSD curve method only utilises presence / absence data, and is therefore insensitive to differences in community structure.

Table 6.3 shows that nine of the thirteen species used in the SSD curves were present in both communities, thus accounting for the similarity in the estimates of HC5. However, many of these species are represented by very few individuals. Only four taxa had more than 50 individuals across the four mesocosms stocked with the River Wheeler, namely *E. ignita, Hydropsyche* spp, Oligochaeta and *R. semicolorata.* Three taxa were present in these abundances in the River Tame community, *A. aquaticus, E. octoculata,* and Oligochaeta. If the SSD curve's were able to utilise information on the relative abundance of different taxa then a more accurate estimate of the 'safe concentration' may be derived as dominant species would have a greater affect on the shape of the distribution.

Single-species data from short-term tests are not able to determine the effect of toxicant exposure on species sensitivities in mesocosms over extended periods of time. To make a true comparison between the two systems would require the sensitivities of a wide range of indigenous taxa in longer-term single-species tests to be known. This is currently not practical due to difficulties in maintaining such species under laboratory conditions for extended periods of time (e.g. Elliott *et al.*, 1988). Species sensitivity distribution (SSD) curves are also unable to differentiate between communities with similar species composition, but different community structures, whereas stream mesocosms can. However, as SSD curves tend to be over- rather than under-protective of community sensitivities (Emans *et al.*, 1993; Okkerman *et al.*, 1993), then they are of value in determining 'safe concentrations' where no long term multispecies data are available.

7 General discussion

7.1 Single-species tests and SSD curves

Despite the existence of a large volume of single-species data for high use compounds such as LAS, data were available for a very limited number of species (e.g. Painter & Zabel, 1988). This was due to a few standard test species (i.e. *Daphnia magna, Lepomis machrochirus, Pimephales promelas*) being over-represented, with 63% of invertebrate tests conducted using *Daphnia magna* (BKH Consulting Engineers, 1992). These surrogate species offer many advantages in effects assessment by allowing the relative sensitivity of toxicants to be determined in Tier I and Tier II effects assessments (Van Leeuwen & Hermens, 1996). Where comparisons of the predicted environmental concentration and the predicted no effect concentration indicate large safety margins exist, further testing may not be required (e.g. Rand, 1995). Where a sufficiently large safety margin is not identified, or where the compound is used in very large volumes, higher tier risk assessments may be required. Higher tier effects assessments are conducted to determine the hazard associated with a compound with greater accuracy, by reducing uncertainties associated with the use of short-term single-species tests (Van Breukelen & Brock, 1993). The overall aim of this study was to examine the effect of species composition on the responses of indigenous communities to toxicants, as determined by species sensitivity distribution (SSD) curves generated from single-species tests, and multispecies exposures in mesocosms. This aim was addressed using the surfactant linear alkylbenzene sulphonate (LAS) as a reference compound, and benthic macroinvertebrates as study organisms.

Interspecific variation in species sensitivity has been demonstrated for compounds from many different classes including surfactants, pesticides and metals (e.g. Main & Mulla, 1992; Van Wijngaarden *et al.*, 1993; Forget *et al.*, 1999; Jorgensen & Christoffersen, 2000). The use of indigenous species in single-species toxicity tests would minimise uncertainties associated with extrapolating from species not found within the exposed communities (Greve *et al.*, 1998; Greve *et al.*, 1999).

The sensitivity of 15 indigenous lotic macroinvertebrate species were experimentally determined in short-term laboratory tests and supplemented with data for non-Daphniidae macroinvertebrate taxa from the literature. The range of indigenous macroinvertebrate sensitivities to LAS covered almost four orders of magnitude. Vaal *et al.* (1997) demonstrated that compounds with a non-specific modes of action, such as LAS, typically have smaller ranges of interspecific variability than compounds with more specific modes of action. The high variability observed in the sensitivity of test species to LAS may be due to differences in methods used for determining test concentrations; variation in LAS chain length; the application of different statistical methods; or different exposure conditions (Divo & Cardini, 1980; Waters & Garrigan, 1983; Martinez *et al.*, 1989; Rapaport & Eckhoff, 1990; Scott & Jones, 2000; Verge *et al.*, 2001). The novel toxicity data generated for indigenous species had a smaller range of values, differing by less than two orders of magnitude. The mean value for the standard invertebrates test species *Daphnia magna* was found to be less sensitive than half of the indigenous taxa, and was therefore considered to be representative of the sensitivity of indigenous taxa to LAS.

An application factor of ten was applied to the *Daphnia magna* data to account for interspecific variability, and the resulting value was lower than the L(E)C50 value for 32 of the 35 non-standard test species. The exceptions (*Tubifex tubifex, Lymnaea vulgaris, Culex pipiens*) were all sourced from the same reference (Lal *et al.*, 1983), and were more than an order of magnitude more sensitive than any other test species. In reviews of the sensitivity of LAS it has been suggested that these data were inaccurate (e.g. Painter & Zabel, 1988). However, as no other tests have been conducted on these species, and the data met the selection criteria set out in Section 2.2.1 these data were included in this study.

As species differ in their sensitivity to toxicants, communities composed of different species may also differ in their toxicant sensitivity. Single-species toxicity data for indigenous taxa were used to determine the variability in community sensitivities due to differences in species composition using species sensitivity distribution (SSD) curves. SSD curves extrapolate the concentration of a toxicant hazardous to a proportion of all species from the distribution of sensitivities for tested species (Kooijman, 1987). This method is normally used to determine the hazardous

concentration to 5% of species (HC5) (Stephan, 1977; Van Straalen & Denneman, 1989), although other hazardous concentrations may be determined (e.g. Solomon *et al.*, 2001).

Initially, an SSD curve was plotted using all the single-species toxicity data for indigenous macroinvertebrate species. This SSD curve used all the available data to determine a hazardous concentration for the total data set ($HC5_{TOT}$). Communities will only contain a subset of the species used in the SSD curve. If communities exist that are composed predominantly of sensitive taxa, then the $HC5_{TOT}$ may underestimate the concentration hazardous to 5% of species in that community ($HC5_{COM}$). In such instances, the calculated $HC5_{TOT}$ would be under-protective of the exposed community. However, as the $HC5_{TOT}$ will, by definition, include the widest possible range of species sensitivities, it was predicted that the HC_{TOT} would be lower than the $HC5_{COM}$ for 95% of natural communities.

The $HC5_{COM}$ values for 60 indigenous macroinvertebrate communities from low-order circumneutral streams were calculated, and were found to vary by an order of magnitude. The $HC5_{COM}$ values were compared to the $HC5_{TOT}$ and it was shown that the $HC5_{TOT}$ was lower than the $HC5_{com}$ values 92% of the time.

These results clearly indicate that species composition influences the sensitivity of a community, as determined by the SSD curve approach. Furthermore, the concentration that is not hazardous for 95% of all taxa appears to be close to the concentration that that is not hazardous for 95% of species in 95% of communities. This suggests that SSD curves, derived from single-species toxicity tests for indigenous taxa may determine HC5 values that are protective of natural communities.

SSD curves have been criticised for assuming that protecting 95% of species is adequate (Hopkin, 1993). The assumption that some species loss may be tolerated due to toxicant stress is based on the ecological theory of ecosystem redundancy (Naeem, 1998). This theory states that where more than one species is present within a functional group, the loss of one species may be compensated for by the remaining species within that group. It has been argued that the presence of ecosystem redundancy is an important attribute of a communities structure, providing stability in the face of stochastic variability (Lewontin, 1969; Naeem, 1998).

Van Straalen (1993) addressed the critisism of determining a concentration that is protective of 95% of species by explaining that a species that is affected at the HC5 value will not necessarily become extinct, and a range of adaptive mechanisms have been identified that may reduce the effect of exposure on community structure and function. Communities exposed to toxicants may adapt to toxicant exposure through physiological adaptation (Cairns, 1986); selection of tolerant genotypes (e.g. Maltby & Crane, 1994); and the replacement of sensitive taxa with more tolerant ones (Blanck *et al.*, 1988; Millward & Grant, 1995).

In order to determine whether the HC5 values generated from SSD curves would be sufficiently low to prevent measurable effects due to toxicant exposure, two communities were exposed to LAS in a stream mesocosm. The sensitivity of these communities was assessed using community-level endpoints (total number of individuals, species richness and diversity). SSD curves were then generated for the communities present in the stream mesocosms prior to LAS exposure, thus allowing a direct comparison to be made between HC5 values and the concentration at which effects were observed in the stream mesocosms.

7.2 Multispecies exposures in mesocosms

Multispecies tests, such as stream mesocosm studies are used to determine the effect of community exposure under semi-natural conditions. These tests address many of the criticisms of single-species tests, by exposing whole communities to the toxicant under semi-natural conditions (Lamberti & Steinman, 1993). One limitation of the method is the complexity of the data generated, making data interpretation difficult (e.g. Van Wijngaarden *et al.*, 1995). Also, the inherent noisiness of the data due to temporal and stochastic variation mean that significant effects due to toxicant exposure may be missed (Van den Brink *et al.*, 1996). This thesis determined the effect of species composition and community structure on the sensitivity of communities exposed in a stream mesocosm by comparing the responses of two structurally distinct communities. Meosocosm studies using different communities exist for a number of compounds including surfactants (Fairchild *et al.*, 1993; Belanger *et al.*, 1995; Dorn *et al.*, 1996) and atrazine (Dewey, 1986; Van den Brink

et al., 1995; Gruessner & Watkin, 1996). However, no standardised test design exists for mesocosm studies, therefore differences may exist in mesocosm design; chemical formulation of the toxicant; exposure concentrations; stocking methods; sampling methods; study duration; and physiochemical parameters such as water quality; temperature; flow rate; and substrate composition (Swift *et al.*, 1993; Guckert, 1996). Any of these factors could affect the level of observed response of a community to toxicant exposure (e.g. Craig, 1993; Kiffney *et al.*, 1997). In order to make a direct comparison of the response of two structurally distinct communities to LAS exposure in a stream mesocosm it was therefore necessary to expose distinct communities in the same system at the same time.

As the habitat in the stream mesocosms will be different to the natural habitats of the communities selected, some loss of species would be expected due to differences in physiochemical variables such as substrate type, water quality and temperature (Heimbach & Pflueger, 1992; Craig, 1993), resulting in a shift in community structure. If the stream mesocosms were only able to support a small proportion of the species found in natural communities, structurally distinct communities may converge to a 'generic mesocosm community' through time. Therefore, the ability of the stream mesocosms to support structurally distinct communities had to be established prior to exposing the communities to a toxicant.

Three structurally distinct communities were used to stock the stream mesocosms, and changes in community composition and structure were monitored over ten weeks. Despite the initial losses of some species during the first two to four weeks of the study, the communities did not converge over time, but remained remarkably distinct. Having established that structurally distinct communities could be maintained in the stream mesocosms it was possible to test the hypothesis that species composition would affect community responses in a stream mesocosm. This was done by exposing two structurally distinct communities simultaneously. It was predicted that the community composed of more sensitive dominant taxa would respond to LAS exposure at lower concentrations than a community dominated by more tolerant taxa. These predictions were verified, with the NOEC values for species richness and diversity found to be significantly lower in the community dominated by sensitive taxa.

These results have implications for the use of mesocosm studies in effects assessments. As communities have been demonstrated to differ in their sensitivity to a toxicant, and those differences were detectable within a mesocosm study, the selection of communities used for stocking these systems must be carefully considered. It has been proposed that an application factor of 1 may be appropriate for NOEC data from mesocosm studies (Belanger, 1997). The results presented here suggest that this is inappropriate, as the NOEC concentrations for one community were shown to significantly affect species richness and community diversity of the second community.

7.3 Comparison of single-species and multispecies methods

Having established that the effect of species composition on community sensitivity can be detected using both SSD curves and stream mesocosms, the final objective of this thesis was to directly compare the two methods and determine whether single-species tests for indigenous taxa, and SSD curves derived from those data, were comparable to the responses of species and communities exposed in a stream mesocosm.

No significant correlation was detected between the ranked sensitivity of species exposed in single-species and multispecies exposures. This suggests that the ranked sensitivity of species in the single-species toxicity tests was different to that measured in the mesocosm study. This lack of correlation appeared to be due to an increase in the sensitivity of crustacean taxa in the stream mesocosm study, relative to the sensitivity of insects and non-arthropod species.

SSD curves were unable to detect differences in the sensitivity observed in the two communities exposed in the stream mesocosms. These two communities, although structurally distinct, were very similar in their species composition, with nine of the thirteen taxa represented by single-species toxicity data occurring in both communities. However, the HC5 estimates generated from the SSD curves were equal to, or lower than, the concentration at which effects were observed. This study showed that the HC5 calculated from an SSD curve for indigenous taxa would have been low enough to ensure no effects were observed for either community exposed in

the mesocosm study. The SSD curves were therefore found to be either protective or over-protective of community responses in stream mesocosms.

Incorporating some measure of community structure into the SSD curves may improve the methods ability to determine the sensitivity of natural communities to toxicant exposure. Methods have been developed to try and incorporate food-web structure into effects assessments (Hanratty & Stay, 1994; Klepper *et al.*, 1999). However, these methods require large amounts of data on single-species effects, and species interactions. An alternative, and simpler method would be to weight species relative importance within a community according to it's relative abundance. This would result in lower estimates of $HC5_{COM}$ for communities dominated by sensitive taxa and higher $HC5_{COM}$ for communities dominated by tolerant taxa. This method of weighting species could be applied to parameters other than abundance. Theoretically, this method could incorporate weighting for particular species of interest such as keystone species (Tanner *et al.*, 1994), or species of recreational value. However, the existing 'food-web' based methods, designed to incorporate species interactions, may be more suited to situations where the effect of exposure on particular communities or ecosystems are of interest.

7.4 Conclusions

In order to determine whether the effect of a toxicant on communities was affected by species composition in extrapolations from species sensitivity distribution (SSD) curves and exposures in stream mesocosms five key objectives were addressed. The conclusion for these objectives are as follows;

1. The sensitivity of non-standard macroinvertebrate taxa to LAS varied by up to four orders of magnitude. The standard test species *Daphnia magna* was less sensitive than half of the indigenous taxa tested.

2. Changes in sensitivity of natural communities due to differences in species composition were detected by SSD curves. Communities composed of similar species, found in similar habitats differed in their sensitivity to LAS by an order of magnitude. An SSD curve using all the data for indigenous macroinvertebrate

species determined an HC5 value that was protective for at least 95% of taxa in 95% of communities.

3. It was possible to maintain structurally distinct macroinvertebrate communities in an experimental streams for the duration of a ten week study. Despite considerable overlap in the composition of these communities, the communities did not converge to a 'generic mesocosm community'. Replicate streams, stocked with the same community, remained similar to one another for the duration of the study.
4. Structurally distinct communities differed in their response to toxicant exposure in a stream mesocosm study. These differences were measured as changes in species richness and community diversity relative to control streams. Principal response curves identified different taxa to be driving these changes in the two communities.
5. A comparison of the ranked sensitivity of taxa in single-species and multispecies tests were not significantly correlated. SSD curves were not able to detect significant differences in the sensitivity of two structurally distinct macroinvertebrate communities. However, the HC5 estimate generated from SSD curves was lower than the concentrations at which an effect was observed, and would therefore have been protective of these communities.

Appendix 2.1: Composition of artificial pond water (APW). 50 ml of each compound is added to 10 litres of reverse osmosis filtered water.

Stock	Compound	Concentration (mg/l)
1	$CaCl_2.2H_2O$	294
2	$MgSO_4.7H_2O$	123.25
3	$NaHCO_3$	64.5
4	KCl	5.75

Appendix 2.2a: The Development of a Micro MBAS Method for the Determination of Anionic Surfactants in Environmental Test Liquors

Test Facility	-	SEAC Environment, Analytical Unit
Sponsor	-	UNILEVER RESEARCH
Study class	-	BACKGROUND
STUDY NUMBER	-	AU/Micrombas/02
Compiled by	-	Jim Melling
Date	-	26/04/99

Purpose of the Study

To investigate a small scale Methylene Blue method, previously described by *Hayashi (1975),* for the analysis of anionic surfactants in environmental test samples.

Reference Substance

Manoxol OT - Dioctylsulfosuccinate sodium salt (97EO78).

Persons Participating in the Study

Project Manager	:	M Burford
Analysts	:	D Cooper and J Melling

Study Dates

Starting Date	:	Week beginning 1/3/99
Finish Date	:	Week ending 28/4/99
Target Reporting Date	:	4/5/99

Summary

A robust method using cheap and easily attainable apparatus was found for the range 0-1 mg/l as test substance. The method was statistically comparable with the current method but used less chloroform, time and cost.

1. Introduction

1.1 Objective

To determine the acceptability of a small scale methylene blue test for the analysis of anionic surfactants in environmental test samples. The test should reduce chloroform usage, time and cost when compared with the current method.
The method must be robust enough to quantify samples in elendt and effluent media, at ranges routinely tested.

1.2 Reference Compounds

The following compound was used to produce a standard calibration and spike solution for analysis.

Manoxol OT - Dioctylsulfosuccinate sodium salt (97EO78)

2. Experimental

2.1 Apparatus

6 and 8 dram vials with caps
250 ml separating funnel
Hitachi UV 3100 spectrophotometer [1]
Hearus Megafuge 1.0R centrifuge [2]
Adapters for centrifuge to fit 6 and 8 dram vials
Whirlimixer
Gilson pipettes [3]
Rainin EDP pipette [4]

2.2 Chemicals

Chloroform - Baker HPLC Grade

2.3 Reagents

HPLC grade Chloroform (Baker)
Millipore water [6]
Manoxol OT (97E078)
Alkaline Borate Buffer
Methylene blue solution

2.4 UV Spectrophotometer Conditions

Wavelength	650 nm
Slit Width	2 nm

3. Method

3.1 Preparation of Stock and Working Solutions

1000 mg/l Manoxol OT Aqueous Stock Solution
Accurately weigh 1 g of Manoxol OT into a 1000 ml volumetric flask and make up to the mark with Millipore water. This solution contains 1000 µg/ml Manoxol OT.

5 mg/l Manoxol OT Working Solution
Quantitatively dilute 5 ml of the Manoxol stock solution to 1000 ml with Millipore water. This gives a working solution of 5 mg/l Manoxol OT.

1 mg/l Manoxol OT Working Solution
Quantitatively dilute 20 ml of the 5 mg/l working solution to 100 ml with Millipore water. This gives a working solution of 1 mg/l Manoxol OT.

3.2 Experimental method

Evaluation of 6 and 8 dram vials for use in the UV spectrophotometer.
Turn on the spectrophotometer and allow too warm up. Create a method to measure absorbence at 650 nm. Zero the spectrophotometer against air in both the reference and sample sides.
Measure the absorbence of 10 different vials and calculate the mean and standard deviation.
Prepare 5 vials of both types containing 10 ml of chloroform and measure absorbence, calculating mean and standard deviation.

Evaluation of Sensitivity of Blanks
Cleaning of Methylene Blue Solution - Add 10 ml methylene blue solution, 10 ml of borate solution and 10 ml of chloroform to a 250 ml separating funnel. Stopper and shake for 30 seconds then discard chloroform layer. Add a further 10 ml aliquot of chloroform and repeat until extract is clear.

Sample Preparation
To ten, 6 dram vials add 12 ml of Millipore water, 0.6 ml of cleaned methylene blue / borate solution and 6.4 ml of chloroform.
To ten, 8 dram vials add 18 ml Millipore water, 1 ml of cleaned methylene blue / borate solution and 8 ml of chloroform.
Mix on a Whirli mixer for 30 seconds and centrifuge at 2000 rpm for 3 mins at 4°C. Allow the vials to warm to room temperature (approx. 1 hour). Measure the absorbence at 650 nm of each vial and calculate mean and relative standard deviation.

Detection Limits
i) Pipette the following volumes of Manoxol OT 5 mg/l stock solution into 6 dram Vials; 0, 3, 6, 9 and 12 ml. Make the volume of sample up to a total of 12 ml with Millipore water. This gives concentrations of 0, 15, 30, 45 and 60 µg Manoxol OT in the vials.

ii) Pipette the following volumes of Manoxol OT 1 mg/l stock solution into 6 dram Vials; 0, 3, 6, 9 and 12 ml. Make the volume of sample up to a total of 12 ml with Millipore water. This gives concentrations of 0, 3, 6, 9 and 12 μg Manoxol OT in the vials.
Prepare 1 other blank for use as reference and for zeroing.
Add 64 ml chloroform followed by 0.6 ml of cleaned methylene blue solution.
Mix on a Whirli mixer for 30 seconds and centrifuge at 2000 rpm for 3 mins at 4°C.
Although vials to warm to room temperature (approx. 1 hour). Measure the absorbence of each vial and use UV solutions [7] software to calculate regression coefficient, slope and intercept.
If the regression coefficients are >0.9970 further calibrations should be performed with decreasing concentrations of manoxol OT in the vials.
Repeat with the 8 dram vial, using 18 ml of sample, 1 ml of cleaned methylene blue and 8 ml of chloroform.

Linearity of Response and Repeatability
Choose an applicable range (the lowest range possible giving a regression coefficient of >0.9960) for both vials and repeat the calibration three times with 5 samples containing a mid range concentration of manoxol OT.
Measure the absorbence of each vial and use the UV Solution software to calculate μg surfactant in vials and mg/l surfactant in the samples.

Robustness
Perform a calibration with mid-range samples in for both 6 and 8 dram vials but use Elendt and Iso Algal media instead of Millipore water.
Using freshly cleaned methylene blue prepare vials of both sizes containing a mid range concentration of manoxol OT, the normal amount of chloroform but double the amount of methylene blue normally used i.e. 1.2 ml for 6 dram, 2 ml for 8 dram.
Prepare vials of both sizes containing 50% extra chloroform than normally used with normal amounts of methylene blue and a mid range concentration of manoxol OT.
Measure the absorbence of each vial.
After leaving the cleaned methylene blue prepared earlier for approximately 5 hours prepare a further set of vials containing the increased amounts of cleaned methylene blue as outlined previously.
Measure the absorbence of each vial and compare.
Prepare cleaned methylene blue and using the volumes from linearity experiments prepare a calibration in both sets of vials.
Once prepared mix each vial for only 15 seconds before centrifugation.
Measure the absorbence of each vial and compare.
Prepare cleaned methylene blue and using the volumes from linearity experiments prepare a calibration in both sets of vials. Mix for 30 seconds and centrifuge at 2000 rpm for 3 mins at room temperature.

4. Results and Discussion

4.1 Vial Evaluation

empty vial v air: 6 dram

Sample	Run 1	Run 2	Diff
1	0.063	0.061	0.002
2	0.062	0.063	-0.001
3	0.066	0.068	-0.002
4	0.058	0.059	-0.001
5	0.062	0.061	0.001
6	0.062	0.062	0.000
7	0.057	0.059	-0.002
8	0.060	0.060	0.000
9	0.067	0.065	0.002
10	0.064	0.064	0.000
Mean	0.062	0.062	
Std Dev	0.003	0.003	

empty vial v air: 8 dram

Sample	Run 1	Run 2	Diff
1	0.067	0.067	0.000
2	0.075	0.070	0.005
3	0.077	0.074	0.003
4	0.077	0.075	0.002
5	0.069	0.069	0.000
6	0.071	0.071	0.000
7	0.066	0.067	-0.001
8	0.062	0.065	-0.003
9	0.069	0.065	0.004
10	0.067	0.067	0.000
Mean	0.070	0.069	
Std Dev	0.005	0.003	

Both sets of vials gave low and consistent absorbances (run 2 uses the same vial as run 1).

Chloroform v air: 6 dram

Sample	Turn 1	Turn 2	Turn 3	Turn 4	Mean	Std Dev	Confidence limits
1	0.357	0.449	0.462	0.35	0.405	0.051	0.32 - 0.49
2	0.382	0.366	0.314	0.372	0.359	0.026	0.32 - 0.40
3	0.447	0.54	0.287	0.28	0.389	0.110	0.21 - 0.56
4	0.315	0.363	0.486	0.345	0.377	0.065	0.27 - 0.48
5	0.326	0.39	0.308	0.366	0.348	0.032	0.30 - 0.40
				Std Dev	0.020		
				CI	0.35 - 0.40		

Chloroform v's air: 8 dram

Sample	Turn 1	Turn 2	Turn 3	Turn 4	Mean	Std Dev	Confidence limits
1	0.121	0.089	0.137	0.167	0.129	0.028	0.08 - 0.17
2	0.137	0.139	0.143	0.11	0.132	0.013	0.11 - 0.15
3	0.218	0.212	0.148	0.119	0.174	0.042	0.11 - 0.24
4	0.138	0.086	0.162	0.152	0.135	0.029	0.09 - 0.18
5	0.157	0.127	0.174	0.107	0.141	0.026	0.10 - 0.18
				Std Dev	0.017		
				CI	0.12 - 0.16		

The vials were turned to ascertain whether this made any difference to absorbance. A small difference was seen with each turn however the difference was such that by zeroing using chloroform blanks the error would become negligable.
The absorbance was generally low and showed good consistency between vials.

Water/Chloroform blanks v air

Sample	Abs
1	0.694
2	0.686
3	0.676
4	0.68
5	0.68

Blue aqueous layer/Chloroform blanks v air

Sample	Abs
1	0.745
2	0.759
3	0.75
4	0.763
5	0.759

4.2 0-1 mg/l Calibration and Sample Analysis

Calibration

Curve no.	Slope	R value
1	8.2	0.9810
2	7.5	0.9817
3	7.9	0.9905
Std Dev	0.29	

Sample Analysis

	Curve no.		
Sample	1	2	3
1	0.5	0.46	0.72
2	0.66	0.69	0.67
3	0.55	0.62	0.61
4	0.48	0.47	0.54
5	0.57	0.55	0.59
Mean	0.55	0.56	0.63
Std Dev	0.06	0.09	0.06
Max % diff from mean	20%	24%	15%
Max % diff from nominal	32%	38%	44%

4.3 0-5 mg/l Calibration and Sample Analysis

Calibration in Millipore Water

Curve no.	Slope	R value
1	8.13	0.9992
2	8.27	0.9997
3	8.3	0.9996
4	8.31	0.9988
5	8.55	0.9992
6	8.46	0.9990
Std Dev	0.1340	

Sample Analysis in Millipore Water

	Curve no.		
Sample	4	5	6
1	2.56	2.53	2.5
2	2.6	2.52	2.53
3	2.48	2.55	2.53
4	2.5	2.55	2.46
5	2.43	2.48	2.46
6	2.58	2.5	2.46
7	2.53	2.55	2.53
8	2.49	2.53	2.54
9	2.48	2.43	2.45
10	2.44	2.42	2.44
Mean	2.51	2.51	2.49
Std Dev	0.05	0.05	0.04
Max % diff from mean	3.63 %	3.43 %	2.01 %
Max % diff from nominal	4.00 %	3.20 %	2.40 %

Calibration in Elendt

Curve no.	Slope	R value
1	8.20	0.9990
2	8.33	0.9999
3	8.35	0.9999
Std Dev	0.0663	

Sample Analysis in Elendt

	Curve no.		
Sample	1	2	3
1	2.52	2.59	2.56
2	2.48	2.56	2.47
3	2.46	2.49	2.50
4	2.59	2.56	2.52
5	2.52	2.54	2.52
Mean	2.51	2.55	2.51
Std Dev	0.04	0.03	0.03
Max % diff from mean	3.02 %	2.28 %	1.83 %
Max % diff from nominal	3.60 %	3.60 %	2.40 %

Calibration in Elendt exposed to daphnia and algae

Curve no.	Slope	R value
1	8.51	0.9999
2	8.68	0.9993
3	8.33	0.9993
Std Dev	0.1449	

Sample Analysis in Elendt exposed to daphnia and algae

	Curve no.		
Sample	1	2	3
1	2.58	2.58	2.53
2	2.62	2.56	2.52
3	2.49	2.54	2.50
4	2.53	2.60	2.56
5	2.56	2.55	2.52
Mean	2.56	2.57	2.53
Std Dev	0.04	0.02	0.02
Max % diff from mean	2.58 %	1.33 %	1.35 %
Max % diff from nominal	4.80 %	4.00 %	4.00 %

5. Conclusion

High blank values due to refraction in the glass meant detection at low levels gave unsatisfactory regression coefficients and high errors in concentration detection.
The 0-5 mg/l range however showed good correlation and only a small error in concentration detection. It also proved to be robust with no difference in error due to the use of different media. This range, although of greater sensitivity than previously seen, is unlikely to be used routinely.
The Micro MBAS method uses only 6 % of the chloroform used in the current method (60 ml compared to 1 litre) and takes approximately half the time.
If the method is to be used routinely greater sensitivity must be achieved. A larger vial with higher volume of sample should also give lower blanks due to a reduction in refraction.

6. References

[1] Study Plan AU-MicroMBAS-01
[2] Ecotoxicology SOP 258 01 Use of Hitachi Model U-3000 UV/VIS Spectrophotometer
[3] Ecotoxicology SOP 253 02 Use of the Heraeus Megafuge 1.0R for Centrifugation
[4] Ecotoxicology SOP 160 04 Maintenance and operation of the Gilson Displacement Pipettes
[5] Ecotoxicology SOP 176 03 Maintenance and Operation of Rainin EDP Plus Electronic Pipettes
[6] Ecotoxicology SOP 204 02 Operation and Maintenance of the Milli-Q Plus Water Purification System
[7] UV solutions, Version 1.1 Build 55, Hitachi instruments. Unilever Macros revised by J.L.Melling 1999.

Appendix 2.2b: Modifications made to the microMBAS method.

The following modifications were made to the method in development (Appendix 2.2a) for LAS samples analysed at the University of Sheffield.

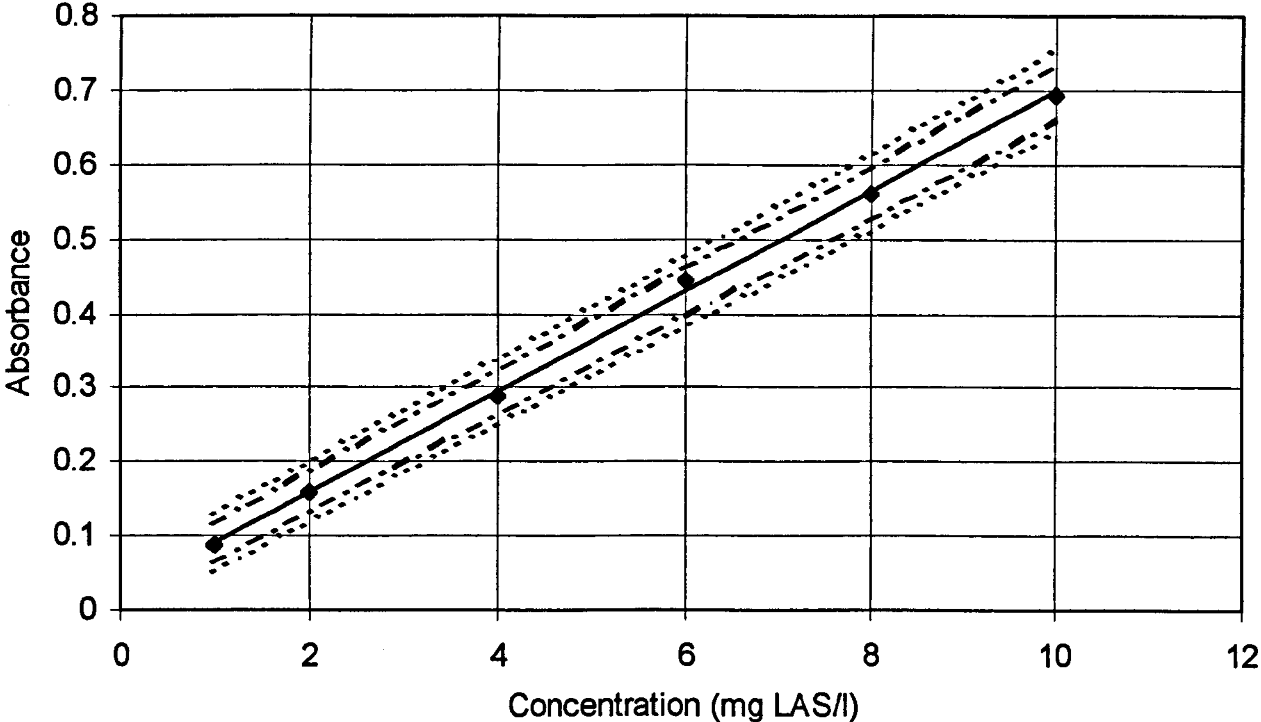
Modification to reference compound

The reference chemical used was NANSA HC80/S, dissolved in APW.

Modifications to apparatus

Varian Cary I spectrophotometer replaced the Hitachi UV 3100 spectrophotometer.
One dram vials with screw caps were used in place of the 6 and 8 dram vials.

Appendix 2.2c: Calibration curve for LAS in APW using the modified miroMBAS method.



Appendix 2.3: L(E)C50 (mortality or immobility) data from the literature for freshwater macroinvertebrates (excluding Daphniidae).

Genus / species	LAS spec	Test duration	Conc mg/l	Reference
Dugesia sp.	C11.8	48h	1.8	(Lewis & Suprenant, 1983)
Planaria sp.	C12	48h	1.8	31340*
Rhadbitis sp.	C11.8	48h	16	(Lewis & Suprenant, 1983)
Branchiura sowerbyi	LAS	96h	4.4	(Casellato & Negrisolo, 1989)
Branchiura sowerbyi	LAS	96h	4.8	(Casellato & Negrisolo, 1989)
Branchiura sowerbyi	LAS	96h	4.4	(Bressan *et al.*, 1989)
Dero sp.	C11.8	48h	1.7	(Lewis & Suprenant, 1983)
Limnodrilus hoffmeisteri	C12.3	96h	1.8	34845*
Limnodrilus hoffmeisteri	LAS	96h	2.0	(Bressan *et al.*, 1989)
Tubifex rivulorum	LAS	48h	0.06	(Lal *et al.*, 1983)
Goniobasis sp.	C12 LAS	24h	19.4	(Hendricks *et al.*, 1974)^
Goniobasis sp.	C13 LAS	24h	92.0	(Hendricks *et al.*, 1974) ^
Lymnaea vulgaris	LAS	48h	0.06	(Lal *et al.*, 1983)
Physa integra	LAS	96h	9.0	(Arthur, 1970)
Anodonta cygnea	LAS	96h	200.0	(Bressan *et al.*, 1989)
Unio elongatulus	LAS	96h	182.5	(Bressan *et al.*, 1989)

* referenced as in BKH (1992b) as no published reference available.

^ referenced after (Lewis & Suprenant, 1983), reference unavailable.

Appendix 2.3 (contd.): L(E)C50 (mortality or immobility) data from the literature for freshwater macroinvertebrates (excluding Daphniidae).

Genus / species	LAS spec	Test duration	Conc mg/l	Reference
Hyalella azetec	C12.3	96h	3.5	34845*
Asellus sp.	C11.8	48h	270.0	(Lewis & Suprenant, 1983)
Gammarus pulex	C14,	72h	1.5-36.6	AT/778026B*
Gammarus pulex	C10-13	72h	9.2	AT/73/15G (after BKH, 1992)
Gammarus pulex	C10-13	72h	11.0	AT/80/4G (after BKH, 1992)
Gammarus sp.	C11.8	48h	3.3	(Lewis & Suprenant, 1983)
Aedes aegypti	LAS	24h	2.0	(Van Emden *et al.*, 1974)
Aedes aegypti	LAS	24h	6.0	(Van Emden *et al.*, 1974)
Aedes aegypti	LAS	48h	78	(Canton & Slooff, 1982)
Chironomus riparius	C12.3	96h	6.5	34845*
Chironomus tentans	LAS	48h	12.9	(Ziegenfuss *et al.*, 1986)
Chironomus thummi	LAS	72h	2.8	(Pittinger *et al.*, 1989)
Culex pipieus	LAS	48h	0.08	(Lal *et al.*, 1983)
Paratanytarsus parthenogenica	C11.8	48h	23.0	(Lewis & Suprenant, 1983)
Isonychia sp.	C12 LAS	96h	5.3	(Dolan *et al.*, 1974)

* referenced as in BKH (1992b) as no published reference available.

Appendix 2.4: LC50 values for freshwater macroinvertebrate species (literature and novel data). Where more than one value was available the geometric mean has been calculated.

Genera / species	mean LC50 values (mg/l)
Dugesia sp.	1.8
Planaria sp.	1.8
Polycelis tenticulata	1.8
Rhadbitis sp.	16.0
Branchiura sowerbyi	4.5
Dero sp.	1.7
Limnodrilus hoffmeisteri	1.9
Lumbriculus variegatus	1.9
Tubifex rivulorum	0.06
Erpobdella octoculata	7.8
Goniobasis sp.	42.2
Lymnaea vulgaris	0.06
Physa integra	9.0
Anodonta cygnea	200.0
Unio elongatulus	182.5
Asellus sp.	81.3
Gammarus pulex	6.3
Hyalella azetec	3.5
Aedes aegypti	9.8
Agapetus fuscipes	14.4
Baetis rhodani	4.1
Chironomus riparius	9.9
Chironomus tentans	12.9
Chironomus thummi	2.8
Culex pipiens	0.08
Ecdyonurus dispar	3.9
Elmis aenea	70.2
Ephemerella ignita	4.9
Hydropsyche angustipennis	62.2
Isonychia sp.	5.3
Leuctra sp.	2.8
Paratanytarsus parthenogenica	23
Rhithrogena semicolorata	4.3
Rhyacophila dorsalis	13.5

Appendix 3.1: HC5 calculations for taxa with L(EC)50 data from 60 communities (see Section 3.2.2 for community selection criteria.

	all data	Set 1	Set 2	Set 3	Set 4	Set 5	Set 6	Set 7	Set 8	Set 9	Set 10	Set 11	Set 12	Set 13	Set 14	Set 15
count	25	12	10	8	10	11	8	9	11	11	11	7	13	10	11	8
mean	0.9	0.8	0.8	0.7	0.8	0.7	0.9	0.7	0.9	0.7	0.9	0.8	0.9	0.8	0.6	0.9
sd	0.8	0.6	0.6	0.6	0.6	0.5	0.5	0.5	0.6	0.4	0.6	0.6	0.5	0.5	0.7	0.6
k_L 95%*	2.5	2.9	3.1	3.4	3.1	3.0	3.4	3.2	3.0	3.0	3.0	3.6	2.8	3.1	3.0	3.4
k_L 50%*	1.7	1.7	1.7	1.8	1.7	1.7	1.8	1.8	1.7	1.7	1.7	1.8	1.7	1.7	1.7	1.8
HC5 95%	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.2	0.2	0.1	0.2	0.2	0.0	0.1
HC5 50%	0.3	0.6	0.6	0.5	0.6	0.7	0.8	0.7	0.8	0.8	0.8	0.6	0.9	0.8	0.2	0.7

	Set 16	Set 17	Set 18	Set 19	Set 20	Set 21	Set 22	Set 23	Set 24	Set 25	Set 26	Set 27	Set 28	Set 29	Set 30
count	10	11	7	8	14	11	9	8	7	11	13	10	11	10	10
mean	0.9	0.7	0.8	0.8	0.8	0.8	0.8	0.7	1.0	1.0	0.8	1.0	0.9	0.8	0.7
sd	0.6	0.8	0.5	0.6	0.5	0.5	0.6	0.9	0.5	0.6	0.8	0.5	0.6	0.6	0.8
k_L 95%*	3.1	3.0	3.6	3.4	2.7	3.0	3.2	3.4	3.6	3.0	2.8	3.1	3.0	3.1	3.1
k_L 50%*	1.7	1.7	1.8	1.8	1.7	1.7	1.8	1.8	1.8	1.7	1.7	1.7	1.7	1.7	1.7
HC5 95%	0.2	0.0	0.1	0.1	0.2	0.3	0.1	0.0	0.1	0.2	0.1	0.3	0.1	0.1	0.0
HC5 50%	0.9	0.2	0.8	0.7	0.7	1.1	0.6	0.2	1.0	1.0	0.3	1.3	0.8	0.5	0.2

* k_L values sourced from (Aldenberg & Slob, 1993)

Appendix 3.1 (contd.): HC5 calculations for taxa with L(EC)50 data from 60 communities (see Section 3.2.2 for community selection criteria.

	Set 31	Set 32	Set 33	Set 34	Set 35	Set 36	Set 37	Set 38	Set 39	Set 40	Set 41	Set 42	Set 43	Set 44	Set 45
count	7	7	6	10	6	8	6	6	6	6	7	7	6	8	6
mean	0.7	0.4	0.6	0.9	0.6	0.7	0.9	0.9	0.9	0.9	0.9	0.9	0.8	0.9	0.9
sd	0.4	0.7	0.3	0.5	0.3	0.4	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.5	0.6
k_L 95%*	3.6	3.6	3.9	3.1	3.9	3.4	3.9	3.9	3.9	3.9	3.6	3.6	3.9	3.4	3.9
k_L 50%*	1.8	1.8	1.8	1.7	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
HC5 95%	0.2	0.0	0.3	0.3	0.3	0.3	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1
HC5 50%	1.1	0.1	1.1	1.1	1.1	1.2	0.8	0.8	0.8	0.8	0.8	0.8	0.6	0.9	0.8

	Set 46	Set 47	Set 48	Set 49	Set 50	Set 51	Set 52	Set 53	Set 54	Set 55	Set 56	Set 57	Set 58	Set 59	Set 60
count	6.0	7.0	8.0	8.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	7.0	8.0	6.0	6.0
mean	0.8	0.7	0.7	0.8	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.7	0.7	0.6	0.7
sd	0.6	0.4	0.3	0.4	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.4	0.3	0.3	0.4
k_L 95%*	3.9	3.6	3.4	3.4	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.6	3.4	3.9	3.9
k_L 50%*	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
HC5 95%	0.0	0.2	0.4	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.4	0.2	0.1
HC5 50%	0.6	1.1	1.2	1.1	0.8	0.8	0.8	0.8	0.8	0.8	0.8	1.1	1.2	1.0	0.8

* k_L values sourced from (Aldenberg & Slob, 1993)

Appendix 4.1: Total counts of macroinvertebrates in stream mesocosms stocked from the River Wheeler (WH), the River Tame (TA) and the River Wye (WY). Counts from 12 sediment samplers were pooled to obtain these data.

Stream	TA1	TA1	TA1	TA1	TA1	TA1	TA2	TA2	TA2	TA2	TA2	TA2	TA3	TA3	TA3	TA3	TA3	TA3
Sample Day	**0**	**14**	**28**	**42**	**56**	**70**	**0**	**14**	**28**	**42**	**56**	**70**	**0**	**14**	**28**	**42**	**56**	**70**
Tricladida										2	1	2						
Polycelis sp.																2	1	
Ancylus fluviatilis									1									
Physa fontinalis										1		1						
Segmentina complanata			1															1
Sphaeriidae												1						
Lumbricidae							3	1	5	3	6	5						
Oligochaeta (other)	14	31	40	36	35	28	46	50	34	59	63	80	23	30	40	45	39	26
Erpobdella octoculata	52	66	57	48	72	73	62	99	57	59	88	56	55	63	57	50	60	57
Glossiphonia complanata							1			1							1	
Haemopis sanguisuga												1						
Asellus aquaticus	45	93	68	49	64	147	32	65	50	49	81	77	43	79	48	40	56	108
Gammarus pulex	5	1	5	5	3	2	3	1	1	1		1	1	3			1	2
Baetis rhodani	1	1	1		1	1	4		1				3		1			
Ecdyonurus dispar			2	1			1		2	1	2						1	
Ecdyonurus sp.	1						1											
Ephemerella ignita								1							1	2		
Nemoura avicularis											1							
Nemoura sp.													1					
Protonemura sp.					2				1									
Elmis aenea					1													
Esolus parallelipipidus								1			1							
Glossosoma boltoni								1										
Hydropsyche pellucidula			2	1	3	1	3	3		6	5	6		1	1	1		3
Hydropsyche siltalai	3	2	3	2	3	5	2			3	2		2	3	3	3	4	4

Appendix 4.1 continued: Total counts of macroinvertebrates in stream mesocosms stocked from the River Wheeler (WH), the River Tame (TA) and the River Wye (WY). Counts from 12 sediment samplers were pooled to obtain these data.

Stream	**TA1**	**TA1**	**TA1**	**TA1**	**TA1**	**TA1**	**TA2**	**TA2**	**TA2**	**TA2**	**TA2**	**TA2**	**TA3**	**TA3**	**TA3**	**TA3**	**TA3**	**TA3**
Sample Day	**0**	**14**	**28**	**42**	**56**	**70**	**0**	**14**	**28**	**42**	**56**	**70**	**0**	**14**	**28**	**42**	**56**	**70**
Potamophylax latipennis		2																
Rhyacophila dorsalis	5	1	3	2	4	1		4	3	4	3	1	2	2		2	3	1
Ceratopogonidae				1				1			1			1	1			
Chironomidae	2	82	115	160	221	204	14	46	86	121	94	73	3	60	107	75	102	93
Dicranota sp.		2	1		2	2									1	3	1	
Empididae								1			1							
Simulidae				1			1	1										
no. of individuals	128	281	298	306	411	464	173	275	241	308	348	302	133	242	261	223	269	295
no.of species	9	10	12	11	12	10	13	14	11	12	13	11	9	9	10	10	11	9

Appendix 4.1 continued: Total counts of macroinvertebrates in stream mesocosms stocked from the River Wheeler (WH), the River Tame (TA) and the River Wye (WY). Counts from 12 sediment samplers were pooled to obtain these data.

Stream	WH 1	WH 1	WH 1	WH 1	WH 1	WH 1	WH 2	WH 2	WH 2	WH 2	WH 2	WH 2	WH 3	WH 3	WH 3	WH 3	WH 3	WH 3
Sample day	0	14	28	42	56	70	0	14	28	42	56	70	0	14	28	42	56	70
Tricladida							1			1						1		
Polycelis sp.			1				1	2				1	1		1	1		
Ancylus fluviatilis	2	2		3	4	1	2	2	1	5		2		1	2	1		1
Physa sp.			1	1		1												
Potamopyrgus jenkinsi								1						1	1	2		
Pisidium									1				1			1	2	
Lumbricidae								1	1	3	7	3	1			3	5	3
Oligochaeta (other)	92	60	80	85	80	62	153	155	98	165	121	99	100	122	80	77	100	72
Erpobdella octoculata	1	1					2				1				1	1		
Asellus aquaticus		2	1			1					2		1		1		1	1
Gammarus pulex	37	36	50	41	32	45	42	31	42	50	35	58	22	29	23	14	18	22
Baetis rhodani	9	2					7	1					3	1				
Ecdyonurus dispar	1	5	12	5	1	6	4	8	5	2	3	2		1	4	1	2	2
Isoperla grammatica	1				3	2	3	2	1	3	2	6	1	3	1		1	
Leuctra fusca				1														
Leuctridae										1								
Nemoura sp.				2	1													
Protonemura montana	1																	
Elmis aenea	6	5	7	8	2	16	8	9	7	6	6	10	4	1	6	5	4	6
Esolus parallelipipidus													1	1			1	
Hydraena gracilis											2							
Limnius volckmari	9	8	16	9	19	16	13	6	11	13	13	13	6	6	7	11	13	12

Appendix 4.1 continued: Total counts of macroinvertebrates in stream mesocosms stocked from the River Wheeler (WH), the River Tame (TA) and the River Wye (WY). Counts from 12 sediment samplers were pooled to obtain these data.

Stream	WH 1	WH 1	WH 1	WH 1	WH 1	WH 1	WH 2	WH 2	WH 2	WH 2	WH 2	WH 2	WH 3	WH 3	WH 3	WH 3	WH 3	WH 3
Sample day	**0**	**14**	**28**	**42**	**56**	**70**	**0**	**14**	**28**	**42**	**56**	**70**	**0**	**14**	**28**	**42**	**56**	**70**
Agapetus fuscipes															1			
Hydropsyche instabilis		4	8	12	4	15	9	8	13	12	10	16	7	6	6	8	5	8
Hydropsyche siltalai								1										
Hydropsyche sp.	4	4																
Limnephilidae							1						1	1				
Rhithrogena semicolorata	46	9	44	52	25	8	27	5	19	20	12	6	16	7	4	12	10	8
Rhyacophila sp.				1														
Rhyacophila dorsalis	2	3		3	2		1	1	2	1	1	2	2		2	1		
Sericostoma personatum				2														
Silo spp							3											
Silo pallipes	4	4	3	1	4	2		4	1	3	2	2	3	3	2	3	4	6
Chironomidae	3	55	73	71	106	74	4	42	37	82	80	70	3	55	64	96	78	71
Dicranota sp.	7	8	9	9	14	6	8	8	12	6	9	2	5	4	7	11	9	5
Diptera							1					1			1			
Tipulidae									1									
no. of individuals	**225**	**208**	**305**	**306**	**297**	**255**	**289**	**287**	**252**	**372**	**306**	**293**	**178**	**242**	**214**	**248**	**253**	**217**
no.of species	**16**	**16**	**13**	**17**	**14**	**14**	**18**	**18**	**16**	**15**	**16**	**16**	**18**	**16**	**19**	**17**	**15**	**13**

Appendix 4.1 continued: Total counts of macroinvertebrates in stream mesocosms stocked from the River Wheeler (WH), the River Tame (TA) and the River Wye (WY). Counts from 12 sediment samplers were pooled to obtain these data.

Stream	**WY1**	**WY1**	**WY1**	**WY1**	**WY1**	**WY1**	**WY2**	**WY2**	**WY2**	**WY2**	**WY2**	**WY2**
Sample day		**14**	**28**	**42**	**56**	**70**	**0**	**14**	**28**	**42**	**56**	**70**
Polycelis sp.	61	33	42	42	2	25	69	94	64	232	75	58
Ancylus fluviatilis	2		1	1	2		2	1	2	4	2	
Potamopyrgus jenkinsi	1									2		
Segmentina complanata								1				
Pisidium									1		1	
Lumbricidae							4	5	5	3	2	5
Oligochaeta (other)	67	82	125	189	133	141	138	227	156	344	420	163
Erpobdella octoculata		1	1	1	1	1						1
Glossiphonia complanata	4						1	2	2	1		
Hydracarina	2						1	1			1	
Asellus aquaticus		1			1			1	1	2	1	1
Gammarus pulex	559	530	489	340	421	369	828	791	739	709	725	626
Baetis rhodani							2		1			
Elmis aenea				1		1						1
Agapetus fuscipes	3	2	1	1	1		3	4	2	4	1	3
Limnephilidae							2					
Potamophylax latipennis				1					1	1		
Rhyacophila dorsalis	1	1	3	1		1	2	1	4	2	2	1
Chironomidae	4	5	9	42	30	21	3	14	26	74	63	46
Dicranota sp.				1					1			
Empididae									1			
Tipulidae		1								1	1	
no. of individuals	**704**	**656**	**671**	**620**	**591**	**559**	**1055**	**1142**	**1006**	**1379**	**1294**	**905**
no.of species	**10**	**9**	**8**	**11**	**8**	**7**	**12**	**12**	**15**	**13**	**12**	**10**

Appendix 5.1: Practitioners report for the analysis of LAS samples from the experimental stream study.

THE EFFECTS OF LAS ON TWO DISTINCT COMMUNITIES IN AN EXPERIMENTAL STREAM FACILITY

Date Started: 19/04/1999

Date Practical Work Completed: 01/02/2000

Date Reported: 13/07/2000

Contents Include:

(i) Analytical Practitioner Report

(ii) COSHH Risk Assessments

(iii) Sediment Weights

(iv) Preparation Of Test/Reference Stock Standards

(v) Calculations for homologue distribution in test samples

Analytical Supervisor: **M Burford**

Analyst: **P Blanco**

ANALYTICAL PRACTITIONER REPORT

THE EFFECTS OF LAS ON TWO DISTINCT COMMUNITIES IN AN EXPERIMENTAL STREAM FACILITY

1. Analytical Methodology

Three types of sample were provided for analysis for this study, namely stream, interstitial water and sediment samples.

Stream Samples

On each sampling day of the study test samples from the artificial streams were obtained in either glass measuring cylinders or the appropriate glass vials. For the 0 and 0.8 mg/l test samples clean 50 ml measuring cylinders were used to collect 50-ml samples. For the 2.5 and 8.0 mg/l test samples 20 and 7 ml glass vials were used to collect 20 and 6 ml samples respectively. All the samples were supplied slightly in excess, so that the exact volume could be manually adjusted by the analyst on receipt of the samples.

On days 0, 3, 7, and 10 single aliquots and days 13, 17, 20, 24 and 27 duplicate aliquots of the test samples were obtained from the top of the stream riffle sections. On Days 3, 17 and 27 of the study test samples were also obtained from the bottom of the riffle sections, the day 3 being undertaken as a single aliquot and day 17 and 27 undertaken in duplicate. All the samples were preserved with 3% v/v of a 40% formaldehyde solution and stored at the SEAC Environment Centre or the D1 refrigerator prior to analysis [a].

Interstitial Water Samples

On Days 3, 13 and 27 of the study duplicate 40 ml samples of interstitial water (namely water taken at a depth of ca., 6 cm within the channel sediments) from each of the streams were collected in 40 ml EPA vials. An additional interstitial water was obtained from one of the control streams on each of the sampling days and this was used to determine the analytical recovery of the method. All the samples were preserved with 3% v/v of a 40% formaldehyde solution and stored at the SEAC Environment Centre or the D1 refrigerator prior to analysis [a].

Sediment Samples

On Days 13 and 27 of the study twenty four sediment samples were collected in plastic bags. These samples were stored at ca., -15°C prior to analysis [a].

SEAC Environment pond water

A 1 litre aliquot of SEAC Environment pond water was obtained on each sampling day of the study, preserved and stored at ca., 4°C prior to analysis [a]. This sample was used in triplicate for the analytical recovery determination.

1.1 Sample Concentration / Extraction Procedure for Stream Samples

For the 0.0 and 0.8 mg/l test samples the 50 ml sample volume was applied directly from the 50 ml measuring cylinder to methanol/water pre-conditioned C_{18} SPE (1g/6ml) cartridges and allowed to pass through at a flow rate of 1-2 ml/min. However, for day 0 and 3 of the study, the 0.0 and 0.8 mg/l stream samples were stored in 100 ml glass bottles due to shortage of 50 ml stoppered measuring cylinders. The required volumes of these stream samples were transferred into clean 50 ml measuring cylinders and the excess material in the sample bottle discarded. The test sample was then transferred to the conditioned C_{18} cartridge. For all the 0.0 and 0.8 mg/l test samples the measuring cylinders, SPE reservoirs and C_{18} cartridges were rinsed with 5 ml Millipore Q water and the SPE cartridges then washed with 2 ml 80:20 water/methanol. The SPE cartridges were air dried under vacuum [b,c].

For the 2.5 and 8.0 mg/l stream samples an EDP pipette was used to transfer the 20 ml and 6 ml sample volumes test volume from the 20 ml and 7 ml vials to the conditioned C_{18} cartridge [b]. However, for day 0 and 3 of the study, the 2.5 and 8.0 mg/l stream samples were stored in error in 100 ml glass bottles instead of the 20 ml and 7 ml vials respectively. For all 2.5 and 8.0 mg/l test samples the EDP pipette tips were retained for the following SAX SPE step. The vials (except for day 0 and 3), SPE reservoirs and C_{18} cartridges were rinsed with 5 ml Millipore Q water and the SPE cartridges then washed with 2 ml 80:20 water/methanol. The SPE cartridges were air dried under vacuum [b,c].

Each C_{18} SPE cartridge was connected to the top of a hexane/methanol pre-conditioned SAX (500mg/3ml) SPE cartridge. For the 0.0 and 0.8 mg/l test samples 2 x 10 ml aliquots of methanol were used to wash the 50 ml measuring cylinder, and these washings were then used to elute the corresponding LAS from the C_{18} cartridge onto the SAX cartridge. Though for day 0 and 3, 0.0 and 0.8 mg/l test samples the

100 ml glass bottle used to transport the sample also needed to be washed with two aliquots of methanol.

For the 2.5 and 8.0 mg/l test samples 2 x 5 ml aliquots of methanol were used to wash the 20 and 7 ml glass vials using the corresponding EDP tip which was used to measure the original test sample [b]. These washings were transferred to the C_{18} cartridge to elute the LAS onto the SAX cartridge. Though for day 0 and 3, 2.5 and 8.0 mg/l test samples only the corresponding EDP tips were washed with the methanol aliquots. The 100 ml glass bottles used to transport these test samples was not extracted with methanol.

The methanol was allowed to pass through the SAX cartridges at a flow rate of 1-2 ml/min and the cartridges air dried under vacuum. The C_{18} cartridges were removed and the SAX cartridges eluted with 3 ml of methanolic HCl directly into a 10 ml glass vials. The samples are then evaporated to dryness at 75°C under a stream of nitrogen before being resuspended in 1 ml of methanol and transferred to 2 ml autosampler vials.

To determine percent LAS recovery from SEAC Environment pond water, 50 µg of LAS (0.5 ml of the 100 mg/l LAS solution) was added to 50 ml of pond water [b]. The spiked pond water was then extracted as outlined above and the procedure was undertaken in triplicate.

1.2 Sample Concentration / Extraction Procedure for the Interstitial Water

The 40 ml EPA vials containing the interstitial water samples were placed in a Heraeus Megafuge and centrifuged at 3500 rpm for 10 minutes [d]. The resulting supernatants were decanted into SPE reservoirs connected to pre-conditioned C_{18} cartridges and the samples extracted as outlined for the stream samples.

The sediment in the vials was sonicated with 20 ml of methanol for 1 hour. The methanol extracts were then carefully decanted onto pre-conditioned C_{18}/SAX cartridges attached to each other and drawn through the cartridges under vacuum until the methanol had passed through the cartridges. The sediments were sonicated with two additional 20-ml aliquots of methanol for 30 minutes. These sequential extracts were decanted to the C_{18}/SAX cartridges and drawn through the systems.

The C_{18} cartridges were removed and the SAX cartridges and eluted as described for the stream samples [e].

In error the day 3 interstitial water samples were supplied in glass bottles and had to be transferred to 40 ml vials for sample preparation. In this case a 40 ml aliquot of the shaken sample was transferred to a 40 ml EPA vial via a measuring cylinder and centrifuged as described above. The remaining sample in the glass bottle was discarded and the bottles and measuring cylinders rinsed with 2 × 10 ml aliquots of methanol. The resulting methanol aliquots were used to elute the LAS from the supernatant on the C_{18} cartridge to the SAX column [d].

An additional interstitial water sample from one of the control streams was obtained for a spiking recovery experiment. The sample was centrifuged as described above and the resulting supernatant spiked with 50 μg LAS (0.5 ml of the 100 mg/l aqueous LAS stock) and extracted as outlined for the stream samples. The resulting sediment was spiked with 50 μg of LAS and extracted as described above [b,d].

1.3 Sample Concentration / Extraction Procedure for Sediment Samples

The sediment samples were spread over evaporation dishes and placed in an oven at 80°C. When dry, the samples were removed from the oven and allowed to cool. Note as the sediment samples consisted of stones and pebbles they were not ground up in a pestle and mortar and sieved.

A pre-weighed, methanol washed cellulose acetate extraction thimble was filled with ca., 55 g of dried sediment. A spiked sample was prepared by pipetting 100 μg of LAS (1 ml of the 100 mg/l LAS aqueous spiking solution) into an extraction thimble containing a control sediment, i.e. sediment from the 0 mg/l stream [b].

The thimble containing the sample was extracted in a Soxhlet extractor with 200 ml methanol for approximately 6 hours. After extraction the Soxhlet extractor was removed and rinsed with methanol, which was allowed to run into the respective methanol extract.

The Soxhlet extracts were evaporated to ca., 2 – 4 ml using a rotary evaporator and resuspended in 100 ml of 95:5 water/methanol. These sample were analysed using the SPE extraction procedure undertaken for the stream water samples.

1.4 Calibration Standards

A set of Nansa HS 80/S calibration standards containing 0, 15, 30, 45, 60, and 75 mg/l Nansa HS 80/S were prepared by diluting 0.0, 1.5, 3.0, 4.5, 6.0, and 7.5 ml of the 1000 mg/l methanol working solution to 100 ml with methanol. Each standard was transferred to a HPLC vial in duplicate [b].

All test samples, spikes and standards were then analysed by High Performance Liquid Chromatography (HPLC) together with 45 mg/l Nansa HS 80/S standard checks under the following conditions:-

Injector	:	Kontron 360A Autosampler [f]
		Injection volume 20 ul
Pump	:	Perkin Elmer Series 200 [g]
		Eluent 22:78 water/methanol containing 0.0875M sodium perchlorate
		Flow 2.0 ml/min
Column Heater	:	7990 column space heater [h]
		Temperature set at 35°C
Guard Column:	Packed with BondaPak C18 Corasil 2mm id × 2cm	
Column	:	uBondaPak C18 10 um 300 mm × 3.9 mm
Detector	:	Perkin Elmer LC240 fluorescence [i]
		λ excitation 232 nm, λ emission 290 nm
Data Handling	:	Perkin Elmer Turbochrom [j]

2. Results

2.1 Calibration Data for Stream, Interstitial and Sediment Samples

A calibration curve for Nansa HS 80/S was produced for each analysis day of the Study. The results are given in Table 1 below.

Table 1 Gradient and Correlation Coefficient Data for All Samples

Day	Slope	R^2
0, 3	9581.826417	0.9982
3, 3 (Int.) 7	10173.228278	0.9955
7, 10, 13	9684.676808	0.9943
13, 13 (Int.& Sed.), 17	10790.181057	0.9924
13 (Sed.)	9437.061075	0.9966
20	9943.772763	-
20, 24	10450.484452	0.9993
27	13257.904972	0.9978
27	14711.004753	0.9993
27	12849.133527	0.9991
13 (Sed.), 27 (Sed./Int.)	15042.214081	0.8932

Sed. = Sediment samples

Int. = Interstitial water samples

Note, following the upgrade of the Turbochrom Data handling system to version 6 all calibration data for Day 20 were, in error, deleted on transfer to the G drive. Therefore to quantify all Day 20 and Day 17 stream (bottom of the riffle) samples, an average slope value of Days 13 (Sed.) and 24 was used. These slope values were selected from analysis runs immediately before (Day 13 data acquisition date 13/10/1999) and after (Day 24 data acquisition date 22/10/1999) the Day 20 analysis run (data acquisition date 21/10/1999).

A linear regression was applied to each data set to obtain a calibration equation in the form of

$$x = y / m$$

where

x is the raw amount (i.e. conc. of Nansa HS 80S in vial, mg/l)

y is the peak area

m is the slope of the line

2.2 Standard Check Data

The precision of the method was assessed by the repeatability of injecting the 45-mg/l calibration standard at regular intervals throughout each sequence run. The measured concentrations of LAS in the standard checks expressed as raw amount (:g/ml) are given in Table 2 below. Raw amount values are displayed in the order they were injected through the sequence.

Table 2 Measured Concentration of Nansa HS80/S in Standard Checks

Sample day analyzed in sequence	Raw Amount (:g/ml)	Avg. ± SD (RSD)	Variation from nominal value (%)
0, 3	43.99		
	43.01		
	43.48		
	43.58	43.5 ± 0.4 (0.9)	3.3
3, 3 (Int.) 7	44.07		
	43.92		
	43.31		
	44.87		
	43.90	44.0 ± 0.6 (1.4)	2.2
7, 10, 13	43.41		
	45.46		
	45.82		
	43.60		
	44.82	44.6 ± 1.1 (2.5)	0.8
13, 13 (Int.& Sed.), 17	42.25		
	43.37		
	43.55		
	41.98		
	42.48		
	43.53		
	42.01	42.7 ± 0.7 (1.6)	5.0
13 (Sed.)	45.32		
	44.86	45.1 ± 0.3 (0.7)	0.2
20	46.38		
	46.58		
	45.53		
	44.98		
	44.45		
	44.55		
	45.95	45.5 ± 0.9 (2.0)	1.1
20, 24	43.81		
	47.55		
	49.24		
	42.78	45.8 ± 3.0 (6.5)	1.9

Table 2 (contd.) Measured Concentration of Nansa HS80/S in Standard Checks

Sample day analyzed in sequence	Raw Amount (:g/ml)	Avg. ± SD (RSD)	Variation from nominal value (%)
27	43.93		
	42.13		
	43.15		
	41.45		
	41.56		
	42.06	42.4 ± 1.0 (2.4)	5.8
27	No Standard Checks[a]	-	
13 (Sed.), 27 (Sed./Int.)	42.38		
	43.65		
	43.73		
	50.99		
	46.73		
	47.08		
	51.23	46.5 ± 3.6 (7.7)	3.4

[a]Sequence run consisted of only four samples and therefore no standard checks were performed.

SD = Standard deviation

RSD = Relative standard deviation

All the 45 mg/l calibration standard checks results were within 6 % of the nominal concentration value with an analytical repeatability of ca., 8 %. The method is therefore assessed as fit for purpose.

2.3 Recovery and Measured Concentration Data of Stream Samples

The accuracy of the extraction method used for the stream samples was assessed by performing spike recoveries in the test media for each study day. The percent recovery in SEAC Environment pond water (triplicate) was determined based a 50 :g Nansa HS 80S spike in a 50 ml sample volume. The results are given in Table 3. Note, all the recovery checks were calculated after subtraction of Pond Water blank results.

The results show that a quantitative recovery of the Nansa HS 80S was achieved, with the average recovery from the pond water over the whole study being 101.6 ± 4.8 %. This was assessed as quantitative, so no analytical recovery correction was made to the measured Nansa HS 80/S concentrations.

Table 3 Percent Recovery of Nansa HS 80/S in Stream Samples

Day	Environment Centre Pond Water (% Recovery)[a]			
	Spk 1	Spk 2	Spk 3	*Avg.*
0	90.8	93.3	95.3	*93.1*
3	98.9	93.6	98.0	*96.8*
7	101	98.6	104	*101*
10	103	107	103	*104*
13	104	106	DD	*105*
17[b]	106	110	112	*109*
20	103	103	98.3	*101*
24	104	103	108	*105*
27	99.8	101	DD	*100*

[a] % Recovery = $\frac{\text{Raw Amount } (\mu g) \text{ in 'spiked' sample - Raw Amount } (\mu g) \text{ in 'control' sample}}{\text{Spike amount } (\mu g) \text{ in sample volume}} \times 100$

[b] % Recovery results for Day 17 were calculated using the average slope value as determined in Table 1 for Day 20.

DD = Data Deleted. Following the upgrade of the Turbochrom Data handling system to version 6 the data for these samples was, in error, deleted on transfer to the G drive.

2.4 Test Sample Concentrations

Test samples were supplied for analysis from the bottom and top of the riffle section in the artificial streams. The measured Nansa HS 80/S test concentrations for the individual streams are given in Table 4 and the corresponding average concentrations are given in Table 5.

Table 4 Measured Concentrations of Nansa HS 80/S in Stream Samples (mg/l)

Stream No. (aliquot number)	Nom. Nansa Conc. (mg/l)	Measured Concentrations (mg/l) at top or bottom of stream											
		Day 0 Top	Day 3 Top	Day 3 Bottom	Day 7 Top	Day 10 Top	Day 13 Top	Day 17 Top	Day 17 Bottom	Day 20 Top	Day 24 Top	Day 27 Top	Day 27 Bottom
S3 (01)	0	<0.01	<0.01	0.05	<0.01	0.01	<0.01	<0.01	<0.01	0.05	<0.01	<0.01	0.01
S3 (02)	0	N/S	N/S	N/S	N/S	N/S	<0.01	<0.01	<0.01	0.03	<0.01	0.01	<0.01
S8 (01)	0	0.02	0.01	<0.01	<0.01	<0.01	0.8	<0.01	<0.01	0.04	0.06	0.01	<0.01
S8 (02)	0	N/S	N/S	N/S	N/S	N/S	0.06	<0.01	<0.01	0.03	0.03	<0.01	0.01
S1 (01)	0.8	0.6	0.7	0.6	0.8	0.7	0.6	0.6	0.5	0.6	0.5	0.5	0.4
S1 (02)	0.8	N/S	N/S	N/S	N/S	N/S	0.6	0.5	0.5	0.6	0.6	0.5	0.4
S5 (01)	0.8	0.7	0.6	0.6	0.6	0.7	0.6	0.7	0.6	0.7	0.7	0.6	0.5
S5 (02)	0.8	N/S	N/S	N/S	N/S	N/S	0.7	0.7	0.6	0.7	0.8	0.6	0.5
S2 (01)	2.5	2.0	1.5	1.6	2.2	1.8	1.9	2.0	1.7	1.8	1.9	1.6	1.5
S2 (02)	2.5	N/S	N/S	N/S	N/S	N/S	1.8	1.9	1.7	1.9	2.0	1.7	1.6
S6 (01)	2.5	1.9	1.9	1.8	2.4	2.0	2.5	1.9	1.7	1.8	2.0	1.6	1.3
S6 (02)	2.5	N/S	N/S	N/S	N/S	N/S	1.9	1.9	1.8	1.7	2.0	1.6	1.5
S4 (01)	8.0	6.7	5.2	6.5	6.0	5.1	4.8	5.7	5.8	3.3	5.0	4.5	4.3
S4 (02)	8.0	N/S	N/S	N/S	N/S	N/S	5.6	5.6	5.8	4.7	5.6	3.9	4.1
S7 (01)	8.0	7.7	6.1	6.9	6.4	6.7	6.7	6.3	6.1	4.5	5.6	5.1	4.7
S7 (02)	8.0	N/S	N/S	N/S	N/S	N/S	6.3	6.5	4.9	4.3	5.5	4.6	4.8

The detection limit of the analysis is 0.01 mg/l

N/S = no sample supplied for analysis

It can be seen in Table 5 that the exposure concentration in the top riffle section of the streams was about 63 to 85% of the nominal Nansa value. A slight decrease was observed in these concentrations over the course of the study, particularly with the higher anionic dose concentrations. See Table 4. However, overall, the amount of Nansa present remained relatively constant over the 27-day period as the measured concentrations had relative standard deviations in the region of 10 to 20% (excluding the control samples). Furthermore the anionic content in the streams was maintained as there was only ca., an 11% decrease in the Nansa concentration between the top and bottom riffle sections. See Table 5.

Table 5 Average Measured Concentrations of Nansa HS 80/S in Stream Samples

Stream No. (aliquot number)	Nominal Nansa HS 80/S (mg/l)	Top of riffle section in stream		Bottom of riffle section in stream		Percentage decrease in Nansa from top to bottom
		Measured Nansa HS 80/S mg/l ± SD (RSD)	Percentage of nominal value (%)	Measured Nansa HS 80/S mg/l ± SD (RSD)	Percentage of nominal value (%)	
S3 (01)	0	0.01 ± 0.01 (209)	N/A	0.01 ± 0.02 (181)	N/A	N/A
S8 (01)	0	0.08 ± 0.21 (277)	N/A	<0.01 ± 0.00 (224)	N/A	N/A
S1 (01)	0.8	0.61 ± 0.09 (15)	76	0.48 ± 0.08 (17)	60	-21
S5 (01)	0.8	0.67 ± 0.07 (10)	83	0.56 ± 0.05 (10)	70	-16
S2 (01)	2.5	1.84 ± 0.19 (10)	74	1.62 ± 0.08 (5)	65	-12
S6 (01)	2.5	1.94 ± 0.28 (14)	78	1.62 ± 0.22 (13)	65	-17
S4 (01)	8	5.03 ± 0.91 (18)	63	5.30 ± 1.05 (20)	66	+5
S7 (01)	8	5.79 ± 1.04 (18)	72	5.48 ± 0.98 (18)	69	-5

N/A = not applicable

The detection limit of the analysis is 0.01 mg/l

SD = Standard deviation

RSD = Relative standard deviation

2.5 Recovery and Measured Concentration Data of Interstitial Water Samples

The percent recovery of Nansa HS 80/S from the interstitial water samples was assessed by spiking 50 :g Nansa into the supernatant and sediment from the 40 ml test sample. The results are in Table 6 and show that the recoveries are very variable from both the supernatant and sediment fractions of the test sample. The reasons for this are unclear as the extraction methodology is based on the same clean-up procedure as that used for the stream samples that were quantitative as shown in Table 3. The results therefore suggest the problem may be related to the spike procedure, in that it may not be representative or reproducible. Thus, the recovery values will not be used in this study and the measured Nansa concentrations in the interstitial water will be recorded 'as is'.

Table 6 Percent Recovery of LAS in Interstitial Water Samples

Day	% Recovery	
	Supernatant	Sediment
3	SL	SL
13	SL	247
27	47	4

[a] % Recovery = Raw Amount (μg) in 'spiked' sample - Raw Amount (μg) in 'control' sample × 100 / Spike amount (μg) in sample volume

SL = Sample Lost through spillage or vial breakage in centrifuge.

The measured Nansa HS 80/S concentration in the interstitial water samples and the corresponding percentage associated with the supernatant and sediment fraction are given in Table 7 and 8 respectively. It can be seen that the measured concentrations are relatively consistent between streams of the same nominal exposure concentration and that the levels in the interstitial water increase with corresponding surface water concentration. See Table 7. The majority (on average 75%) of the anionic was associated with these suspended solids in the interstitial waters, the remainder being present in the aqueous phase. See Table 8. This distribution generally seems independent of exposure concentration and sample day.

Table 7 Measured Concentrations of Nansa HS 80/S Corresponding to the Supernatant and Suspended Sediment in the Interstitial Water Samples

Stream No.	Nom. Nansa Conc. (mg/l)	Measured Concentrations (mg/l)[a]								
		Day 3			Day 13			Day 27		
		Super.	Sed.	Total	Super.	Sed.	Total	Super.	Sed.	Total
IS3	0.0	<0.01	<0.01	*<0.01*	SL	SL	*SL*	0.1	0.3	*0.4*
IS8	0.0	<0.01	<0.01	*<0.01*	1.2	5.6	*6.8*	0.03	0.1	*0.1*
IS1	0.8	0.3	0.6	*0.9*	0.1	0.5	*0.6*	0.2	1.0	*1.2*
IS5	0.8	0.3	0.7	*1.0*	0.1	0.4	*0.5*	0.3	1.2	*1.5*
IS2	2.5	1.2	2.3	*3.5*	0.2	2.1	*2.3*	1.4	3.3	*4.7*
IS6	2.5	1.0	2.6	*3.6*	0.3	1.5	*1.8*	SL	SL	*SL*
IS4	8.0	4.6	10.8	*15.4*	SL	SL	*SL*	1.6	5.3	*6.9*
IS7	8.0	5.1	8.9	*14.0*	1.3	3.2	*4.5*	2.5	4.0	*6.5*

[a] Measured Conc (mg/l) = $\frac{\text{Raw Amount } (\mu g)}{\text{Sample Vol. Taken (ml)}}$

The detection limit of the analysis is 0.01 mg/l

Super. = Supernatant

Sed. = Sediment. The concentration value of mg/l equates to sediment associated with a 1-litre interstitial sample volume. No attempt was made to determine the concentration based on the weight of the sediment.

Total = The sum of the measured concentration in the supernatant and sediment samples.

SL = Sample Lost through spillage or vial breakage in centrifuge.

Table 8 Percentage of Nansa 80/S Associated with the Supernatant and Sediment Fractions of the Interstitial Water

Stream No.	Nom. Conc. of stream (mg/l)	Percent Nansa associated with the supernatant and sediment fraction of the interstitial water (%)					
		Day 3		Day 13		Day 27	
		Super.	Sed.	Super.	Sed.	Super.	Sed.
IS3	0.0	N/A	N/A	N/A	N/A	25	75
IS8	0.0	N/A	N/A	18	82	23	77
IS1	0.8	33	67	17	83	17	83
IS5	0.8	30	70	20	80	20	80
IS2	2.5	34	66	9	91	30	70
IS6	2.5	28	72	17	83	N/A	N/A
IS4	8.0	30	70	N/A	N/A	23	77
IS7	8.0	36	64	29	71	38	62
Average ± SD		*32 ± 3*	*68 ± 3*	*18 ± 6*	*82 ± 6*	*25 ± 7*	*75 ± 7*

SD = Standard deviation

N/A = not applicable due to sample loss or concentration below the detection limit, i.e. <0.01 mg/l

By comparing the average Nansa values for the interstitial water with those of the corresponding surface water, it can be seen that the anionic concentration in the interstitial water is generally higher. However the interstitial water values are quite variable, so the two data sets given in Table 9 are not totally discrete. The data does suggest that there may be a concentration effect occurring in the interstitial water, mainly associated with the suspended matter. See Table 8 and 9.

Table 9 Average concentration of Nansa HS 80/S in the surface and interstitial water of the artificial streams

Nominal stream concentration (mg/l)	Average surface water stream concentration (mg/l ± SD)[A]	Average interstitial water stream concentration (mg/l ± SD)[B]
0.0	0.02 ± 0.04	0.12 ± 0.19
0.8	0.58 ± 0.08	0.95 ± 0.37
2.5	1.75 ± 0.16	3.18 ± 1.15
8.0	5.40 ± 0.32	9.46 ± 4.89

[A]Average value of top and bottom riffle section

[B]Average value of all the interstitial measurements for the corresponding exposure concentration

SD = Standard deviation

2.6 Recovery and Measured Concentration Data of Sediment Samples

The percent recovery of the Nansa HS 80/S from the sediment obtained from the bed of the artificial streams was determined based on a 100 :g spike. The results are given in Table 10 and demonstrate that the recoveries are quantitative and that the method is fit for purpose. No correction for analytical recovery was made to the corresponding measured Nansa concentrations in the sediment samples.

Table 10 Percent Recovery of LAS in Sediment Sample

Day	% Recovery[a]		Avg. % Recovery
	Replicate 1	Replicate 2	
13	103	80.4	91.7

[a] $\% \text{ Recovery} = \dfrac{\text{Raw Amount } (\mu g) \text{ in 'spiked' sample - Raw Amount } (\mu g) \text{ in Soxhlet blank}}{\text{Spike amount } (\mu g) \text{ in sample}} \times 100$

Note, in error, no percent recovery check was performed for day 27 samples.

The concentration of Nansa HS 80/S was measured in the artificial stream bed sediment and the results for the individual streams are given in Table 11 and the corresponding averages in Table 12. It can be seen that the anionic sediment concentration appears to be independent of the stream exposure concentration. Furthermore, the levels of anionic were relatively low and may reflect the low surface area of the sediment, which mainly consisted of large pebbles, rather than fine particulates.

Table 11 Measured Concentrations of Nansa HS 80/S in Dried Sediment Samples (:g/g)

Stream No.	Nom. Conc. (mg/l)	Day 13		Day 27	
		Bag no.	Measured Conc. (:g/g)[a]	Bag no.	Measured Conc. (:g/g)[a]
SS3	0.0	39	0.04	32	0.06
SS3	0.0	43	0.03	41	DD
SS8	0.0	39	0.03	32	0.53
SS8	0.0	43	0.04	41	0.77
SS8	0.0	44	DD	47	0.71
SS1	0.8	39	0.50	32	DD
SS1	0.8	43	0.15	41	DD
SS1	0.8	44	0.20	47	DD
SS5	0.8	39	0.22	32	0.14
SS5	0.8	43	0.15	41	0.29
SS5	0.8	44	0.15	47	0.08
SS2	2.5	39	0.32	32	0.48
SS2	2.5	43	0.35	41	0.87
SS2	2.5	44	BDL	47	DD
SS6	2.5	39	0.50	32	0.18
SS6	2.5	43	0.38	41	0.19
SS6	2.5	44	0.34	47	0.25
SS4	8.0	39	1.0	32	0.61
SS4	8.0	43	0.72	41	0.45
SS4	8.0	44	3.2	47	0.72
SS7	8.0	39	1.1	32	0.09
SS7	8.0	43	1.2	41	0.15
SS7	8.0	44	1.1	47	0.19

[a] Measured Conc (:g/g) = $\frac{\text{Raw Amount (:g)}}{\text{Wt. of Dried Sediment (g)}}$

DD = Data Deleted. Following the upgrade of the Turbochrom Data Handling system to version 6 these samples were in error deleted on transfer to the G drive.

BDL = below the detection limit of 0.1 ug injected on column

Table 12 Average Nansa concentration in the stream bed sediments

Nominal Nansa HS 80/S concentration in stream (mg/l)	Day 13 average measured Nansa HS 80/S in dried sediment (ug/g ± SD)	Day 27 average measured Nansa HS 80/S in dried sediment (ug/g ± SD)
0.0	0.04 ± 0.01	0.52 ± 0.32
0.2	0.23 ± 0.14	0.17 ± 0.11
2.5	0.32 ± 0.17	0.39± 0.29
8.0	1.39 ± 0.90	0.37± 0.26

SD = standard deviation

2.7 Nansa HS 80/S Homologue Distribution in Test Samples

The homologue distribution of the Nansa HS 80/S anionic was determined in terms of the C10, C11, C12 and C13 alkyl chain homologues that were resolved on the HPLC system. This alkyl chain distribution was assessed for the stream, interstitial and sediment test samples from the top exposure concentration (8.0 mg/l) obtained near the beginning of the study (Day 3) and at the end of the study (day 27). Note, that as no sediment samples were collected on Day 3, the sediments from Day 13 were used instead.

The results are given in Table 13 and 14 and show that throughout the study the alkyl chain distribution of Nansa HS 80/S in the top and bottom riffle section of the 8 mg/l streams is comparable to the anionic calibration standards. Thus the Nansa in the surface water of the streams was indistinguishable from the test material dosed into the system. Conversely, for the interstitial water there was a significant shift in the homologue distribution, with the more hydrophobic, higher alkyl chain homologues being the major components in the suspended sediment fraction, whereas the shorter alkyl chains were mainly associated with the supernatant or dissolved fraction of the sample. For the stream bed sediments a similar pattern of higher alkyl chain homologues was associated with the sample.

Table 13 **Homologue distribution of Nansa HS 80/S in the various test samples from the artificial streams obtained near the beginning of the study on day 3 and 13**

Sample description for Day 3 samples	Homologue Distribution (% ± SD[A])			
	C10	C11	C12	C13
45 mg/l STD[B]	18	41	29	12
8 mg/l stream – top[C]	19	41	28	12
8 mg/l stream – bottom[D]	18	41	28	12 ± 1
Supernatant interstitial water[E]	21 ± 1	42 ± 2	26 ± 1	11 ± 2
Sediment interstitial water[F]	4	21 ± 2	37	38 ± 3
Stream bed sediment (Day 13)[G]	4	22	37	37

[A]SD = standard deviation. Only standard deviations greater than 1 are reported

[B]Average of four 45 mg/l Nansa HS 80/S calibration standards run in the day 3 HPLC sequence

[C]Stream top samples D3/S4/T/01 – D3/S7/T/01

[D]Stream bottom samples D3/S4/B/01 – D3/S7/B/01

[E]Interstitial supernatant samples SNIW, D3/IS4/SN – D3/IS7/SN

[F]Interstitial sediment samples SDIW, D3/IS4/SD – D3/IS7/SD

[G]Stream bed sediments SBS (Day13), D13/SS4/01 (Bag 44) - D3/SS7/01 (Bag 43) - D3/SS7/01 (Bag 44)

Table 14 **Homologue distribution of Nansa HS 80/S in the various test samples from the artificial streams obtained at the end of the study on day 27**

Sample description for Day 27 samples	Homologue Distribution (% ± SD[A])			
	C10	C11	C12	C13
45 mg/l STD[B]	17	42	29	12
8 mg/l stream – top[C]	19	42	29	10
8 mg/l stream – bottom[D]	18	43	28	11
Supernatant interstitial water[E]	28	45	20	7
Sediment interstitial water[F]	5	26	37	32
Stream bed sediment[G]	10 ± 6	28 ± 13	32 ± 1	29 ± 18

[A]SD = standard deviation. Only standard deviations greater than 1 are reported

[B]Average of four 45 mg/l Nansa HS 80/S calibration standards run in the day 3 HPLC sequence

[C]Stream top samples, D27/8.0/S4/T/01, D27/8.0/S4/T/02, D27/8.0/S7/T/01, D27/8.0/S7/T/02

[D]Stream bottom samples, D27/8.0/S4/B/01, D27/8.0/S4/B/02, D27/8.0/S7/B/01, D27/8.0/S7/B/02

[E]Interstitial supernatant samples SNIW, D27/IS4/SN – D27/IS7/SN

[F]Interstitial sediment samples SDIW, D27/IS4/SD – D27/IS7/SD

[G]Stream bed sediment samples SBS, D27/SS4/02, D27/SS4/03, D27/SS7/01 - 03

References

[a]. Fridges, Freezers and Ovens (Ecotoxicology SOP 026 01)

[b]. EDP Plus Pipette (Ecotoxicology SOP 176 04)

[c]. Milli-Q Plus Water Purification System (Ecotoxicology SOP 204 03)

[d]. Heraeus Megafuge 1.0R (Ecotoxicology SOP 253 02)

[e]. Decon Ultrasonic Bath (Ecotoxicology SOP 203 06)

[f]. Kontron 360A Autosampler (Ecotoxicology SOP 238 03)

[g] Perkin Elmer Series 200 (Ecotoxicology SOP 218 02)

[h]. Jones Chromatography 7990 Column Space Heater (Ecotoxicology SOP 256 02) Serial No. 13202-B

[i]. Perkin Elmer LC 240 Fluorescence (Ecotoxicology SOP 233 02)

[j]. Perkin Elmer Turbochrom (Ecotoxicology SOP 208 03)

Appendix 5.2: Total macroinvertebrate counts for samples taken from the River Wheeler and the River Tame during the stocking phase.

Taxa	River Wheeler	River Tame
Bivalvia	19	1
Elmis aenea	84	1
Esolus parallelepipedus	6	0
Hydraena gracilis	1	0
Limnius volckmari	254	0
Oreodytes sanmarkii	0	2
Riolus subviolaceus	1	0
Asellus aquaticus	1	119
Gammarus pulex	455	20
Ceratopogonidae	0	90
Chironomidae	184	814
Diptera - unidentified	70	34
Simuliidae	9	0
Baetis sp.	425	1187
Ecdyonurus dispar	2	6
Ephemera danica	1	0
Ephemerella ignita	20	0
Mayfly- unidentified	1	0
Rhithrogena semicolorata	2068	2
Ancylus fluviatilis	12	6
Potamopyrgus jenkinsi	29	0
Erpobdella octoculata	5	206
Glossiphonia complanata	2	7
Piscicola geometra	1	0
Hydracarina	0	50
Sialidae	3	0
Oligochaeta	1463	4540
Isoperla grammatica	84	0
Leuctra fusca	4	0
Leuctra inermis	0	1
Nemoura cinerea	3	0
Plecoptera - unidentified	3	0
Protonemura praecox	2	6
Glossosomatidae	0	1
Hydropsyche instabilis	222	10
Hydropsyche siltali	1	15
Limnephilidae	7	0
Psychomyiidae	0	1
Rhyacophila dorsalis	24	14
Sericostoma personatum	19	0
Silo pallipes	7	0
Tinodes waeneri	0	2
Unidentified caseless caddis	8	3
Tricladida	23	17
Total individuals	**5523**	**7155**
Total species	**37**	**26**

Appendix 5.3: Total counts of macroinvertebrates in artificial streams stocked from the River Wheeler. Counts from 24 sediment samplers were pooled to obtain these data.

Dose (mg/l)	0.0 mg/l			0.8 mg/l			2.5 mg/l			8.0 mg/l		
Sample day	**-1**	**13**	**27**	**-1**	**13**	**27**	**-1**	**13**	**27**	**-1**	**13**	**27**
Physa sp.									1			
Sphaeriidae		2	9		3	5		2	9		1	3
Agabus sp.						1						
Coleoptera sp.						1						1
Elmis aenea	31	22	18	31	17	20	31	25	19	32	19	17
Esolus parallelepipidus		1	4		1	2	2		1		1	
Helophorus brevipalpis		1	2			3			2	1		3
Limnius volckmari	64	44	76	60	49	56	54	55	56	61	51	60
Oulimnius sp.											1	
Asellus aquaticus	1	10	4	2	4	9	1	17	3			
Gammarus pulex	12	89	24	19	115	43	4	57	31	46	2	
Ceratopogonidae	1							1	1			
Chironomidae	357	1742	1665	259	1826	1754	260	1133	721	521	1019	655
Dicranota sp.	3	8	7	1	3	3	3	6	1	1	5	2
Diptera		2	1			1		2	4	1	2	3
Empididae		6	8	3	1	2	7	3	2	1	3	2
Simuliidae		14			12		12	1		3	1	
Baetis rhodani	2									1		
Ephemera danica										1		
Ephemerella ignita	9	32	112	3	8	46	3	21	41	9	2	
Rhithrogena semicolorata	46	9		15	6		10		1	54		

Appendix 5.3 (contd.): Total counts of macroinvertebrates in artificial streams stocked from the River Wheeler. Counts from 24 sediment samplers were pooled to obtain these data.

Dose (mg/l)	0.0 mg/l			0.8 mg/l			2.5 mg/l			8.0 mg/l		
Sample day	**-1**	**13**	**27**	**-1**	**13**	**27**	**-1**	**13**	**27**	**-1**	**13**	**27**
Ancylus fluviatilis		1	3	1		2				1		
Gastropoda	1	1										
Potamopyrgus jenkinsi	8	11	26	9	8	15	5	4	8	1	1	2
Erpobdella octoculata	1	2		6	6	6	1		1			
Glossiphonia complanata			1					1		1		
Hydracarina				1						1		
Oligochaeta	12	37	35	27	66	89	65	49	70	45	2	5
Amphinemura sulcicollis										1		
Isoperla grammatica	1			1	1		1	2			1	
Leuctra hippopus		3	2			2			3	1		3
Leuctra sp.					1			1				
Hydropsyche instabilis	26	26	22	34	16	6	28	13	6	34	25	20
Lasiocephala basalis				1								
Limnephilidae							3					
Micropterna lateralis						1						
Rhyacophila dorsalis		1									1	
Rhyacophila obliterata							2					
Rhyacophila septentrionis								1				
Rhyacophila sp.				4	2							
Sericostoma personatum	4	9	2	3	6	11	1		4	2	4	3
Tricladida	2	1				2	1		2			
Total no. of individuals	**581**	**2074**	**2021**	**480**	**2151**	**2080**	**494**	**1394**	**987**	**819**	**1141**	**779**
Total no. of species	**18**	**24**	**19**	**19**	**20**	**23**	**20**	**19**	**22**	**22**	**18**	**14**

Appendix 5.4: Total counts of macroinvertebrates in artificial streams stocked from the River Tame. Counts from 24 sediment samplers were pooled to obtain these data.

Dose (mg/l)	0.0	0.0	0.0	0.8	0.8	0.8	2.5	2.5	2.5	8	8	8
Sample day	**-1**	**13**	**27**	**-1**	**13**	**27**	**-1**	**13**	**27**	**-1**	**13**	**27**
Sphaeriidae			6			1						1
Coleoptera sp.						1		4				1
Elmis aenea					1					1	1	
Helophorus brevipalpis			1			5		1	4			6
Hydroporinae												1
Limnius volckmari				1		1	1					
Asellus aquaticus	31	102	852	43	130	399	62	166	497	21	60	128
Gammarus pulex		1	6									
Ceratopogonidae	2			4			3	2	1	2		1
Chironomidae	430	1074	1516	408	1011	2249	370	1289	822	446	1834	1457
Dicranota sp.											1	
Diptera			1		1					2		13
Empididae	5	18	4	6	7	5	5	4	4	5	33	7
Simuliidae	15	8	13	9	8					9	30	5
Baetis rhodani	5			2			1			2	1	
Ecdyonurus sp.							1					
Ephemerella ignita		5	42		6	13		8	37			1
Potamopyrgus jenkinsi		1	3	1		4	1		1		5	6
Erpobdella octoculata	37	45	49	42	42	35	53	36	39	30	12	16
Glossiphonia complanata	3		1	3	1				1	1		3
Hirudinea sp.									1			
Hydracarina	1	1			1		5					
Oligochaeta	24	35	79	2	22	71	22	16	11	12	3	

Appendix 5.4 (contd.): Total counts of macroinvertebrates in artificial streams stocked from the River Tame. Counts from 24 sediment samplers were pooled to obtain these data.

Dose (mg/l)	**0.0**	**0.0**	**0.0**	**0.8**	**0.8**	**0.8**	**2.5**	**2.5**	**2.5**	**8**	**8**	**8**
Sample day	**-1**	**13**	**27**	**-1**	**13**	**27**	**-1**	**13**	**27**	**-1**	**13**	**27**
Amphinemura sulcicollis	2			2			3			1	1	
Leuctra sp.					1					1		
Hydropsyche pellucidula				3								
Hydropsyche siltalai	2	1	2		4	2	1	1	2	9	5	4
Rhyacophila dorsalis								1				
Rhyacophila septentrionis								1				
Rhyacophila sp.				2								
Tricladida	2	1		2	1		1					
Total no. of individuals	**559**	**1292**	**2575**	**530**	**1236**	**2786**	**529**	**1529**	**1420**	**542**	**1986**	**1650**
Total no. of species	**13**	**12**	**14**	**15**	**14**	**12**	**14**	**12**	**12**	**14**	**12**	**15**

Appendix 5.5: Total number of Chironomidae in each tribe or sub-tribe in 3 sediment samplers. 'Field' counts are the total number of Chironomidae added to each stream during the stocking phase.

Source	River Tame													River Wheeler												
Dose (mg/l)		0.0 mg/l			0.8 mg/l			2.5 mg/l			8.0 mg/l				0.0 mg/l			0.8 mg/l			2.5 mg/l			8.0 mg/l		
Sample day	Field	-1	13	27	-1	13	27	-1	13	27	-1	13	27	Field	-1	13	27	-1	13	27	-1	13	27	-1	13	27
Tanytarsini	55	9	12	5	0	39	73	47	130	160	109	50	28	6	34	17	6	52	60	63	20	36	86	89	54	31
Chironomini	13	13	25	24	18	7	5	39	89	10	15	16	126	52	41	42	15	56	86	64	18	63	9	20	3	54
Orthocladiinae	562	1	12	3	8	10	71	79	20	4	1	0	22	80	11	11	18	3	41	32	56	29	1	2	0	4
Tanypodinae	4	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Chironominae unidentified	0	0	0	0	0	6	1	9	3	3	1	1	2	0	0	0	0	0	0	0	0	1	0	0	0	0
Chironomidae unidentified	103	3	4	2	0	0	4	0	10	0	0	0	7	23	3	6	11	5	21	14	1	2	2	0	0	2

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