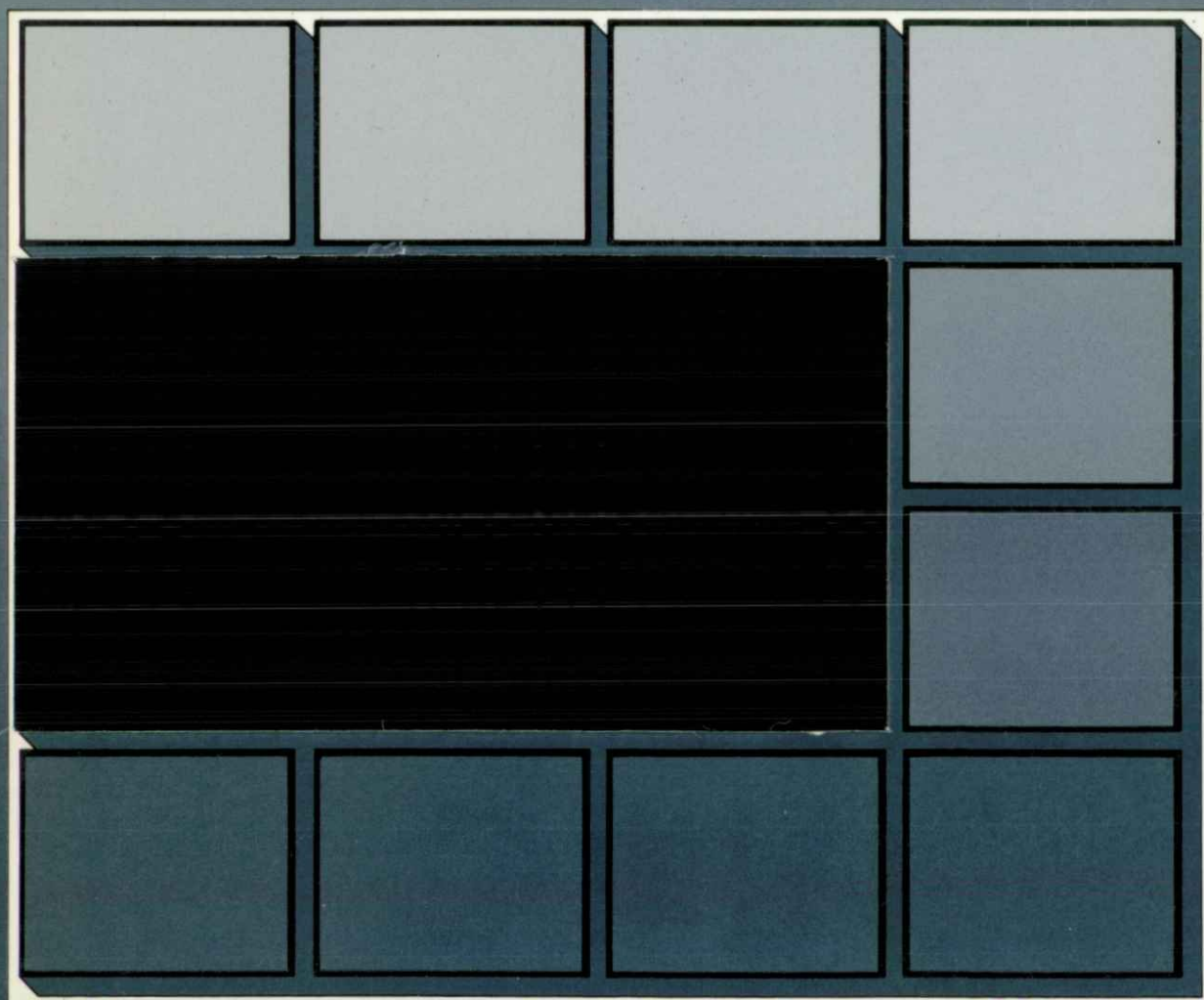




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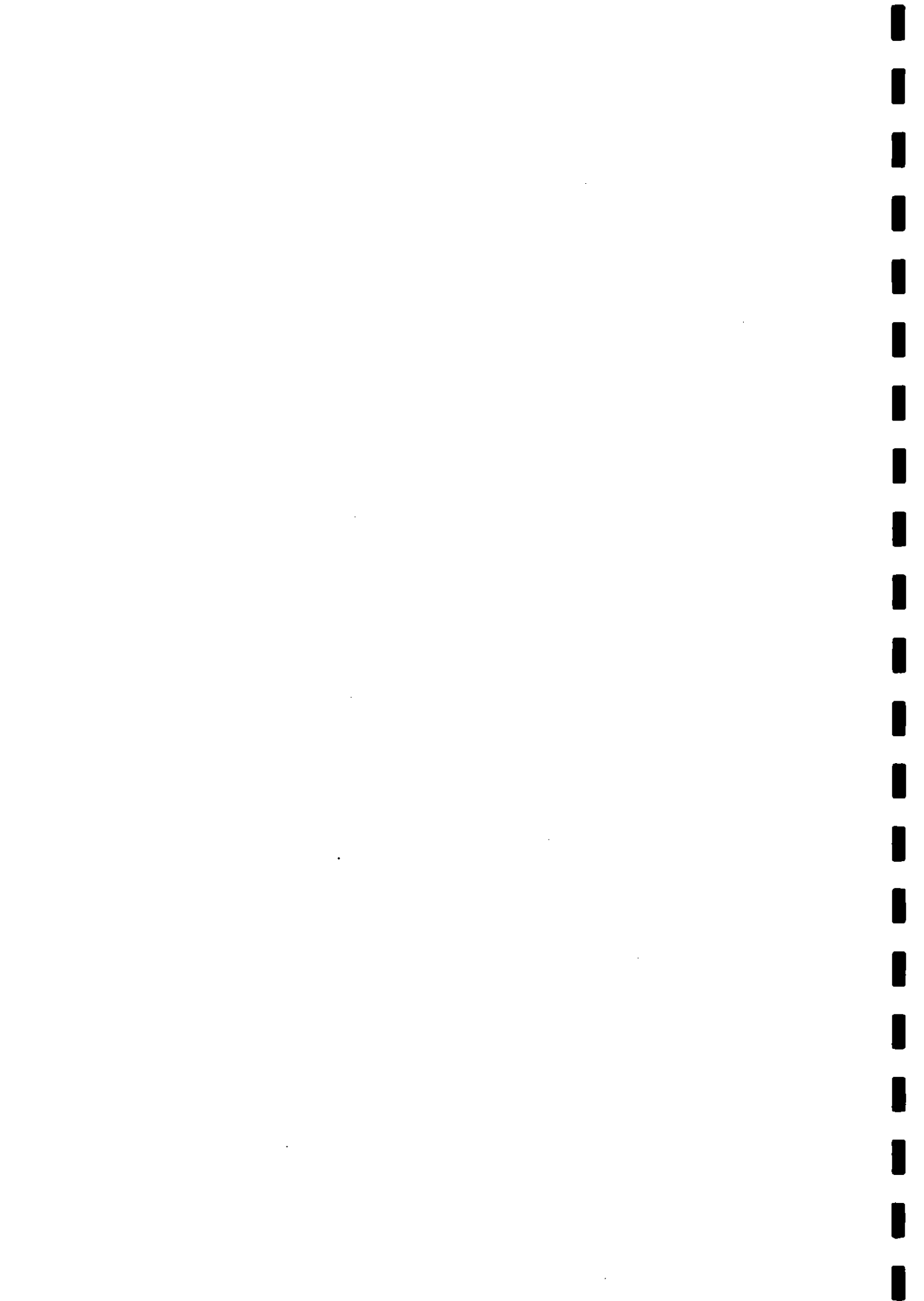
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Faunal characteristics of a site
subject to high loadings of
linear alkylbenzene sulphonates
(LAS)

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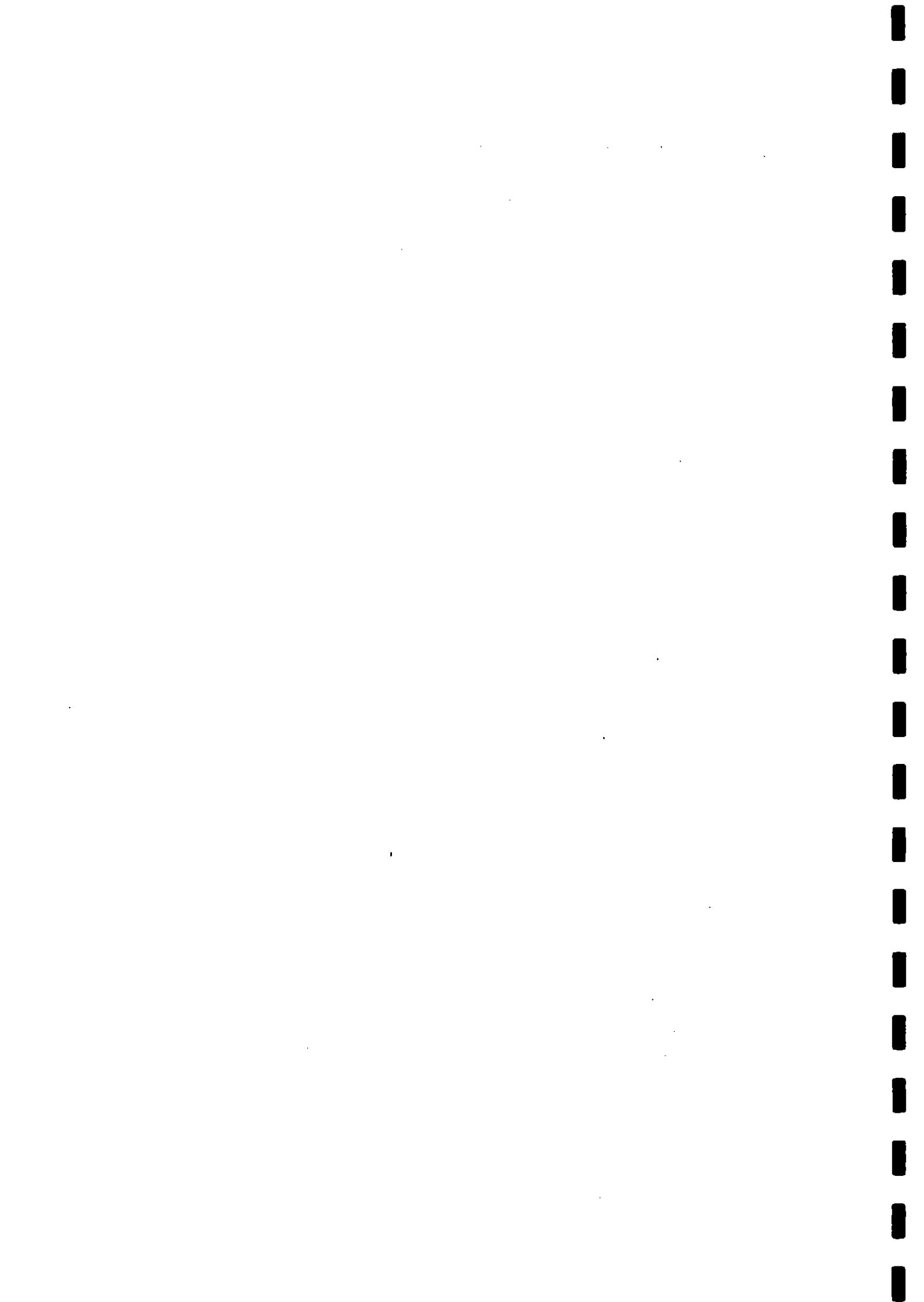
FAUNAL CHARACTERISTICS OF A SITE SUBJECT TO HIGH LOADINGS
OF LINEAR ALKYL BENZENE SULPHONATES (LAS)

Final Report
ECOSOL TECHNICAL SUB-GROUP OF C.E.F.I.C.

M. Ladle, W.A. House, P.D. Armitage & I.S. Farr

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1. INTRODUCTION

Linear alkylbenzene sulphonates (LAS) are widely used synthetic detergents that enter water bodies by discharge from sewage works. LAS are a class of important compounds with various chain lengths (mainly C10-C14 linear chain alkylates) and isomers whose biodegradation and toxicity for aquatic organisms have been studied extensively. There is much less information about the fate of LAS after entering streams and rivers or about the ecological impact of these compounds on the invertebrate macrofauna.

The objective of the present study is to assess changes in aquatic invertebrate communities associated with elevated levels of LAS. Since the work was carried out in a field situation it is clear that factors other than LAS concentration may have influenced the fauna. However, by careful site selection and the application of established techniques of faunal analysis it should be possible, by inference, to demonstrate any overriding influence of the LAS component.

2. SITE SELECTION

A field location was sought to enable an assessment of the impact of an LAS load downstream of a sewage outflow. It was proposed that the location should consist of a control site upstream of the discharge, an impact site immediately downstream of the discharge and a recovery site about 200 metres downstream of the impact site. To select a suitable location for the main study the following criteria were applied:

- a) The presence of a sewage treatment plant receiving essentially domestic effluent and likely to have a relatively large LAS component.
- b) A comparatively small dilution by the receiving stream and no obvious complicating inflows.
- c) Similar conditions and stream bed sediments at impact, control and recovery sites.
- d) A receiving stream having a rich and varied fauna in order to permit a detectable response to effluent input.
- e) A stable situation in terms of stream discharge and effluent input.
- f) Good accessibility, permitting installation and operation of sampling equipment.

A selection of potentially suitable sites was considered in terms of the above criteria. Water Authorities and land owners were contacted and permissions obtained to visit eight locations in Somerset, Dorset and Devon. On each site visit the suitability of the sewage treatment plant and its associated river was assessed. If the above criteria were satisfied and the location appeared to be suitable, sediment samples were taken from control, impact and recovery sites along the watercourse to be dried and retained for LAS determination. They were then used to evaluate and refine sampling techniques. Invertebrate (kick) samples from control, impact and recovery sites were examined and the nature of the invertebrate communities present were recorded.

Samples were taken at six of the sites visited (Table 1), the remaining two being rejected because of difficult access. The reasons for rejection of five of the six were as follows:-

- a) Lounds Mill, Dorchester, SY 714903. Rich and diverse fauna and large input of LAS contaminated sewage. Massive dilution and non-homogeneous substrate in the receiving stream made it potentially very difficult to interpret results.
- b) Wray Brook, SX 708849. High levels of LAS recorded in impact sediment. Unfortunately the stream bed sediments were very heterogeneous with high proportions of fine sediment only at the impact site.
- c) Corfe River, Corfe Castle, SX 961831. Rather low values of LAS in impact site sediments, a very 'flashy' stream with a silty bed and impoverished fauna (probably due to the instability of the sediments).
- d) River Cerne, SY 665999. Access to this site involved crossing fields and fences and traversing a sewage works with locked gates. The stream itself was a chalk stream with a diverse and abundant fauna but the effluent was quite small and it was difficult to establish a downstream recovery site because of dense marginal vegetation.
- e) River Parrett, ST 460100 was originally suggested as a possible site following a SRC study which demonstrated $5.9 \mu\text{g g}^{-1}$ of LAS in sediment. However, subsequent examination showed that the input was far from being a point source, the discharge of the stream was very variable, the sediment was very fine and sparsely distributed over a bed of hard clay. Being unstable, the stream bed had an impoverished fauna of pollution resistant forms.

The location chosen was the Drimpton Stream, Broadwindsor, ST 435028. Although the LAS values were not the highest ones obtained in the initial survey all other factors were satisfactory. There was easy access to control, impact and recovery sites, the stream flow was consistent, dilution of the (domestic) effluent was normally small, the substratum was similar throughout and the fauna included both resistant and sensitive groups of organisms giving a rich and diverse association. This site was chosen for detailed study after discussion with Sittingbourne Research Centre (SRC) scientists.

3. BIOLOGICAL MONITORING TECHNIQUES

Ideally a fully quantitative study of invertebrate distribution, coupled with studies on growth, survival and reproduction of selected groups of invertebrates, would be carried out at the chosen study area. However, in the time available it was necessary to apply a standardised, quasi-quantitative approach to invertebrate sampling to assess invertebrate community structure and abundance. By applying this approach on three occasions in each year of study (spring, summer and autumn) it has been shown that an adequate indication of water quality can be obtained (Armitage et al 1983).

On each occasion three sites were sampled; an upstream control site 20m above the effluent and well clear of any possible influence from the treatment plant; an impact site 2-3m downstream of the main effluent. Chemical observations showed that there was a tendency for effluent contaminated water to remain separate from the main flow of the stream so samples were not taken immediately downstream of the effluent pipe; lastly, a recovery site was examined about 200m downstream of the effluent.

All sites had beds of flint gravel with a relatively large component of fine sediment. The amount of fine sediment was visibly greatest at the impact site. Biological material was collected by a standard, timed, kick-netting approach (Furse et al., 1981) applied for three minutes, with the objective of covering the main marginal and midstream habitats in the proportions in which they were present. Invertebrates were transferred immediately to polythene bags and were preserved within two hours of collection. Subsequent examination of collected material was for purposes of identification and assessment of numerical abundance. Many groups were identified to species although, in general, biotic indices based on identification to family level are totally satisfactory.

The biotic indices described and tested by Armitage et al. (1983) are as follows:-

3.1 Score

Scores do not employ abundance categories. The various taxa (essentially families) of invertebrates are assigned scores according to their supposed tolerance of pollution. Thus Oligochaeta and Chironomidae, of which some species are resistant to high levels of enrichment associated with severe pollution, are scored as 1 and 2 respectively, while many families of Ephemeroptera, Plecoptera and Trichoptera are known to survive only in pristine water conditions with score values of 10 (see Table-2).

3.2 Cumulative score

By summing the scores of all taxa present in a particular sample an index which combines the tolerance of individual taxa with the overall diversity of the invertebrate community is obtained.

3.3 Average score per taxon

By dividing the cumulative score by the number of scoring taxa it is possible to obtain a value for ASPT. This simple index is possibly the best characteristic for assessing levels of perturbation (including pollution) in streams. In general the score obtained for a given gravel stream bed on a particular date is very stable and in an unpolluted situation ASPT values of 5-6 are normally to be expected (Pinder et al, 1982). ASPT is almost unaffected by size of sample and Pinder et al (1982) concluded that such scores had "much to commend them in making comparisons between sites or data". He also concluded that ASPT gave "a much better indication of water quality" than aggregate scores in a chalk stream headwater.

Together, the combination of aggregate score and ASPT provide a useful indication of water quality and results are expressed in this way in the present study.

3.4 The FBA River Laboratory data base and associated computer package (RIVPACS)

This system permits the classification and prediction of macroinvertebrate communities in running water (Wright et al., 1984; Furse et al., 1987; Moss et al., 1987; Armitage et al. 1987) and was used to analyse the results obtained during this survey.

Over the past 10 years about 600 species of macroinvertebrate have been identified from more than 400 substantially unpolluted sites throughout Great Britain. The species lists are being used to construct a national classification of running-water sites and to develop a

technique for predicting the probabilities of occurrence of individual taxa at sites of known environmental characteristics based on data collected in spring, summer and autumn. This large data base provides a standard against which to assess the fauna of new sites and also places the site in a national context.

RIVPACS was used in this survey to predict the faunal composition of all sites using environmental data collected for each site. When the program is running a warning message is shown on the screen and printout if, on the basis of the physical and chemical data, the site has a probability of less than 5% of belonging to any of the classification groups. Families or species are listed on a printout, together with their probability of occurrence at the site. The list is terminated at 50% probability but could be extended to 0% probability if required. If the site was unperturbed by pollution most families or species at the head of the column would be present but at the 50% level only 1 in 2 of the families listed could be expected after the standard sampling effort. The sum of probabilities for each taxon is the expected number of families. If this is compared with the observed number of families or species an index (I) can be derived which provides a measure of the site's deviation from the expected. If the observed results agree with the expected, the values of the index will be 1.0 but this value can be exceeded if more than the expected number of taxa are captured. Conversely, values less than unity indicate that fewer families or species were captured than expected. A comparison of predicted and observed fauna provides an objective indication of the 'biological' water quality of each site.

4. CHEMICAL METHODS

At each visit to the Drimpton Stream at Broadwindsor sediment and water samples were taken for analysis as well as some in situ field measurements which were made at each site.

4.1 Linear alkylbenzene sulphonate analysis in sediments

4.1.1 Equipment cleaning

All glassware was soaked in chromic acid (CrO_3 solid (100 g), distilled water (100 ml), sulphuric acid (2.5 l conc.)), for at least 16 hours prior to washing.

All equipment used in sample collection, processing, extraction and storage was washed in hot tap water, then several changes of distilled water followed by rinsing with at least two changes of fresh Methanol (Rathburn's Chemicals HPLC grade).

Care was taken to avoid contact with detergents at all stages.

4.1.2 Sample collection

At each stream location sediment samples were taken from three sites;

above the effluent discharge to the stream of a sewage treatment plant (Control site C), within the area receiving the effluent (Impact site I) and at a site suitable for macroinvertebrate sampling, 200 m downstream of the impact site (Recovery site R). In addition, for the July sampling date, a sample was taken 500 m above the control site to avoid the influence of a minor input from a domestic source.

Stream sediments were taken for analysis on five occasions. Six locations were sampled during a preliminary survey followed by three seasonal collections at the selected monitoring location (Table 1).

Samples were taken from the stream bed with stainless steel (SS) scoops and sieved wet (2 mm SS, BS 410, Endicotts Ltd) into 90 mm deep SS trays (320 x 470 mm).

For the preliminary survey of locations, this material was taken for drying and extraction. Dry sieving of the preliminary survey samples showed these to contain a high proportion of coarse sand (Table 3). Particles of this size are unlikely to be ingested by the invertebrate fauna of the stream. An initial assumption was made that the organisms most likely to be influenced by adsorbed LAS were those ingesting particles. In addition, the relatively low surface to weight ratio of larger particles was initially assumed to lead to a low adsorption potential for LAS. Subsequent analysis suggests that this is not the case (Table 4).

For the three main sampling dates, sediment "fines" ($<125\ \mu\text{m}$) were separated from coarse sediment by wet sieving on site, only the fine material being taken for analysis. The slurry collected in the SS trays was allowed to settle for several minutes to concentrate the solids. The supernatant liquid was transferred to cleaned tobacco jars and sealed with lids lined with methanol rinsed aluminium foil for transport.

4.1.3 Sample processing

At the laboratory the contents of the jars were spread out in the appropriate SS trays and heated to 80°C for approximately three hours with the jars. This process was intended to evaporate the bulk of the liquid and pasteurise the samples rapidly. Subsequently the oven temperature was reduced to 60°C and samples left to dry overnight. After about 16 hours, the dried and crusted samples were broken up with nickel spatulas and allowed to dry for a further 2 to 4 hours. Dried samples were crushed with a glass pestle and mortar and resieved ($125\ \mu\text{m}$) before being returned to the original jars for storage. Samples in this form were assumed to be stable over periods of several weeks.

Where sufficient sample was available, four subsamples of 10 g were taken for extraction of LAS and a further four for spiking and measurement of extraction efficiency. In some cases sufficient sample for only two or three replicates was available for efficiency analysis.

4.1.4 Extraction procedures

The procedures used for sediment and liquid extraction followed closely the Unilever methods and those recommended by Sittingbourne Research Centre (SRC) with slight modifications to adapt to locally available equipment. Unspiked solids were extracted before spiked samples. Extraction blanks were run with unspiked samples.

Sediment samples were extracted in batches of four. One sample from each of the three sites at a given location, together with a blank extraction or additional site, were included in each batch. Extraction position for any sample site was rotated to each of the available heating mantle places in turn. 500 ml round bottom (RB) flasks were set in heating mantles with soxhlet heads (Quickfit Ex5/55) and condensers. Cellulose thimbles (27 x 80 mm single thickness, Whatman 2800.258) were placed in the heads and methanol (200 ml) in each RB flask. Initially, thimbles were refluxed for one hour but reflux rate varied depending on which heating mantle was used. For the final two sampling dates thimbles were extracted for 25 reflux intervals. After thimble extraction, the apparatus was allowed to cool and the methanol discarded. The flasks were rinsed with fresh methanol and a further 200 ml added.

Sediment samples (10 g) were transferred to the thimbles which were then returned to the extraction heads. The weighing vials were washed

out with 3 x 10 ml fresh methanol and the washings transferred to the thimbles. For a blank extraction, equivalent volumes of methanol were added to the thimble.

Samples were initially extracted for four hours but this was later modified to 100 reflux intervals giving total extraction times of between 5 and 6.5 hours depending on the heating mantle used. After extraction,

the apparatus was allowed to cool, then excess solvent in the thimble compartment drained carefully into the RB flask ensuring no transfer of solids. The contents of the flasks were then reduced to approximately 50 ml by rotary evaporation under low pressure and a bath temperature of $<60^{\circ}\text{C}$ with care being taken to avoid bumping or foaming of contents. The reduced sample was then passed through a SAX (quaternary amine) column (Bondelut 3CC) previously washed with 10 ml methanol. Anionic compounds retained on the column were eluted with methanolic HCl (20:80 conc. HCl:methanol) followed by 0.2 ml methanol at a flow rate $<2\text{ ml min}^{-1}$. The eluent collected in 100 ml glass beakers was diluted to about 60 ml with distilled water. The pH of the eluent was adjusted to 7.02 ± 0.02 with a 0.5 M NaOH for coarse adjustment and 0.1 M NaOH and HCl for fine. The neutralised solution was transferred to a C8 column (Bondelut 3CC) previously washed with methanol (10 ml) followed by distilled water (10 ml). The column and reservoirs were then washed with distilled water. The column alone was rinsed with 3 ml 30:70 methanol:water at a flow rate $<2\text{ ml min}^{-1}$. Adsorbed compounds were eluted with 5 ml methanol and the eluted samples reduced to dryness under nitrogen.

Dried samples were transferred to HPLC injection vials with measured quantities of methanol using a 500 μl capacity syringe (Unimetrics). Two additions of approximately 500 μl were made to the dried sample vial and the volume measured on transfer. Volumes of diluted samples ranged from about 800 μl to 1400 μl and were recorded for each sample. Vials were sealed with teflon coated septa and were assumed to be stable for up to two weeks prior to chromatography. Checks on liquid levels in the vial and close agreement between analyses of contents of a single vial over periods of several months suggests this to be valid.

4.1.5 Spiking of samples

10 g samples were weighed into polycarbonate centrifuge tubes (40 ml capacity fitted with screw caps; methanol rinsed and dried) and the following were added: Formaldehyde (40% v/v Analar BDH) 1 ml, distilled water 7.5 ml, Marlon 'A' (100 mg l⁻¹ active substances in distilled water) 1.5 ml.

The tubes were capped and shaken vigorously for one hour on an automatic shaker then centrifuged at 10,000 rpm for 20 minutes.

(a) Spiked liquid processing

The majority of the centrifuged liquid phase was transferred to a C8 column previously rinsed with methanol (10 ml) and subsequently water (10 ml) taking care to avoid disturbance of the solids. The column was washed with distilled water (10 ml) then 2 ml 30:70 methanol:water at a flow rate $<2\text{ ml min}^{-1}$ and sucked dry. Adsorbed anionics were then eluted with 5 ml methanol and dried under nitrogen. Samples were diluted to a similar volume to that used for solids extracts to allow automatic injection into the HPLC.

(b) Spiked solids processing

The wet solids in the centrifuge tube were oven dried overnight at $65^{\circ}\text{C} \pm 5^{\circ}\text{C}$. The following morning the dried crust was broken and the sample returned to the oven for a further 2 to 4 hours.

In order to mimic the solvent penetration during extraction of the original 125 μm sieved samples, the spiked solids were crushed and ground with small glass pestles and mortars before transfer to the extraction thimbles. Residues on all equipment were washed into the centrifuge tubes with four separate 10 ml aliquots of methanol. Transfer of traces of spiked solids adhering to the centrifuge tubes to the extraction thimbles proved difficult. A standardised procedure involving scraping and shaking of the tubes with the four aliquots of methanol washings was adopted. Thereafter the extraction, clean up and dilution of spiked solids was identical to that for unspiked solids.

4.1.6 Chromatographic analysis

HPLC conditions:

Column: Waters SS, 25 cm x 4.5 mm I.D.

Packing: u-Bondapak C18 (10 μm)

Guard column: None

Eluent: 0.0875 M NaClO₄ in 80:20 methanol:water

Flow rate: 1.0 ml min⁻¹

Detection: Absorption 230 nm, 10 mm path length flow cell

Sensitivity: 0.01 (max sensitivity: 0.001)

Band width: 5 nm

Injection volume: 50 μl (flushed loop) auto injection

Flushed loop injection was found to give replicate values within a precision of 1%. Comparison of integrated peak areas given by flushed loop with those obtained using a micro-metering pump injection (Fig. 1) suggested the former may be 1.3 times the stated volume of 50 μl (66 μl). The latter method is assumed to give greater accuracy but lower precision injections. All analyses were performed with flushed loop 50 μl injections for replicate precision. Integrated areas were converted to concentration values (rather than active substances per injection) with reference to freshly prepared Marlon A standards interspersed with samples on each run.

HPLC equipment, supplied by LDC/Milton Roy was used for all analyses. The modular equipment comprised a Promis automatic injection system, two ConstaMetric III pumps, and a variable wavelength HV/visible absorption detector SpectroMonitor D with wavelength drive through an accessory control module (ACM). The system is controlled by an MP3000 computer with twin disk drives allowing storage of data slices from the chromatograms for later reintegration and replotting.

A FluroMonitor III filter cutoff fluorescence detector was also available but required a separate lamp assembly and filter set to allow detection of LAS. Possible delays in supply and fitting and the additional cost precluded the use of this detector.

Absorption detection using the SpectroMonitor D with wavelength set at 230 nm, band width c. 5 nm, was adopted for all analyses. With this detector, high background absorption was detected in the early part of chromatograms of all extracted sediment samples (Figs. 2 & 8). Most peaks detected in the region of the standard Marlon A peaks registered as 'fused'. For low concentration extracts some peaks appeared to be integrated to a baseline corresponding to the baseline for the early contaminants. By introducing variations in the threshold parameter by

which the integration file recognises baseline shifts, it was found that low values, together with low noise recognition values, were required to give suitable integration.

Insufficient RAM was available in the MP3000 to allow all chromatograms to be integrated as a whole with these parameters set low during chromatography. Data slices were therefore saved and chromatograms replotted and reintegrated over individual LAS peak groups at a later time (Figs. 6 & 7).

The tail of the early adsorption peaks caused baseline shift in following chromatograms. The effect was minimised by extending the interval between injections to more than 40 minutes. LAS peaks are eluted in the time window 7 to 25 mins.

Following initial tests to establish suitable conditions for resolving and integrating LAS peak groups, all subsequent analyses were performed under the above conditions with the exception of the Autumn 1988 series.

All previous analyses which were performed automatically overnight were subject to an upward baseline drift for several of the initial injections. This drift precluded direct use of the peak areas for the initial standard series without preliminary reintegration. It was assumed that the baseline drift was due to machine "warm up" or to changing atmospheric conditions (eg temperature variation). However, it was noticed that during column clean up, which was performed after each overnight run, helium clean up of the eluent (50% aqueous methanol) consistently resulted in baseline shifts.

Helium purging was thought to be prudent for both the clean up and standard eluent because considerable degassing of dissolved air occurs on mixing methanol and water. This may result in a solution supersaturated with air and such supersaturation can lead to degassing and formation of bubbles in the detector cuvette during a run, resulting in highly unstable baselines. Investigations suggested that the positive baseline drifts during the initial phases of a run were in fact due to resolution of air. It was considered that continuous purging would be too expensive.

Normally the rising baseline stabilises after about four hours and there is no further subsequent detector interference suggestive of bubble formation.

For the Autumn 1989 series, eluents were first purged with helium and then shaken with air and allowed to stand for 2 hours before use. Figure 9 shows the close match of calibration data from all four calibration series during subsequent analyses.

It should be noted that although no reintegration was found necessary for the calibration standard series, pretreatment of the eluent does not obviate the need for reintegration of the extract samples.

4.1.7 Calculations

The total active substances (LAS) in each injection vial 'Q' is given

by the expression

$$Q = AV/K (1)$$

where A is the integrated area under LAS peaks, V is the total volume of diluted extract in the HPLC vial (μ l) and K is the factor used to convert integrated areas to concentration values in $\text{mg} \cdot \text{l}^{-1}$ taken from calibration graphs of Marlon A standards.

If Q1, Q2 and Q3 are the total active substances in the injection vials containing extracts of 1) unspiked solids, 2) spiked solids and 3) supernatant liquid from one particular site, then extraction efficiency 'E' for an individual sample is given by

$$E = (Q2 - Q1)/(S - Q3) \quad (2)$$

where S is the known quantity of active substances used to spike each 10 g sediment sample (150 µg), Q1 is the mean quantity of active substances extracted from a 10 g sediment sample at the given site.

Thus the total concentration of active substances (LAS) in each sediment sample extracted 'Q' is

$$Q = Q1/E \quad (3)$$

However, this assumes no variation in calculated extraction efficiency between samples. In practice a mean value for efficiency at a given site was used ie

$$Q = Q1/E \quad (4)$$

Results are expressed as $Q \pm \text{S.D.}$ in µg for each site.

It should be noted that conversion factors vary from one analysis series to another and that unspiked samples were run in different series from spiked.

4.2 Water and sediment analysis

4.2.1 Sediment organic content

This was limited to the measurement of the total organic content of the sediment. Dried sediment samples were placed in weighed porcelain crucibles, carefully pre-combusted over a bunsen burner to prevent flash ignition and heated to 550°C overnight. The ashed samples were coated in a desiccator and reweighed to enable the calculation of the percentage organic content.

4.2.2 Stream water analysis

At each of the visits to Broadwindsor, one litre of stream water was collected at each of the sites. This was returned to the laboratory for chemical analysis using the methods described by Casey & Newton (1973). In addition, field measurements of stream temperature, electrical conductivity and pH were taken at each site. Appropriate instrument and electrode calibration was performed on each visit.

The total ion analysis data were used to estimate the chemical speciation in the stream using the Fortran program RIVEREQ developed by Howard et al. (1984). The chemical speciation data were used to calculate the charge balance i.e. total equivalents of cations and anions taking into account ion-pairs, to predict the partial pressure of CO2 in equilibrium with the stream water and to check for instabilities

in the water chemistry caused by the sewage effluent. The sewage effluent was also analysed and the data used to calculate the ratio of effluent to stream flow using the equation:

$$qI/qB = (cB - cA)/(cI - cA) \quad (5)$$

where q is the flow rate, c the concentration or some other conservative parameter and the subscripts I, A and B refer to the effluent, upstream (or control) and downstream (or recovery) sites respectively.

5. PERFORMANCE AND VERIFICATION

5.1 Sample stability

The stability of samples during transport between the field site and laboratory is uncertain. A potential loss over this period is indicated by the different active substance analysis of R. Parrett sediment dried immediately on return to the laboratory or stored for two days at 5°C.

Sample dried $5.71 \pm 0.39 \mu\text{g g}^{-1}$

Sample stored wet 1.46, 1.59 $\mu\text{g g}^{-1}$ (two values only)

The stored sample values are not corrected for extraction efficiency. The dried subsample was analysed at the SRC.

Loss of active substances from Drimpton Stream sediment during short term storage has been investigated. Four subsamples from the impact site were spiked with Marlon A. Two of these were dried immediately and the others incubated for 24 hours at 20°C before drying.

	LAS concentration in sediment / $\mu\text{g g}^{-1}$	Mean / $\mu\text{g g}^{-1}$
Non incubated samples	89.1 78.5	. 83.3
Incubated samples	74.0 79.6	76.6

This could be interpreted in either of two ways.

1) An 8% decrease over 24 hours, indicating a half-life of about 8.8 days, hence a small potential loss in the <3 hours between collection and drying.

2) Alternatively this may indicate the potential to decay $0.28 \mu\text{g g}^{-1}\text{h}^{-1}$. The question remains unresolved and further investigation is needed to establish the sample stability prior to drying. Dried samples were assumed to be stable over the period of the project.

5.2 Calibration

Freshly prepared standard dilutions of Marlon 'A' were included in each HPLC analysis series. The linearity of response to varying standard concentrations within a given analysis series is indicated in Fig. 3.

The slopes of calibration graphs were not consistent between analysis series. Varying slopes used as area-to-concentration conversion factors may reflect varying chromatographic conditions or inaccuracies in standard preparation. Conversion factors (calibration graph slopes) for the main surveys are shown in Table (5).

5.3 Accuracy

Verification of the reliability of the present method relative to that used at SRC rests on the comparison of analyses of the three sediments sampled in April (Table 6). These data show excellent agreement. Another indication of reliability is the level and consistency of total recovery of spike additions. Data from the seasonal surveys are shown in Table (7).

Recoveries are significantly lower than those reported by SRC for the spring samples, indicating significant loss in the system. Recoveries also vary between sites. However within-site variation is not high. Low recoveries are primarily due to poor extraction from the solid samples.

Measured extraction efficiencies for the main surveys are shown in Table 8. These data show a similar pattern of between-site variation but within-site consistency.

5.4 Blank corrections

Injectons of distilled water or fresh methanol produced no detectable peaks in the LAS region of chromatograms. Single blanks within a chromatographic series showed trace peaks particularly after a high concentration sample. These were assumed to be due to injection needle contamination. A second blank in series showed minimal peaks. Hence, for the purposes of calibration, graphs were assumed to pass through the origin.

Levels of contamination in the methanol were measured by running blank extractions. These followed identical procedures to those for solids extraction, sample clean up and analysis. Active substances (LAS) measured in blank extracts are listed below.

Date	Methanol batch no.	$\mu\text{g LAS}$ per extract	mean S.D.	
April	5423	1.25 0.89	1.07 ± 0.18)))	1.17 ± 0.34 n=6
April	5423	1.46 1.10 0.71 1.61	1.22 ± 0.40)	
July	7130	0.69	0.69))	
October	7130	0.58 1.50 1.96 1.40	1.36 ± 0.57)	1.23 ± 0.58 n=5

This is the level of contamination to be expected in extracts of a 10 g sample. Mean corrections for blank contamination are approximately $0.12 \pm 0.04 \mu\text{g LAS g}^{-1}$ sediment with no significant variation between the batches of methanol.

6. RESULTS FOR THE INITIAL SURVEY

The results from the preliminary survey of six sites, listed in Table (1), are collected in Table (9). As noted in paragraph 4.1.2, the pretreatment of the sediment was different from the procedures developed later for the treatment of the Drimpton Stream sites during the spring, summer and autumn surveys. Low values of the concentration of LAS in the total sediment (<2 mm wet sieved) at all the sites is evident. The highest value obtained was for the R. Parrett ($5.71 \mu\text{g g}^{-1}$ SRC). The Louds Mill, Dorchester, site gave a high concentration of LAS at the impact site but a very low value at the recovery site when compared with the concentration measured at the control site. This could indicate the presence of some source of LAS

upstream of the impact site. The Wray Brook site produced similar results but with little difference between the control and recovery sites. The results from the Drimpton Stream indicated a relatively low concentration at the control site when compared with the impact and recovery sites. The results from the Corfe River are surprising with the concentration determined at the impact site lower than both the control and recovery sites.

In view of later results and checks on the performance of the extraction and analytical procedures, it is likely that the sensitivity of the method is limited to $0.1 \mu\text{g g}^{-1}$. Therefore values below this figure may be considered to be below the limits of detection for the present analytical procedure.

During the initial survey samples of river-bed fauna were collected at the main sites under consideration. Because of time constraints a rapid field analysis of the fauna was carried out and biotic scores, including ASPT values, were determined on the basis of these figures (Table 10). Obviously, factors other than effluent characteristics influence the scores obtained from such simple, one-off, field samples but, in general, all sites except the Corfe River, Semington Brook and Wray Brook, had some ASPT values above 5 and, although the numbers of scoring taxa recorded were generally low, they invariably included some organisms indicative of high water quality. Values at both impact and recovery sites on the Drimpton Stream were lower than the control site but no significance should be attached to these differences.

7. RESULTS OF SURVEYS 1987 & 1988

7.1 Chemical analyses

The results of the total ion analyses in the seasonal samples from the Drimpton stream are shown in Table (11) together with the calculated partial pressure of CO_2 , (PCO_2), and ionic strength I . The concentration of Cd and Pb was found to be $<0.01 \text{ mg dm}^{-3}$ for all the samples. The calculated charge balance is shown in Table (12).

The main difference in the water chemistry is that the concentration of all the ions increased downstream of the discharge (impact site) with the largest difference in Na^+ , K^+ , Cl^- , NO_3^- and phosphate. These changes are also reflected in the increase in ionic strength (10%) and conductivity of the stream water at the recovery site. The difference in PCO_2 between the control and impact site was relatively small and within the margin of natural variability. The charge balance results highlight changes in the chemical speciation caused by the effluent input to the stream. The control site gives excellent agreement whereas the data from the impact site produces particularly poor agreement. The balance improves considerably at the downstream recovery site. These large charge balance differences were attributed to changes in the chemical speciation caused by dissolved organic material in the effluent. The organic anions effectively complex a large proportion of the cations. Similar behaviour is also observed in soft waters containing humic and fulvic acid components.

In the Autumn samples of both years the water chemistry was slightly different to the two preceding samples. Both pH and ionic strength were lower than before, probably because of the inputs from rain storms which occurred prior to the survey but data are within the natural range of variation expected for such a small stream. This change in ionic strength may be attributed to the lower calcium concentration and alkalinity of the stream water at the control site.

The calculated PCO₂ values were also substantially higher than on previous visits. These differences are probably caused by the storms and rainfall which occurred before the survey but, again, the data are within the range of values expected from natural variability for such a small stream.

In this case charge balance calculations correspond much more closely although the same trends as for the spring and summer surveys are evident (Table 12). The reduced differences in cation and anion sums of the effluents (~3%) relative to the summer surveys (~25%) must reflect some changes in composition.

The percentage difference between the cation and anion sums, ie Σ^+ and Σ^- , for the sewage effluent is similar to that calculated for the impact site. The flow ratios for July 1987 were calculated according to equation (5) using the chemical concentrations at the control, recovery and in sewage discharge. The results are shown in Table (13) and indicate a flow-ratio of approximately 12% although serious anomalies are evident for both the Ca²⁺ and Mg²⁺ results. The concentration of Mg²⁺ was unaffected by the sewage

input. The calculations for Mg²⁺ and Ca²⁺ are less reliable because of the small difference in the concentration of these cations at the three sites. In the Autumn of the same year the effluent flow was estimated as 140·dm³·min⁻¹ at the time of the visit. The flow ratio was calculated using the method described above and gave an average value (excluding those values derived from Mg²⁺ and Ca²⁺ concentrations) of 9.6%. This is close to the 12% calculated from the analysis of the summer data. This flow ratio leads to an estimate of the total stream flow of 1.5 m³ min⁻¹ and discharge rate of LAS of 4.2·g·h⁻¹.

7.2 LAS Analyses

Table (14) shows the results of the LAS analysis of the sediments collected at the three sites. The inter-laboratory comparisons of these determinations have already been discussed in paragraph 5.3. The results show very little difference between the contents of LAS in the sediment at control, impact and recovery sites in the spring 1987 survey.

The results from the spring 1987 survey indicated the presence of LAS in the control site sediment, so it was decided to investigate sediment at a site upstream of the control site above, the point where investigation indicated the presence of a previously unsuspected small domestic inflow (upstream control in Tables 11 & 14). The effluent at the point of discharge into the stream from the sewage works was also analysed for the major chemical constituents, and the results are shown in Table (11).

Subsequent samples showed similar values at the control and recovery sites but much higher concentrations at the impact site. However, the final sample in the Autumn of 1988 had a high concentration only at the control site presumably related to an input of essentially domestic effluent some distance upstream (as noted above). Such anomalous results are inevitable in natural stream situations where no control over inputs is possible. There was some association between the organic content of the sediments and the LAS concentration (Figure 4) and by leaving out analyses from the control site (Figure 5) a value of r^2 of 73.1% was obtained.

The influent and effluent were analysed for LAS by SRC and found to contain

5.6 mg dm⁻³ and 0.5 mg dm⁻³ of LAS respectively. This decrease represents about 91% biodegradation of the LAS in the sewage treatment and is typical for UK treatment plants, ie >90% removal for LAS. The influent concentration is also typical (4-10 mg dm⁻³ LAS) of sewage treatment units (Berna, 1987). The effluent concentration is slightly greater than expected from the range given by Waters & Garrigan (1983) for UK treatment plants, ie 0.5-1.0 mg dm⁻³ MBAS corresponding to \approx 0.2-0.45 mg dm⁻³ LAS. The concentration of LAS in the sediment at the three sites is shown in Table (14)

The SRC analysis of the sewage sludge from the settling tank in the sewage plant gave a LAS concentration of 6.7 mg g⁻¹. A similar analysis of the dried sewage gave 11.1 mg g⁻¹. These values are in the range reported for sewage plants, eg Henau et al. (1986) with average values \approx 6 mg g⁻¹ in Switzerland, USA and Germany.

7.3 Biological surveys

The biotic indices for the spring surveys are all high with ASPT values ranging from 5.2 to 6.1 (Table 15). These results are comparable with similar samples taken from pristine streams (Pinder et al., 1982) and there is no evidence of any change in faunal characteristics related to either sewage effluent or the associated LAS.

High values were also characteristic of most other samples with the exception of occasional samples taken at impact and recovery sites. Values of ASPT of 4.9 & 4.6 (Impact and Recovery Autumn 1987), 4.3 (Recovery Summer 1988) and 4.5 (Impact Autumn 1988) suggest that there may have been relatively large sewage inputs in the preceding months. This factor, possibly combined at times with disturbance of the river bed due to natural increases in discharge prior to sampling could have affected the benthic fauna (Table 15). The presence of the larvae of Psychodidae and Ceratopogonidae (both groups being associated with sewage filter beds) in quite large numbers at the impact and recovery sites would support this hypothesis (Tables 17 a, b & c).

8. ANALYSIS OF BIOLOGICAL SURVEY RESULTS USING RIVPACS

The FBA data base and associated computer package was used in this survey to predict the faunal composition of Drimpton sites using the environmental data collected. A value of 1 for a given index indicates agreement between the observed and expected numbers of taxa captured.

Table (16 a & b) shows the physical and chemical characteristics of control, impact and recovery sites used in the present analysis. Elevated levels of oxidised nitrogen and of chloride are indicative of contamination by domestic effluent.

Table (17 a, b & c) indicates the observed relative abundance of all taxa identified from control, impact and recovery sites on the three occasions sampled. In each case the fourth column shows the sum (combined) of the values for the three dates. This is a total fauna list on which comparisons were based and permits comparisons of changes over the three sampling sites. Some of the observed differences simply reflect the normal seasonal fluctuations of the particular taxon. Thus, Leptophlebiidae, present at all sites in spring, were virtually absent on succeeding dates and various subfamilies of Chironomidae, of which some species are opportunist colonisers, show large variations in relative abundance.

Table (17D) draws together the combined samples at the three sites and illustrates that most of the common taxa were well represented at all of them. Notable features are the relative scarcity of Tubificidae, Chironomidae and Asellidae at the control site; all three are tolerant of organic enrichment and tend to flourish where other taxa may be less abundant. Heptageniid mayflies and leuctrid stoneflies which are not tolerant of pollution, show the converse pattern.

Application of the RIVPACS analysis to the above information using 11 physical variables or 11 physical and chemical variables (Table-18) shows excellent agreement between observed and predicted values. Twelve values are less than 0.9 (ie deviate from perfect agreement by more than 0.1). The poorest agreement is for the recovery site in terms of total score but all sites show relatively poor relationships for this index. Despite this, even the lowest value of 0.694 would place the site in the National Water Council's Class 2 and the great majority of observations would lie in Class 1.

9. CONCLUSION

In conclusion, it can be said that the methods applied in this study show no discernible effect of LAS in effluent or in stream bed sediments on the biota even though the concentrations in sediments were, at times, quite high. The major problem of such a study (as was anticipated even before the work commenced) lies in the unpredictable changes and unforeseen inputs which almost invariably affect natural streams. The anomalous values obtained for LAS in the final sample set of the present investigation almost certainly represent inputs from the small domestic effluent which was discovered in the course of the work while the low biological indices detected in some (mostly Autumn) samples were probably the result of flood disturbances and sewage inputs.

10. FUTURE RESEARCH

There is scope for further work on the fate and biological impact of LAS in rivers. A review of the recent literature shows that few studies have examined the effects of LAS on the environment in field conditions. A lot of research has been done on the interaction of LAS with different types of sediment in laboratory conditions and also on the toxicity and persistence in very controlled environments although the toxicity information in relation to benthic invertebrates is extremely limited. The IFE have a wide range of expertise, including invertebrate zoology, botany and chemistry, which permits a multi-disciplinary approach to the assessment of the effects of chemicals in sewage effluents on aquatic ecology. The research on LAS could be continued in several ways:

- 1) One of the problems raised in the present work is that the sewage effluent contains many materials which might have an effect on aquatic life. In a field study of this nature, and considering the variability in the sewage composition, it is impossible to relate any observed improvement or deterioration in river fauna or flora to particular components such as LAS. The only possible conclusions which can be drawn from this type of study are that the sewage effluent itself has some or no effect.

One method which could be used to address this problem is to evaluate LAS independently of other sewage components. This can be done using experimental streams of the type designed and built at the IFE River Laboratory and used for a variety of ecological and impact studies over recent years. The experimental recirculation streams are approximately 70-m in length, of race-track shape with a trapezoidal cross-section. They are fed with a high quality water abstracted from a chalk aquifer. The infeed water is at approximately 10.8°C throughout the year and of very uniform composition. The impact of LAS could be assessed with the experimental stream containing a varied biota with algae and macrophytes and sediments. An identical control system would be used for comparison. The study of such a "river channel macrocosm" would permit: (a) the measurement of the direct effect of LAS on the community ecology, diversity and dynamics in field conditions; (b) an independent assessment of the fate and distribution of LAS in the sediment.

2) An experimental study of the direct effect on benthic invertebrates of the ingestion of particulate matter pretreated with LAS or minor impurity compounds associated with the manufacture of LAS detergents, eg linear alkylbenzenes (LAB). This could be done using well defined clay minerals containing preadsorbed concentrations of the compounds of interest. Experiments investigating the degradation, accumulation, toxicity of particular LAS fractions could be done at the same time. The question of the reversibility of LAS uptake by sediments has also been recently raised and this could also be investigated using well-defined types of sediment as well as natural sediment mixtures.

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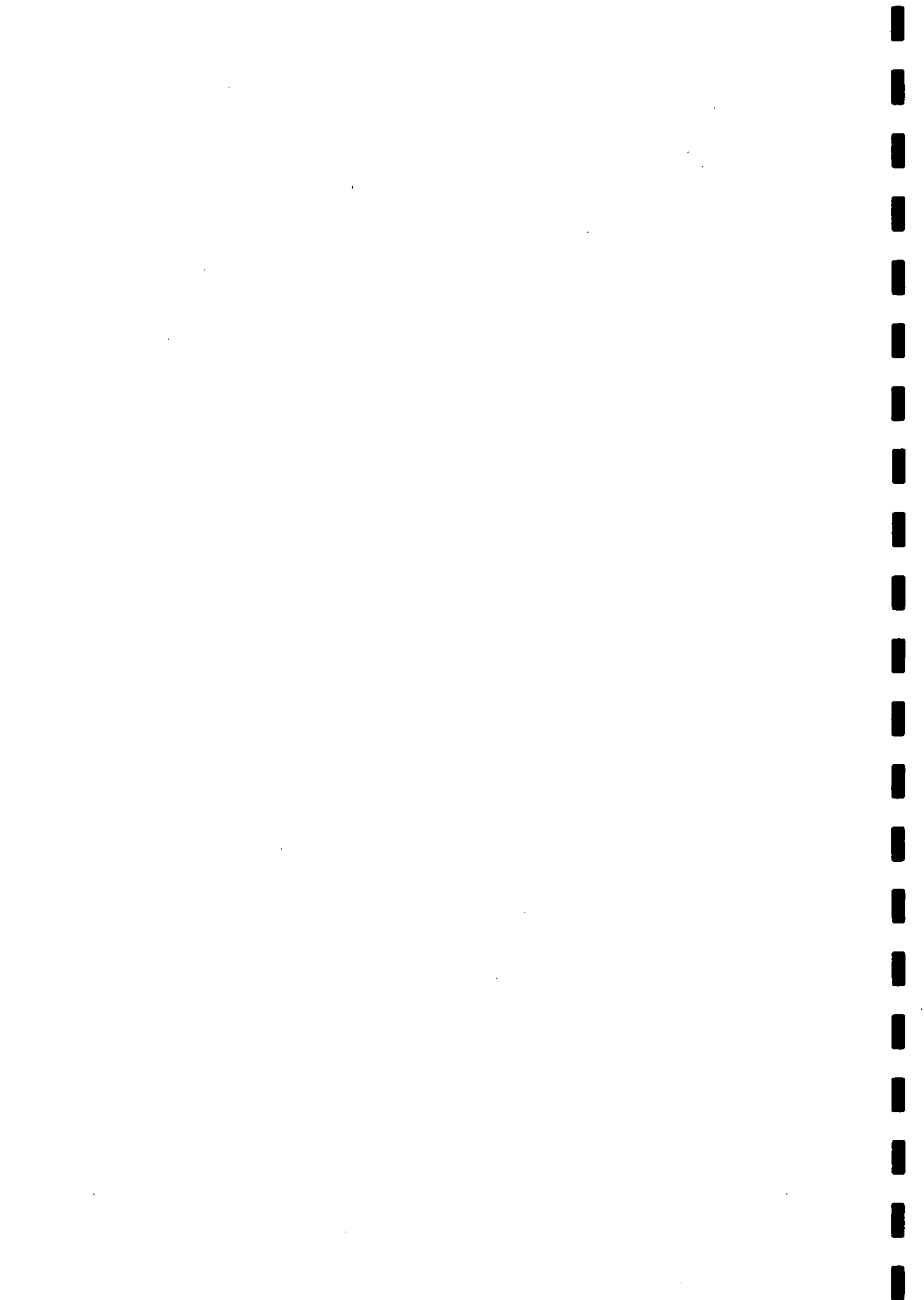


Table 1 Sampling dates, locations and sites.

Sampling date	Location	NGR	Sites sampled		
			C	I	R
03.02.87	River Parrett	ST 460100	↓		
13.02.87	River Frome Louds Mill, Dorchester	SY 714903	↓	↓	↓
26.02.87	Drimpton Stream, Broadwindsor	ST 435028	↓	↓	↓
18.03.87	Wray Brook	SX 708849	↓	↓	↓
18.03.87	Corfe River, Corfe Castle	SX 961831	↓	↓	↓
18.03.87	River Cerne	SY 665999		↓	
28.04.87	Drimpton Stream	ST 435028	↓	↓	↓
27.07.87	Drimpton Stream	ST 435028	↓	↓	↓
			(+ upstream sample)		
15.10.87	Drimpton Stream	ST 435028	↓	↓	↓

Table 2. The BMWP amended score system

Families	Score
Siphonuridae, Heptageniidae, Leptophlebiidae, Ephemerellidae, Potamanthidae, Ephemeridae	
Taeniopterygidae, Leuctridae, Capniidae, Perlodidae, Perlidae, Chloroperlidae	
Aphelocheiridae	10
Phryganeidae, Molannidae, Beraeidae, Odontoceridae, Leptoceridae, Goeridae, Lepidostomatidae, Brachycentridae, Sericostomatidae	
Astacidae	
Lestidae, Agriidae, Gomphidae, Cordulegasteridae, Aeshnidae, Corduliidae, Libellulidae	8
Psychomyiidae, Philopotamidae	
Caenidae	
Nemouridae	7
Rhyacophilidae, Polycentropodidae, Limnephilidae	
Neritidae, Viviparidae, Ancyliidae	
Hydroptilidae	
Unionidae	6
Corophilidae, Gammaridae	
Platycnemididae, Coenagriidae	
Mesovelidae, Hydrometridae, Gerridae, Nepidae, Naucoridae, Notonectidae, Pleidae, Corixidae	
Haliplidae, Hygrobiidae, Dytiscidae, Gyrinidae, Hydrophilidae, Glambidae, Helodidae, Dryopidae, Elminthidae, Chrysomelidae, Curculionidae	5
Hydropsychidae	
Tipulidae, Simuliidae	
Planariidae, Dendrocoelidae	
Baetidae	
Sialidae	4
Piscicolidae	
Valvatidae, Hydrobiidae, Lymnaeidae, Physidae, Planorbidae, Sphaeriidae	3
Glossiphoniidae, Hirudidae, Erpobdellidae	
Asellidae	
Chironomidae	2
Oligochaeta (whole class)	1

Table 3. % coarse sediment $>125\text{-}\mu\text{m}$ in 1-mm sieved sediment samples
(preliminary survey March 1987). Key: C, control I, impact R, recovery

Location	Site	% coarse particles $>125\text{ }\mu\text{m}$
R. Frome	C	67
	I	79
	R	82
Drimpton Stream	C	76
	I	86
	R	88
Wray Brook	C	96.7
	I	69
	R	98.6
Corfe River	C	83
	I	96
	R	87
R. Cerne	I	76
R. Parrett	I	77

Table 4. Comparison of 'active substances' (LAS) content of coarse particles and total sediment for preliminary survey samples (March 1987).

Location	Site	Concentration of LAS in sediment/ $\mu\text{g g}^{-1}$	
		Coarse particles ($>125 \mu\text{m}$)	Total sediment
River Frome	Impact	2.21, 2.48	0.92
Drimpton Stream	Impact	0.38, 0.30	0.34

(N.B. Values not corrected for extraction efficiency.)

Table 5. Variation in calibration graph slopes. These are used as conversion factors for integrated areas to injected sample concentrations (mg active substances l⁻¹).

Analysis date	Sampled analysed	Conversion factors /area l mg ⁻¹
10.04.87	Preliminary survey	169809.62
22.05.87	Spring survey unspiked	158905.48
22.05.87	Spring survey spiked	156752.92
24.07.87	Coarse sediment and blanks	165522.91
27.08.87	Summer survey unspiked	160765.44
12.09.87	Summer survey spiked	159011.29
18.11.87	Autumn survey unspiked	168770.23
19.11.87	Autumn survey spiked	166657.57

Mean C.F. 163274.43 \pm 5007.5 (3.1%) n = 8

Table 6. Comparison of LAS sediment concentrations analysed by SRC and FBA. The samples were obtained from the Drimpton Stream at the spring visit.

Site	Concentration of LAS in the sediment / $\mu\text{g g}^{-1}$		n
	SRC	FBA	
Control	1.31, 1.38	1.30 ± 0.34	4
Impact	1.23, 1.98	0.98 ± 0.08	4
Recovery	1.07	1.01 ± 0.06	4

Table 7. Total recovery of spike additions from spiked Drimpton Stream sediments (including liquid phase)

Site	Spring survey	% recovery Summer survey	Autumn survey
Control	80.1	80.0	82.3
Impact	68.5	73.8	Insufficient data
Recovery	72.1	83.3	77.6

Table 8. Recovery of spike adsorbed onto Drimpton Stream sediments.

Site	% recovery		Autumn survey
	Spring survey	Summer survey	
Control	79.8	78.54	82.1 \pm 16.4 (n=4)
Impact	67.7	71.38	Insufficient data
Recovery	78.4	61.05	78.4 \pm 13.9 (n=4)

Table 9. Results of the analysis of sediments for some sites in the initial assessment survey. For dates of sampling see Table 1.

Key: C, control I, impact R, recovery.

Site	Location	Concentration of LAS in total solids/ $\mu\text{g g}^{-1}$	% of sediment mass $< 125 \mu\text{m}$
Louds Mill, Dorchester	SY 714903		
C		0.50, 0.84	33
I		5.55, 3.39	21
R		0.28, 0.06	18
Drimpton Stream, Broadwindsor		ST 435028	
C		0.09, 0.49	24
I		1.84, 1.06	14
R		2.12, 1.94	12
Wray Brook	SX 768849		
C		0.95, 0.47	3.3
I		3.98, 4.53	31
R		5.19, 2.11	1.4
Corfe River, Corfe Castle	SX 961831		
C		0.99, 0.04	17
I		0.32, 1.82	4
R		0.11, 0.57	13
River Cerne	SY 665999		
I		0.44, 1.36	24
River Parret*	ST 460100		
I		1.46, 1.59	23

(*Note. This sample was treated differently from the rest. The sediment was left - 2 days at 5qC before drying. This may account for the low concentration of LAS compared with the result from the SRC study, ie - $5.71 \mu\text{g} \cdot \text{g}^{-1}$.)

Table 10. Results of "bankside" faunal analysis - BMWP biotic score and averagescore per taxon.

Site		Date	No. scoring taxa	Score	ASPT
Dorchester	Control	13.2.87	11	58	5.3
	Impact	13.2.87	12	62	5.2
	Recovery	13.2.87	14	77	5.5
Drimpton Stream (= Broadwindsor)	Control	26.2.87	9	52	5.7
	Impact	26.2.87	7	34	4.8
	Recovery	26.2.87	10	47	4.4
Corfe River	Control	18.3.87	13	56	4.3
	Impact	18.2.87	11	35	3.2
	Recovery	18.2.87	12	48	4.0
Cerne Abbas	Impact	18.3.87	10	58	5.8
	Recovery	18.3.87	10	58	5.8
Back data (3 min. kick/sweep samples laboratory sorted)					
Semington Brook	Impact	3.86	16	64	4.0
Otter	Impact	3.86	23	118	5.13
Wray Brook	Impact	3.86	13	54	4.15

Table 11. Drimpton Stream water chemistry during 1987 and 1988.

Sampling Date	Temp /°C	pH	Cond /S cm ⁻¹	Na ⁺	K ⁺	Ca ²⁺	Mg ²⁺	SO ₄ ²⁻ mmol dm ⁻³	Cl ⁻	NO ₃ ⁻	PO ₄ ³⁻	I	⁺ Alk /meq dm ⁻³	P _{CO2} /atm
28.4.87														
Control	13.1	8.06	335	0.56	0.08	1.96	0.15	0.35	0.54	0.18	0.005	6.86	3.34	1.45
Impact	13.5	8.06	484	3.13	0.20	2.19	0.44	0.58	2.28	0.44	0.06	9.84	2.11	0.89
Recovery	12.7	8.04	369	1.20	0.08	1.96	0.28	0.37	1.18	0.23	0.02	7.49	2.75	1.24
27.7.87														
Upstream control	14.0	7.79	303	0.41	0.04	1.53	0.15	0.46	0.42	0.05	0.003	5.76	2.70	2.25
Control	14.9	8.08	441	0.64	0.08	2.30	0.21	0.41	0.38	0.14	0.004	7.97	4.06	1.70
Impact	16.0	7.94	706	0.96	0.15	1.70	0.11	0.51	0.62	0.36	0.03	7.18	4.06	2.40
Recovery	14.6	7.96	495	0.91	0.16	1.82	0.21	0.49	0.61	0.33	0.03	7.55	4.12	2.28
Effluent	17.2	7.95	917	3.94	0.46	2.18	0.17	1.35	2.75	1.54	0.27	12.3	4.14	2.26
15.10.87														
Control	10.1	7.68	305	0.40	0.11	1.70	0.16	0.41	0.42	0.22	0.004	6.07	2.76	2.84
Impact	10.1	7.55	465	0.44	0.11	1.75	0.08	0.42	0.48	0.24	0.009	6.10	2.76	3.82
Recovery	10.1	7.55	322	0.50	0.13	1.78	0.17	0.51	0.47	0.29	0.02	6.52	2.80	3.86
Effluent	10.1	7.52	541	2.19	0.33	2.44	0.07	0.98	1.54	0.79	0.19	10.0	3.20	4.46
18.4.88														
Control	11.2	8.07	321.1	0.32	0.07	2.10	0.16	0.36	0.49	0.14	0.003	7.11	3.56	1.48
Impact	11.1	7.90	431.5	0.70	0.12	2.12	0.06	0.51	0.95	0.34	0.048	7.73	3.69	2.25
Recovery	10.6	8.08	353.8	0.42	0.12	2.18	0.07	0.41	0.63	0.17	0.024	7.28	3.48	1.40
Effluent	12.3	7.82	736.4	2.37	0.31	2.44	0.07	1.09	2.31	1.16	0.233	11.26	4.41	3.14
19.7.88														
Control	14.8	8.18	395.6	0.46	0.09	2.32	0.14	0.36	0.47	0.09	0.007	7.90	4.38	1.43
Impact	14.5	8.10	430.8	0.85	0.13	2.34	0.15	0.44	0.76	0.19	0.158	8.52	4.34	1.68
Recovery	14.4	7.96	447.6	0.80	0.17	2.31	0.16	0.46	0.52	0.16	0.146	8.42	4.44	2.39
Effluent	18.0	8.52	801.8	2.63	0.31	2.52	0.15	1.09	2.45	0.90	0.20	11.7	4.86	0.70
16.10.88														
Control	12.1	7.40	340.2	0.44	0.84	2.11	0.14	0.36	0.52	0.14	0.003	7.35	3.86	7.69
Impact	12.1	7.40	353.2	0.93	0.10	2.04	0.14	0.43	0.85	0.04	0.013	7.70	3.76	7.46
Recovery	12.2	7.40	366.9	0.71	0.13	2.01	0.15	0.49	0.39	0.24	0.026	7.55	4.03	8.00
Effluent	13.2	7.33	715.7	3.48	0.45	2.66	0.15	1.25	1.90	0.30	0.028	12.4	4.61	10.63

⁺Alk is the total alkalinity

I is the total ionic strength

Table 12. Comparison of charge balances at each sample site calculated using RIVEREQ.

Date:	$\Sigma+$ /meq dm ⁻³	$\Sigma-$ dm ⁻³	Percentage difference from mean
28.4.87			
Control	4.68	4.58	2.0
Impact	8.32	5.78	35.0
Recovery	5.55	4.74	15.8
18.4.88			
Control	4.70	4.73	0.6
Impact	4.93	5.83	16.7
Recovery	4.81	4.92	2.3
Effluent	7.17	9.84	31.4
18.7.88			
Control	5.21	5.41	3.8
Impact	5.60	6.04	7.6
Recovery	5.56	5.90	5.9
Effluent	7.54	9.98	27.9
16.10.88			
Control	4.85	5.08	4.6
Impact	5.21	5.34	2.5
Recovery	4.93	5.46	10.2
Effluent	9.05	8.85	2.2

Table 13. Flow ratios calculated from chemical data (27 July 1987) using equation [5].

Quantity used in calculation	Flow ratio qI/q8
Na ⁺ concentration	0.08
K ⁺ concentration	0.20
NO ₃ ⁻ concentration	0.13
Phosphate concentration	0.11
Mg ²⁺ concentration	0.00
Ca ²⁺ concentration	4.20
Cl ⁻ concentration	0.10
SO ₄ ²⁻ concentration	0.09
Electrical conductivity	0.11

Table 14. LAS Analyses

Site	Concentration of LAS in sediment / $\mu\text{g g}^{-1}$ (SD)	Extraction efficiency %	Number of samples	Percentage organic material <125- μm size
28.4.87				
Control	1.30 (0.34)	79.8	4	4.1
Impact	0.98 (0.08)	67.7	4	2.7
Recovery	1.00 (0.06)	78.4	4	2.9
27.7.87				
Upstream	0.46 (0.50)	74.4	4	-
Control	2.39 (0.31)	78.5	4	10.8
Impact	41.0 (4.1)	71.4	3	13.7
Recovery	1.41 (0.80)	61.1	4	7.0
15.10.87				
Control	1.93 (0.07)	82.1	4	5.5
Impact	28.4 (1.90)	-	4	11.5
Recovery	0.65 (1.70)	78.4	4	3.0
18.4.88				
Control	22.6 (0.06)	74.7	4	6.9
Impact	15.3 (0.48)	98.0	4	6.4
Recovery	0.65 (0.13)	80.1	4	3.7
18.7.88				
Control	0.40 (0.22)	66.9	4	6.9
Impact	7.44 (0.50)	71.2	4	5.6
Recovery	0.39 (0.17)	66.2	4	5.0
16.10.88				
Control	14.77 (0.47)	73.28	4	7.1
Impact	2.99 (1.58)	83.26	2	9.1
Recovery	0.34 (0.36)	61.20	4	3.6

Table 15. Seasonal and annual variation in total number of families; number of families used in the BMWP score system; the BMWP score and average score per taxon (ASPT) at the control, Impact and Recovery sites on the Drimpton Stream.

		Control		Impact		Recovery	
		1987	1988	1987	1988	1987	1988
No. of families	Sp	29	34	33	29	33	32
	Su	25	26	31	25	32	12
	Au	30	32	33	26	27	29
	Co	39	43	44	37	46	37
No. of BMWP families							
	Sp	23	23	22	21	25	22
	Su	18	21	22	18	20	7
	Au	24	23	23	19	15	20
	Co	28	29	30	25	28	25
BMWP score	Sp	135	132	130	111	152	113
	Su	99	119	133	101	106	30
	Au	139	132	113	86	69	102
	Co	172	170	177	146	162	134
ASPT							
	Sp	5.87	5.74	5.91	5.29	6.08	5.23
	Su	5.50	5.67	6.05	5.61	5.30	4.29
	Au	5.79	5.74	4.91	4.53	4.60	5.10
	Co	6.14	5.86	5.90	5.84	5.79	5.36

Table 16a. The physical and chemical characteristics of sites on the Drimpton Stream above and below the effluent from a sewage treatment works 1987. (1 = $<10 \text{ cm s}^{-1}$, 2 = $>10-25$, 3 = $>25-50$; 2 1 = $<0.31 \text{ m}^3 \cdot \text{s}^{-1}$, 2 = $>0.31-0.62$). [Superscript c indicates the 11 physical and chemical variables and p indicates the 11 physical variables used in the RIVPACS prediction program.]

Site name Grid reference	Control ST432024	Impact ST432024	Recovery ST432024
Water width (m)cp	1.5	2	1.5
Mean depth (cm)cp	9	10	17
Surface velocity (category)*1	3	3	3
Substratum cover %cp			
Boulders and cobbles	43	27	45
Pebbles and gravel	34	43	33
Sand	13	13	12
Silt and clay	10	17	10
Altitude (m)cp	136	134	132
Longitude (o,')p	2o48'W	2o48'W	2o48'W
Latitude ({o},')p	50{o}50'N	50{o}50'N	50{o}50'N
Distance from source (km){cp}	1.9	2.0	2.1
Slope (m km ⁻¹){cp}	25	25	25
Discharge (category) {*2p}	1	1	1
Air temperature range ({o}C){cp}	11.28	11.28	11.28
Mean air temperature ({o}C){cp}	10.55	10.55	10.55
Total oxidised nitrogen (mg l ⁻¹ N){c}	2.51	4.85	3.99
Total alkalinity (mg l ⁻¹ CaCO ₃){c}	169.3	148.7	161.2
Chloride (mg l ⁻¹ Cl){c}	15.8	39.9	26.7

Table 16b. The physical and chemical characteristics of sites on the Drimpton Stream above and below the effluent from a sewage treatment works 1988. (1 = $<10 \text{ cm s}^{-1}$, 2 = $>10-25$, 3 = $>25-50$; 2 1 = $<0.31 \text{ m}^3 \text{ s}^{-1}$, 2 = $>0.31-0.62$). [Superscript c indicates the 11 physical and chemical variables and p indicates the 11 physical variables used in the RIVPACS prediction program.]

Site name Grid reference	Control ST432024	Impact ST432024	Recovery ST432024
Water width (m)cp	1.6	1.7	1.2
Mean depth (cm)cp	10.5	7.5	15
Surface velocity (category)*1	3	3	3
Substratum cover %cp			
Boulders and cobbles	32	33	43
Pebbles and gravel	45	37	30
Sand	16	18	18
Silt and clay	7	12	9
Altitude (m)cp	136	134	132
Longitude (o,')p	2o48'W	2o48'W	2o48'W
Latitude ({o},')p	50{o}50'N	50{o}50'N	50{o}50'N
Distance from source (km){cp}	1.9	2.0	2.1
Slope (m km ⁻¹){cp}	25	25	25
Discharge (category) {*2p}	1	1	1
Air temperature range ({o}C){cp}	11.28	11.28	11.28
Mean air temperature ({o}C){cp}	10.55	10.55	10.55
Total oxidised nitrogen (mg l ⁻¹ N){c}	1.74	3.31	2.66
Total alkalinity (mg l ⁻¹ CaCO ₃){c}	197.7	196.5	199.2
Chloride (mg l ⁻¹ Cl){c}	17.5	30.2	18.2

Table 17A. The relative abundance of fauna in spring (Sp), summer (Su), Autumn (Au) and Combined seasons (Co) samples at the Control site on the Drimpton Stream.

Fauna	Sp		Su		Au		Co	
Hydriidae	-	-	8	-	-	8	8	8
Planariidae	-	-	8	8	1	1	9	9
Hydrobiidae	133	255	50	1049	443	507	626	1311
Lymnaeidae	-	-	-	1	-	-	-	1
Ancylidae	5	11	33	104	79	51	117	166
Succineidae	-	1	-	-	-	-	-	1
Sphaeriidae	12	16	-	9	17	-	29	25
Naididae	6	-	-	-	1	8	7	8
Tubificidae	303	475	116	150	529	93	948	718
Enchytraeidae	-	44	-	-	12	-	12	44
Lumbriculidae	39	3	70	21	61	38	170	62
Lumbricidae	-	-	1	-	1	-	2	-
Glossiphoniidae	1	1	2	2	3	2	6	5
Erpobdellidae	3	2	-	-	-	10	3	12
Hydracarina	-	32	-	-	-	1	-	33
Asellidae	1	1	-	1	10	18	11	20
Gammaridae	61	69	90	80	64	94	215	243
Baetidae	128	119	115	-	114	38	357	157
Heptageniidae	15	43	18	9	62	28	95	80
Leptophlebiidae	70	179	-	15	-	21	70	215
Ephemereidae	-	-	169	17	1	2	170	19
Ephemeridae	1	-	-	-	-	-	1	-
Nemouridae	1	1	-	-	2	1	3	2
Leuctridae	-	-	24	46	1	-	25	46
Perlodidae	1	1	-	-	-	-	1	1
Dytiscidae	20	28	29	-	1	3	50	31
Hydrophilidae	-	-	-	2	-	-	-	2
Elminthidae	53	112	219	16	252	141	524	269
Rhyacophilidae	-	12	68	2	28	3	96	17
Polycentropodidae	4	4	-	-	4	-	8	4
Hydropsychidae	17	46	1	2	90	40	103	38
Limnephilidae	35	33	16	4	2	1	53	38
Goeridae	3	5	-	-	53	9	56	14
Sericostomatidae	2	15	-	5	9	19	11	39
Tipulidae	2	17	356	-	167	60	525	77
Psychodidae	-	8	-	8	-	-	-	1
Ceratopogonidae	-	9	-	-	2	-	2	9
Tanypodinae	10	4	8	4	-	-	18	8
Prodiamesinae	3	2	-	10	-	1	3	13
Orthocladiinae	1	1	217	-	44	67	262	68
Chironomini	8	8	-	1	-	9	8	18
Tanytarsini	9	60	80	53	33	35	122	148
Simuliidae	-	-	81	8	47	706	128	714
Stratiomyidae	-	-	-	-	-	8	-	-
Empididae	-	16	16	-	9	8	25	24

Table 17B. The relative abundance of fauna in Spring (Sp), Summer (Su), Autumn (Au) and Combined seasons (Co) samples at the Impact site on the Drimpton Stream.

Fauna	Sp		Su		Au		Co	
Hydridae	-	-	-	192	36	218	36	410
Hydrobiidae	144	39	1028	304	189	279	1361	622
Lymnaeidae	-	1	-	-	-	66	-	67
Ancylidae	1	3	1	88	51	20	53	111
Zonitidae	-	-	4	-	-	-	4	-
Sphaeriidae	4	2	130	16	10	26	144	44
Naididae	33	318	-	-	4	-	37	318
Tubificidae	1637	282	1490	1184	377	711	3504	2177
Enchytraeidae	-	31	-	-	-	-	-	31
Lumbriculidae	65	106	129	416	67	151	261	673
Lumbricidae	-	3	-	-	-	-	-	-
Glossiphoniidae	3	3	-	-	10	3	13	6
Erpobdellidae	4	1	1	3	24	11	29	15
Hydracarina	72	8	64	-	12	-	148	8
Asellidae	6	1	3	1	29	5	38	7
Gammaridae	28	30	298	49	34	74	360	153
Baetidae	92	-	-	-	32	12	124	49
Heptageniidae	5	6	1	2	9	-	15	8
Leptophlebiidae	104	26	5	-	1	-	110	26
Ephemerellidae	1	-	96	27	-	-	97	27
Ephemeridae	-	-	1	1	-	-	1	1
Leuctridae	2	-	2	9	-	-	4	9
Perlodidae	-	1	-	-	-	-	-	1
Veliidae	-	-	1	-	-	-	1	-
Corixidae	-	-	1	-	-	-	1	-
Halipidae	-	-	-	-	1	-	1	-
Dytiscidae	55	10	130	72	6	12	191	94
Hydrophilidae	-	-	1	-	-	-	1	-
Elminthidae	16	10	64	128	35	128	115	266
Sialidae	-	-	-	-	2	-	2	-
Rhyacophilidae	2	1	1	9	8	3	11	13
Polycentropodidae	-	-	1	-	-	-	1	-
Psychomyiidae	9	-	-	-	-	-	9	-
Hydropsychidae	2	7	-	-	17	2	19	9
Limnephilidae	13	2	11	1	2	2	26	5
Goeridae	1	-	-	-	6	-	7	-
Sericostomatidae	3	1	1	-	3	6	7	7
Tipulidae	14	5	2	3	38	5	54	13
Psychodidae	8	-	-	-	-	-	8	-
Ceratopogonidae	1	-	-	-	1	-	2	-
Tanypodinae	2	-	346	11	16	64	364	75
Prodiamesinae	27	5	262	131	9	3	298	139
Orthoclaadiinae	82	58	515	3	54	130	651	191
Chironomini	8	16	266	905	99	3107	373	4028
Tanytarsini	1	26	835	321	84	64	920	411
Simuliidae	-	-	-	64	8	218	8	282
Empididae	-	-	-	-	6	1	6	1
Muscidae	-	-	64	2	-	-	64	2

Table 17C. The relative abundance of fauna in Spring (Sp), Summer (Su), Autumn (Au) and Combined (Co) seasons samples at the recovery site on the Drimpton Stream.

Fauna	Sp		Su		Au		Co	
Hydriidae	-	-	25	-	-	-	25	-
Planariidae	-	8	8	-	-	16	8	24
Hydrobiidae	107	200	222	-	19	171	348	371
Ancylidae	1	9	9	-	-	8	10	17
Succineidae	-	-	1	-	-	-	1	-
Zonitidae	-	-	-	-	1	-	1	-
Sphaeriidae	2	17	24	-	-	-	26	17
Naididae	117	3082	72	-	34	24	223	3106
Tubificidae	2055	555	138	93	63	57	2256	705
Lumbriculidae	39	5	124	18	60	172	223	195
Lumbricidae	-	2	8	-	-	1	8	3
Glossiphoniidae	4	2	17	-	4	2	25	4
Erpobdellidae	2	1	1	-	1	1	4	2
Hydracarina	48	96	-	-	-	-	48	96
Asellidae	16	22	74	3	6	84	96	109
Gammaridae	43	91	48	-	2	51	93	142
Baetidae	110	50	476	33	92	165	678	248
Heptageniidae	1	3	1	-	1	3	3	6
Leptophlebiidae	138	113	-	-	-	1	138	114
Ephemereilidae	2	-	13	2	-	2	15	4
Nemouridae	6	-	-	-	-	-	6	-
Leuctridae	1	-	1	-	-	-	2	-
Dytiscidae	16	36	24	-	20	51	60	87
Hydrophilidae	1	-	32	1	-	-	33	1
Elminthidae	3	27	-	-	-	24	8	51
Rhyacophilidae	-	12	11	-	1	-	12	12
Polycentropodidae	2	1	-	-	-	-	2	1
Psychomyiidae	8	-	-	-	-	-	8	-
Hydropsychidae	3	21	-	-	2	1	5	22
Limnephilidae	60	4	-	-	4	10	64	14
Leptoceridae	1	-	-	-	-	-	1	-
Sericostomatidae	2	4	2	-	-	-	4	4
Tipulidae	5	4	90	-	1	10	96	14
Psychodidae	-	-	-	-	93	26	93	26
Ptychopteridae	2	-	1	-	-	-	3	-
Ceratopogonidae	-	13	-	-	35	-	35	13
Tanypodinae	16	89	550	5	1	4	567	98
Prodiamesinae	51	23	177	7	5	12	233	42
Orthocladiinae	111	579	1476	49	101	80	1688	708
Chironomini	38	37	660	21	60	9	758	67
Tanytarsini	8	185	1192	2	162	107	1362	294
Simuliidae	-	1	691	20	74	119	765	140
Empididae	-	-	241	-	2	-	243	-
Bibionidae	-	-	1	-	-	-	1	-
Syrphidae	-	-	-	-	2	-	2	-
Stratiomyidae	-	-	-	-	1	1	1	1

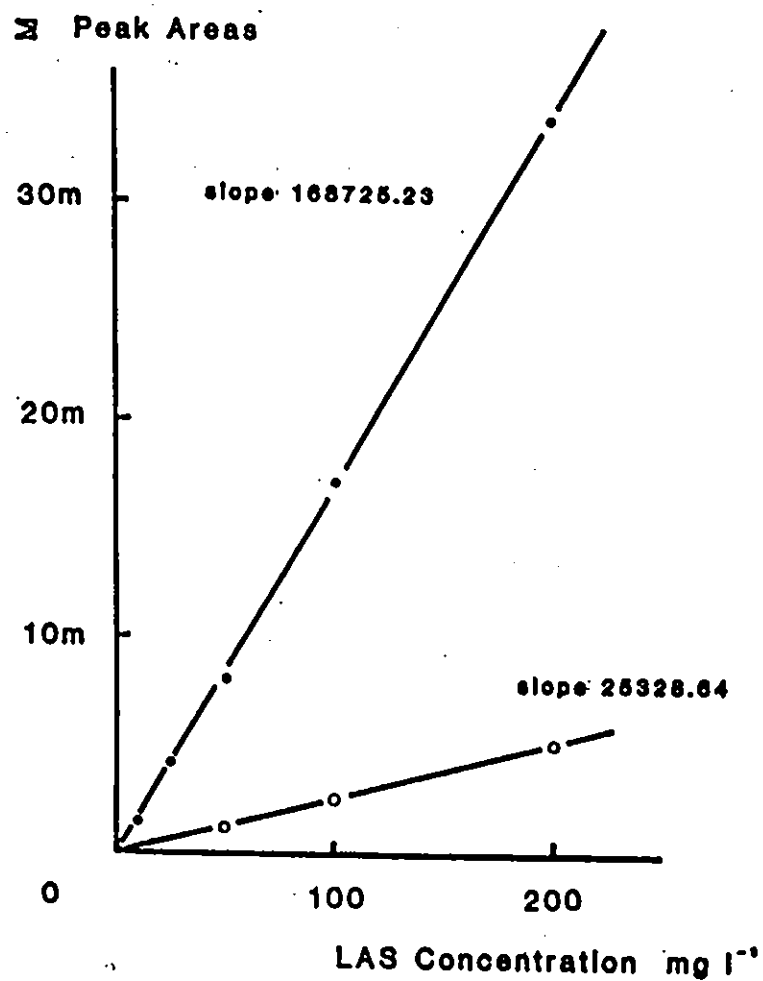
Table 17D. The relative abundance (sum of 3 seasons samples) of fauna at Control, Impact and Recovery sites on the Drimpton Stream.

Fauna	Control		Impact		Recovery	
Hydriidae	8	8	36	410	25	-
Planariidae	9	9	-	-	8	24
Hydrobiidae	626	1811	1361	622	348	371
Lymnaeidae	-	1	-	67	-	-
Ancylidae	117	166	53	111	10	17
Succineidae	-	1	-	-	1	-
Zonitidae	-	-	4	-	1	-
Sphaeriidae	29	25	144	44	26	17
Naididae	7	8	37	318	223	3106
Tubificidae	948	718	3504	2177	2256	705
Enchytraeidae	12	44	-	31	-	-
Lumbriculidae	170	62	261	673	223	195
Lumbricidae	2	-	-	3	8	3
Glossiphoniidae	6	5	13	6	25	4
Erpobdellidae	3	12	29	15	4	2
Hydracarina	-	33	148	8	48	96
Asellidae	11	20	38	7	96	109
Gammaridae	215	243	360	153	93	142
Baetidae	357	157	124	49	678	248
Heptageniidae	95	80	15	8	3	6
Leptophlebiidae	70	215	110	26	138	114
Ephemerellidae	170	19	97	27	15	4
Ephemeridae	1	-	1	1	-	-
Nemouridae	3	2	-	-	6	-
Leuctridae	25	111	4	1	2	-
Perlodidae	1	1	-	1	-	-
Veliidae	-	-	1	-	-	-
Corixidae	-	-	1	-	-	-
Haliplidae	-	-	1	-	-	-
Dytiscidae	50	31	191	94	60	87
Hydrophilidae	-	2	1	-	33	1
Elminthidae	524	269	115	266	8	51
Sialidae	-	-	2	-	-	1
Rhyacophilidae	96	17	11	13	12	12
Polycentropodidae	8	4	1	-	2	1
Psychomyiidae	-	-	9	-	8	-
Hydropsychidae	108	88	19	9	5	22
Limnephilidae	53	38	26	5	64	14
Goeridae	56	14	7	-	-	-
Leptoceridae	-	-	-	-	1	-
Sericostomatidae	11	39	7	7	4	4
Tipulidae	525	77	54	13	96	14
Psychodidae	-	16	8	-	93	26
Ptychopteridae	-	-	-	-	3	-
Ceratopogonidae	2	9	2	-	35	13
Tanypodinae	18	8	364	75	567	98
Diamesinae	-	-	-	-	-	2
Prodiamesinae	3	13	298	139	233	42
Orthocladiinae	262	68	651	191	1688	708
Chironomini	8	18	373	4028	758	68
Tanytarsini	122	148	920	411	1362	294
Simuliidae	128	714	8	282	765	140
Empididae	25	24	6	1	243	-
Muscidae	-	-	64	2	-	-
Bibionidae	-	-	-	-	1	-
Syrphidae	-	-	-	-	2	-
Stratiomyidae	-	8	-	-	1	1

Table 18. Observed/predicted ratios by four monitors of biological water quality. (See text for details) (Values are shown for predictions based on 11 physical (11P) and 11 physical and chemical (11PC) site variables.)

Monitors of biological water quality	Control		Impact		Recovery	
	11P	11PC	11P	11PC	11P	11PC
Total families	0.986	1.002	0.914	0.948	0.956	0.956
BMWP families	0.979	1.008	0.920	0.970	0.935	0.942
ASPT	0.992	1.003	0.959	1.025	0.928	0.970
Score	0.891	0.900	0.908	0.946	0.839	0.848

Calibration graphs Marlon 'A' Standards



- 50 μl 'Flushed loop' injection
- 10 μl micro-metering pump injection

Fig. 1. Detector response calibration for automatic injection

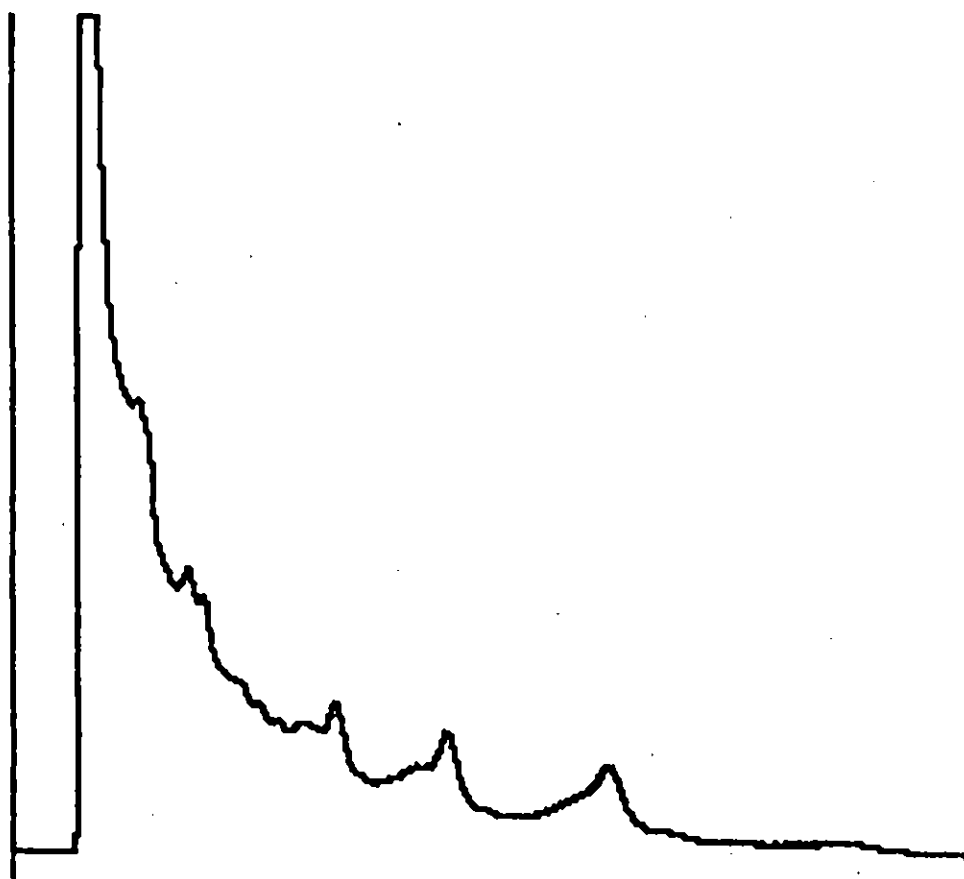


Fig. 2. Replot of chromatogram of recovery site extract showing high background interference in early phase

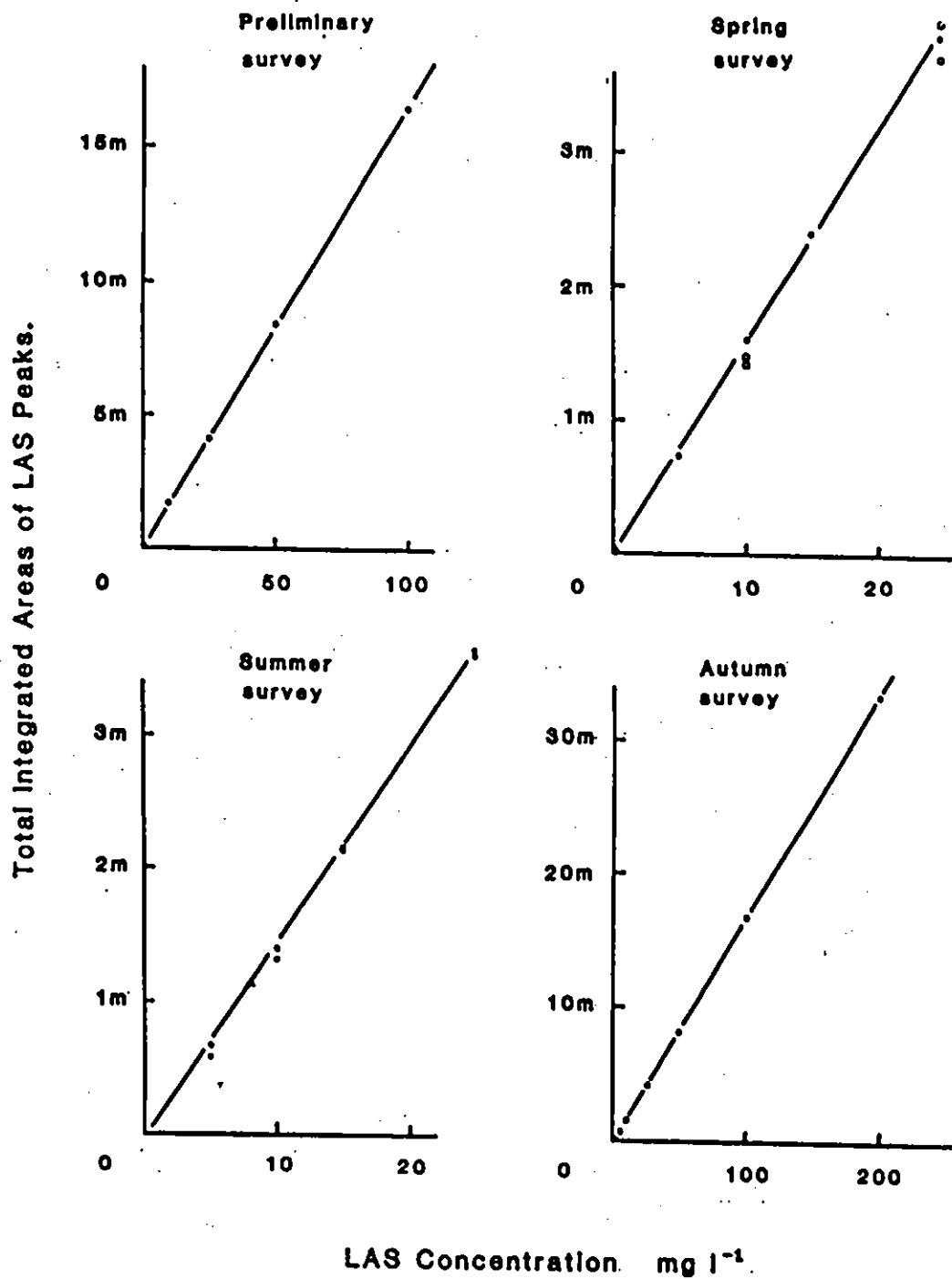


Fig. 3. Calibration graphs for LAS measurement during the four survey periods

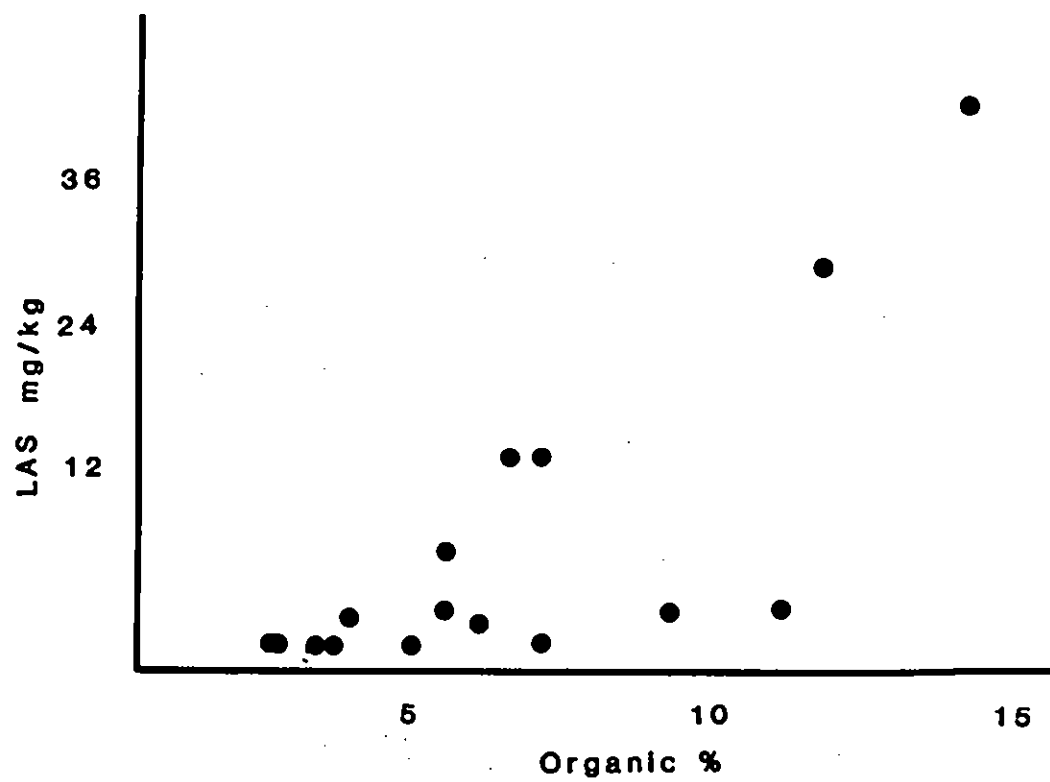


Fig. 4. Association between LAS concentration and organic content of sediments (control values included)

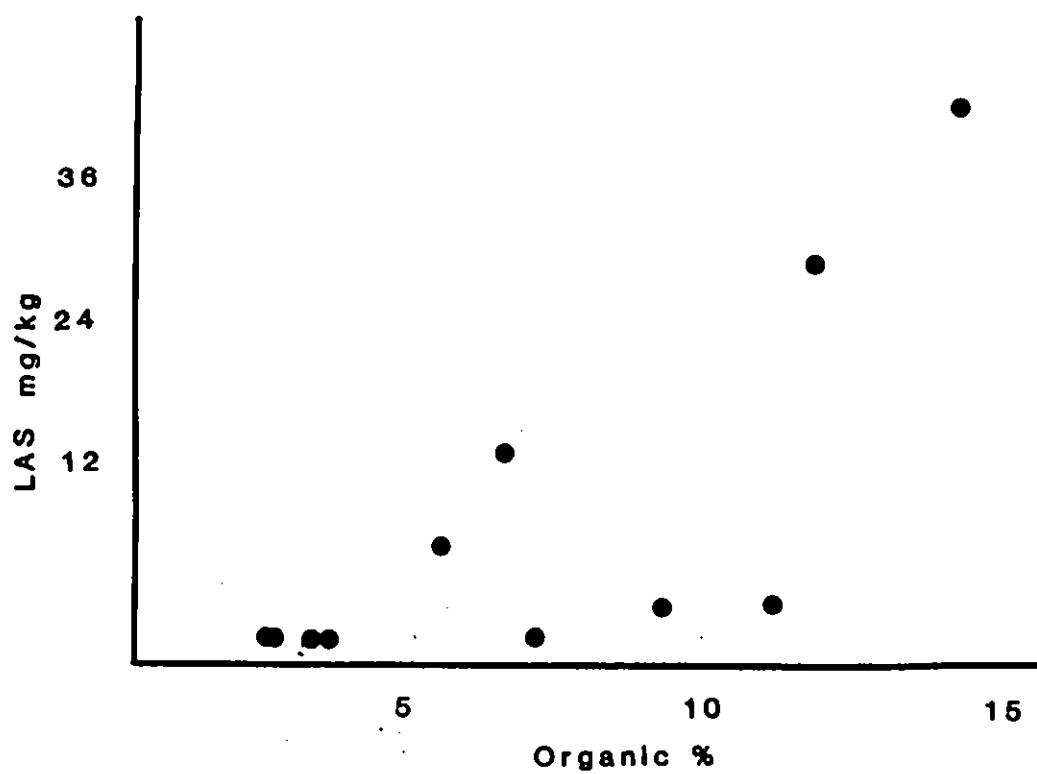


Fig. 5. Association between LAS concentration and organic control of sediment (control values omitted).

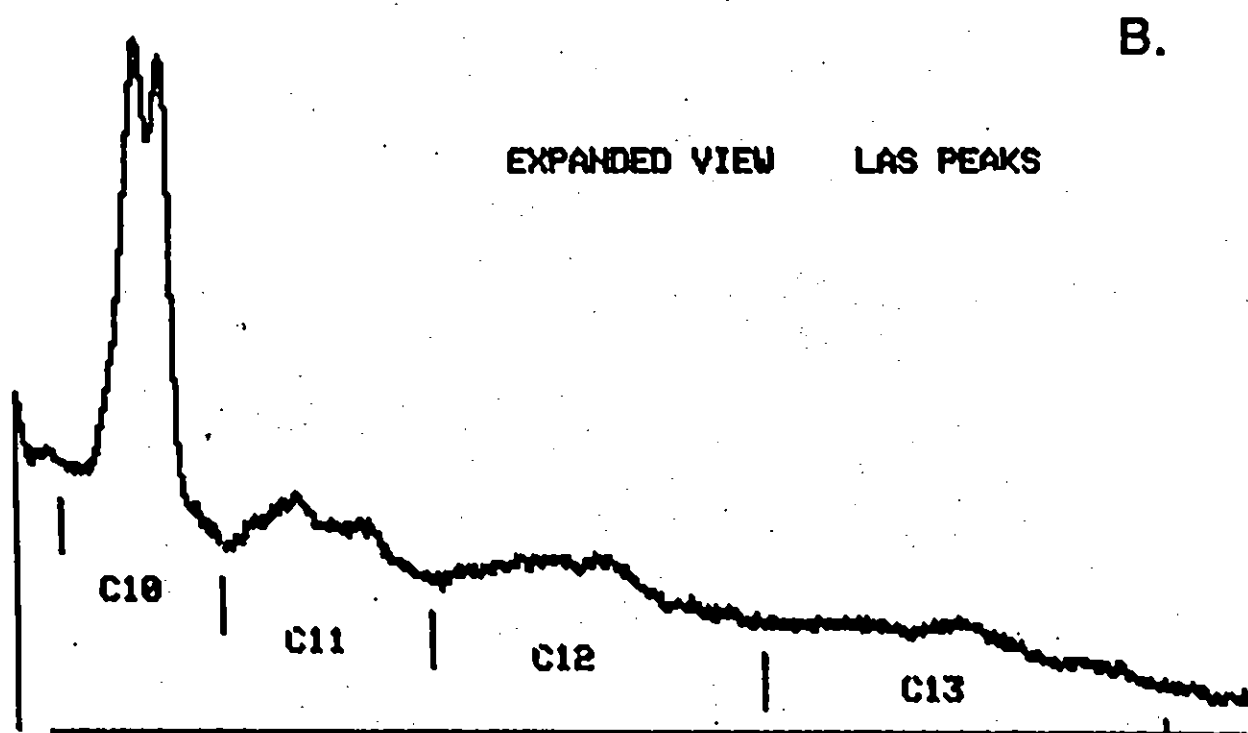
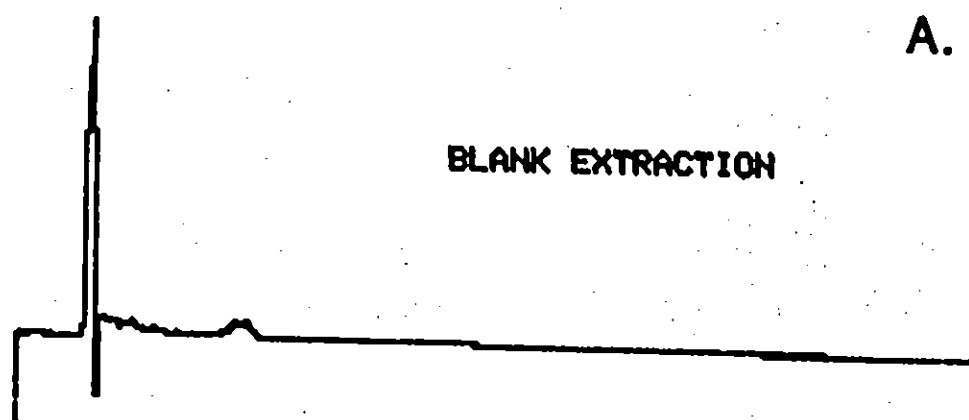


Fig. 6. Replot of chromatogram of an extraction blank sample.
Sample A direct replot at attenuation 8.
B expanded plot over the LAS peaks

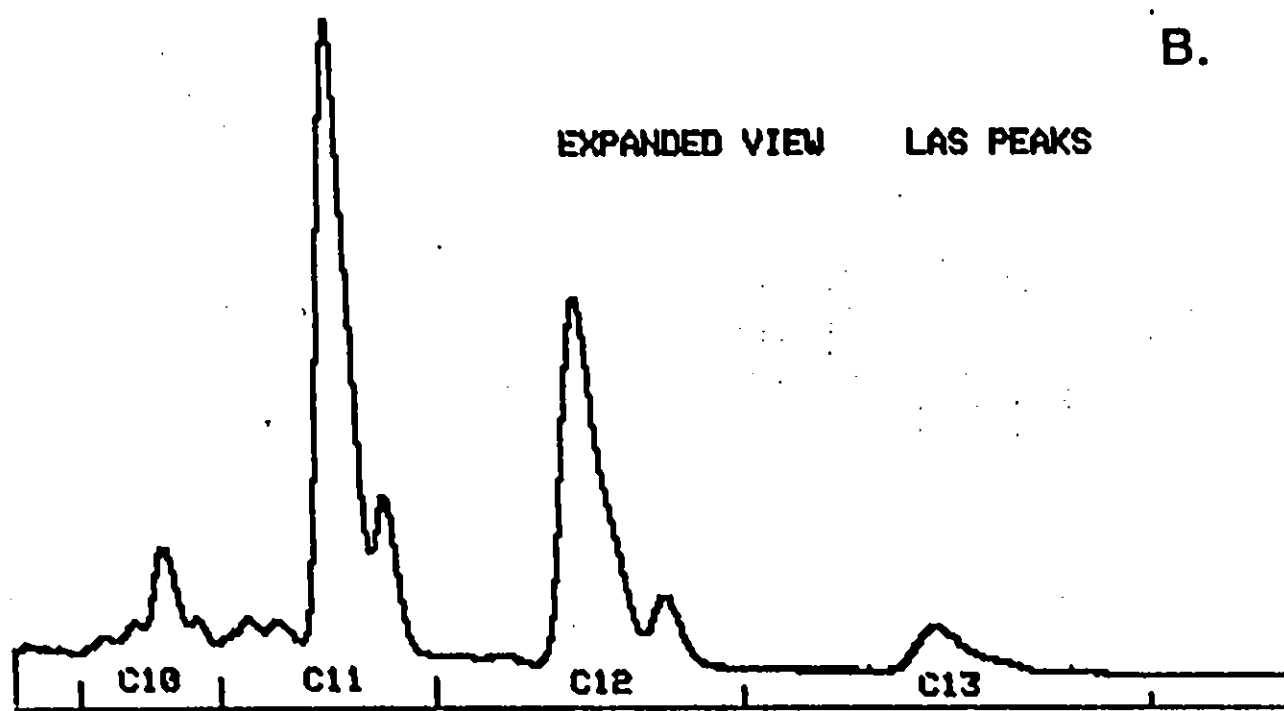
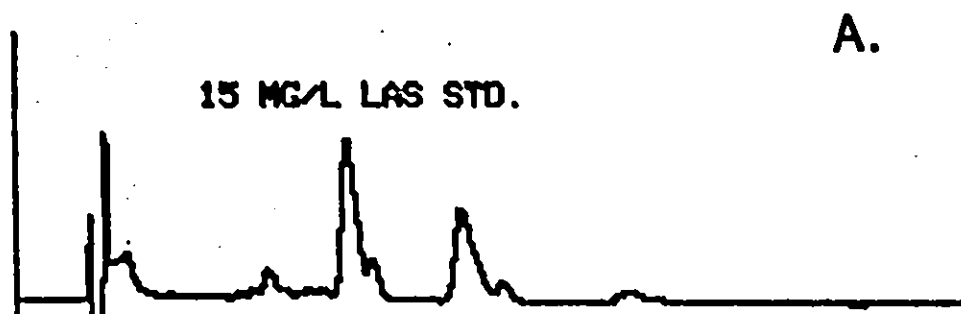


Fig.7. Replot of chromatogram of 15 mg l⁻¹ LAS standard.
A. direct plot at attenuation 8.
B. expanded plot over LAS peaks.

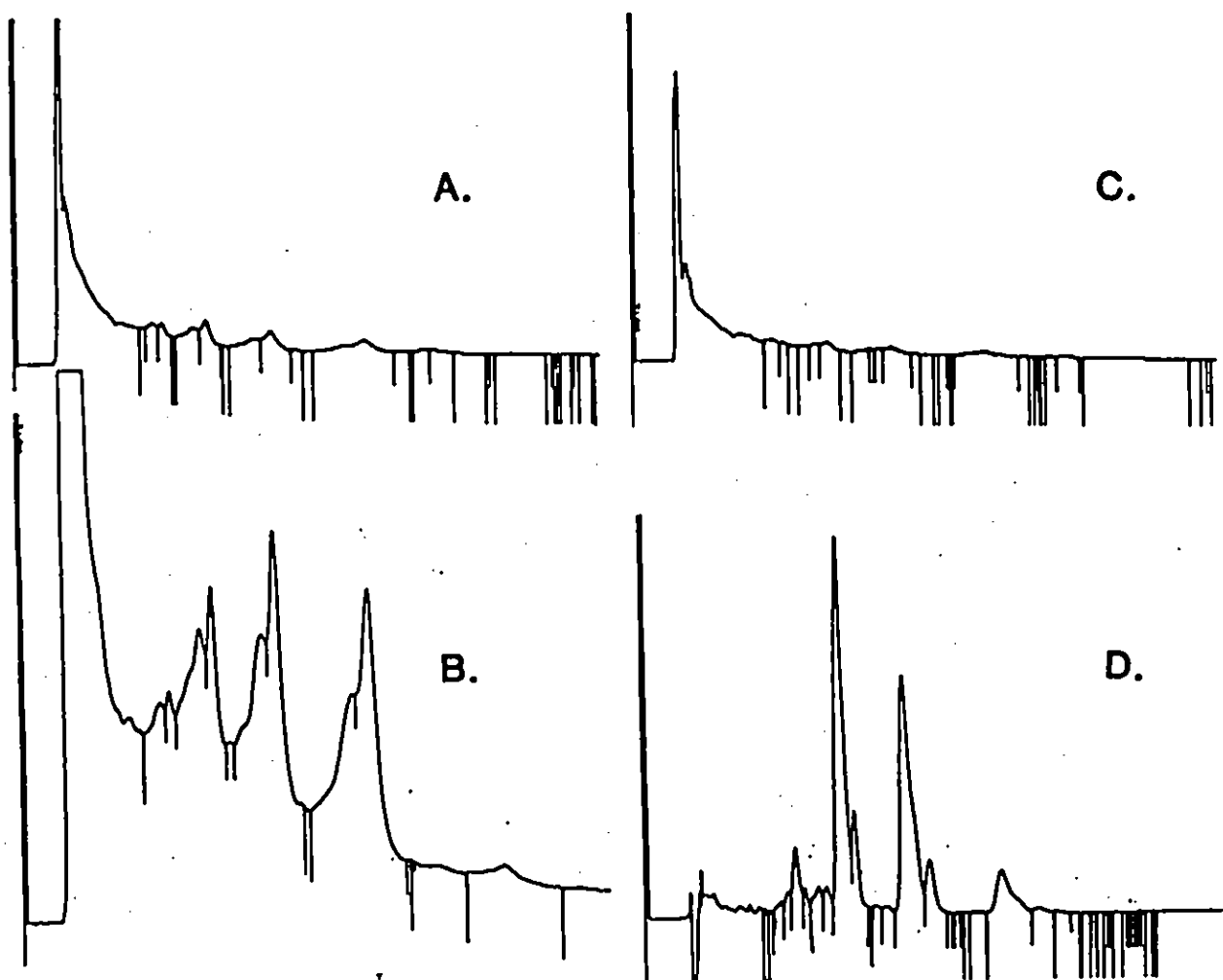


Fig. 8. Chromatograms of autumn 1987 survey samples.
A. Control site B. Impact site C. Recovery site
D. 100 mg l⁻¹ active substances Marlton 'A' standard.

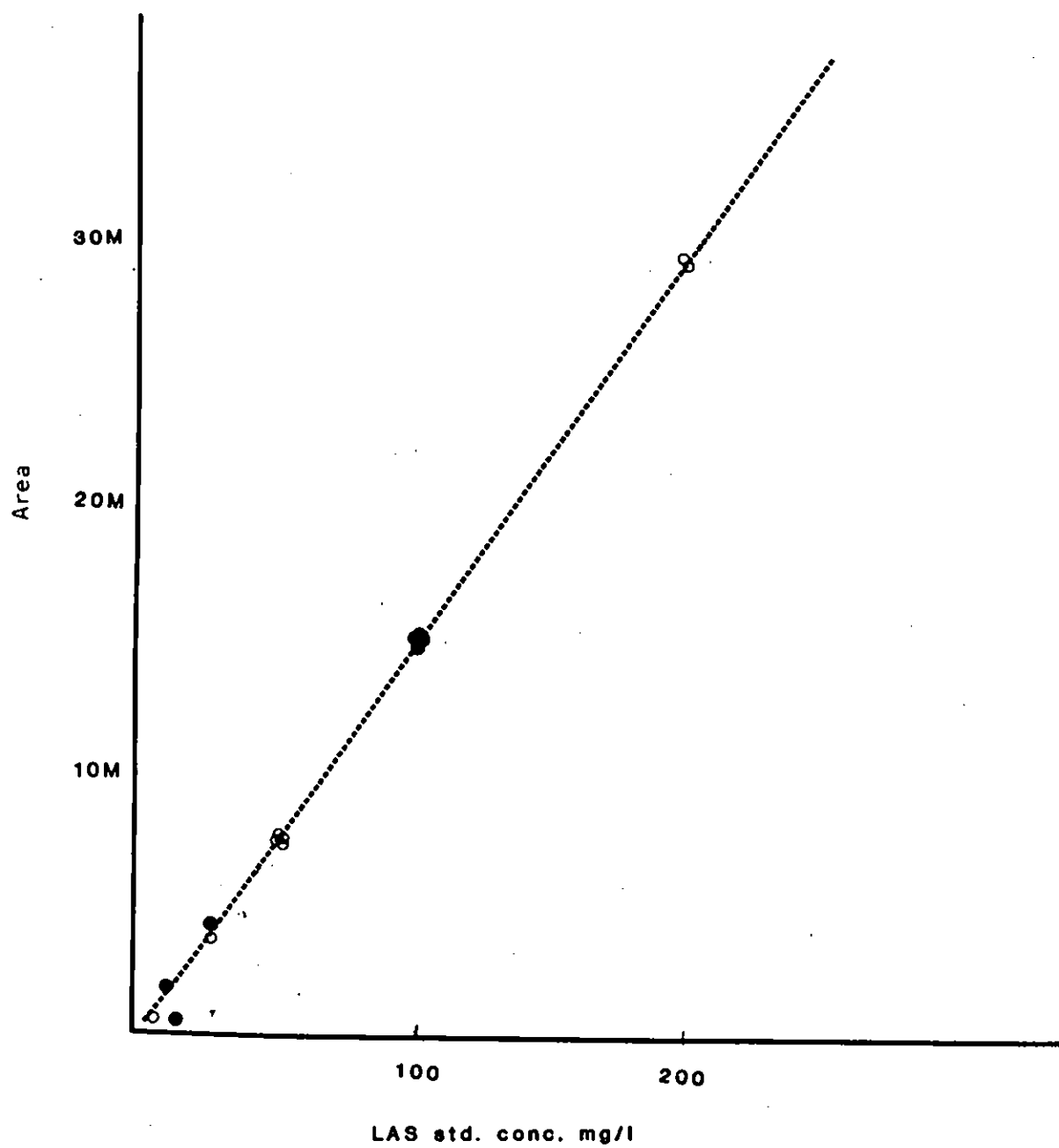


Fig. 9. Calibration graphs for the analysis of autumn 1988 extracts.

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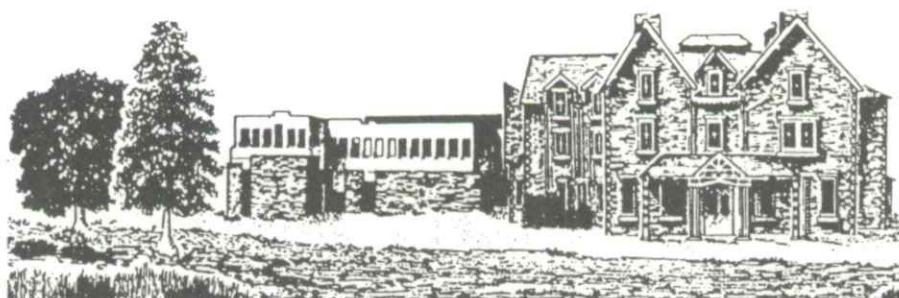
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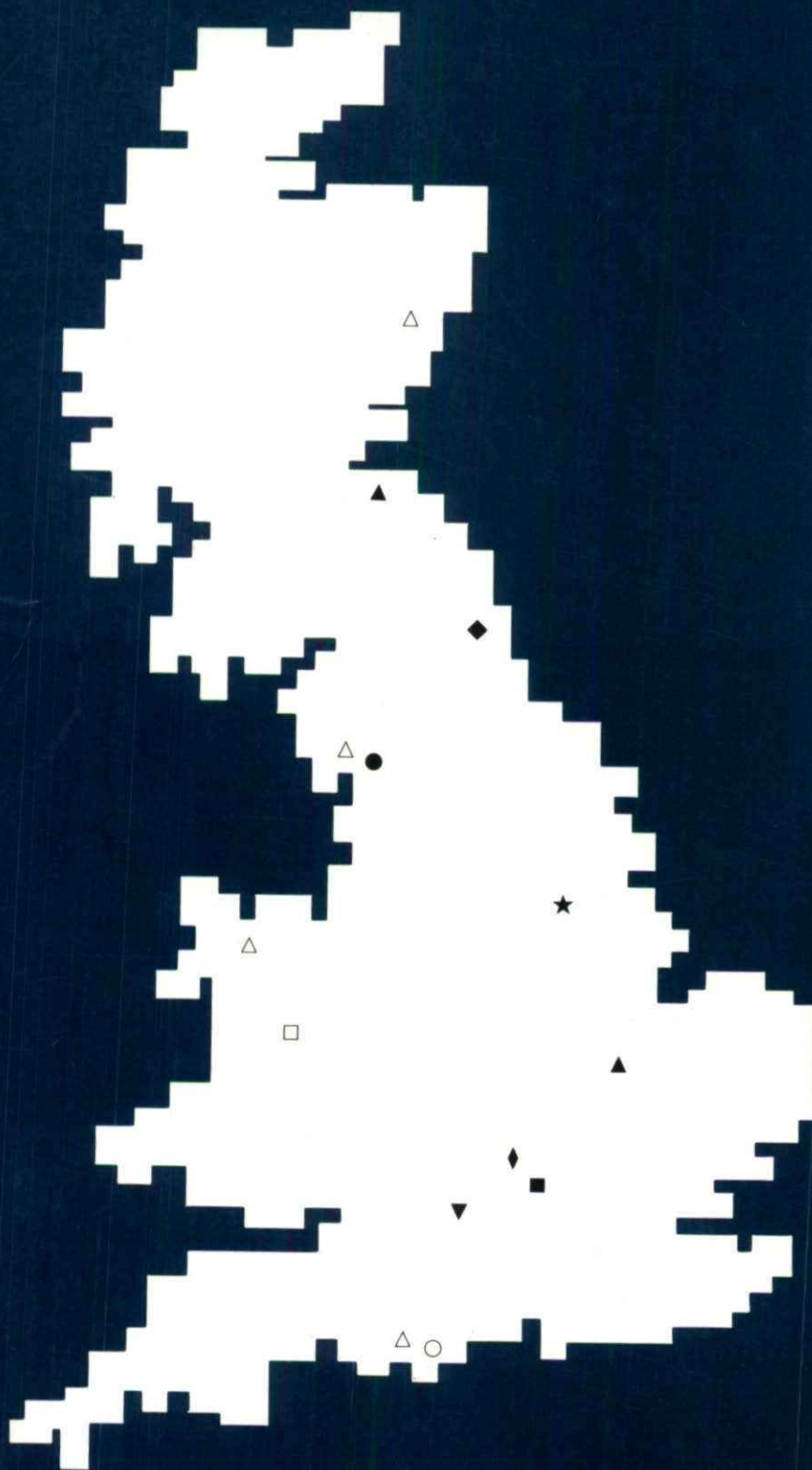
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