ECOTOXICOLOGY OF OIL DERIVED POLLUTANTS IN URBAN RECEIVING WATERS

ROBERT HUW JONES

Urban Pollution Research Centre
Middlesex University

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ABSTRACT

The Silk Stream in north London is one of two principal surface feeders into the Welsh Harp reservoir. The reservoir represents an important amenity and recreational site and is internationally recognized for its wintering wildfowl. However, the larger part of the catchment area lies within a highly urbanized zone and is therefore subjected to a variety of point and non-point pollution sources rendering the ecological balance considerably more delicate than corresponding rural areas. The construction of a litter screen and oil boom on the lowest reaches of the Silk Stream has highlighted the particular problem of oil inputs from urban runoff.

In order to assess the ecotoxicological damage resulting from hydrocarbon pollution in the Silk Stream and at the receiving basin, two common, physiologically contrasting macroinvertebrates, *Asellus aquaticus* and *Lymnaea peregra* were used as caged biomonitors. Both organisms were found to be bioaccumulators of hydrocarbons when transferred from rural sites to the caged urban sites although tissue levels were substantially lower at the receiving basin site. Elevated mortality rates of both organisms were also observed at the three stream sites indicating the considerable recovery that occurs at the receiving basin.

Hydrocarbon tissue concentrations were found to mirror those of the surrounding sediments in which the combustion derived compounds, fluoranthene and pyrene, were consistently the most abundant polycyclic aromatic hydrocarbons (PAHs). The alkane profiles were generally unimodal, peaking in the C_{20}-C_{23} range. Inputs of biogenically derived hydrocarbons and of lubricating oils were also identified in the abiotic environment as well as in the organism tissues. The application of Principal Component Analysis revealed an association between a lubricating hydrocarbon source and mortality in *L. peregra* at two sites and with *A. aquaticus* at one site. Links between mortality and rainfall were also established at the Silk Stream for *L. peregra* and to a lesser extent for *A. aquaticus*. Neither hydrocarbon tissue burdens nor rainfall were linked to the low mortalities at the receiving basin.

Laboratory toxicity tests showed that both organisms accumulate and depurate hydrocarbons rapidly and that lower mortalities were attained for similar tissue burdens compared with the field results. The depuration patterns indicated that a substantial proportion of the measured hydrocarbons were in non-assimilated forms.

CRYNODEB

Y Ffrwd Sidan yw un o'r ddwy brif afon sy'n bwydo cronfa ddwr yng ngogledd Llundain. Mae'r gronfa, sydd oherwydd ei siap wedi cael yr enw "Y Delyn Gymreig", yn safle boblogaidd ar gyfer adloniant ac yn cael ei chydnabod yn rhyngwladol fel cyrchfan aeafu i adar gwyllt. Mae rhan helaeth o ddalgylch yr afon, fodd bynnag, yn ardal drefol ac o'r herwydd yn agored i'w difwyno o ffynonellau lleol a rhai mwy gwasgaredig. Mae cydbwysedd ecolegol y cylch, felly, yn llawer mwy sigledig nag yw mewn ardal wledig gyfatebol. Mae gosod rhwydlen dal ysbwriel ac atalfa olew yng ngennau'r ffrwd yn awgrymmu mai olew yn cael ei gario gan lif dwr glaw oedd achos pennaf y difwyno.

I asesu'r difrod ecolegol, sy'n dilyn o'r difwyno gan hidrocarbonau yn yr afon ac yn y gronfa, dewiswyd dau bryfyn, o'r macroinvertebrata, sydd yn gyffredin ond yn ffisiolegol wahanol, sef *Asellus aquaticus* a *Lymnaea peregra*. Cawellwyd y ddau hyn a'u defnyddio fel bio-fonitorion ar dair safle yn yr afon ac un yn y gronfa. Canfuwyd, ar ol symud yr organebau o safle wledig i'w cewyll yn y safleoedd trefol, fod yr hidrocarbonau yn ymgasglu yn eu meinweoedd. Ymddengys, fodd bynnag, fod y crynodiad yn sylweddol is wedi i'r afon gyrraedd y gronfa. Gwelwyd graddfa marwoldeb uwch yn y ddau organeb mewn safleoedd yn yr afon - hyn yn dangos fod adferiad sylweddol yn digwydd wrth gyrraedd y gronfa.

Gwelwyd fod crynodiad yr hidrocarbonau yn y meinweoedd yn adlewyrchiad o'r hyn ganfuwyd yn y gwaddod o gwmpas safleoedd y cewyll. O'r hidrocarbonau aromarig poliamgylchredol (HAP), y cyfansoddion sy'n deillio o losgi, sef fluoranthin a pyrin yw'r uchaf eu crynodiad. Yr oedd dosraniad yr alcanau yn unfodd a'i uchafbwynt yn y rhediad C_{20} i C_{23}. Adnabuwyd hidrocarbonau bio-gynyrchiedig ac olew iro yn yr amgylchedd di-fywyd yn ogystal a meinweoedd yr organebau. Trwy wneud Dadansoddiad Prif Elfennau (DPE) darganfuwyd fod perthynas rhwng olew iro a graddfa marwoldeb *L. peregra* ar ddwy safle a *A. aquaticus* ar un. Gwelwyd fod perthynas rhwng swm y glaw a marwoldeb *L. pererga* yn yr afon ac i raddau llai yn *A. aquaticus* hefyd. Ni chanfuwyd unrhyw berthynas rhwng naill ai crynodiad yr hidrocarbonau na'r glaw a'r raddfa marwoldeb isel yn y gronfa.

Dangosodd profion labordy ar wenwyndra fod y ddau organeb yn sydyn eu gallu i gasglu hidrocarbonau a'u gwaredu a bod graddfa marwoldeb yn is yn y labordy nac yn y maes er fod crynodiad yr hidrocarbonau yn debyg. Ymddengys, yn ol cyflymdra'r gwaredu, fod cyfran helaeth o'r hidrocarbonau fesurwyd wedi eu casglu ond heb eu cyfuno yn y meinwe.

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ABBREVIATIONS

Where individual PAH compounds appear in figures or tables, the following abbreviations are used.

N	naphthalene
M-N	methyl-naphthalene
Fl	fluorene
Ph	phenanthrene
M-Ph	methyl-phenanthrene
A	anthracene
M-A	methyl-anthracene
Fa	fluoranthene
M-Fa	methyl-fluoranthene
Py	pyrene
M-Py	methyl-pyrene
BA	benzo(a)anthracene
C	chrysene
BFs	benzo(b)fluoranthene and benzo(k)fluoranthene
BP	benzo(a)pyrene
IPy	indeno(1,2,3-c,d)pyrene
DBA	dibenzo(a,h)anthracene
BPe	benzo(g,h,i)perylene

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Finally, I thank my parents for their unfailing support and, accordingly, it is to them that I dedicate this work.

Chapter 1

INTRODUCTION

1.1 BACKGROUND

In recent decades, incidents of oil pollution have become increasingly familiar and of growing concern to the general public. More recently, concern has been expressed at the potential effects of oil pollution that enters freshwater streams in urban areas (NRA, 1995). However, environmental research has generally mirrored public concerns by focusing on acute marine incidents and their ecological effects (Royal Commission, 1981).

It has been established that in most oils, a small number of organic compounds (e.g. benzo(a)pyrene) of the polycyclic aromatic hydrocarbon (PAH) group are present which can induce cancers in mammals and that occupational cancers in humans could be related to exposure to these compounds (WHO, 1972). The alkane component of oil has also been shown to disrupt important cell functions. Only in the 1970s, however, with the increasing use of high resolution techniques, was it possible to demonstrate the ubiquitous distribution of organic compounds, including PAH in the aquatic environment (e.g. Blumer, 1976). It became apparent, therefore, that a potential for ecological or human health damage was present. It is also known that sites exposed to chronic inputs of weathered oils in urban runoff may attain hydrocarbon concentrations that equal or even exceed those measured during spillages (Vandermeulen & Hrudey, 1987). Despite this, investigations into the ecological effects of hydrocarbons at such freshwater sites are surprisingly rare and much of the field and laboratory work into biological effects of hydrocarbons continues to focus on marine organisms. Moreover, investigations conducted at freshwater sites have tended to focus on the effects of heavy metals rather than hydrocarbons.

The value of traditional methods for the *in situ* assessment of biological water quality such as benthic macroinvertebrate community structure (e.g. BMWP, 1979) must also be considered. Such biological indicators are sensitive to low oxygen levels as may be

caused by sewage pollution. In built-up areas, urban runoff may exert greater ecological harm through the toxic effects of organic compounds and heavy metals rather than simple deoxygenation (Ellis, 1989). Under such conditions, the commonly used biological indices may therefore be inappropriate. Also, with increasingly efficient sewage treatment, urban runoff is likely to become the primary source of pollution in lowland UK rivers and receiving water systems.

Thus, the increasing importance of urban runoff pollution in which hydrocarbons are primary constituents combined with the relative paucity of research on these compounds in freshwater and the limitations of conventional biological indices suggest that substantial gaps remain in our understanding of the role of hydrocarbon pollution in stream ecology. It was against this background that this work, by the Urban Pollution Research Centre (UPRC) at Middlesex University, in collaboration with English Nature (formerly the Nature Conservancy Council), was undertaken and findings of which are presented in this thesis. The work also complements and further develops previous studies undertaken within the UPRC which have investigated the distribution and biological effects of heavy metals in urban watercourses (Mulliss, 1994; Bascombe, 1991; Beckwith, 1989; Harrop, 1984). Work currently being undertaken involves the further analysis of heavy metal bioaccumulation in macroinvertebrates as well as bioaccumulation of heavy metals and hydrocarbons by aquatic macrophytes.

1.2 AIMS

The principal question which the research programme addresses is whether hydrocarbon compounds could be linked to mortalities of common freshwater invertebrate species. The ecotoxicological component of the work applies methods of chemistry to the study of the occurrence and effects of hydrocarbons in an aquatic system. The approach does not exclude the synergistic or antagonistic effects of other pollutant such as heavy metals but attempts to focus upon hydrocarbon impacts. Two common and physiologically contrasting organisms, *Asellus aquaticus* and *Lymnaea peregra* were chosen as biomonitors for the investigation. The organisms were captured at clean sites and transfered to the test sites where an apparatus, designed for in-stream use, housed the organisms and enabled a closely monitored field trial to be conducted. Laboratory

trials were also undertaken to assess under controlled conditions the rate of uptake and effect of bioaccumulated hydrocarbons. A multivariate statistical procedure, commonly used in air pollution studies, but rarely applied to aquatic pollution, was used to analyze the field data. The use of marker compounds allowed an assessment of the sources of the measured hydrocarbons and also allowed the more *in situ* toxic fractions to be identified.

1.3 OUTLINE OF THESIS

The thesis is presented in eight chapters including this introduction. Chapter 2 presents an overview of our understanding of hydrocarbons in freshwaters and includes sections on hydrocarbon sources, their distribution in freshwater and sediments and a discussion of their biological effects and distributions in organism tissues. In Chapter 3, site descriptions and field methodologies are given together with laboratory analytical techniques. The results of surveys of sediment and water quality in the study catchment as well as the use of marker compounds used to identify hydrocarbon sources are given in Chapter 4. Chapter 5 presents the results and analysis of the *in situ* trials with caged macroinvertebrates, in which over thirty five individual hydrocarbons were analyzed in field trials covering 2 years. Laboratory toxicity trials were also undertaken on the same monitor organisms. These provide a greater understanding of the rates and modes of hydrocarbon bioaccumulation and are presented in Chapter 6. In Chapter 7, a multivariate statistical procedure is presented which attempts to identify associations between the measured parameters such as invertebrate mortality and tissue concentrations of hydrocarbons. Chapter 8 summarizes the findings and identifies further interesting areas of research, indicated by the thesis, as well as those in the broader areas of freshwater pollution and aquatic toxicology.

1.4 ASSOCIATED PUBLICATIONS AND CONFERENCES

During the period of this research a number of conferences were attended and, where possible, presentations outlining the preliminary analysis of the research data were given. A selection of these conferences is listed overleaf.

- "GC/MS for the chromatographer" (organized by Hewlett Packard), Windsor (February 1989).
- "Urban Wetland Management" Workshop at Middlesex University (March 89).
- "Urban Stormwater Quality and Ecological Effects on Receiving Waters" *2nd International Conference*, Wageningen, Holland. [poster presentation, September 1989].
- "New Horizons in Ecotoxicology" *British Ecological Society* Southampton University (December 1989).
- "Biological Standards for Water Quality Assessment" Middlesex Polytechnic. [poster presentation, February., 1990].
- "Ecological Impacts of Urban Runoff" *1st European Postgraduate Workshop* Middlesex Polytechnic. [paper presentation, July, 1990].
- "Invertebrate Communities of Rivers: Structure and Function" *Freshwater Biological Association Annual Scientific Meeting*, The Royal Society, London (July, 1990).
- "Urban Runoff and Effects upon Receiving Basins" *2nd European Postgraduate Workshop*, University of Essen, Germany. [paper presentation, July 1991].
- "Joint Meeting of the Society of Environmental Toxicology and Chemistry (SETAC, Europe) and the Aquatic Ecosystem Health and Management Society (AEHMS)" Potsdam, Germany. [joint paper presentation, June, 1992].
- "Technology, Man and Science" *2nd European Meeting*, University Polytechnic of Valencia, Spain. [poster presentation, October, 1992].
- "Urban Storm Drainage" *6th International Conference*, Niagara Falls, Ontario, Canada. [joint paper presentation, September, 1993].

Additionally, a series of research reports (listed below) were presented to the project collaborators, English Nature.

Ellis J.B., Jones R.H., Revitt D.M. & Shutes R.B.E. "Ecotoxicological Modelling of Oil Pollution in Urban Receiving Waters" Inception Report submitted to English Nature (formerly Nature Conservancy Council) March 1989.

Ellis J.B., Revitt D.M., Shutes R.B.E. & Jones R.H. "Ecotoxicological Modelling of Oil Pollution in Urban Receiving Waters" Report No.1 November 1989.

Jones R.H. Ellis J.B., Revitt D.M. & Shutes R.B.E. "Ecotoxicological Modelling of Oil Pollution in Urban Receiving Waters" Report No.2 October 1990.

Jones R.H., Ellis J.B., Revitt D.M. & Shutes R.B.E. "Ecotoxicological Modelling of Oil Pollution in Urban Receiving Waters" Report No.3 June 1991.

Jones R.H. & Revitt D.M. "Ecotoxicological Modelling of Oil Pollution in Urban Receiving Waters" Report No.4 March 1992.

Jones R.H., Ellis J.B., Revitt D.M. & Shutes R.B.E. "Ecotoxicological Modelling of Oil Pollution in Urban Receiving Waters" Report No.5 June 1992.

Jones R.H. "Oil Pollution Monitoring and Impacts in the Silk Stream and Welsh Harp Catchment" *Welsh Harp Conservation Group Newsletter* **4** pp.24-27 May 1992.

Chapter 2

HYDROCARBONS AND BIOTA IN THE AQUATIC ENVIRONMENT

2.1 INTRODUCTION

The presence of anthropogenic organic compounds in freshwater systems has been of growing concern because of possible harmful effects to man and other organisms. Many classes of organic compounds, in particular the polycyclic aromatic hydrocarbons (PAHs) have, in laboratory studies, demonstrated toxic, mutagenic or carcinogenic effects (WHO, 1972; Lavoie *et al.*, 1982; Durant *et al.*, 1994). When hydrocarbons enter streams or lakes, adsorption to fine particles may occur followed by settlement to the bottom sediment. Thus, an accumulation of pollutants may occur such that subsequent disturbance, for example, during high river flow, may result in their release and an increased exposure of aquatic organisms to hydrocarbons. Contaminated sediments therefore act as a virtually permanent reservoir or potential source of hydrocarbons and other hydrophobic pollutants with respect to the stream fauna. In urban rivers, increased impervious catchment areas result in more pronounced changes in volume and flow and therefore in sediment resuspension compared to corresponding rural streams. This effect, combined with the increased number and magnitude of sources of hydrocarbons and other oil or combustion derived pollutants, results in a greater possibility of ecological damage in urban watercourses.

2.2 CLASSIFICATION

True hydrocarbons contain only hydrogen and carbon atoms although sulphur, oxygen nitrogen and chlorine-containing polycyclic aromatic compounds (PAC) form a general group of which the polycyclic aromatic hydrocarbons (PAH) are important members. In general terms, hydrocarbons may be divided into four classes:

i) straight chained (e.g. *n*-alkanes)
ii) branched chained (e.g. pristane)

iii) cyclic (e.g. cyclopentane)

iv) aromatic (e.g. monoaromatic, benzene; polyaromatic, polycyclic aromatic or polynuclear, benzo(a)pyrene)

In environmental samples from polluted locations, a mixture of all four classes of compound as well as other organic compounds is likely to be present. In this study, attention was focused upon straight and branched chained compounds and the PAHs since these are generally the most abundant compounds at contaminated sites and because of their recognized potential to cause environmental damage (Clark & Brown, 1977).

2.3 SOURCES

Hydrocarbons may enter freshwater from a number of sources but three distinct source groups exist which relate to their initial mode of formation. Each group will be subjected to changes occurring through chemical and physical weathering and biodegradation, but will retain distinct characteristics that will enable it to be recognized even when it is present in complex environmental samples (see Section 2.6). A summary of ancient (diagenic) and contemporary (biogenic and pyrolytic) sources is given in Table 2.1.

The three modes of initial formation and introduction of hydrocarbons to the environment are:

i) **Biosynthesis.** This process is the recent formation through bacterial, algal, higher plant, zooplankton or insect metabolic activity of relatively simple and limited numbers of usually straight or branched chained hydrocarbons. Bacteria, for example, are known to produce vast quantities of methane (Sahm, 1981). Algae predominantly synthesize relatively short chained *n*-alkanes chiefly C_{15} and C_{17} (Douglas & Eglinton, 1966; Philp *et al.*, 1972). In the higher plants, addition of C_2 units to palmitic acid C_{16} produces long-chained fatty acids. Decarboxylation of the fatty acids leads to the formation of *n*-alkanes in the range C_{25}-C_{33} (Eglinton *et al.*, 1962; Kolattukudy, 1976). The marine copepod *Calanus* sp. produces large quantities of pristane (a branched isoprenoid alkane)

TABLE 2.1

SOURCES OF HYDROCARBON INPUTS TO THE FRESHWATER ENVIRONMENT (FROM MULLER, 1987)

Contemporary hydrocarbons $^{14}C/^{12}C$- ratio as in contemporary organic matter		*Fossil Hydrocarbons $^{14}C/^{12}C$- ratio significantly lower than in contemporary organic matter*	
Natural input (biosynthesis)	*Anthropogenic input (pyrolysis)*	*Natural input (diagenesis)*	*Anthropogenic input (diagenesis)*
Products of recent biosynthesis release by aquatic or terrestrial organisms	Contemporary hydrocarbons via industrial and domestic wastewater	Natural erosion of sedimentary rocks	Fossil hydrocarbons via industrial and domestic wastewater (e.g. machine lubrication, cooking)
Production from natural combustion processes (e.g.forest fires) via atmospheric input by rain and dustfall	Combustion products from contemporary organic matter (e.g. wood, cigarettes) via atmospheric input	Natural oil seeps	Losses from handling and transport of fossil fuels via street runoff
			Losses of fossil fuels and combustion products from motorboat traffic, direct input
			Oil spills, via ground water or direct input
			Erosion from asphalt pavements and tyres, via street runoff
			Combustion products from fossil fuels (e.g. coal, diesel) via atmospheric input

from chlorophyll in its diet. Pristane is concentrated in the lipid tissue and similarly elevated concentrations of pristane have been found in the fatty tissues of fishes and whales which feed on zooplankton (Blumer *et al.*, 1963). In early studies, evidence supporting biosynthesis of PAH by bacteria has been presented. Much of this has been disputed, the PAH "production" being attributed to bioaccumulation from low or undetectable initial concentrations in the culture media. Similarly, initial work on plants suggested PAH production by trees e.g. in Graff & Diel (1966) a value of 10 -20 μg kg^{-1} benzo(a)pyrene in leaves was given as evidence of PAH production. Grimmer & Duvel (1970) demonstrated that much, if not all, of the recorded levels was the result of surface contamination by deposited background particles. Currently, it is accepted that *de novo* production and input of PAH into aquatic systems is negligible. However,

on a global scale, total hydrocarbon production by biosynthesis exceeds that of all other sources.

ii) **Diagenesis.** In contrast to recent biosynthesis, long term geochemical transformations of biological precursors results in the formation of a wide range of organic compounds in extremely complex mixtures containing at least 10,000 organic compounds. Such mixtures may enter aquatic systems through natural seepage or bedrock erosion or as a result of human extraction and subsequent use of crude or refined oils. In petroleum, hydrocarbons typically represent about 75% of the total mass, containing a 5-40% aromatic content and a 20-70% content of cyclic, branched or straight chained aliphatic compounds. Sulphur, nitrogen and oxygen containing compounds constitute the remainder. The introduction of diagenic compounds represents the major input of "man-made" aliphatic compounds into the aquatic environment and also represents significant inputs of aromatics. Originally, PAHs were believed to be synthesized only during high temperature (>400°C) burning of organic matter. Blumer (1976) identified PAHs in coal and oil demonstrating their possible formation at much lower temperatures (100-150°C) albeit over much longer time periods. Diagenic formation of PAHs results in a predominance of alkylated compounds compared with the parent the molecules. Table 2.2 summarizes the contributions of the various sources of petroleum hydrocarbons to the marine environment.

iii) **Pyrolysis.** The incomplete combustion of many organic compounds leads to the release of hydrocarbons. The resulting mixture may again be highly complex, containing hundreds of hydrocarbons of various classes. Pyrolysis is believed to be the major source of PAHs in the environment. PAH production is greatest at high temperatures and in a reducing atmosphere with cyclic precursors, although combustion of any organic material under virtually any conditions will result in some PAH production. PAHs formed by burning are almost completely associated with the particulate fraction composed of soots and carbon blacks. Thus, local and long range transport of hydrocarbons produced in this way will occur. In urban areas, motor vehicles are likely to be the major sources with diesel vehicles important as sources of a wide range of PAHs and aliphatic coumpounds (Williams *et al.*, 1986). The relative contributions of various sources of petroleum hydrocarbons (see Table 2.2) indicate that

on a global scale the contribution of urban runoff is relatively small but accounts for high localized concentrations (see Table 2.6). It should also be noted that pyrolytically and biogenically derived hydrocarbon production processes are not included as at present the magnitudes of these sources cannot be quantified with accuracy.

TABLE 2.2
GLOBAL INPUT OF PETROLEUM HYDROCARBONS INTO THE MARINE ENVIRONMENT (FROM NATIONAL RESEARCH COUNCIL, 1985)

Source	*Probable Range (million tons/annum)*	*Best Estimate (million tons/annum)*
Natural sources		
Marine seeps	0.02-2.0	0.2
Sediment erosion	0.005-0.5	0.05
(Total natural sources)	(0.025-2.5)	(0.25)
Offshore production	0.04-0.06	0.05
Transportation		
Tanker operations	0.4-1.5	0.7
Dry-docking	0.02-0.05	0.03
Marine terminals	0.01-0.03	0.02
Bilge and fuel oils	0.2-0.6	0.3
Tanker accidents	0.3-0.4	0.4
Non-tanker accidents	0.02-0.04	0.02
(Total Transportation)	(0.95-2.62)	(1.47)
Atmosphere	0.05-0.5	0.3
Municipal and industrial wastes and runoff		
Municipal wastes	0.4-1.5	0.7
Refineries	0.06-0.6	0.1
Nonrefining industrial wastes	0.1-0.3	0.2
Urban runoff	0.01-0.2	0.12
River runoff	0.01-0.5	0.04
Ocean dumping	0.005-0.02	0.02
(Total wastes and runoff)	0.585-3.12	1.18
Total	1.7-8.8	3.2

2.4 PHYSICO-CHEMICAL PROPERTIES OF HYDROCARBONS

In order to assess the possible ecological impact of hydrocarbons it is necessary to consider their physical and chemical properties. Aqueous solubility is an important factor since bioaccumulation is known to be far more efficient from solution compared to adsorbed or otherwise bonded chemicals. In general, all hydrocarbons are considered hydrophobic but large differences exist between various hydrocarbon classes and within the same class. Generally, within a given class of compound, solubility is inversely related to the number of carbon atoms. For the main classes, solubility increases in the order: *n*-alkanes < iso-alkanes < cyclo-alkanes < aromatics (Wheeler, 1978).

The addition of alkyl groups results in reduced solubility in proportion to the size of the substituent group. Multiple alkylation also results in a proportional decrease in solubility. The presence of nitrogen, oxygen and sulphur-containing polar functional groups increases aqueous solubility.

The presence of dissolved organic matter (DOM) is believed to increase the solubility of hydrocarbons. Up to 50% of DOM may be composed of humic and fulvic acids much of which may be in colloidal form but is classified as soluble material. According to Hiemenz (1977) colloids refer to any particle with a linear dimension between 10^{-7}cm and 10^{-4} cm. Colloidal organic material in this size range has been shown to efficiently sorb hydrophobic pollutants, particularly the PAHs. In spite of apparently increasing hydrocarbon solubility the bioavailability of DOM-associated hydrocarbons is known to decrease. This would further support the claim that, in the presence of DOM, close binding of hydrocarbons with the colloidal material occurs as opposed to an increase in true solubility.

May (1980) reported that the solubility of specific PAHs show 2-5 fold increases with increasing temperature in the range 5 to 30°C. An inverse relationship has been reported between salinity and solubility for the PAHs but changes are only by a factor of 2 or less over a 0-36% salinity range (Whitehouse, 1985). However, in field studies, Readman (1988) found no correlation between salinity and PAH solubility. Mackay

(1987) demonstrated that surface slick behaviour, mousse formation and sedimentation mechanisms are virtually identical in sea and freshwater. Similarly, the range of effects, toxic mechanisms and biodegradative pathways of the two systems are comparable which has meant that much of the early research undertaken on hydrocarbons in the open sea has been applied to the freshwater environment.

2.5 REMOVAL PROCESSES

2.5.1 Sorption

As a result of the generally low aqueous solubilities of hydrocarbons, sorption to solid surfaces occurs readily. Large amounts of compounds derived from oil pollution are present in association with particulate matter (e.g Carey *et al.*, 1990; Saliot *et al.*, 1990) and these associations will have important influences on the likelihood of uptake and toxicity of hydrocarbons in aquatic organisms.

When the concentration of the sorbed material is much lower than the sorptive capacity of the material the equilibrium ratio of the sorbed concentration to dissolved concentration is a constant (K_p) which can be expressed by the equation:

$$K_p = S/C$$

where K_p = partition coefficient
S = solid phase concentration (hydrocarbon mass per dry mass sorptive material).
C = liquid phase concentration (hydrocarbon mass in solution per volume liquid)

An additional partition coefficient K_{OC} describes the pollutant distribution in relation to the organic content of the sorbent. The ratio between pollutant concentration on a hypothetical sorbent of 100% carbon and the concentration in water gives the K_{OC}. Further, a positive linear relationship has been established between K_{OC} and another partition coefficient, the octanol/water partition coefficient, K_{OW}. Examples of K_{OW} together with solubility data for selected hydrocarbons are shown in Table 2.3. The K_{OW}

TABLE 2.3
AQUEOUS SOLUBILITIES AND OCTANOL-WATER PARTITION COEFFICIENTS OF SELECTED HYDROCARBON COMPOUNDS (FROM HUTCHINSON *et al.*, 1980)

Compound	*Solubility (mg l^{-1})*	*log Kow*
Decane	0.052	5.01
Dodecane	0.0038	6.10
Tetradecane	0.0023	7.20
Cyclooctane	7.9	3.28
Naphthalene	31.7	3.35
Phenanthrene	1.29	4.63
Pyrene	0.135	5.22

is an important parameter which has been used for the prediction of the bioaccumulative potential of organic compounds since it reflects the tendency of organic compounds to accumulate in lipids. Thus, the relationship between K_{OC} and K_{OW} has biological significance since both are likely to indicate biologically active compounds. In the field, sorption, however, as a major removal process, is likely to reduce the bioaccumulative capacity of recently introduced or desorbed organic compounds. The intrinsic likelihood of hydrocarbons to accumulate as predicted from laboratory derived K_{OW} are therefore complicated by a number of exogenous factors in the field (see Section 2.8.3). This matter will be re-addressed in Chapter 6 where the relationship between bioaccumulation and K_{OW} in laboratory tested invertebrates will be discussed.

2.5.2 Volatilization

The lighter and more soluble compounds, though potentially the most biologically damaging because of their higher aqueous solubility also have the lowest boiling points and therefore when introduced to water as a mixture will be the first to be removed from the system by evaporation. Theoretical half lives of PAHs in water, based on losses by evaporation, vary from a few days (naphthalene) to hundreds of days for benzo(a)pyrene (Royal Commission, 1981; Southworth *et al.*, 1978). Similarly, half-lives for alkanes may vary from octane (hours) to eicosane (hundreds of days). Henry's law constants [H] for a range of PAHs shown in Table 2.4 further illustrate the large

differences in physical characteristics displayed among common PAHs.

TABLE 2.4

HENRY'S LAW CONSTANTS FOR SELECTED PAHS (FROM SOUTHWORTH, 1978)

Compound	*H (Molar concentration in the gaseous phase / molar concentration in the aqueous phase)*
Naphthalene	1.9×10^{-2}
Phenanthrene	2.0×10^{-4}
Pyrene	4.3×10^{-4}
Benzo(a)anthacene	1.0×10^{-4}
Benzo(a)pyrene	2.1×10^{-5}

2.5.3 Photolysis

Generally, oxidation reactions are accelerated by ultra-violet light and the resultant oxidised products are usually more soluble and more toxic than the original compound. Typical photolysis products from aromatic and unsaturated hydrocarbons are phenolic and acidic compounds. High molecular weight compounds may form relatively inert polymeric asphaltic type materials. For the PAHs, photolysis has been described as a major process occurring in the atmosphere and in the upper parts of the water column. Lee & Ryan (1983) have suggested that photolysis of PAHs leads to increased rates of bacterial breakdown. Although they are more mobile in the environment, sediment bound photolytic products are known to be relatively stable (Hinga *et al.*, 1987).

2.5.4 Microbial degradation

At least 40 genera of bacteria are able to utilize hydrocarbons as a food source (CONCAWE, 1979; Atlas, 1981) and following the introduction of oils to an aquatic environment, microbial populations increase massively. For complete breakdown to occur, a mixed population as opposed to a single strain is normally required. Alkanes are most susceptible to bacterial attack followed by aromatics, heterocyclics and asphaltenes. The differing rates of hydrocarbon breakdown that exist may therefore enable an assessment of the age of contaminated samples to be assessed. Breakdown of oils may be restricted by nutrient availability but when nutrients are available, oxygen

demand may exceed availability resulting in the formation of anaerobic conditions. This results in a virtual cessation of breakdown since anaerobic activity is much slower (Ward & Brock, 1978).

2.6 SOURCE APPORTIONMENT AND FINGERPRINTING TECHNIQUES

In environmental samples it is often the case that a mixture of compounds representing various sources of origin are present. Source apportionment is useful because it enables an assessment to be made on the potential ecological damage which may be caused by hydrocarbon mixtures since different source classes may have increased effects due to intrinsic properties e.g. certain source types may contain a greater proportion of biologically active components. Identification of major harmful groups may also allow a more targeted management strategy for the control of hydrocarbon pollution. This approach will be returned to in the assessment of abiotic contamination (Chapter 4) and in the interpretation of the invertebrate tissue contamination (Chapter 7).

A number of fingerprinting techniques exist which, following High Performance Liquid Chromatography (HPLC) or Gas Chromatography/Mass Spectrometry (GC/MS) analyses of organic extracts, may be applied to the results. These rely on quantitative or semi-quantitative analysis of specific compounds or classes in terms of ratios or in terms of their presence or absence. The Gas Chromatogram of the aliphatic fraction of many environmental samples will display a hump on which resolved peaks may be present. The hump is composed of many hundreds of cyclic and branched compounds in many homologous series which, having overlapping retention times, cannot be resolved by conventional GC techniques. This so-called unresolved complex mixture (UCM) is believed to be a reliable indicator of petrogenic pollution since crude and weathered oils always display this feature. Moreover, biogenic assemblages are notably absent in an UCM since synthetic pathways in *de novo* production result in a restricted assemblage of resolvable compounds. The UCM may also be used as an indicator of the extent of physico-chemical weathering of aliphatic mixtures, as the greater the proportion of UCM compared with the resolved total aliphatics, the greater the number of weathered products. Some workers (Matsumoto, 1982; Eganhouse & Kaplan, 1981) have suggested that some UCM is derived from bacterial action although there is

limited evidence to support this claim.

Comparison of odd:even carbon chain length alkanes between C_{20} and C_{33} in an environmental sample from a pristine location may give an odd:even ratio or Carbon Preference Index (CPI) of 10 or more, as a result of the production of odd numbered alkanes in higher plant waxes. Similarly, in the C_{14}-C_{20} range, algal odd-numbered chain length alkane synthesis will result in a high odd:even ratio. In contrast, petrogenic samples display no prevalence of odd or even carbon numbered alkanes, thus, the CPI is expected to approximate to unity. Increasing inputs of petroleum derived hydrocarbon pollution to an unpolluted site will result in a decrease of the CPI until biogenic inputs are effectively "drowned" and the CPI approaches unity with no odd:even predominance. The index therefore gives an estimation of the relative proportion of biogenic and anthropogenic hydrocarbons in an environmental sample with respect to the aliphatic assemblage.

The relative amounts of *n*-C_{17} to pristane and *n*-C_{18} to phytane can give an indication of the amount of weathering or degradation which has occurred in an environmental sample, as the branched compounds (pristane and phytane) are more resistant to bacterial action than the corresponding straight chained alkanes. Phytane is believed to be of petroleum origin only, whereas pristane may be derived from both anthropogenic and biogenic sources. The origin of pristane may be confirmed by stereochemical analysis, since diagenesis leads to equal production of all stereochemical isomers while biogenic production results in specific isomer formation (Muller, 1987).

Further use of specific isomers or compounds such as squalene or hopanes present in oils can identify pollution sources in water and wastewaters. Specific crude oils may also be characterized by particular proportions of specific hopanes (Crompton, 1985).

Analysis of the PAH composition of environmental samples also provides information on source discrimination. In samples of crude oils the PAH assemblage will be dominated by relatively low boiling point PAHs of 2 and 3 rings which are predominantly alkylated. In contrast, PAHs formed by pyrolysis are likely to have a predominance of 4 and 5 ringed compounds which are generally non-alkylated. Thus,

predominance of 2/3 or 4/5 ringed compounds together with extent of alkylation provides valuable information on the relative contributions of diagenic or petrogenic aromatics to an environmental sample of water or sediment (Neff, 1979).

Clearly, these fingerprinting techniques are most effective when combined together e.g. in the case of the CPI a relatively high index will indicate low anthropogenic input. However, if aromatic groups are omitted from the analysis it may be the case that significant quantities of long range transported pyrolytic PAHs are present which have relatively low levels of aliphatics. Thus, the hydrocarbon suite would be composed chiefly of biogenic aliphatics and pyrogenic parent PAHs. The use of only one fingerprinting technique in this instance would almost certainly lead to an incorrect conclusion regarding the sources of the overall assemblage. Thus, analysis of the same environmental sample but using different fingerprinting techniques at the exclusion of others may result in entirely different conclusions being drawn. Muller (1987) discusses the investigations of a number of workers on Lake Constance to illustrate this problem.

In turbulent aquatic systems, such as urban streams, where sediment resuspension occurs, an historical record of pollution may be impossible to obtain due to vertical mixing and lateral shifts of sediment. The information derived from such sediments would be of a composite sample representing hydrocarbons accumulated over a number of years, the exact time period depending on rate of deposition an extent of mixing. Although not necessarily directly reflecting the current type of pollution input in the sediments, such samples represent the assemblage to which organisms are exposed and together with water and wastewater analysis still allow an assessment of magnitude, sources and related bioavailability of the hydrocarbon pollution.

2.7 URBAN RUNOFF AND HYDROCARBON POLLUTION

2.7.1 Introduction

Although recent biogenesis on a global scale is the biggest source of hydrocarbons, human activity in local urbanized areas may result in hydrocarbon levels being far in excess of those encountered in unpopulated areas and may approach levels experienced

in acute marine incidents. Such acute effects of large marine or estuarine oil spills on birds and mammals are familiar and well documented. In urban freshwaters, however, contamination usually occurs as a result of long-term chronic inputs of which stormwater runoff is a prime contributor.

With increasing effluent limitation by environmental protection agencies and water industries, refinery and other industrial effluents together with municipal effluent have become less important loading factors of hydrocarbon inputs to aqueous systems. Conversely, urban runoff has become an increasingly important contributory factor (Whipple & Hunter, 1979; Andoh, 1994).

In the UK, 96% of the population is connected to the foul sewer system. Combined sewers represent 70% of the overall sewer system and half of these were constructed before 1938 (Ellis 1986). Storm-associated sewered discharges, particularly in urban areas, have been identified as the major water quality problem in the UK by the Water Industry (Crabtree 1986) and at least a third of UK storm sewer overflows have been considered unsatisfactory, resulting in infringements of water quality goals (Ellis, 1989).

2.7.2 Sources

All major pollution groups including nutrients, organic matter (as BOD and COD), suspended solids and metals have been identified in urban runoff (Bennett *et al.*, 1981; Hoffman *et al.* 1985). Hydrocarbon compounds are also important constituents of urban runoff (e.g. Wakeham *et al.* 1980; Eganhouse *et al.*, 1981; Gavens *et al.*, 1982; Muller, 1987) and are principally derived from spilled petroleum products, partially combusted petroleum products, leachate and particles from road surface materials and tyres. These may enter watercourses directly as a slick or in a dissolved state but, more commonly, in particulate association with street dust, roadside vegetation, roadside soil or through atmospheric fallout. Typically, chromatograms of the aliphatic component of hydrocarbon extracts from various urban sites including commercial, residential, industrial and highway locations resemble those of sump (crankcase) oils as well as displaying significant inputs of odd carbon chain numbered higher plant derived

alkanes. Latimer *et al.* (1990) also established a close relationship between relative abundances of PAHs in urban runoff and their distribution in weathered sump oils.

2.7.3 Influence of land use

Land use within an urban area exerts an important influence on the relative and absolute abundances of hydrocarbons in urban runoff. Increases in impervious area clearly increase mobility of hydrocarbons not only through transport to streams but also resuspension of sediment associated compounds. Generally, industrial and highway containing catchment areas have elevated concentrations of all hydrocarbon groups compared with residential or commercial zones. Aliphatic hydrocarbon concentrations are proportionally more elevated in the low to mid-range alkanes (C_{12}-C_{22}) in samples from industrial or commercial areas, whereas the aromatic component becomes more dominated by fluoranthene and higher molecular weight compounds such as chrysene, (Hoffman *et al.*, 1983, 1984).

2.7.4 Concentrations and controlling factors

The concentrations of hydrocarbons in urban runoff are highly variable: changes of over four orders of magnitude are commonly reported. At the same sampling location large differences in hydrocarbon levels have been observed during single storm events. The range of hydrocarbon concentrations which have been recorded are shown in Table 2.5.

The general environmental factors which control hydrocarbon concentrations in runoff may be described as:

a) the source strength of the pollutant.

b) the transport dynamics of the pollutant in the runoff.

Traffic densities, population densities and land use will clearly influence source strength, as will the extent of removal processes such as temperature on volatilization,

TABLE 2.5

HYDROCARBON CONCENTRATIONS IN URBAN RUNOFF FROM VARYING LAND USE LOCATIONS

Compound	*Concentration Range (mg l^{-1})*	*Location (Land Use)*	*Reference*
Hydrocarbons	-	Rhode Island - USA	Hoffman & Quinn (1984)
"	0.02-3.93	(Residential)	" "
"	0.04-5.71	(Commercial)	" "
"	0.03-6.85	(Highway)	" "
"	1.03-58.4	(Industrial)	" "
"	1.42-19.9	Los Angeles - USA	Eganhouse & Kaplan (1981)
"	0.95-5.58	Delaware - USA	Whipple & Hunter (1979)
"	0-6	Rhode Island - USA	Hoffman *et al.* (1985)
"	1.0-8.0	Philadelphia - USA	Hunter *et al.* (1979)
"	-	Colorado - USA	Bennett *et al.* (1981)
"	1.7-25.9	(Residential)	" "
"	6-24	(Commercial)	" "
"	0.2-24	Seattle - USA	Wakeham (1977)
"	-	Switzerland	Zurcher *et al.* (1978)
"	1.7-10.0	(Highway)	" "
n-alkanes	0.068-0.82	Los Angeles	Eganhouse *et al.* (1981)
PAH	0-0.005	Rhode Island - USA	Hoffman *et al.* (1984)
PAH	0.0045-0.022	USA	Horkeby & Malmquist (1977)
Benzo(a)pyrene	0-0.004	Germany	Herrmann (1981)

ultraviolet radiation on photolysis and bacterial community composition and numbers on biodegradation. Duration of antecedent dry period will also affect source strength (Whipple *et al.*, 1977). Pollutant transport dynamics are governed by size of particle association but principally by catchment flow characteristics such as flow and physical nature of surfaces as well as rainfall intensity.

2.8 HYDROCARBON DISTRIBUTION IN SEDIMENTS, WATER AND BIOTA

The ranges of hydrocarbon concentrations which have been found in selected freshwater and marine sediments are presented in Table 2.6. Total aliphatic levels vary from the

low ppm range to a few thousand ppm for highly contaminated areas. Alkane levels vary from less than 0.1 ppm in unpolluted areas to about 100 ppm at contaminated sites. Aromatic concentrations, although generally lower, display similar ranges in concentrations. Many studies e.g. Wakeham (1976) have compared polluted locations

TABLE 2.6

HYDROCARBON CONCENTRATIONS IN FRESHWATER AND MARINE SEDIMENTS

Compound	*Concentration* ($\mu g\ g^{-1}$)	*Location*	*Reference*
Aliphatic Hydrocarbons	30-385	L. Huron. (Canada)	Myers *et al.* (1980)
Aliphatics	6-28	L. Quinalt (USA)	Wakeham (1976)
"	99-520	L. Sammamish (USA)	"
"	26-1600	L. Washington (USA)	"
Total Aliphatics	30-80	Narragansett Bay (USA)	Teal & Farrington (1977)
" "	1180-2660	Buzzards Bay (USA)	" "
Total Saturates	6-230	Green Duwamish River (USA)	Hamilton *et al.* (1984)
Saturates	0.4-24	Port Valdez (USA)	Shaw & Baker (1978)
Resolved Hydrocarbons	0.26	Great Barrier Reef (Australia)	Coates *et al.* (1986)
n-paraffins	12-56	Terminus Lagoon (Mexico)	Botello & Mandelli (1978)
Alkanes	10-41	Rostherne Lake (UK)	Cardoso *et al.* (1983)
Alkanes	0.03-3	N.Sea (UK)	Middleditch & Basile (1978)
Hydrocarbons	50-900	L. Zug (Switzerland)	Giger *et al.* (1974)
Aliphatics	30-100	L. Lucerne (Switzerland)	Wakeham (1979)
Aromatics	6-60	" "	"
Aromatics	3.8-50	L. Washington (USA)	Wakeham *et al.* (1980)
"	7.5-100	L. Zurich (Switzerland)	"
"	3.2-133	L. Greifersee (Switzerland)	"
"	14.7-25	L. Lucerne (Switzerland)	"
PAH (19)	0.288	Colin Scott Lake (Canada)	Brown & Starnes (1988)
PAH (17)	5-213	L. Ontario (Canada)	Metcalfe *et al.* (1990)
PAH	0.02-210	River Derwent (UK)	Evans *et al.* (1991)
PAH (9)	0.8-290	Pensacola Bay (USA)	Elder & Dressler (1988)
PAH	0.4-3.64	Sagamore Lake (USA)	Heit *et al.* (1988)
PAH (12)	8.4-150.4	Lake Erie (USA)	Plowchalk & Zagorski (1986)
PAH (18)	0.2-13	Adirondack Lake (USA)	Tan & Heit (1981)
Fluoranthene	792	Black River (USA)	West *et al.* (1988)

with sites considered to be in pristine locations. Comparisons of recent and ancient levels of hydrocarbons are possible using sediment cores and lead dating. Even at "unpolluted" sites some elevation in surface sediment hydrocarbon concentration compared with deep core (ancient) levels are often recorded, emphasising the importance of long range atmospheric transport and the ubiquity of hydrocarbons in the environment.

2.8.1 Hydrocarbon accumulation in aquatic organisms

In spite of the greater global quantities of total hydrocarbons originating from biogenic sources, hydrocarbons of anthropogenic origin are invariably associated with the greatest tissue concentrations measured in aquatic organisms. The impacts of large oil spills on marine organisms, particularly wildfowl, have been documented but for the invertebrate community of urban freshwaters, more elevated tissue burdens and potentially longer term ecological damage may occur as a result of chronic input of hydrocarbons in urban runoff. In the freshwater environment, many important processes such as dilution and evaporation are, as a consequence of its reduced dimensions, less important removal mechanisms compared with the marine environment. In undisturbed seawater, the hydrocarbons will initially be retained in the top 10 m of the water column (Mackay, 1987). Marine waters below this depth are considered free of immediate impact, and dispersive mechanisms will eventually reduce the local concentration before benthic communities are exposed to potentially high levels. Conversely, in freshwater systems, the whole water column and entire biological community is likely to be exposed to the hydrocarbon input. In particular, in lentic receiving waters, accumulation of contaminated sediments results in long term contamination as a result of low rates of removal. Lotic freshwater environments may be expected to remove oil residues as a result of greater flows but, in urban areas, chronic inputs throughout a catchment resulting in pulse shifts of already contaminated sediments and the increased hydraulic stress and scouring effects exerted by such streams result in an increased threat to the stream ecology.

2.8.2 Laboratory studies

A number of workers have demonstrated in laboratory studies that hydrocarbons can be readily taken up by aquatic organisms (e.g. Stegeman & Teal, 1973; Cravedi & Tulliez, 1981; Reichert *et al.*, 1985). The bioconcentration of hydrocarbons is due to their affinity for the lipophilic components of biological membranes and cellular materials. Cravedi & Tulliez (1981, 1982) demonstrated that a broad range of aliphatic hydrocarbons including C_{14} to C_{24} *n*-alkanes, isoprenoids and cyclics were readily accumulated through the gastrointestinal mucosa of the rainbow trout, *Salmo gairdneri*.

Subsequent analysis of body tissues during depuration phases revealed a retainment of hydrocarbons in fatty tissues while in the liver, significant reductions in levels were reported as a result of metabolic activity. Predictions of bioaccumulation of specific compounds by use of the octanol:water partition coefficient are possible and would, for the PAH group, predict an increase in bioaccumulation with increasing ring number. This is supported by laboratory test results on *Daphnia pulex* (Southworth *et al.*, 1978) as shown in Table 2.7.

TABLE 2.7

BIOACCUMULATION OF PAHs BY *Daphnia pulex* (FROM SOUTHWORTH *et al.*, 1978)

Compound	*Molecular Weight*	*Ring Number*	*Bioconcentration Factor* $(24h)^{-1}$
naphthalene	128	2	131
phenanthrene	178	3	325
anthracene	178	3	917
9-methylanthracene	192	3	4583
pyrene	202	4	2702
benzo(a)anthracene	228	4	10,109
perylene	252	5	7191

The data indicate a general trend of increasing bioaccumulation with increasing PAH ring number. However, these data refer to a highly controlled laboratory situation with little or no interfering factors. Trucco *et al.* (1983), carried out experiments in which PAHs were introduced in both algal food and water and found naphthalene to be bioaccumulated to a greater extent than phenanthrene, benzo(a)anthracene or benzo(a)pyrene by *D. magna*. Such data illustrate the conflicting patterns that exist in terms of hydrocarbon distributions between biota and their habitats. Work by Neff (1976) on the estuarine clam *Rangia cuneata* showed naphthalene to be accumulated and released most rapidly while benzo(a)pyrene was accumulated more slowly but retained for longer. The differences in aqueous solubilities and lipid water partitioning coefficients were used to explain the differences and these issues will be considered in greater detail in Chapter 6.

To further understand the potential for trophic transfer or biomagnification of hydrocarbons it is necessary to investigate uptake from food. Rossi (1977) and Rossi & Anderson (1977) were unable to demonstrate any uptake of 2-methyl naphthalene

from a dosed powdered alfalfa food source in the polychaete worm *Neanthes arenaceodentata*. Other laboratory studies on higher animals such as crustacea and fish have demonstrated variable extents of accumulation from food. e.g. Corner *et al.* (1976) showed efficient uptake of ^{14}C-naphthalene from a spiked food source (copepod nauplii *Elminius* sp.) in *Calanus helgolandicus*. In cod, *Gadus morrhua*, however much of the detected compounds remained in an unassimilated state in gut contents. Neff (1979) suggested that in all cases where rapid assimilation of hydrocarbons from food occurred, metabolism and excretion was also rapid and that food chain biomagnification would therefore be limited. Again, the important issue of the extent of biological assimilation of hydrocarbons will be re-addressed in Chapter 6.

Bioaccumulation from marine sediments has been investigated by a number of workers and the results are summarized in Table 2.8. The data indicate the generally low extent to which hydrocarbons may be accumulated from sediments. Particularly notable is the large extent to which high organic sediment content reduces PAH bioavailability and which, in these cases mask other possible influencing factors (see Section 2.8.4).

TABLE 2.8

BIOACCUMULATION OF SELECTED PAHs FROM SEDIMENTS (FROM NEFF, 1984)

Species		*Compound*	*Bioaccumulation factor*
Arenicola marina		naphthalene	4.1
Abarenicola pacifica		phenanthrene	5.4
		chrysene	6.7
		benzo(a)pyrene	5.8
Macoma inquita	High organic sediment	phenanthrene	0.096
		chrysene	0.308
		dimethylbenzathrcene	0.297
		benzo(a)pyrene	0.059
M. inquita	Low organic sediment	phenanthrene	7.9
		chrysene	11.6
		benzo(a)pyrene	5.2

2.8.3 Field studies

Levels of specific hydrocarbons in aquatic organisms vary enormously e.g. for the PAH group, concentrations of individual compounds may vary by six orders of magnitude from barely detectable levels of approximately 0.01 ng g^{-1} to 10 μg g^{-1} (dry mass concentration). Concentrations of total PAHs may be a further order of magnitude higher. The aliphatic hydrocarbons also display large concentration variations in biota, individual alkanes achieving maximum values similar to PAHs in the low μg g^{-1} range although the lowest levels may be one or two orders of magnitude higher than the PAHs as a result of the alkanes' greater natural ubiquity in the environment. Total aliphatic concentrations in biota may approach 1 mg g^{-1} in heavily polluted areas (Fossato *et al.* 1978). The ranges of hydrocarbon concentrations that have been found in various aquatic organisms are presented in Table 2.9 .

Of the many factors which have an influence on hydrocarbon concentrations, a clear link can be established between elevated PAH and alkane tissue concentrations and the extent and proximity of human industrial activity which results (through entry by the routes shown in Table 2.1) in elevated hydrocarbon levels in aquatic habitats. Gosset *et al.* (1983) considered sediments the primary source of a number of organic micropollutants to aquatic organisms. Positive correlations were found between sediment and tissue levels and K_{ow} but the correlations were negative with concentrations in effluent from a wastewater treatment outfall. A number of other potential sources of hydrocarbons to the stream fauna exist: they may be taken directly from solution through the gill or body wall, in food as micro-droplets or from particulates as well as directly from the sediments. However, as previously discussed the likelihood of sorption of hydrocarbons from solution will substantially reduce the bioavailabilty of hydrocarbons entering in this form.

Correlations between tissue hydrocarbon concentrations and organism habitat have been demonstrated by Eadie *et al.* (1982a,b) in midges collected at contaminated sites in Lake Erie. Further experiments on worms, however, revealed a substantial depletion of PAH concentrations relative to ambient sediment levels for which an enhanced metabolic ability to eliminate the PAHs was offered as an explanation. The amphipod

TABLE 2.9

HYDROCARBON CONCENTRATIONS IN SELECTED FRESHWATER AND MARINE BIOTA

Organism	*Location*	*Concentration ($\mu g\ g^{-1}$ dry mass)*		*Reference*
FUNGI	Great Barrier Reef	Total resolved	0.19*	Coates *et al.* (1986)
ALGA		aliphatics	66*	
ANNELIDA	Lake Erie,	Ph	0.02-0.04*	Eadie *et al.* (1982b)
	Michigan	A	0.01-0.02*	
		Fl	0.01*	
		Py	0.075*	
		Ch	0.06-0.07*	
		BP	0.02-0.1*	
Nephthys sp.	New York Bight	Fl	0.01-0.055*	Farrington *et al.* (1983)
		Py	0.025-0.09*	
MOLLUSCA				
Mytilus edulis	Seine estuary	BaP	ND-0.38	from Neff (1979)
	New York Bight	Fl	0.05-0.2	Farrington *et al.* (1983)
		Py	0.05-0.6	
M. galloprovincialis	Ebro Delta, Spain	saturate fraction	18-740	Riseborough *et al.*
		aromatic fraction	0.4-66	(1983)
M. californianus	California Coast	saturates	7-115	Disalvo *et al.*(1975)
		aromatics	3-290	
Thais haemostoma	Florida	Na	0.035-0.055	Elder and Dressler
		Ph	0.03-0.19	(1988)
		Fl	0.06	
		Py	0.04	
Mussels	Hershey River,	resolved aliphatics	1.8-10.9*	Wise *et al.* (1980)
Oysters	Michigan	Ph	0.50	Black *et al.* (1980)
		Fl	0.06	
		n-paraffins	10-160	Anderson *et al.* (1974a)
Tridacna sp.		paraffins	3.3-8.4*	Botello and Mandelli (1978)
CRUSTACEA				
Crayfish	Lake Michigan	Ph	0.50*	Black *et al.* (1980)
		BA	0.05*	
		BaP	0.01*	
Pontoporeia hoyi	Lake Erie	Ph	0.2-1.5*	Eadie *et al.* (1982a)
		A	0.1-0.15*	
		Fl	1.0-2.0*	
		Py	0.25-3.0*	
		Ch	0.15-0.60*	
		BaP	0.3-0.9*	
INSECTA				
mixed	Hershey River,	Ph	6.0*	Black *et al.* (1980)
	Michigan	BA	3.0*	
Midges		Ph	0.03-0.04*	Eadie *et al.* (1982b)
		A	0.01-0.02*	
		Fl	0.02-0.125*	
		Py	0.08-0.13*	
		Ch	0.04-0.10*	
		BaP	0.075-0.15*	
		BeP	0.03-0.14*	
VERTEBRATA				
Scarus sp.	North Sea	n-alkanes	1.5-2.2*	Hardy *et al.* (1974)

* concentration as $\mu g\ g^{-1}$ wet mass

Pontoporeia hoyi displayed bioaccumulation ratios of 0.1 to 10 compared to surrounding sediments. The PAH ratio between organisms and interstitial water was of the order of 100-1000.

2.8.4 Factors influencing bioaccumulation of hydrocarbons

2.8.4.1 Original source of hydrocarbons

Farrington *et al.* (1983) provided important circumstantial evidence to suggest that not all hydrocarbons are equally available for accumulation. In a survey of coastal sediments, fingerprinting techniques suggested that the hydrocarbons present were predominantly of pyrolytic origin. However, mussels and polychaetes collected from the same site revealed profiles characteristic of both pyrogenic and diagenic (unburnt) sources. Thus, hydrocarbons of pyrolytic origin were considered less bioavailable than diagenic ones as a result of the tighter binding to particulate matter that would occur during their high temperature formation. The differing bioavailability hypothesis has also been applied to the results of the U.S.E.P.A. Mussel Watch Program (Farrington *et al.*, 1982) in which similar disparities were observed between organism hydrocarbon assemblages and the sediments in which they lived. Recognition of source groups with greater tendencies to bioaccumulate represent an important step in providing effective environmental protection and such an approach will be returned to in subsequent chapters.

2.8.4.2 In-situ source of hydrocarbons

In laboratory studies, hydrocarbons have generally been shown to be accumulated most rapidly from water > food > sediment but in field studies the quantification of the relative importance of each source is extremely complex. Landrum & Scava (1983) working on the amphipod *Hyalella acteca* estimated that 77% of the anthracene body burden was derived from sediment or pore waters. The presence of sediment had no effect on initial uptake rate while depuration rates were faster in the presence of sediment. It is generally believed that sediment associated PAHs are only available to organisms following desorption to the dissolved phase. However, the presence of fine

suspended particulate matter leads to colloidal associations with hydrocarbons. Clearly, detailed knowledge on the relative concentrations of hydrocarbons in each of these compartments is required before accumulation can be predicted accurately.

2.8.4.3 Temperature

The effect of increasing temperatures has generally been found to decrease the bioaccumulation of hydrocarbons (Varanasi *et al.*, 1981). Gerould *et al.* (1983) demonstrated that the bioconcentration factor of chironomid worms exposed to anthracene was more strongly affected by the temperature induced changes on biotransformation (elimination) rather than temperature induced changes on uptake. The bioconcentration was found to be highest at 16°C because biotransformation rate was lowest even though uptake was also lowest at this temperature.

2.8.4.4 pH

The physico-chemical properties of alkanes or PAHs would not be expected to change during acidification of freshwater because these molecules do not contain polar groups that may ionize during pH changes. However, Wildi *et al.* (1994) have demonstrated that pH changes resulted in variations in bioaccumulation of the PAH pyrene by the larval midge *Chironomous riparius*. The variations were considered behaviour dependent: at pH 4 an increase in mucus secretion led to a denser construction of larval dwelling tubes, whereas at pH 8, less mucus was secreted and consequently the tube composition was less dense which resulted in an increase in pyrene accumulaton.

A further indirect effect of pH changes on possible accumulation of PAHs has been shown by Schlautman & Morgan (1993) in which hydrocarbon binding to dissolved humic material (see following section) was found to increase at lower pHs.

2.8.4.5 Dissolved organic matter

The presence of dissolved organic matter (DOM) is acknowledged to greatly reduce the bioavailability of hydrocarbons (e.g. Lee *et al.*, 1993). Large reductions in naphthalene

accumulation by the shrimp *Pandalus platyceros* on addition of bovine serum albumin have been shown (Sanborn & Malins, 1977). McCarthy (1983) working with *Daphnia magna* demonstrated a reduction of 97% in uptake and accumulation of benzo(a)pyrene due to sorption of PAH to naturally occuring DOM.

Antagonistic effects relating to specific hydrocarbon uptake occurring as result of the presence of other organic compounds have been reported. Fortner & Sick (1985) demonstrated several cases in which the accumulation of individual PAH compounds were reduced in a PCB, naphthalene and benzo(a)pyrene multiple component mixture compared with accumulation in its absence. This work appears to be of considerable significance given the large cocktail of pollutants to which organisms in urban locations are likely to be exposed.

2.8.4.5 Endogenous factors

A number of factors, related to the organism physiological and behavioural make-up, are known to affect hydrocarbon bioaccumulation.

Boehm (1980) investigated petroleum uptake by two contrasting bivalves, *Macoma bathica* (deposit-feeder) and *Mytilus edulis* (suspension feeder) and showed clear differences between the uptake and depuration patterns in the two organisms. *M. balthica's* assemblage was controlled by sediment levels and *M. edulis'* by those in the water. There is no evidence for similar distinctive differences between species, based on feeding strategies in the freshwater environment although it is possible that co-habiting organisms such as *Gammarus pulex*, *Asellus aquaticus* and *Lymnaea peregra*, which possess quite different feeding strategies, may reflect this in the accumulated hydrocarbons. The act of feeding also influences accumulation: within the same species, feeding by *Chironomous riparius* has been demonstrated by Leversee *et al.*, (1982) to increase the elimination rate of benzo(a)pyrene.

As a result of the known affinity of hydrocarbons for lipids, the lipid concentration of an organism or of a specific tissue will in general terms be related to bioaccumulative potential. Stegeman & Teal (1973) demonstrated positive correlations between

petroleum accumulation by oysters and their fat content. However, work by Mix *et al.* (1982) has shown that spawning and gametogenesis of mussels did not alter total PAH body burdens in spite of the associated changes in body lipid levels. Differences in hydrocarbon bioaccumulation attributed to the sex of a species have been reported mainly as functions of varying metabolic activity. Work on the polychaete, *N. arenaceodentata* (Rossi & Anderson, 1977) showed males to release hydrocarbons faster than females although uptake was similar. During spawning a rapid depuration of hydrocarbons was observed in the females and the differences were suggested to be associated with the yolk lipid levels of the worms.

2.9 METABOLISM OF HYDROCARBONS

2.9.1 Toxicity and sub-lethal effects

As with bioaccumulation, freshwater organisms vary considerably in their sensitivity to hydrocarbon compounds. Many of the factors which are known to influence bioaccumulation also affect toxicity. In the laboratory, great differences in the types of toxicity tests performed have led to the gathering of substantial amounts of non-standardized data. Test organism type, life cycle, method of pollutant introduction and test criteria vary enormously. Lethality tests are relatively clear but sub-lethal effects have been measured with a large number of parameters, including neoplasm formation, pathogenesis, growth rates, fecundity, respiration, feeding rates, avoidance behaviour and the induction of Mixed Function Oxidase (MFO) systems.

Variabilities in response may be due to organism nutritional status, life cycle and sex. Generally, developmental stages particularly juveniles of aquatic organisms display a greater sensitivity to hydrocarbons than adult stages. Larval stages may display increased tolerances as a result of initial elevated yolk or lipid levels which compartmentalize, and, with ongoing development and subsequent fat reductions, eliminate hydrocarbons.

2.9.2 Cytochrome P-450 mediated mixed function oxidase activity

The biological turnover and elimination of hydrocarbons is determined by the presence and extent of activity of a cytochrome P450 dependent mixed function oxidase (MFO) system. Normally this enzyme system is involved in steroid catabolism but on introduction of hydrocarbon compounds, conjugation with glucorinic acid or hydroxylation leads to an increase in hydrocarbon solubility enabling increased excretion. The MFO system has been detected to varying extents in virtually all organisms. The system may be induced to enhanced activity above baseline levels through the introduction of hydrocarbons, most notably aromatics, and has been demonstrated in a wide range of organisms but most commonly and pronouncedly in fish (e.g. Varanasi, 1989). The enhanced MFO activity in fish is therefore considered as a major controlling factor in the generally lower concentrations of hydrocarbons recorded in fish compared with co-habiting or prey invertebrates and is therefore important in reducing trophic transfer and possible biomagnification effects.

2.9.3 Mutagenic and carcinogenic effects

Several PAH compounds have, in laboratory studies, demonstrated mutagenicity and carcinogenicity in a wide range of organisms ranging from bacteria to mammals. Neff (1979) compiled a list of relative carcinogenicity of a range of PAHs shown in Table 2.10. Large differences in carcinogenicity exist between closely related compounds (e.g. benzo(e)- and benzo(a)-pyrene) suggesting that molecular structure is of primary importance in inducing cancer. Carcinogenicity is believed to be related to angular arrangements of benzene rings, and the extent and position of alkylated groups. In field studies, significant positive correlations have been established between malformations and neoplasms in invertebrates and fish and levels of PAHs in surrounding sediments. A causal relationship, however, is difficult to establish because of the large number of known carcinogens present in highly contaminated sediment.

TABLE 2.10

RELATIVE CARCINOGENICITY OF SELECTED PAHs TO LABORATORY MAMMALS (FROM NEFF, 1979).

Compound	*Carcinogenicity*
Phenanthrene	-
Dibenzo(ah)anthracene	+++
Fluorene	-
Fluoranthene	-
Benzo(b)fluoranthene	++
Benzo(k)fluoranthene	-
Pyrene	-
Benzo(a)pyrene	+++
Benzo(e)pyrene	-
Indeno(1,2,3,-cd)pyrene	+

- not carcinogenic

+ carcinogenic

++ +++ strongly carcinogenic

2.9.4 Transformation of parent compounds to metabolites

The values presented for hydrocarbon concentrations in aquatic organisms (Table 2.9) do not take into account the uptake and transformation of hydrocarbons: they represent tissue levels at a given time. Clearly, full understanding of the mechanisms of hydrocarbon toxicity or sublethal effects requires a knowledge of the nature of metabolites and their turnover rates. MFO mediated transformation of parent compounds is required in carcinogenesis since the parent compounds are intrinsically non-carcinogenic (Sims & Grover, 1974). For example, oxidative metabolism of benzo(a)pyrene yields arene oxide intermediates which bind covalently to DNA, RNA and proteins. The carcinogenicity of benzo(a)pyrene is due chiefly to 7,8-dihydroxy 9,10-epoxy-7,8,9,10 tetrahydrobenzo(a)pyrene.

Krahn *et al.* (1987) have detected levels of metabolites of fluorene, phenanthrene, anthracene, biphenyl and dimethylnaphthalene at concentrations of 90 - 19,000 ng g^{-1} wet weight in the bile of English sole (*Parophtys vetulus*) which were ten times the levels of the reference sites. Other workers e.g. Gossett *et al.* (1984) have suggested that PAH metabolites are ubiquitously distributed in marine sediments. The possibility

of trophic transfer of "activated" compounds must therefore exist although knowledge on this matter is currently limited.

2.10 SUMMARY

Hydrocarbons including alkanes and PAHs have been demonstated as important components of urban runoff pollution and have been measured in receiving waters, sediments and organisms therein. As a result of high hydrophobicity and tendency to sorb to particles, concentrations in water are three to five orders of magnitude lower than those in the underlying sediments. Concentrations in the tissues of organisms are generally lower or in the same order of magnitude as the sediments. Thus, excessive bioaccumulation has not been reported.

The mechanisms that control the extent to which organisms accumulate hydrocarbons are many and complex but may be subdivided into exogenous and endogenous factors. Exogenous factors include all physical factors relating to a given compound including solubility, original mode of formation (extent of sorption), temperature and DOM content of the medium. Endogenous factors relate to the organism's characteristics including sex, lipid content, and feeding behaviour.

There appears to be little evidence that biomagnification with increasing trophic levels occurs with hydrocarbons and in the case of fish many reports indicate that levels are lower than those of invertebrates inhabiting the same site. Such observations are believed to be the result of the enhanced removal mechanisms of fish compared with invertebrates. However, the mixed function oxidases that effect the removal of xenobiotic compounds also modify aromatic compounds to potentially carcinogenic arene oxide intermediates.

Chapter 3

SAMPLING STRATEGIES, METHODOLOGY AND ANALYTICAL TECHNIQUES

3.1 INTRODUCTION

Data from field studies in the Dean's Brook, the Silk Stream and their receiving basin the Welsh Harp in NW London, and laboratory trial results have been collected over a 30 month period. The study catchment drains a total area of 5239 hectares and within it approximately 65% of the total area is urbanized and about 25% of the catchment area is impermeable. Approximately 60% of the annual flow volume is derived from impermeable surfaces (Hall, 1977)

Ten sampling sites have been established during the research programme, including 2 reference sites, 5 test sites on the Silk Stream and 2 test sites in the Welsh Harp Reservoir. Caged macroinvertebrates have been placed at Sites 5, 6, 7 and 9 and it is at these downstream strategic sites that the most intensive sampling strategies have been employed. The progressive downstream location sequence of the sites.is shown in Fig. 3.1 with the exception of Sites 1 and 1a which are background reference sites located outside the Silk Stream catchment. At all of the sampling sites with the exception of Site 6, water clarity was generally good during normal dry weather flow and suspended solid levels were less than 30 mg l^{-1}. However, during storm events, the fine loose nature of the lower Silk Stream sediment results in considerable resuspension of the substrate and associated pollutants. The study and chemical features characterizing the selected sampling sites within the Silk Stream catchment (Fig. 3.1) have been recorded on special data sheets of which a specimen is included (Fig. 3.2).

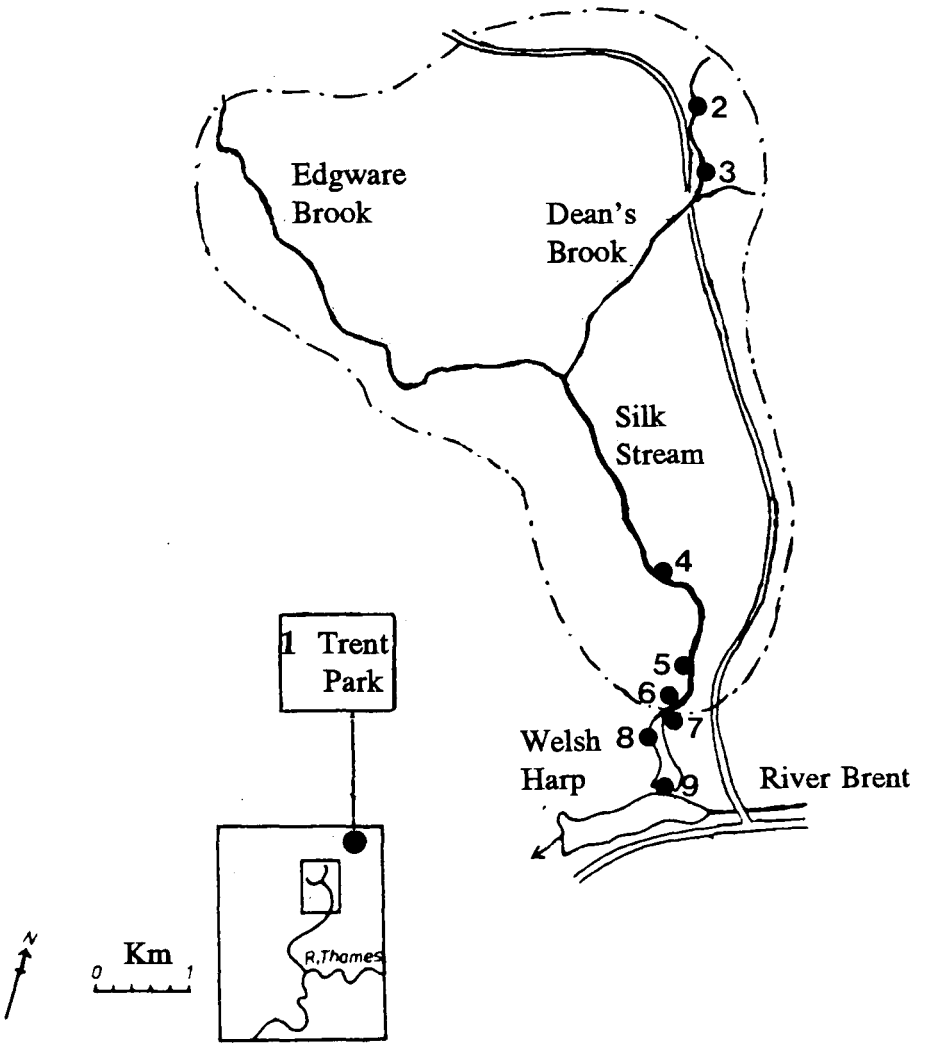


Fig. 3.1 Sampling sites in the Silk Stream catchment

TABLE 3.1

CODES, NAMES, AND GRID REFERENCES OF THE SILK STREAM CATCHMENT SAMPLING SITES

Site Code	*Site Name*	*Grid Reference*
1	Dew Pond, Trent Park	TQ 293973
1a	Salmon's Brook, Hadley Road	TQ 303980
2	Stoneywood Lake	TQ 204936
3	Dean's Brook (Stoneyfield Park)	TQ 203930
4	Silk Stream (Rushgrove Park)	TQ 218893
5	Silk Stream (relocated above boom site)	TQ 219885
6	Silk Stream (original above boom site)	TQ 217883
7	Silk Stream (below boom)	TQ 217883
8	Welsh Harp (north)	TQ 216882
9	Welsh Harp (Cool Oak Lane)	TQ 219877

DATE:
SITE:
G.R.:
TIME:

SITE DESCRIPTION

Width:	
Distance in:	
Depth:	
Flow:	

Floating debris:	
Oil sheen:	
Scums/stains:	
Odours:	

Surfactant presence:	
Plume from pipe:	
Sewage fungus:	
Macrophyte coating:	

Other comments:

Propeller:

Calculation:

FLOW:

Temp.:	
% Shading:	
pH	
Conductivity:	
D.O.:	
B.O.D.:	
Hardness:	
Alkalinity:	
Ammonia:	
S.S.:	

CLARITY

Clear	
Slightly Turbid	
Turbid	

COLOUR

Clay Brown	
Green	
Other	

SUBSTRATE COMPOSITION

% Gravel	
% Sand	
% Silt	
% Mud	

Detritus (1-5)	

MANAGEMENT

Weed Cutting	
Dredging	
Bankside Maintenance	
Channel straightening	
Flow Management	

INVERTEBRATE SPECIES:

MACROPHYTE SPECIES

HYDROBIOLOGICAL INDICES

Chandler Score	
BMWP Score	
ASPT	
Lincoln Index	

DATA ACCEPTABILITY

Weather:

Rainfall:

A.D.P.:

Other Comments:

Fig. 3.2 Data sheet used for site descriptions

3.2 SAMPLING LOCATIONS

The Dew Pond (Site 1), is situated within the Middlesex University campus in Trent Park Country Park (Grid Reference 293973). This site supports a diverse population of macroinvertebrates and has extensive marginal colonisation by *Typha latifolia* and *Juncus effusus* as well as by *Salix* spp. and *Quercus* sp. which overhang the water body (Plate 1a). The location was selected as a reference site for the measurement of background concentrations of extractable organics and for the determination of sediment oxygen demand rate. Reference specimens of relatively uncontaminated water lice, *Asellus aquaticus* were initially collected from this site for use in caged macroinvertebrate experiments.

A further reference site (Site 1a) located on the Salmon's Brook in Hadley Road (Grid Reference 303980) was used for the main collection of unpolluted *A. aquaticus*. This site, (Plate 1b) has a coarse gravel substrate and dense mats of *Cladophora* were observed during the summer months in which *A. aquaticus* were abundant. Riffle zones are predominant at this site and during the winter months the asellids were found in the gravel substrate.

Site 2 (Grid Reference 204936) which is located near the headwater of the Dean's Brook, a tributary of the Silk Stream, temporarily replaced the Trent Park Dew Pond as a reference site for *A. aquaticus* collection, owing to their abundance here throughout the sampling period. The marginal areas of the lake have been extensively colonized by *Typha latifolia* and the presence of a dense algal bloom which was observed to persist throughout the summer together with the high levels of detritus suggested that the water is of an eutrophic nature. The dense macrophytic margins of this shallow ornamental lake are clearly illustrated in Plate 2a.

The Dean's Brook at Stoneyfield Park (Site 3; Grid Reference 203930) was the furthest upstream location for chemical and biological monitoring. This site shows little if any artificial channelization and significant erosion (steep cut banks) and deposition (gravel shoals and point bars) characterize this meander section (Plate 2b). The substrate at this site is essentially composed of medium and coarse gravel, as can be seen from the

skewed distribution in the sediment particle size mass distribution in Table 3.2 with Md_{50} sizes approximating to 4 mm. The organic component of the sediment is consistently less than 10% (Table 3.2). There is no substrate attached vegetation in the deeper fast flowing mid section of the stream but an approximate 15% cover by marginal moss exists at depths of less than 10 cm. In the late summer period, attached algae of *Cladophora* sp. were abundant in areas upstream of the *Typha* reed beds. At the sampling site there is an approximate 30% shading cover due to overhanging vegetation, chiefly *Quercus* sp.

TABLE 3.2

SEDIMENT PARTICLE SIZE MASS DISTRIBUTION AT SITES 3, 4 AND 7

Particle Size Range (mm)	*% Mass*		
	Site 7	*Site 4*	*Site 3*
>40	-	-	3
20-40	-	5	2
10-20	-	10	16
4-10	-	12	30
2-4	1	14	24
1-2	3	19	12
0.5-1	2	14	7
0.25-0.5	6	10	4
0.125-0.25	22	8	1
0.063-0.125	44	6	1
0.02-0.063	14	2	1
0.01-0.02	8	1	-

In the Rushgrove Park site of the Silk Stream (Site 4; Grid Reference 218893) the stream bed has been artificially straightened and channelized (Plate 3a). There is a small increase in benthal sediment organic content and a clear establishment of attached filamentous algae, dominated by *Cladophora* sp. At this site there is a wider and more Gaussian distribution of sediment particle size in comparison to Site 3 as can be seen from Table 3.2. However, the size distribution suggests that there exists a sufficient minimum stream velocity at both Sites 3 and 4 to prevent the accumulation of the fine silt particles which are associated with the sludges found in the lower Silk Stream.

TABLE 3.3

ESTIMATION OF SEDIMENT ORGANIC CONTENT BY LOSS ON IGNITION

Site	*% Loss on Ignition*
3 Stoneyfield Park	8.5
4 Rushgrove Park	13.1
7 Below the boom	64.8
9 Cool Oak Lane	9.3

The detailed location of the lower Silk Stream and Welsh Harp sites together with detail of supporting land use, bank vegetation and channel form and structure are shown in Figs. 3.3 and 3.4. Sampling sites on the lower Silk Stream were originally established immediately upstream (Site 6; Grid Reference 217883; Plate 5) and downstream (Site 7; Grid Reference 217883; Plate 4b) of the newly installed oil boom and rubbish collector. Both these sites are situated at locations where considerable canalization and channel management has taken place. Upstream of the A5 Edgware Road bridge the channel banks are also constrained by a wooden palisade with piled concrete beams. Downstream of the road bridge however, although the channel has been straightened there is an absence of any bank constraining structures. On the south eastern bank of the oil boom some erosion of the clay bank has occurred which may have been caused by lateral deflection of the current by the boom during storm events. Subsequent storm events led to further damage, particularly on the south eastern bank and in May 1990 caged stones were placed by Thames NRA to reinforce the damaged banks. During high river flow part of the channel flows over the stones and around the control structure.

The initial Site 6 sampling location between the boom and the A5 Edgware Road Bridge crossing was initially discontinued as it was discovered that the site was effectively being "drowned" by excessive oil emissions from the adjacent surface water pipe (pipe No.2 in Fig. 3.4) which also caused a permanent odorous surface sheen and high turbidity. Not only did this dry weather discharge cause excessive and early mortality of the caged organisms, it was also considered that it caused conditions unrepresentative of the general ambient water quality upstream of the oil boom.

At Site 7, below the boom (Grid Reference 217883) there is an approximate 90% vegetation cover, dominated by *Salix* sp. which overhang the channel and form an almost continuous canopy shading the water channel as shown in Plate 4b. During normal dry weather conditions there is only a very low flow velocity maintained at this site. The sediment composition is of a fine organic nature with more than 60% of the total mass comprised of particles finer than 125 μm (Table 3.2).

As a consequence of the problems encountered at the original above boom site (Site 6) an alternative location, (Site 5, Grid Reference 219885) was selected on 9/8/89 to represent above-boom conditions. This new site differs from the original site in that there is:

1) no direct or immediate exposure to any pipe discharges.
2) a presence of aquatic macrophytes and algae, *Elodea* sp. and *Cladophora* sp. being the most abundant genera. These species are gradually eliminated downstream and are absent below the Edgware Road bridge.
3) a marked change in sediment composition. At the new site the bed sediment is composed of consolidated clay with a few patches of coarse gravel. Below the Edgware Road bridge, and at each downstream site (Sites 6, 7 and 8), through to the Welsh Harp Reservoir, the bed sediment (excluding the large detritus and urban refuse) is a fine, highly organic substrate (Table 3.3).

As a consequence of the important differences between the two above-boom sites a further site was established at Site 6 close to the original location but on the opposite bank and not directly exposed to the pipe discharge. This relocated site provides a direct comparison with Site 7 in terms of sediment composition, canopy cover, general water quality as well as biota.

Site 8 (Fig.3.3; Plate 5a) is situated on the edge of the *Typha* reed beds which face the most western inlet of the Silk Stream into the Welsh Harp (Grid Reference 216882). The site is characterised by thick, fine, organic sludges which when disturbed release highly odorous oil slicks, globules and bubbles. Due to the excessive sedimentation suffered within the relocated macroinvertebrate cages very high mortality rates were

encountered at this site which was abandoned during later trials.

Site 9 is located at the downstream end of the Welsh Harp near Cool Oak Lane bridge (Grid Reference 219877) where there is a notable decrease in organic sediment composition (to about 20%) and an absence of emergent macrophytes which appear to be associated with the northern arm of the Welsh Harp (Fig.3.3) and the lowest reaches of the Silk Stream. This site is also exposed to the open waters of the Brent Reservoir and consequently there is some limited wave erosion of the adjacent bank. The sampling site location is shown in Plate 5b.

3.2.1 Pipe discharges

An inventory of the various pipe outfalls that have been found in the lower Silk Stream section including their location, construction material, level and diameter is included in Fig. 3.4. Five pipes, discharging at a high level relative to the river surface, two surface level outfalls and one low level submerged pipe have been identified in the important downstream region. Discharges from outfalls 1, 3, 7 and 8 have been observed only during storm events, but continuous discharges occur from outfalls 4 and 5 which are small, open ditches. Pipe 2 discharges at an average rate of 2 to 2.5 l s^{-1} under normal dry weather conditions and is a prime and obvious pollutant source in the vicinity of the oil boom. It is the most probable cause of the acute deterioration in local water quality between the Edgware Road bridge and the Welsh Harp basin. Its impact is illustrated by the occasional presence of whitish sheets of sewage fungus, dominated by *Sphaerotilus natans* which is attached to the substrate near the pipe outfall but which is notably absent upstream and further downstream of the outfall. There is an absence of algae and submerged macrophytes and additionally *A. aquaticus* has not been found in the immediate vicinity of the outfall. The relatively pollution tolerant leech *Glossiphonia complanata*, which has been identified at all sampling sites on the Silk Stream, is absent downstream of this outfall (Sites 6, 7 and 8) and is present at Site 9.

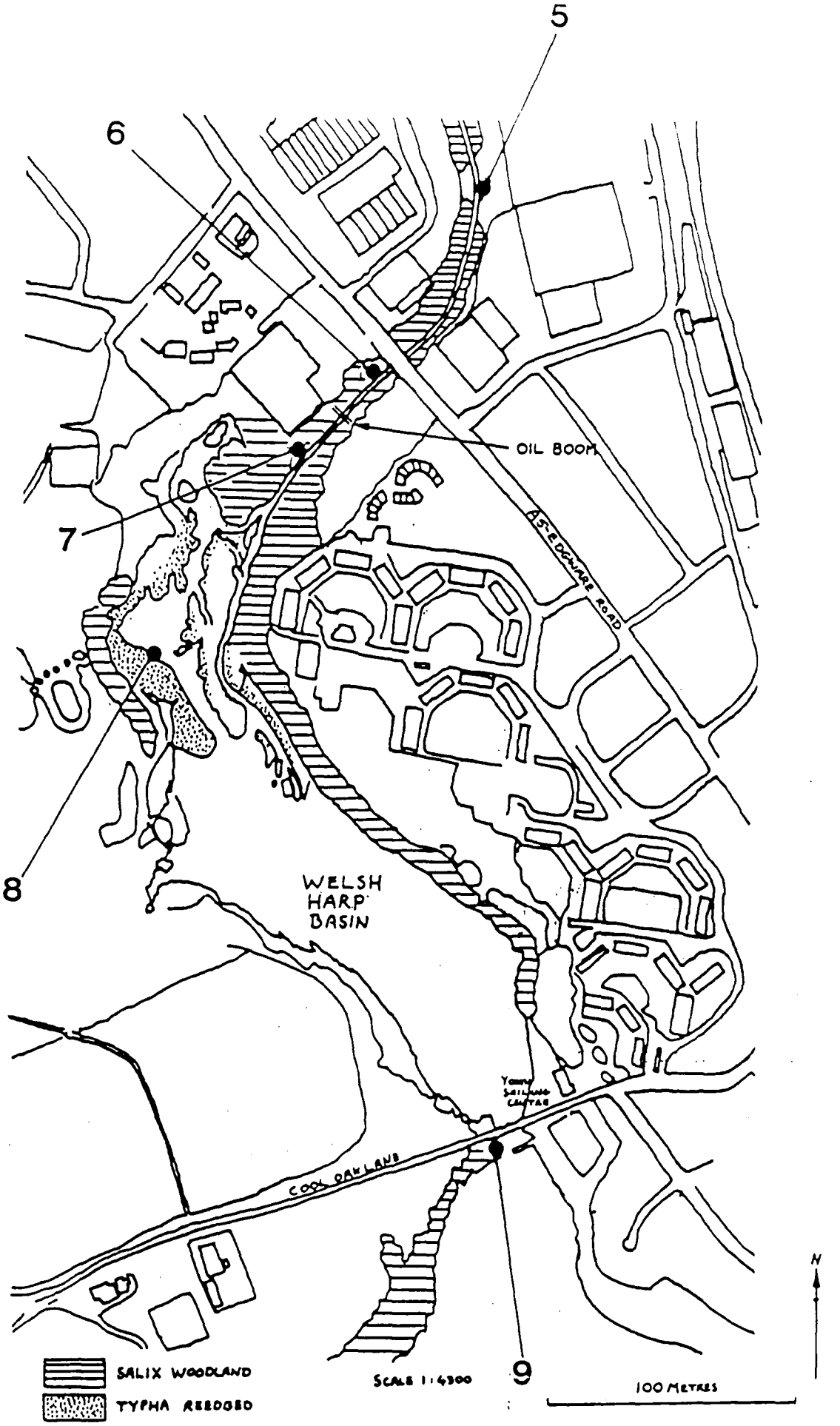


Fig. 3.3 Lower Silk Stream and Welsh Harp sampling locations (Sites 5-9).

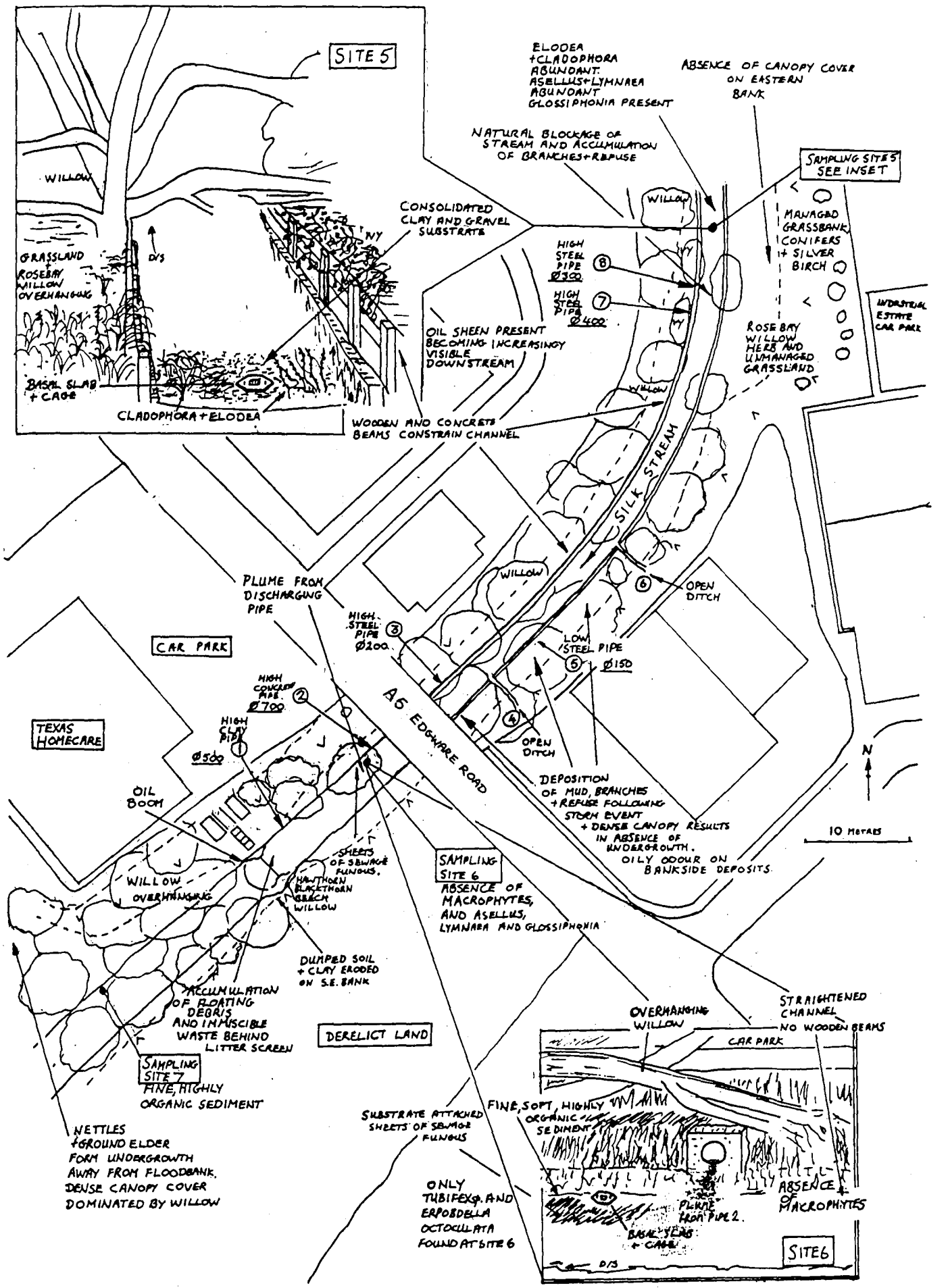


Fig. 3.4 Detail of supporting land, outfalls 1 - 8 and the lower Silk Stream sampling Sites 5 - 7.

Plate 1a Site 1 - Dew Pond, Trent Park.

Plate 1b Site 1a - Salmon's Brook (Hadley Rd.)

Plate 2a Site 2 - Stoneywood Lake

Plate 2b Site 3 - Dean's Brook (Stoneyfield Park)

Plate 3a Site 4 - Silk Stream (Rushgrove Park)

Plate 3b Site 5 - Silk Stream (relocated, above boom)

Plate 4a Site 6 - Silk Stream (above boom)

Plate 4b Site 7 - Silk Stream (below boom)

Plate 5a Site 8 - Welsh Harp (north)

Plate 5b Site 9 - Welsh Harp (Cool Oak Lane)

3.3 FIELD METHODOLOGIES

3.3.1 Introduction

Over the 30 month sampling period physico-chemical and biological data relating to a range of parameters were collected. The levels of dissolved oxygen in particular were closely monitored as this was considered to be an important parameter governing caged organisms mortality rate as well as influencing stream community structure. Other measurements including pH, temperature, biochemical, chemical and sediment oxygen demands, suspended solids, sediment organic content and conductivity were monitored. Biological indices (BMWP, ASPT and Lincoln index) were calculated from 5 minute kick sample collections using a 1mm mesh size square headed net (width 25cm).

3.3.2 Analytical methods

3.3.2.1 Sediment oxygen demand

Sediment cores were collected from the various field sites using a steel sediment corer of 10cm diameter. The corer was carefully positioned on the bed sediment, at all the sampling sites with the exception of Site 2, such that minimum disturbance of the top slurry took place. Inevitably however, a limited loss of the fluidised top fraction occurred. Analyses were performed on fresh sediment where possible. Otherwise, sediment cores were frozen prior to subsequent analysis. The technique was based on the methods of Hatcher (1987).

Approximately 150 ml of the sediment core was placed in a 1 l plastic reservoir and distilled water, which was aerated to saturation, was carefully added until the reservoir was full. The DO probe was inserted through a plastic lid which itself screwed downwards on to the container to seal the water reservoir. On the bottom of the DO probe a stage was attached to accommodate a magnetic flea which, after placing the whole apparatus on a magnetic stirrer, ensured thorough mixing of the reservoir without disturbance of sediment.

The dissolved oxygen changes were initially recorded every 5 minutes and then at 10 and 15 minute intervals as incremental changes became smaller. A replicate sample of water and sediment was prepared in a separate flask and the supernatant water was removed and placed in a similar container to enable DO of any suspended material to be measured in the same way. The difference between the oxygen demand of sediment and water, and water with suspended particles alone provides a indication of the sediment oxygen demand.

3.3.2.2 Dissolved oxygen and temperature

The percentage oxygen saturation, dissolved oxygen concentration and temperature were measured in the field by immersion of the probe of a PHOX oxygen meter which was calibrated against ambient air taken as 100% saturation. Basal and surface readings were taken at each sampling location.

3.3.2.3 Suspended solids

Water samples were initially filtered through a Whatman No.1 filter paper then a 0.45μm Millipore filter. The filters were heated to dryness and were weighed before and after filtration and the result expressed as the suspended solid concentration mg l^{-1}.

3.3.2.4 pH

pH was determined in the field using a Whatman model 62 pH meter.

3.3.2.5 Sediment organic content

Sediment samples (5g) were air dried and weighed accurately, then placed in a furnace at 700°C for 30 minutes. After cooling carbonate was replaced by ammonium carbonate solution before final heating and weighing.

3.3.3 In-stream biomonitoring methods

In order to assess *in-stream* macroinvertebrate response to pollutant exposure, an apparatus was constructed consisting of a basal concrete slab which was impaled to the bed sediment by two stakes, and to which was attached by wire and clips a small plastic cage with nylon mesh windows in which macroinvertebrates could be placed (Fig.3.5). As a result of the smothering effect on organisms by the deposition of sediment within the cages, modifications were subsequently made to those cages located at sites possessing fine organic sediments. The cages were either attached by wire to the securing wire on the basal slab so that the natural buoyancy of the cage held it above the bottom sediments or suspended by means of wire tied from overhanging *Salix* present in the close vicinity (5m) of the initial basal slab locations. A weight was fitted to the base of the cages to ensure that the cages would not rise from the water column. Initially replicate tests of suspended and basal caged organisms were carried out at all the test sites, the results of which led to the abandoning of basal cages in favour of a duplicate suspended cage system at Sites 6 and 7. At these sites two cages were suspended at 30 and 80 cm depths respectively. This system offered the advantage of controlling the cage depth as well as allowing access to the cages without entering the stream, which could cause sediment disturbance and turbidity that may result in organism stress.

Following the addition of 50 - 100 organisms, cages were inspected and the number of live and dead organisms were carefully counted and any dead organisms were removed. Live animals (5 - 8) depending on size were removed from the cages at intervals of between 3-18 days. At Site 9, where the lowest mortality rates were recorded, the longest durations between sampling visits were employed. At the lower Silk Stream sites inspections were made at weekly or more frequent intervals. On return to the laboratory the organisms were frozen and subsequently extracted as described in Section 3.4.1.3.

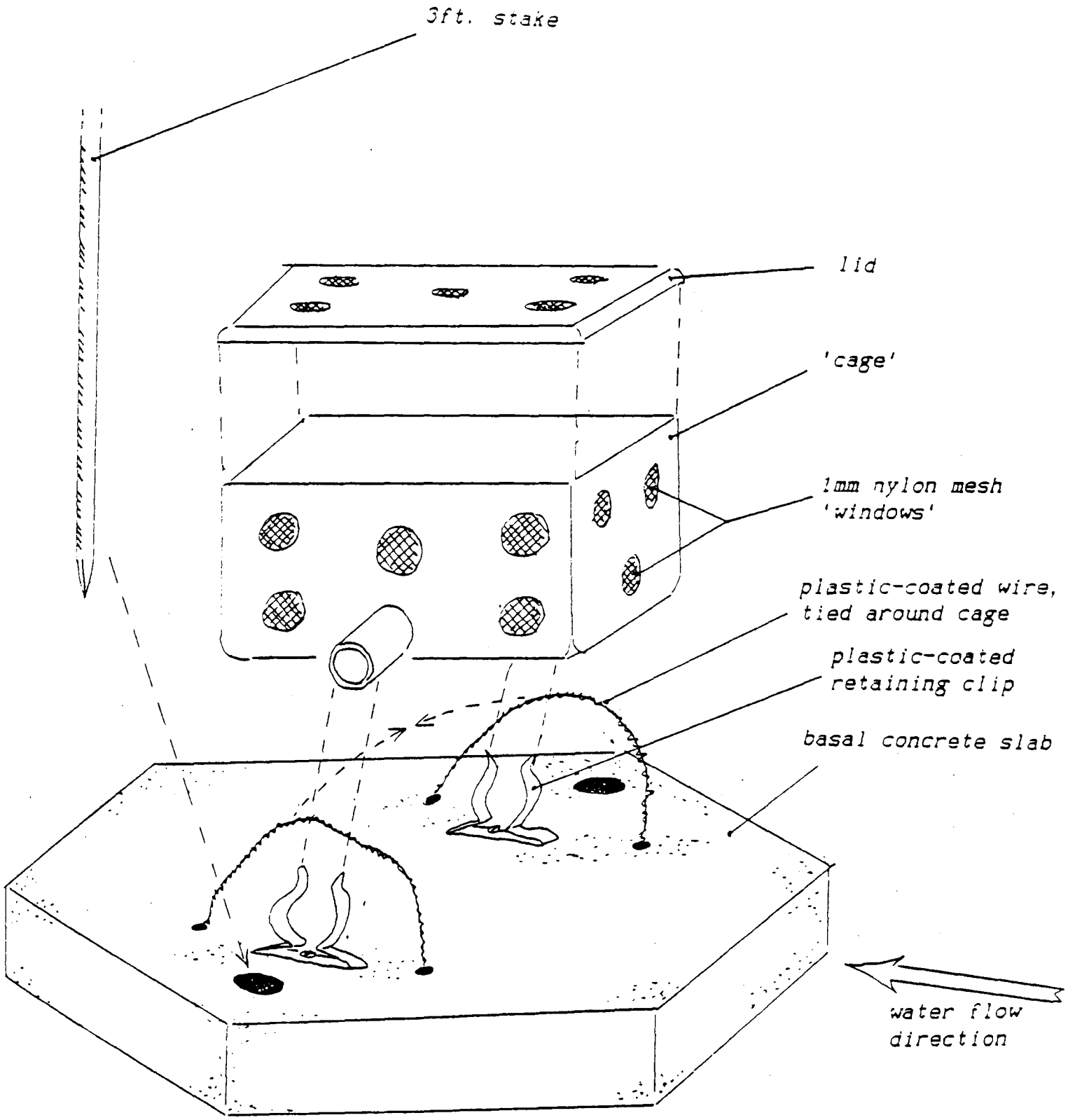


Fig. 3.5 Apparatus used for in-stream experiments

Plate 6a Suspended and basal cages containing *Asellus aquaticus*.

Plate 6b Cages for in-situ macroinvertebrate tests with DO probe (Site 5)

3.4 SAMPLE PREPARATION AND ANALYSIS

3.4.1 Extraction procedure

3.4.1.1 Water

Water samples were collected in clean, dichloromethane-rinsed glass bottles (1.5 l) and were transferred to the laboratory and either extracted immediately or refrigerated. Water (100-500 ml) was placed in a round bottomed flask and dichloromethane (50 ml) was added. The flask was fitted to a condensing column and placed in an ultrasonic bath for 2 hours. Any compounds present on the inner surface of the condenser or round bottomed flask neck were carefully rinsed back with dichloromethane. The resulting mixture was separated in a separating funnel and the water was extracted again by further addition of dichloromethane (50 ml) and 2 further hours ultrasonication. In both cases the ultrasonic bath water temperature was carefully monitored to prevent evaporative losses by overheating. Following separation of the second extraction, the two organic extracts were combined in a clean round-bottomed flask and placed on a rotary evaporator operating under vacuum. A water bath temperature of 35°C was sufficient to reduce the extract relatively rapidly (10 - 15 mins) to the required 0.5-1 ml volume. The extract was transferred by pasteur pipette to a small glass sample tube, together with subsequent dichloromethane rinsing aliquots. The sample tube was allowed to stand in a fume cupboard to allow the air flow to produce a final dry extract which was cleaned up as described in Section 3.6.

3.4.1.2 Sediment

Sediment samples were dried at 40°C for 48hrs and either sieved and analyzed as separate size fractions or as a bulk sample. The sample (1 - 5 g) was weighed accurately and placed in a 100 ml round bottomed flask to which dichloromethane (50 ml) was added. The flask was fitted to a condensor and placed in an ultrasonic bath for 3hrs. The filtrate was collected by filtering through a glass fibre filter paper. The residue was re-extracted for 3 hrs with a further 50 ml dichloromethane and following filtration the filtrates were combined and reduced under rotary evaporation as described above (Section 3.4.1.1). The clean-up procedure (Section 3.6) was employed for sediment samples and repeated in the case of highly contaminated samples.

3.4.1.3 Biota

Macroinvertebrates collected by kick sampling or recovered from test cages were transferred to the laboratory, placed on a nylon mesh and loosely attached particles were rinsed away from the organisms using distilled water and a pasteur pipette. The animals were then frozen before being dried carefully at temperatures not exceeding 40°C for approximately 48hrs. In the cases of *A. aquaticus* and *G. pulex*, no attempt was made to differentiate tissue fractions and therefore only whole body determinations were performed. For *L. peregra*, before drying, the soft tissue was separated from the shell using tweezers. In all cases the dried tissues from 5-8 organisms were ground to a fine powder by solvent rinsed pestle and mortar and weighed accurately. The sample was then transferred to a round bottomed flask, extracted ultrasonically and cleaned-up as described in Section 3.6.

3.5 LABORATORY TOXICITY TEST METHODS

The laboratory based toxicity tests were carried out in plastic containers (50 cm x 35 cm x 20 cm) under static conditions. Macroinvertebrate species (*A. aquaticus* and *L. peregra*) were placed in plastic cages within the tank. The cages were similar to those used in the field tests except larger 1mm nylon mesh windows at the base of the cage were used together with legs to raise the cage from the bottom in order to allow faecal and other particles to fall through and thus be isolated from the test organisms to prevent re-ingestion. Fifty live organisms collected from the Trent Park and Salmon's Brook reference sites (Sites 1 and 1a) were placed in each of the cages which were immersed in 10 l of water collected from the respective sites. The water was dosed with alkanes and PAHs according to the concentrations indicated by mixtures A to F. These concentrations represent the ranges of individual compound concentrations found in environmental samples from the Silk Stream catchment. For the alkane mixtures (A, B and C) the increased concentrations in the mid-range C_{16}-C_{22} alkanes relative to the rest of the suite represent a simplified simulation of the approximately normal distribution in the alkane range observed in environmental samples (see Chapter 4). For the PAH mixtures (D, E and F) the generally dominant compounds, fluoranthene,

pyrene, phenanthrene and benzo(a)anthracene found in environmental samples, have accordingly been represented at relatively elevated levels.

Mixture A alkanes (C_{10}-C_{15}, 5μg l^{-1}; C_{16}-C_{22}, 10μg l^{-1}; C_{22}-C_{30}, 5μg l^{-1})
Mixture B alkanes (C_{10}-C_{15}, 10μg l^{-1}; C_{16}-C_{22}, 20μg l^{-1}; C_{22}-C_{30}, 10μg l^{-1})
Mixture C alkanes (C_{10}-C_{15}, 20μg l^{-1}; C_{16}-C_{22}, 40μg l^{-1}; C_{22}-C_{30}, 20μg l^{-1})

Mixture D PAHs (fluorene, anthracene, benzo(b)fluoranthene, benzo(k)fluorathene, benzo(a)pyrene, benzo(g,h,i)perylene, 5μg l^{-1}; fluoranthene, pyrene, phenanthrene, benzo(a)anthracene, 10μg l^{-1})
Mixture E PAHs (fluorene, anthracene, benzo(b)fluoranthene, benzo(k)fluorathene, benzo(a)pyrene, benzo(g,h,i)perylene, 10μg l^{-1}; fluoranthene, pyrene, phenanthrene, benzo(a)anthracene, 20μg l^{-1})
Mixture F PAHs (fluorene, anthracene, benzo(b)fluoranthene, benzo(k)fluorathene, benzo(a)pyrene, benzo(g,h,i)perylene, 20μg l^{-1}; fluoranthene, pyrene, phenanthrene, benzo(a)anthracene, 40μg l^{-1})

The hydrocarbon mixtures were introduced to the tanks as solutions in methanol (2 ml) in order to improve dispersion and hydrocarbon miscibility with the aqueous phase. Each tank contained a mechanical stirrer and an air pump to assist mixing and aeration of the water. The DO and temperature of the water was regularly monitored by means of a DO probe. The DO levels did not fall below 40% saturation during the test periods. The water temperature was 13°C (+/-2°C). A control tank containing reference water and organisms and fitted with the same equipment was run concurrently with the dosed tanks. The animals were examined at intervals of 2-5 days and any dead organisms were counted and removed. Additionally, live animals (5-8) were removed for hydrocarbon tissue analysis.

3.6 CLEAN-UP PROCEDURE

Following initial sample extraction a clean-up procedure, based on the methods of Giger & Schaffner (1978), was performed on most samples. This was primarily to remove phthalate esters which were present in sufficiently high concentrations, particularly in

the water samples, to prevent the accurate quantification of the aliphatic and aromatic compounds of interest. Sediment and biotic samples from the reference sites could be satisfactorily analyzed with or without clean-up.

The clean-up procedure involved dissolving the dried dichloromethane extracts from the water and sediment samples in a 100 μl dichloromethane/0.5 ml hexane mixture. This concentrated mixture was applied to 15 cm^3 of activated silica gel (grade 40, 35-70 mesh, 0.25-0.50 mm particle size Kiesel Gel) contained in a chromatography column (20 cm x 1 cm i.d.) fitted with a sintered glass disc. The silica gel was saturated with redistilled AnalaR hexane and a constant head of hexane was maintained over the silica gel prior to sample addition. After the addition of the dissolved extract, hexane (100 ml) was added as four 25ml aliquots and passed through the column at a rate of 2-4 ml min^{-1}. More than 80% of the low molecular weight (MW) alkanes (C_{10}-C_{20}) were eluted in the first 25 ml of hexane as well as the lower MW aromatics. In the next fraction (26-50 ml), the remainder of the low MW alkanes, the bulk of the longer chained alkanes (C_{21}-C_{32}) as well as the intermediate PAHs were found. Further hexane additions (51-75 and 76-100 ml) yielded the higher MW PAHs and the remainder of the longer chained alkanes (Table 3.4).

TABLE 3.4

ALKANE AND PAH RECOVERY FROM CLEAN-UP PROCEDURE.

Fraction (ml)	*Hydrocarbon group % recovered*			
	Alkanes (C_{10}-C_{20})	*Alkanes (C_{21}-C_{32})*	*PAH (2/3 rings)*	*PAH (4/5 rings)*
0-25	85	22	56	18
26-50	7	55	23	24
51-75	3	15	10	38
76-100	3	8	9	15

In column elutions of extracts from spiked sediment further additions of hexane did not elute significant amounts of aliphatic or aromatic compounds. A final addition of dichloromethane, however, eluted 5 - 10% of the total amounts of dibenzo(a,h)anthracene, indeno(1,2,3-cd)pyrene and benzo(g,h,i)perylene applied to the

column. However, in contaminated samples the dichloromethane rinse resulted in the elution of phthalate esters such that the presence of the above compounds would be masked. In environmental samples the final dichloromethane rinse was therefore not used and as a result a small unavoidable loss of the high MW PAHs may have occurred at the clean-up stage.

3.7 EXTRACTION EFFICIENCY

The efficiencies of the extraction procedures were tested by using blank water and sediment samples which had been spiked with known concentrations of hydrocarbons.

3.7.1 Aqueous Samples

a) *n*-alkanes

Spiked water samples at a concentration of 20 $\mu g\ l^{-1}$ for each component in the C_{10} - C_{32} *n* -alkane range were prepared in distilled water by the addition of the appropriate *n* -alkane mixture (C_{10}-C_{32}) in methanol (2 ml). These water samples (100 ml) were extracted twice with dichloromethane (50 ml) as described in Section 3.2.1.1. The extraction efficiencies over the full alkane range were found to be good (mean extraction efficiency 84.8%; standard deviation, 19.3%). The efficiencies for the range C_{15} - C_{32} were consistently high (93.7% $\pm$ 7.3%) as shown in Table 3.5. However,

TABLE 3.5

ALKANE EXTRACTION EFFICIENCIES FROM SPIKED WATER AND SEDIMENT SAMPLES

Alkane	*Extraction Efficiency %*		*Alkane*	*Extraction Efficiency %*		*Alkane*	*Extraction Efficiency %*	
	Water	*Sediment*		*Water*	*Sediment*		*Water*	*Sediment*
C_{10}	39	64	C_{18}	102	86	C_{26}	98	85
C_{11}	42	68	C_{19}	97	83	C_{27}	96	88
C_{12}	55	68	C_{20}	101	86	C_{28}	106	81
C_{13}	62	71	C_{21}	93	88	C_{29}	90	80
C_{14}	65	78	C_{22}	89	85	C_{30}	87	83
C_{15}	78	75	C_{23}	103	91	C_{31}	93	78
C_{16}	92	83	C_{24}	94	90	C_{32}	88	81
C_{17}	95	82	C_{25}	91	83			

extraction efficiencies for the alkanes C_{10} - C_{14} were considerably lower (52.6% ± 11.7%).The losses during the extraction procedure, particularly for the lower MW alkanes, were almost certainly due to evaporation of the more volatile hydrocarbons. The possible loss of alkanes below C_{15} has been noted by Ajayi & Poxton (1987) who used a mixture of pentane and dichloromethanol for extraction followed by vacuum evaporation at temperatures not exceeding 40°C. A number of tests were therefore undertaken in this study to determine at which stage the evaporative losses might be occurring. These tests included modifying the previously described extraction procedure (Section 3.4.1.1) according to the following experimental conditions:

- increasing the length of the condensing column fitted to the round-bottomed flask during ultrasonic extraction.

- maintaining a temperature of below 40°C in the water bath during ultrasonic extraction.

- ensuring the temperature of the water bath did not exceed 40°C during rotary evaporation.

- employing a less severe vacuum during the evaporation stage.

Evaporative losses of hydrocarbons were also possible during the final sample concentration. It was found that the application of a stream of nitrogen over the mouth of the small sample tube, to accelerate solvent evaporation, resulted in greater losses of the C_{10}-C_{14} alkanes in comparison to those samples which were either allowed to stand in quiescent air at room temperature or placed in the gentle air flow produced within a fume cupboard. It was also considered that the observed alkane losses may have been the result of the deposition during solvent evaporation of the alkanes on the upper surface of the inside of the sample tube during the final concentration procedure. In order to eliminate this possible cause, 0.5 ml aliquots of dichloromethane were first used to wash the entire inner glass surface of the sample tube. Following the evaporation of this solvent rinse, the final 100 μl of dichloromethane was added, again ensuring that any deposits on the glass surface were dissolved.

The n-alkane analysis of spiked mixtures processed under combinations of these different extraction conditions showed that the temperature during rotary evaporation was the most important factor responsible for losses by evaporation. The critical temperature appeared to be 40°C above which over 90% of the C_{10}-C_{14} alkanes were lost. It was not possible however, to determine the exact cause of the n-alkane losses which still occurred under optimal extraction conditions and consequently the values recorded from the extracted samples have been adjusted to take into account the lower extraction efficiencies for the range C_{10} - C_{14}.

b) PAHs

Mixtures of PAH in water ranging from 10-100 μg l^{-1} for individual compounds were also prepared from a Supelco Ltd. PAH standard mixture (no. 610-M) in a methanol: methylene chloride (50:50) mixture or from solid individual PAHs dissolved in dichloromethane. The PAH concentrations in water given below represent 1000 fold dilutions of the standard 610-M mixture. In both cases, as for the alkanes the mixtures were introduced with methanol (2 ml) to improve miscibility with water. The extraction efficiencies were found to be consistently over 80% except for naphthalene (b.p 70°C) for which some evaporative losses are likely to have occured (Table 3.6). However, this most volatile PAH still achieved an overall extraction efficiency of 74%.

3.7.2 Sediment samples

The extraction efficiency from sediment was determined by spiking a sediment sample collected from Site 7 below the oil boom on the lower Silk Stream which had previously been extracted twice with dichloromethane to a condition in which the presence of hydrocarbons was not detectable. Sediments collected from the reference Site 1 were also spiked with PAHs. The PAH and alkane spiking levels are given in Tables 3.5 and 3.6. These spiking concentrations represent levels which were within the range of concentrations determined in environmental samples collected from the catchment (see Sections 3.1 and 3.2). The hydrocarbons were introduced into the sediment using Hamilton microsyringe injections of dichloromethane-based PAH and alkane solutions. The solution was distributed as evenly as possible over the sediment

surface and the sediment was thoroughly mixed. This method would however be unlikely to result in an absolutely homogenous distribution of the introduced hydrocarbons and may possibly lead to elevated overall extraction efficiencies as a result of the formation of concentrated pockets of hydrocarbons which are more easily extracted. The spiked sediment (1-5 g) was then extracted by ultrasonication with dichloromethane as described in Section 3.4.1.2. The extraction efficiencies are satisfactory for the entire PAH range although generally slightly lower than those obtained from the water samples. The majority of the alkanes were also efficiently extracted from sediment although as in the case of water samples this was decreased for the lower molecular weight range C_{10}-C_{15} (60-75%).

TABLE 3.6

PAH EXTRACTION EFFICIENCIES FROM SPIKED WATER AND SEDIMENT SAMPLES

Compound	*Code*	*Spiking Concentration*		*Extraction Efficiency %*	
		Water ($\mu g\ l^{-1}$)	*Sediment ($\mu g\ g^{-1}$)*	*Water*	*Sediment*
Naphthalene	N	100	100	74	80
Fluorene	F	20	20	85	83
Phenanthrene	P	10	10	92	80
Fluoranthene	Fa	20	20	106	94
Pyrene	Py	10	10	96	85
Benzo(a)anthracene	BA	10	10	87	77
Chrysene	C	10	10	87	77
Benzo(b)fluoranthene	B(b)Fa	20	20	84	85
Benzo(k)fluoranthene	B(k)Fa	10	10	84	85
Benzo(a)pyrene	BP	10	10	88	82
Indeno(1,2,3-cd)pyrene	IPy	10	10	102	87
Dibenzo(a,h)anthracene	DBA	20	20	102	87
Benzo(g,h,i)perylene	BPe	20	20	93	80

3.7.3 Standard Reference Sediment

A marine sediment reference material for PAHs was obtained from the National Research Council of Canada (sample code HS-3). The sample was extracted, cleaned

up and analyzed as described in Sections 3.4.1.2, 3.6 and 3.8. The determined concentrations for 10 PAHs are given in Table 3.7 and are compared with those authenticated through the Canadian Marine Analytical Chemistry Standards Program. Table 3.7 shows the determined values in $\mu g\ g^{-1}$ against means of previously determined values.

TABLE 3.7

COMPARISON OF PAH CONCENTRATIONS IN STANDARD SEDIMENT REFERENCE MATERIAL

Compound	*Concentration ($\mu g\ g^{-1}$)*		*% Recovery*
	This work	*Authenticated (± 90% C.I.)*	
Naphthalene	6.2	9.0 ± 0.7	68.9
Fluorene	10.1	13.6 ± 3.1	74.3
Phenanthrene	69.7	85 ± 20	82.0
Fluoranthene	54.0	60 ± 9	90.0
Pyrene	49.5	39 ± 9	109.9
Benzo(a)anthracene	11.0	14.6 ± 2.0	75.3
Chrysene	12.8	14.1 ± 2.0	85.1
Benzo(a)pyrene	8.0	7.4 ± 3.6	108.1
Benzo(b+k)fluoranthene	14.4	10.5 ± 3.2	137.3
Dibenzo(a,h)anthracene	1.8	1.3 ± 0.5	138.4

With the exception of the fluorene and benzo(b+k)fluoranthene determinations, all PAH values were within the 90% confidence intervals around the means given for the 22-40 determinations previously made. Benzo(b+k)fluoranthene and dibenzo(a,h)anthracene values appear relatively elevated but, given the large confidence limits quoted, remain acceptable. The recovered values obtained for the reference material are in broad agreement with those obtained from the spiked sediment experiments and indicate good overall extraction efficiencies for PAHs.

3.7.4 Biotic Samples

To further assess the nature and extent of aliphatic hydrocarbon contamination at the sampling sites and to investigate methods of hydrocarbon extraction from biota, samples of the stickleback, *Gasterosteus aculeatus* were collected from Sites 3 (Dean's brook) and 5 (Silk Stream, above the oil boom). The greater mass of these organisms enabled different tests of the extraction techniques to be performed in comparison to the macroinvertebrates which were used as the caged test organisms. Three extraction methods were tested:

Method 1: Fish were dried at 40°C for 48hrs, ground and extracted ultrasonically for three hours using dichloromethane as solvent as described for sediment samples (Section 3.4.1.2).

Method 2: A modified version of the method of saponification described by Law *et al.* (1988) was tested. Potassium hydroxide (0.5 g) and methanol (15 ml) were added to approximately 3 g of ground dried fish placed in a round bottomed flask. The mixture was then placed in an ultrasonic bath for 2 hrs. Hexane (50 ml) was added and the flask placed in the ultrasonic bath for a further 2 hours. The mixture was then filtered and the residue rinsed with hexane (30 ml). The hexane layer was isolated from the methanol layer using a separating funnel and evaporated to dryness.

Method 3: This was identical to method 2 except that dichloromethane was used for the extraction in place of hexane.

Method 2 resulted in a mean extraction efficiency of only 32% of n-alkanes compared with Method 1, the direct extraction by dichloromethane (Table 3.8). Addition of dichloromethane to the saponified material (Method 3) yielded approximately 57% of the n-alkanes extracted by direct ultrasonication. The direct ultrasonic extraction technique with dichloromethane as employed for sediment extraction (Method 1) was therefore considered the most satisfactory for dried biological samples.

TABLE 3.8

EXTRACTION EFFICIENCIES (PERCENTAGE RECOVERY) OF ALKANES BY THREE EXTRACTION PROCEDURES USED ON BIOLOGICAL MATERIAL

	Method				*Method*				*Method*		
alkane	*1*	*2*	*3*	*alkane*	*1*	*2*	*3*	*alkane*	*1*	*2*	*3*
C_{10}	40	ND	ND	C_{17}	89	31	69	C_{24}	80	27	41
C_{11}	36	ND	21	C_{18}	78	26	99	C_{25}	85	28	42
C_{12}	45	ND	30	C_{19}	95	30	74	C_{26}	92	34	74
C_{13}	67	ND	30	C_{20}	83	20	54	C_{27}	82	27	40
C_{14}	61	20	27	C_{21}	108	35	58	C_{28}	78	23	34
C_{15}	74	26	39	C_{22}	82	26	43	C_{29}	66	18	30
C_{16}	84	29	40	C_{23}	90	32	46	C_{30}	94	ND	140

3.8 GC/MS OPERATING CONDITIONS

Following sample extraction and clean-up, the dry extract was dissolved by dichloromethane (20 - 200 μl) and analyzed immediately. The extracted samples were analyzed using a Hewlett Packard model 5995B Gas Chromatograph/Mass Spectrometer (GC/MS) fitted with a fused silica capillary column (25 m, crosslinked methyl - silicone). Initially, samples were screened with the instrument in peakfinder mode to identify compounds present. Select Ion Monitoring (SIM) enabled subsequent quantification of the aliphatic and aromatic compounds of interest.

Samples were introduced in the splitless injection mode, using 1 μl Hamilton microsyringe injections. Rapid volatilization of the sample was achieved at the injection port set at 350°C. The helium carrier gas was set at a flow rate of 1 ml $min.^{-1}$ and the column pressure was 20 psi. A solvent elute time of 2.8 minutes was used to avoid dichloromethane peaks. The initial column temperature was 30°C which was held for 30 seconds with a further 30 seconds purge followed by a fast ramp to 120°C. This temperature was held for 1 minute before a 10°C $min.^{-1}$ increase to 300°C which was maintained for up to 20 minutes. Under these conditions all of the compounds were eluted within 35 mins. The electron multiplier was set at 1600 volts and a 0.2 amu window size around the selected peaks was used. A temperature of 305°C was used for

the transfer line to reduce possible compound condensation during transfer to the MS chamber and the ion source and analyzer were maintained at 150 and 180°C respectively.

In the SIM mode, variable values were used for the summed and single ion scale and normally 12-20 ions were selected for SIM monitoring. The dwell times were set according to relative abundances of ions e.g. alkanes (57.1) were set at 50 milliseconds and benzo(a)pyrene to 250 milliseconds. Increasing the dwell time increases the signal to noise ratio and improves the ion statistics but integration accuracy should be preserved by keeping the dwell time reasonably low to increase the number of samples (optimally 20) per GC peak. SIM fat peaks were also used in order to improve sensitivity and produce a more rounded peak apex which facilitates finding the peak maximum. All ion option was used as the trigger for peak integration. A maximum smoothing factor of 1.00 was employed in order to improve chromatogram appearance and slope sensitivity detection. The integration sensitivity was set at a value of 0.05 meaning that peak integration began and ended when the rate of change of the signal was 5% or more for 4 consecutive data points.

Optical tuning of the instrument was carried out daily at mass 502. Examples of alkane (Scan) and PAH (SIM reconstruction)standard traces are given in Figs. 3.6 and 3.7.

Environmental samples were compared with alkane and PAH standards obtained from Phase Separation Ltd. and Supelco Ltd. The standards were composed of individual compounds and mixtures at concentrations ranging from 1 to 200 mg l^{-1}. For the alkanes standard concentrations were prepared in dichloromethane as C_8-C_{19} and C_{20}-C_{40} mixtures. Concentrations of pristane and phytane were determined by reference to the peak areas of the *n* - alkanes C_{17} and C_{18} respectively in the standard mixture. The PAH standards were introduced as one mixture ranging from a neat to a 1:100 dilution in dichloromethane of the Supelco 610-M mixture. Concentrations of the methyl derivatives of PAH compounds were determined by reference to the peak areas of the respective standard parent compound.

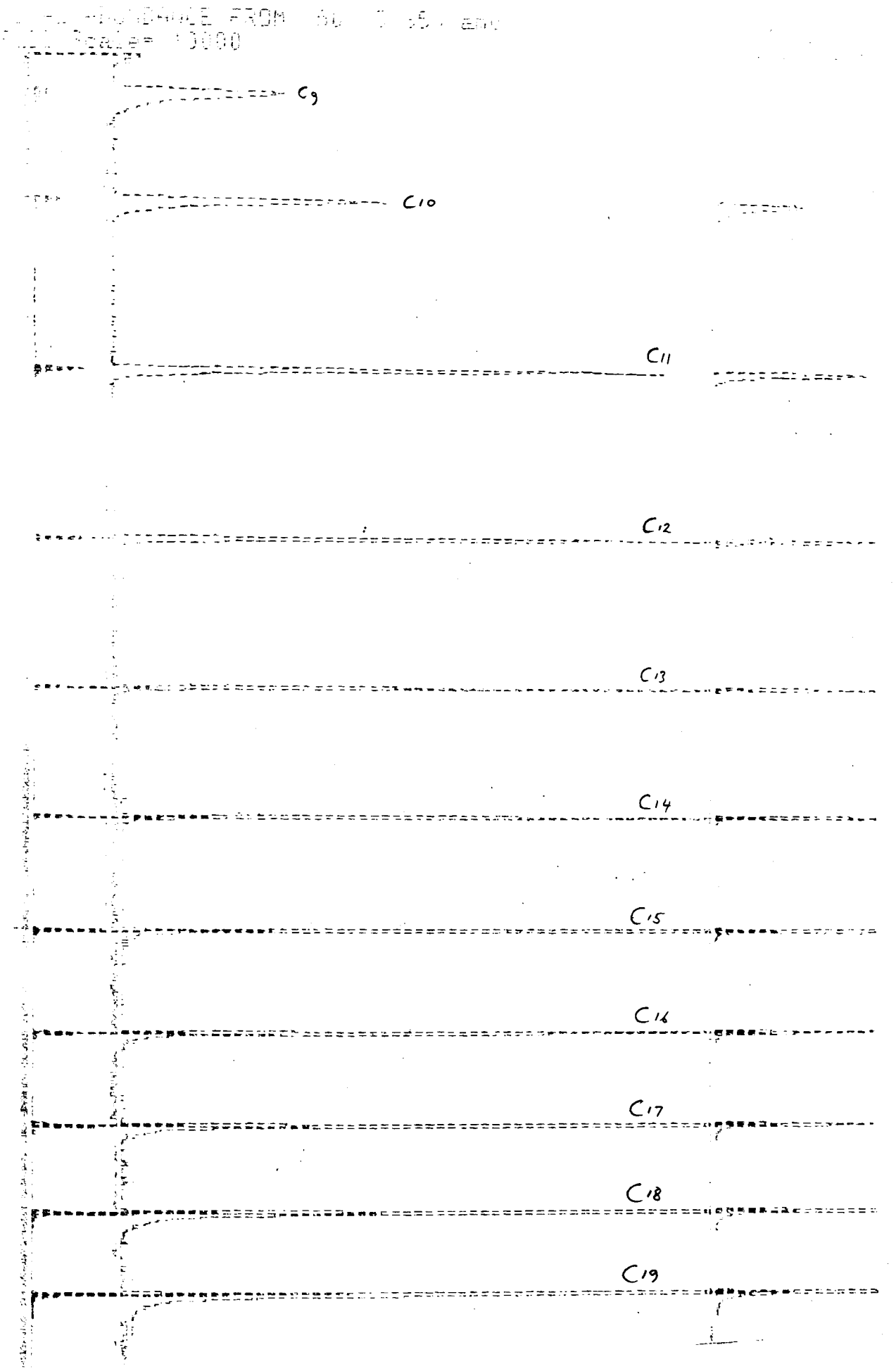


Fig. 3.6. GC/MS trace of alkanes C_9-C_{19}

ION MASSES	202.10	216.10	226.20	242.20	252.20
DWELL TIMES (msec)	50.00	50.00	50.00	50.00	50.00
MAXIMUM ABUNDANCES	159.05	2.79	27.39	3.12	22.55

ION MASSES	276.20	278.20	166.30
DWELL TIMES (msec)	50.00	50.00	50.00
MAXIMUM ABUNDANCES	1.40	1.47	44.79

FILE SIM 2 TOTAL RUN TIME =33.49 SAMPLE AMOUNT = 1.00

ION	165.10	178.10	202.10	228.30	252.20
TRACE	1	2	3	4	5
FULL SCALE	50	91	159	27	23

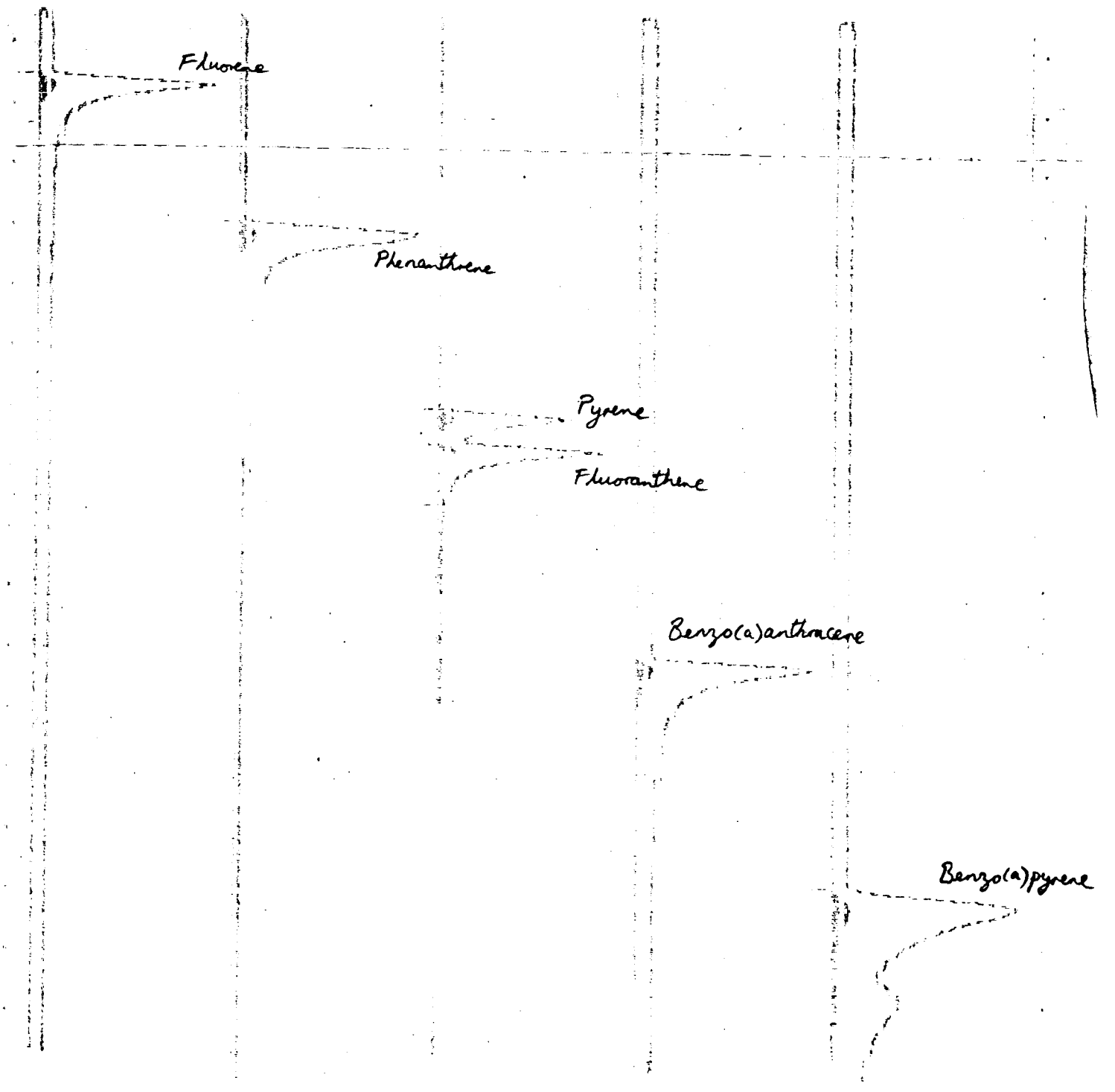


Fig. 3.7 GC/MS trace of PAHs fluorene, phenanthrene, pyrene, fluoranthene, benzo(a)anthracene and benzo(a)pyrene.

3.9 SUMMARY

The sampling sites were selected in order to examine potential changes in water, sediment and biological quality that occurred within the Silk Stream catchment. Photographs and site descriptions are provided in this section. A particular emphasis was placed upon the lowest reaches of the Silk Stream since it was here that the possible effects of oil pollution were perceived to be greatest and as such required detailed surveys including surrounding land use, vegetation and pipe discharges.

A review of the methods used for general field physico-chemical measurements is provided including a description of the in-stream apparatus. The preparation and analysis of environmental samples, including water, sediment and biota for hydrocarbon analysis is covered in greater depth. The clean-up procedure was assessed and a particular emphasis was placed on the important aspect of the efficiency of the complete preparation and extraction processes. These were satisfactory for the majority of the measured hydrocarbons for the three sample types and the results were found to be consistent with measurements on standard reference material. For the low molecular weight alkanes, unsatisfactory extraction efficiencies were reported and evaporative losses were considered the main cause for the reduced concentrations.

A summary of the GC/MS operating conditions with examples of output from the instrument are provided.

Chapter 4

SPATIAL AND TEMPORAL VARIATIONS IN SEDIMENT AND WATER QUALITY IN THE SILK STREAM CATCHMENT

4.1 INTRODUCTION

In order to provide the relevant background information to the data presented in Chapters 5 and 6 on in-stream and laboratory responses of invertebrates to hydrocarbon pollution, this chapter discusses the results of investigations of the sediment and water chemistry and associated ecology at the sampling locations described in Chapter 3.

4.2 PHYSICO-CHEMICAL CHARACTERISTICS

The physico-chemical measurements taken from the control and sampling locations are presented and discussed in Sections 4.2.1 to 4.2.3 The determinations for suspended solids, dissolved oxygen and sediment oxygen demand were carried out as described in Section 3.2. Many of the determined variables display changes which can be attributed to increasing urbanization at the downstream sampling locations with the poorest water quality indicators, without exception, being recorded at the most downstream sites (Sites 6 and 7). In contrast, all measured parameters show substantial improvements at the receiving basin (Site 9).

4.2.1 Dissolved oxygen

The spatial and temporal variations observed in percentage dissolved oxygen saturations at the Silk Stream sampling sites during April to October 1989 are illustrated in Fig. 4.1. For each sampling site, surface and basal DO readings were recorded. The basal readings have been taken, where applicable, close to the bed location of the caged macroinvertebrates, which were at depths of between 70 and 100 cm below the surface. With the exception of Site 3, surface DO was significantly greater than the basal value ($p < 0.05$, paired t-test) which is believed to be a consequence of both the existence

of a substantial sediment oxygen demand and a poorly mixed water column. Using a two-way analysis of variance test, significant differences ($p < 0.05$) were found between all sites, with the exception of Sites 6 and 7 where the difference was marginal at the 5% level. At Site 9 the DO levels are typical of those of the euphotic zone associated with a lentic environment. The oxygen saturation at this site was consistently the highest of all the sites visited and can be partly attributed to photosynthetic oxygen production by resident *Cladophora* since the recorded values appeared to be closely related to the abundance of this alga. In particular, the supersaturated values recorded during the summer sampling times must be attributed to algal production. Some of the saturation is also undoubtedly due to the good surface aeration generated by wind and wave action at this open site.

At Sites 6 and 7, oxygen saturation rarely exceeded 30/40% and during the mid-summer sampling period, there appeared to be a pronounced sag in oxygen saturation with levels of 3% or less recorded at Site 7. Such low levels must be important contributory factors to the limited macroinvertebrate diversities recorded at these sites. The lower DO levels recorded here, compared to Site 9 can be attributed to the relatively poor aeration by mixing in this slow flowing section of the river. The scarcity or absence of macrophytes and algae at these sites also results in an overall decrease in the potential for photosynthetic oxygen production. Thus, increasing temperatures and light intensity do not result in any increased rate of oxygen input, but conversely, result in increased organic sediment decomposition rates which superimpose an increased oxygen burden on the water column.

At the upstream site (Site 3) the dissolved oxygen saturation level remained fairly constant at around 60% throughout the six month sampling period. At this sampling location, the absence of macrophytes, the low sediment organic content and the relatively high flow rate results in low autochthonous production and demand of oxygen, thus limiting possible temperature induced variance in dissolved oxygen saturation. Turbulence at an adjacent weir, approximately 30 m upstream of the sampling site is also responsible for maintaining the consistent DO levels recorded.

4.2.2 Sediment oxygen demand (SOD)

The sediment oxygen demand rate has been defined as the rate that oxygen is removed from the water column in receiving waters as a result of the decomposition of settled organic matter in the bottom sediments (Hatcher, 1987). Table 4.1 shows the SOD rates determined for the various test sites described in Section 3.1.

TABLE 4.1
SEDIMENT OXYGEN DEMAND VALUES AT THE SILK STREAM, WELSH HARP AND BACKGROUND SAMPLING LOCATIONS

Site name	*Site code*	*Mean SOD ± S.D. g m^2 d^{-1} (Number of samples)*
Dew Pond (Trent Park)	1	1.65 ± 0.21 (4)
Silk Stream (Stoneyfield Park)	3	0.56 ± 0.06 (3)
Silk Stream (Rushgrove Park)	4	0.76 ± 0.10 (4)
Silk Stream (Above boom, relocated)	5	1.10 - (1)
Silk Stream (Above boom, original)	6	2.57 ± 0.42 (7)
Silk Stream (below boom)	7	2.37 ± 0.40 (7)
Welsh Harp (North)	8	2.44 ± 0.23 (6)
Welsh Harp (Cool Oak Lane)	9	0.91 ± 0.19 (5)

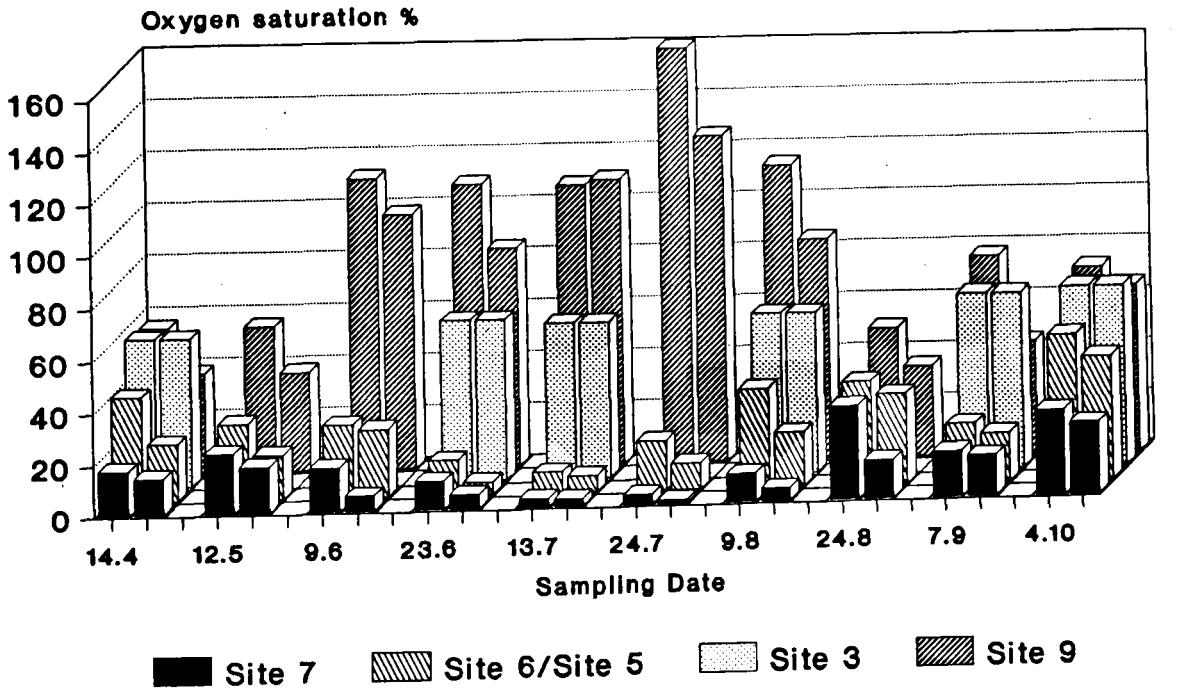


Fig. 4.1 Variations in surface (left) and basal (right) DO levels at the Silk Stream and Welsh Harp sampling sites.

The SOD data show the mean analytical values for sediment samples collected between February and August 1989. The values lie within the range of 0.15 - 2.75 g m^2 d^{-1} which has been quoted by Ellis (1989) as being typical of undisturbed urban benthal sediments. The values obtained did not show any significant variation with sampling time, and are consistent with the trends noted in the dissolved oxygen survey (Section 4.2.1). The mean SOD rates are significantly higher at the downstream sites (Sites 6, 7 and 8) compared to the upstream sites, and the SOD rate at the relocated above-boom site (Site 5) is less than half that of the original site (Site 6). This further illustrates the localized nature of the deterioration that is typical of the Silk Stream sediment and water quality and which appears to be related to specific outfall discharges (see Section 3.2.2)

At Sites 6 and 7 which are adjacent to the oil boom, the high measured SOD rates could account for a permanent deficit in in-stream dissolved oxygen of up to 2.5 mg l^{-1} and therefore represent an important contributing factor towards the permanently low oxygen saturations that have been recorded at these sites. The SOD value obtained for the reference control site in Trent Park (Site 1) is also relatively high and would indicate a high sediment organic content at this site. This site however, differs from the test sites in that there is no flow within the control pond compared to the intermittent high flows which occur in the generally depositing substrate of the lower Silk Stream and the northern Welsh Harp. At Site 9 the continuous outlet flow and wind and wave action contribute to reduce both organic substrate content and consequent SOD rate. The sedimenting capacity of the northern Welsh Harp is also important in reducing the amount of settling organic sediments at Site 9.

4.2.3 Suspended solids

The mean and standard deviation values for suspended solid concentrations are listed in Table 4.2. Proportionally, downstream changes in suspended solids are much more pronounced in samples taken during storm events such as on 24/10/90. These results illustrate the increases in resuspended material which occur during high flow at the predominantly slow flowing Sites 6 and 7. The much greater proportion of fine sediments at these downstream sites (Section 3.1.2) is clearly an extremely important

factor in the spatial variations observed. A large fall in mean suspended solid levels also occurs in the receiving basin compared with the lower Silk Stream levels as a result of particulate settling at the basin inlet. The suspended solid loadings during baseflow conditions (30-40 mg l^{-1}) are typical of values obtained from urban streams (Hamilton *et al.*, 1984). High suspended solid loadings have important ecological consequences as smothering of filter feeders such as *Gammarus pulex* may occur. In Chapter 5 the importance of the settling of suspended particles within the test cages will be discussed.

TABLE 4.2
SUSPENDED SOLID LEVELS IN THE SILK STREAM CATCHMENT

Site	*Suspended solids (mg l^{-1})*
1	18.8 ± 6.9
3	29.0 ± 20.1
4	32.5 ± 15.3
5	45.1 ± 24.7
6	88.6 ± 60.2
7	77.4 ± 49.2
8	50.2 ± 28.5
9	15 ± 4.5

4.3. BIOLOGICAL WATER QUALITY

4.3.1 Macroinvertebrate diversity and hydrobiological indices

Standard "kick samples" (see Section 3.3) were collected at monthly intervals during April 1989 to October 1990. The surveys were carried out to assess the overall water quality in the Silk Stream catchment as a complement to the chemical analyses also undertaken. Information on macroinvertebrate distribution was also used to assess the suitability of organisms for use in *in-situ* experiments. The species present in samples collected at each site are shown in Table 4.3. At Site 3, 14 species were recorded in total, dominated largely by Annelids (5 species) and Molluscs (6 species). Although eight and seven species were recorded at Sites 4 and 5 respectively, the communities were dominated by *Glossiphonia complanata, Erpobdella octoculata, Lymnaea peregra*

and *Asellus aquaticus*. With the exception of *Lymnaea peregra* the most notable change between the headwater site (Site 3) and Sites 4 and 5 was the exclusion of molluscs. At Site 6 only *Tubifex* sp. and *Erpobdella octoculata* have been recorded. Five species have been recorded at Site 7 below the oil boom, including *Asellus aquaticus*, which represent a partial recovery at this site. Species richness was greatest at Site 9 where a substantial recovery occurs.

The British Monitoring Working Party score (BMWP, 1979) and Average Score Per Taxon (ASPT) have been calculated from standard macroinvertebrate collections undertaken at each sampling site. The changes in water quality within the Silk Stream catchment as reflected by the biotic scores are illustrated in Figs. 4.2 and 4.3. The highest scores were consistently obtained at Site 9. However, it is not appropriate to compare the ASPT and BMWP between this relatively lentic site and the other lotic sites because of the widely differing habitats and macroinvertebrate populations which would be found under unpolluted conditions. It can be stated, however, that the higher macroinvertebrate species richness and the greater proportion of relatively pollution sensitive species found at Site 9 (as indicated by the enhanced BMWP score and ASPT, respectively) support the data for water and sediment quality and highlights the improvement in water quality within the filtered Welsh Harp outlet waters compared to the influent flows of the lower Silk Stream. The highest ASPT/BMWP scores on the Silk Stream were obtained at Site 3, where there appeared to be little temporal variation in macroinvertebrate population, except that the high scoring *Gammarus pulex* was absent on one of the summer sampling occasions. This may have been caused by the high water temperatures encountered during the height of the 1989 drought.

The mean BMWP score and ASPT presented in Figs. 4.2 and 4.3 further illustrate the downstream decline in water quality with lowest scores at Sites 6 and 7 in the Silk Stream as well as the substantial recovery which occurs in the receiving basin (Site 9).

In terms of community diversity, using a scheme introduced by Bascombe *et al.* (1988) which follows recommendations proposed by Thames Water relating water quality class as described by the NWC classification with the recorded BMWP scores, the Silk

TABLE 4.3
INVERTEBRATE SPECIES FOUND AT THE SILK STREAM AND WELSH HARP SAMPLING SITES

Phylum	*Species*	*Site* 3	4	5	6	7	9
ANNELIDA	*Nais* sp.	*	*	*			
	Tubifex sp.	*	*	*	*	*	*
	Erpobdella octoculata	*	*	*	*	*	*
	Erpobdella testacea	*					
	Glossiphonia complanata	*	*	*			*
PLATYHELMINTHES	*Polycelis nigra*						*
MOLLUSCA	*Vivaparus* sp.	*					
	Potamopyrgus jenkinsii	*					*
	Lymnaea stagnalis	*					*
	Lymnaea peregra	*	*	*		*	*
	Planorbarius vorneus	*					*
	Planorbis carinatus						*
	Acroloxus lacustris	*					*
	Sphaerium sp.						*
CRUSTACEA	*Gammarus pulex*	*	*				*
	Asellus aquaticus	*	*	*		*	*
	Daphnia sp.						*
	Cyclops sp.						*
UNIRAMIA	*Corixa* sp.						*
	Dytiscus sp.						*
	Maliplus sp.						*
	Chironomous thummi	*	*	*		*	
Species richness		**14**	**8**	**7**	**2**	**5**	**19**

Stream locations would be classed as follows. Site 3 would fall across the Class II/III boundary, Sites 4 and 5 would be a Class III. Site 7 represents an area of poor river quality being on the boundary of Class III/IV or within the Class IV boundary while Site 6 would be a Class IV river. This further emphasises the grossly polluted nature of the lowest reaches of the Silk Stream. The changes in species richness which exist between the relatively adjacent Sites 5 and Sites 6 and 7 can be attributed to a number of factors. The seasonal presence of the alga *Cladophora* sp., notably absent at Sites 6 and 7 offers increased colonization opportunities for invertebrates such as *L. peregra*

and *A. aquaticus*. At Site 5 there is also a significant difference in sediment composition compared with the downstream sites. Here, consolidated clays and gravel, resulting from increased stream flow, form the bed sediment. In the case of *L. peregra*, the presence of filamentous algae and coarser sediment and stones provide sites for algal films to develop which provide an important food source for grazer feeders, as well as increasing shelter and providing suitable egg laying sites.

In contrast, the slower flowing depositing environments of Sites 6 and 7 have led to the accumulation of fine organic silts which are known to impose severe stress particularly on organisms respiration. A number of workers (e.g. Chutter, 1969; Nuttall & Bielby, 1973; Luedtke *et al.*, 1976) have associated heavy silting with decreases in diversity or productivity. Clearly the heavy silting of the lower Silk Stream would exert severe stress on many macroinvertebrate groups irrespective of any differences or decreases in water quality.

The sludges at Sites 6 and 7 with their associated bacterial communities also exhibited high SODs and reduced water column DO values below the normal tolerances of most macroinvertebrates leading to organism mortality or migration. It must be stressed, however that there does not exist a specific value for DO below which a given organism will die and even among so-called intolerant groups, great variations in DO requirements exist especially with respect to current velocity (Olson & Rueger, 1968). Organisms adapted to high flow rates e.g. *Rhithrogena* sp. have elevated lethal DO levels at low velocities in comparison to organisms morphologically adapted to increase water circulation when flow is low. Such organisms have lower and more constant lethal DO levels during reduced flow rates. Each organism found in the lower Silk Stream has some adaptation to tolerate low DO. *A. aquaticus* has a relatively low oxygen requirement in comparison with *G. pulex* and is also capable of increasing water circulation across its gills; *L. peregra* is a pulmonate mollusc and is therefore capable of utilizing atmospheric oxygen. The three remaining dominant species found in the lower Silk Stream are *G. companata, E. octoculata* and *Tubifex* sp. The leech species are highly tolerant of low DO and high organic loadings. *Tubifex* sp. are capable of rapid repopulation of deoxygenated areas and are capable of concentrating DO in haemoglobin.

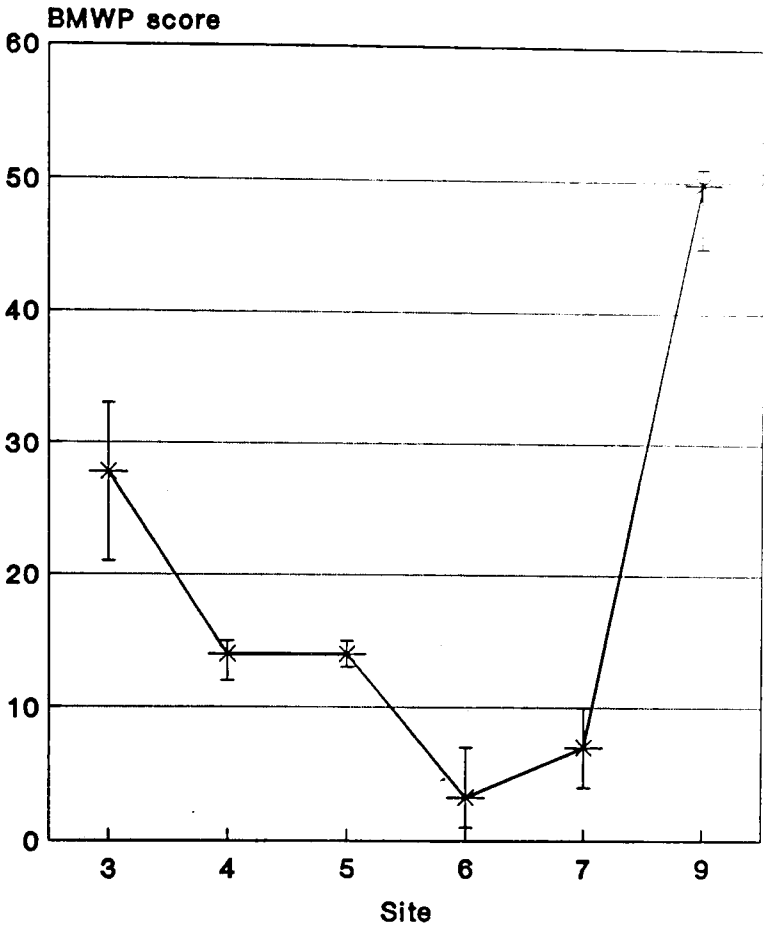


Fig. 4.2 Mean and range of BMWP scores for the Silk Stream and Welsh Harp sampling sites.

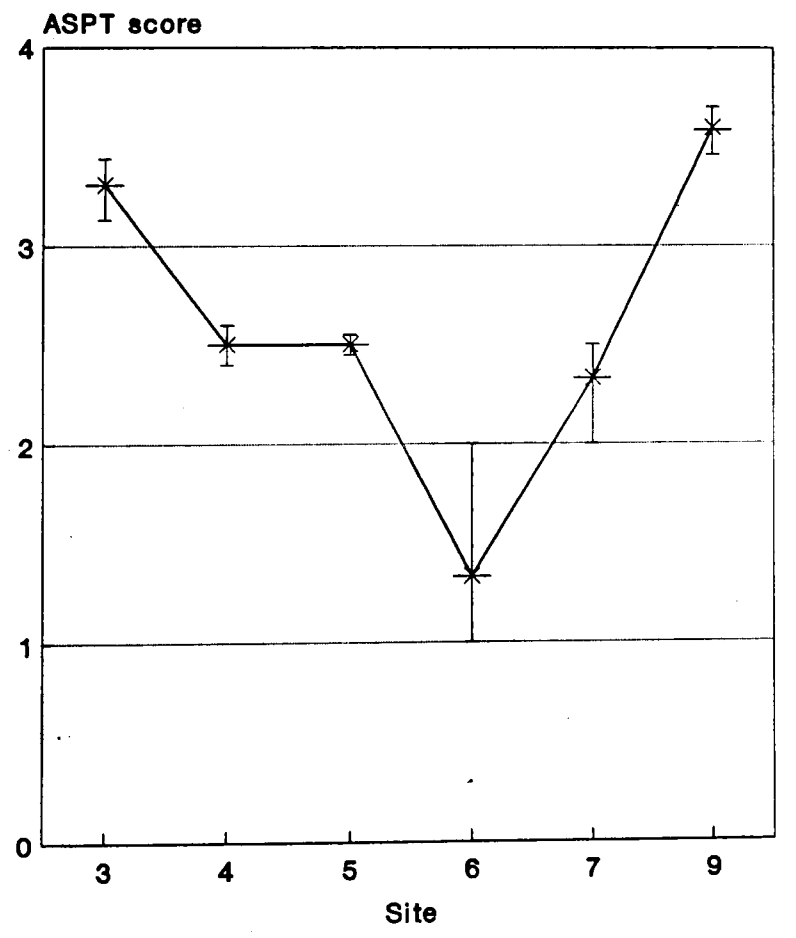


Fig.4.3 Mean and range of the average score per taxon (ASPT) for the Silk Stream and the Welsh Harp sampling locations.

4.4. HYDROCARBON CONCENTRATIONS AND DISTRIBUTION IN WATER AND SEDIMENT SAMPLES

A survey of hydrocarbons in sediments and water in the Silk Stream catchment was undertaken. The data obtained were to be used to assess the extent of hydrocarbon contamination and to consider changes in contamination with increasing urbanization. Thus, spatial variations between the control, headwater, Silk Stream and receiving basin sites will be discussed. A number of fingerprinting techniques have also been applied to the collected data in order to assess the nature of the hydrocarbon pollution, and probable sources.

4.4.1 Total alkane levels

The mean values obtained from 15 determinations (Table 4.4) are at least an order of magnitude higher than those reported by Wakeham & Farrington (1980) and by Teal & Farrington (1977). This may, in part, be due to the Silk Stream catchment being dominated by urban runoff to a greater extent than other reported sites (Gavens *et al.*, 1981, 1982). Wakeham (1976a) however, reported relatively high (1600 $\mu g\ g^{-1}$) concentrations for the total aliphatic fraction which was dominated by a large UCM. The UCM concentration was not determined in the currently reported analyses but would undoubtedly contribute significantly to the total aliphatic fraction. The presence of an UCM which is composed of a large number of compounds with similar, overlapping GC retention times is believed to be a reliable indicator of petrogenic pollution (Chapter 2, Section 2.6.). Hydrocarbon mixtures of recent biological origin are composed of relatively few compounds and consequently tend not to have overlapping GC peaks.

4.4.2 Individual alkane concentrations and profiles

The levels of n-alkanes found in water and sediment samples from the reference site in Trent Park Dew Pond (Site 1) and Sites 3, 4, 5, 6, 7, 8, and 9 within the Silk Stream catchment are shown in Figs.4.4 and 4.5. The site locations have been identified and described in Chapter 3. Typical GC/MS traces for extracted water and sediment

samples are shown in Fig.4.6.

The main feature of the recorded hydrocarbon profiles is a series of resolved n-alkane peaks superimposed on a generally small unresolved complex mixture (UCM). The water phase alkane concentration distributions (Figs.4.4 a-h) show a generally unimodal profile which is partially disturbed by fall in values at around C_{22}. The highest alkane concentrations occur in the C_{18}, C_{19}, C_{20} range. In general however, the sediment profiles (Figs. 4.5 a-h) differ from the water profiles in that the greatest concentrations are found in the higher molecular weight range C_{25} - C_{27} and are thus negatively skewed. The sediment profiles also display a clearer unimodal profile than water. Another difference can be noticed in the lowest chain length alkanes which, in general, are relatively more abundant in the water samples than in sediment.

TABLE 4.4
TOTAL ALKANE LEVELS IN SEDIMENT AND WATER IN THE SILK STREAM CATCHMENT

Site		*Mean concentration*	
Code	*Name*	*Water ($\mu g\ l^{-1}$)*	*Sediment ($\mu g\ g^{-1}$)*
1	Dew Pond (Trent Park)	110.3	26.5
3	Dean's Brook (Stoneyfield Park)	261.3	37.4
4	Silk Stream (Rushgrove Park)	596.3	135.1
5	Silk Stream (relocated)	741.6	146.3
6	Silk Stream (above boom original)	884.3	250.3
7	Silk Stream (below boom)	665.4	250.6
8	Welsh Harp (north)	600.4	227.0
9	Welsh Harp (Cool Oak Lane)	322.7	70.6

These different features reflect the physical properties (low density and high volatility) which render the lower MW alkanes more susceptible to removal from aqueous systems and prevent long term sediment accumulation i.e hydrocarbons present in water are likely to be of more recent introduction to the system than those present in the sediment. It is likely, however, that many of the hydrocarbons in the water column, particularly during storms are from the resuspension of sediment bound compounds.

The profiles at all the sites also display increases to varying degrees of odd numbered carbon chain length alkanes relative to the even numbered compounds in the range C_{20}-C_{32} which are superimposed on the lower unimodal distribution. These carbon number preferences are indicative of biogenic inputs and are discussed more fully in Section 4.4.1.2. The total alkane concentrations in sediment and water phases (Table 4.3 and 4.4) indicate clearly that downstream increases in aliphatic concentrations occur corresponding to the downstream increase in urbanized area which exists in this catchment. These represent approximately tenfold mean concentration increases from the background control site (Site 1) to the lower Silk Stream sites in both sediment and water, although proportionally the changes appear to be more pronounced in sediment than in water. At each site, however, large temporal variations in concentrations were observed which were related to weather conditions. The highest levels were always recorded in storm samples containing high suspended solid loadings. The results for basal sediment concentrations show that there are significant step increases from Site 3 on the Dean's Brook to Sites 4 and 5 followed by a further increase to the lowest Silk Stream sites (Site 6, 7 and 8). There was no significant difference however, between the sediment values at Sites 6, 7 or 8. In water samples however, a drop of 25% and 10% in total alkane concentration between sites 6 and 7 and Sites 5 and 7 occurs. The results therefore indicate that during the sampling period the oil boom is reducing immediate downstream water phase alkane concentrations but does not appear to significantly affect sediment levels.

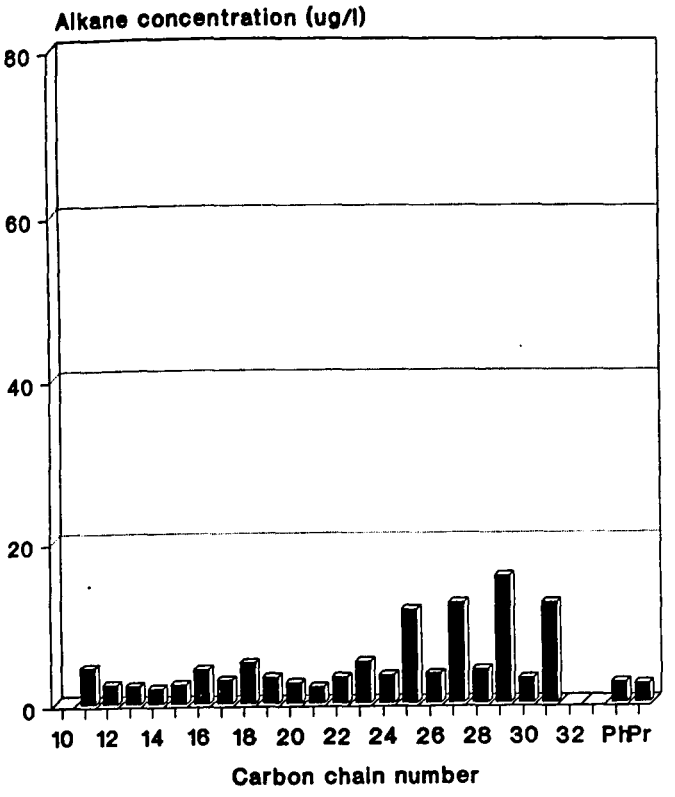


Fig. 4.4a Site 1

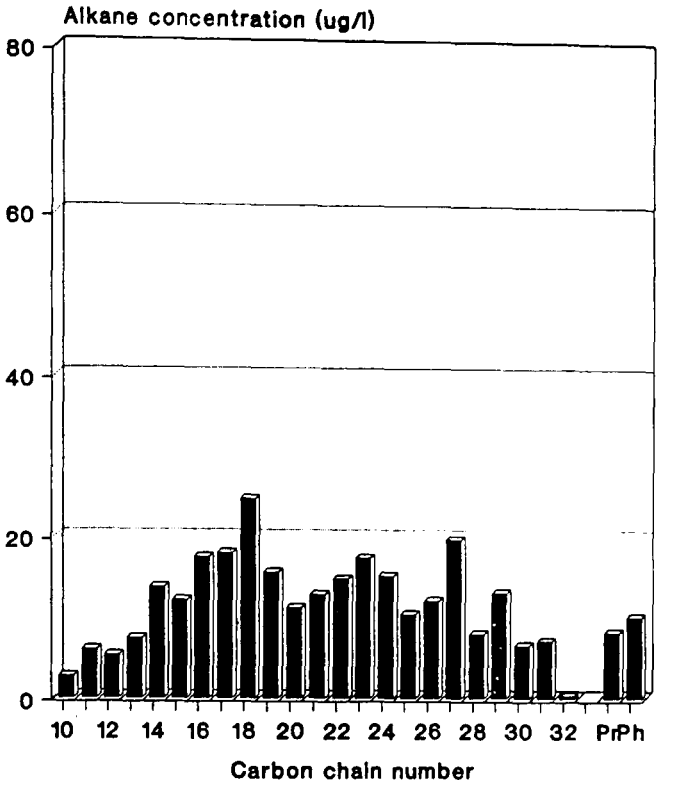


Fig. 4.4b Site 3

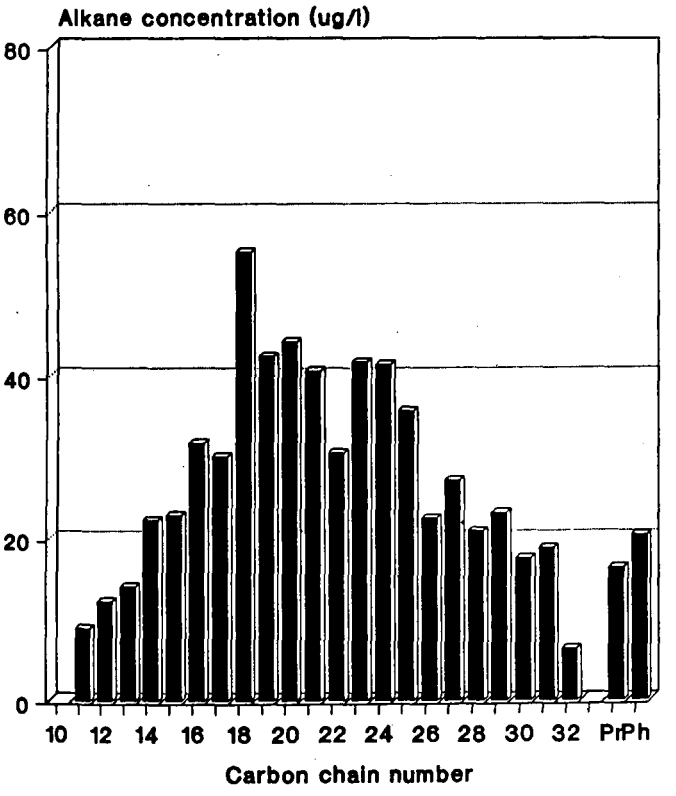


Fig. 4.4c Site 4

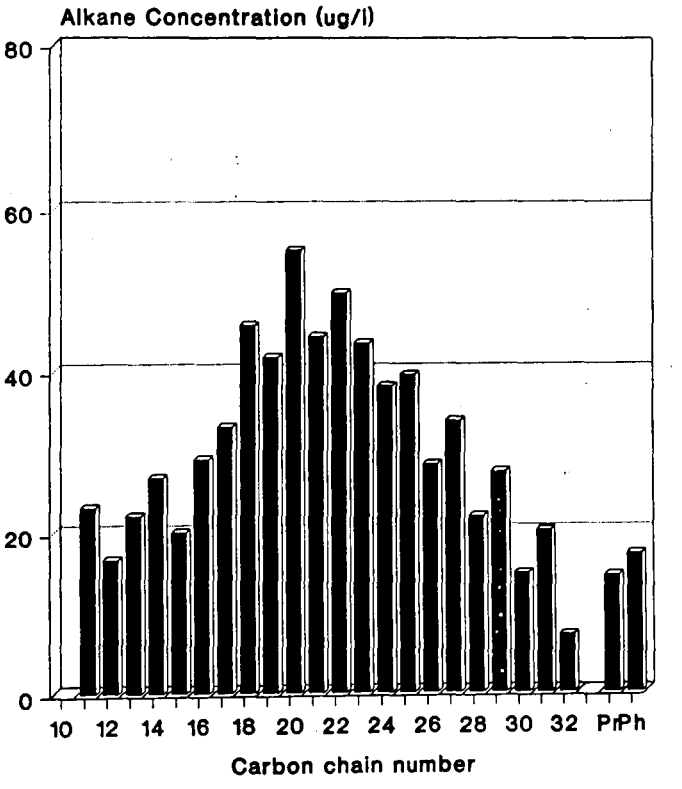


Fig. 4.4d Site 5

Figs. 4.4a-d Alkane, pristane and phytane levels in water samples from Sites 1, 3, 4 and 5.

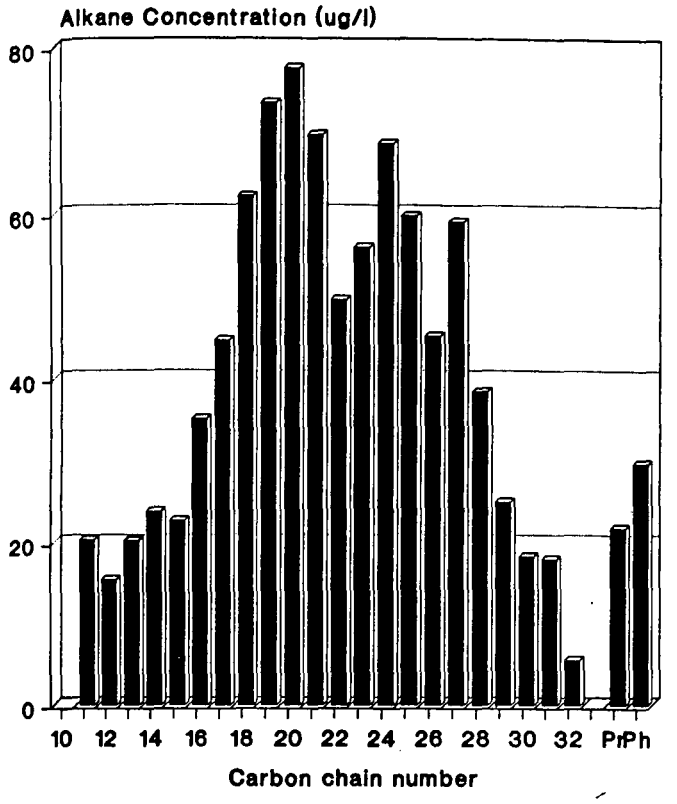


Fig. 4.4e Site 1

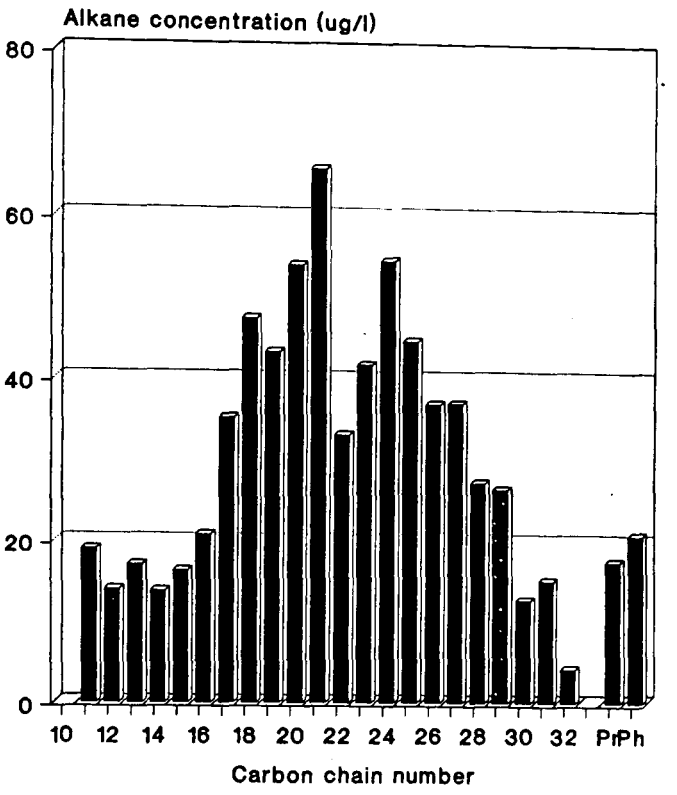


Fig. 4.4f Site 3

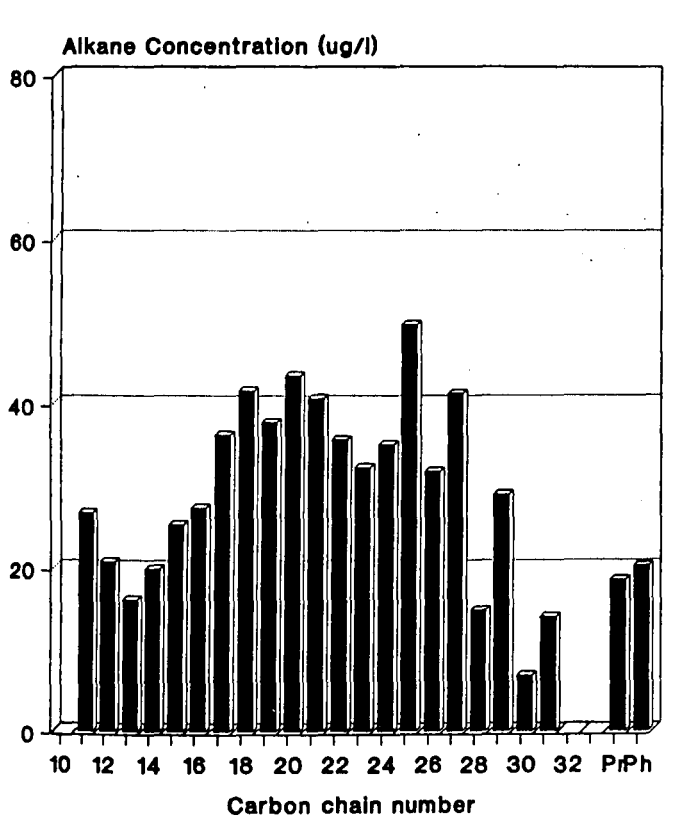


Fig. 4.4g Site 4

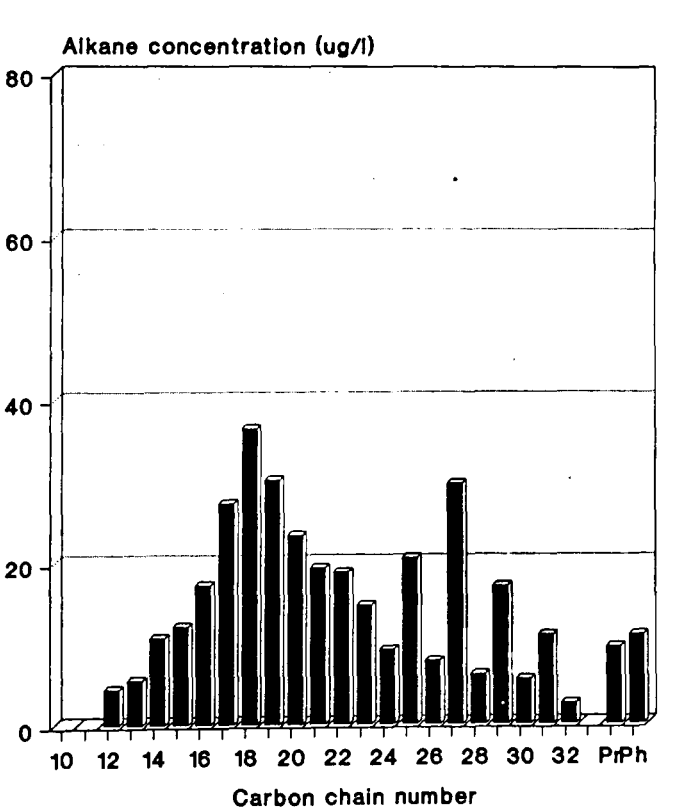


Fig. 4.4h Site 5

Figs. 4.4e-h Alkane, pristane and phytane levels in water samples from Sites 6, 7, 8 and 9.

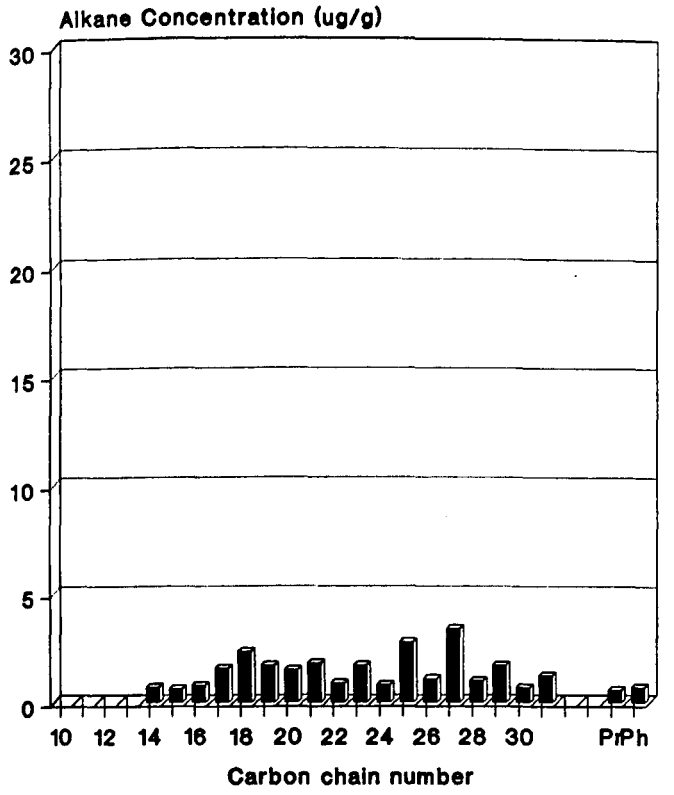


Fig. 4.5a Site 1

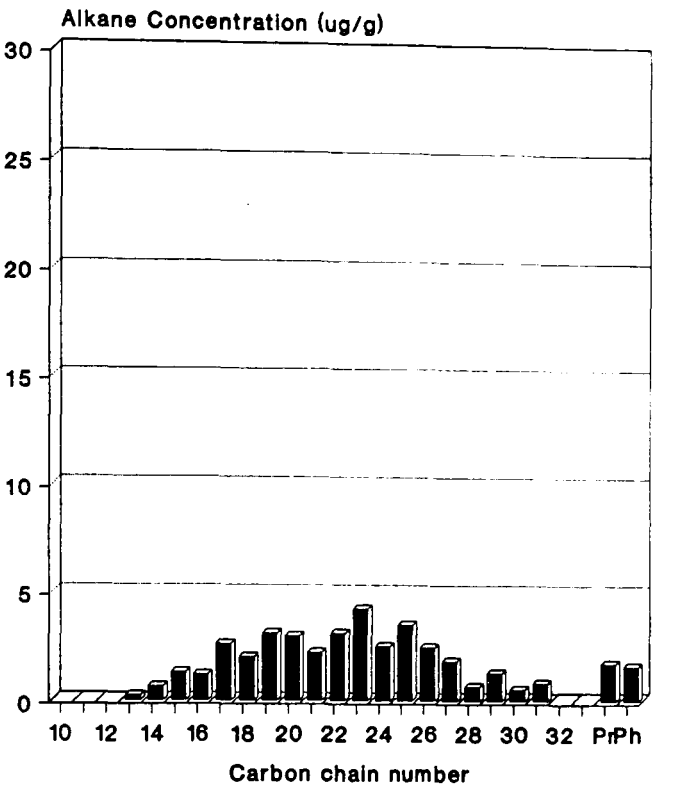


Fig. 4.5b Site 3

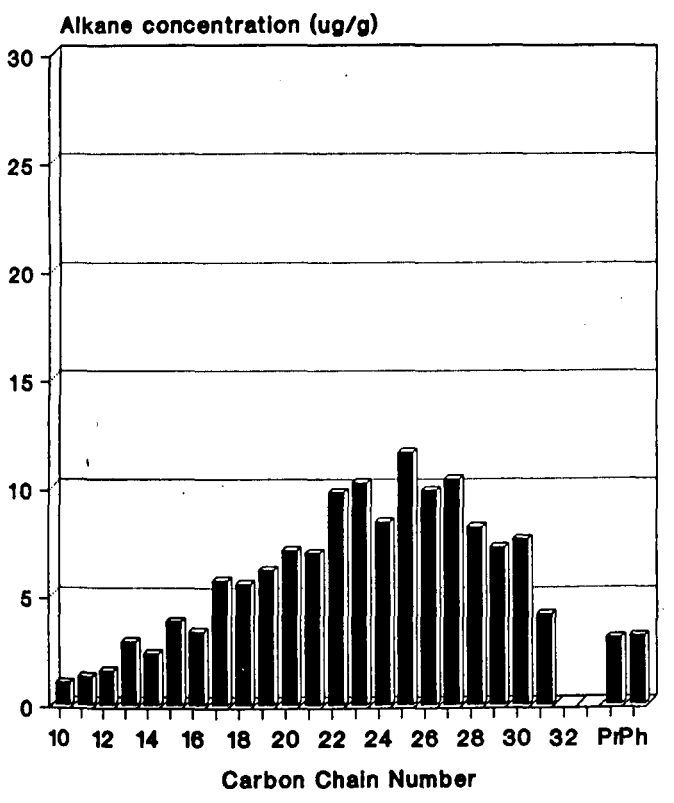


Fig. 4.5c Site 4

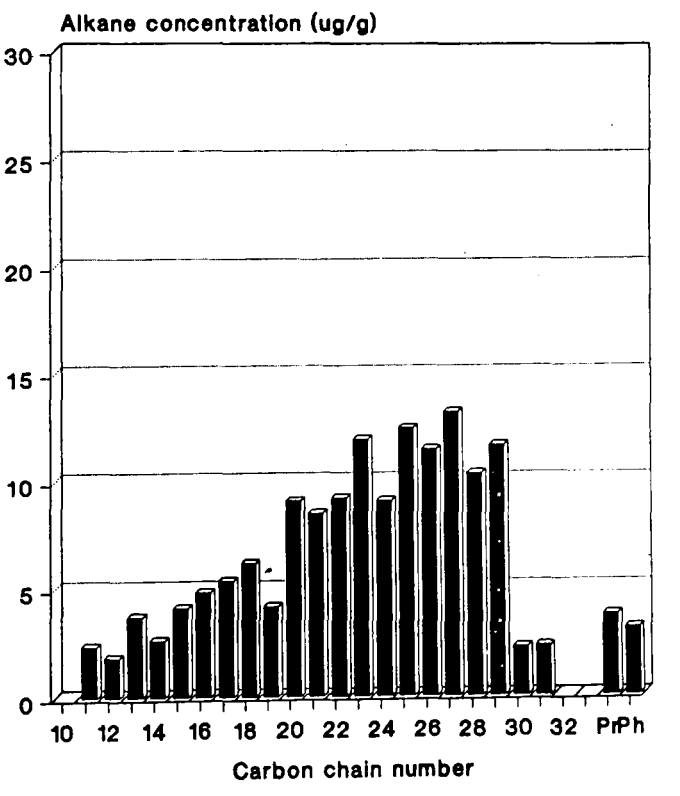


Fig. 4.5d Site 5

Figs. 4.5a-d Alkane, pristane and phytane levels in sediment samples from Sites 1, 3, 4 and 5.

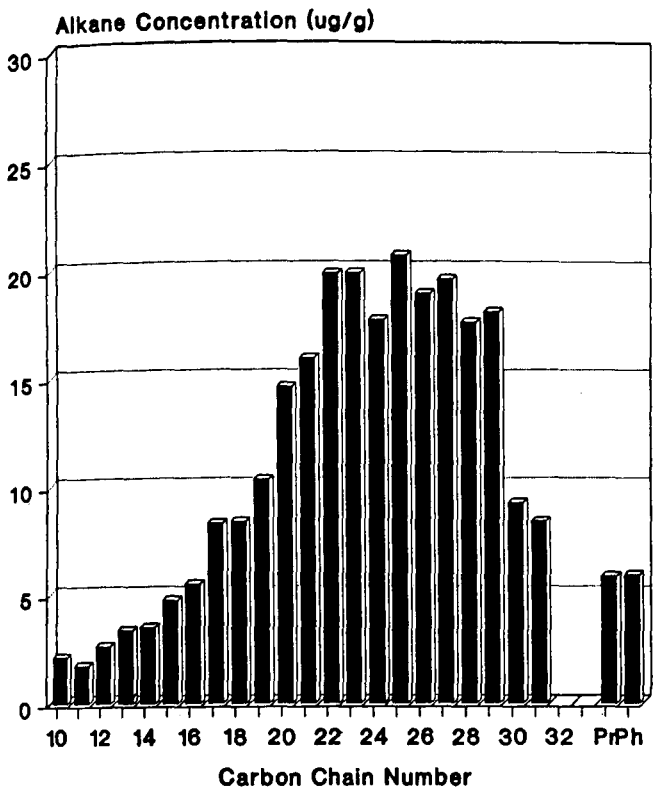


Fig. 4.5e Site 6

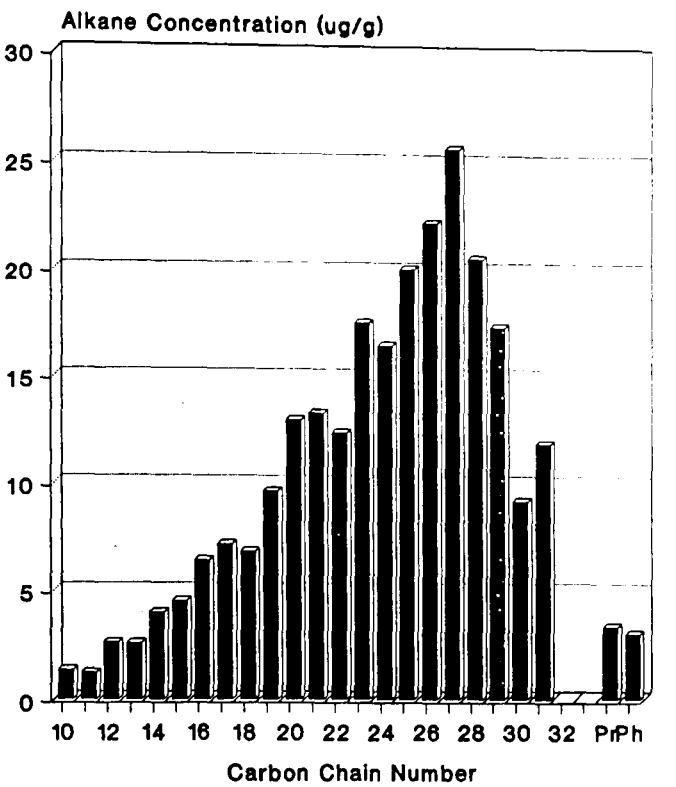


Fig. 4.5f Site 7

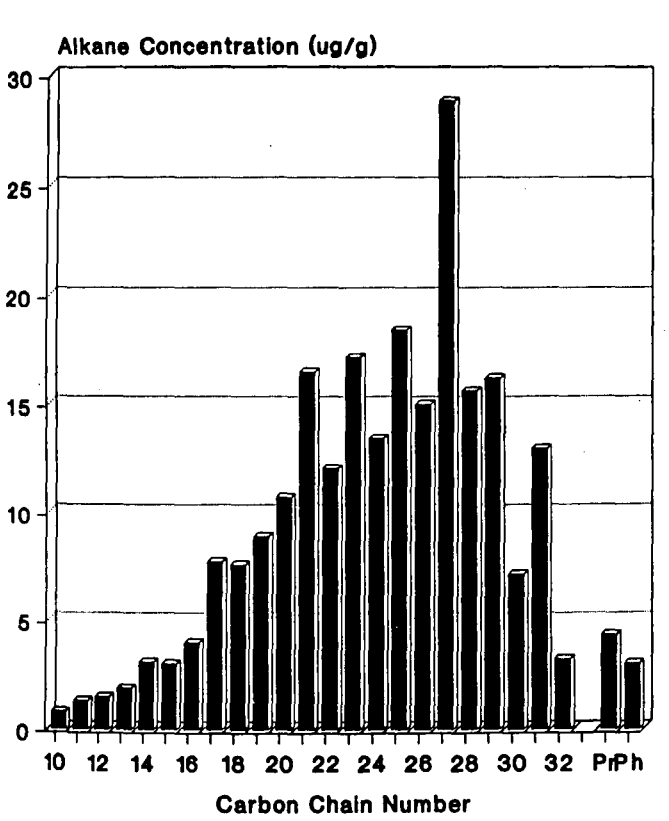


Fig. 4.5g Site 8

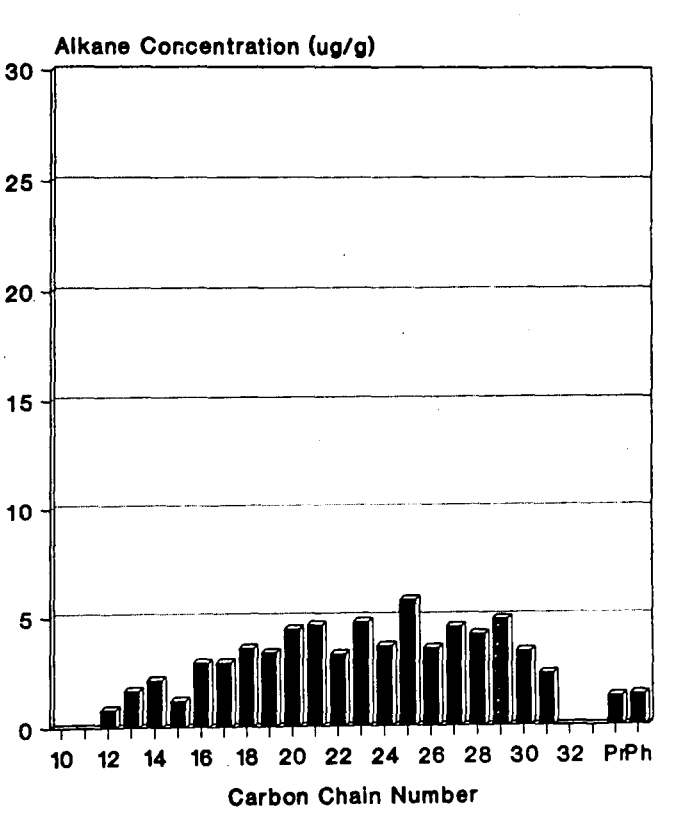
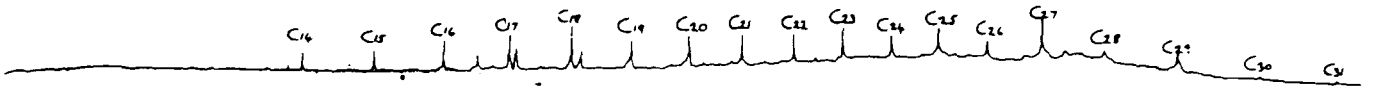


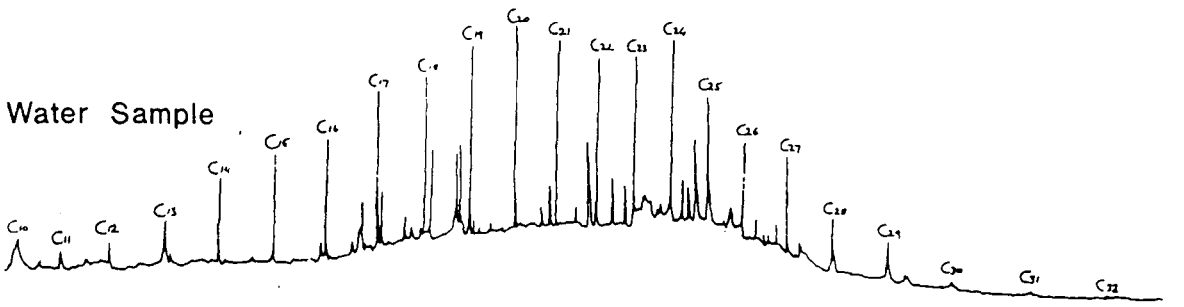
Fig. 4.5h Site 9

Figs. 4.5e-h Alkane, pristane and phytane levels in water samples from Sites 6, 7, 8 and 9.

(i) Site 1, Water Sample

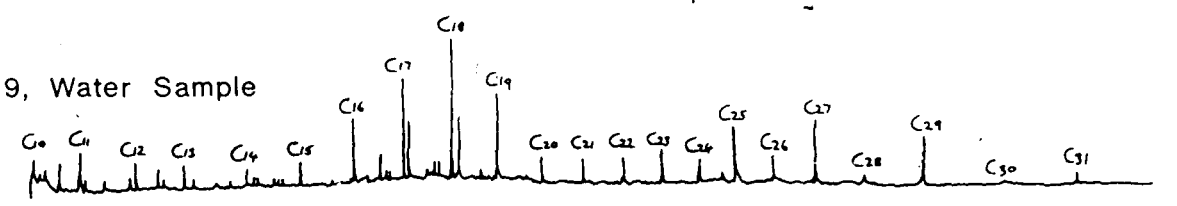


(ii) Site 6, Water Sample

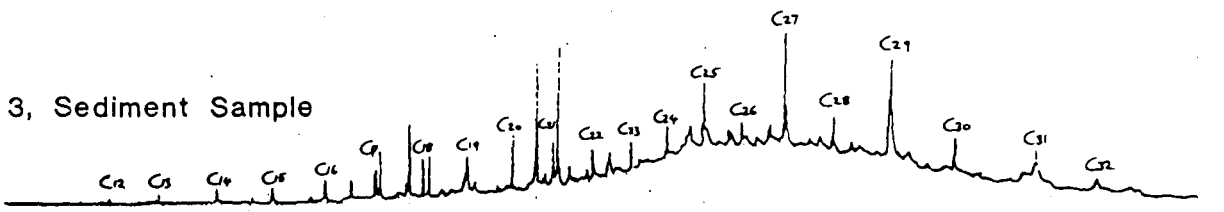


(iii) Site 8, Water Sample

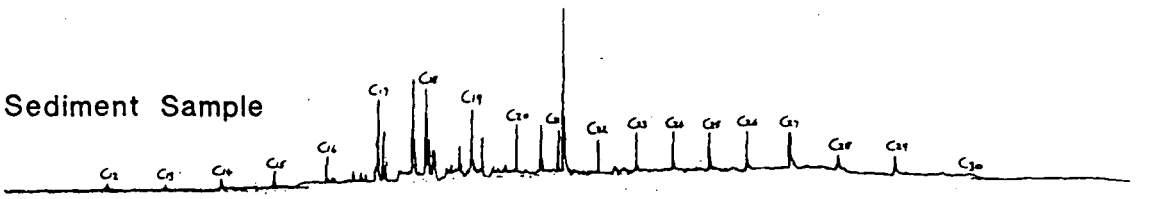
(iv) Site 9, Water Sample



(v) Site 3, Sediment Sample



(vi) Site 4, Sediment Sample



(vii) Site 6, Sediment Sample

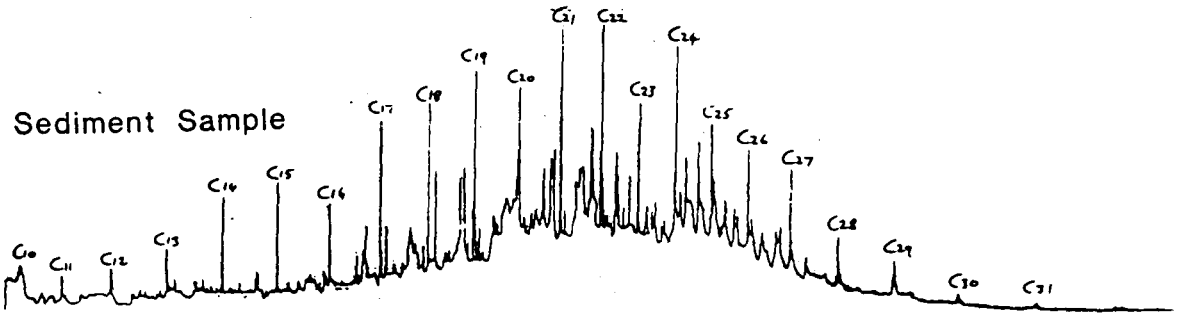


Fig. 4.6 GC/MS traces for water and sediment samples collected from the reference, Silk Stream and Welsh Harp sites.

4.4.3 Carbon Preference Index

The ratio of odd to even carbon number chain length alkanes or Carbon Preference Index (CPI) may be used to assess the relative contributions of recent biogenic production and that of petroleum derived aliphatics to the sample mixture (see Chapter 2, Section 2.6). In this study, two indices have been used (the C_{14}-C_{20} and the C_{20}-C_{32} CPIs) in order to distinguish between possible algal and higher plant contributions. The C_{14}-C_{20} CPI will indicate the extent of algal inputs while the C_{20}-C_{32} CPI represents a higher plant wax presence. The calculated CPIs for the various water and sediment samples are listed in Tables 4.5 and 4.6 respectively together with relevant phytane (Ph) and pristane (Pr) ratios.

The $CPI_{14\text{-}20}$ values lie in the range 0.83 to 1.12 for the analyzed water samples (Table 4.5) and 0.77 to 1.37 for the sediment samples (Table 4.5). The lack of any preference for odd numbered alkanes between C_{14} and C_{20} suggests that algal inputs do not contribute significantly to the hydrocarbon assemblages in sediment or water at any of the sites. The most surprising result is the low value at Site 9 in the Welsh Harp receiving basin indicating a slight even carbon preference in the water phase at a location where algal inputs would be expected to be highest.

Ajayi & Poxton (1987) used the $CPI_{14\text{-}20}$ value to assess three sites on the Forth Estuary and also found the ratios close to unity. The sites studied had lower sediment alkane concentrations (4.7- 40.7 $\mu g\ g^{-1}$ total alkanes) than those recorded in the Silk Stream catchment which would be expected to result in an increase in the relative contribution of biogenic alkanes to the hydrocarbon suite. However, the results suggest that the $CPI_{14\text{-}20}$ may in relatively clean conditions still have ratios close to unity. It is perhaps not surprising, therefore, that values close to one were found at all the Silk Stream sites and the control site.

TABLE 4.5

ALIPHATIC PARAMETERS CALCULATED FROM WATER SAMPLES FROM THE REFERENCE, SILK STREAM AND WELSH HARP SITES

Site	*CPI*		*Ph:Pr*	C_{17}:*Pr*	C_{18}:*Ph*
	C_{14}-C_{20}	C_{20}-C_{32}			
1	0.83	3.40	1.19	1.16	2.17
3	0.91	1.41	1.51	2.20	2.46
4	0.83	1.34	1.29	1.85	1.45
5	0.81	1.34	1.29	2.29	2.66
6	1.12	1.27	1.36	2.05	2.08
7	0.93	1.40	1.19	2.01	2.27
8	1.00	1.67	1.09	1.97	2.05
9	0.95	2.37	1.23	2.87	3.12

TABLE 4.6

ALIPHATIC PARAMETERS CALCULATED FROM SEDIMENT SAMPLES FROM THE REFERENCE, SILK STREAM AMD WELSH HARP SITES

Site	*CPI*		*Ph:Pr*	C_{17}:*Pr*	C_{18}:*Ph*
	C_{14}-C_{20}	C_{20}-C_{32}			
1	0.98	2.79	1.24	2.74	3.25
3	1.37	1.50	0.94	1.53	1.25
4	1.14	1.81	1.03	1.88	1.78
5	1.08	1.42	0.83	1.44	1.99
6	0.97	1.22	1.01	1.42	1.42
7	0.94	1.23	0.90	1.13	2.26
8	1.03	1.66	0.93	1.78	1.89
9	0.77	1.48	1.05	2.19	2.53

The $CPI_{20\text{-}32}$ values calculated for the sampling sites range from 1.27 to 3.40 in water samples and from 1.22 to 2.79 in sediment samples (Table 4.6). No significant temporal variation was found in the calculated indices. The lowest $CPI_{20\text{-}32}$ values were obtained at Sites 5, 6 and 7 in the lower Silk Stream indicating that higher plant alkane input is proportionally least significant at the sites where the highest overall alkane

concentrations were obtained. Conversely, the highest $CPI_{20\text{-}32}$ values were obtained at the control Site 1 where the total alkane concentration was lowest. The values recorded indicate that biogenic inputs from higher plants are evident at all the sites but do not dominate the profiles to the very large extents that have been reported in pristine environments where the CPI may be 15 or more (Douglas & Eglinton, 1966).

The progressive decrease in sediment CPI from Sites 3 and 4 to Sites 5 and 6 suggests that there is a downstream decrease in the relative contributions of plant wax components to the total alkanes present. At Site 8 on the northern inlet to the receiving basin the index is higher and may be related to alkane synthesis from the extensive *Typha* reed beds present at this site. The sediment and water CPI values are in broad agreement but a discrepancy appears to exist between these values at the least polluted sites (Sites 1 and 9). The relatively elevated water phase values suggest an initial impact of biogenically derived hydrocarbons in the water phase when hydrocarbon contamination is less pronounced. In association with the high overall alkane concentrations recorded at the the lower Silk Stream sites (Sites 5, 6 and 7) the slight odd carbon predominance which still exists in both water and sediment phases suggests significant biogenic alkane inputs in absolute terms. Epicuticular leaf-fall waxes and stem debris from the abundant overhanging *Salix* sp. (Chapter 3, Section 3.2) in this downstream region must therefore be primary contributors to the relevant biogenic inputs which seem to be ubiquitously present.

4.4.4 Pristane and phytane levels and ratios

The isoprenoid compounds pristane and phytane may be used to assess possible hydrocarbon sources. Pristane may be derived from zooplankton but phytane is reported to be only of petrogenic origin (Brassel *et al.*, 1978). At all the monitored sites phytane is present in both water and sediment (Figs. 4.4 and 4.5) and the phytane to pristane ratios are close to one. Values in this range have been reported as being characteristic of sewage derived hydrocarbons (Hamilton *et al.*, 1984). However, since separate sources for pristane and phytane exist it is clear that such ratios may occur without sewage input, which may explain the value (close to one) calculated at the background reference site (Site 1).

The C_{17}:Pr and C_{18}:Ph ratios can also be used to indicate the extent of biodegradation of hydrocarbons in aquatic environments since the branched isoprenoids are more resistant to bacterial action than the n-alkanes (Eganhouse *et al.*, 1981; Eganhouse & Kaplan, 1982b). In general, the ratios in sediment and in water are in agreement. However, with the exception of Site 1, the values tend to be slightly higher in the water phase (Sites 3, 5, 6, 8 and 9) indicating that some biodegradation may have occurred in the sediment associated alkanes relative to those in the water phase. The mean C_{17}:Pr and C_{18}:Ph ratios at all the sites are, however, greater than one, indicating that, in general, biodegradation has not occurred to any significant degree. There are two possible explanations for this:

a) the hydrocarbons measured may be of recent input to the system and therefore there has been an insufficient time period for bacterial action to result in detectable degradation of the aliphatic assemblage.

b) anaerobic conditions may exist in the substrate which will severely reduce the rate at which biodegradation occurs (see Chapter 2, Section 2.5.4).

Anaerobic conditions are more commonly associated with deep sediments. However the DO and SOD levels recorded at the lower Silk Stream sampling sites (Section 4.2) and the highly organic nature of the sediments (Chapter 3, Section 3.2) strongly suggest that anoxic conditions may exist in sediment layers very close to the water interface at Sites 6, 7 and 8. The apparently low level of degradation as indicated by the C_{17}:Pr and C_{18}:Ph ratios are consistent with the relatively low UCM:n-alkane proportions which exist and which have also been used as an index of degradation (Matsumoto, 1982).

4.5 POLYCYCLIC AROMATIC HYDROCARBON DISTRIBUTION

A wide assemblage of PAHs which are typical of recent sediments have been recorded at the various Silk Stream and Welsh Harp receiving basin sites (Tables 4.7-4.9; Figs. 4.7 and 4.8). The results show that a similar distribution in sediment and water phases exists at all the sites, although actual concentrations of individual PAHs are higher by a factor of around 1000 in the sediment. At the downstream sites the PAH in the water

phase are present at levels close to or above their saturation concentration in freshwater. The hydrophobic nature of PAHs and their known affinity to particles (Neff, 1979) would indicate however, that the bulk of the PAHs are particulate associated.

TABLE 4.7

TOTAL PAH CONCENTRATIONS AT THE SILK STREAM AND WELSH HARP SAMPLING SITES

		Mean concentration	
Code	*Site Name*	*Water ($\mu g\ l^{-1}$)*	*Sediment ($\mu g\ g^{-1}$)*
1	Dew Pond (Trent Park)	3.2	2.8
3	Dean's Brook (Stoneyfield Park)	33.2	4.1
4	Silk Stream (Rushgrove Park)	83.4	5.6
5	Silk Stream (relocated)	97.2	74.8
6	Silk Stream (above boom original)	140.0	120.6
7	Silk Stream (below boom)	128.8	110.9
8	Welsh Harp (north)	117.1	97.4
9	Welsh Harp (Cool Oak Lane)	36.8	25.9

The values shown for total sediment PAHs show marked downstream increases from 4$\mu g\ g^{-1}$ at Site 3 to 120 $\mu g\ g^{-1}$ at Site 6 (Table 4.7) although maximum values at these sites (8.8 - 210 $\mu g\ g^{-1}$) show more pronounced differences. In the water phase a less pronounced but significant downstream increase occurs. The values in water samples were also highly variable compared with sediment values as can be seen from the higher standard deviations for individual PAHs in water samples (Tables 4.8 and 4.9). The difference in downstream changes between the two phases is the result of the elevated PAH concentration in water relative to sediment at Sites 3 and 4. This may be related to the sediment composition at these sites which is known to have lower amounts of fine organic particles compared with lower Silk Stream values resulting in a relatively low capacity for retention of particulate associated hydrocarbons. Following the maximum PAH concentration values recorded at Site 6 there are successive reductions at Sites 7 and 8. Again however, the changes in sediment and water PAH concentrations above and below the oil boom cannot be said to be significant. A marked reduction in sedimentary PAH concentrations occurs at Site 9, indicating that effective particulate settlement and filtration occurs within the Welsh Harp as well as dilution of the Silk

Stream inflow. A similar drop in water phase PAH concentration also occurs between these sites further confirming the particulate associated nature of the hydrocarbons.

TABLE 4.8

PAH CONCENTRATION IN SEDIMENT AT THE REFERENCE, SILK STREAM AND WELSH HARP SAMPLING SITES (MEAN ± S.D)

Compound	*Concentration ($\mu g\ g^{-1}$)*							
	Site							
	1	*3*	*4*	*5*	*6*	*7*	*8*	*9*
N	ND	0.61±0.32	1.01±0.76	1.59±0.46	2.30±1.03	3.41±1.39	2.45±1.58	0.25±0.08
M-N	ND	0.40±0.54	1.21±0.58	1.63±0.67	2.05±0.38	2.10±1.48	2.32±1.59	ND
F	ND	ND	0.74±0.67	1.21±0.38	1.46±0.27	1.18±0.23	1.56±2.23	0.36±0.38
Ph	0.81±0.36	0.28±0.09	3.34±1.59	4.83±1.82	7.50±2.69	6.95±2.54	7.30±5.78	2.48±1.01
M-Ph	ND	ND	1.08±0.31	1.59±0.39	2.07±0.68	1.32±0.18	1.35±1.09	ND
M-A	ND	ND	1.68±0.73	1.98±0.78	1.19±0.29	1.02±0.32	1.14±1.03	ND
Fa	1.12±0.32	1.50±0.21	8.72±2.86	12.07±7.39	19.55±6.38	22.60±7.23	17.83±10.43	5.38±2.30
M-Fa	ND	ND	0.34±0.19	1.41±0.30	2.09±0.58	2.31±0.29	3.41±1.48	ND
Py	0.85±0.19	1.31±0.45	10.74±3.78	13.85±4.25	19.81±8.45	20.35±8.12	18.49±12.93	4.91±1.69
M-Py	ND	ND	0.21±0.11	1.28±0.36	2.42±0.57	2.28±0.45	1.78±1.32	ND
BA	ND	ND	6.36±2.80	6.18±2.06	12.32±	10.43±3.28	9.11±3.67	2.07±1.03
C	ND	ND	4.72±2.67	5.00±2.67	8.85±2.89	8.03±2.03	6.54±2.41	1.68±0.58
BFs	ND	ND	6.52±3.99	8.41±2.69	16.75±5.91	14.31±4.47	15.80±5.80	3.82±1.49
BP	ND	ND	5.40±2.58	6.26±1.54	9.39±3.96	8.55±4.68	5.15±2.78	3.42±1.59
IPy+DBA	ND	ND	3.34±0.88	7.23±1.21	12.34±2.59	5.78±2.39	3.40±2.40	1.50±2.33
BPe	ND	ND	ND	0.28±0.34	0.51±0.19	0.30±0.45	0.92±0.69	0.12±0.23

The PAH values recorded in sediments are an order of magnitude higher than those quoted for recent surface sediments in many freshwaters. Eadie *et al.* (1982a; 1982b) reported total concentrations (by GC analysis) for 7 PAHs in Lake Michigan of up to 3.33 $\mu g\ g^{-1}$. Bates *et al.* (1987) give values in the range 1-26 $\mu g\ g^{-1}$ for 9 PAHs in an urban estuary and Wakeham *et al.* (1980) reported values for the sum of major PAHs in lakes in Switzerland and the United States in the range 5-15$\mu g\ g^{-1}$ although these workers remark that the GC measured PAHs are only a fraction of the total weighable aromatic fraction (measured at 25-100 $\mu g\ g^{-1}$). Other workers have reported comparable PAH concentrations in sediments e.g. Metcalfe *et al.* (1990) reported values of 38.5 - 212 $\mu g\ g^{-1}$ for total measured PAHs in Lake Ontario and Plowchalk & Zagorski (1986) found sediment PAHs in the range 8.4 to 150.4 $\mu g\ g^{-1}$ in Lake Erie.

The Lake Ontario study area received industrial effluent, treated municipal sewage and discharges from shipping, whilst the Lake Erie sites were described as being in heavily industrialized watersheds. The most polluted Lake Erie site received discharges from industry, combined sewer/storm overflows, road runoff and other municipal sources. The values obtained in this present study place the lower Silk Stream sites towards the upper end of the range of PAH concentrations reported in the literature for highly urbanized sites.

Fluoranthene and pyrene are the most abundant PAHs in the sediment and water phases at all the sampling sites (Tables 4.8 and 4.9). Fluoranthene is known to be associated with combustion related anthropogenic pollution (Heit, 1977) and on a global scale fluoranthene and pyrene are considered to be the most abundant combustion derived

TABLE 4.9

MEAN PAH CONCENTRATION ($\mu g\ l^{-1}$) IN WATER FROM THE REFERENCE, SILK STREAM AND WELSH HARP SAMPLING SITES

	Concentration ($\mu g\ l^{-1}$)							
Compound	*Site*							
	1	*3*	*4*	*5*	*6*	*7*	*8*	*9*
N	ND	3.8±1.4	3.5±2.4	4.0±3.5	4.9±2.7	5.0±1.3	3.7±0.7	4.0±2.5
M-N	ND	2.7±1.8	3.0±2.1	2.3±1.5	2.5±1.8	3.0±1.4	3.4±1.6	2.1±0.78
F	ND	ND	0.50±0.3	0.90±0.5	0.8±0.3	1.1±0.6	0.6±0.4	0.2±0.1
Ph	0.50±0.28	3.8±2.4	4.7±2.8	4.0±3.6	8.5±5.8	5.3±1.9	4.2±2.6	2.0±1.6
M-Ph	ND	0.2±0.2	0.7±1.1	1.3±0.8	3.6±3.0	2.8±1.5	3.4±2.7	0.6±0.4
M-A	ND	0.5±0.2	1.50±0.8	1.7±0.6	2.9±2.0	2.7±0.7	2.7±1.0	0.7±0.3
Fa	1.5±0.96	4.4±2.8	17.0±11.9	25.3±15.9	34.8±22.7	30.2±19.3	28.2±24.7	6.8±4.2
M-Fa	ND	0.5±0.3	1.9±0.6	2.8±2.6	3.9±2.4	3.5±2.6	3.3±2.8	0.9±0.3
Py	1.2±0.86	4.6±2.5	19.5±13.6	23.9±14.7	22.5±12.4	24.0±11.3	30.0±23.0	7.2±4.6
M-Py	ND	ND	2.0±2.1	2.5±1.6	2.7±1.9	2.6±2.5	3.2±1.5	0.8±0.7
BA	ND	5.1±3.6	8.0±6.4	8.3±4.9	15.3±4.8	13.4±5.9	8.6±5.7	2.9±0.8
C	ND	3.9±2.9	5.7±2.4	6.2±3.6	10.3±6.8	11.2±3.6	6.6±2.1	2.2±0.9
BFs	ND	0.5±0.4	6.5±2.8	5.5±2.8	12.8±2.5	8.0±3.5	6.4±4.1	5.0±2.7
BP	ND	0.2±0.1	3.8±1.9	4.0±3.7	7.2±3.6	8.2±4.6	6.1±2.7	0.6±0.2
IPy+DBA	ND	2.9±0.9	4.8±2.7	4.2±2.4	5.6±2.7	7.0±2.8	5.6±2.8	0.9±0.5
BPe	ND	ND	ND	ND	0.2±0.1	0.2±0.2	0.1±0.1	ND

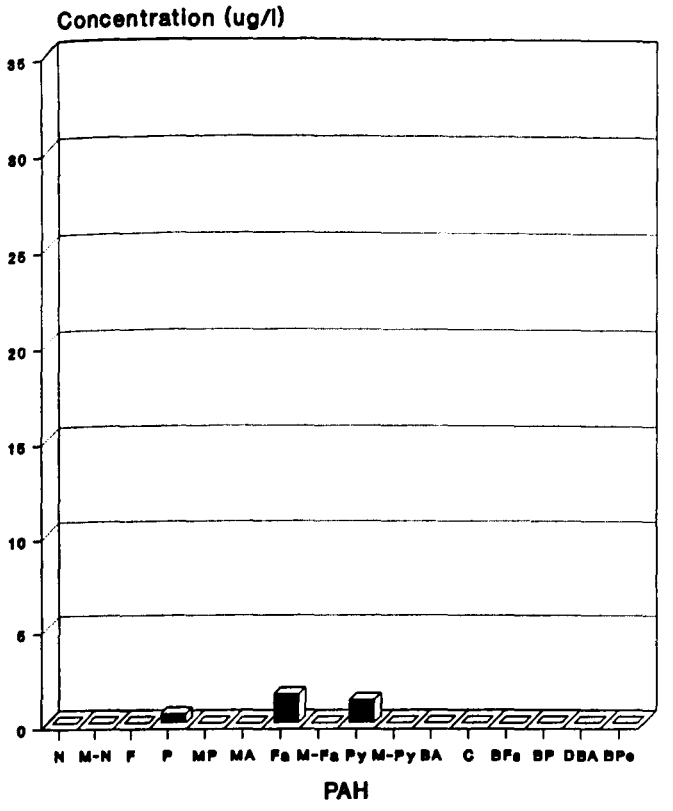


Fig. 4.7a Site 1

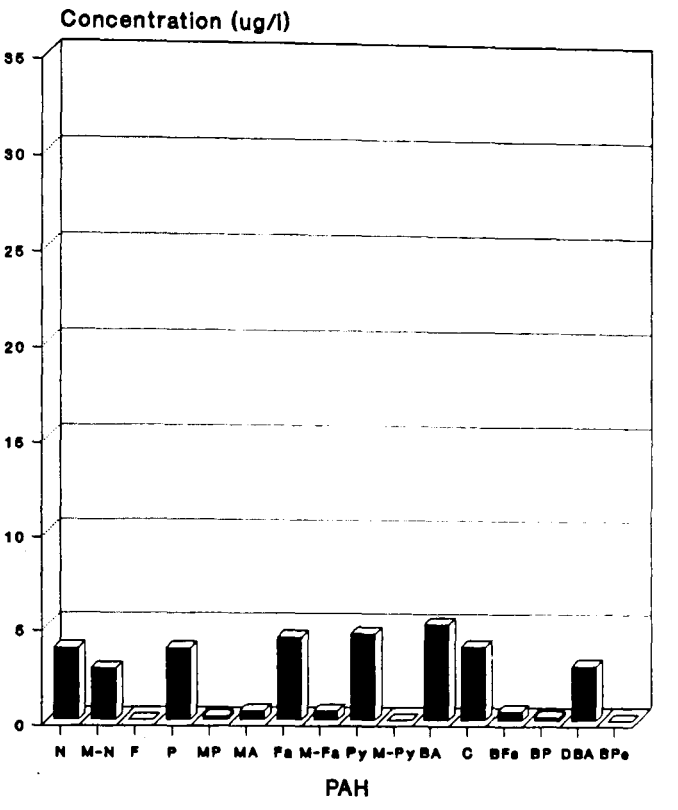


Fig. 4.7b Site 3

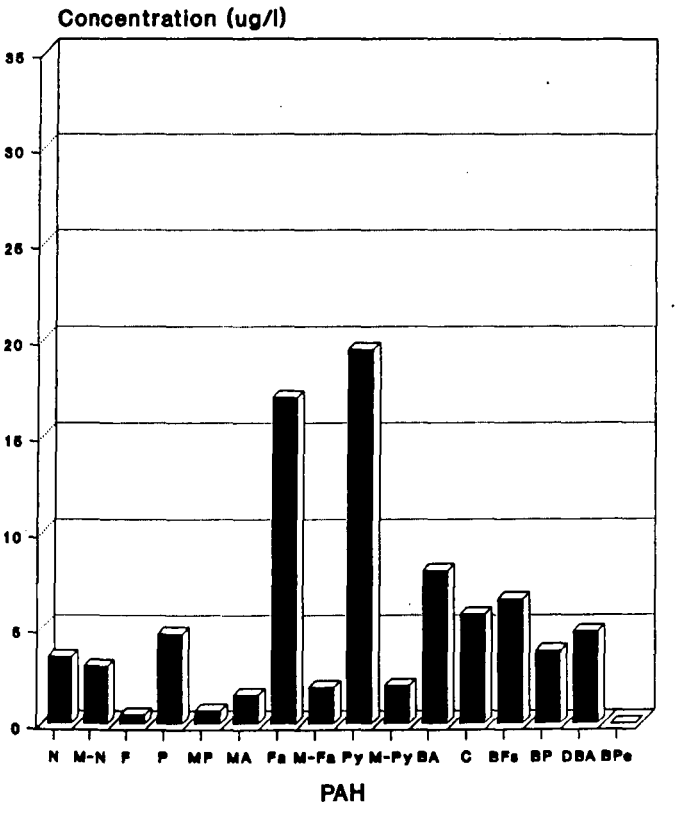


Fig. 4.7c Site 4

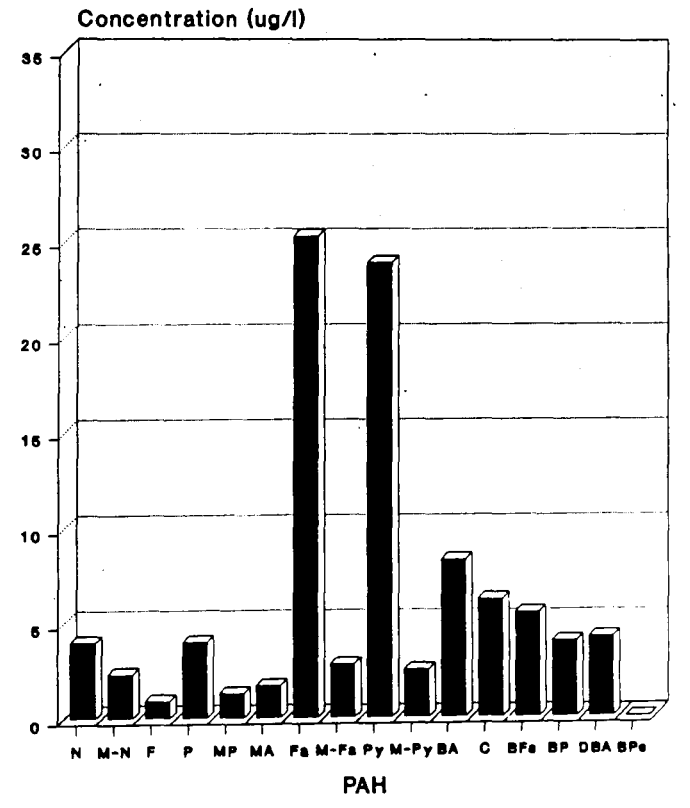


Fig. 4.7d Site 5

Figs. 4.7a- 4.7d PAH levels in water samples from Sites 1, 3, 4 and 5.

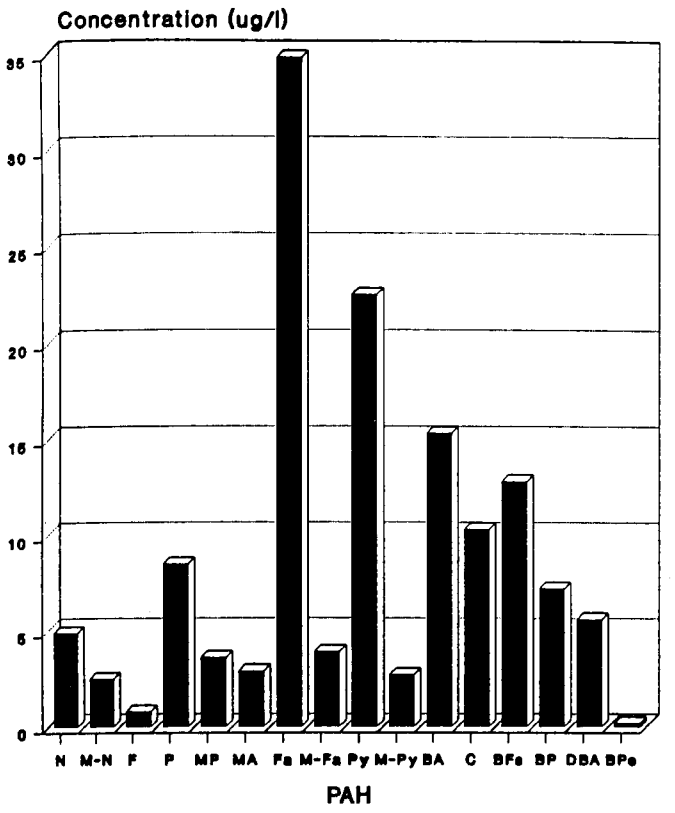


Fig. 4.7e Site 1

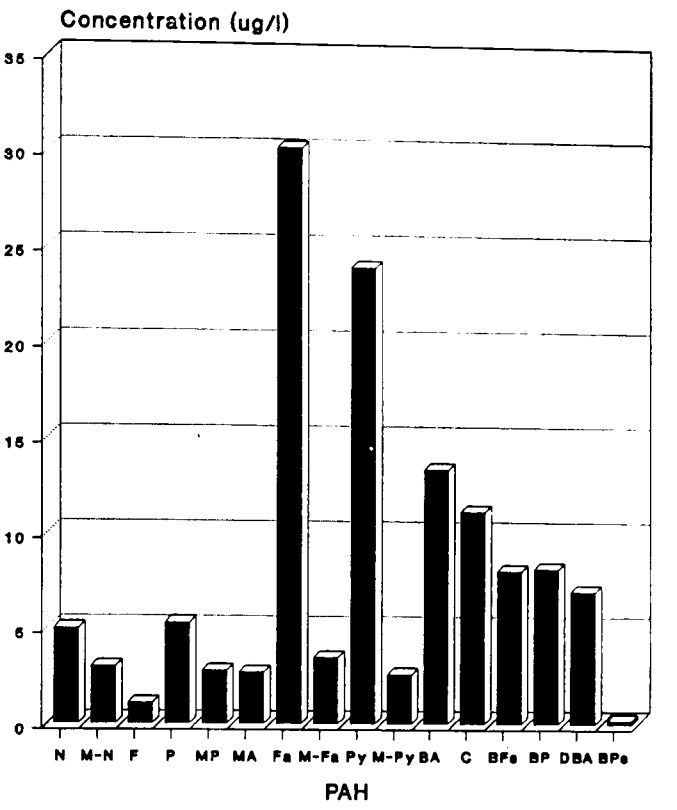


Fig. 4.7f Site 3

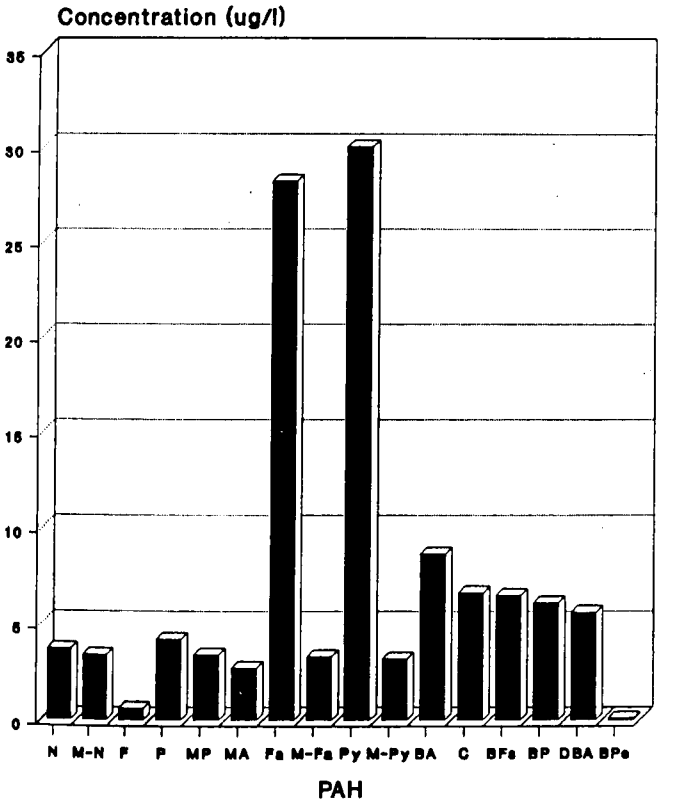


Fig. 4.7g Site 4

Fig. 4.7h Site 5

Figs. 4.7e-h PAH levels in water samples from Sites 6, 7, 8 and 9.

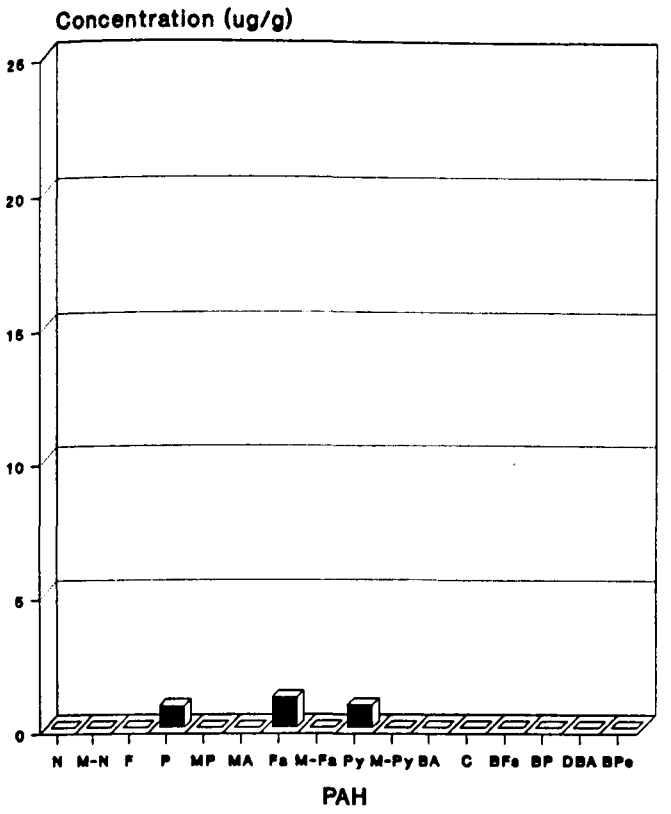


Fig. 4.8a Site 1

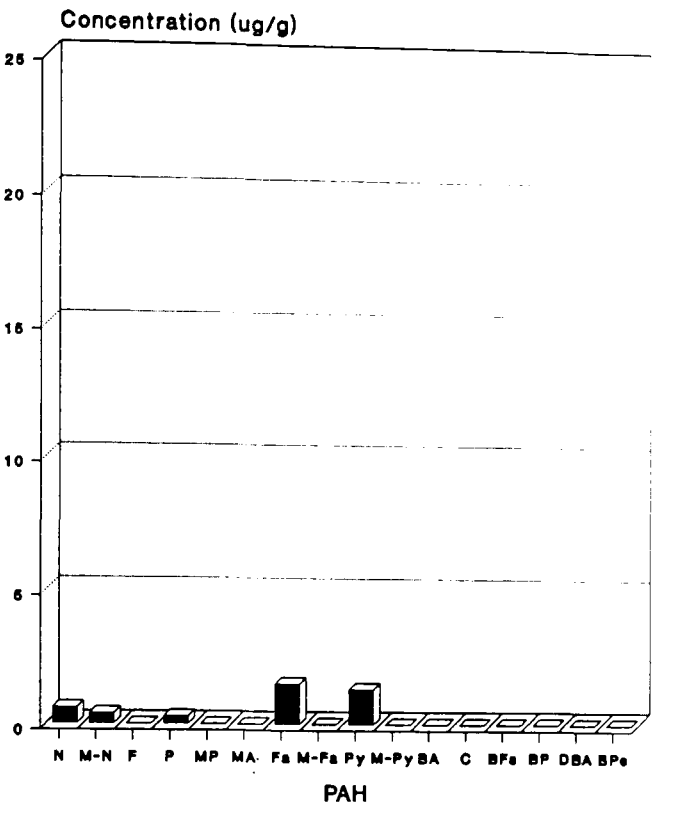


Fig. 4.8b Site 3

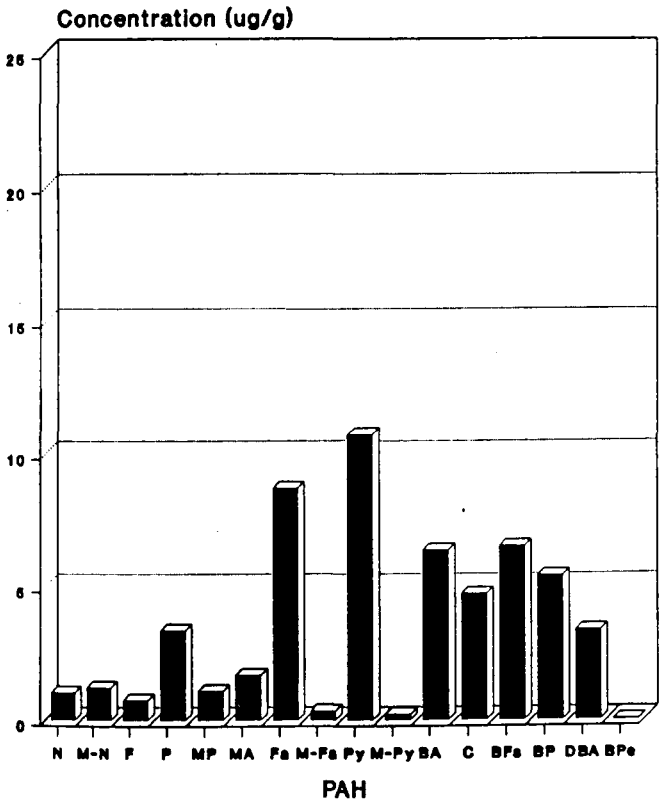


Fig. 4.8c Site 4

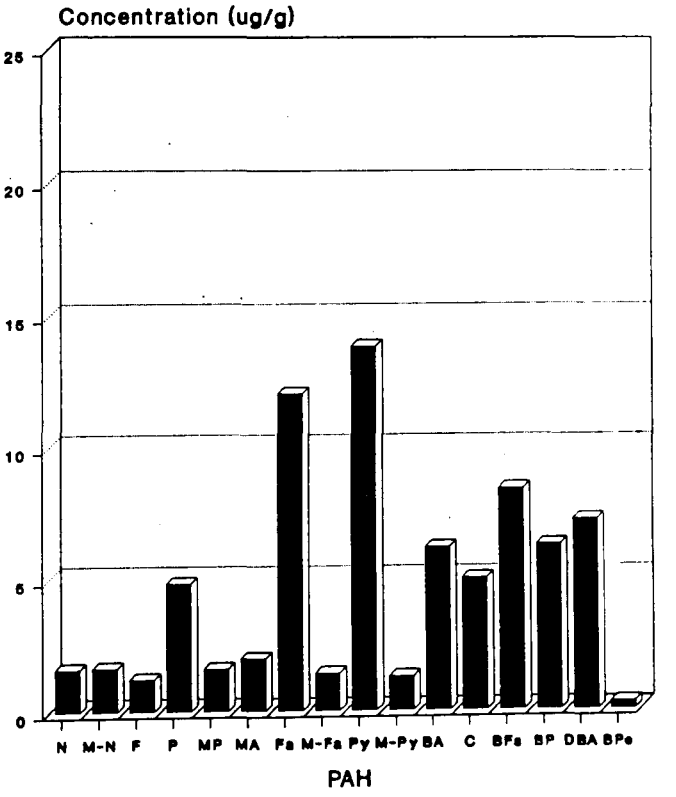


Fig. 4.8d Site 5

Figs. 4.8a-d PAH levels in sediment samples from Sites 1, 3, 4 and 5.

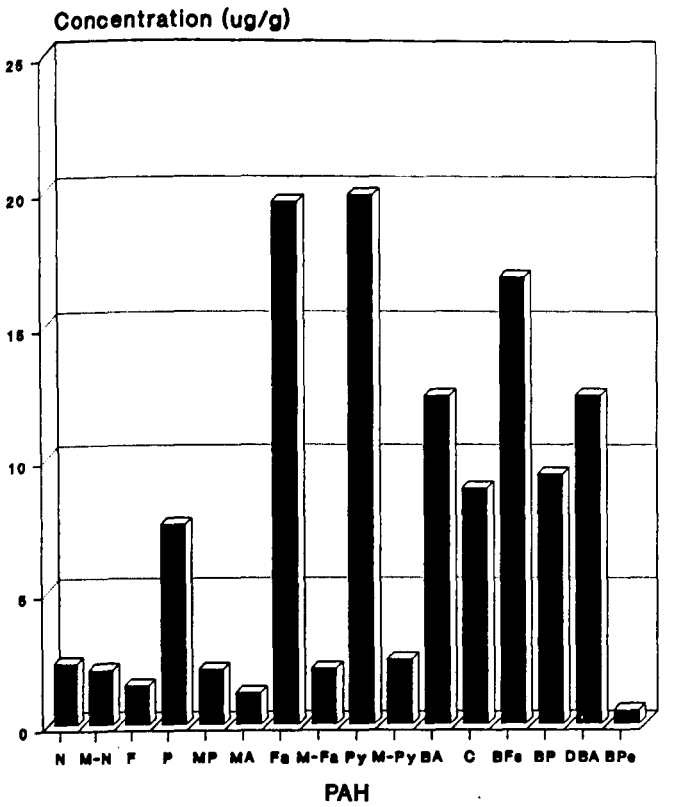
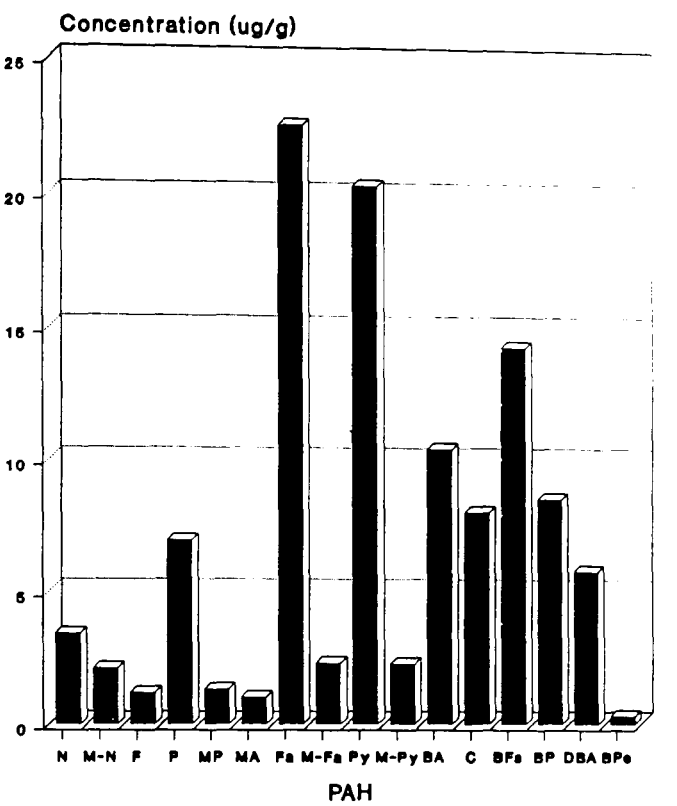


Fig. 4.8e Site 6



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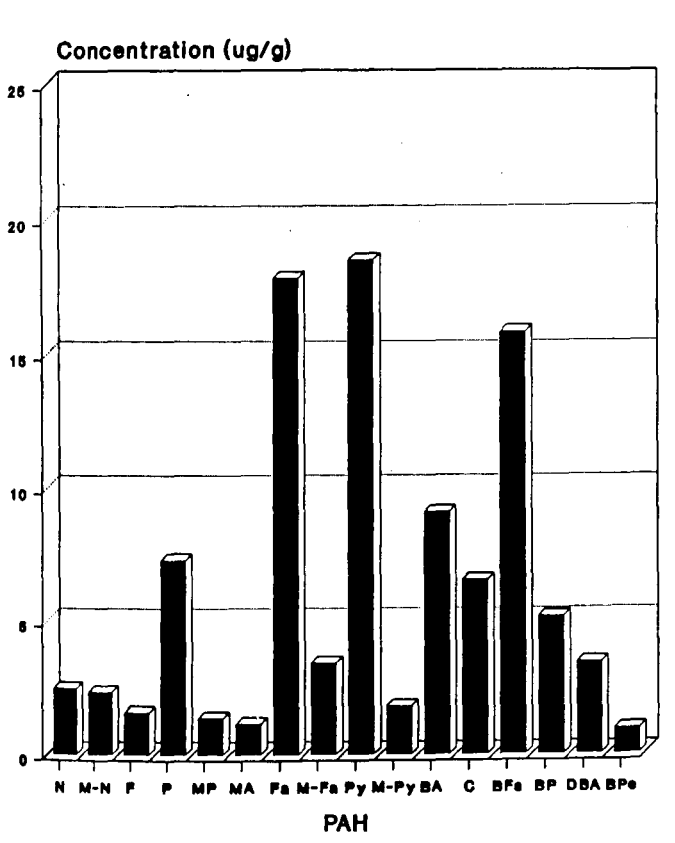


Fig. 4.8g Site 8

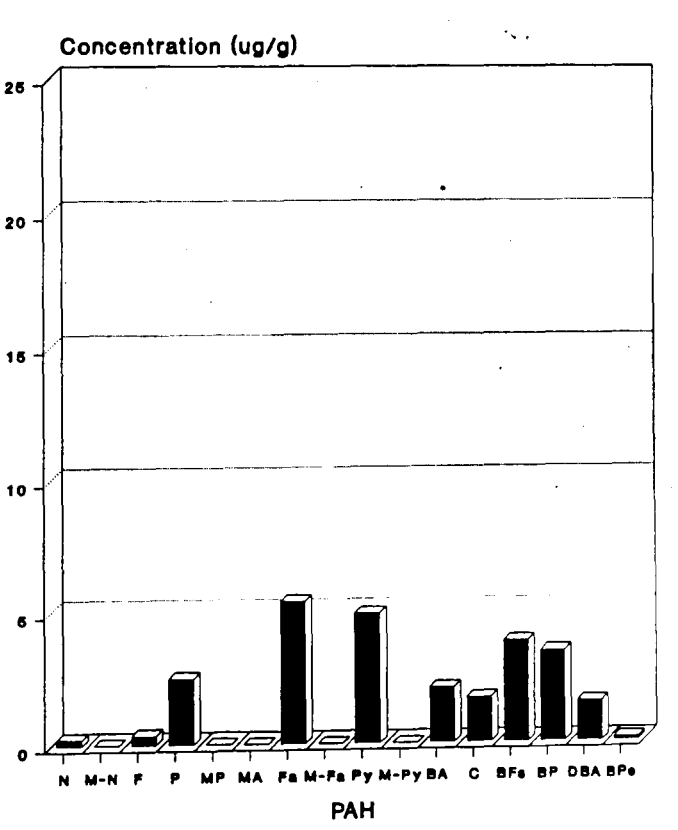


Fig. 4.8h Site 9

Figs. 4.8e-h PAH levels in water samples from Sites 6, 7, 8 and 9.

PAHs (Laflamme & Hites, 1980). The results therefore suggest that the PAHs detected in the catchment are primarily derived from combustion sources and not fromunburnt petroleum. The low relative abundances of naphthalene recorded are consistent with this observation since low concentrations of this compound are also considered a feature of combustion derived PAH mixtures (Sporstol *et al.*, 1985).

The methyl phenanthrene/phenanthrene ratio has been used to assess PAH sources (Prahl *et al.*, 1984) because of the known association between assemblages of PAHs with limited alkylation and high temperature derivation (Giger & Schaffner, 1975; Smith & Levy, 1990). Heit *et al.* (1981) have also presented the high ratio of fluoranthene and pyrene to their respective alkyl derivatives as evidence for recent combustion PAH input. In the current study all the Silk Stream and Welsh Harp sites exhibited PAH distributions in the water phase and surface sediment in which the parental PAHs predominated over their methylated homologues. The methyl derivatives of fluoranthene and pyrene were not detected at Sites 1, 3 or 9 but were present in the sediment in mean ratios of 1:35 at Site 4 and at 1:8 at the other sites (5, 6, 7 and 8) relative to the parent compounds. No other alkyl groups were recorded at any of the sites. In comparison, Heit *et al.* (1981) reported values of 1:20-50 (methyl - fluoranthene:fluoranthene combined with methyl-pyrene:pyrene) in sediment from one field site and of 1:80 from another site thus showing in general a greater predominance of the parent compound over the alkyl derivatives than that observed in the Silk Stream catchment. A possible explanation of the ratios obtained at the Silk Stream and Welsh Harp sites is that the combustion derived PAHs, while dominating the PAH assemblage may be masking a lower level fossil fuel (unburnt) fluoranthene and pyrene signature. This argument is supported by the fact that phenanthrene is found in absolute terms in high concentrations which is indicative of unburnt petroleum input (Plowchalk & Zagorski, 1986). The absence of alkyl substituted PAHs at Site 3 and the differences in ratio of parent to methylated derivatives between Site 4 (Rushgrove Park) and the lower Silk Stream suggest that unburnt petroleum products may become proportionately of greater significance to the overall values obtained at the downstream sites. At the upstream site (Site 3) where methyl: parent ratios are lowest combustion sources must be relatively highest. Thus at this site relatively long range airbourne sources are more dominant than at the other sites. Hydrocarbon profiles intermediate between unused oil

(high alkyl derivative concentration) and straight combustion products (low alkyl derivative concentration) are often present in sump oils due to the gradual build up of pyrogenic PAH and may therefore be important contributors to the lower Silk Stream hydrocarbon levels since this is the general characteristic of these sites. Latimer *et al.*, (1990) have suggested sump oils to be the principal source of hydrocarbons in stream sediments in urban areas. In most environmental samples however, PAHs from both petroleum products and pyrogenic processes are present and therefore, as in the currently reported findings the profile reflects the mixed origins of the contaminating products.

4.6 SUMMARY

The chemical and biological surveys undertaken illustrated a deterioration in the water quality of the Silk Stream with downstream progression. The DO readings provided examples of the decline: at the upstream sites, saturations of 70% were consistently found whereas in the lower reaches of the Silk Stream, values rarely exceeded 35%. Sediment oxygen demand measurements were also consistent with these findings. Biological water quality also mirrored the changes in chemical measurements: a decline in species richness from 14 at Site 3 to 2 at Site 6 was recorded. For all the measured parameters, an improvement in water quality was found at Site 9 due to the effects of the likely precipitation of particulate-associated pollutants at the inlet of the Silk Stream to the receiving basin.

Surveys of hydrocarbons in sediments and water revealed that high levels of contamination were present, particularly at the downstream Silk Stream sites, which were about an order of magnitude higher than the headwater sites. Again, at the receiving basin, a fall in hydrocarbon concentrations was recorded which represented a substantial improvement in water quality. The use of the Carbon Preference Index (CPI) showed that biogenic contributions were present at all sites and were derived from higher plant waxes while contributions from algae were probably negligible. Analysis of the PAH assemblage showed that the measured compounds were chiefly of combustion origin although contributions of lubricating oils increased at the lower Silk Stream sites.

Chapter 5

HYDROCARBON BIOACCUMULATION BY CAGED MACROINVERTEBRATES

5.1 INTRODUCTION

In Chapter 4, a clear trend of downstream reduction in invertebrate taxa in the Silk Stream catchment was reported. These observations were consistent with previous observations at similar sites in London (Shutes, 1984, 1985; Bascombe *et al.*, 1988) and elsewhere (e.g. Garie & McIntosh, 1986). Hydrocarbon concentrations found in the water and sediment phases have also been shown to be associated with the known increase in urbanization which occurs within the catchment (Hall, 1977). In this chapter, data will be presented relating to the mortality rates and hydrocarbon accumulation levels found in three invertebrate and one fish species, with particular attention being paid to the responses of *Asellus aquaticus* and *Lymnaea peregra*.

5.2 SELECTION OF TEST ORGANISM

5.2.1 Macroinvertebrate species

Previous aquatic biomonitor studies have used the mollusc group extensively because of their sedentary lifestyle, wide distribution (including polluted areas), ability to accumulate hydrocarbons and low depuration rates (e.g. Fossato & Siviero, 1974; Boom, 1987; Moore *et al.*, 1989). Two principal reasons for the high hydrocarbon accumulation potentials in this group are the high levels of lipids in mollusc tissue and the absence or scarcity of mixed function oxidase removal systems (see Section 2.6). Other invertebrate groups, e.g. Crustacea, have been used less extensively as biomonitors. In the present study, three invertebrate species, *A. aquaticus*, *G. pulex* and *L. peregra* and one fish species, the stickleback, *Gasterosteus aculeatus* were considered for use in biomonitor tests.

Using the cage system described in Section 3.2, field trials showed *A. aquaticus* to be

tolerant of prevailing conditions encountered in the lower Silk Stream compared with both *L. peregra* and *G. pulex* as indicated by their respective mortality rates. In initial feasibility trials carried out at the lower Silk Stream sites, median survival durations of between 5 - 24 days for *G. pulex*, 16 - 28 days for *A. aquaticus* and between 10 - 25 days for *L. peregra* were recorded. A general feature of the mortality trials was the high rates recorded for each of the three test species during wet weather. During these conditions, 100% mortality occurred within 14 days for *A. aquaticus* and *L. peregra*. With *G. pulex*, 100% mortality occurred within 7 days. Although survival rates for *G. pulex* at the receiving basin (Site 9) were high, the results obtained from the lower Silk Stream locations (Sites 5, 6 and 7) were generally much lower and, as a result, *G. pulex* was considered inappropriate as a biomonitor. Survival rates of *A. aquaticus* were also high at Site 9 but, in contrast to *G. pulex*, this species also displayed good survival rates at the lower Silk Stream sites. Populations of *A. aquaticus* were recorded throughout the Silk Stream catchment including, albeit on only two sampling occasions, the most highly polluted Site 6 and this species was also abundant at the background collection sites. An extensive body of literature also exists on its ecology (e.g. Williams, 1962; Babula, 1979; Murphy & Learner, 1982). *A. aquaticus* was therefore considered a suitable biomonitor.

The trials with *L. peregra* suggested it to be of intermediate tolerance between *A. aquaticus* and *G. pulex* as indicated by its mortality rate. In order to provide a contrasting biomonitor to *A. aquaticus* in terms of physiology, feeding strategies and life cycle, *L. peregra* was therefore considered the most suitable second organism. This organism was also widely distributed throughout the Silk Stream catchment.

5.2.2 Fish species

Mortality rate trials using *G. aculeatus* resulted in 100% mortality within 5 days of emplacement at Sites 5, 6, 7 and 9 despite the fact that feral *G. aculeatus* were distributed throughout the Silk Stream catchment. The lower flow rates and reduced food availability within the cages must therefore have lowered tolerances to the low DO levels encountered at these sites (see Section 4.4). As a consequence of these results for the fish and invertebrate species, modifications to the cage design were made in

order to reduce siltation. These are discussed fully in Chapter 3.2. At this time, however, the use of *G. aculeatus* as a biomonitor species was discontinued.

5.3 USE OF BASAL AND SUSPENDED CAGES

The general pattern of survival rate for *A. aquaticus* in basal and suspended cages simultaneously located at two contrasting sites (Sites 7 and 9) can be seen in Fig. 5.1. The results show that mortality rate was clearly highest at Site 7 but also that intra-site differences were clearly observable. At both sites, the mortality rate (excluding the final Site 7 visit) was lower in the suspended cage compared to the basal cage. At both sites this differentiation becomes distinct after approximately 7 days. At Site 7, the difference is probably the consequence of a reduction in suspended material entering the suspended cage resulting in reduced organism stress by smothering. At Site 9, basal cages were not exposed to such fine organic sediments but two other factors may be important at this site. Firstly, occlusion of the cage nylon mesh windows by resident *Cladophora* sp. appeared to be more pronounced in basal cages and secondly, predation of *A. aquaticus* by the flatworm *Polycelis nigra* which was seasonally abundant at Site 9 was possible since these flatworms were found to penetrate the cage windows to a greater extent in substrate attached cages.

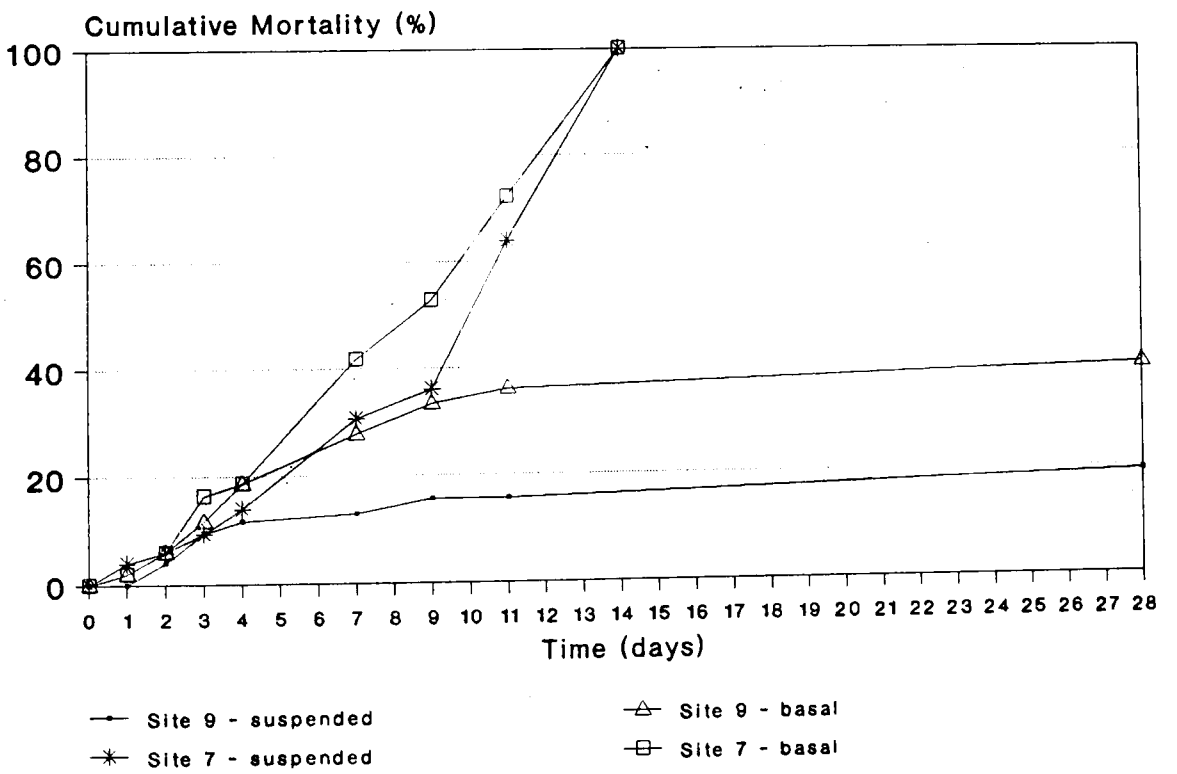


Fig. 5.1 Mortality rates in basal and suspended cages at Sites 7 and 9 for Trial 1.

5.4 HYDROCARBON CONCENTRATIONS IN CAGED *G. PULEX* AND FERAL *G. ACULEATUS*

G. pulex (25 individuals) collected from the background site, were exposed to the polluted waters of the Silk Stream in a cage placed at Site 7, below the oil boom, for two weeks between 12 July and 25 July 1989. The organisms were all dead when collected but were relatively undecayed and were analyzed as described in Method 1, Section 3.3. The measured tissue alkane and PAH levels are presented in Tables 5.1 and 5.2 respectively.

TABLE 5.1

n - ALKANE CONCENTRATIONS ($\mu g\ g^{-1}$) IN WHOLE BODY

G. pulex EXPOSED FOR 14 DAYS AT SITE 7.

alkane	*concentration*	*alkane*	*concentration*	*alkane*	*concentration*
C_{12}	ND	C_{19}	1.11	C_{26}	1.06
C_{13}	ND	C_{20}	0.44	C_{27}	3.87
C_{14}	ND	C_{21}	0.48	C_{28}	2.81
C_{15}	0.19	C_{22}	0.99	C_{29}	3.22
C_{16}	0.29	C_{23}	1.72	C_{30}	0.64
C_{17}	0.43	C_{24}	1.17	C_{31}	1.59
C_{18}	0.80	C_{25}	1.73	C_{32}	0.71

The most notable feature of the analyzed alkane assemblage is the odd carbon predominance in the high molecular weight range (C_{23} -C_{32}) that exists despite the sediment and water phases in this area exhibiting CPIs that are much lower. The alkanes detected would undoubtedly represent biogenic inputs, and on initial examination of the data, it would seem as though these compounds have a greater bioavailability than the even carbon chain numbered alkanes in this range. The PAH distribution (Table 5.2) also differs from the ambient concentrations in water and sediment at this site. Fluoranthene and pyrene, the most abundant PAHs in the non-biological samples, are present at lower concentrations than both benzo(a)anthracene and the benzofluoranthenes indicating a lower bioavailability of the former compounds. These features will be further discussed in later sections with respect to *A. aquaticus* and *L. peregra*.

G. aculeatus individuals were captured using a standard 1mm sampling net. Analyses of tissue samples were performed as described in Section 3.3. The alkane concentrations found in the stickleback show higher levels in the fish caught at Site 3 compared to Site 5 (Figs. 5.2 and 5.3) although this did not represent a significant difference at the 5% level (paired t-test). Therefore, despite there being significantly higher alkanes in water and sediment phases at Site 5, this is not mirrored by the alkane levels in fish. High mobility and avoidance behaviour would be important factors in explaining the lack of correlation between the recorded species concentrations and those of the surrounding environment. Also, since whole body samples were analyzed (as opposed to tissues and organs) it may be the case that a large proportion of the hydrocarbons were surface adsorbed and therefore the surface area of the sample and not ambient hydrocarbon concentration (beyond a threshold value) would determine the final tissue concentration.

TABLE 5.2

PAH CONCENTRATIONS IN WHOLE BODY *G. pulex* EXPOSED AT SITE 7 (14 DAYS)

Compound	*Concentration ($\mu g\ g^{-1}$)*
Fluorene	0.35
Phenanthrene	0.49
Fluoranthene	1.30
Pyrene	0.79
Benzo(a)anthracene	1.51
Chrysene	1.03
Benzo(b)fluoranthene and Benzo(k)fluoranthene	3.90
Benzo(a)pyrene	0.96

The PAH concentrations recorded in *G. aculeatus* show higher overall concentrations in those specimens caught at Site 5 compared with Site 3 although the results were highly variable. The overall values also tend to be generally lower than those obtained in invertebrate species and may be attributed to the more efficient removal mechanisms known to exist in fish (Varanasi *et al.*, 1989). These findings have some significance in terms of reducing the possibility of food web biomagnification within the freshwater environment. Contrary to the values obtained from the caged *G. pulex* samples,

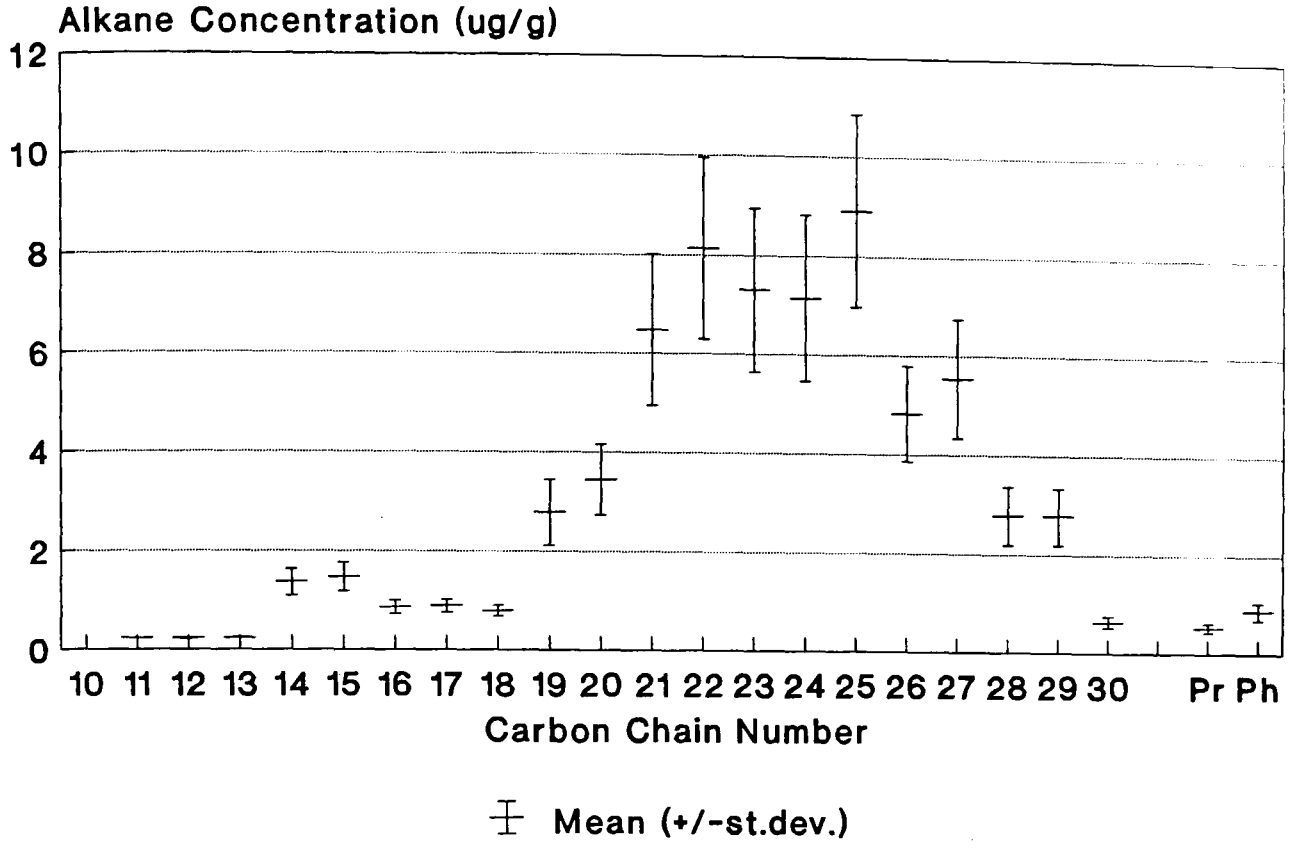


Fig. 5.2 Alkane concentrations in *G. aculeatus* (Site 3).

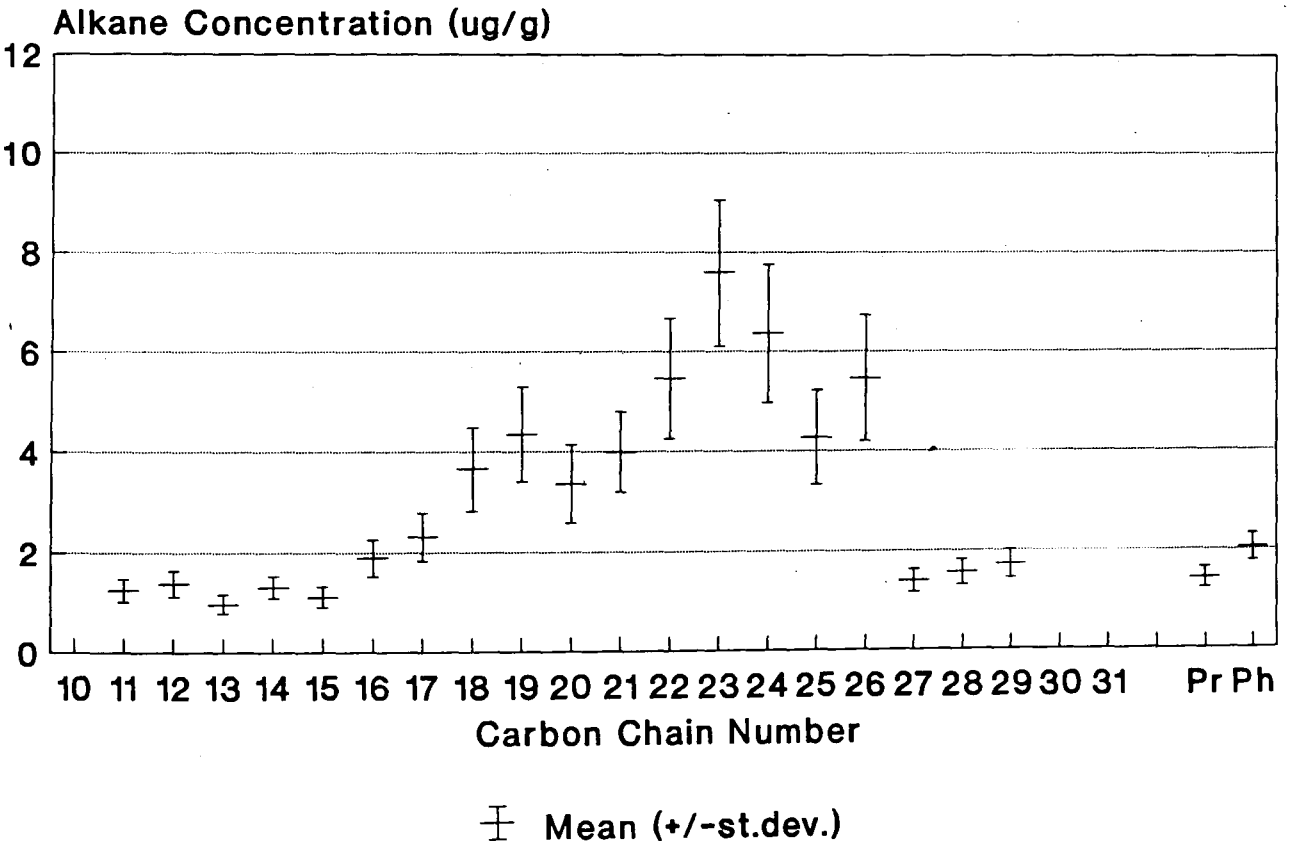


Fig. 5.3 Alkane concentrations in *G. aculeatus* (Site 5).

benzo(a)anthracene and the benzofluoranthenes were not detected or were present at much lower concentrations in *G. aculeatus* samples (Table 5.3). Also, in contrast with the sediment and water samples, the aromatic component was dominated by naphthalene and methyl naphthalene. As a result of the abandonment of *G. aculeatus* as a biomonitor species the changes between levels and distribution of the hydrocarbons between water/sediment phases and fish tissue is unclear and would require further investigation. Future work using *G. aculeatus* would require a new cage design, of larger volume and larger mesh size.

TABLE 5.3

PAH CONCENTRATION IN WHOLE BODY *Gasterosteus aculeatus* ($\mu g\ g^{-1}$)

Compound	Site 3	Site 5
Naphthalene	3.6	1.5
Methyl - naphthalene	1.4	8.7
Fluorene	ND	0.2
Phenanthrene	ND	0.6
Methyl - phenanthrene	1.3	0.8
Fluoranthene + pyrene	0.1	0.1
Benzo(a)anthracene + chrysene	0.3	ND

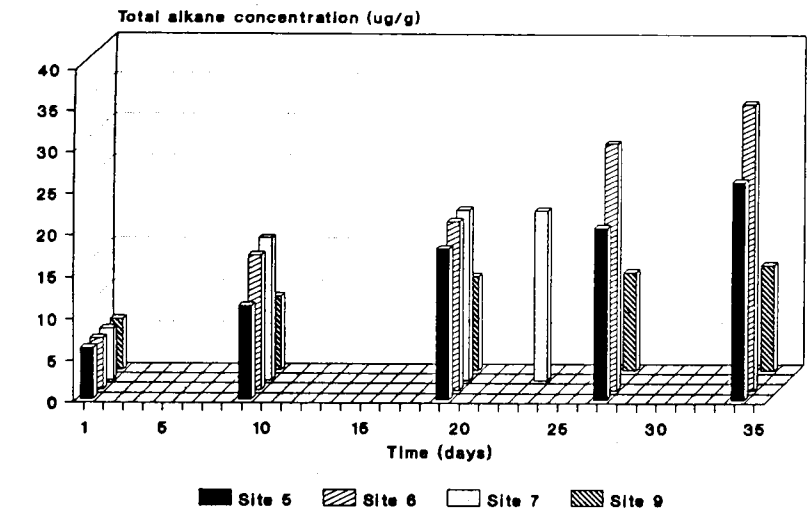
5.5 SPATIAL AND TEMPORAL VARIATIONS IN HYDROCARBON LEVELS IN CAGED MACROINVERTEBRATES

This section covers the results of hydrocarbon analyses from field trials using *A. aquaticus* and *L. peregra*. The exposure periods are described as two separate trial series, Trials 1 - 2 and Periods A - E. Both sets of exposure trials concentrated on the important downstream region of the Silk Stream (Sites 5, 6 and 7) and the receiving basin (Site 9). In the first series of trials (Trials 1 and 2), only *A. aquaticus* was used as a biomonitor and in this series, paired exposures using a combination of basal and suspended cages were used. In the second series of trials (Periods A - E), both *L. peregra* and *A. aquaticus* were used in duplicate suspended cages at Sites 5, 6 and 7 and in duplicate basal cages at Site 9.

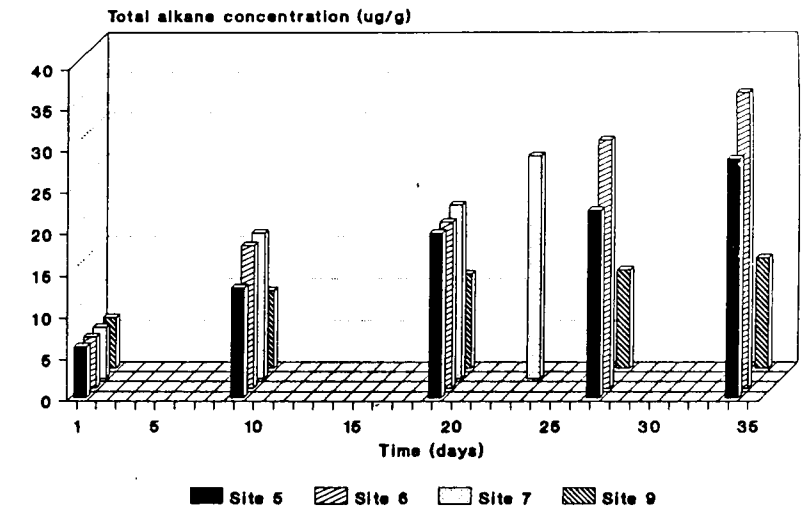
5.5.1 General patterns in total PAH and alkane levels in *A. aquaticus* during Trials 1 and 2

Following the initial feasibility tests on the four test organisms, two field trials involving full hydrocarbon analyses of exposed *A. aquaticus* tissue were undertaken in the winter period of October 1990 to January 1991. In these trials (Trials 1 and 2) an important aim was to establish the importance of the position of the cages (suspended or basal) in terms of its effect on organism survival and the accumulation of hydrocarbons. Thus, organisms were placed in basal and suspended cages simultaneously at each sampling location. The overall spatial and temporal variations in total PAH and alkane organism concentrations are presented in Figs 5.4 and 5.5 for the basal and suspended cages for the measurement periods 24/10/90 - 3/12/90 (Trial 1) and 5/12/90 - 17/1/91 (Trial 2).

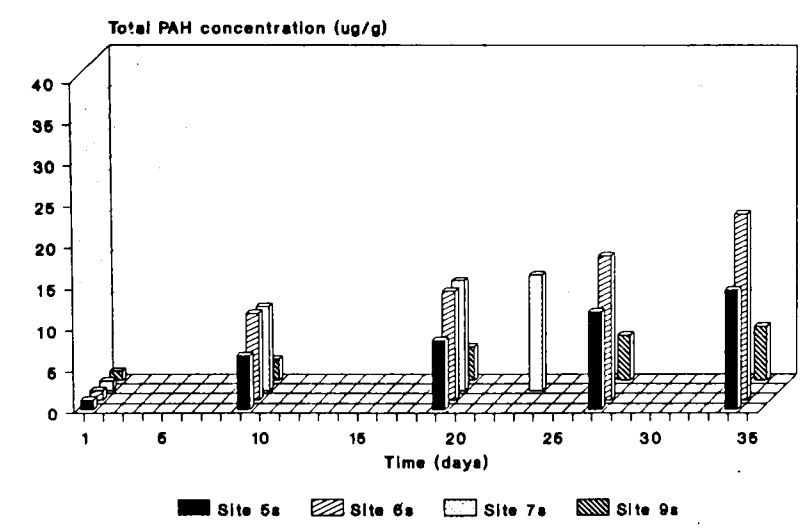
A. aquaticus can be seen to accumulate both aliphatic and aromatic compounds at all the sampling locations. The recorded levels are generally in a higher concentration range compared to those presented in Chapter 2 (Table 2.9). It must be considered, however, that much of the previously reported data refer to wet weight concentrations of hydrocarbons and therefore given the moisture content of *A. aquaticus* (80%) a factor of 5 may be applied to give greater comparability. As discussed in Section 4.1., the high discharge of urban runoff in the Silk Stream catchment and the resultant elevated hydrocarbon concentrations in sediment and water result in the organisms in the Silk Stream being exposed to generally higher concentrations than those reported elsewhere. The results obtained for Trial 1, shown in Fig. 5.4, indicate that the total alkane body concentrations rise during the initial (9 day) exposure to achieve their highest value at Site 7 immediately below the oil boom (mean value for suspended and basal cages; 17.2 $\mu g\ g^{-1}$) with mean values of 16.6 $\mu g\ g^{-1}$ at Site 6, 12.3 $\mu g\ g^{-1}$ at Site 5 and 9.3 $\mu g\ g^{-1}$ at Site 9. After 19 days however, the organisms at Sites 5, 6 and 7 have attained similar concentrations in the range 18-21 $\mu g\ g^{-1}$ while the lake site (9) has a reduced mean value of 11.3 $\mu g\ g^{-1}$.



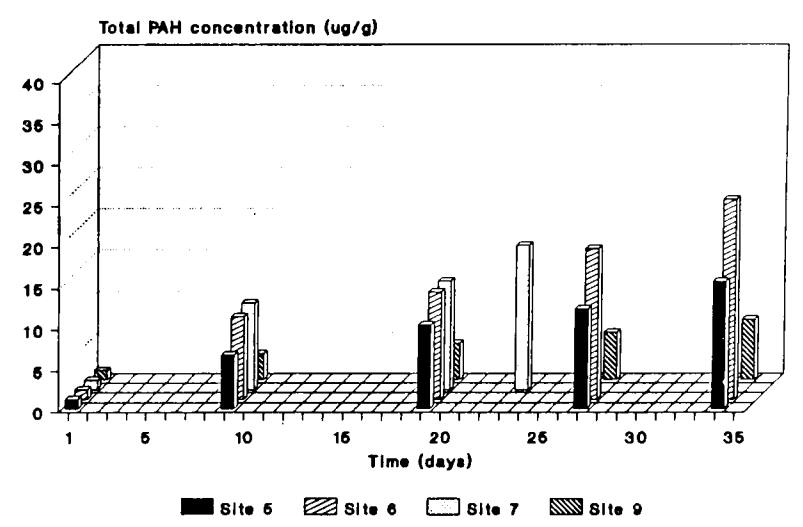
ALKANES - SUSPENDED



ALKANES - BASAL

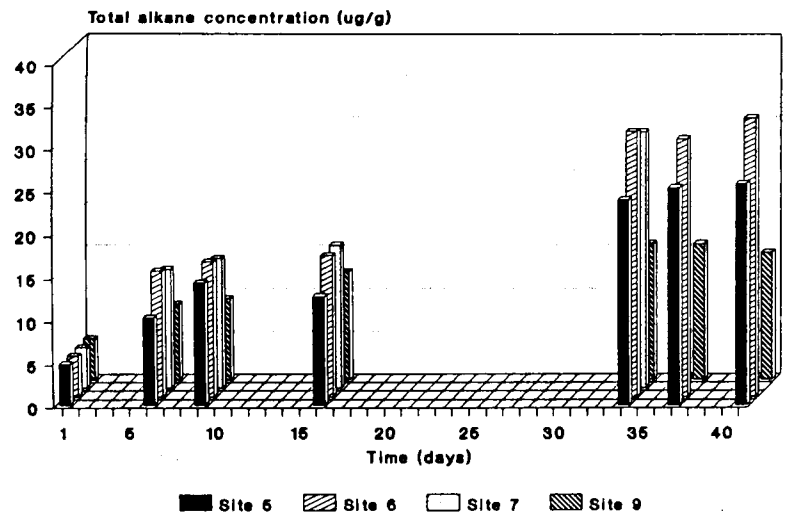


PAH - SUSPENDED

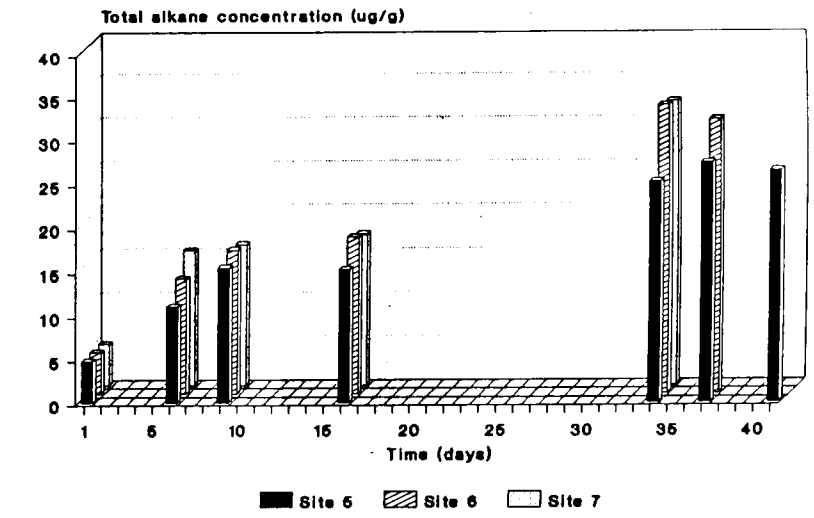


PAH - BASAL

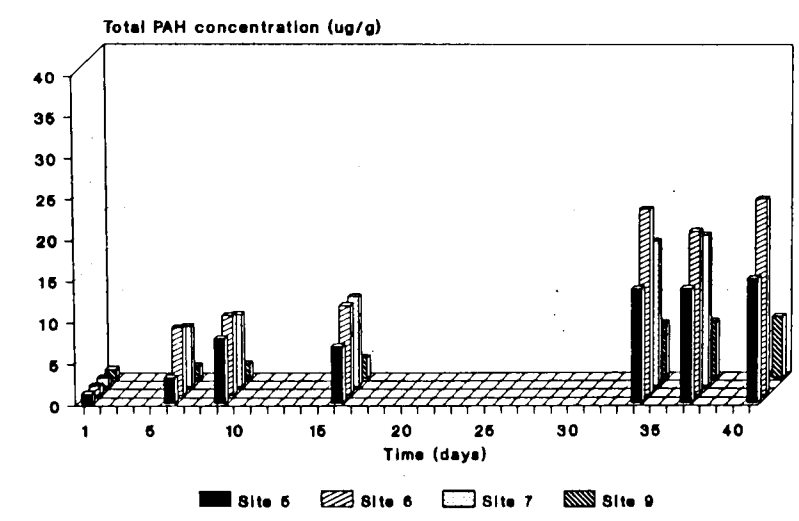
Fig. 5.4 Temporal and spatial variation of total alkanes and PAH levels in *A. aquaticus* in basal and suspended cages at Sites 5, 6 7 and 9 during Trial 1.



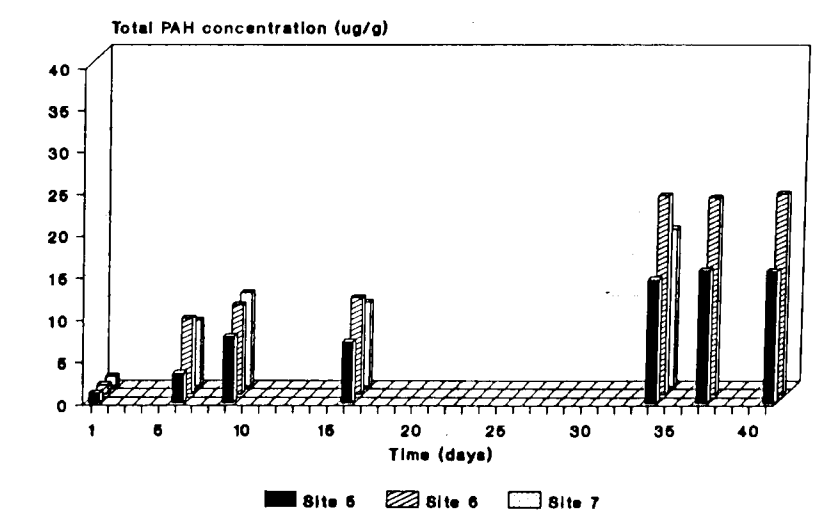
ALKANES - SUSPENDED



ALKANES - BASAL



PAH - SUSPENDED



PAH - BASAL

Fig. 5.5 Temporal and spatial variation of total alkanes and PAH levels in *A. aquaticus* in basal and suspended cages at Sites 5, 6 7 and 9 during Trial 2.

As described in Section 3.4, the animals were normally removed live for analysis. The levels shown at Day 23 at Site 7, however, represent concentrations recorded for dead organisms at this site as 100% mortality had occurred at this time. There is no significant difference between levels in live organisms after 19 days and dead organisms after 23 days in the suspended organisms but an increase to 26.7 $\mu g\ g^{-1}$ occurs in the basal cages. The dead organisms removed from Site 7 at this time were found to be decayed to a greater extent (as indicated by coloration) in the basal cage. It appeared therefore, that little if any loss of aliphatics occurred immediately following organism death at this site.

The ratio of mean total aliphatic levels after 34 days compared with background *A. aquaticus* concentrations were 4.5, 5.8 and 2.1 at Sites 5, 6 and 9, respectively. After 19 days the ratios were 3.1, 3.3, 3.4 and 1.8 at Sites 5, 6, 7 and 9, respectively. Similar relative ratio values can be observed in the PAH results. The ratios of final concentration (34 days) to initial background levels were 12.9, 20.1 and 6.0 at Sites 5, 6 and 9 respectively. In comparison, the values after 19 days were 8.0, 11.2 11.3 and 3.6 at Sites 5, 6, 7 and 9, respectively. The higher overall ratios for the PAH group are due to the lower background concentrations detected in *A. aquaticus* ($\sim 1\ \mu g\ g^{-1}$) in comparison with values of approximately 5 $\mu g\ g^{-1}$ for the aliphatics. The major trend differences between PAHs and aliphatics is that the *A. aquaticus* PAH levels at Site 5 are consistently lower than at Site 6 and 7 throughout Trial 1, including Day 19. At Site 7 a small increase was recorded in dead suspended organisms. It is unclear as to what extent this apparent retainment of PAH in *A. aquaticus* is significant in terms of PAH cycling.

The overall pattern of hydrocarbon levels suggest that in Trial 1, equilibrium levels may not have been attained. However, examination of the temporal changes of individual compounds suggests that a levelling off of some compound concentrations does occur towards the end of the exposure period.

Similar overall patterns were found in the second trial period (Trial 2). However, there was little change in the overall alkane and PAH levels between days 34 to 41 and at Site 9 there was a small decrease in body burdens of suspended organisms during this

period (Fig. 5.5). Equilibrium levels, therefore, appear to have been established during this trial. The general rates of hydrocarbon level increases at the various sites are comparable for the two initial trials. However, the concentrations after equivalent exposure periods are marginally higher at Sites 5, 6 and 7 in the first trial but higher in the second trial at Site 9.

5.5.2 Temporal and spatial variation for individual alkanes

Levels for individual alkanes in Trials 1 and 2 are presented in Tables 5.4 - 5.7. The individual temporal changes for the C_{22} and C_{27} alkanes have been selected to compare the concentration variations of alkanes that are likely to have different sources. C_{22} is probably entirely of anthropogenic origin while C_{27} is likely to be of both anthropogenic and biogenic origin. Initially, as a result its higher background concentrations, C_{27} exceeds C_{22}, and during Trials 1 and 2, there is a tendency for C_{27} levels to remain higher than C_{22} levels at all sites. If tissue concentrations reflect those in the surrounding sediment, the concentrations of C_{22} might be expected to be similar to C_{27} as was found in the sediment at Sites 5, 6 and 7. At Site 7, in Trial 1, the alkane concentrations appear to approach each other but there is no evidence of a similar effect at Sites 5, 6, or 9 (Fig. 5.6 - 5.7). In Trial 2, surprisingly, the effect is not repeated at Site 7 but can be seen at Sites 5 and 6. In both trials at Site 9 the relative concentrations of the two compounds remain distinct for the entire test duration with C_{22} levels remaining relatively low and constant ($< 0.80\ \mu g\ g^{-1}$). Thus, with increasing exposure time the difference between C_{22} and C_{27} concentrations at Site 9 does not lessen. In contrast, the three Silk Stream sites each display a convergence of the two compounds to a certain extent, reflecting the prevailing ambient levels. A more thorough discussion of the uptake of biogenic and non-biogenic hydrocarbons can be found in the section on Carbon Preference Index (5.5.8.1) which incorporates all hydrocarbons of carbon chain lengths greater than C_{20}, including C_{22} and C_{27}. Typical examples of the overall profiles for individual alkanes monitored during Trial 1 are shown in Figs. 5.8 - 5.10. The alkane concentrations are generally low in the range C_{11}-C_{13} followed by a progressive increase to a more level profile which occurs between C_{18} and C_{24}. The biogenically derived odd-carbon numbered alkanes are prevalent (C_{25},

TABLE 5.4

INDIVIDUAL ALKANE CONCENTRATIONS IN *ASELLUS AQUATICUS* AT SITES 5, 6, 7 AND 9 DURING TRIAL 1 (SUSPENDED CAGES).

	SITE 5 SUSPENDED TRIAL 1 (Days)					SITE 6 SUSPENDED TRIAL 1 (Days)				
alkane	0	8	18	26	33	0	8	18	26	33
C_{10}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C_{11}	ND	ND	ND	ND	0.05	ND	0.05	0.05	0.05	0.07
C_{12}	ND	ND	ND	ND	0.07	ND	0.06	0.11	0.39	0.05
C_{13}	0.07	0.08	0.12	0.05	0.11	0.07	0.10	0.32	0.48	0.68
C_{14}	0.05	0.09	0.34	0.05	0.27	0.05	0.19	0.48	0.75	1.01
C_{15}	0.08	0.15	0.47	0.35	0.46	0.08	0.47	0.65	1.17	1.39
C_{16}	0.17	0.28	0.65	0.79	0.79	0.17	0.58	0.69	1.11	1.62
C_{17}	0.28	0.50	0.93	0.95	1.07	0.28	0.74	0.82	1.44	1.71
Pr	0.18	0.33	0.57	0.59	0.65	0.18	0.49	0.50	0.83	1.12
C_{18}	0.32	0.55	0.98	0.98	0.95	0.32	0.78	0.96	1.39	1.61
Ph	0.13	0.34	0.64	0.63	0.68	0.13	0.37	0.47	0.81	1.21
C_{19}	0.31	0.68	1.07	1.21	0.35	0.31	0.76	0.93	1.52	1.86
C_{20}	0.19	0.80	1.18	1.29	0.58	0.19	0.89	1.10	1.50	1.84
C_{21}	0.18	0.79	1.13	1.23	0.35	0.18	0.95	1.10	1.60	1.76
C_{22}	0.17	0.68	0.90	1.19	1.44	0.17	0.93	1.28	1.72	1.92
C_{23}	0.16	0.43	0.89	1.18	1.40	0.16	1.01	1.17	1.74	1.90
C_{24}	0.16	0.56	0.90	1.37	1.30	0.16	0.92	1.11	1.68	2.03
C_{25}	0.24	0.65	0.89	1.25	1.68	0.24	1.06	0.86	1.52	1.96
C_{26}	0.22	0.44	0.81	1.12	1.58	0.22	0.65	0.95	1.16	1.64
C_{27}	1.18	1.28	2.03	2.31	2.87	1.18	1.71	2.06	2.79	3.00
C_{28}	0.17	0.45	0.63	0.93	0.82	0.17	0.79	0.94	1.35	1.63
C_{29}	1.25	1.37	2.17	2.40	3.41	1.25	1.67	2.04	2.52	3.67
C_{30}	0.19	0.36	0.45	0.65	0.82	0.19	0.75	0.69	1.25	1.39
C_{31}	0.61	0.91	1.32	1.32	2.34	0.61	0.84	1.42	1.59	1.78
C_{32}	0.15	0.21	0.26	0.15	0.12	0.15	0.29	0.54	0.93	0.92
C_{33}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

	SITE 7 SUSPENDED TRIAL 1 (Days)					SITE 9 SUSPENDED TRIAL 1 (Days)				
	0	8	18	26	33	0	8	18	26	33
C_{10}	ND	0.05	ND	0.05	ND	ND	ND	0.05	ND	ND
C_{11}	ND	0.07	0.05	0.10	0.13	ND	ND	0.05	0.05	0.05
C_{12}	ND	0.08	0.11	0.17	0.39	ND	0.05	0.11	0.06	0.06
C_{13}	0.07	0.22	0.17	0.23	0.40	0.07	0.05	0.15	0.09	0.08
C_{14}	0.05	0.20	0.24	0.41	1.16	0.05	0.13	0.14	0.12	0.24
C_{15}	0.08	0.38	0.41	0.35	1.25	0.08	0.25	0.22	0.35	0.39
C_{16}	0.17	0.55	0.65	0.56	1.48	0.17	0.39	0.44	0.49	0.64
C_{17}	0.28	0.77	0.90	0.83	1.78	0.28	0.43	0.65	0.55	0.77
Pr	0.18	0.53	0.63	0.57	1.30	0.18	0.42	0.32	0.28	0.43
C_{18}	0.32	0.75	0.89	0.97	1.50	0.32	0.50	0.61	0.68	0.73
Ph	0.13	0.46	0.65	0.64	1.10	0.13	0.46	0.34	0.35	0.48
C_{19}	0.31	0.82	0.89	0.90	1.75	0.31	0.49	0.77	0.72	0.80
C_{20}	0.19	1.05	1.17	1.23	1.81	0.19	0.38	0.69	0.71	0.82
C_{21}	0.18	1.23	1.45	1.13	1.86	0.18	0.52	0.58	0.61	0.65
C_{22}	0.17	1.10	1.30	0.98	1.94	0.17	0.38	0.38	0.59	0.54
C_{23}	0.16	0.91	1.51	1.28	2.24	0.16	0.52	0.43	0.49	0.64
C_{24}	0.16	0.94	1.18	1.40	2.12	0.16	0.51	0.40	0.48	0.49
C_{25}	0.24	1.10	1.23	1.25	1.98	0.24	0.46	0.38	0.59	0.47
C_{26}	0.22	0.88	1.11	1.35	1.92	0.22	0.46	0.32	0.31	0.45
C_{27}	1.18	1.64	1.90	2.03	2.48	1.18	1.50	1.76	1.54	1.66
C_{28}	0.17	1.09	1.23	1.17	1.75	0.17	0.39	0.33	0.51	0.39
C_{29}	1.25	1.52	2.14	1.85	3.40	1.25	1.34	1.64	1.75	1.89
C_{30}	0.19	0.66	0.78	1.03	1.47	0.19	0.45	0.42	0.41	0.51
C_{31}	0.61	0.78	0.76	0.71	1.41	0.61	0.66	0.78	0.59	0.92
C_{32}	0.15	0.24	0.42	0.40	0.56	0.15	0.05	0.12	0.20	0.10
C_{33}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE 5.5

INDIVIDUAL ALKANE CONCENTRATIONS IN *ASELLUS AQUATICUS* AT SITES 5, 6, 7 AND 9 DURING TRIAL 1 (BASAL CAGES).

	SITE 5 BASAL TRIAL 1 (Days)					SITE 6 BASAL TRIAL 1 (Days)				
alkane	0	8	18	26	33	0	8	18	26	33
C_{10}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C_{11}	ND	ND	ND	ND	0.05	ND	0.05	ND	ND	0.13
C_{12}	ND	0.07	0.05	0.05	0.08	ND	0.05	ND	0.09	0.39
C_{13}	0.07	0.09	0.16	0.05	0.17	0.07	0.10	ND	0.30	0.40
C_{14}	0.05	0.36	0.21	0.16	0.23	0.05	0.26	0.31	0.86	1.16
C_{15}	0.08	0.78	0.52	0.61	0.53	0.08	0.35	0.60	1.13	1.25
C_{16}	0.17	0.49	0.45	0.87	0.85	0.17	0.69	0.73	1.21	1.48
C_{17}	0.28	0.57	0.81	1.18	1.20	0.28	0.84	0.87	1.43	1.78
Pr	0.18	0.37	0.52	0.60	0.60	0.18	0.53	0.58	0.81	1.30
C_{18}	0.32	0.55	1.17	1.26	1.12	0.32	0.88	0.95	1.58	1.50
Ph	0.13	0.34	0.68	0.65	0.69	0.13	0.50	0.46	0.94	1.10
C_{19}	0.31	0.55	1.15	1.12	1.35	0.31	0.95	0.82	1.48	1.75
C_{20}	0.19	0.70	1.20	1.34	1.56	0.19	0.76	0.95	1.69	1.81
C_{21}	0.18	0.72	1.40	1.20	1.45	0.18	1.17	1.18	1.64	1.86
C_{22}	0.17	0.73	1.22	1.16	1.50	0.17	1.26	1.34	1.67	1.94
C_{23}	0.16	0.66	0.89	1.38	1.61	0.16	1.32	1.26	1.87	2.24
C_{24}	0.16	0.52	1.14	1.22	1.59	0.16	1.10	1.13	1.71	2.12
C_{25}	0.24	0.47	1.33	1.49	1.63	0.24	1.19	0.89	1.85	1.98
C_{26}	0.22	0.81	0.97	1.48	1.68	0.22	0.78	1.09	1.47	1.92
C_{27}	1.18	1.34	2.43	2.22	3.69	1.18	1.67	2.17	2.40	3.26
C_{28}	0.17	0.54	0.66	0.91	1.44	0.17	0.81	0.85	1.48	1.75
C_{29}	1.25	1.50	2.14	2.55	2.99	1.25	1.55	1.97	2.30	3.40
C_{30}	0.19	0.49	0.59	0.75	1.19	0.19	0.36	0.71	1.15	1.47
C_{31}	0.61	0.99	1.18	1.14	2.56	0.61	0.81	1.56	1.72	1.41
C_{32}	0.15	0.18	0.19	0.51	0.22	0.15	0.24	0.60	0.80	0.56
C_{33}	ND	ND	ND	0.23	ND	ND	ND	ND	ND	ND

	SITE 7 BASAL TRIAL 1 (Days)					SITE 9 BASAL TRIAL 1 (Days)				
alkane	0	8	18	23	33	0	8	18	26	33
C_{10}	ND	ND	ND	ND	NA	ND	ND	ND	ND	ND
C_{11}	ND	0.05	0.12	0.19	NA	ND	ND	ND	0.05	0.05
C_{12}	ND	0.17	0.30	0.23	NA	ND	0.05	0.05	0.05	0.05
C_{13}	0.07	0.15	0.21	0.41	NA	0.07	0.04	0.05	0.07	0.09
C_{14}	0.05	0.24	0.27	0.66	NA	0.05	0.12	0.11	0.11	0.152
C_{15}	0.08	0.36	0.45	0.52	NA	0.08	0.19	0.34	0.40	0.56
C_{16}	0.17	0.58	0.76	0.87	NA	0.17	0.35	0.55	0.52	0.65
C_{17}	0.28	0.78	0.85	1.22	NA	0.28	0.39	0.63	0.27	0.77
Pr	0.18	0.50	0.63	0.87	NA	0.18	0.24	0.30	0.65	0.34
C_{18}	0.32	0.82	0.88	1.21	NA	0.32	0.46	0.61	0.23	0.78
Ph	0.13	0.57	0.65	0.82	NA	0.13	0.21	0.28	0.69	0.38
C_{19}	0.31	0.89	1.05	1.35	NA	0.31	0.38	0.62	0.72	0.72
C_{20}	0.19	0.91	1.28	1.28	NA	0.19	0.42	0.54	0.53	0.65
C_{21}	0.18	1.14	1.19	1.48	NA	0.18	0.37	0.57	0.45	0.50
C_{22}	0.17	0.91	1.30	1.19	NA	0.17	0.50	0.44	0.64	0.53
C_{23}	0.16	1.20	1.42	1.55	NA	0.16	0.39	0.50	0.44	0.60
C_{24}	0.16	1.27	1.38	1.33	NA	0.16	0.37	0.45	0.37	0.47
C_{25}	0.24	1.12	1.36	1.74	NA	0.24	0.36	0.38	0.51	0.56
C_{26}	0.22	0.87	0.94	1.80	NA	0.22	0.38	0.40	0.38	0.48
C_{27}	1.18	1.56	2.07	2.34	NA	1.18	0.33	1.75	1.83	1.75
C_{28}	0.17	1.13	1.17	1.53	NA	0.17	0.20	0.35	0.47	0.53
C_{29}	1.25	1.48	1.71	2.27	NA	1.25	0.40	1.62	1.64	1.70
C_{30}	0.19	0.89	0.81	1.47	NA	0.19	0.75	0.32	0.46	0.21
C_{31}	0.61	0.75	0.80	1.56	NA	0.61	0.07	0.91	0.68	0.81
C_{32}	0.15	0.14	0.56	0.54	NA	0.15	0.05	0.10	0.12	0.14
C_{33}	ND	0.23	ND	ND	NA	ND	ND	ND	ND	ND

TABLE 5.6

INDIVIDUAL ALKANE CONCENTRATIONS IN *ASELLUS AQUATICUS* AT SITES 5 ,6, 7 AND 9 DURING TRIAL 2 (SUSPENDED CAGES).

	SITE 5 SUSPENDED TRIAL 2 (DAYS)							SITE 6 SUSPENDED TRIAL 2 (DAYS)						
Alkane	0	5	8	15	33	36	40	0	5	8	15	33	36	40
C_{10}	ND	ND	ND	ND	ND	ND	ND	ND	0.05	ND	ND	ND	ND	0.05
C_{11}	0.05	ND	ND	ND	ND	0.13	0.12	0.05	0.14	0.05	0.10	0.16	ND	ND
C_{12}	ND	ND	0.09	0.07	0.07	0.29	0.37	ND	0.21	0.17	0.38	0.46	0.11	0.16
C_{13}	0.06	0.07	0.17	0.11	0.31	0.22	0.49	0.06	0.40	0.45	0.66	0.57	0.20	0.43
C_{14}	0.18	0.34	0.28	0.19	0.62	0.74	0.88	0.18	0.44	0.49	0.52	0.71	0.39	0.76
C_{15}	0.15	0.48	0.63	0.58	0.78	0.96	1.05	0.15	0.62	0.75	0.73	1.04	0.85	1.10
C_{16}	0.21	0.45	0.74	0.71	1.58	1.45	1.33	0.21	0.56	0.79	0.78	1.33	1.13	1.32
C_{17}	0.24	0.59	0.98	0.89	1.69	1.68	1.72	0.24	0.73	0.75	0.60	1.45	1.20	1.54
Pr	0.09	0.53	0.57	0.52	0.71	0.71	0.84	0.09	0.54	0.68	0.47	0.72	1.42	1.03
C_{18}	0.21	0.58	0.92	0.94	1.67	1.67	1.68	0.21	0.85	0.80	0.99	1.78	0.78	1.80
Ph	0.05	0.44	0.61	0.49	0.90	0.90	0.89	0.05	0.72	0.53	0.76	0.90	1.67	1.11
C_{19}	0.18	0.73	1.02	0.94	1.76	1.76	1.40	0.18	0.68	0.93	1.14	1.64	0.96	1.75
C_{20}	0.19	0.48	0.85	0.76	1.47	1.47	1.70	0.19	0.59	0.72	0.81	1.74	1.75	1.82
C_{21}	0.19	0.60	0.77	0.59	1.66	1.41	1.34	0.19	0.65	1.46	0.78	1.98	1.71	2.05
C_{22}	0.16	0.62	0.69	0.63	1.30	1.66	1.25	0.16	0.67	0.84	0.58	1.80	1.94	1.67
C_{23}	0.18	0.47	0.66	0.65	0.92	1.30	1.26	0.18	0.81	0.65	0.65	1.72	1.54	1.71
C_{24}	0.11	0.46	0.48	0.42	1.04	0.92	1.14	0.11	0.68	0.67	0.70	1.45	1.78	1.86
C_{25}	0.17	0.46	0.50	0.60	0.74	1.04	0.82	0.17	1.44	0.81	0.64	1.78	1.59	2.11
C_{26}	0.05	0.21	0.39	0.35	2.30	0.74	2.42	0.05	0.74	0.72	0.66	1.42	1.82	1.59
C_{27}	0.74	1.16	1.58	1.33	0.68	2.30	0.97	0.74	1.20	1.46	1.52	2.86	1.38	2.78
C_{28}	0.12	0.38	0.58	0.38	2.38	0.68	2.44	0.12	0.58	0.84	0.92	1.13	2.70	1.54
C_{29}	0.83	1.09	1.46	1.36	0.59	2.38	0.79	0.83	0.41	1.69	1.66	1.21	1.65	2.95
C_{30}	0.15	0.27	0.54	0.49	0.88	0.59	0.83	0.15	0.17	0.78	0.87	0.19	2.43	1.28
C_{31}	0.64	0.71	0.87	0.64	0.07	0.88	0.20	0.64	ND	0.62	0.79	ND	1.05	1.84
C_{32}	0.05	0.07	0.13	0.08	ND	0.07	ND	0.05	ND	0.10	0.05	ND	1.42	0.35
C_{33}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.37	ND

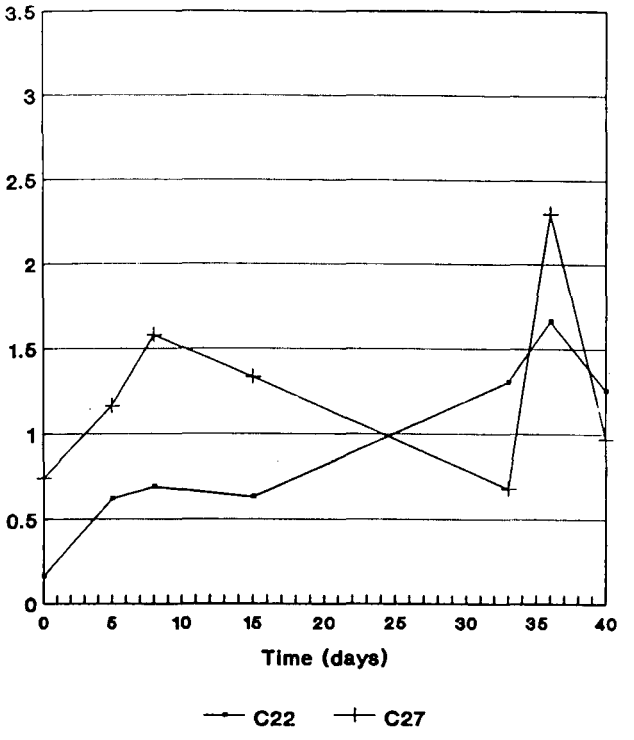
	SITE 7 SUSPENDED TRIAL 2 (DAYS)							SITE 9 SUSPENDED TRIAL 2 (DAYS)						
Alkane	0	5	8	15	33	36	40	0	5	8	15	33	36	40
C_{10}	ND	ND	ND	ND	ND	NA	NA	ND	ND	ND	ND	ND	ND	ND
C_{11}	ND	0.13	0.07	0.05	0.05	NA	NA	ND	0.06	0.05	0.08	0.21	0.10	0.14
C_{12}	ND	0.29	0.12	0.09	0.05	NA	NA	ND	0.10	0.08	0.12	0.20	0.27	0.48
C_{13}	0.05	0.38	0.21	0.29	0.41	NA	NA	0.05	0.23	0.21	0.33	0.47	0.22	0.33
C_{14}	0.18	0.50	0.35	0.58	1.07	NA	NA	0.18	0.31	0.24	0.22	0.46	0.31	0.36
C_{15}	0.15	0.40	0.45	0.65	1.15	NA	NA	0.15	0.30	0.24	0.36	0.42	0.66	0.35
C_{16}	0.21	0.63	0.69	0.73	1.44	NA	NA	0.21	0.35	0.25	0.45	0.53	0.78	0.45
C_{17}	0.24	0.85	0.78	0.84	1.66	NA	NA	0.24	0.58	0.30	0.63	0.75	0.84	0.67
Pr	0.09	0.50	0.43	0.59	0.93	NA	NA	0.09	0.21	0.14	0.32	0.36	0.48	0.55
C_{18}	0.21	0.72	0.75	0.83	1.54	NA	NA	0.21	0.43	0.44	0.64	0.89	0.95	0.81
Ph	0.05	0.45	0.56	0.63	0.89	NA	NA	0.05	0.14	0.17	0.21	0.41	0.53	0.52
C_{19}	0.18	0.86	0.94	0.80	1.52	NA	NA	0.18	0.42	0.53	0.76	0.87	0.92	0.72
C_{20}	0.19	0.62	0.87	0.76	1.60	NA	NA	0.19	0.55	0.52	0.74	0.95	0.81	0.89
C_{21}	0.19	0.70	0.81	0.88	1.46	NA	NA	0.19	0.61	0.57	0.62	0.75	0.72	0.60
C_{22}	0.16	0.61	0.73	0.83	1.48	NA	NA	0.16	0.39	0.44	0.66	0.86	0.83	0.71
C_{23}	0.18	0.76	0.79	0.75	1.55	NA	NA	0.18	0.44	0.42	0.54	0.87	0.79	0.76
C_{24}	0.11	0.52	0.69	0.74	1.52	NA	NA	0.11	0.35	0.40	0.67	0.74	0.62	0.73
C_{25}	0.17	0.71	0.83	0.91	1.69	NA	NA	0.17	0.41	0.59	0.75	0.88	0.70	0.81
C_{26}	ND	0.60	0.71	0.79	1.37	NA	NA	ND	0.47	0.26	0.54	0.40	0.46	0.55
C_{27}	0.74	1.32	1.45	1.42	2.82	NA	NA	0.74	0.97	1.27	1.35	1.72	1.62	1.49
C_{28}	0.12	0.79	0.75	0.89	1.58	NA	NA	0.12	0.24	0.36	0.37	0.48	0.49	0.62
C_{29}	0.83	1.20	1.30	1.52	2.43	NA	NA	0.83	0.91	1.28	1.26	1.59	1.80	1.60
C_{30}	0.15	0.53	0.69	0.79	0.78	NA	NA	0.15	0.13	0.28	0.35	0.48	0.61	0.52
C_{31}	0.64	0.54	0.78	0.86	1.97	NA	NA	0.64	0.42	0.59	0.86	0.99	1.12	0.90
C_{32}	ND	0.22	0.41	0.68	0.79	NA	NA	ND	0.16	0.18	0.28	0.38	0.21	0.30
C_{33}	ND	ND	ND	ND	ND	NA	NA	ND	ND	ND	ND	ND	ND	ND

TABLE 5.7

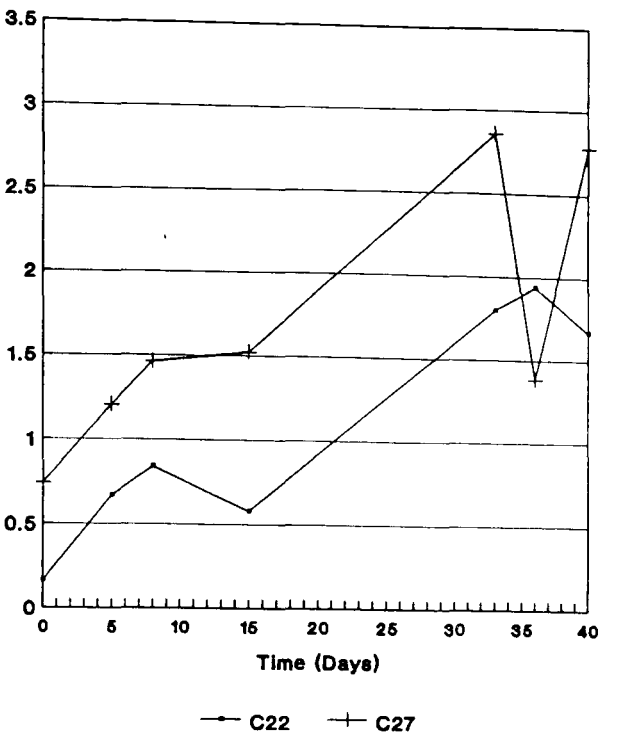
INDIVIDUAL ALKANE CONCENTRATIONS IN *ASELLUS AQUATICUS* AT SITES 5 ,6, 7 AND 9 DURING TRIAL 2 (BASAL CAGES).

	SITE 5 BASAL TRIAL 2 (DAYS)							SITE 6 BASAL TRIAL 2 (DAYS)						
Alkane	0	5	8	15	33	36	40	0	5	8	15	33	35	40
C_{10}	ND	ND	ND	ND	ND	ND	0.05	ND	ND	ND	ND	ND	ND	NA
C_{11}	0.05	ND	ND	ND	ND	0.14	0.15	0.05	ND	0.08	0.13	0.22	0.13	NA
C_{12}	0.05	ND	0.08	0.06	0.07	0.15	0.11	0.05	0.07	0.22	0.26	0.41	0.35	NA
C_{13}	0.05	ND	0.19	0.10	0.21	0.21	0.23	0.05	0.30	0.36	0.49	1.03	0.28	NA
C_{14}	0.18	0.42	0.34	0.26	0.23	0.64	0.39	0.18	0.52	0.54	0.47	0.45	0.76	NA
C_{15}	0.15	0.50	0.75	0.31	0.56	0.97	0.86	0.15	0.70	0.52	0.77	1.38	0.95	NA
C_{16}	0.21	0.55	0.84	0.60	0.82	1.35	1.32	0.21	0.62	0.70	0.80	1.46	1.31	NA
C_{17}	0.24	0.67	1.13	0.85	1.46	1.76	1.60	0.24	0.65	0.68	1.05	1.57	1.59	NA
Pr	0.09	0.53	0.69	1.01	0.95	0.90	0.80	0.09	0.43	0.54	0.78	0.84	0.94	NA
C_{18}	0.21	0.69	1.20	0.64	1.88	1.75	1.76	0.21	0.86	0.92	1.16	1.72	1.70	NA
Ph	0.05	0.44	0.63	1.12	1.17	1.10	0.85	0.05	0.76	0.60	0.73	0.91	1.11	NA
C_{19}	0.18	0.68	1.07	0.59	1.72	1.85	1.90	0.18	0.72	0.85	1.21	1.84	1.90	NA
C_{20}	0.19	0.58	0.86	0.87	1.64	1.78	1.73	0.19	0.66	1.17	0.94	2.01	1.82	NA
C_{21}	0.19	0.67	0.77	0.62	1.73	1.69	1.88	0.19	0.52	0.96	0.74	1.75	1.95	NA
C_{22}	0.16	0.52	0.72	0.73	1.69	1.56	1.50	0.16	0.58	0.83	0.66	1.91	1.76	NA
C_{23}	0.18	0.46	0.83	0.87	1.50	1.66	1.55	0.18	0.60	0.88	0.88	1.86	1.68	NA
C_{24}	0.11	0.32	0.70	0.63	1.55	1.53	1.69	0.11	0.79	0.73	0.64	1.58	1.62	NA
C_{25}	0.17	0.49	0.75	0.65	1.49	1.77	1.38	0.17	0.48	1.19	0.84	1.79	1.76	NA
C_{26}	0.05	0.34	0.41	0.56	0.65	1.14	1.17	0.05	1.27	0.62	0.72	1.36	1.28	NA
C_{27}	0.74	1.30	1.53	1.47	2.10	2.33	2.26	0.74	0.73	1.54	1.72	2.55	2.47	NA
C_{28}	0.12	0.46	0.50	0.46	0.59	0.75	0.84	0.12	1.14	0.97	1.06	1.67	1.87	NA
C_{29}	0.83	1.23	1.41	1.44	2.04	2.46	2.13	0.83	0.67	1.43	1.61	2.94	2.69	NA
C_{30}	0.15	0.35	0.53	0.40	0.61	0.81	0.67	0.15	0.38	0.56	0.81	1.81	1.33	NA
C_{31}	0.64	0.62	0.71	0.92	0.75	0.91	1.02	0.64	0.08	0.61	0.90	1.30	1.64	NA
C_{32}	0.05	0.08	0.09	0.21	0.05	0.11	0.21	0.05	ND	0.08	0.10	0.32	0.41	NA
C_{33}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	NA

	SITE 7 BASAL TRIAL 2 (DAYS)							SITE 9 (BASAL) NOT ANALYZED						
Alkane	0	5	8	15	33	36	40	0	5	8	15	33	35	40
C_{10}	ND	ND	ND	ND	ND	NA	NA							
C_{11}	ND	0.24	0.17	0.07	ND	NA	NA							
C_{12}	0.05	0.34	0.35	0.17	0.08	NA	NA							
C_{13}	0.05	0.41	0.43	0.35	0.37	NA	NA							
C_{14}	0.18	0.67	0.56	0.44	0.95	NA	NA							
C_{15}	0.15	0.45	0.68	0.71	1.18	NA	NA							
C_{16}	0.21	0.62	0.49	0.99	1.53	NA	NA							
C_{17}	0.24	0.88	0.78	0.88	1.74	NA	NA							
Pr	0.09	0.65	0.55	0.63	0.96	NA	NA							
C_{18}	0.21	0.89	0.65	0.84	1.60	NA	NA							
Ph	0.05	0.68	0.49	0.72	0.89	NA	NA							
C_{19}	0.18	0.88	0.90	0.73	1.83	NA	NA							
C_{20}	0.19	0.73	0.85	0.86	1.94	NA	NA							
C_{21}	0.19	0.62	0.73	0.62	1.60	NA	NA							
C_{22}	0.16	0.76	0.78	0.79	1.56	NA	NA							
C_{23}	0.18	0.74	0.66	0.88	1.46	NA	NA							
C_{24}	0.11	0.66	0.53	0.72	1.41	NA	NA							
C_{25}	0.17	0.75	0.92	0.96	1.67	NA	NA							
C_{26}	0.05	0.87	0.66	0.67	1.55	NA	NA							
C_{27}	0.74	1.14	1.43	1.71	2.82	NA	NA							
C_{28}	0.12	0.79	0.80	0.84	1.74	NA	NA							
C_{29}	0.83	1.25	1.65	1.52	2.72	NA	NA							
C_{30}	0.15	0.67	0.66	0.64	1.48	NA	NA							
C_{31}	0.64	0.66	0.87	1.34	2.29	NA	NA							
C_{32}	0.05	0.38	0.48	0.56	0.88	NA	NA							
C_{33}	ND	ND	ND	ND	ND	NA	NA							



SITE 5



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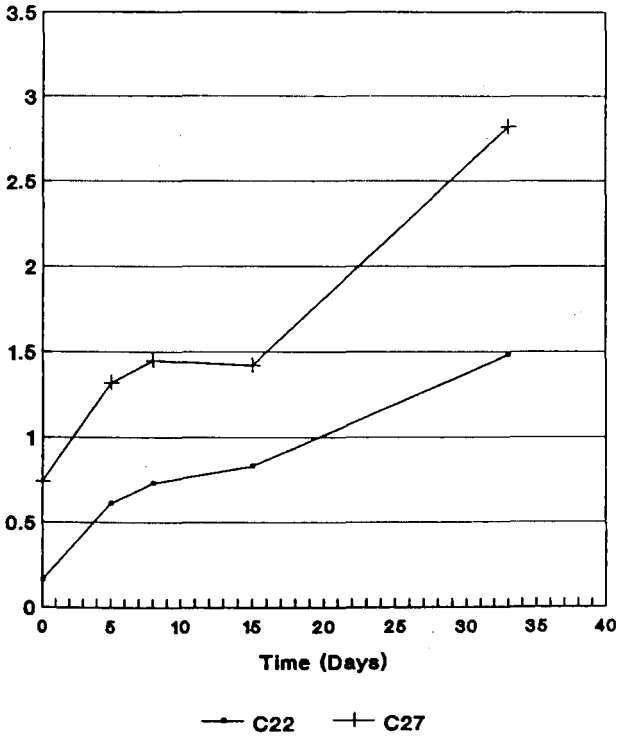

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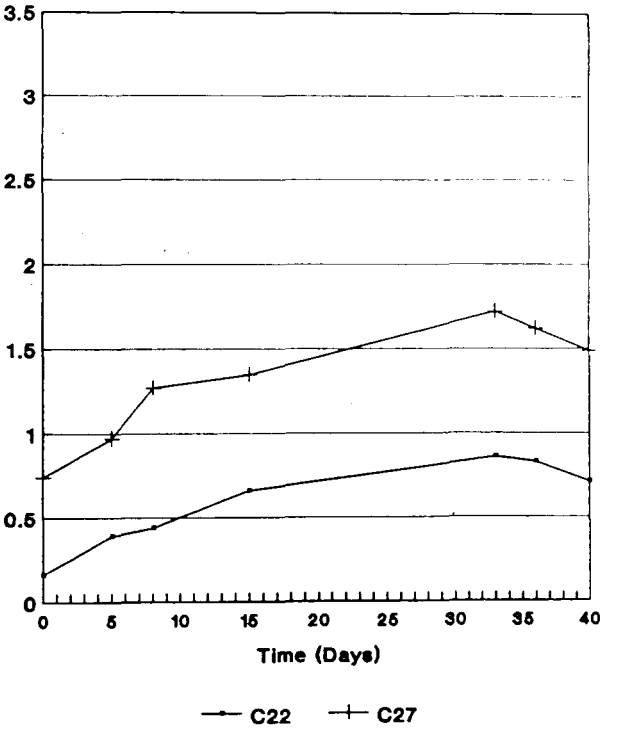
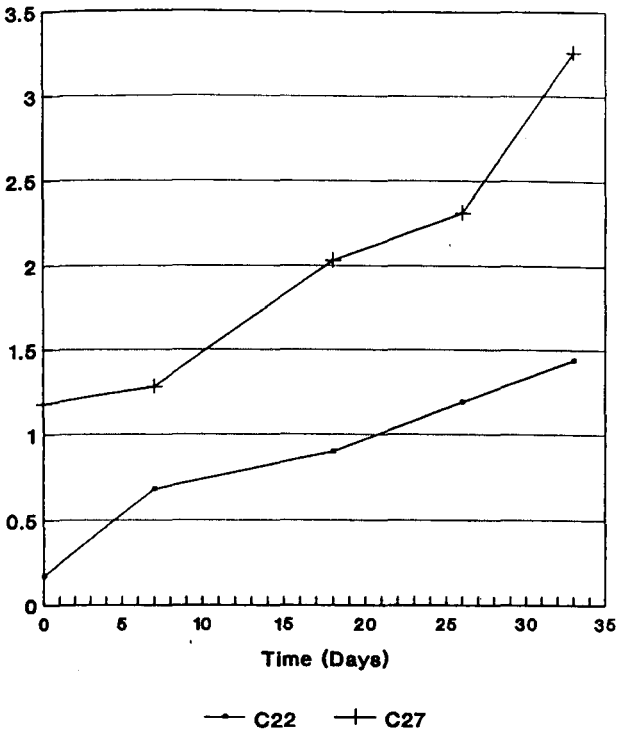
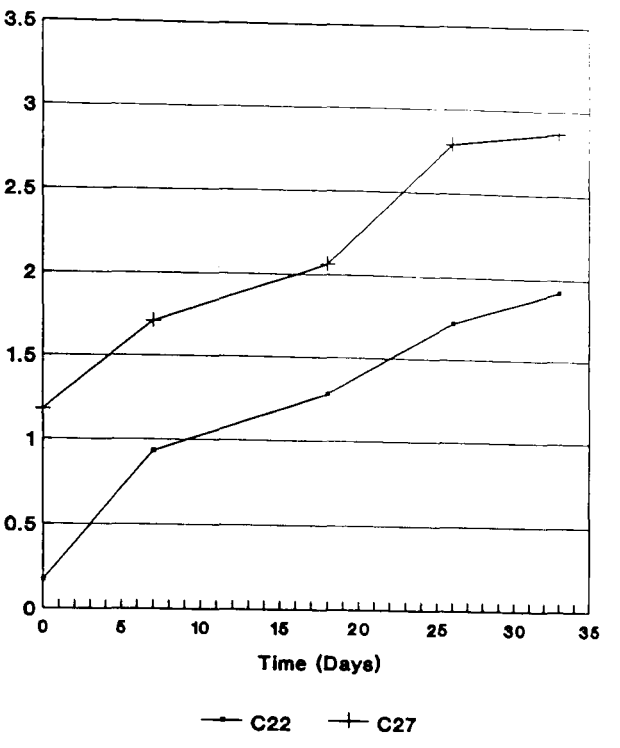


SITE 9

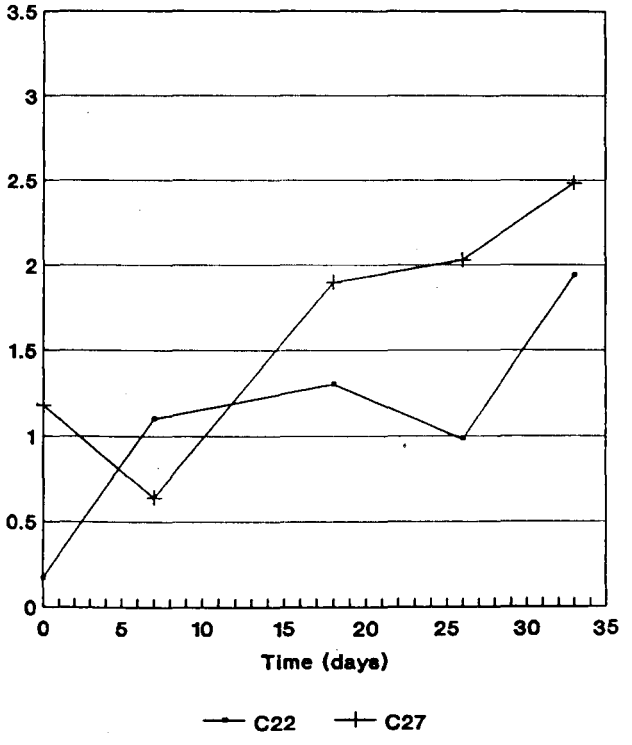
Fig. 5.6 Temporal variation of C_{22} and C_{27} alkanes in *A. aquaticus* in suspended cages during Trial 1.



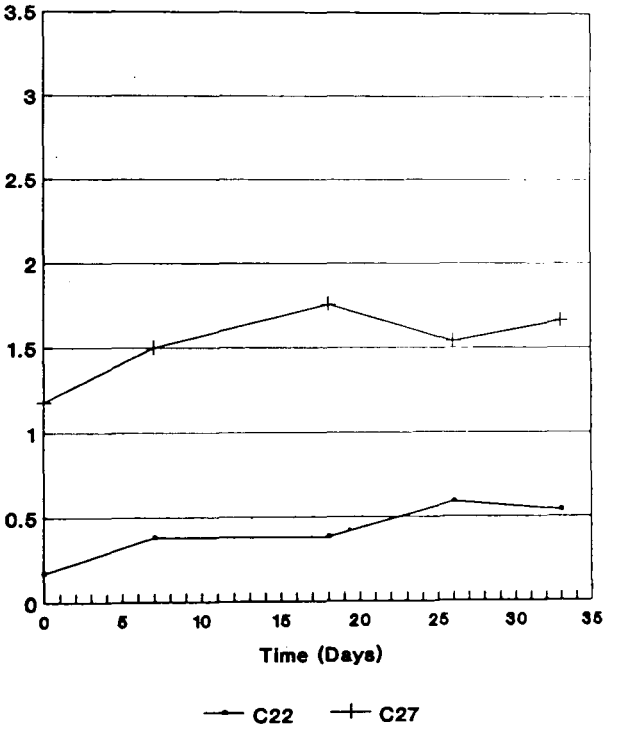
SITE 5



SITE 6



SITE 7



SITE 9

Fig. 5.7 Temporal variation of C_{22} and C_{27} alkanes in *A. aquaticus* in suspended cages during Trial 2.

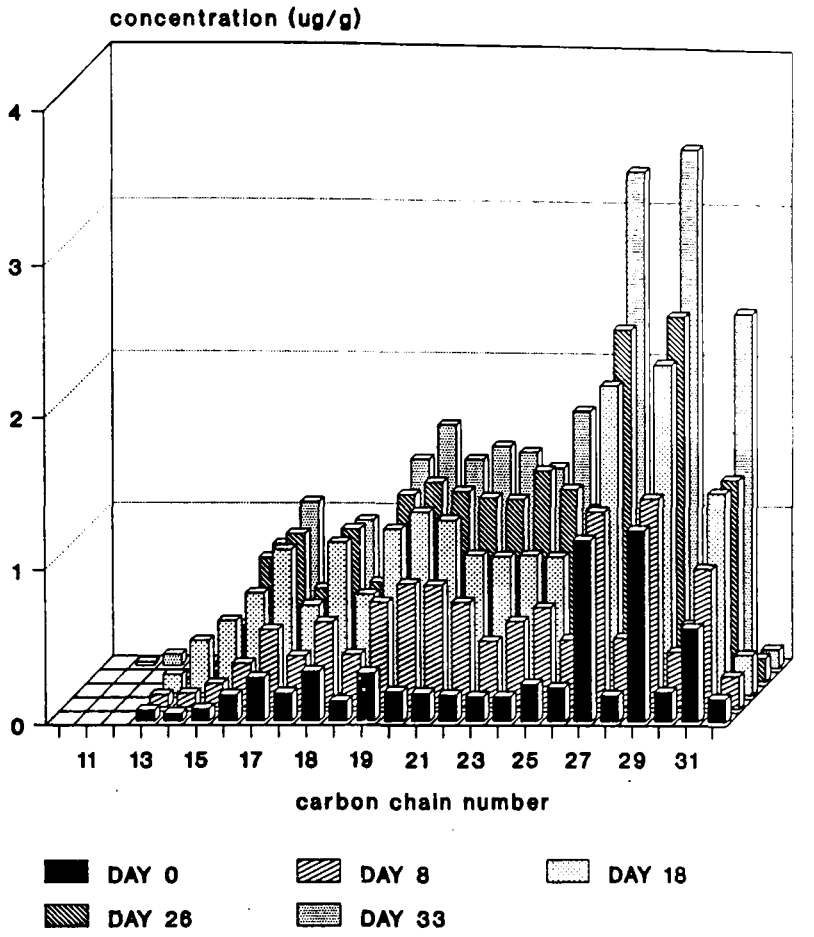


Fig.5.8 Alkane distribution in *A. aquaticus* at Site 5 during Trial 1 (suspended cages).

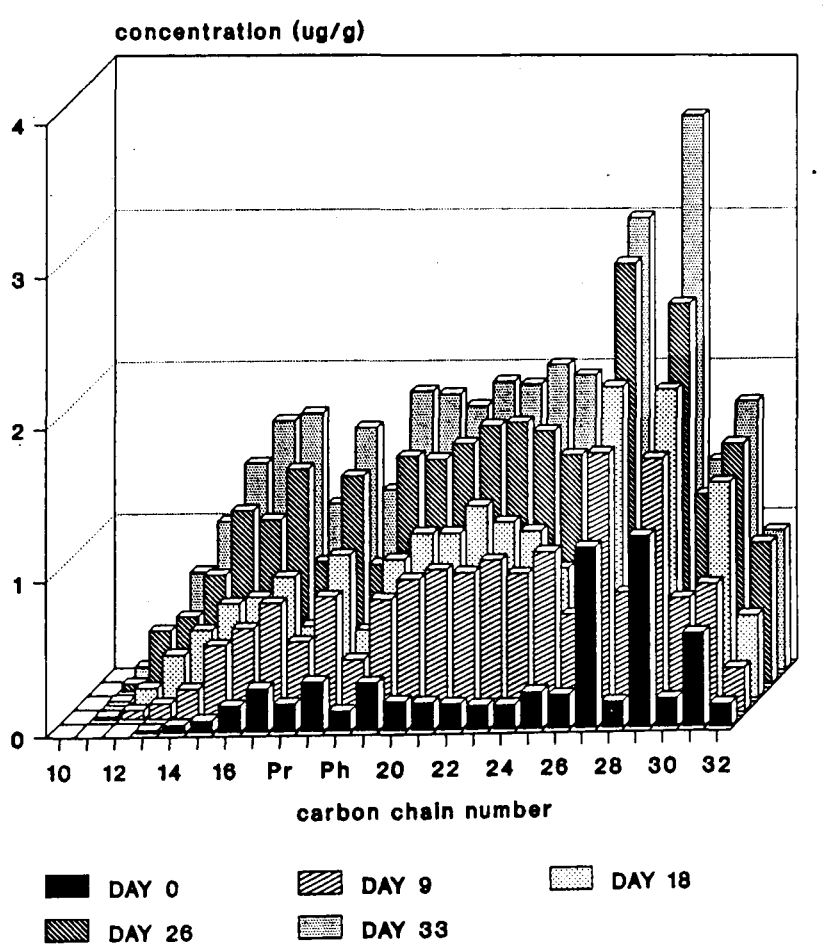


Fig.5.9 Alkane distribution in *A. aquaticus* at Site 6 during Trial 1 (suspended cages).

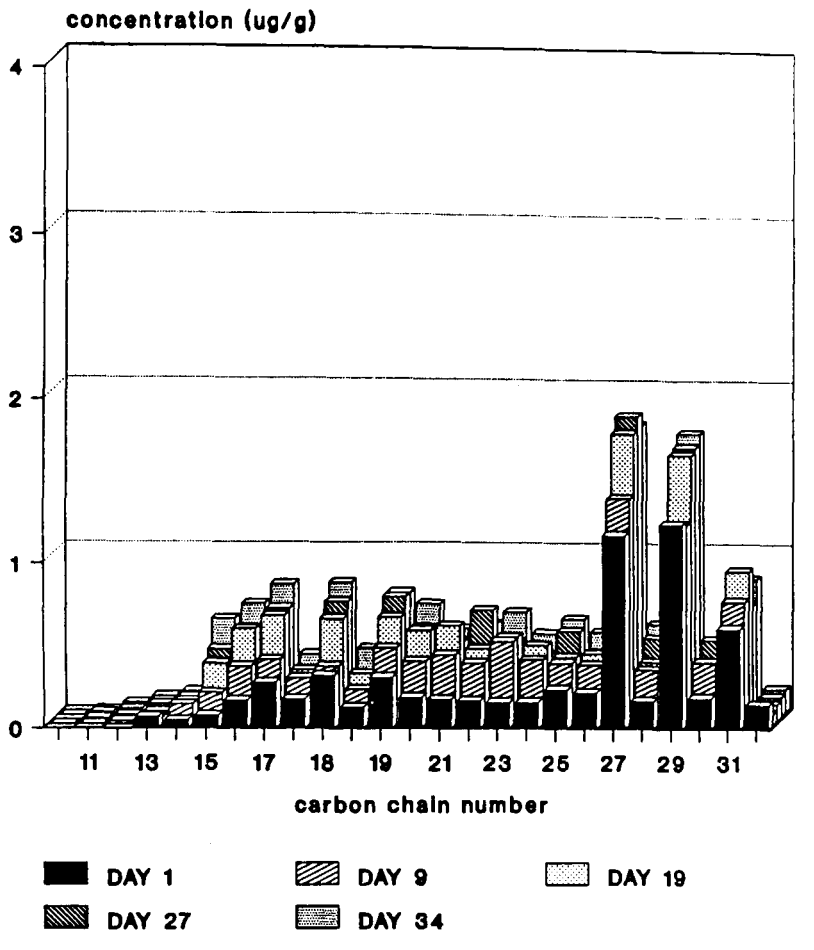


Fig.5.10 Alkane distribution in *A. aquaticus* at Site 9 during Trial 1 (suspended cages).

C_{27} and C_{29}) and these compounds dominate the profile at Site 9. Also, these odd-numbered alkanes dominate the profile in *A. aquaticus* to a greater extent than they are dominant in water and sediment at the respective field sites. This was also a feature of the distribution reported for *G. pulex* from Site 7 (Section 5.3) and may be the result of the feeding habits of the organisms studied. *G. pulex* and *A. aquaticus* are detritivores and are known to feed on partially decayed organic matter, including leaf litter. Their selection of *Salix* sp. leaves which have béen implicated as a primary source of biogenic inputs in this area could lead to increased biogenic alkane levels through ingestion.

5.5.3 Comparison of alkane accumulation in basal and suspended cages

In both of the monitored test periods there is a slight tendency for higher body burdens to be recorded in the basal cages. Any recorded difference between basal and suspended

cages was not proportional to the overall recorded hydrocarbon values. This suggests that the increase is not a feature of higher biouptake but probably the result of an increase in macroinvertebrate surface contamination. As previously mentioned, basal cages revealed a far higher input of silt and detritus compared to suspended cages. Some of this material may have remained bound to the organisms during cleaning. In addition, ingestion of sediment hydrocarbon sources will dominate uptake in basal caged organisms when the ambient levels (suspended and dissolved hydrocarbons) are low. As water source levels increase, for example during a storm event, the influence of the sediment source of uptake may proportionally decrease.

5.5.4 Individual variations in PAH levels

The individual PAH concentrations for *A. aquaticus* during Trials 1 and 2 in suspended and basal cages are presented in Tables 5.9 - 5.12. Fluoranthene and pyrene are present in the greatest concentrations of the PAHs determined in this work as was found by Eadie *et al.* (1982a; 1982b) for PAH levels in worms, midges and the amphipod *Pontoporeia hoyi*. Benzo(a)anthracene and chrysene and the benzofluoranthenes are also represented in high concentrations. Comparison of the concentrations of fluoranthene and pyrene against their methylated homologues for the background site against the test sites supports the hypothesis that these compounds have differing origins (Table 5.8). At the background collection sites, where no point sources of hydrocarbons were identified, lubricating oils would be expected to be present in low concentrations and any hydrocarbons detected are likely to have been combustion derived compounds, transported over long distances. The high ratio of [Fl+Py]: [M-Fl+M-Py] at the control site supports this view. At the test sites, where unburnt oil contamination is far more likely, the relative contribution of [M-Fl+M-Py] increases and this is reflected in the lower ratios recorded at these sites.

Benzo (g,h,i) perylene was rarely detected even at the most polluted sites and, during Trial 1, indeno (1,2,3 - c d) pyrene was only detected once, at Site 7. The tissue

TABLE 5.8

RATIO OF FLUORANTHENE AND PYRENE TO METHYLATED HOMOLOGUES IN *A. AQUATICUS* AT THE BACKGROUND, SILK STREAM AND WELSH HARP SAMPLING SITES.

SITE	[Py+Fl]:[M-Py+M-Fy]
Background	>10
5	4.71
6	3.09
7	2.96
9	3.10

distributions reported here can broadly be related to those found in associated basal and suspended sediments. However, there tends to be a greater variation in relative levels of individual compounds within tissues compared with sediment determinations.

5.5.5 Temporal and spatial variations in total PAH tissue concentrations in *A. aquaticus* and *L. peregra* during Periods A-E

Following Trials 1 and 2, in which basal and suspended cages were used simultaneously at each site and in which suspended cages resulted in generally lower mortality rates, five further trial periods (Periods A - E) were undertaken. As a result of the higher mortality results from Trials 1 and 2 in basal cages at Sites 5, 6 and 7, the basal design was discontinued at these sites. At Site 9 where, overall, cage position had less influence on mortality, the use of the basal design was continued because of the greater ease of access using this system. As noted in Section 5.1, another, contrasting organism, the mollusc *L. peregra* was introduced and was run concurrently with the three later *A. aquaticus* exposure periods (Periods C - E).

The overall temporal and spatial variations in PAH levels in *A. aquaticus* for Periods A (7/6/91 - 25/6/91), B (27/6/91 - 27/7/91), C (8/8/91 - 9/9/91), D (12/9/91 - 2/10/91) and E (20/11/91 - 12/12/91) are presented in Figs. 5.11 and 5.12. The same information for *L. peregra* for Periods C - E is presented in Fig. 5.13. In general, there is a marked accumulation of compounds during the first 3 to 12 days exposure for each species. After this period there appears to be no consistent pattern of elevation. This

TABLE 5.9

INDIVIDUAL PAH CONCENTRATIONS IN *ASELLUS AQUATICUS* AT SITES 5 ,6, 7 AND 9 DURING TRIAL 1 (SUSPENDED CAGES).

	SITE 5 SUSPENDED TRIAL 1 (Days)					SITE 6 SUSPENDED TRIAL 1 (Days)					SITE 7 SUSPENDED TRIAL 1 (Days)					SITE 9 SUSPENDED TRIAL 1 (Days)				
PAH	0	8	18	26	33	0	8	18	26	33	0	8	18	23	33	0	8	18	26	33
N	0.04	0.18	0.20	0.26	0.25	0.04	0.25	0.40	0.50	0.65	0.04	0.23	0.25	0.25	NA	0.04	0.07	0.10	0.09	0.10
M-N	0.05	0.07	0.12	0.13	0.16	0.05	0.11	0.14	0.14	0.40	0.05	0.17	0.02	0.20	NA	0.05	0.05	0.05	0.05	0.05
F	0.26	0.62	0.85	0.95	1.01	0.26	1.00	0.88	1.54	0.82	0.26	0.35	1.01	1.01	NA	0.26	0.24	0.31	0.13	0.45
Ph+A	ND	0.05	0.21	0.25	0.47	ND	0.06	0.26	0.23	0.41	ND	0.82	0.48	0.48	NA	ND	0.18	0.40	0.05	0.17
M-Ph	ND	ND	ND	0.05	0.07	ND	0.05	0.02	0.09	0.13	ND	0.35	0.05	0.05	NA	ND	0.05	0.05	0.05	0.05
M-A	ND	ND	ND	0.05	0.05	ND	0.05	0.02	0.05	0.05	ND	0.02	0.05	NA	NA	ND	ND	ND	1.21	ND
Fl	0.28	1.21	1.52	2.81	3.53	0.28	1.73	2.07	3.73	4.91	0.28	1.93	2.50	NA	NA	0.28	0.30	0.65	1.17	1.49
Py	0.22	0.70	1.30	2.32	3.09	0.22	1.28	1.95	3.47	5.25	0.22	0.59	1.20	NA	A	0.22	0.40	0.10	0.18	1.64
M-Fl	0.05	0.28	0.34	0.34	0.68	0.05	0.49	0.52	0.84	1.53	0.05	1.20	0.67	NA	NA	0.05	0.06	0.73	0.25	0.22
M-Py	ND	0.22	0.32	0.49	0.71	ND	0.37	0.45	0.59	1.52	ND	0.41	0.75	NA	NA	ND	0.33	0.51	0.74	0.31
BA+Ch	0.05	1.20	1.31	1.62	1.85	0.05	2.01	2.83	2.45	2.70	0.05	2.00	2.34	NA	NA	0.05	0.40	0.20	0.65	0.82
BFs	0.16	1.08	1.45	1.73	1.50	0.16	1.56	1.67	2.01	2.05	0.16	1.13	1.83	NA	NA	0.16	0.33	0.43	0.14	0.80
BPs	ND	0.18	0.24	0.28	0.39	ND	0.27	0.33	0.37	0.47	ND	1.01	0.81	NA	NA	ND	0.05	0.09	0.48	0.16
DBA	0.12	0.80	0.65	0.74	0.82	0.12	1.40	1.68	1.53	1.74	0.12	ND	1.23	NA	NA	0.12	0.20	0.53	ND	0.35
BPe	ND	ND	ND	ND	ND	ND	0.05	0.05	0.05	0.05	ND	ND	0.05	NA	NA	ND	ND	ND	ND	ND
IPy	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.05	NA	NA	ND	ND	ND	ND	ND

TABLE 5.10

INDIVIDUAL PAH CONCENTRATIONS IN *ASELLUS AQUATICUS* AT SITES 5 ,6, 7 AND 9 DURING TRIAL 1 (BASAL CAGES).

	SITE 5 BASAL TRIAL 1 (Days)					SITE 6 BASAL TRIAL 1 (Days)					SITE 7 BASAL TRIAL 1 (Days)					SITE 9 BASAL TRIAL 1 (Days)				
PAH	0	8	18	26	33	0	8	18	26	33	0	8	18	23	33	0	8	18	26	33
N	0.05	0.06	0.21	0.30	0.24	0.05	0.28	0.45	0.74	0.68	0.05	0.31	0.42	0.21	NA	0.05	0.06	0.08	0.07	0.11
M-N	0.05	0.07	0.15	0.14	0.18	0.05	0.13	0.18	0.32	0.25	0.05	0.19	0.31	0.19	NA	0.05	0.05	0.05	0.06	0.05
F	0.26	0.74	0.85	1.34	1.25	0.26	0.65	0.82	1.60	1.40	0.26	0.71	0.94	1.21	NA	0.26	0.26	0.35	0.37	0.48
Ph+A	ND	0.05	0.24	0.28	0.41	ND	0.10	0.24	0.43	0.60	ND	0.41	0.59	0.35	NA	ND	0.18	0.21	0.22	0.34
M-Ph	ND	ND	0.05	ND	0.05	ND	ND	0.05	0.08	0.12	ND	0.05	ND	0.05	NA	ND	0.05	0.05	0.05	0.05
M-A	ND	ND	ND	ND	0.05	ND	ND	ND	0.05	0.05	ND	ND	ND	ND	NA	ND	0.05	ND	ND	ND
Fl	0.28	1.06	2.22	2.56	3.48	0.28	1.60	2.30	3.89	5.70	0.28	1.75	2.30	2.69	NA	0.28	0.32	0.71	1.25	1.56
Py	0.22	0.83	1.59	2.10	3.21	0.22	1.40	2.05	2.90	5.50	0.22	1.35	0.59	4.39	NA	0.22	0.05	0.68	1.23	1.83
M-Fl	0.05	0.37	0.41	0.52	0.63	0.05	0.40	0.57	0.90	1.74	0.05	0.55	1.60	0.72	NA	0.05	0.96	0.11	0.15	0.30
M-Py	ND	0.27	0.34	0.55	0.74	ND	0.42	0.51	0.63	1.37	ND	0.49	0.79	1.21	NA	ND	0.25	0.24	0.27	0.34
BA+Ch	0.05	1.40	1.50	1.59	1.91	0.05	1.57	2.00	2.80	2.70	0.05	1.90	1.63	2.84	NA	0.05	0.43	0.59	0.71	0.75
BFs	0.16	1.11	1.59	1.62	2.10	0.16	1.80	1.72	1.94	2.34	0.16	1.28	1.54	1.99	NA	0.16	0.39	0.82	0.75	0.83
BPs	ND	0.19	0.25	0.31	0.36	ND	0.31	0.43	0.48	0.50	ND	0.21	1.02	0.95	NA	ND	0.05	0.13	0.11	0.14
DBA	0.12	0.35	0.76	0.89	0.92	0.12	1.40	1.68	1.53	1.37	0.12	ND	1.40	0.69	NA	0.12	0.17	0.45	0.49	0.59
BPe	ND	ND	ND	ND	ND	ND	ND	0.05	0.05	0.05	ND	ND	0.05	0.05	NA	ND	ND	0.05	ND	ND
IPy	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	NA	ND	ND	ND	ND	ND

TABLE 5.11

INDIVIDUAL PAH CONCENTRATIONS IN *ASELLUS AQUATICUS* AT SITES 5 ,6, 7 AND 9 DURING TRIAL 2 (SUSPENDED CAGES).

	SITE 5 SUSPENDED TRIAL 2 (DAYS)							SITE 6 SUSPENDED TRIAL 2 (DAYS)						
	0	6	9	16	34	37	41	0	6	9	16	34	37	41
N	0.05	0.07	0.05	0.06	0.28	0.27	0.30	0.05	0.18	0.17	0.14	0.34	0.36	0.41
M-N	0.05	0.05	ND	0.05	0.11	0.13	0.16	0.05	0.05	0.06	0.06	0.17	0.21	0.25
F	0.21	0.52	0.87	0.75	1.30	1.51	1.28	0.21	0.65	0.91	0.81	1.75	1.74	2.10
Ph+A	0.05	0.12	0.27	0.17	0.51	0.41	0.58	0.05	0.16	0.22	0.42	0.81	0.93	1.21
M-Ph	ND	0.05	0.05	0.05	0.10	0.10	0.14	ND	ND	ND	0.12	0.25	0.24	0.29
M-A	ND	ND	ND	0.05	0.05	0.05	0.05	ND	ND	ND	0.05	0.11	0.10	0.15
Fl	0.25	0.38	1.75	1.48	2.78	3.01	3.30	0.25	1.49	1.60	1.70	3.71	3.37	4.52
Py	0.31	0.53	1.87	1.72	3.20	3.28	3.15	0.31	1.69	1.74	1.91	4.67	4.18	4.67
M-Fl	ND	0.08	0.45	0.39	0.75	0.81	0.76	ND	0.29	0.41	0.53	0.92	0.48	1.11
M-Py	ND	0.11	0.28	0.25	0.65	0.68	0.60	ND	0.32	0.38	0.44	1.30	1.24	1.58
BA+Ch	0.05	0.44	0.71	0.67	1.45	1.59	1.65	0.05	0.75	1.21	1.30	2.30	2.47	1.48
BFs	0.16	0.43	0.83	0.75	1.61	0.82	1.70	0.16	1.31	1.30	1.57	2.58	1.39	1.71
BPs	ND	0.10	0.24	0.14	0.31	0.35	0.38	ND	0.42	0.45	0.61	1.51	1.21	0.05
DBA	0.05	0.24	0.44	0.40	0.74	0.84	1.00	0.05	0.81	1.14	1.08	2.07	1.85	ND
BPe	ND	0.05	ND	0.05	ND	ND	ND	ND	0.05	0.05	0.07	0.05	0.05	ND
IPy	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

	SITE 7 SUSPENDED TRIAL 2 (DAYS)							SITE 9 SUSPENDED TRIAL 2 (DAYS)						
PAH	0	6	9	16	34	37	41	0	6	9	16	34	37	41
N	0.03	1.00	0.21	0.29	0.34	0.32	0.41	0.03	0.08	ND	0.05	0.10	0.13	0.10
M-N	ND	0.08	0.14	0.21	0.21	0.28	0.25	ND	0.05	0.05	0.05	0.07	0.5	0.10
F	0.21	0.30	0.58	0.61	1.00	0.95	2.10	0.21	0.13	0.20	0.21	0.46	0.45	0.57
Ph+A	ND	0.21	0.40	0.83	1.30	1.43	1.21	ND	0.07	0.05	0.09	0.21	0.21	0.24
M-Ph	ND	0.05	0.08	0.12	0.17	0.16	0.29	ND	ND	ND	ND	ND	0.07	0.08
M-A	ND	ND	ND	0.05	ND	0.05	0.15	ND	ND	ND	ND	ND	ND	ND
Fl	0.25	1.55	1.88	1.95	2.99	3.39	4.52	0.25	0.25	0.26	0.40	1.45	1.67	1.60
Py	0.31	1.23	1.72	1.87	2.85	0.79	3.65	0.31	0.27	0.20	0.51	1.59	1.57	1.62
M-Fl	ND	0.21	0.31	0.42	0.76	2.91	1.11	ND	0.05	0.06	0.08	0.24	0.32	0.29
M-Py	ND	0.30	0.35	0.48	0.77	0.78	1.58	ND	ND	0.09	0.10	0.34	0.34	0.40
BA+Ch	ND	0.67	1.00	1.35	2.43	2.40	2.71	ND	0.23	0.40	0.49	0.89	0.74	0.79
BFs	0.16	0.82	0.87	1.11	2.3	2.39	1.58	0.16	0.17	0.24	0.31	0.73	0.70	1.08
BPs	ND	0.27	0.24	0.41	0.75	0.80	1.48	ND	0.05	0.05	0.05	0.10	0.11	0.15
DBA	ND	0.64	0.96	1.21	1.72	1.71	1.71	ND	0.13	0.15	0.18	0.46	0.49	0.45
BPe	ND	ND	ND	0.05	0.05	0.05	0.05	ND	0.05	ND	0.05	ND	ND	ND
IPy	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE 5.12

INDIVIDUAL PAH CONCENTRATIONS IN *ASELLUS AQUATICUS* AT SITES 5 ,6, 7 AND 9 DURING TRIAL 2 (BASAL CAGES).

	SITE 5 BASAL TRIAL 2 (DAYS)							SITE 6 BASAL TRIAL 2 (DAYS)						
	0	6	9	16	34	37	41	0	6	9	16	34	37	41
N	ND	ND	0.05	0.07	0.29	0.25	0.27	ND	0.11	0.18	0.14	0.31	0.35	0.41
M-N	ND	ND	0.05	0.05	0.14	0.11	0.15	ND	0.05	0.07	0.09	0.18	0.17	0.25
F	0.21	0.64	0.83	0.72	1.40	1.49	1.61	0.21	0.84	1.11	0.95	1.90	2.24	2.10
Ph+A	ND	0.15	0.25	0.19	0.57	0.81	0.63	ND	0.16	0.30	0.34	0.71	0.85	1.21
M-Ph	ND	ND	ND	0.05	0.10	0.12	0.16	ND	ND	0.05	0.11	0.20	0.21	0.29
M-A	ND	ND	ND	0.05	0.05	0.05	0.05	ND	ND	ND	0.09	0.14	0.12	0.15
Fl	0.25	0.65	1.79	1.62	3.29	3.24	3.45	0.25	1.55	1.75	1.85	4.21	4.22	4.52
Py	0.31	0.42	1.92	1.75	3.31	3.36	3.52	0.31	1.70	0.42	1.99	4.52	4.69	4.67
M-Fl	ND	0.13	0.38	0.41	0.83	0.75	0.71	ND	0.31	1.90	0.64	1.17	1.20	1.11
M-Py	ND	0.12	0.27	0.23	0.59	0.61	0.67	ND	0.28	0.33	0.52	1.27	1.26	1.58
BA+Ch	ND	0.51	0.75	0.60	1.47	1.78	1.34	ND	1.09	1.31	1.45	2.63	2.45	2.71
BFs	0.16	0.32	0.89	0.82	1.34	1.78	1.65	0.16	1.26	1.42	1.69	3.21	2.30	1.58
BPs	ND	0.10	0.19	0.16	0.32	0.38	0.42	ND	0.41	0.50	0.51	1.14	1.36	1.48
DBA	0.05	0.31	0.45	0.49	0.83	0.89	0.91	0.05	1.13	1.21	1.05	1.92	1.91	1.71
BPe	ND	0.05	ND	0.05	0.05	ND	0.05	ND	0.06	0.05	ND	0.05	0.05	0.05
IPy	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

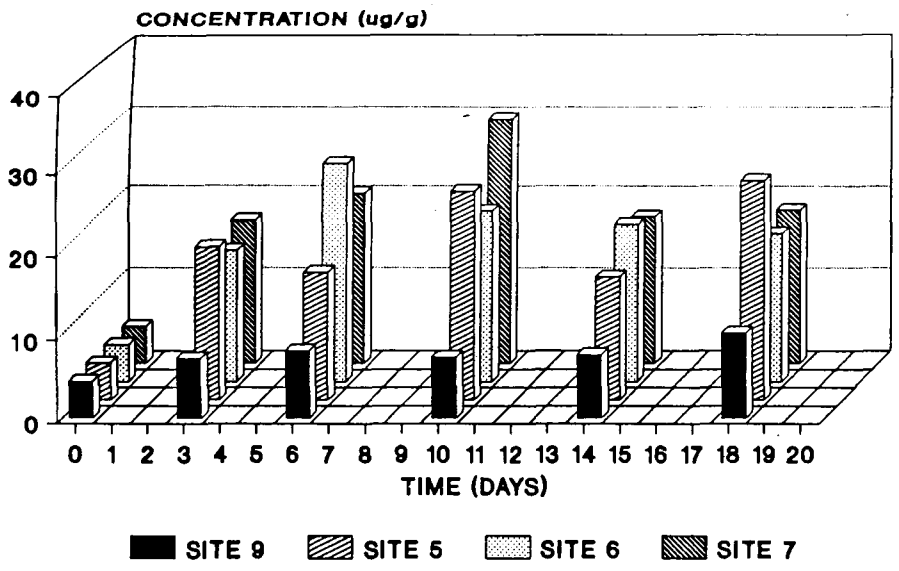
	SITE 7 BASAL TRIAL 2 (DAYS)							SITE 9 BASAL TRIAL 2 (DAYS)						
	0	6	9	16	34	37	41	0	6	9	16	34	37	41
N	ND	0.72	0.23	0.28	0.45	0.32	0.41	NA	NA	NA	NA	ND	ND	ND
M-N	ND	0.09	0.18	0.11	0.30	0.28	0.25	NA	NA	NA	NA	ND	ND	ND
F	0.21	0.34	0.65	0.54	1.07	1.15	2.10	NA	NA	NA	NA	ND	ND	ND
Ph+A	ND	0.21	0.46	0.74	1.21	1.43	1.21	NA	NA	NA	NA	ND	ND	ND
M-Ph	ND	ND	0.10	0.08	0.20	0.16	0.29	NA	NA	NA	NA	ND	ND	ND
M-A	ND	ND	0.05	ND	ND	0.05	0.15	NA	NA	NA	NA	ND	ND	ND
Fl	0.25	1.71	2.24	1.54	3.08	3.39	4.52	NA	NA	NA	NA	ND	ND	ND
Py	0.31	1.46	2.10	1.75	3.41	2.91	3.65	NA	NA	NA	NA	ND	ND	ND
M-Fl	ND	0.31	0.48	0.42	0.83	0.79	1.58	NA	NA	NA	NA	ND	ND	ND
M-Py	ND	0.32	0.37	0.40	0.97	0.78	2.71	NA	NA	NA	NA	ND	ND	ND
BA+Ch	ND	0.64	1.39	1.50	2.52	2.40	1.58	NA	NA	NA	NA	ND	ND	ND
BFs	0.16	0.84	1.20	1.06	2.15	2.39	1.48	NA	NA	NA	NA	ND	ND	ND
BPs	ND	0.36	0.41	0.42	0.81	0.80	1.71	NA	NA	NA	NA	ND	ND	ND
DBA	ND	0.71	1.20	1.01	1.59	1.71	0.05	NA	NA	NA	NA	ND	ND	ND
BPe	ND	ND	0.05	ND	0.05	0.05	ND	NA	NA	NA	NA	ND	ND	ND
IPy	ND	ND	0.05	ND	ND	ND	ND	NA	NA	NA	NA	ND	ND	ND

trend can be clearly observed for the total PAH variation in *A. aquaticus* during the summer trial of Period A (Fig. 5.11). Here, there is a general increase in body burdens at Sites 5, 6 and 7 to Day 10, after which the levels fall slightly. Therefore, during Period A the three Silk Stream sites have very similar levels but at Site 9 in the Welsh Harp the levels remain relatively low and constant.

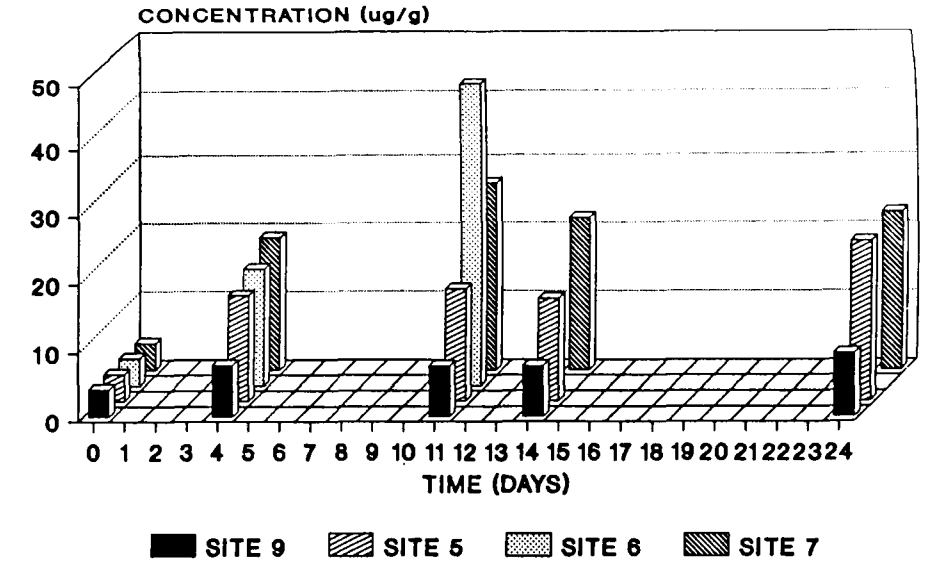
A less clear pattern in total PAH variation occurs during another summer exposure period (Period B). Following four days of exposure the values at three of the sites are very similar, but a marked increase in levels occurs at Site 6 which corresponds to a high mortality count (>70%) recorded between Days 4 and 11 at this site. A smaller elevation in total levels during this period can also be observed at Site 7 during which a 40% increase in mortality rate was recorded.

The overall trend during the late summer/early autumn exposure period (Period C) for *A. aquaticus* is broadly similar to Period A, with a general increase occurring at all sites until Day 16, after which a small fall occurs at the three Silk Stream sites. At Site 7, 100% mortality had occurred by Day 26 but no significant corresponding elevation in body burdens was recorded between days 15 and 20. During the same time period, *L. peregra* displays similar patterns in total PAH variation although continued elevations up to Day 32 at Sites 5 and 6 can be observed (Fig 5.13). Again, there is little difference between the three Silk Stream sites and the values at Site 9 remain low and relatively constant.

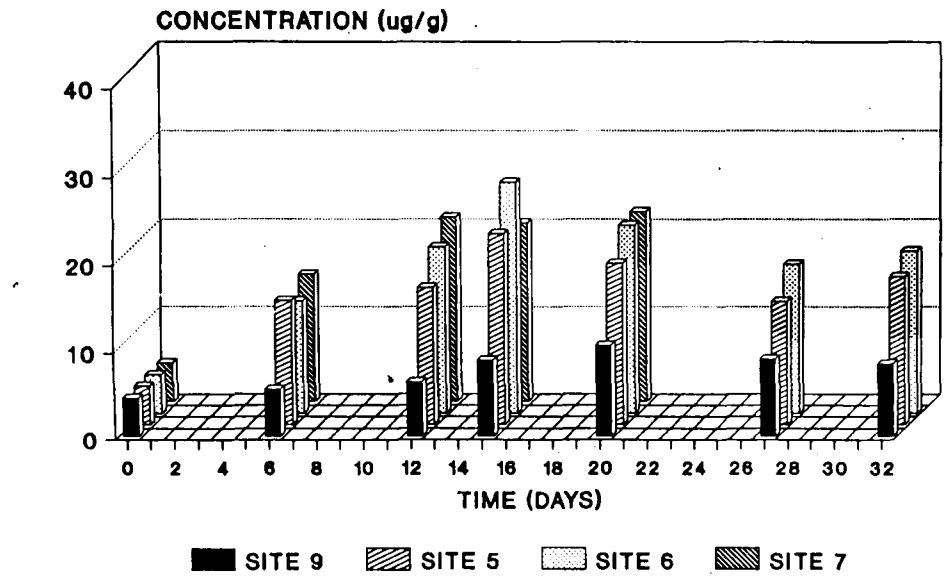
During the autumn exposure period (Period D) there is a less rapid rise in concentrations for *A. aquaticus*. During this relatively wet sampling period, 100% mortalities were reached at Sites 6 and 7 within 19 days; a relatively high value was also attained at Site 7 by Day 10. Similar rapid mortality rates were recorded for *L. peregra* during this period and comparatively elevated PAH levels were recorded at Sites 5, 6 and 7 after 7 days. The final winter monitoring exposure period (Period E) displays similar variations for *A. aquaticus* and *L. peregra* although total PAH levels were generally slightly higher in *L. peregra*. For both organisms the highest levels were clearly recorded at Site 6.



PERIOD A

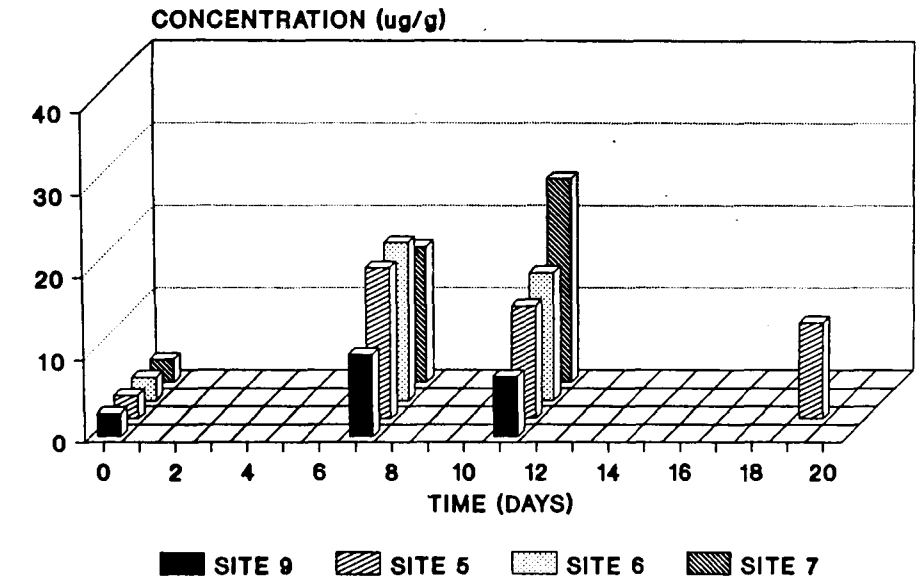


PERIOD B

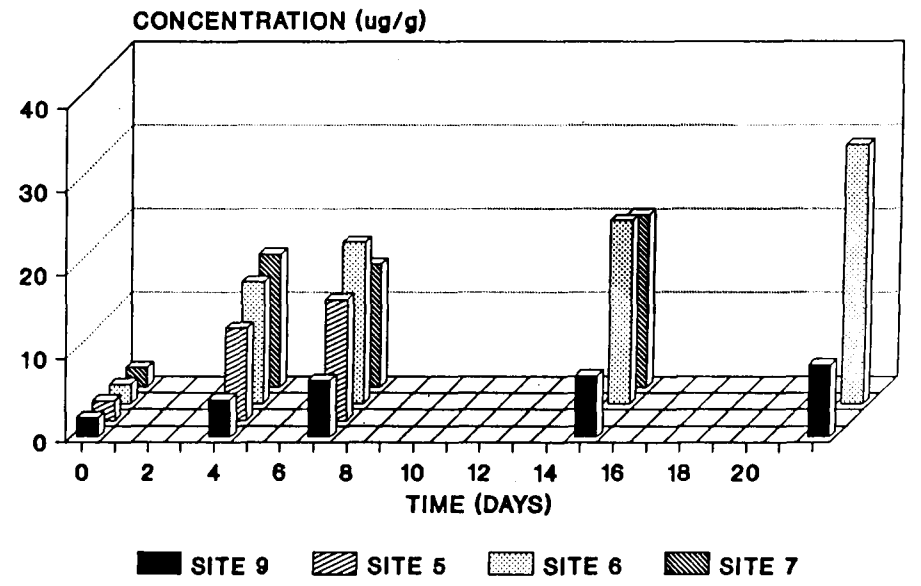


PERIOD C

Fig. 5.11 Temporal and spatial variation of total PAH levels in *A. aquaticus* in suspended cages at Sites 5, 6, 7 and 9 during Periods A - C.

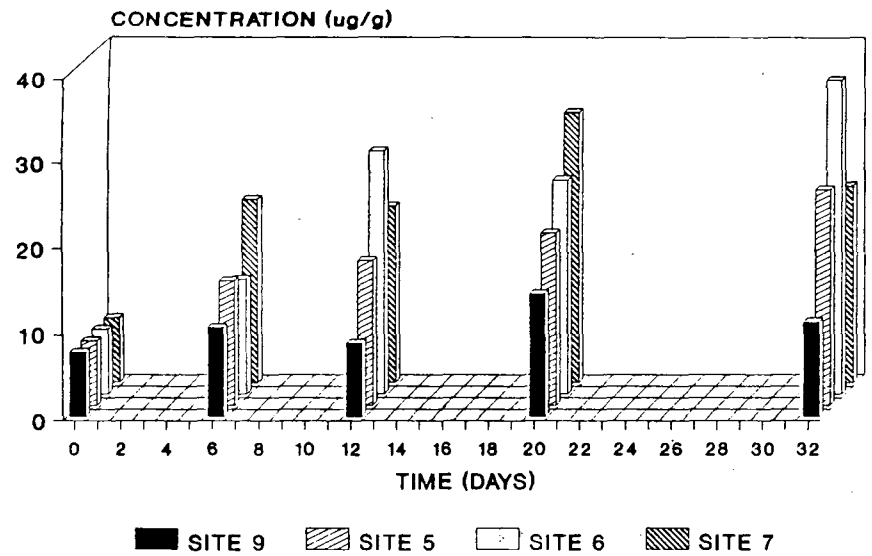


PERIOD D

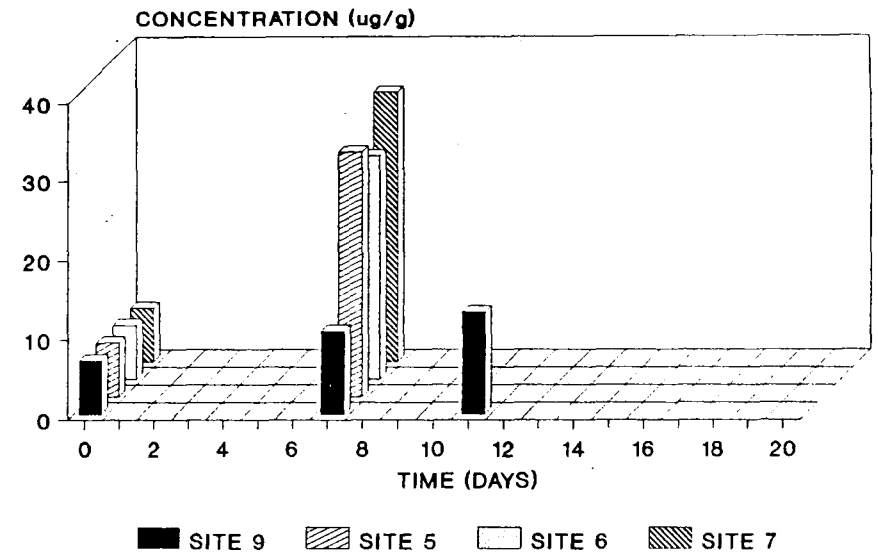


PERIOD E

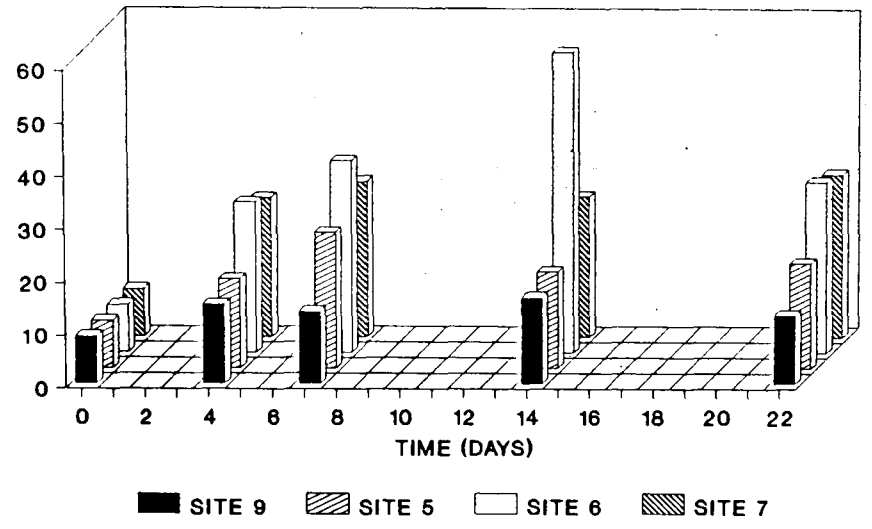
Fig. 5.12 Temporal and spatial variation of total PAH levels in *A. aquaticus* in suspended cages at Sites 5, 6, 7 and 9 during Periods D - E.



PERIOD C



PERIOD D



PERIOD E

Fig. 5.13 Temporal and spatial variation of total PAH levels in *L. peregra* in suspended cages at Sites 5, 6, 7 and 9 during Periods C - E.

The ratios of maximum exposed concentrations to background levels in *A. aquaticus* are between 4 and 6 for the Silk Stream sites and are less than 2 at Site 9. Slightly higher ratios were obtained for Periods C, D and E with *L. peregra*. These ratios are generally lower than those reported for the initial *in-situ* tests (Trials 1 and 2) even though the exposed organisms attained equal or higher levels in Periods A - E. The differences can be explained by the lower background reference site (Hadley Road) concentration of PAHs detected during Trials 1 (24/10/90-3/12/90) and 2 (5/12/90-17/1/90) compared to the later trials (Periods A-E).

TABLE 5.13

MAXIMUM TOTAL PAH CONCENTRATION IN *A. AQUATICUS* TISSUE AT SAMPLING LOCATIONS 5, 6, 7 AND 9 DURING TEST PERIODS A - E.

SITE	EXPOSURE PERIOD				
	A	B	C	D	E
5	26.63	23.20	22.04	18.40	14.51
6	29.90	44.03	26.48	19.17	31.00
7	32.42	27.38	28.12	24.77	20.67
9	12.31	9.22	9.97	10.08	8.71

[concentrations in $\mu g\ g^{-1}$ dry weight)

TABLE 5.14

MAXIMUM TOTAL PAH CONCENTRATION IN *LYMNAEA PEREGRA* TISSUE AT SAMPLING LOCATIONS 5, 6, 7 AND 9 DURING TEST PERIODS C - E.

SITE	EXPOSURE PERIOD		
	C	D	E
5	25.26	30.86	25.73
6	36.96	28.17	56.77
7	31.68	34.22	30.93
9	14.36	12.90	16.44

[concentrations in $\mu g\ g^{-1}$ dry weight].

The maximum total PAH levels achieved at each site during the five test periods are summarized in Tables 5.13 and 5.14. The maximum PAH tissue value is consistently found at Sites 6 or 7 emphasising the relatively high contamination at these sites and subsequent uptake by the monitored macroinvertebrates.

Total maximum PAH tissue values at Site 9 are typically only 25 to 40% of those achieved at the Silk Stream sites and are much more constant, confirming the substantial water quality recovery that occurs within the receiving basin with regard to PAH removal. The maximum PAH levels reached in *A. aquaticus* tissue at Site 5 closely approach those of Sites 6 and 7 for three of the five monitoring periods. Generally, however Site 5 levels can be said to be intermediate between the lowest Silk Stream sites (Sites 6 and 7) and the receiving basin (Site 9).

PAH levels monitored in *L. peregra*, without exception, exceed those for *A. aquaticus* at the same site and during the same exposure period, further indicating the greater capacity of the mollusc group for hydrocarbon accumulation.

5.5.6 Individual PAH Variations in *A. aquaticus* and *L. peregra* during Periods A-E

Representative examples of PAH profiles obtained for exposure Periods C - E for *L. peregra* and Periods A - E for *A. aquaticus* are presented in Figs. 5.14 and 5.15, respectively. The examples given demonstrate three general differences in bioaccumulation patterns between the two organism.

1) Total PAH accumulation is highest in *L. peregra*.

2) The assemblage in *L. peregra* is flat whereas in *A. aquaticus* the PAH distribution is more peaked in the central portion.

3) In *L. peregra*, methyl substituted homologues are more prominent than in *A. aquaticus* in which the parental PAHs dominate.

Individual values for each sampling period and for both species are provided in Tables 5.15 - 5.22. The results show that, again, fluoranthene and pyrene are generally the most abundant compounds in organism tissue. Phenanthrene, benzo(a)anthracene and the benzo(b+k)fluoranthenes are also present in relatively high concentrations. The relative abundances of the PAHs in *A. aquaticus* broadly mirrors the PAH composition

of the surrounding sediments. This has been demonstrated in other studies eg Eadie *et al.* (1982a) where, as in the currently presented work, fluoranthene and pyrene were found to be predominant in organisms and ambient sediments. In urban areas, where there are a large number of diffuse hydrocarbon sources and where combustion sources are prevalent, fluoranthene and pyrene are likely to be principal components of the abiotic and biotic hydrocarbon assemblage. Where this is not the case, specific pollution sources or incidents are likely to be present e.g. Elder & Dressler (1988) reported that discharges from a wood preserving facility led to elevated phenanthrene concentrations in the resident mollusc *Thais haemastoma*. The discharge was dominated by creosote wastes of which phenanthrene was the single most abundant PAH. Similarly, Boom (1987) reported high anthracene concentrations in *Mytilus edulis* along the Dutch coast, but did not consider specific potential sources of this compound.

Where tissue assemblages differ from those in ambient sediments, differing bioavailabilities of specific compounds are probable. In the currently reported work, tissue methyl-naphthalene levels are proportionally present in far higher concentrations in tissues than in sediments. This phenomenon occurs at every site during each sampling period for *L. peregra*. Similar, though slightly less pronounced increases in other methylated PAHs (methyl-phenanthrene, methyl-fluoranthene and methyl-pyrene) can also be observed in tissue samples. The differing bioavailability hypothesis (see Section 2.8.4.1) proposed by Farrington *et al.* (1983) and applied to the USEPA Mussel Watch Program (Farrington *et al.*, 1982) can be applied to these results. This hypothesis suggests that the mode of formation of the methyl homologues i.e. through diagenesis leads to less tightly particle bound compounds that may be more readily bioaccumulated. This hypothesis will be returned to in Chapter 7.

Examples of the temporal variation for the four predominant PAHs (phenanthrene, pyrene, fluoranthene, benzo(a)anthracene) measured in *A. aquaticus* tissue are presented in Figs. 5.16 and 5.17. In *A. aquaticus* these compounds represent about 60% of the total PAH suite. In *L.peregra* they represent 40-50% of the total value. The results show a consistent initial elevation in PAH concentrations at all the sampling sites relative to the background collection site levels. The most significant temporal increase occurs during the first 4-6 days of exposure after which the levels tend to remain fairly

constant except for phenanthrene which in the the selected example ultimately approaches the starting level.

At Site 9 the PAH concentration increases are most pronounced during Period C, but there is little elevation in levels during the other monitoring periods. Sites 6 and 7 display similar profiles of PAH levels with the exception of Site 6 during period B, where there is a marked increase in levels particularly for phenanthrene which approaches 10 μg g^{-1} dry weight. These high levels correspond to a high mortality count during Period B.

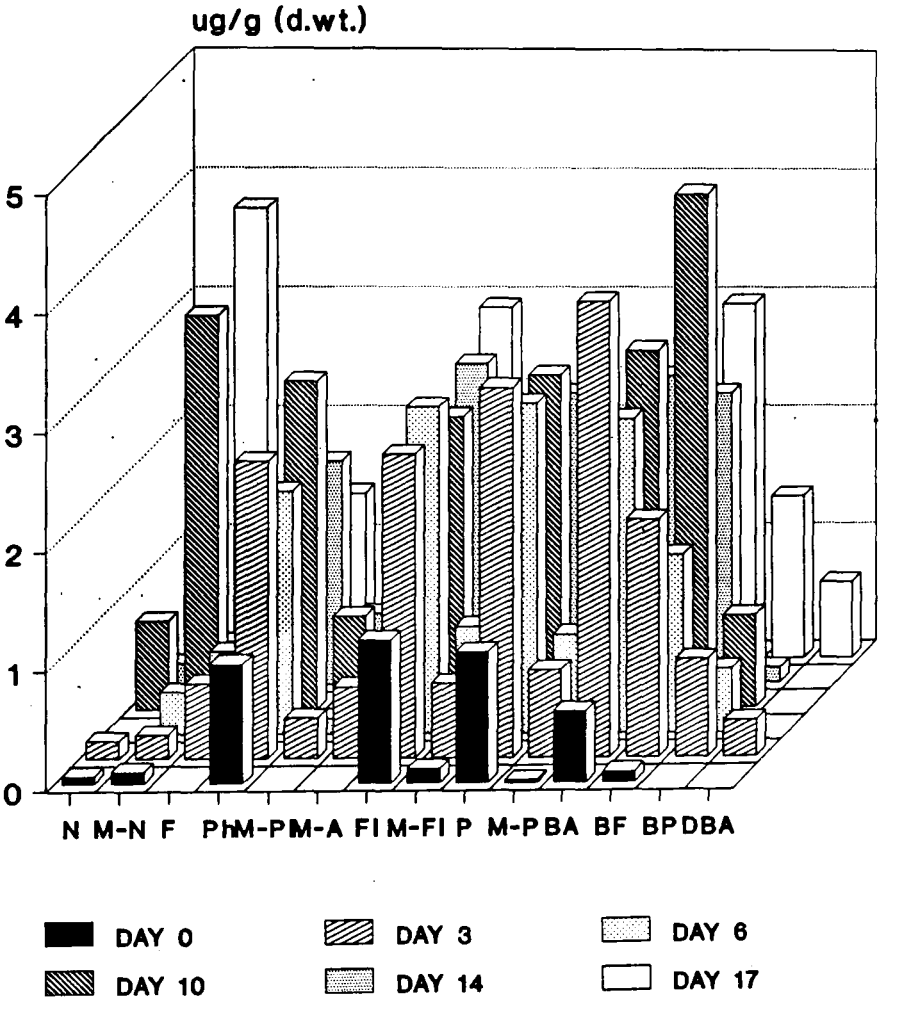


Fig. 5.14 Spatial and temporal individual PAH distribution in *L. peregra* at Site 5 during Period C.

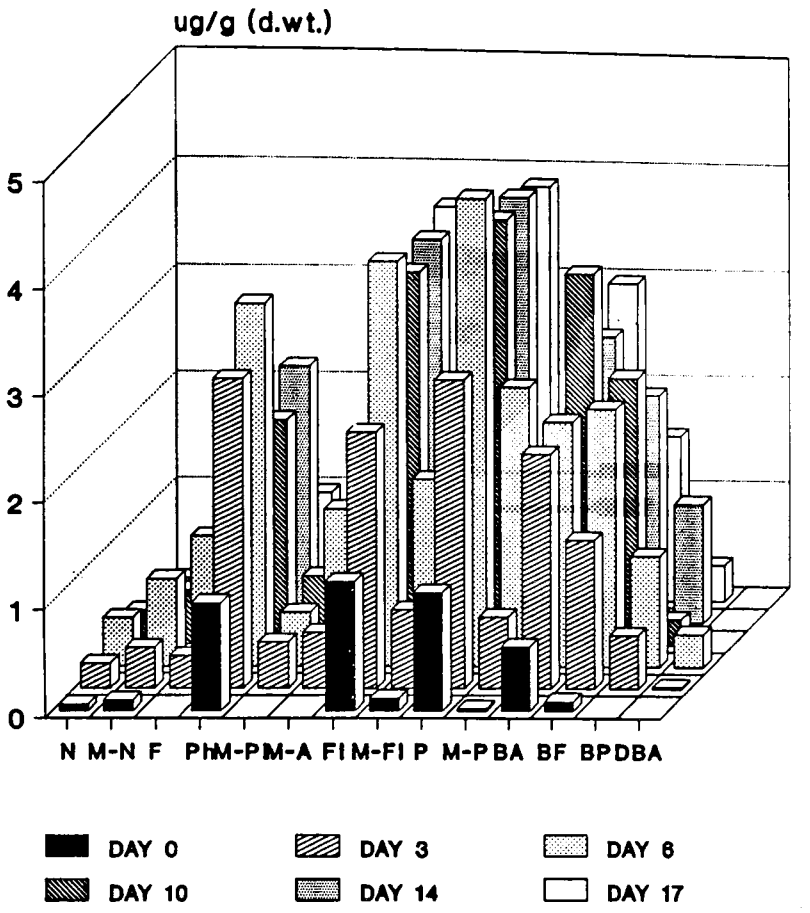


Fig. 5.15 Spatial and temporal individual PAH distribution in *A. aquaticus* at Site 5 during Period A.

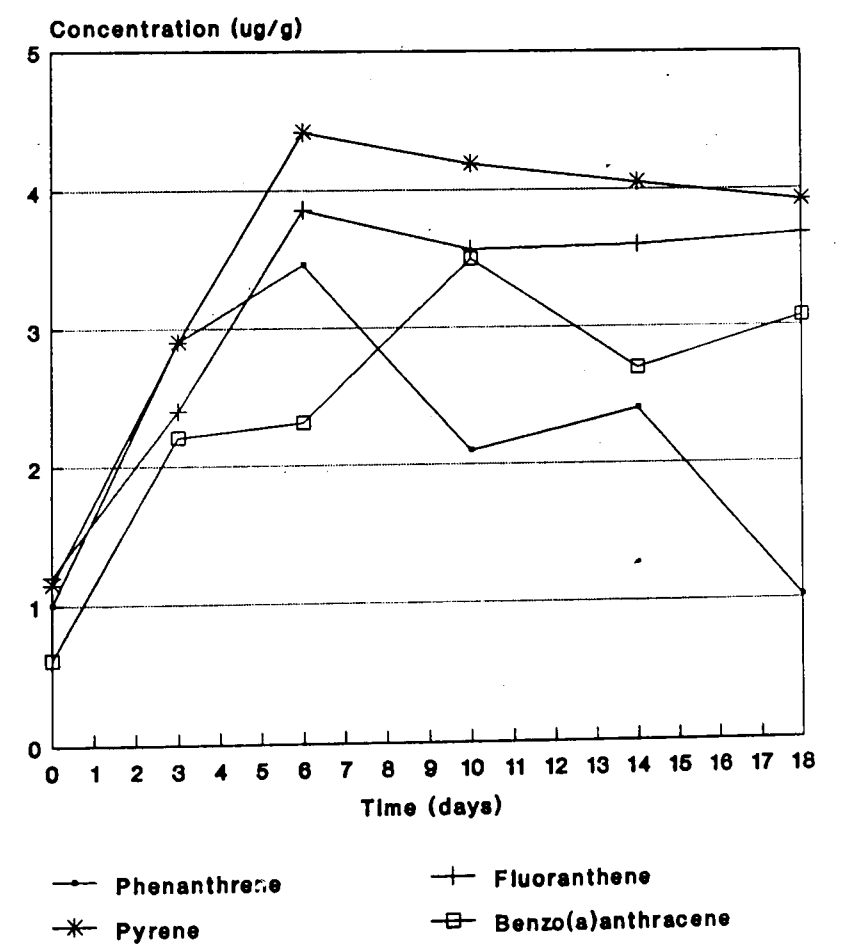


Fig. 5.16 Temporal variation in the 4 principal PAHs in *A. aquaticus* tissue at Site 6 during Period B.

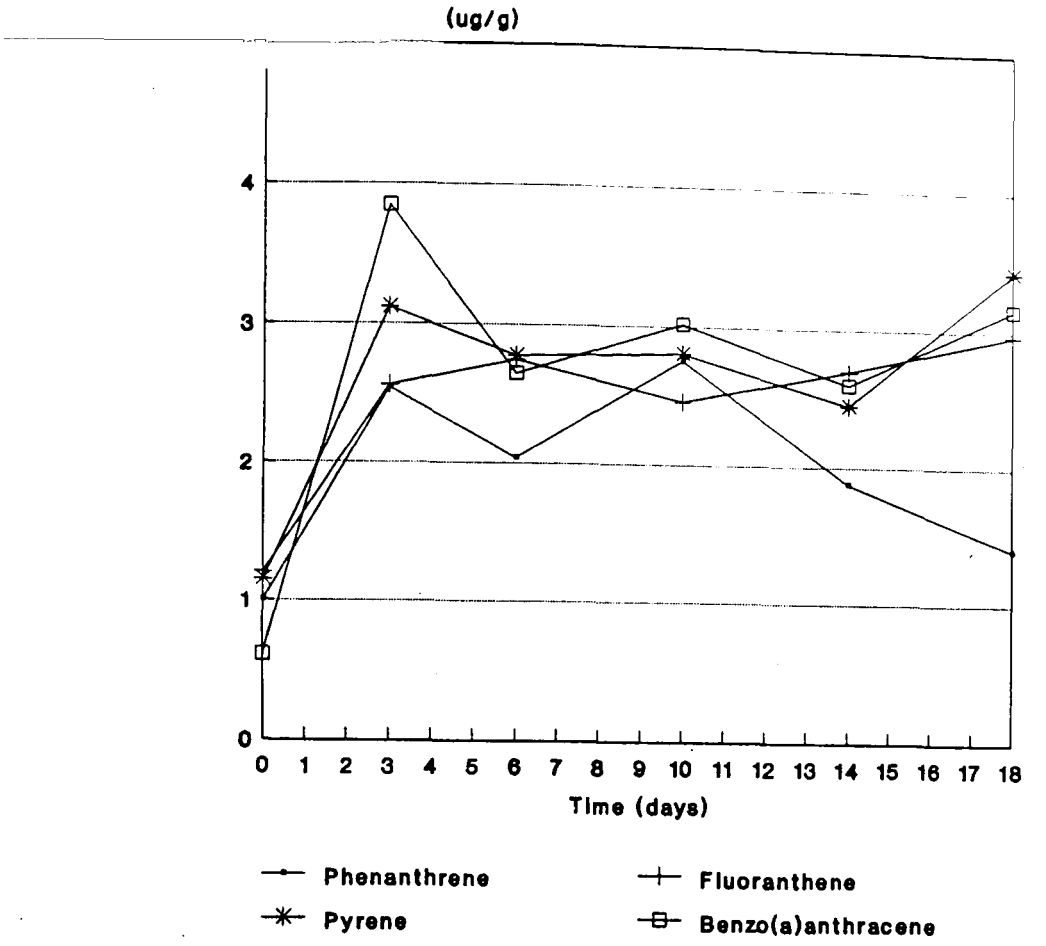


Fig. 5.17 Temporal variation in the 4 principal PAHs in *A. aquaticus* tissue at Site 5 during Period A.

The results also show that *L. peregra* PAH tissue levels are generally greater than *A. aquaticus* values with the highest values again being consistently obtained at Sites 6 and 7 (Tables 5.15 - 5.22). Particularly high levels were detected on day 15 of monitoring Period E at Site 6 (Table 5.21). In *L. peregra*, phenanthrene levels appear proportionally higher in comparison with pyrene and fluoranthene than is the case for *A. aquaticus*. Thus, the proportion of the four principal PAHs (phenanthrene, pyrene, fluoranthene, benzo(a)phenanthrene compared to the total PAH level is reduced to between 35 and 50%. Other differences in overall profiles include an elevation in methyl substituted PAH levels in some *L. peregra* samples (e.g. methyl-naphthalene during days 10 and 17, Fig 5.14). These species differences may be the result of the lack or scarcity of mixed function oxidase (MFO) enzymes in molluscs which effect the removal of many organic compounds. It would seem likely, therefore, that in view of the tissue PAH data reported and what is known of MFO status in molluscs that *L. peregra*, although able to eliminate PAHs (see Chapter 6), is less efficient at doing so than the Crustacean *A. aquaticus*. While further experiments would be needed to test the hypothesis, it would appear that the species differences observed are more likely to be governed by differences in removal mechanisms rather than uptake mechanisms.

TABLE 5.15

INDIVIDUAL PAH CONCENTRATIONS IN *A. AQUATICUS* AT SITES 5, 6, 7, AND 9 DURING PERIOD A.

	SITE 5 PERIOD A (Days)						SITE 6 PERIOD A (Days)					
	0	3	6	10	14	18	0	3	6	10	14	18
N	0.06	0.15	ND	0.74	0.18	0.84	0.06	0.23	0.45	0.31	ND	0.20
M-N	0.10	0.30	0.35	3.31	0.36	3.80	0.10	0.38	0.81	0.50	ND	0.40
F	ND	0.63	0.69	0.54	0.37	0.64	ND	0.30	1.21	0.40	0.12	0.30
Ph+A	1.00	2.54	2.04	2.76	1.88	1.40	1.00	2.90	3.46	2.10	2.40	1.02
M-Ph	ND	0.34	0.13	0.78	0.60	0.83	ND	0.43	0.50	0.63	0.40	0.26
M-A	ND	0.60	0.14	1.91	0.35	0.94	ND	0.52	1.47	0.35	0.69	0.53
Fl	1.20	2.56	2.75	2.46	2.70	2.97	1.20	2.40	3.86	3.57	3.60	3.68
Py	1.15	3.12	2.78	2.81	2.45	3.43	1.15	2.90	4.42	4.19	4.05	3.92
M-Fl	0.12	0.63	0.90	1.21	1.03	1.58	0.12	0.73	1.75	1.64	0.95	1.30
M-Py	0.02	0.74	0.83	0.65	0.72	2.03	0.02	0.67	2.63	0.92	0.83	1.39
BA+Ch	0.61	3.85	2.65	3.02	2.60	3.16	0.61	2.21	2.31	3.50	2.70	3.07
BFs	0.09	2.01	1.50	4.34	2.45	3.00	0.09	1.39	2.43	2.52	2.15	1.56
BPs	ND	0.83	0.54	0.78	0.13	1.37	ND	0.50	1.04	0.24	1.12	0.34
DBA	ND	0.21	ND	ND	ND	0.64	ND	0.02	0.31	ND	ND	ND
BPe	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
IPy	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

	SITE 7 PERIOD A (Days)						SITE 9 PERIOD A (DAYS)					
	0	3	6	10	14	18	0	3	6	10	14	18
N	0.06	0.12	0.10	0.37	0.05	ND	0.06	ND	ND	ND	ND	ND
M-N	0.10	ND	0.05	2.29	0.71	0.10	0.10	ND	ND	ND	ND	0.40
F	ND	0.11	0.41	0.73	ND	0.31	ND	ND	ND	ND	ND	ND
Ph+A	1.00	2.59	3.27	2.51	2.35	2.08	1.00	1.58	1.27	1.63	1.52	1.46
M-Ph	ND	0.19	0.39	2.24	0.10	0.39	ND	0.33	0.25	0.20	ND	ND
M-A	ND	0.39	0.63	1.38	0.72	0.91	ND	0.62	0.74	0.59	0.34	ND
Fl	1.20	2.86	3.93	3.74	3.99	3.75	1.20	1.02	1.30	1.72	1.41	1.58
Py	1.15	3.10	4.18	4.02	4.58	4.20	1.15	1.13	1.26	1.93	1.37	1.63
M-Fl	0.12	0.74	1.34	1.95	0.83	1.24	0.12	0.23	0.35	0.29	0.39	1.45
M-Py	0.02	0.96	1.86	1.94	0.95	1.03	0.02	0.17	0.42	0.30	0.61	0.54
BA+Ch	0.61	2.94	2.84	3.11	2.03	2.65	0.61	0.75	0.68	1.01	0.90	1.30
BFs	0.09	2.35	1.35	2.87	1.02	1.63	0.09	0.48	0.56	0.34	0.81	0.13
BPs	ND	0.84	0.29	2.64	0.40	0.25	ND	0.18	0.36	0.13	0.05	0.17
DBA	ND	ND	ND	0.25	ND	ND	ND	ND	ND	ND	ND	ND
BPe	ND	ND	ND	0.18	ND	ND	ND	ND	ND	ND	ND	ND
IPy	ND	ND	ND	0.21	ND	ND	ND	ND	ND	ND	ND	ND

TABLE 5.16

INDIVIDUAL PAH CONCENTRATIONS IN *A. AQUATICUS* AT SITES 5, 6, 7 AND 9 DURING PERIOD B.

	SITE 5 PERIOD B (DAYS)					SITE 6 PERIOD B (DAYS)				
	0	4	11	14	24	0	4	11	14	24
N	ND	0.26	0.34	0.19	0.45	ND	0.39	0.38	NA	NA
M-N	ND	0.65	0.87	0.49	0.63	ND	1.96	2.85	NA	NA
F	ND	1.42	0.39	0.37	0.59	ND	0.42	0.63	NA	NA
Ph+A	0.75	1.82	2.83	2.43	2.15	0.75	2.10	9.69	NA	NA
M-Ph	0.10	0.05	0.17	0.65	0.75	0.10	0.38	2.02	NA	NA
M-A	ND	0.30	0.21	0.39	1.14	ND	0.59	2.19	NA	NA
Fl	0.80	2.14	2.47	2.70	2.96	0.80	1.82	7.14	NA	NA
Py	1.21	2.16	4.03	2.41	3.45	1.21	2.16	8.49	NA	NA
M-Fl	0.05	1.02	1.11	0.69	2.17	0.05	1.05	2.04	NA	NA
M-Py	0.45	0.49	0.38	0.54	2.31	0.45	1.21	2.08	NA	NA
BA+Ch	0.66	2.88	2.38	2.15	2.62	0.66	2.29	0.62	NA	NA
BFs	ND	2.03	1.04	1.56	2.09	ND	2.03	3.95	NA	NA
BPs	ND	0.51	0.12	0.39	1.34	ND	0.67	2.89	NA	NA
DBA	ND	0.05	ND	ND	0.12	ND	ND	ND	NA	NA
BPe	ND	ND	ND	ND	0.06	ND	ND	ND	NA	NA
IPy	ND	ND	ND	ND	0.12	ND	0.21	ND	NA	NA

	SITE 7 PERIOD B (Days)					SITE 9 PERIOD B (Days)				
	0	4	11	14	24	0	4	11	14	24
N	ND	0.10	0.12	ND	1.03	ND	ND	ND	ND	0.10
M-N	ND	0.40	0.92	0.38	2.64	ND	0.38	ND	0.21	0.05
F	ND	0.24	0.98	0.90	0.19	ND	0.35	0.26	0.14	ND
Ph+A	0.75	2.45	3.41	2.10	2.25	0.75	0.91	0.52	1.04	0.51
M-Ph	0.10	0.63	1.04	0.25	0.15	0.10	0.64	0.50	0.62	0.19
M-A	ND	0.44	0.92	0.63	0.45	ND	0.13	1.29	1.28	0.31
Fl	0.80	3.20	4.17	4.72	3.96	0.80	1.36	1.61	1.40	1.54
Py	1.21	3.49	6.24	4.90	4.78	1.21	1.52	0.25	0.51	1.69
M-Fl	0.05	0.69	1.41	1.82	1.02	0.05	0.46	0.31	0.42	0.64
M-Py	0.45	0.73	1.79	1.06	1.39	0.45	0.34	0.65	0.29	0.40
BA+Ch	0.66	3.04	2.01	2.83	2.90	0.66	1.26	1.72	1.07	2.05
BFs	ND	3.01	2.94	1.84	1.35	ND	0.15	0.29	0.39	0.45
BPs	ND	0.90	1.43	1.01	0.85	ND	0.10	0.08	0.21	0.22
DBA	ND	ND	ND	ND	0.16	ND	0.05	ND	ND	ND
BPe	ND	ND	ND	ND	0.06	ND	ND	ND	ND	ND
IPy	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE 5.17

INDIVIDUAL PAH CONCENTRATIONS IN *A. AQUATICUS* AT SITES 5, 6, 7 AND 9 DURING PERIODS C.

	SITE 5 PERIOD C (Days)							SITE 6 PERIOD C (DAYS)						
	0	6	12	15	20	27	32	0	6	12	15	20	27	32
N	0.11	0.10	0.06	0.18	0.13	0.10	0.13	0.11	0.17	0.25	0.08	0.35	0.12	0.19
M-N	0.32	0.34	0.09	0.29	0.69	0.39	0.45	0.32	0.19	0.21	0.84	0.75	0.13	0.34
F	0.19	0.17	0.46	0.29	0.24	0.34	0.26	0.19	0.05	ND	0.62	0.39	0.06	0.34
Ph+A	0.42	0.82	2.45	3.02	2.72	2.69	3.91	0.42	0.86	3.24	1.72	2.63	2.80	2.60
M-Ph	0.24	0.26	0.43	0.42	0.79	0.37	0.45	0.24	0.43	0.39	0.49	0.38	0.29	0.45
M-A	0.69	0.89	0.39	0.84	0.74	0.87	0.63	0.69	0.31	0.12	0.64	0.54	0.30	0.09
Fl	0.38	2.92	2.95	3.60	2.82	2.77	3.02	0.38	2.69	4.36	5.01	3.45	4.22	4.20
Py	0.52	3.03	2.60	3.72	3.42	2.46	3.20	0.52	0.34	4.24	4.27	4.72	3.50	4.52
M-Fl	0.29	0.75	1.83	1.13	3.21	0.85	0.39	0.29	3.05	1.75	2.63	1.71	1.03	1.21
M-Py	0.36	0.96	0.72	1.21	2.40	0.74	0.62	0.36	0.97	1.44	2.53	2.01	1.23	1.36
BA+Ch	0.24	2.32	1.77	1.64	1.93	1.44	1.36	0.24	1.98	1.30	2.29	3.04	1.65	1.94
BFs	0.39	1.24	1.54	1.28	2.28	1.01	2.65	0.39	1.24	1.54	2.02	1.02	1.24	3.01
BPs	0.42	0.56	0.92	1.01	0.67	0.10	ND	0.42	0.65	0.31	2.98	0.49	0.56	0.53
DBA	ND	ND	ND	0.10	ND	0.12	ND	ND	0.05	ND	0.35	0.09	0.08	ND
BPe	ND	ND	ND	0.12	ND	ND	ND	ND	ND	ND	ND	0.05	0.06	ND
IPy	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.12	ND	ND

	SITE 7 PERIOD C (Days)							SITE 9 PERIOD C (Days)						
	0	6	12	15	20	27	32	0	6	12	15	20	27	32
N	0.11	0.22	0.14	0.10	0.24	NA	NA	0.11	0.21	ND	ND	0.21	0.05	ND
M-F	0.32	0.36	0.39	ND	0.29	NA	NA	0.32	0.06	0.32	0.10	0.12	0.10	0.34
F	0.19	0.26	0.84	0.63	0.39	NA	NA	0.19	0.14	0.09	0.08	0.13	0.09	0.20
Ph+A	0.42	0.92	3.36	3.63	1.45	NA	NA	0.42	0.38	1.14	1.08	2.24	1.42	1.12
M-Ph	0.24	0.77	0.38	1.63	0.30	NA	NA	0.24	0.24	0.47	0.46	0.83	0.34	0.47
M-A	0.69	0.64	0.84	1.83	0.93	NA	NA	0.69	0.39	0.06	0.07	0.36	0.49	0.06
Fl	0.38	3.82	5.03	4.30	4.28	NA	NA	0.38	1.55	1.32	1.86	2.28	2.03	1.68
Py	0.52	3.20	5.82	4.08	4.62	NA	NA	0.52	1.20	1.03	1.72	2.32	2.55	2.67
M-Fl	0.29	0.79	0.52	2.34	0.84	NA	NA	0.29	0.26	0.42	0.21	0.30	0.24	ND
M-Py	0.36	0.62	0.59	2.19	1.34	NA	NA	0.36	ND	0.39	0.62	0.31	ND	ND
BA+Ch	0.24	1.67	1.57	4.36	4.20	NA	NA	0.24	1.10	0.56	1.24	0.84	1.02	1.02
BFs	1.39	1.26	0.83	2.97	1.88	NA	NA	1.39	ND	0.54	1.12	0.43	0.63	0.62
BPs	0.42	0.12	0.89	0.08	0.56	NA	NA	0.42	0.24	ND	0.30	0.20	0.09	0.09
DBA	ND	0.09	ND	0.31	0.12	NA	NA	ND	ND	0.05	ND	ND	ND	ND
BPe	ND	ND	ND	ND	ND	NA	NA	ND	ND	ND	ND	ND	ND	ND
IPy	ND	ND	ND	ND	ND	NA	NA	ND	ND	ND	ND	ND	ND	ND

TABLE 5.18

INDIVIDUAL PAH CONCENTRATION IN *A. AQUATICUS* AT SITES 5, 6, 7 AND 9 DURING PERIOD D.

	SITE 5 PERIOD D (Days)				SITE 6 PERIOD D (Days)				SITE 7 PERIOD D (Days)				SITE 9 PERIOD D (Days)			
	0	7	11	19	0	7	11	19	0	7	11	19	0	7	11	19
N	ND	0.30	0.13	0.10	ND	0.61	ND	NA	ND	0.21	0.13	NA	ND	0.06	0.06	0.05
M-N	ND	2.45	0.63	0.25	ND	1.21	0.42	NA	ND	0.36	0.07	NA	ND	0.23	0.14	0.13
F	0.05	0.34	0.38	0.15	0.05	0.44	0.13	NA	0.05	ND	0.41	NA	0.05	0.12	0.07	0.05
Ph+A	0.32	1.24	1.50	1.72	0.32	1.34	1.60	NA	0.32	1.56	1.92	NA	0.32	1.00	1.27	1.09
M-Ph	0.05	1.43	0.87	0.75	0.05	0.84	0.69	NA	0.05	0.84	1.26	NA	0.05	0.39	0.34	0.25
M-A	0.09	0.74	0.63	0.54	0.09	0.34	0.39	NA	0.09	0.69	1.54	NA	0.09	0.50	0.28	0.38
Fl	0.59	2.76	2.50	2.23	0.59	2.88	2.94	NA	0.59	3.24	3.70	NA	0.59	1.52	1.09	1.63
Py	0.24	1.04	1.03	0.87	0.24	3.42	3.49	NA	0.24	3.06	4.67	NA	0.24	1.97	1.24	1.54
M-Fl	0.72	2.04	2.82	2.64	0.72	1.49	1.38	NA	0.72	1.06	2.20	NA	0.72	0.96	0.42	0.49
M-Py	0.21	0.83	0.59	0.98	0.21	2.03	1.54	NA	0.21	1.21	2.09	NA	0.21	0.76	0.38	0.63
BA+Ch	0.45	1.62	1.94	1.04	0.45	1.72	1.70	NA	0.45	2.03	2.42	NA	0.45	1.43	1.15	1.17
BFs	0.10	2.43	0.60	0.45	0.10	0.94	0.62	NA	0.10	1.74	2.35	NA	0.10	0.84	0.69	0.74
BPs	0.05	0.69	ND	ND	0.05	1.34	0.60	NA	0.05	0.45	0.77	NA	0.05	0.21	0.24	0.07
DBA	ND	0.23	ND	ND	ND	0.59	ND	NA	ND	ND	0.21	NA	ND	ND	ND	ND
BPe	ND	ND	ND	ND	ND	ND	ND	NA	ND	ND	ND	NA	ND	ND	ND	ND
IPy	ND	ND	ND	ND	ND	ND	ND	NA	ND	ND	ND	NA	ND	ND	ND	ND

TABLE 5.19

INDIVIDUAL PAH CONCENTRATION IN *A. AQUATICUS* AT SITES 5, 6, 7 AND 9 DURING PERIOD E.

	SITE 5 PERIOD E (Days)					SITE 6 PERIOD E (Days)					SITE 7 PERIOD E (Days)					SITE 9 PERIOD E (Days)				
	0	4	7	15	22	0	4	7	15	22	0	4	7	15	22	0	4	7	15	22
N	0.12	0.18	0.39	NA	NA	0.12	0.13	0.10	ND	0.60	0.12	0.12	0.30	0.12	NA	0.12	0.10	0.13	0.32	0.46
M-N	0.12	0.22	0.46	NA	NA	0.12	0.24	0.45	0.34	1.12	0.12	0.32	0.40	0.36	NA	0.12	0.30	0.26	0.65	0.30
F	ND	0.36	0.43	NA	NA	ND	0.18	0.32	0.28	0.50	ND	0.48	0.30	ND	NA	ND	0.30	0.49	0.36	0.34
Ph+A	0.25	1.21	1.34	NA	NA	0.25	2.63	3.22	2.78	4.31	0.25	1.72	1.55	1.65	NA	0.25	0.32	0.62	0.80	0.62
M-Ph	0.09	0.39	0.65	NA	NA	0.09	1.39	1.42	1.66	2.30	0.09	0.64	0.90	0.36	NA	0.09	0.42	0.39	0.20	0.52
M-A	0.12	0.46	0.39	NA	NA	0.12	0.86	0.87	0.94	0.76	0.12	0.65	0.65	0.79	NA	0.12	ND	0.36	ND	0.41
Fl	0.40	2.40	2.32	NA	NA	0.40	2.03	2.87	4.01	3.04	0.40	2.70	3.05	3.24	NA	0.40	0.74	0.97	1.25	1.74
Py	0.62	2.55	0.93	NA	NA	0.62	2.10	3.49	1.63	5.21	0.62	1.10	3.78	3.58	NA	0.62	0.89	1.21	1.73	1.80
M-Fl	0.04	0.69	3.25	NA	NA	0.04	0.85	1.03	3.45	2.65	0.04	2.95	2.10	1.34	NA	0.04	0.13	0.42	0.34	0.40
M-Py	0.11	0.74	1.50	NA	NA	0.11	0.54	1.70	1.78	2.94	0.11	1.09	2.73	1.98	NA	0.11	0.50	0.39	0.28	0.39
BA+Ch	0.18	0.70	1.44	NA	NA	0.18	1.55	0.82	1.90	3.08	0.18	1.42	2.04	1.75	NA	0.18	0.38	0.84	0.57	0.26
BFs	0.24	0.65	0.99	NA	NA	0.24	1.03	0.59	1.67	2.76	0.24	1.12	1.21	0.64	NA	0.24	0.21	0.63	0.64	0.54
BPs	0.10	0.34	0.42	NA	NA	0.10	1.08	2.36	1.39	1.73	0.10	0.49	0.43	0.65	NA	0.10	0.24	0.20	0.28	0.48
DBA	ND	0.21	ND	NA	NA	ND	ND	0.10	0.24	ND	ND	ND	0.23	ND	NA	ND	ND	ND	ND	0.49
BPe	ND	ND	ND	NA	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND	NA	ND	ND	ND	ND	ND
IPy	ND	ND	ND	NA	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND	NA	ND	ND	ND	ND	ND

TABLE 5.20

INDIVIDUAL PAH CONCENTRATIONS IN *L. PEREGRA* AT SITES 5, 6, 7 AND 9 DURING PERIOD C.

	SITE 5 PERIOD C					SITE 6 PERIOD C				
	0	6	12	20	32	0	6	12	20	32
N	ND	0.43	ND	ND	0.13	ND	ND	0.73	0.60	0.32
M-N	ND	1.69	1.24	1.03	1.75	ND	0.36	2.58	2.02	3.14
F	ND	ND	ND	ND	0.12	ND	0.49	0.39	0.59	0.42
Ph+A	1.62	1.59	2.50	2.32	2.86	1.62	2.69	3.05	3.10	4.20
M-Ph	0.14	0.74	0.79	0.91	2.04	0.14	0.58	1.03	1.24	2.30
M-A	0.20	1.36	0.85	0.98	1.45	0.20	0.76	1.09	0.86	2.45
Fl	1.45	1.25	2.32	2.68	2.59	1.45	1.98	4.03	3.75	4.49
Py	2.02	1.09	2.45	3.58	3.51	2.02	3.87	4.5	4.39	5.69
M-Fl	0.12	1.36	0.52	1.78	1.98	0.12	0.32	2.69	1.58	2.94
M-Py	0.26	2.28	1.94	2.36	2.49	0.26	0.48	2.04	1.39	3.89
BA+Ch	1.12	0.85	1.75	1.92	2.00	1.12	1.71	3.06	3.71	3.22
BFs	0.39	1.29	1.64	1.38	2.32	0.39	ND	2.54	1.28	3.04
BPs	0.20	0.49	0.68	1.74	2.02	0.20	0.14	0.63	0.58	0.89
DBA	ND	ND	0.13	0.06	ND	ND	ND	0.13	0.12	ND
BPe	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
IPy	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

	SITE 7 PERIOD C					SITE 9 PERIOD C				
	0	6	.12	20	32	0	6	12	20	32
N	ND	ND	0.23	0.65	0.07	ND	0.10	0.09	1.01	0.45
M-N	ND	O.64	0.75	2.99	1.38	ND	0.63	0.23	2.03	0.94
F	ND	ND	0.18	0.54	0.39	ND	0.23	0.69	0.62	0.36
Ph+A	1.62	1.42	2.95	3.17	2.49	1.62	1.94	1.02	1.61	1.72
M-Ph	0.14	0.59	0.84	1.85	0.86	0.14	0.39	0.28	0.84	0.98
M-A	0.20	1.03	0.39	0.74	0.73	0.20	0.45	0.73	0.92	0.84
Fl	1.45	4.02	3.70	3.84	4.59	1.45	1.20	1.20	1.30	1.61
Py	2.02	4.10	3.92	4.63	5.04	2.02	2.09	1.34	0.18	1.29
M-Fl	0.12	2.00	1.42	1.69	1.35	0.12	0.49	0.36	1.72	0.63
M-Py	0.26	1.76	1.23	2.84	2.09	0.26	0.54	0.38	0.54	0.24
BA+Ch	1.12	0.86	3.19	3.19	1.23	1.12	1.03	0.41	0.94	0.05
BFs	0.39	3.34	1.38	3.76	2.20	0.39	1.02	1.23	1.34	0.99
BPs	0.20	1.08	0.39	1.79	0.57	0.20	0.30	0.15	0.62	0.38
DBA	ND	0.54	ND	ND	ND	ND	ND	0.06	0.09	ND
BPe	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
IPy	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE 5.21

INDIVIDUAL PAH CONCENTRATIONS IN *L. PEREGRA* AT SITES 5, 6, 7 AND 9 DURING PERIOD D.

	SITE 5 PERIOD D		SITE 6 PERIOD D		SITE 7 PERIOD D		SITE 9 PERIOD D		
	0	7	0	7	0	7	0	7	11
N	ND	ND	ND	0.24	ND	0.60	ND	ND	ND
M-N	ND	3.46	ND	2.39	ND	3.13	ND	1.34	2.36
F	ND	0.64	ND	0.21	ND	0.46	ND	0.69	0.49
Ph+A	1.01	2.77	1.01	2.82	1.01	3.63	1.01	1.24	1.40
M-Ph	0.09	1.75	0.09	1.65	0.09	1.78	0.09	0.23	0.76
M-A	0.15	1.65	0.15	1.39	0.15	0.94	0.15	0.46	0.74
Fl	1.20	3.46	1.20	3.40	1.20	4.03	1.20	1.62	1.81
Py	1.63	3.81	1.63	4.09	1.63	4.58	1.63	1.82	2.04
M-Fl	0.36	2.63	0.36	2.04	0.36	1.75	0.36	0.49	0.45
M-Py	0.48	2.04	0.48	2.06	0.48	3.90	0.48	0.32	0.54
BA+Ch	0.94	1.72	0.94	3.25	0.94	3.84	0.94	1.24	1.30
BF	0.64	3.85	0.64	2.69	0.64	3.26	0.64	0.72	0.62
BP	0.29	2.94	0.29	1.36	0.29	2.32	0.29	0.30	0.44
DBA	ND	0.14	ND	ND	ND	ND	ND	ND	ND
BPe	ND	ND	ND	ND	ND	ND	ND	ND	ND
IPy	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE 5.22

INDIVIDUAL PAH CONCENTRATIONS IN *L. PEREGRA* AT SITES 5 and 6 DURING PERIOD E.

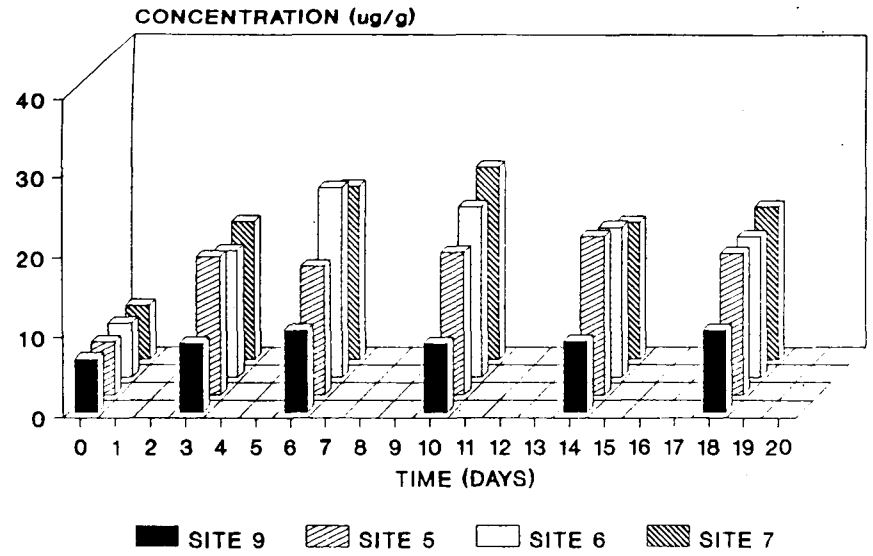
	SITE 5 PERIOD E					SITE 6 PERIOD E				
	0	4	7	14	22	0	4	7	14	22
N	ND	ND	0.86	0.24	0.56	ND	0.36	0.78	2.18	0.06
M-N	0.16	0.30	2.36	1.39	1.42	0.16	0.36	2.58	2.02	3.14
F	0.24	0.12	0.56	0.40	0.36	0.24	0.49	0.39	0.59	0.42
Ph+A	2.21	1.80	2.56	2.32	1.70	2.21	2.69	3.05	3.10	4.20
M-Ph	0.19	0.43	1.39	1.02	0.35	0.19	0.58	1.03	1.24	2.30
M-A	0.30	0.60	1.48	1.19	0.63	0.30	0.76	1.09	0.86	2.45
Fl	1.08	2.12	2.51	2.03	2.76	1.08	1.98	4.03	3.75	4.49
Py	2.43	2.56	2.80	2.07	2.98	2.43	3.87	4.5	4.39	5.69
M-Fl	0.20	0.79	2.80	1.01	1.97	0.20	0.32	2.69	1.58	2.94
M-Py	0.25	1.76	4.22	1.65	1.50	0.25	0.48	2.04	1.39	3.89
BA+Ch	1.41	2.13	3.13	2.79	2.68	1.41	1.71	3.06	3.71	3.22
BFs	0.45	3.48	2.36	1.09	2.32	0.45	ND	2.54	1.28	3.04
BPs	0.10	0.82	1.01	0.68	0.72	0.10	0.14	0.63	0.58	0.89
DBA	ND	ND	ND	ND	ND	ND	ND	0.13	0.12	ND
BPe	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
IPy	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE 5.22 (cont.)
INDIVIDUAL PAH CONCENTRATIONS IN *L. PEREGRA*
AT SITES 7 AND 9 DURING PERIOD E

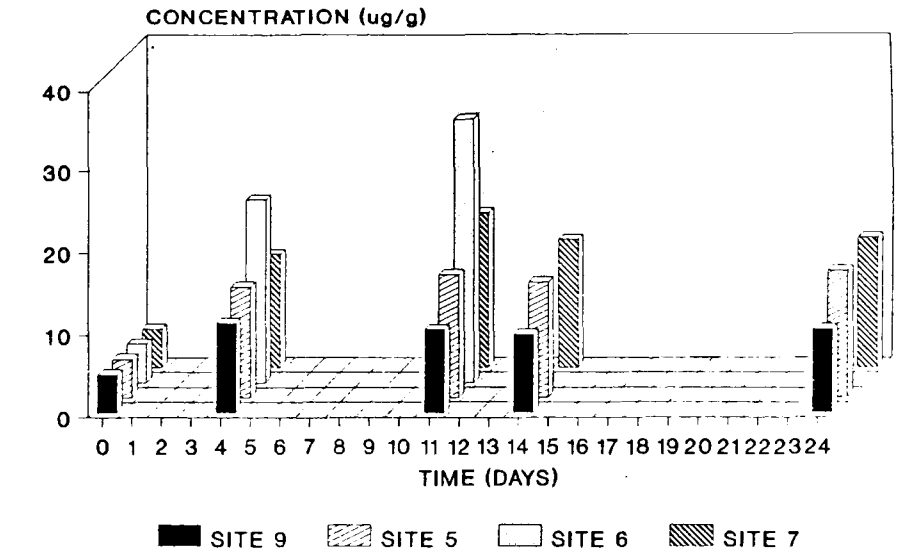
	SITE 7 PERIOD E					SITE 9 PERIOD E				
	0	4	7	14	22	0	4	7	14	22
N	ND	ND	0.23	0.65	0.07	ND	0.35	0.42	0.29	ND
M-N	0.16	O.64	0.75	2.99	1.38	0.16	1.03	1.69	1.85	0.36
F	ND	ND	0.18	0.54	0.39	ND	0.35	0.29	0.51	0.81
Ph+A	2.21	1.42	2.95	3.17	2.49	2.21	1.62	1.84	1.94	2.01
M-Ph	0.19	0.59	0.84	1.85	0.86	0.19	0.92	0.39	1.23	0.69
M-A	0.30	1.03	0.39	0.74	0.73	0.30	0.66	0.54	0.69	0.42
Fl	1.08	4.02	3.70	3.84	4.59	1.08	2.30	1.91	2.04	2.62
Py	2.43	4.10	3.92	4.63	5.04	2.43	2.69	2.44	2.80	2.74
M-Fl	0.20	2.00	1.42	1.69	1.35	0.20	1.13	1.03	0.74	1.09
M-Py	0.25	1.76	1.23	2.84	2.09	0.25	0.82	0.64	0.98	0.74
BA+Ch	1.41	0.86	3.19	3.19	1.23	1.41	0.82	1.34	1.11	1.10
BFs	0.45	3.34	1.38	3.76	2.20	0.45	2.21	1.01	1.29	0.64
BPs	0.10	1.08	0.39	1.79	0.57	0.10	0.23	0.19	0.34	0.26
DBA	ND	0.54	ND	ND	ND	ND	ND	ND	0.16	ND
BPe	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
IPy	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

5.5.7 Spatial and temporal variations in total alkanes in *A. aquaticus* and *L. peregra* during Periods A - E

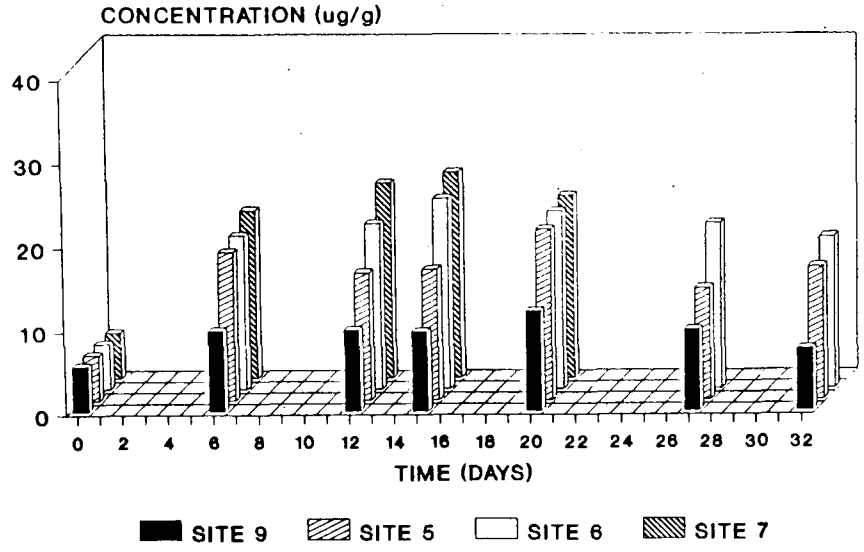
The variations in total alkane levels for *A. aquaticus* during Periods A - E are shown in Figs. 5.18 - 5.19. The general patterns are similar to those previously discussed for the PAHs (Section 5.5.4), in that rises occur within 3-10 days of organism exposure followed by a relatively stable period. Initial levels at the exposure sites are between 2-8 $\mu g\ g^{-1}$ which are higher than those for the PAHs because of the higher aliphatic hydrocarbon concentrations in organisms collected at the background site. At Sites 5, 6 and 7 the levels rise to approximately 20 $\mu g\ g^{-1}$ and remain close to this value for the rest of the trial. During Period B at Site 6, high aliphatic concentrations were recorded which coincided with rainfall and high a mortality count. During Trial D the levels remain lower at each sampling site and during this period, 20 $\mu g\ g^{-1}$ was not reached at any site. These recorded levels, therefore, did not reflect the high mortality count recorded during Period D. The alkane variation in *L. peregra* for Periods C - E is presented in Fig. 5.20.



PERIOD A

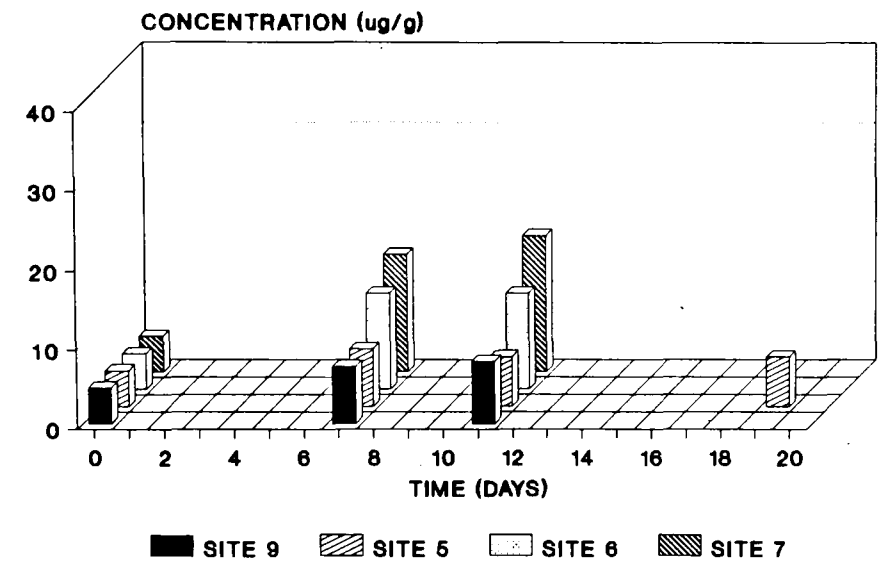


PERIOD B



PERIOD C

Fig. 5.18 Temporal and spatial variation of total alkane levels in *A. aquaticus* in suspended cages at Sites 5, 6, 7 and 9 during Periods A - C.



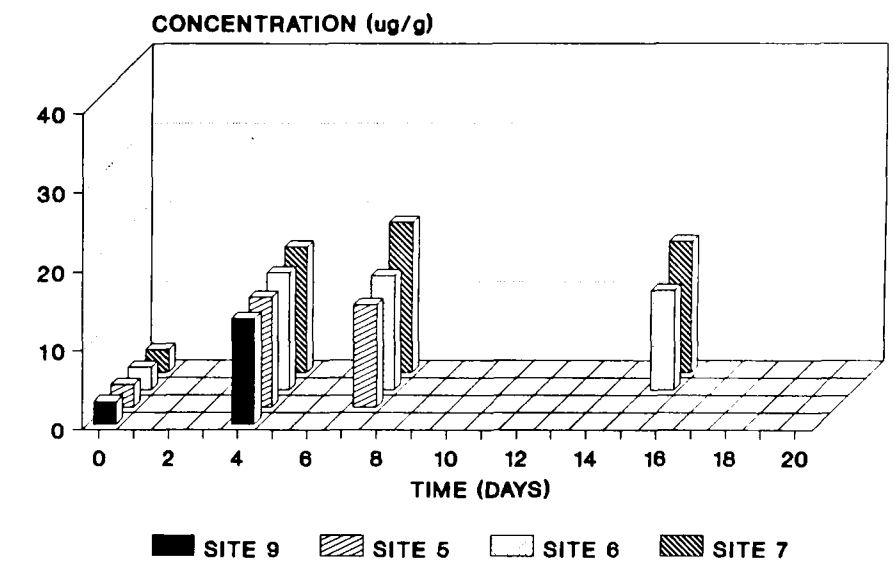
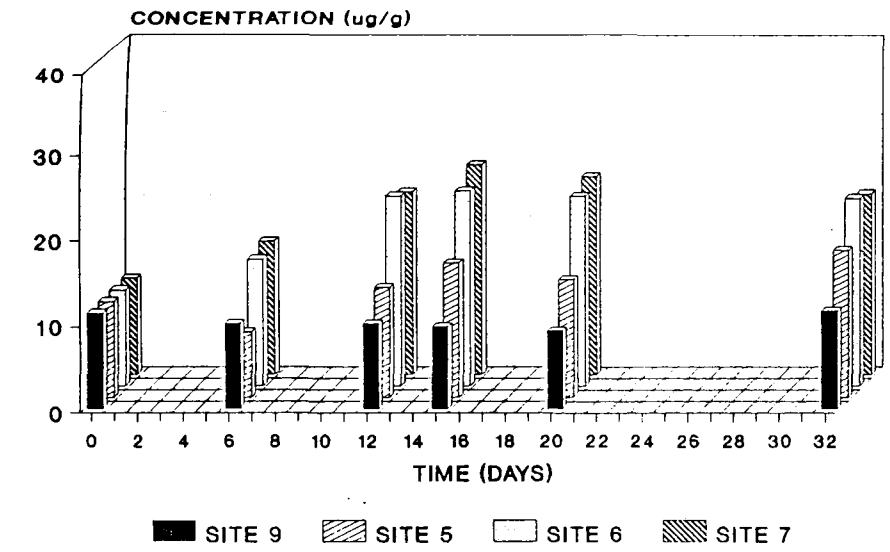
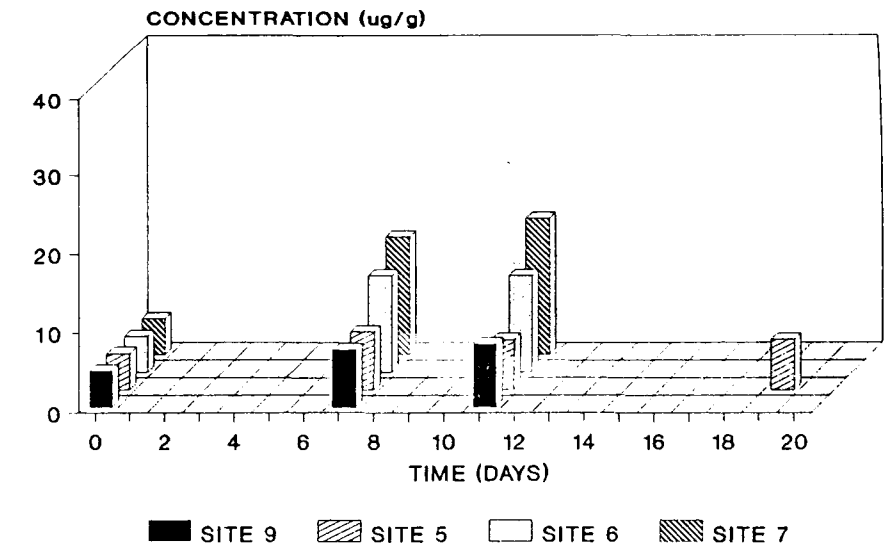


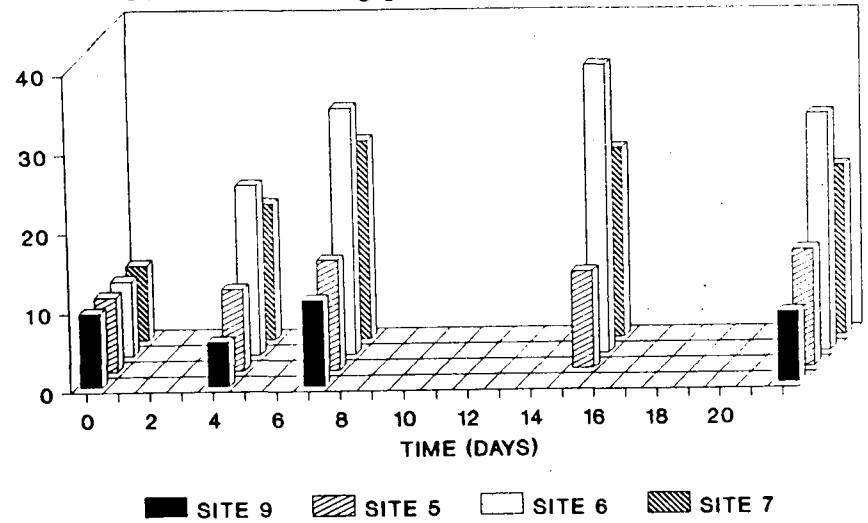
Fig. 5.19 Temporal and spatial variation of total alkane levels in *A. aquaticus* in suspended cages at Sites 5, 6, 7 and 9 during Periods D - E.



PERIOD C



PERIOD D



PERIOD E

Fig. 5.20 Temporal and spatial variation of total alkane levels in *L. peregra* in suspended cages at Sites 5, 6, 7 and 9 during Periods C - E.

In *L. peregra* a typical trend can be observed in Period C, where, from an initial value of 12 $\mu g\ g^{-1}$, a stable plateau of about 20 $\mu g\ g^{-1}$ at the Silk Stream sites occurs. At Site 9, nosignificant change occurs. A similar pattern can be observed during Period E. During Period D values were recorded only to Day 7 after which 100% mortality occurred at Sites 5, 6 and 7.

5.5.8 Spatial and temporal variations in individual alkanes in *A. aquaticus* and *L. peregra* during Periods A-E

The alkane and isoprenoid aliphatic distributions for Periods A - E for *A. aquaticus* show rapid accumulation of aliphatics at each of the Silk Stream sampling sites. The individual alkane values are presented in Tables 5.23 - 5.27. The general pattern is of a unimodal but rather flattened profile with relatively low levels below C_{16} rising to peak values between C_{20} and C_{24} followed by a fall in values with the exception of the superimposition to varying extents of biogenically derived odd numbered long chained alkanes e.g C_{27} (Fig. 5.21). The odd-numbered long chained peaks are most prominent during Period A in *A. aquaticus* but tend to be less important in the other trials. It is possible that either changes in odd-numbered alkane input to the Silk Stream or differences in their uptake by *A. aquaticus* during varying periods of its life-cycle could account for such temporal variations. In Britain, *A. aquaticus* tends to have two major breeding seasons, in late spring and in late summer. Organisms display elevated growth rates when the water temperature exceeds 9^0C. Thus, juvenile or young adult asellids caged during Period A (June) could have distinctly different growth patterns and, consequently, could possess different bioaccumulative patterns to those caged later in the season.

In *L. peregra* at Sites 5, 6 and 7 a more peaked, leptokurtic distribution can be observed representative of a greater dominance of the mid-range C_{20}-C_{24} alkanes (Figs. 5.21 and 5.22). The biotic individual alkane profiles tend to reflect those of surrounding sediments to a greater extent than in the PAH profile. A general exception to this is the slightly elevated CPI values in the long carbon chain length range which are discussed in the following section.

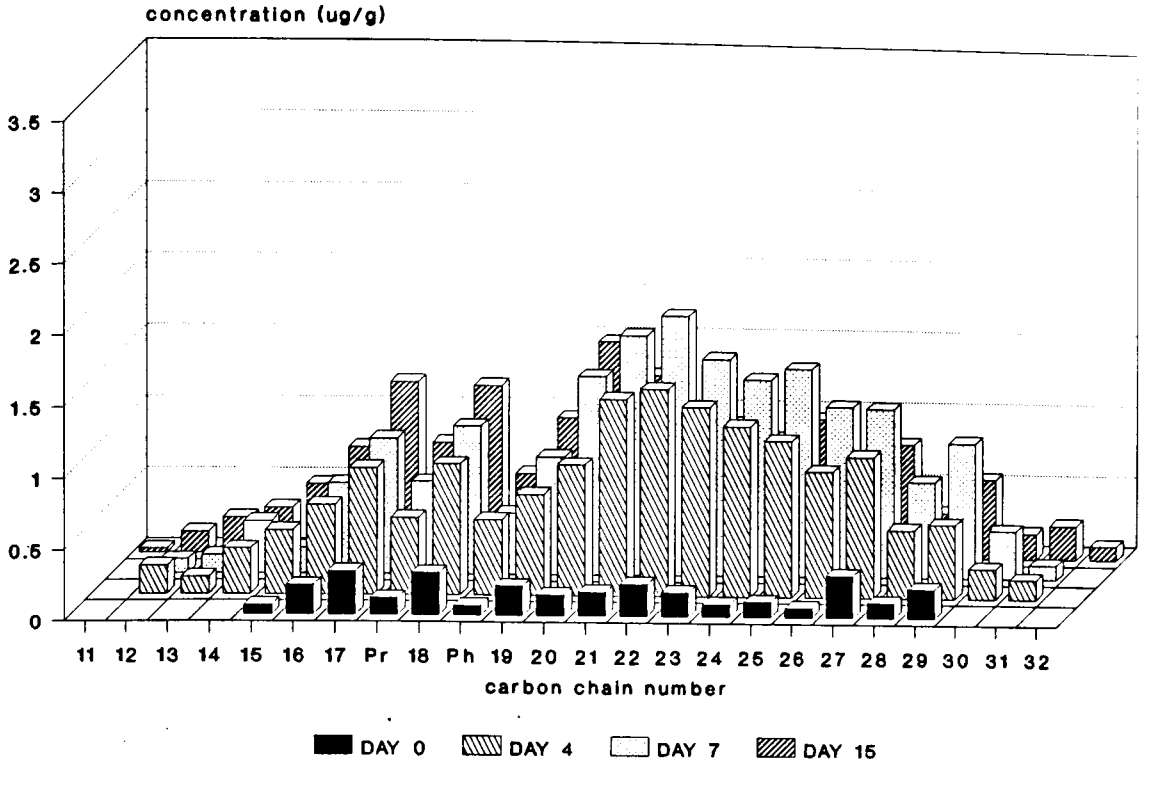


Fig. 5.21 Aliphatic profile for *A. aquaticus* at Site 7 during Period E.

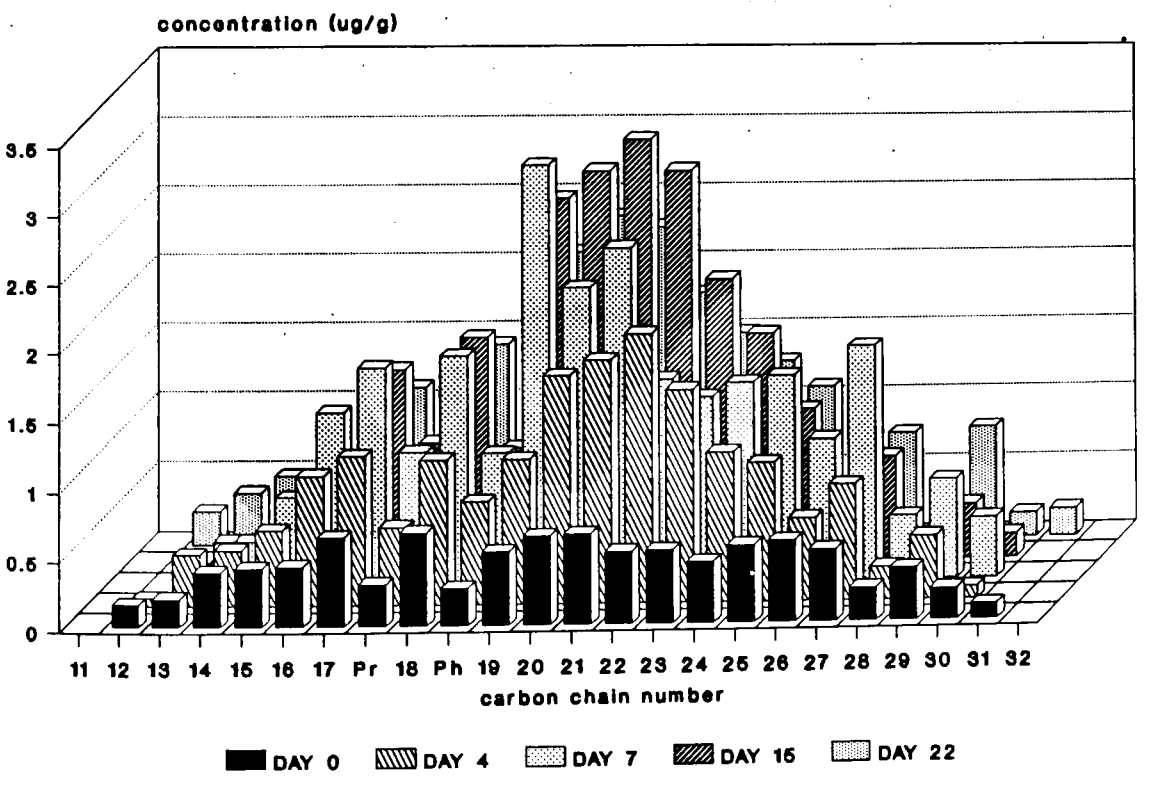


Fig. 5.22 Aliphatic profile for *L. peregra* at Site 7 during Period E.

Teal & Farrington (1977) have noted that the alkane profile in benthic animals tends to reflect the sediment more than the PAH profile, particularly in areas of low pollution. When oil pollution increases, the hydrocarbons become an important carbon source for microorganisms and, as a result of microbial degradation, composition changes occur which may be reflected in the tissue distribution. In the currently reported data there appear to be no changes in discrepancy between abiotic and biotic samples that can be related to the overall amount of contamination at different sites. However, this apparent paradox may be explained by the previous findings relating to biodegradation that were reported in Section 4.4.4. Here, the C_{17}/Pristane and C_{18}/Phytane ratios show that hydrocarbons had been degraded to a very limited extent as a result of recent input and the anoxic sediment conditions present at the most polluted sites. Thus, bacterial related composition changes of the hydrocarbon assemblage prior to incorporation into macroinvertebrates is unlikely to be a major effect at the Silk Stream locations. Fossato & Siviero (1974) noted that biotic aliphatic assemblages contained lower levels of alkanes in the range above C_{25} at oil contaminated sites in Venice. Teal & Farrington (1977) suggested that these types of observations may be explained by the longer chained alkanes being more closely bound with humic substances in sediments with sediment age. Continued and relatively fresh inputs of hydrocarbons in the Silk Stream catchment would help to explain why alkanes $>C_{25}$ were regularly recorded in both sediments and animals.

TABLE 5.23

INDIVIDUAL ALKANE CONCENTRATIONS IN *A. AQUATICUS* AT SITES 5, 6, 7 AND 9 DURING PERIOD A.

	SITE 5 PERIOD A (Days)						SITE 6 PERIOD A (Days)					
alkane	0	3	6	10	14	18	0	3	6	10	14	18
C_{10}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C_{11}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C_{12}	ND	ND	0.08	ND	0.21	ND	ND	0.17	0.10	ND	0.07	0.15
C_{13}	ND	0.21	0.36	0.31	0.34	0.21	ND	0.18	0.39	0.25	0.09	0.24
C_{14}	0.34	0.49	0.52	0.47	0.63	0.25	0.34	0.35	0.43	0.33	0.23	0.21
C_{15}	0.39	0.85	0.78	0.46	0.62	0.49	0.39	0.79	0.65	0.65	0.62	0.36
C_{16}	0.53	0.92	0.79	0.98	0.69	0.34	0.53	0.84	1.30	0.72	0.45	0.49
C_{17}	0.41	1.17	0.69	0.92	1.21	0.84	0.41	1.34	1.87	1.44	1.01	0.69
Pr	0.12	1.34	0.42	0.74	0.92	0.64	0.12	0.59	1.94	1.01	0.76	0.53
C_{18}	0.40	1.35	1.32	0.95	1.13	1.21	0.40	1.39	1.94	1.59	1.23	0.74
Ph	0.18	1.08	1.01	0.79	0.84	0.72	0.18	0.74	1.40	0.93	0.84	0.44
C_{19}	0.39	1.39	1.25	1.01	0.99	1.15	0.39	1.19	1.75	1.69	1.39	1.24
C_{20}	0.52	0.92	1.19	1.25	1.21	1.69	0.52	1.02	1.60	1.29	1.44	1.80
C_{21}	0.41	1.01	1.24	1.12	0.87	1.56	0.41	1.24	1.63	1.34	1.67	1.75
C_{22}	0.40	0.79	1.21	1.57	0.89	1.23	0.50	0.84	1.28	1.80	1.45	1.69
C_{23}	0.32	0.82	0.99	1.39	1.24	1.74	0.32	0.76	1.43	1.75	1.39	1.49
C_{24}	0.21	0.81	0.63	0.83	1.12	0.85	0.21	0.79	1.89	1.24	1.03	0.93
C_{25}	0.48	0.35	0.44	0.94	1.35	1.21	0.48	0.44	1.11	1.15	1.24	1.43
C_{26}	0.28	0.25	0.23	0.24	0.37	0.39	0.28	0.29	0.49	0.63	0.45	0.12
C_{27}	0.69	1.60	1.24	1.96	1.79	1.98	0.69	1.35	1.10	1.94	2.34	1.97
C_{28}	0.21	0.30	0.21	0.30	0.49	0.40	0.21	0.43	0.23	0.57	0.63	0.50
C_{29}	0.23	1.30	1.39	1.21	1.32	0.82	0.23	1.01	1.14	1.02	0.33	0.45
C_{30}	0.05	ND	0.10	0.12	0.40	ND	0.11	0.05	0.10	ND	0.11	0.23
C_{31}	0.11	0.32	ND	0.30	1.21	0.12	ND	ND	ND	ND	ND	0.21
C_{32}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C_{33}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

	SITE 7 PERIOD A (Days)						SITE 9 PERIOD A (DAYS)					
alkane	0	3	6	10	14	18	0	3	6	10	14	18
C_{10}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C_{11}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C_{12}	ND	ND	ND	ND	ND	0.05	ND	ND	ND	ND	ND	ND
C_{13}	ND	0.20	0.36	0.36	0.17	0.14	ND	0.12	0.25	0.14	0.20	0.10
C_{14}	0.34	0.34	0.32	0.43	0.36	0.18	0.34	0.17	0.39	0.20	0.32	0.32
C_{15}	0.39	0.92	0.49	0.72	0.46	0.46	0.39	0.63	0.32	0.30	0.50	0.49
C_{16}	0.53	0.90	0.95	1.30	0.49	0.63	0.53	0.54	0.43	0.40	0.79	0.53
C_{17}	0.41	1.41	1.34	1.74	1.12	0.94	0.41	0.69	0.53	0.72	0.62	0.61
Pr	0.12	0.75	0.88	1.12	0.93	0.61	0.12	0.44	0.40	0.53	0.45	0.23
C_{18}	0.40	1.04	1.59	1.63	1.07	1.39	0.40	0.73	0.93	0.54	0.73	0.62
Ph	0.18	0.63	1.13	1.12	0.63	0.91	0.18	0.56	0.84	0.49	0.60	0.53
C_{19}	0.39	1.11	1.84	1.43	1.26	1.20	0.39	0.53	0.64	0.39	0.56	0.84
C_{20}	0.52	1.34	1.59	1.53	1.34	1.14	0.52	0.69	0.86	0.64	0.64	0.70
C_{21}	0.41	1.23	1.74	1.29	1.66	1.54	0.41	0.56	0.72	0.63	0.60	0.83
C_{22}	0.40	0.95	1.63	1.92	1.50	1.35	0.40	0.44	0.75	0.49	0.57	0.69
C_{23}	0.32	1.18	1.57	1.59	NA	1.36	0.32	0.29	0.89	0.50	0.39	0.74
C_{24}	0.21	1.04	1.07	1.92	1.33	0.88	0.21	0.31	0.40	0.36	0.30	0.66
C_{25}	0.48	0.83	1.34	1.82	1.26	1.13	0.48	0.13	0.51	0.35	0.40	0.63
C_{26}	0.28	0.30	0.74	0.84	1.26	1.13	0.28	0.29	0.39	0.42	0.37	0.44
C_{27}	0.69	0.92	1.64	1.45	0.53	1.54	0.69	0.83	0.83	0.69	0.67	0.80
C_{28}	0.21	0.24	0.30	0.39	1.31	1.56	0.21	0.11	ND	0.13	0.20	0.31
C_{29}	0.23	1.35	1.12	1.41	0.44	1.71	0.23	0.28	0.24	0.42	0.20	0.31
C_{30}	0.05	0.63	ND	0.12	1.13	0.84	0.05	ND	ND	0.12	ND	ND
C_{31}	0.11	ND	0.10	ND	ND	0.08	0.11	ND	ND	ND	ND	ND
C_{32}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C_{33}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE 5.24

INDIVIDUAL ALKANE CONCENTRATIONS IN *A. AQUATICUS* AT SITES 5, 6, 7 AND 9 DURING PERIOD B.

	SITE 5 PERIOD B (Days)					SITE 6 PERIOD B (Days)				
alkane	0	4	11	14	24	0	4	11	14	24
C_{10}	ND	ND	ND	ND	ND	ND	ND	ND	NA	NA
C_{11}	ND	ND	ND	ND	ND	ND	ND	ND	NA	NA
C_{12}	ND	ND	0.09	ND	ND	ND	ND	0.22	NA	NA
C_{13}	ND	ND	0.40	0.10	0.18	ND	0.39	0.68	NA	NA
C_{14}	0.11	0.63	0.26	0.19	0.20	0.11	0.92	1.19	NA	NA
C_{15}	0.31	0.38	0.63	0.38	0.48	0.31	0.84	1.12	NA	NA
C_{16}	0.27	0.75	0.72	0.41	0.59	0.27	1.12	1.40	NA	NA
C_{17}	0.31	0.80	0.95	0.78	1.18	0.31	1.30	1.75	NA	NA
Pr	0.14	0.64	0.54	0.39	0.30	0.14	1.12	1.03	NA	NA
C_{18}	0.24	0.73	0.90	0.97	0.98	0.24	1.59	1.93	NA	NA
Ph	0.10	0.34	0.47	0.65	0.26	0.10	1.74	1.04	NA	NA
C_{19}	0.20	0.97	1.47	1.30	1.24	0.20	1.73	2.48	NA	NA
C_{20}	0.23	1.12	1.24	1.05	1.39	0.23	1.48	3.35	NA	NA
C_{21}	0.27	0.85	1.11	1.34	1.60	0.27	1.92	3.90	NA	NA
C_{22}	0.31	1.21	1.23	1.36	1.32	0.31	1.76	3.09	NA	NA
C_{23}	0.24	1.14	1.24	1.25	1.21	0.24	1.64	2.10	NA	NA
C_{24}	0.20	0.84	1.25	1.14	1.12	0.20	1.13	2.17	NA	NA
C_{25}	0.35	0.73	0.89	0.80	1.33	0.35	1.23	1.55	NA	NA
C_{26}	0.23	0.32	0.40	0.45	0.76	0.23	0.85	0.76	NA	NA
C_{27}	0.56	0.60	0.58	0.97	0.76	0.57	0.80	0.83	NA	NA
C_{28}	0.20	0.48	0.28	0.33	0.34	0.20	0.30	0.72	NA	NA
C_{29}	0.21	0.73	0.31	0.21	0.24	0.21	0.43	0.91	NA	NA
C_{30}	0.09	0.10	0.07	ND	ND	0.09	ND	0.07	NA	NA
C_{31}	0.1	0.17	ND	ND	ND	0.10	ND	ND	NA	NA
C_{32}	ND	ND	ND	ND	ND	ND	ND	ND	NA	NA
C_{33}	ND	ND	ND	ND	ND	ND	ND	ND	NA	NA

	SITE 7 PERIOD B (Days)					SITE 9 PERIOD B (Days)				
alkane	0	4	11	14	24	0	4	11	14	24
C_{10}	ND	ND	ND	ND	ND	ND	ND	D	ND	ND
C_{11}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C_{12}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C_{13}	ND	ND	0.32	0.19	0.29	ND	0.26	ND	0.14	0.16
C_{14}	0.11	0.41	0.60	0.36	0.28	0.11	0.11	ND	0.21	0.19
C_{15}	0.31	0.58	0.25	0.40	0.37	0.31	0.23	0.31	0.34	0.24
C_{16}	0.27	0.80	0.76	0.88	0.75	0.27	0.38	0.42	0.52	0.34
C_{17}	0.31	0.85	1.26	0.80	0.70	0.31	0.49	0.67	0.63	0.54
Pr	0.14	0.64	0.74	0.73	0.45	0.14	0.27	0.39	0.43	0.29
C_{18}	0.24	0.87	1.40	1.00	0.93	0.24	0.72	0.79	0.54	0.88
Ph	0.10	0.91	1.11	0.67	0.63	0.14	0.46	0.63	0.19	0.54
C_{19}	0.20	1.1	1.63	1.45	1.35	0.20	0.80	0.49	0.68	0.83
C_{20}	0.23	1.33	1.68	1.40	1.52	0.23	1.04	0.88	0.72	0.94
C_{21}	0.27	0.95	1.69	1.53	1.30	0.27	0.91	0.70	1.20	1.11
C_{22}	0.32	1.02	1.38	1.22	1.28	0.31	1.23	0.86	0.94	0.86
C_{23}	0.24	1.10	1.22	1.15	1.21	0.24	1.06	1.04	0.73	0.52
C_{24}	0.20	0.91	1.21	1.37	1.13	0.20	0.94	0.72	0.65	0.49
C_{25}	0.35	0.74	1.10	1.11	1.33	0.35	0.81	0.81	0.47	0.48
C_{26}	0.23	0.54	0.89	0.42	0.76	0.23	0.49	0.24	0.54	0.32
C_{27}	0.56	0.80	0.63	0.62	0.96	0.56	0.59	0.72	0.44	0.93
C_{28}	0.20	0.27	0.55	0.13	0.34	0.20	0.13	0.04	0.12	0.12
C_{29}	0.21	nd	0.42	0.42	ND	0.21	ND	0.40	ND	0.21
C_{30}	0.09	ND	ND	ND	ND	0.09	ND	ND	ND	0.05
C_{31}	0.10	ND	ND	ND	ND	0.10	ND	ND	ND	0.08
C_{32}	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.05
C_{33}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE 5.25

INDIVIDUAL ALKANE CONCENTRATIONS IN *A. AQUATICUS* AT SITES 5, 6, 7 AND 9 DURING PERIOD C.

	SITE 5 PERIOD C (Days)							SITE 6 PERIOD C (DAYS)						
alkane	0	6	12	15	20	27	32	0	6	12	15	20	27	32
C_{10}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C_{11}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C_{12}	0.11	0.10	0.12	ND	ND	ND	ND	0.11	1.20	ND	0.33	0.31	0.16	ND
C_{13}	0.12	0.31	0.12	0.21	ND	ND	0.36	0.12	0.40	0.09	0.21	0.48	0.32	0.32
C_{14}	0.29	0.44	0.13	0.40	0.21	0.22	0.52	0.29	0.53	0.42	0.43	0.67	0.31	0.42
C_{15}	0.28	0.63	0.69	0.71	0.56	0.35	0.81	0.28	0.65	0.73	0.50	0.71	0.67	1.09
C_{16}	0.20	0.72	0.74	0.74	1.02	0.40	0.63	0.20	1.18	0.85	0.64	1.00	0.75	1.14
C_{17}	0.44	0.85	0.92	0.92	1.20	0.63	0.83	0.44	0.69	1.16	1.40	0.79	0.70	0.30
Pr	0.12	0.67	0.72	0.66	1.38	0.78	0.19	0.12	1.29	0.84	0.75	1.23	0.43	0.71
C_{18}	0.30	0.85	1.10	1.01	0.65	0.21	0.89	0.30	0.72	1.20	1.30	0.40	0.82	1.43
Ph	0.15	0.52	0.79	0.57	1.49	0.89	0.19	0.15	1.42	0.72	0.73	1.69	0.50	0.71
C_{19}	0.33	0.91	1.12	1.39	0.85	0.32	0.74	0.33	1.38	1.33	1.45	1.68	1.31	1.66
C_{20}	0.35	1.32	1.24	1.01	1.50	1.28	1.71	0.35	1.62	1.44	1.83	1.62	1.50	1.42
C_{21}	0.31	1.14	1.08	1.72	1.63	1.12	1.24	0.31	1.30	1.82	2.02	1.74	1.60	1.23
C_{22}	0.24	1.26	1.23	1.69	1.45	0.96	1.24	0.24	1.40	1.68	1.92	1.55	1.54	1.35
C_{23}	0.28	1.52	0.97	1.02	1.42	1.04	1.26	0.28	0.84	1.46	1.69	1.72	1.88	1.20
C_{24}	0.19	1.18	0.84	1.01	1.20	1.12	1.04	0.19	0.90	1.07	1.25	1.46	1.64	1.27
C_{25}	0.42	1.49	0.91	0.84	1.43	1.24	1.31	0.42	0.72	0.81	1.10	1.34	1.56	1.11
C_{26}	0.26	1.20	0.83	0.24	1.39	0.96	0.68	0.26	1.03	0.65	0.58	1.15	0.93	0.40
C_{27}	0.34	1.78	0.84	0.65	0.97	0.40	0.92	0.34	0.50	1.79	1.76	1.15	1.44	1.24
C_{28}	0.10	0.32	0.21	0.40	0.91	0.73	0.31	0.10	0.52	0.34	1.24	0.74	0.84	0.11
C_{29}	ND	0.41	0.42	0.21	0.24	0.13	0.47	ND	0.10	1.44	1.12	0.80	0.71	ND
C_{30}	ND	0.19	0.21	0.29	0.82	0.51	0.41	ND	0.05	ND	0.53	0.22	0.24	ND
C_{31}	ND	ND	0.07	ND	0.12	0.13	0.09	ND	ND	ND	ND	ND	ND	0.06
C_{32}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C_{33}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

	SITE 7 PERIOD C (Days)							SITE 9 PERIOD C (Days)						
alkane	0	6	12	15	20	27	32	0	6	12	15	20	27	32
C_{10}	ND	ND	ND	ND	ND	NA	NA	ND	ND	ND	ND	ND	ND	ND
C_{11}	ND	ND	ND	ND	ND	NA	NA	ND	ND	ND	ND	ND	ND	ND
C_{12}	0.11	0.10	ND	ND	ND	NA	NA	0.11	ND	ND	ND	ND	ND	ND
C_{13}	0.12	0.40	0.49	0.29	0.13	NA	NA	0.12	ND	0.15	0.10	ND	ND	ND
C_{14}	0.29	0.64	0.43	0.48	0.36	NA	NA	0.29	0.84	0.16	ND	ND	0.19	ND
C_{15}	0.28	0.73	0.64	0.63	0.52	NA	NA	0.28	0.74	0.27	0.21	0.41	ND	ND
C_{16}	0.20	0.96	0.75	0.82	0.25	NA	NA	0.20	0.66	0.33	0.34	0.90	ND	0.34
C_{17}	0.44	1.01	1.67	1.45	1.50	NA	NA	0.44	0.70	0.63	0.59	1.02	0.56	0.79
Pr	0.12	0.54	0.85	0.79	0.39	NA	NA	0.12	0.21	0.41	0.24	0.75	0.40	0.43
C_{18}	0.30	1.22	1.52	1.34	1.76	NA	NA	0.30	0.72	0.75	0.72	1.08	0.69	0.85
Ph	0.15	0.65	0.92	0.93	0.94	NA	NA	0.15	0.59	0.60	0.19	0.65	0.49	0.41
C_{19}	0.33	1.42	2.00	1.63	1.98	NA	NA	0.33	0.85	0.65	0.63	1.33	0.75	0.41
C_{20}	0.35	1.20	2.13	1.83	2.10	NA	NA	0.35	0.54	0.72	0.79	1.02	0.72	0.53
C_{21}	0.32	1.63	2.36	2.85	2.30	NA	NA	0.32	0.63	0.49	0.80	0.94	0.83	0.57
C_{22}	0.35	1.51	1.94	2.73	1.74	NA	NA	0.35	0.72	0.69	1.01	0.69	0.82	0.42
C_{23}	0.31	1.79	1.64	2.10	1.63	NA	NA	0.31	0.73	0.78	0.83	0.52	0.74	0.72
C_{24}	0.24	1.13	1.10	1.79	1.34	NA	NA	0.24	0.60	0.50	0.51	0.71	0.98	0.42
C_{25}	0.28	1.42	1.22	1.03	1.62	NA	NA	0.28	0.59	0.84	0.77	0.63	0.82	0.61
C_{26}	0.19	0.84	0.70	1.11	0.80	NA	NA	0.19	0.24	0.42	0.69	0.41	0.41	0.34
C_{27}	0.42	1.35	1.23	1.30	1.20	NA	NA	0.42	0.30	0.79	0.52	0.82	0.57	0.43
C_{28}	0.26	0.31	0.26	0.49	0.30	NA	NA	0.26	ND	0.21	0.19	0.13	0.33	ND
C_{29}	0.34	0.52	1.19	0.59	0.70	NA	NA	0.34	ND	0.42	0.35	ND	0.39	0.17
C_{30}	0.10	0.38	0.28	0.14	0.12	NA	NA	0.10	ND	ND	0.11	ND	0.09	ND
C_{31}	ND	0.31	ND	0.30	0.14	NA	NA	ND	ND	ND	ND	ND	ND	ND
C_{32}	ND	ND	ND	ND	ND	NA	NA	ND	ND	ND	ND	ND	ND	ND
C_{33}	ND	ND	ND	ND	ND	NA	NA	ND	ND	ND	ND	ND	ND	ND

TABLE 5.26

INDIVIDUAL ALKANE CONCENTRATIONS IN *A. AQUATICUS* AT SITES 5, 6, 7 AND 9 DURING PERIOD D.

	SITE 5 PERIOD D (Days)				SITE 6 PERIOD D (Days)				SITE 7 PERIOD D (Days)				SITE 9 PERIOD (Days)			
alkane	0	7	11	19	0	7	11	19	0	7	11	19	0	7	11	19
C_{10}	ND	ND	ND	ND	ND	ND	ND	NA	ND	ND	ND	NA	0	ND	ND	NA
C_{11}	ND	ND	ND	ND	ND	ND	ND	NA	ND	ND	ND	NA	ND	ND	ND	NA
C_{12}	ND	ND	ND	ND	ND	ND	ND	NA	ND	0.07	ND	NA	ND	0.07	ND	NA
C_{13}	ND	0.06	0.18	0.07	ND	ND	0.08	NA	ND	0.06	0.18	NA	ND	0.06	0.18	NA
C_{14}	ND	0.12	0.12	0.11	ND	0.32	0.30	NA	ND	0.12	0.12	NA	ND	0.12	0.12	NA
C_{15}	0.17	0.30	0.21	0.14	0.17	0.47	0.53	NA	0.17	0.30	0.21	NA	ND	0.30	0.21	NA
C_{16}	0.10	0.30	0.37	0.20	0.10	0.50	0.40	NA	0.10	0.30	0.37	NA	0.17	0.30	0.37	NA
C_{17}	0.30	0.43	0.45	0.31	0.30	0.87	0.75	NA	0.30	0.43	0.45	NA	0.10	0.43	0.45	NA
Pr	0.32	0.13	0.20	0.12	0.32	0.67	0.60	NA	0.32	0.13	0.30	NA	0.30	0.13	0.30	NA
C_{18}	0.23	0.50	0.43	0.39	0.23	0.90	0.89	NA	0.23	0.50	0.43	NA	0.32	0.50	0.43	NA
Ph	0.35	0.38	0.19	0.20	0.35	0.75	0.63	NA	0.35	0.38	0.19	NA	0.23	0.38	0.19	NA
C_{19}	0.22	0.29	0.48	0.66	0.22	1.24	1.12	NA	0.22	0.21	0.48	NA	0.35	0.21	0.48	NA
C_{20}	0.38	0.48	0.40	0.53	0.38	1.15	1.3	NA	0.38	0.48	0.40	NA	0.22	0.48	0.40	NA
C_{21}	0.19	0.78	0.49	0.42	0.19	1.25	1.26	NA	0.19	0.78	0.49	NA	0.38	0.78	0.49	NA
C_{22}	0.28	0.83	0.52	0.71	0.28	0.92	0.96	NA	0.28	0.63	0.52	NA	0.19	0.63	0.52	NA
C_{23}	0.39	0.49	0.50	0.41	0.39	0.84	0.49	NA	0.39	0.49	0.50	NA	0.28	0.49	0.50	NA
C_{24}	0.17	0.39	0.40	0.40	0.17	0.60	0.62	NA	0.17	0.39	0.41	NA	0.39	0.39	0.41	NA
C_{25}	0.69	0.45	0.36	0.39	0.69	0.72	0.59	NA	0.69	0.45	0.36	NA	0.17	0.45	0.36	NA
C_{26}	0.11	0.39	0.21	0.37	0.11	0.39	0.23	NA	0.11	0.39	0.75	NA	0.69	0.39	0.75	NA
C_{27}	0.30	0.51	0.51	0.49	0.30	0.55	0.85	NA	0.30	0.51	0.68	NA	0.11	0.51	0.68	NA
C_{28}	0.21	0.09	0.05	0.11	0.21	ND	0.12	NA	0.21	0.09	0.58	NA	0.30	0.09	0.58	NA
C_{29}	0.08	0.39	0.21	0.21	0.08	ND	0.30	NA	0.08	0.39	0.08	NA	0.21	0.39	0.08	NA
C_{30}	0.05	0.05	0.04	0.11	0.05	ND	0.09	NA	0.05	ND	0.08	NA	0.08	ND	0.08	NA
C_{31}	0.11	ND	ND	ND	0.11	ND	ND	NA	0.11	0.21	0.34	NA	0.05	0.21	0.34	NA
C_{32}	ND	ND	ND	ND	ND	ND	ND	NA	ND	ND	0.08	NA	0.11	ND	0.08	NA
C_{33}	ND	ND	ND	ND	ND	ND	ND	NA	ND	ND	ND	NA	ND	ND	ND	NA

TABLE 5.27

INDIVIDUAL ALKANE CONCENTRATIONS IN *A. AQUATICUS* AT SITES 5, 6, 7 AND 9 DURING PERIOD E.

	SITE 5 PERIOD E (Days)					SITE 6 PERIOD E (Days)					SITE 7 PERIOD E (Days)					SITE 9 PERIOD E (Days)				
alkane	0	4	7	15	22	0	4	7	15	22	0	4	7	15	22	0	4	7	15	22
C_{10}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	NA	ND	ND	ND	ND	NA
C_{11}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.03	NA	ND	ND	ND	0.03	NA
C_{12}	ND	0.05	ND	0.21	ND	ND	0.10	0.06	0.09	0.19	ND	0.20	0.10	0.15	NA	ND	0.10	0.10	0.15	NA
C_{13}	ND	0.13	ND	0.34	0.21	ND	0.35	0.11	0.21	0.32	ND	0.12	0.13	0.25	NA	ND	0.12	0.13	0.25	NA
C_{14}	ND	0.46	0.19	0.63	0.25	ND	0.69	0.26	0.54	0.85	ND	0.32	0.36	0.32	NA	ND	0.22	0.36	0.32	NA
C_{15}	0.07	0.63	0.53	0.62	0.39	0.07	0.80	0.39	0.43	0.74	0.07	0.45	0.18	0.49	NA	0.07	0.35	0.18	0.49	NA
C_{16}	0.21	0.59	0.30	0.69	0.34	0.21	0.63	0.42	0.64	0.67	0.21	0.63	0.64	0.75	NA	0.21	0.53	0.64	0.75	NA
C_{17}	0.31	0.73	0.84	0.21	0.84	0.31	0.59	0.65	0.45	1.54	0.31	0.89	0.95	1.20	NA	0.31	0.69	0.95	1.20	NA
Pr	0.12	0.49	0.63	0.92	0.64	0.12	0.40	0.40	0.23	1.09	0.12	0.51	0.65	0.78	NA	0.12	0.34	0.65	0.78	NA
C_{18}	0.30	0.79	0.94	0.13	1.21	0.30	0.66	0.72	0.56	1.63	0.30	0.92	1.04	1.18	NA	0.30	0.52	1.04	1.18	NA
Ph	0.07	0.53	0.72	0.84	0.72	0.07	0.29	0.83	0.34	1.02	0.07	0.53	0.43	0.57	NA	0.07	0.33	0.43	0.57	NA
C_{19}	0.21	0.83	1.21	0.99	1.15	0.21	0.90	1.40	0.72	2.03	0.21	0.71	0.83	0.96	NA	0.21	0.61	0.83	0.96	NA
C_{20}	0.15	0.92	1.30	1.21	1.69	0.15	0.72	1.03	0.94	2.78	0.15	0.92	1.39	1.49	NA	0.15	0.42	1.39	1.49	NA
C_{21}	0.17	1.21	1.01	0.87	1.56	0.17	1.00	1.28	1.23	2.59	0.17	1.38	1.68	1.26	NA	0.17	0.89	1.68	1.26	NA
C_{22}	0.23	1.35	0.85	0.89	1.23	0.23	0.93	1.35	0.94	2.24	0.23	1.46	1.82	1.03	NA	0.23	1.45	1.82	1.03	NA
C_{23}	0.17	1.09	1.15	1.24	1.74	0.17	1.49	1.19	0.54	2.10	0.17	1.33	1.52	0.93	NA	0.17	1.33	1.52	0.93	NA
C_{24}	0.09	1.07	1.29	1.12	0.85	0.09	1.28	1.18	0.96	2.02	0.09	1.20	1.38	0.83	NA	0.09	1.20	1.38	0.83	NA
C_{25}	0.11	0.83	0.76	1.35	1.21	0.11	1.21	1.21	0.84	1.67	0.11	1.10	1.46	0.97	NA	0.11	1.10	1.46	0.97	NA
C_{26}	0.07	0.75	0.45	0.37	0.39	0.07	1.21	0.69	0.58	0.94	0.07	0.88	1.20	0.67	NA	0.07	0.88	1.20	0.67	NA
C_{27}	0.30	0.80	0.55	1.79	1.98	0.30	0.94	0.72	1.17	1.29	0.30	0.99	1.19	0.80	NA	0.30	0.99	1.19	0.80	NA
C_{28}	0.11	0.23	0.14	0.49	0.40	0.11	0.29	0.12	0.32	0.35	0.11	0.48	0.68	0.32	NA	0.11	0.48	0.68	0.32	NA
C_{29}	0.21	0.29	0.18	1.32	0.82	0.21	0.18	0.34	0.48	0.45	0.21	0.52	0.95	0.56	NA	0.21	0.52	0.95	0.56	NA
C_{30}	ND	0.10	ND	0.40	ND	ND	0.14	0.12	0.11	ND	ND	0.21	0.34	0.18	NA	ND	0.21	0.34	0.18	NA
C_{31}	ND	0.12	ND	1.21	0.12	ND	ND	ND	0.20	ND	ND	0.14	0.10	0.24	NA	ND	0.14	0.10	0.24	NA
C_{32}	ND	ND	ND	ND	ND	ND	ND	ND	0.06	ND	ND	ND	ND	0.10	NA	ND	ND	ND	0.10	NA
C_{33}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	NA	ND	ND	ND	ND	NA

TABLE 5.28

INDIVIDUAL ALKANE CONCENTRATIONS IN *L. PEREGRA* AT SITES 5, 6, 7 AND 9 DURING PERIOD C.

	SITE 5 PERIOD C (Days)							SITE 6 PERIOD C (DAYS)						
alkane	0	6	12	15	20	27	32	0	6	12	15	20	27	32
C_{10}	ND	ND	ND	NA	ND	NA	ND	ND	ND	ND	NA	ND	NA	ND
C_{11}	ND	ND	ND	NA	ND	NA	ND	ND	ND	ND	NA	ND	NA	ND
C_{12}	0.13	0.05	0.12	NA	ND	NA	0.07	0.13	ND	0.19	NA	0.07	NA	0.23
C_{13}	0.34	0.17	0.27	NA	0.12	NA	0.21	0.34	0.31	0.26	NA	0.95	NA	0.25
C_{14}	0.55	0.23	0.35	NA	0.29	NA	0.41	0.55	0.37	0.49	NA	0.25	NA	0.67
C_{15}	0.76	0.40	0.57	NA	0.34	NA	0.51	0.76	0.75	0.85	NA	0.47	NA	0.75
C_{16}	0.68	0.47	0.60	NA	0.75	NA	0.90	0.68	1.01	1.05	NA	0.85	NA	0.85
C_{17}	0.80	0.65	0.79	NA	0.95	NA	0.98	0.80	1.24	1.79	NA	1.53	NA	1.23
Pr	0.62	0.30	0.50	NA	0.34	NA	0.69	0.62	0.76	1.02	NA	0.94	NA	0.75
C_{18}	0.71	0.42	0.85	NA	0.97	NA	1.31	0.71	1.63	1.63	NA	1.45	NA	1.49
Ph	0.44	0.54	0.46	NA	0.69	NA	0.73	0.44	0.69	0.94	NA	0.73	NA	0.84
C_{19}	0.90	0.43	1.24	NA	0.69	NA	1.45	0.90	1.21	1.98	NA	2.16	NA	1.83
C_{20}	0.96	0.51	1.53	NA	0.75	NA	1.23	0.96	1.81	2.71	NA	2.90	NA	2.06
C_{21}	0.87	0.49	0.75	NA	1.09	NA	1.69	0.87	1.06	2.40	NA	2.17	NA	2.47
C_{22}	0.80	0.36	0.65	NA	1.21	NA	1.45	0.80	0.73	1.47	NA	1.76	NA	1.84
C_{23}	0.56	0.50	0.79	NA	1.34	NA	1.43	0.56	0.66	1.32	NA	1.54	NA	1.04
C_{24}	0.45	0.48	0.84	NA	0.94	NA	1.39	0.45	0.78	0.94	NA	1.03	NA	1.23
C_{25}	0.39	0.42	0.79	NA	0.84	NA	0.79	0.39	0.39	1.11	NA	0.92	NA	1.24
C_{26}	0.24	0.23	0.69	NA	0.74	NA	0.64	0.24	0.29	0.65	NA	0.65	NA	0.74
C_{27}	0.35	0.38	0.74	NA	0.84	NA	0.73	0.35	0.48	0.97	NA	0.89	NA	1.02
C_{28}	0.21	0.25	ND	NA	0.30	NA	0.24	0.21	0.14	0.23	NA	0.46	NA	0.45
C_{29}	0.22	0.19	0.11	NA	0.45	NA	0.30	0.22	0.18	0.16	NA	0.34	NA	0.56
C_{30}	0.06	ND	0.17	NA	0.09	NA	ND	0.06	0.10	ND	NA	0.05	NA	0.40
C_{31}	0.14	0.17	ND	NA	0.12	NA	0.08	0.14	0.17	0.06	NA	0.10	NA	0.08
C_{32}	ND	ND	ND	NA	ND	NA	ND	ND	ND	ND	NA	ND	NA	ND
C_{33}	ND	ND	ND	NA	ND	NA	ND	ND	ND	ND	NA	ND	NA	ND

	SITE 7 PERIOD C (Days)							SITE 9 PERIOD C (Days)						
alkane	0	6	12	15	20	27	32	0	6	12	15	20	27	32
C_{10}	ND	ND	ND	NA	ND	NA	ND	ND	ND	ND	NA	ND	NA	ND
C_{11}	ND	ND	ND	NA	ND	NA	ND	ND	ND	ND	NA	ND	NA	ND
C_{12}	0.13	0.12	0.09	NA	ND	NA	0.12	0.13	0.23	0.17	NA	0.09	NA	0.29
C_{13}	0.34	0.21	0.30	NA	ND	NA	ND	0.34	0.21	0.08	NA	0.49	NA	0.38
C_{14}	0.55	0.36	0.42	NA	0.73	NA	0.69	0.55	0.59	0.14	NA	0.65	NA	0.48
C_{15}	0.76	0.49	0.63	NA	0.95	NA	0.95	0.76	0.75	0.12	NA	0.42	NA	0.58
C_{16}	0.68	0.58	0.98	NA	1.27	NA	0.99	0.68	0.86	0.64	NA	0.69	NA	0.63
C_{17}	0.80	1.67	1.43	NA	1.71	NA	1.43	0.80	0.75	0.64	NA	0.60	NA	0.69
Pr	0.62	0.87	1.00	NA	0.63	NA	0.89	0.62	0.41	0.43	NA	0.40	NA	0.38
C_{18}	0.71	1.39	1.53	NA	1.58	NA	1.29	0.71	0.53	0.72	NA	0.74	NA	0.60
Ph	0.44	0.63	1.02	NA	0.98	NA	0.75	0.44	0.40	0.39	NA	0.18	NA	0.40
C_{19}	0.90	1.43	1.69	NA	1.63	NA	1.63	0.90	0.72	0.68	NA	0.53	NA	0.73
C_{20}	0.96	1.40	2.04	NA	1.87	NA	1.75	0.96	0.68	0.54	NA	0.56	NA	0.74
C_{21}	0.87	1.33	2.13	NA	1.84	NA	1.94	0.87	0.69	0.83	NA	0.69	NA	0.73
C_{22}	0.80	1.02	1.80	NA	2.43	NA	1.69	0.80	0.58	0.84	NA	0.55	NA	0.79
C_{23}	0.56	0.98	1.53	NA	2.50	NA	1.75	0.56	0.44	0.69	NA	0.54	NA	0.72
C_{24}	0.45	0.79	1.40	NA	1.74	NA	1.24	0.45	0.37	0.72	NA	0.63	NA	0.50
C_{25}	0.39	0.46	1.00	NA	0.83	NA	1.01	0.39	0.31	0.63	NA	0.48	NA	0.59
C_{26}	0.24	0.34	0.64	NA	0.83	NA	0.63	0.24	0.42	0.49	NA	0.28	NA	0.30
C_{27}	0.35	0.71	1.08	NA	0.74	NA	1.09	0.35	0.25	0.18	NA	0.49	NA	0.79
C_{28}	0.21	0.25	0.19	NA	0.21	NA	0.34	0.21	0.33	0.24	NA	0.10	NA	0.25
C_{29}	0.22	0.40	0.21	NA	0.57	NA	0.64	0.22	0.10	0.31	NA	0.30	NA	0.45
C_{30}	0.06	0.10	0.13	NA	0.05	NA	0.30	0.06	ND	0.11	NA	0.10	NA	0.33
C_{31}	0.14	ND	0.10	NA	0.07	NA	0.10	0.14	ND	ND	NA	0.15	NA	0.10
C_{32}	ND	ND	ND	NA	0.07	NA	ND	ND	ND	ND	NA	ND	NA	ND
C_{33}	ND	ND	ND	NA	ND	NA	ND	ND	ND	ND	NA	ND	NA	ND

TABLE 5.29

INDIVIDUAL ALKANE CONCENTRATIONS IN *L. PEREGRA* AT SITES 5, 6, 7 AND 9 DURING PERIOD D.

	SITE 5 PERIOD D (Days)				SITE 6 PERIOD D (Days)				SITE 7 PERIOD D (Days)				SITE 9 PERIOD (Days)			
alkane	0	7	11	19	0	7	11	19	0	7	11	19	0	7	11	19
C_{10}	ND	ND	NA	NA	ND	ND	NA	NA	ND	ND	NA	NA	0	ND	ND	NA
C_{11}	ND	ND	NA	NA	ND	ND	NA	NA	ND	ND	NA	NA	ND	ND	ND	NA
C_{12}	0.13	ND	NA	NA	0.13	ND	NA	NA	0.13	0.20	NA	NA	ND	0.16	0.24	NA
C_{13}	0.09	0.06	NA	NA	0.09	ND	NA	NA	0.09	0.34	NA	NA	ND	0.21	0.25	NA
C_{14}	0.17	0.12	NA	NA	0.17	0.32	NA	NA	0.17	0.67	NA	NA	ND	0.42	0.31	NA
C_{15}	0.19	0.30	NA	NA	0.19	0.47	NA	NA	0.19	1.02	NA	NA	ND	0.33	0.24	NA
C_{16}	0.27	0.30	NA	NA	0.27	0.50	NA	NA	0.27	0.63	NA	NA	0.17	0.32	0.49	NA
C_{17}	0.41	0.43	NA	NA	0.41	0.87	NA	NA	0.41	2.03	NA	NA	0.10	0.47	0.36	NA
Pr	0.36	0.13	NA	NA	0.36	0.67	NA	NA	0.36	1.13	NA	NA	0.30	0.28	0.49	NA
C_{18}	0.40	0.50	NA	NA	0.40	0.90	NA	NA	0.40	2.70	NA	NA	0.32	0.41	0.39	NA
Ph	0.21	0.38	NA	NA	0.21	0.75	NA	NA	0.21	2.10	NA	NA	0.23	0.22	0.20	NA
C_{19}	0.37	0.29	NA	NA	0.37	1.24	NA	NA	0.37	3.60	NA	NA	0.35	0.33	0.23	NA
C_{20}	0.23	0.48	NA	NA	0.23	1.15	NA	NA	0.23	2.59	NA	NA	0.22	0.46	0.42	NA
C_{21}	0.39	0.78	NA	NA	0.39	1.25	NA	NA	0.39	2.18	NA	NA	0.38	0.46	0.35	NA
C_{22}	0.28	0.83	NA	NA	0.28	0.92	NA	NA	0.28	2.44	NA	NA	0.19	0.63	0.36	NA
C_{23}	0.36	0.49	NA	NA	0.36	0.84	NA	NA	0.36	1.30	NA	NA	0.28	0.55	0.25	NA
C_{24}	0.42	0.39	NA	NA	0.42	0.60	NA	NA	0.42	1.90	NA	NA	0.39	0.34	0.32	NA
C_{25}	0.46	0.45	NA	NA	0.46	0.72	NA	NA	0.46	0.84	NA	NA	0.17	0.37	0.42	NA
C_{26}	0.35	0.39	NA	NA	0.35	0.39	NA	NA	0.35	0.72	NA	NA	0.69	0.37	0.12	NA
C_{27}	0.67	0.51	NA	NA	0.67	0.55	NA	NA	0.67	1.16	NA	NA	0.11	0.47	0.23	NA
C_{28}	0.18	0.09	NA	NA	0.18	ND	NA	NA	0.18	0.76	NA	NA	0.30	0.25	0.21	NA
C_{29}	0.20	0.39	NA	NA	0.20	ND	NA	NA	0.20	1.03	NA	NA	0.21	0.21	ND	NA
C_{30}	ND	0.05	NA	NA	ND	ND	NA	NA	ND	0.20	NA	NA	0.08	ND	ND	NA
C_{31}	0.09	ND	NA	NA	0.09	ND	NA	NA	0.09	0.40	NA	NA	0.05	ND	ND	NA
C_{32}	ND	ND	NA	NA	ND	ND	NA	NA	ND	ND	NA	NA	0.11	ND	ND	NA
C_{33}	ND	ND	NA	NA	ND	ND	NA	NA	ND	ND	NA	NA	ND	ND	ND	NA

TABLE 5.30

INDIVIDUAL ALKANE CONCENTRATIONS IN *L. PEREGRA* AT SITES 5, 6, 7 AND 9 DURING PERIOD E.

	SITE 5 PERIOD E (Days)					SITE 6 PERIOD E (Days)					SITE 7 PERIOD E (Days)					SITE 9 PERIOD E (Days)				
alkane	0	4	7	15	22	0	4	7	15	22	0	4	7	15	22	0	4	7	15	22
C_{10}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	NA	ND	ND	ND	ND	NA
C_{11}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	NA	ND	ND	ND	0.03	NA
C_{12}	0.16	0.13	0.21	0.20	0.10	0.16	ND	ND	0.43	0.30	ND	0.06	ND	ND	NA	ND	0.10	0.10	0.15	NA
C_{13}	0.20	0.30	0.28	0.18	0.27	0.20	0.12	0.44	0.86	0.59	ND	0.37	0.11	0.17	NA	ND	0.12	0.13	0.25	NA
C_{14}	0.39	0.40	0.49	0.53	0.34	0.39	0.65	0.33	0.72	0.58	ND	0.40	0.32	0.20	NA	ND	0.22	0.36	0.32	NA
C_{15}	0.42	0.58	0.43	0.81	0.51	0.42	0.83	0.66	0.99	0.52	0.07	0.54	0.63	0.37	NA	0.07	0.35	0.18	0.49	NA
C_{16}	0.43	0.63	0.43	0.87	0.63	0.43	0.94	0.84	1.46	1.35	0.21	0.93	1.24	0.47	NA	0.21	0.53	0.64	0.75	NA
C_{17}	0.64	0.72	0.64	0.82	0.81	0.64	1.43	1.54	2.54	2.13	0.31	1.07	1.56	1.4	NA	0.31	0.69	0.95	1.20	NA
Pr	0.30	0.34	0.47	0.72	0.52	0.30	0.65	0.94	1.73	1.43	0.12	0.56	0.95	0.87	NA	0.12	0.34	0.65	0.78	NA
C_{18}	0.67	0.84	0.74	0.69	0.80	0.67	1.62	2.12	2.64	2.30	0.30	1.04	1.64	1.63	NA	0.30	0.52	1.04	1.18	NA
Ph	0.27	0.53	0.43	0.73	0.90	0.27	0.94	1.63	1.60	1.10	0.07	0.74	0.94	0.83	NA	0.07	0.33	0.43	0.57	NA
C_{19}	0.53	0.56	0.84	0.82	1.14	0.53	1.73	2.64	4.14	2.76	0.21	1.04	3.03	2.64	NA	0.21	0.61	0.83	0.96	NA
C_{20}	0.64	0.69	1.02	0.64	1.25	0.64	1.94	3.24	4.09	2.54	0.15	1.64	2.13	2.84	NA	0.15	0.42	1.39	1.49	NA
C_{21}	0.65	0.75	0.89	0.65	1.19	0.65	2.34	4.02	3.63	2.42	0.17	1.75	2.42	3.07	NA	0.17	0.89	1.68	1.26	NA
C_{22}	0.52	0.90	0.91	0.68	1.36	0.52	1.67	2.83	2.53	2.13	0.23	1.94	1.46	2.84	NA	0.23	1.45	1.82	1.03	NA
C_{23}	0.53	0.63	1.02	0.74	1.02	0.53	1.20	2.98	2.13	2.01	0.17	1.53	1.33	2.04	NA	0.17	1.33	1.52	0.93	NA
C_{24}	0.44	0.76	1.24	0.67	0.63	0.44	1.20	1.49	2.09	1.37	0.09	1.08	1.43	1.64	NA	0.09	1.20	1.38	0.83	NA
C_{25}	0.56	0.50	0.83	0.56	0.88	0.56	0.80	1.72	1.49	1.52	0.11	1.00	1.48	1.09	NA	0.11	1.10	1.46	0.97	NA
C_{26}	0.59	0.47	1.20	0.27	0.31	0.59	0.63	0.64	0.19	1.40	0.07	0.60	1.02	0.26	NA	0.07	0.88	1.20	0.67	NA
C_{27}	0.52	0.40	0.63	0.35	0.54	0.52	0.98	1.32	1.43	1.20	0.30	0.84	1.69	0.74	NA	0.30	0.99	1.19	0.80	NA
C_{28}	0.24	0.24	0.74	0.99	0.29	0.24	0.54	0.46	0.57	0.86	0.11	0.24	0.46	0.17	NA	0.11	0.48	0.68	0.32	NA
C_{29}	0.38	0.38	0.23	0.34	ND	0.38	0.79	0.83	0.84	0.98	0.21	0.46	0.72	0.39	NA	0.21	0.52	0.95	0.56	NA
C_{30}	0.23	0.23	0.16	ND	ND	0.23	0.14	0.40	0.14	0.30	ND	0.09	0.44	0.17	NA	ND	0.21	0.34	0.18	NA
C_{31}	0.11	0.11	ND	ND	ND	0.11	0.30	ND	ND	0.20	ND	ND	ND	ND	NA	ND	0.14	0.10	0.24	NA
C_{32}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	NA	ND	ND	ND	0.10	NA
C_{33}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	NA	ND	ND	ND	ND	NA

5.5.8.1 Carbon Preference Index

The Carbon Preference Index (CPI) has been applied to the aliphatic data collected for *A. aquaticus* and *L. peregra* during Periods A - E and C - E respectively (Tables 5.31 and 5.32).

These CPI values should give an indication concerning whether the organisms preferentially take up certain source classes of aliphatic compounds. The C_{14}-C_{20} CPI was first applied and the results showed there to be little or no uptake from biogenic algal sources at any sampling locations. This is consistent with the sediment/water surveys which indicated no prevalence of odd chain numbers in this region suggesting insignificant algal inputs. It may be assumed, therefore, that virtually all aliphatic hydrocarbons detected in *A. aquaticus* and *L. peregra* tissue in this carbon chain length range are from anthropogenic sources since there is no indication either in abiotic or tissue extracts of any preference for biogenically derived compounds (e.g. heptadecane). The $CPI_{14\text{-}20}$ is therefore not included in Tables 5.31 and 5.32. The $CPI_{20\text{-}32}$ which may be used for the assessment of higher plant wax inputs indicated the presence of biogenic inputs to abiotic samples at each of the sites. In invertebrate tissue the general trend is that some higher plant waxes are almost always present (i.e. the CPI generally exceeds unity) although there is considerable variation in the actual value. The results for *A. aquaticus* Period A begin with an initial value of 1.95 at the Trent Park reference site which increases rapidly at Site 5 and remains above 2 throughout the sampling period. At this least contaminated site with respect to hydrocarbons, on the lower Silk Stream, biogenic higher plant inputs therefore remain significant. At Site 6 the index remains at a relatively high level during this summer sampling period indicating a possible preferential uptake of odd numbered hydrocarbons as the work on sediments (Chapter 4) showed the $CPI_{20\text{-}32}$ to be much closer to unity in abiotic Site 6 samples. Thus, the index remains at a relatively high level in *A. aquaticus* in spite of the lower figure which prevails in surrounding water and sediment. At Site 7 there is a clear fall in the index towards the end of the exposure period to a figure close to unity that relates more closely to ambient CPIs than the other Silk Stream sites. At Site 9 the index remains consistently high and there is little change in value indicating lower anthropogenic inputs at this site.

TABLE 5.31

CARBON PREFERENCE INDEX (CPI_{20-32}) FOR *A. AQUATICUS* WHOLE BODY TISSUE AT SITES 5, 6, 7 AND 9 DURING SAMPLING PERIODS A-E.

SITE	PERIOD A (DAYS)					
	0	3	6	10	14	18
5	1.95	2.50	2.23	2.26	2.38	2.59
6	1.95	2.00	1.61	1.70	1.90	2.10
7	1.95	1.74	2.00	1.46	1.33	1.13
9	1.95	2.09	2.07	1.85	1.82	1.62

SITE	PERIOD B (DAYS)				
	0	4	11	14	24
5	1.68	1.43	1.28	1.39	1.45
6	1.68	1.49	1.36	NA	NA
7	1.68	1.31	1.25	1.54	1.37
9	1.68	1.28	1.97	1.26	2.01

SITE	PERIOD C (DAYS)						
	0	6	12	15	20	27	32
5	1.46	1.63	1.28	1.22	1.01	1.66	1.44
6	1.46	1.58	1.96	1.39	1.14	1.38	1.55
7	1.46	1.68	1.78	1.31	1.76	NA	NA
9	1.46	1.44	1.82	1.30	1.50	1.27	2.12

SITE	PERIOD D (DAYS)			
	0	7	11	19
5	2.13	1.51	1.74	1.13
6	2.13	1.76	1.73	NA
7	2.13	1.86	1.18	NA
9	2.13	1.89	1.01	NA

SITE	PERIOD E (DAYS)				
	0	4	7	15	22
5	2.05	1.60	1.43	NA	NA
6	2.05	1.50	1.24	1.09	1.17
7	2.05	1.43	1.25	1.22	NA
9	2.05	1.56	1.53	1.45	1.65

NA = Not analyzed.

During Period B the initial *A. aquaticus* tissue value is lower (1.68) and there are overall declines in the index at each of the Silk Stream sampling sites which represent decreases in the biogenic portion of the assemblage. At Sites 5 and 6 the values are lower than those recorded during Period A but are similar at Site 7. Although the index varies considerably at Site 9 during this period, there is ultimately no overall decrease with a final value of 2.01 recorded. This would be expected since Site 9 had the lowest anthropogenic (low CPI) inputs. The initial value (1.46) during Period C is the lowest for *A. aquaticus* during any of the five trials and changes little at any site with the exception of Site 9. In spite of this low starting value the values ultimately reached are similar to those recorded during other periods.

During Period D the initial value (2.13) is the highest of the five *A. aquaticus* trials and there appears to be a clear temporal fall in the index at each of the sampling sites. In contrast to the other trials the lowest levels were recorded at Sites 5 and 9. The unexpectedly low level (1.01) recorded at Site 9 on Day 11 cannot easily be explained in view of the consistently higher scores obtained at this site in other trial periods. A clear decline in the index occurs during Period E where levels approach unity at Sites 5, 6 and 7 and to a lesser extent at Site 9, indicating the uptake of anthropogenically derived hydrocarbons from the Silk Stream with this process occurring to a smaller extent in the receiving basin.

The CPI values for *L. peregra* display marked differences from the *A. aquaticus* values. In general terms, lower CPIs were recorded at all sites including the reference site. Here, initial CPIs of less than 1.5 were recorded on each sampling occasion. At the test sites, only during Period C do any of the CPIs remain consistently higher than one and during this period there are no pronounced inter site (including Site 9) differences. During Periods D and E the index remains close to unity at all sites and at Site 5 a very low score of 0.68 on Day 7 of Period E was recorded as a result of high C_{26} values. The lower general scores for *L. peregra* must be attributed to the elevated uptake of anthropogenically derived compounds and are not considered to be a result of reduced biogenic uptakes. This can be stated because the individual biogenic values are equally as high in *L. peregra* as they are in *A . aquaticus*. Thus, reductions in the CPIs occur as a result of proportionally greater accumulations of non-biogenic compounds. These

TABLE 5.32

CARBON PREFERENCE INDEX (CPI_{20-32}) FOR *L. PEREGRA* SOFT BODY TISSUE AT SITES 5, 6, 7 AND 9 DURING PERIODS C-E.

SITE	PERIOD C (DAYS)				
	0	6	12	20	32
5	1.44	1.63	1.37	1.43	1.35
6	1.44	1.44	1.83	1.51	1.37
7	1.44	1.55	1.45	1.25	1.55
9	1.44	1.38	1.10	1.60	1.53

SITE	PERIOD D (DAYS)		
	0	7	12
5	1.49	1.02	NA[1]
6	1.49	1.13	NA[1]
7	1.49	0.76	NA[1]
9	1.49	1.00	0.97

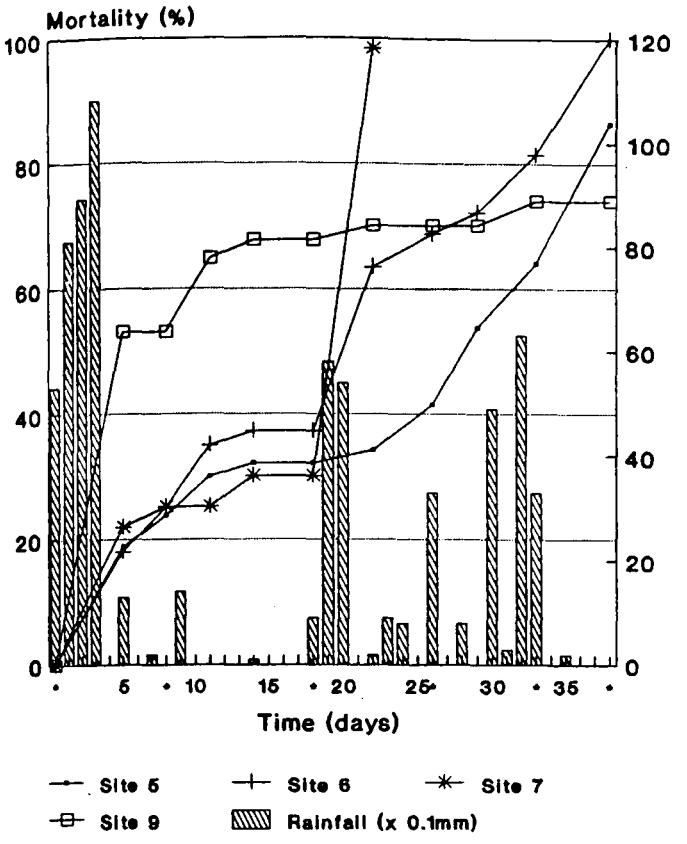
SITE	PERIOD E (DAYS)				
	0	4	7	15	22
5	1.03	0.91	0.68	0.81	1.01
6	1.03	1.05	1.14	1.01	0.97
7	1.03	1.00	1.10	0.93	1.06
9	1.03	1.08	0.83	0.94	NA[2]

NA[1] = Result not available because 100% mortality had occurred.
NA[2] = Result not available because of lost cage.

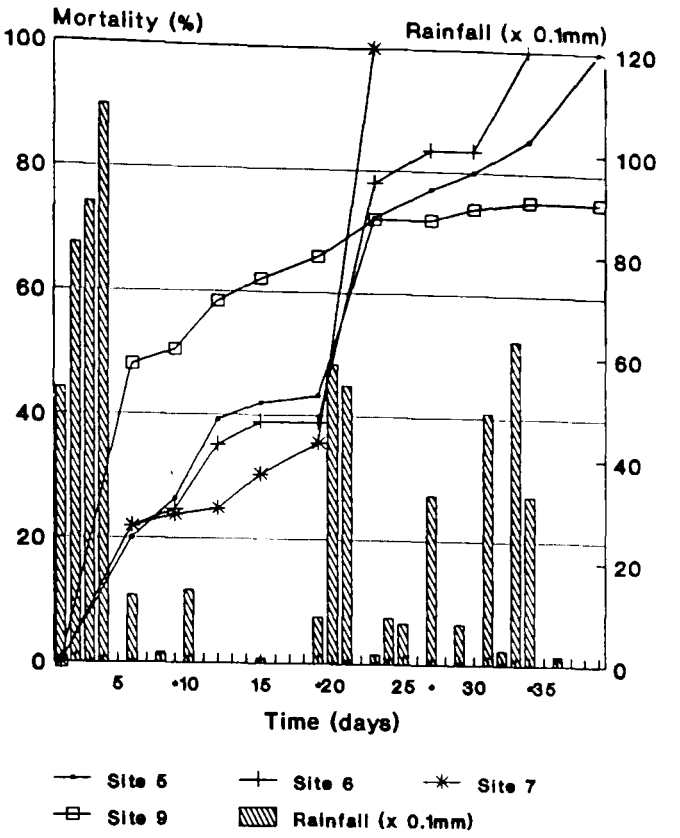
5.6 MORTALITY PATTERNS IN *A. AQUATICUS* AND *L. PEREGRA*

5.6.1 *A. aquaticus* during Trials 1 and 2

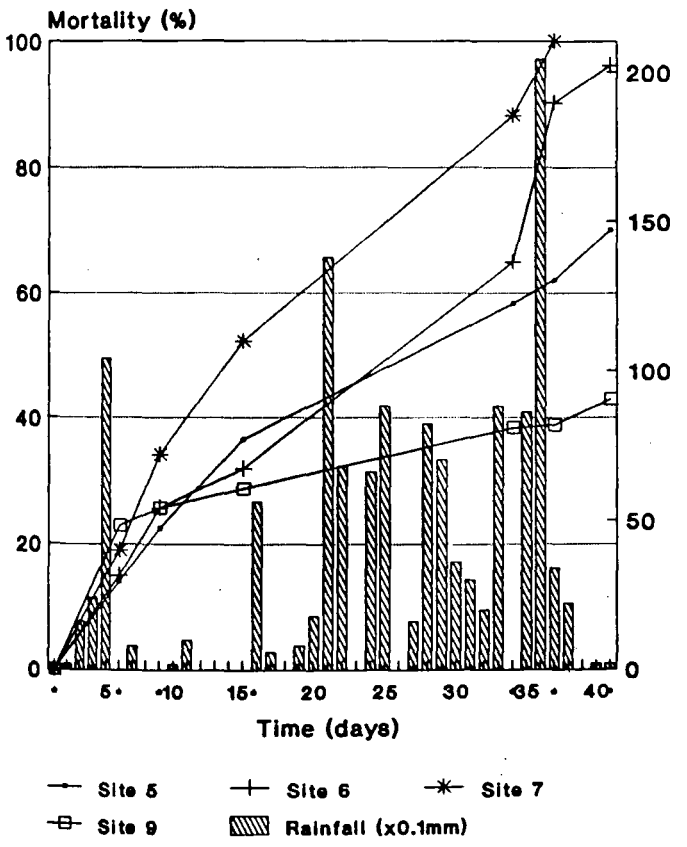
The mortality rates in basal and suspended cages are generally comparable for Sites 6, 7 and 9 during Trial 1 (see Fig. 5.23). Initially (10 days) the rates are similar at Sites 5, 6 and 7 but there is a slightly higher loss at Site 9 in the suspended cages. However, after 20 days there is little if any difference in mortality between basal and suspended organisms at this site. For this reason, and a shortage of control organisms it was considered that a single suspended cage would suffice at Site 9 during Trial 2. The major difference between basal and suspended organisms can be seen at Site 5 where the mortality rate is higher in the basal organisms after 20 days exposure. During the second trial there was relatively little difference in mortality between the suspended and basal organisms except at Site 6 where a lower rate in suspended cages was recorded.



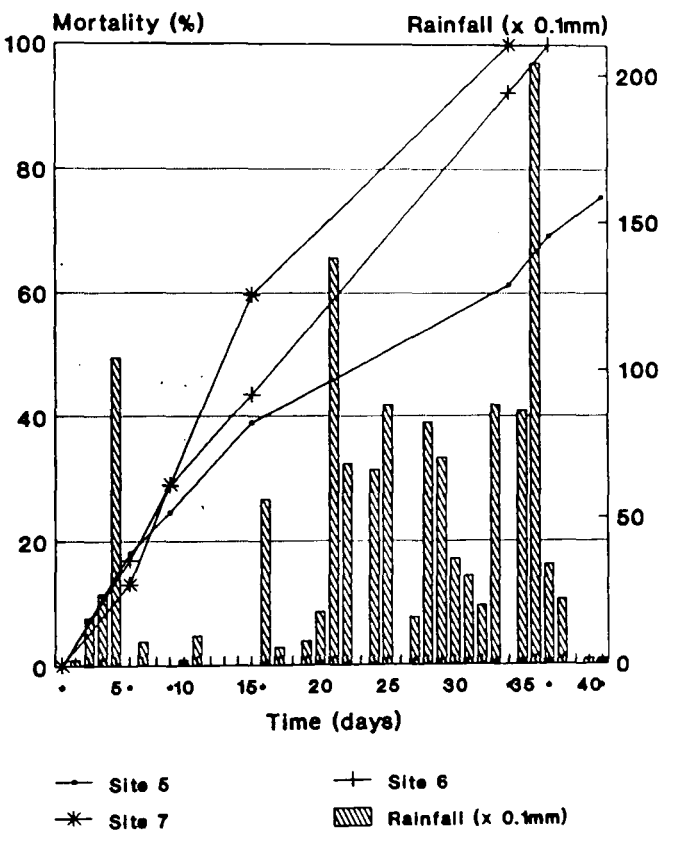
SUSPENDED TRIAL 1



BASAL TRIAL 1



SUSPENDED TRIAL 2



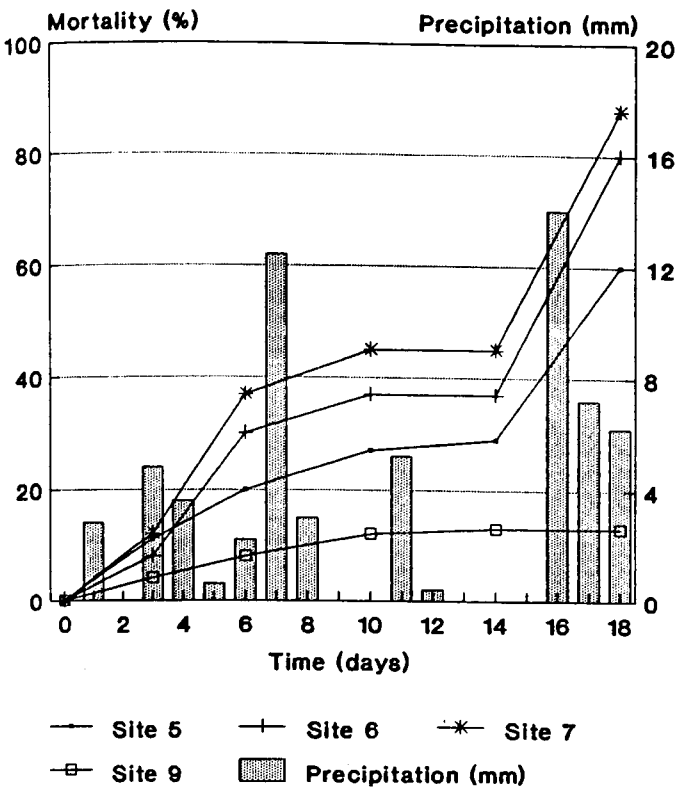
BASAL TRIAL 2

Fig. 5.23 Mortality rates and precipitation data for *A. aquaticus* at Sites 5, 6, 7 and 9 during 24/10/90 - 3/12/90 (Trial 1) and 5/12/90 - 17/1/90 (Trial 2) in suspended and basal cages.

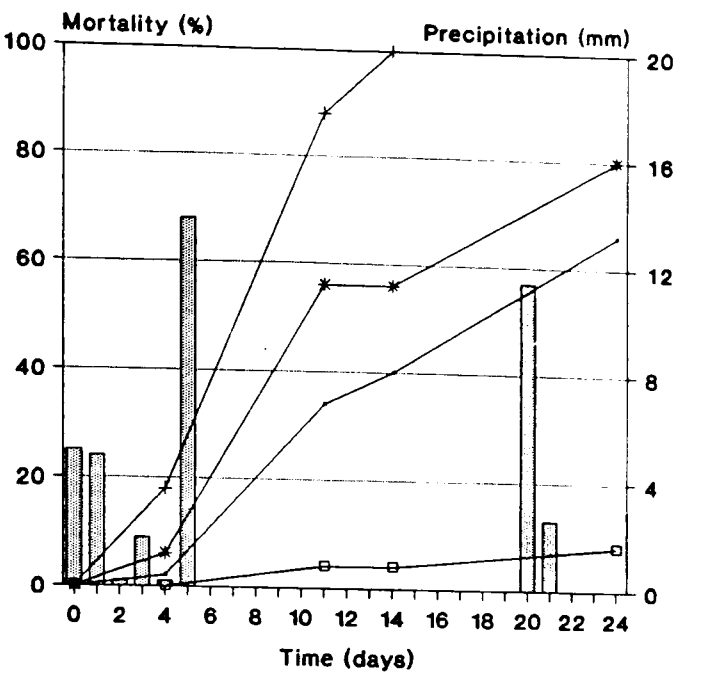
the mortality rate is higher in the basal organisms after 20 days exposure. During the second trial there was relatively little difference in mortality between the suspended and basal organisms except at Site 6 where a lower rate in suspended cages was recorded. Rainfall runoff may be an important factor in increasing pollutant exposure to freshwater organisms. In urban areas pollutant wash-off from impervious surfaces and sediment resuspension are important mechanisms for increasing the potential bioavailability of hydrocarbons. However, it is difficult to relate with any certainty a specific rainfall event with in-stream macroinvertebrate mortality. In Chapter 7 a multivariate statistical procedure will examine these relationships. During the first trial an initial loss of 20-50% of *A. aquaticus* occurs. This may be related to rainfall events of 5-10mm recorded on 4 prior consecutive days as indicated in Fig. 5.23. However, initial mortalities are more likely to be simply the result of a stress effect on weaker individuals while an adaptation to the new habitat occurs in other more robust individuals. A more significant relationship between mortality and rainfall appears to exist after 18-21 days. Here, following 8 virtually dry days there is a sharp increase in mortality rate at Sites 5, 6 and 7 in the basal cages as well as increases at Sites 6 and 7 in suspended cages which can be related to rainfall on days 18, 19 and 20. Since there was no great difference in hydrocarbon concentrations between *A. aquaticus* species contained in basal and suspended cages, other factors such as the lower dissolved oxygen levels which have been recorded near the sediment water interface must be a major reason for these elevated, possibly rainfall runoff related mortalities, particularly those in basal cages. A similar though less pronounced increase occurs between Days 34 and 37 of the second trial which may be related to the heavy rainfall which occurred on Day 36 of this trial.

5.6.2 *A. aquaticus* during Periods A - E

The mortality rates for *A. aquaticus* in Periods A - E are shown in Figs. 5.24 - 5.25. Generally, the mortality rate is highest at Site 7 with 100% mortality being reached earliest at this site during four of the five test periods. Only at Site 6 is the mortality rate at any time higher than at Site 7; 100% mortality occurs earlier at Site 6 during monitoring Period B.



PERIOD A



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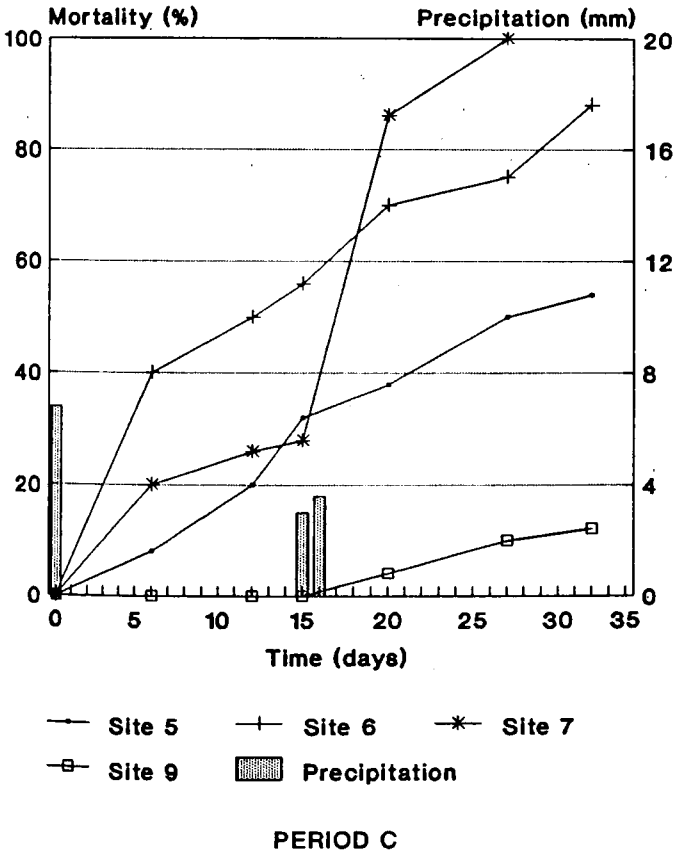


PERIOD C

Fig. 5.24 Mortality rates and precipitation data for *A. aquaticus* at Sites 5, 6, 7 and 9 during Periods A (7/6/91 - 25/6/91), B (26/6/91 - 27/7/91) and C (8/8/91 - 9/9/91)

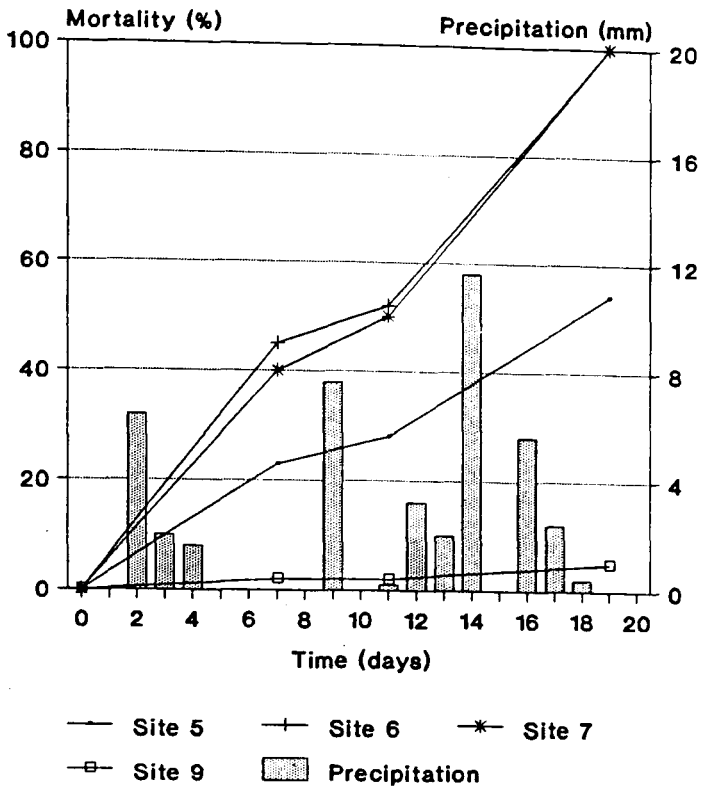


Fig 5.25 Mortality rates and precipitation data for *A. aquaticus* at Sites 5, 6, 7 and 9 during Period D (12/9/91 - 2/10/91) and Period E (20/11/91 - 12/12/91).

and 7.

During Period D the mortality rate patterns at Sites 6 and 7 are virtually identical. The mortality rate at Site 5 is consistently lower than that at the two downstream sites and during monitoring Periods A - D, 50% mortality at Site 5 occurs after that at Sites 6 and 7. Due to the loss of a cage on Day 15 of Period E no further data were obtained for Site 5.

A consistent feature of the data during the five test periods is the extremely low mortality rate at Site 9. These data would suggest that the use of the cage system *per se* imposes low stress on the organisms.

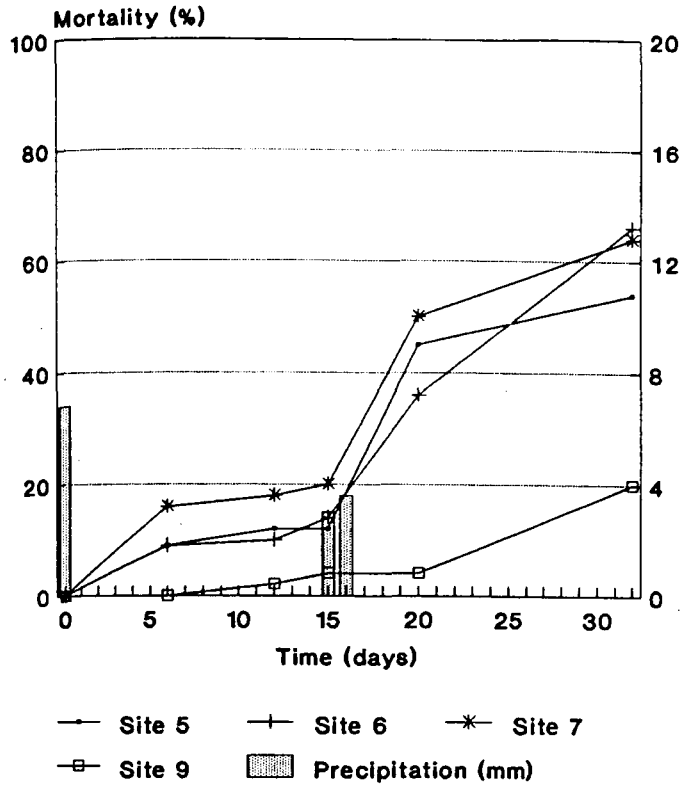
The data also suggest the existence of relationships between precipitation and mortality. During Trials 1 and 2 links between specific rainfall events and sudden increases in mortality were discussed. There is evidence of a similar response in *A. aquaticus* at Site 7 during period C where the percentage mortality rises from 28 to 87% between days 15 and 20 following substantial rainfall events on days 15 and 16. This rainfall was preceded by fourteen dry weather days.

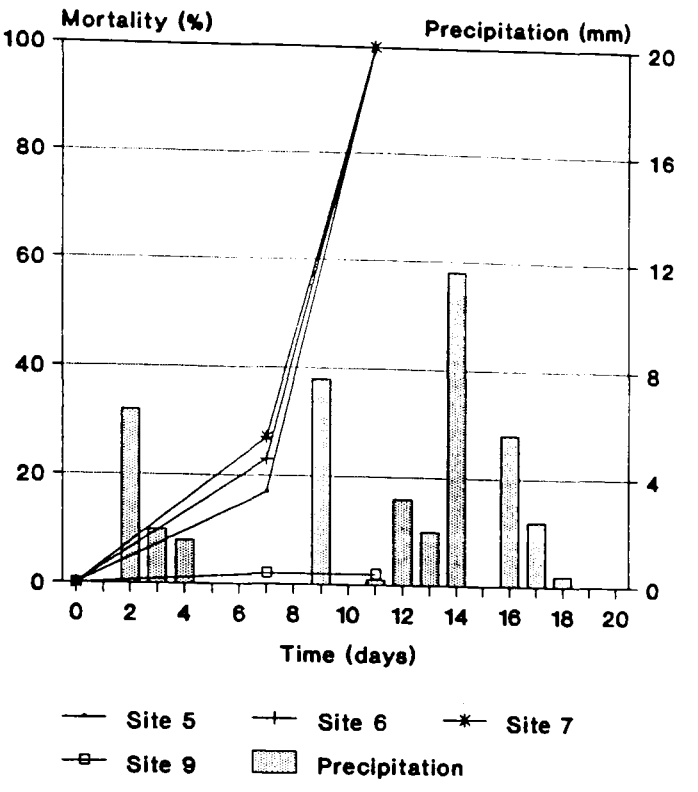
There would also appear to be a correlation between the overall precipitation during the test period and the mortality rates at the Silk Stream sites. In general, the highest mortalities occur when the total rainfall during the trial is highest. During drier sampling periods lower mortality rates are generally recorded.

The relationships between mortality rates, hydrocarbon tissue levels and rainfall during in-stream exposure trials will be investigated by applying principal component analysis to the data (see Chapter 7).

5.6.3 *L. peregra* during Periods C - E

The mortality rate trends for *L. peregra* are illustrated in Fig. 5.26 for the three monitored exposure periods. *L. peregra* generally display similar or lower mortality rates than *A. aquaticus* indicating their suitability as a biomonitor. This organism displays less inter-site distinctions than *A. aquaticus* for the Silk Stream sites and at Site 9 (Welsh Harp) there is again a clear reduction in mortality rates.





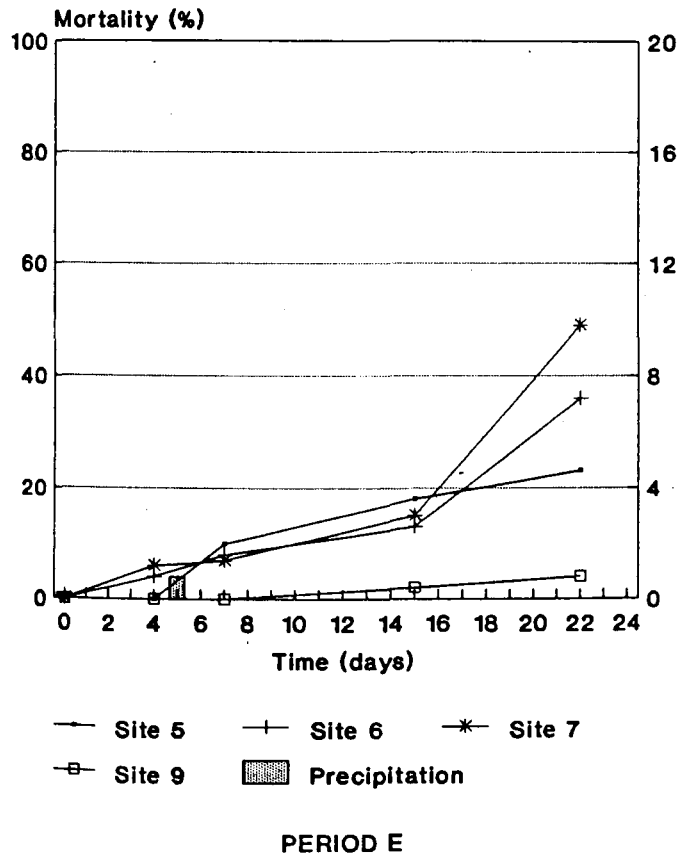


Fig. 5.26 Mortality rates and precipitation data for *L. peregra* during Periods C - E.

A relatively rapid increase in mortality during Period C (Days 15-20) occurs at three sites corresponding to that noted for *A. aquaticus* at Site 7. During Period D, 100% mortality occurred at Sites 5, 6 and 7 between Days 7 and 11. There was no corresponding increase in *A. aquaticus* mortality at this time and the cause is therefore unclear.

The final trial (Period E) showed a similar pattern to Period C. There is generally little distinction between Sites 5, 6 and 7 until Day 22 when mortality is again highest at Site 7 followed by Sites 6 and 5. Site 9 mortality rate remained below 5% throughout the test period.

5.7 SUMMARY

The four test species were each found to be bioaccumulators of hydrocarbons following transfer to the polluted locations. However, the survival rates of two species, *G. aculeatus* and *G. pulex* were found to be poor and biomonitor trials with these species were therefore discontinued. Longer survival durations were attained by *A. aquaticus* and *L. peregra* and these species were selected for extended *in situ* trials.

In both organisms the highest hydrocarbon tissue levels and highest mortalities were consistently recorded at Sites 6 and 7 on the lower Silk Stream. Hydrocarbon levels and mortality rates were substantially lower at Site 9 in the Welsh Harp. At Site 5, measurements were intermediate between the lowest Silk Stream sites and those of the receiving basin. Generally, the composition of the bioaccumulated hydrocarbon suite reflected that of the surrounding sediments. However, elevations in accumulation of methylated homologues were found in both organisms and were particularly prevelant in *L. peregra.* Total body burdens were in the same concentration range for both organisms, but were consistently higher in *L. perergа.* Some storm related effects have been postulated and will be further explored in Chapter 7.

Chapter 6

LABORATORY BIOACCUMULATION AND TOXICITY TESTING

6.1 INTRODUCTION

The aim of this work was to compare bioaccumulation and depuration rates measured under controlled dosing conditions to the field measurements previously described in Chapter 5. Although the *in situ* trials offer a great deal more realism in terms of general organism response to stream pollution, laboratory tests allow greater control over exposure levels as well as other parameters which should result in a greater understanding of the field data. It was hoped therefore that differing rates of assimilation of hydrocarbons or hydrocarbon groups could be identified and that these could be related to the field results. The extent of bioavailability of the hydrocarbons in the field was also intended to be more clearly identified as a result of the laboratory trials. A further aim was to assess the toxicity of hydrocarbons in the laboratory, particularly in relation to hydrocarbon tissue concentration, and to compare this information with the field results for the two test species.

Thus, to complement the *in situ* biological assessments described in Chapter 5, toxicity and bioaccumulation tests were undertaken using the laboratory method previously described in Chapter 3. The test organisms, *A. aquaticus* and *L. peregra,* were collected from the reference site in Trent Park, together with water which was dosed in the laboratory with alkanes and PAHs. The compositions of the dosing mixtures are given below.

Mixture A alkanes (C_{10}-C_{15}, 5μg l^{-1}; C_{16}-C_{22}, 10μg l^{-1}; C_{22}-C_{30}, 5μg l^{-1})
Mixture B alkanes (C_{10}-C_{15}, 10μg l^{-1}; C_{16}-C_{22}, 20μg l^{-1}; C_{22}-C_{30}, 10μg l^{-1})
Mixture C alkanes (C_{10}-C_{15}, 20μg l^{-1}; C_{16}-C_{22}, 40μg l^{-1}; C_{22}-C_{30}, 20μg l^{-1})

Mixture D PAHs (fluorene, anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, 5μg l^{-1}; fluoranthene, pyrene, phenanthrene, benzo(a)anthracene,

10 μg l^{-1})

Mixture E PAHs (fluorene, anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, 10μg l^{-1}; fluoranthene, pyrene, phenanthrene, benzo(a)anthracene, 20 μg l^{-1})

Mixture F PAHs (fluorene, anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, 20μg l^{-1}; fluoranthene, pyrene, phenanthrene, benzo(a)anthracene, 40 μg l^{-1})

In the following sections the individual alkanes have been clustered into three distinct groups (C_{10}-C_{17}, C_{18}-C_{22} and C_{23}-C_{30}) in order to illustrate more clearly overall temporal changes as well as to highlight groups of compounds with varying physico-chemical properties that may influence the extent to which bioaccumulation occurs. The C_{10}-C_{17} group have melting points in the range -30^{0}C to +22^{0}C and can therefore be considered liquids at room temperatures. The melting points of the mid-range alkanes are 28^{0}C-44^{0}C and these compounds are therefore solid at normal temperatures. This alkane range is also often the most abundant in samples from polluted locations as may be seen in the alkane profiles presented in Chapter 5. The highest molecular weight group have melting points in the range 48^{0}C - 66^{0}C and it is also in this range that plant waxes predominate in some environmental samples. It should also be noted that these groupings differ slightly from those used in the initial dosing compositions. This was in order to give each group more equal weighting in terms of the total amount of alkane each contained, thus allowing a fairer representation of the relative extents to which accumulation occurs as represented in Figs. 6.1 - 6.3 and Figs. 6.7 - 6.9 for *A. aquaticus* and *L. peregra*, respectively.

6.2 ALKANE ACCUMULATION IN *A. AQUATICUS*

The concentrations of alkanes in *A. aquaticus* tissue during and after exposure to Mixtures A - C are shown in Figs. 6.1 - 6.3. In general, it can be seen that *A. aquaticus* efficiently assimilates alkanes following exposure to dosed water. Similar patterns of accumulation can be observed in *A. aquaticus* for the three alkane mixtures of differing concentrations in that the bulk of the alkanes are accumulated in the first

4 days of exposure after which no further large increases can be observed. The C_{18}-C_{22} alkane grouping is most rapidly accumulated, followed by those in the C_{23} - C_{30} range. The low molecular weight compounds (C_{10}-C_{17}) are accumulated to the least extent.

The most clearly defined pattern of accumulation is in the mid-range alkane class C_{18}-C_{22} which displays a consistent initial elevation followed by a highly stable plateau beyond 4 days of exposure. During this initial exposure period, the rate of accumulation in *A. aquaticus* exposed to Mixture A (Fig. 6.1) was approximately 0.75 $\mu g\ g^{-1}\ day^{-1}$, 6 $\mu g\ g^{-1}\ day^{-1}$ and 3 $\mu g\ g^{-1}\ day^{-1}$ for the alkanes C_{10} - C_{17}, C_{18} - C_{22} and C_{23} - C_{30}, respectively. In organisms exposed to Mixture B (Fig. 6.2), the rate of accumulation for the equivalent time period ranged from 2 $\mu g\ g^{-1}\ day^{-1}$ for the C_{10} - C_{17} alkanes, to 3.5 $\mu g\ g^{-1}\ day^{-1}$ for the C_{23} - C_{30} alkanes and 8 $\mu g\ g^{-1}\ day^{-1}$ for the range C_{18} - C_{22}. There is a small increase in overall accumulation rate with organisms dosed with Mixture C (Fig. 6.3) but the increase is proportionally far less than might be expected were organism levels to directly mirror dosing levels. For the class C_{10}-C_{17}, the levels in *A. aquaticus* at the higher dosing level even fall slightly ($\simeq$ 1 $\mu g\ g^{-1}\ day^{-1}$). However, the levels rise to 4 $\mu g\ g^{-1}\ day^{-1}$ for the C_{23}-C_{30} alkanes and remain constant at 8 $\mu g\ g^{-1}\ day^{-1}$ for the C_{18}-C_{22} alkanes.

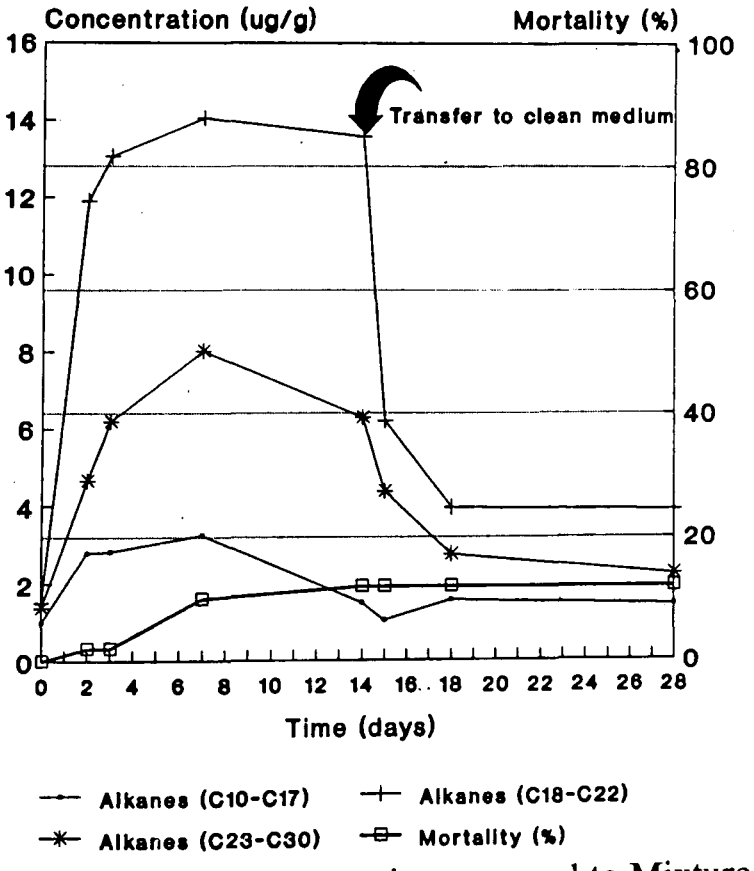


Fig. 6.1 Temporal variation of alkanes in *A. aquaticus* exposed to Mixture A, followed by transfer to clean water.

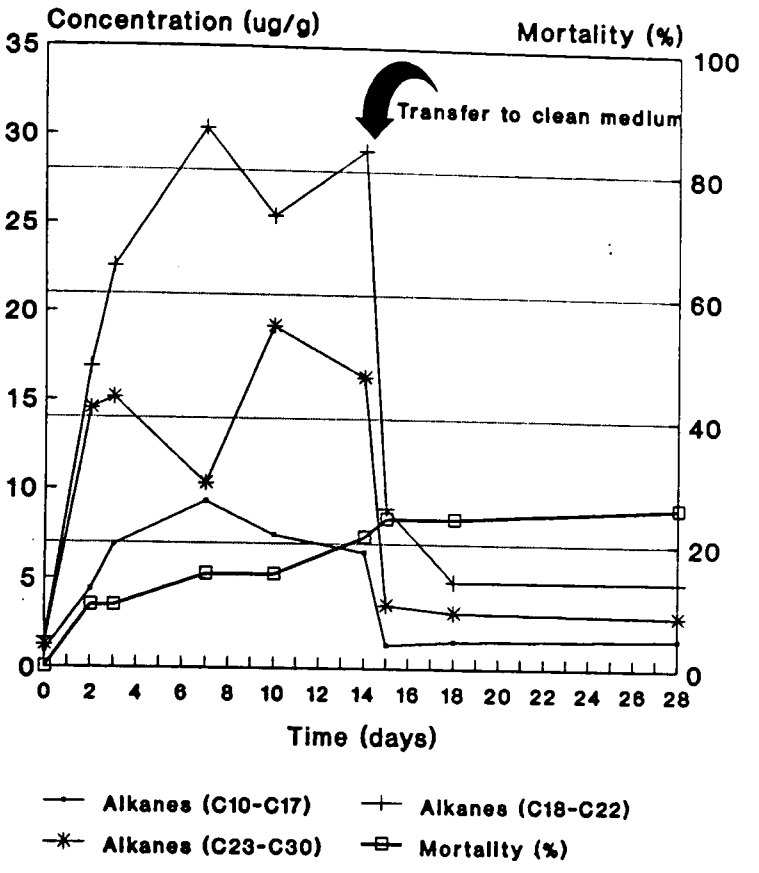


Fig. 6.2 Temporal variation of alkanes in *A. aquaticus* exposed to Mixture B, followed by transfer to clean water.

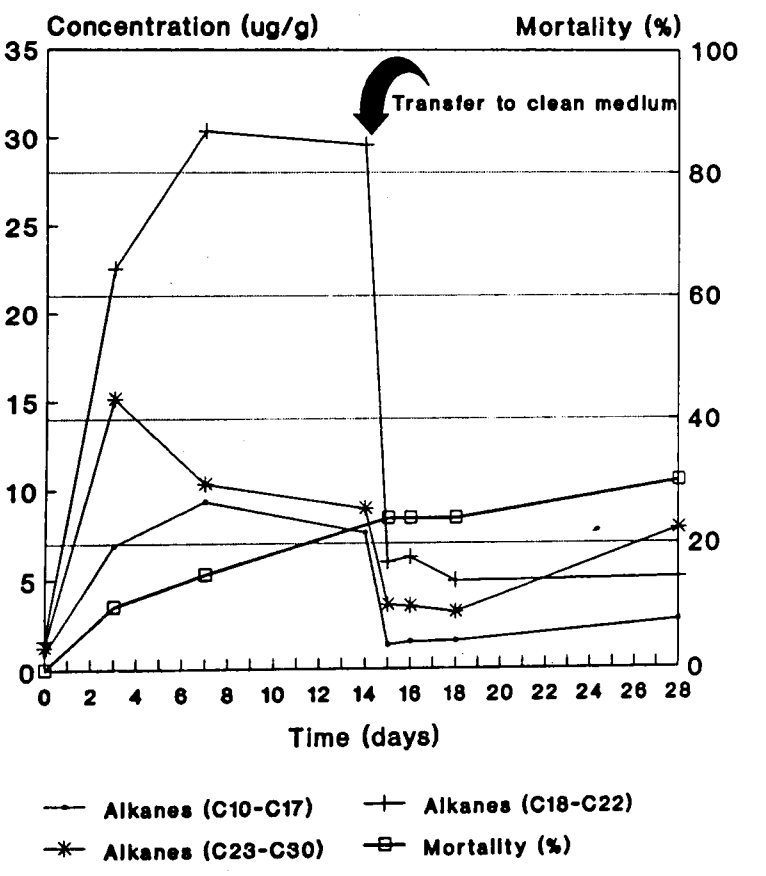


Fig. 6.3 Temporal variation of alkanes in *A. aquaticus* exposed to Mixture C, followed by transfer to clean water.

These results suggest that there is a limit rate beyond which alkanes will not be more rapidly accumulated which is independent of exposure concentrations. This is supported by examination of Table 6.1 which provides the bioconcentration factors after equilibrium was established (mean alkane concentrations following 4 days exposure but before transfer to clean water, divided by dosing concentrations in the water). The data indicate that exposures of *A. aquaticus* to Mixtures A and B result in very similar bioconcentration factors for each alkane group. However, a large reduction in the calculated ratios between Mixtures B and C can be seen suggesting that an accumulation saturation point occurs between these two concentrations. The results also indicate, perhaps surprisingly, that at each concentration, the low range alkanes exhibited the lowest bioconcentration factors. This is unexpected because the lower range alkanes might be expected to be more water soluble and therefore more available for initial accumulation by aquatic organisms i.e. these compounds are more likely to be able to cross gill membranes which may allow further accumulation (see Section 6.3). This apparent paradox is discussed fully with regard to the distribution of the alkane assemblage in water and organisms in Section 6.7.

TABLE 6.1

BIOCONCENTRATION FACTORS (l/g) FROM WATER IN *A. aquaticus*

mixture	*alkane group*		
	C10-C17	*C18-C22*	*C23-C30*
A	48	276	179
B	78	284	191
C	40	137	72

6.3 ALKANE DEPURATION IN *A. AQUATICUS*

On return to uncontaminated water, *A. aquaticus* exhibits extremely rapid clearance of alkanes, particularly in the C_{18}-C_{22} range as can be seen for Mixtures A, B and C in Figs. 6.1 - 6.3. It should also be noted that the body concentrations measured 24 hrs after transfer to the clean medium display very few differences for the three initial

dosing mixtures. This may indicate that the capacity of *A. aquaticus* to remove hydrocarbons is increased by the amount of hydrocarbons previously accumulated. If this feature is genuinely related to an enhancement of metabolic removal capacity by *A. aquaticus* during increased pollutant exposure, it represents an important feature in terms of the ability of the organism to recover from high body burdens. The activity of the MFO enzyme is known to be stimulated by the introduction of pollutants. However, monitoring of the rate of production of metabolites either by the use of ^{3}H labelled hydrocarbons or more sophisticated GC methods would be necessary to more closely identify the depuration pathways.

A further interesting feature of the tissue concentrations post-transfer to clean water is the rapid reduction (24hrs) in levels of the C_{10}-C_{17} alkanes which represent the final elimination level of this class i.e. no further decreases occur after the initial 24 hr elimination. In contrast, within the same period, large falls also occur in the two other classes but, generally, in the post-transfer period, these are accompanied by further albeit smaller falls in body concentrations. Thus, hydrocarbon removal appears most rapid and complete in the lower molecular weight alkanes. The differences in relative aqueous solubilities may be used to explain these results. The lower molecular weight alkanes have a greater solubility in water and therefore on transfer to clean media their elimination from *A. aquaticus* would be facilitated by more favourable partition coefficients compared with the less water soluble heavier molecular weight hydrocarbons.

This explanation, however, is not entirely satisfactory on initial examination of the alkane retention ratios presented in Table 6.2. The alkane retention ratio is calculated from the mean tissue alkane concentrations recorded 7 days or more following transfer to clean water against the mean equilibrium alkane concentration calculated after 4 days exposure. Thus, for the C_{10}-C_{17} alkanes, in Mixture A, it can be seen that 61% of the alkanes measured at dosing equilibrium remained in the tissue after at least 7 days in clean water. Although some of this effect can be related to the amount originally accumulated, the extent to which retention occurs requires further explanation. This may be provided if it is assumed that the amount of hydrocarbon that may be highly assimilated to tissue in a given exposure period is independent of the dosing

concentration. Therefore, any of the additional observed elevations in tissue concentrations which occur between, for example, Mixtures A and B are chiefly composed of unassimilated hydrocarbon e.g as gut contents or contained in micellar layers. If this is the case, then the proportion of hydrocarbons that are most easily eliminated would be greatest in organisms dosed with higher levels and which possess high body burdens. Conversely, where dosing level is lower, the proportion of highly assimilated compounds is higher and therefore the proportion retained on transfer to clean media is higher. This argument is also consistent with the original question regarding the swift reduction in C_{10}-C_{17} alkanes in that the compounds originally eliminated are likely to be unassimilated or not tightly bound and therefore on return to clean water they will more readily partition into the aqueous phase compared with unassimilated mid and high-range alkanes.

TABLE 6.2

ALKANE RETENTION IN *A. aquaticus*

mixture	*alkane group (% retained)*		
	C10-C17	*C18-C22*	*C23-C30*
A	61.0	28.2	31.2
B	21.9	17.3	20.3
C	33.9	18.5	68.0

Almost the reverse situation occurs in the C_{23}-C_{30} alkanes in which the greatest extent of retainment (68%) occurs following exposure to the highest alkane concentration (Mixture C). With no similar effect occurring in Mixture B it is difficult to provide an explanation other than to suggest that the greater initial concentration of higher molecular weight compounds *does* (for this range of alkanes) result in a greater amount of truly assimilated compounds and that these, once assimilated, display a greater incumbency in *A. aquaticus* tissue.

6.4 PAH ACCUMULATION AND DEPURATION IN *A. AQUATICUS*

Accumulation of PAHs by *A. aquaticus* occurs at similar rates to those recorded for aliphatics. *A. aquaticus* exposure to Mixtures D - F indicates that maximum levels are commonly attained within 4 days, although in Mixture E, the maximum level occurs after a slightly longer exposure period. It must be stressed that these time periods for maximum values refer to total PAH accumulation and that considerable variations in individual rates occur as can be observed in Figs. 6.4 - 6.6. In general, however the patterns of accumulation and depuration are comparable for the alkane and PAH mixtures.

Fluoranthene and pyrene are generally the two most abundant compounds in *A. aquaticus* tissue. Of these, fluoranthene tends to accumulate to a greater extent than pyrene. The greatest accumulation rates observed were during exposure to Mixture F where fluoranthene and pyrene displayed initial accumulation rates of 1.6 and 2.0 μg g^{-1} day^{-1} respectively. The same compounds displayed accumulation rates of 0.9 and 0.7μg g^{-1} day^{-1} when exposed to Mixture E and 0.8 μg g^{-1} and 0.7 μg g^{-1} on exposure to Mixture D. Other compounds displaying high accumulation rates include the benzofluoranthenes which approached 1 μg g^{-1} day^{-1} when *A. aquaticus* was exposed to Mixture D.

Examination of Table 6.3 which gives the bioconcentration factors of PAHs in *A. aquaticus* indicates that pyrene and fluoranthene, although achieving the highest body concentrations, did not achieve the highest accumulation relative to the initial dosing concentrations. During exposure to Mixture D, more of the benzofluoranthenes are accumulated compared with any other individual PAH. This is a surprising result in view of the fact that the benzofluoranthenes were introduced at only half the concentration of each of phenanthrene, fluoranthene and pyrene. Less pronounced relative elevations of accumulation of the benzofluoranthenes compared with phenanthrene, pyrene and fluoranthene can be observed in exposures to Mixtures E and F.

The data indicate that for the lowest dosing mixture, fluorene and the

benzofluoranthenes were accumulated to the greatest extent. At this concentration, the benzofluoranthenes display a tissue water ratio of over 800 which far exceeds any other individual PAH. At other exposure concentrations the benzofluoranthenes also display relatively high ratios. On initial examination these results may be considered consistent with the work of Southworth *et al.* (1978; see Section 2.6.2) in that a 5 ringed compound of high molecular weight is accumulated to the greatest extent. Southworth *et al.* (1978) however also found another 5 ringed PAH, benzo(a)pyrene, accumulated to a similarly large extent. This was not the case in the currently reported data in which benzo(a)pyrene was consistently among the most poorly accumulated compounds.

TABLE 6.3

BIOCONCENTRATION FACTORS (l/g) FOR PAH IN *A. aquaticus*

mixture	*PAH*						
	Fl	*Ph+A*	*Fa*	*Py*	*BA*	*BF*	*BP*
D	442	136	268	267	268	848	188
E	335	127	286	249	302	203	92
F	199	85	143	136	88	220	89

The depuration of PAHs by *A. aquaticus* can be observed in Figs. 6.4 - 6.6. In almost all cases substantial reductions in concentrations can be seen following transfer to clean media. Following the exposure of *A. aquaticus* to Mixtures D and F, the rate of depuration is slower than the initial accumulation rate e.g. the highest depuration rate over 4 days was 1.1 $\mu g\ g^{-1}\ day^{-1}$ for fluoranthene in Mixture F whereas accumulation rates of up to 2 $\mu g\ g^{-1}\ day^{-1}$ over 4 days were recorded. Whereas accumulation is relatively stable after 4 - 7 days exposure, depuration continues 10 days or more after transfer. The accumulation rate during exposure to Mixture E is initially comparable to the depuration phase but again depuration takes at least 10 days to stabilize.

In Table 6.4 it can be seen that the individual PAHs display quite consistent retention values, the only exception being the high retention of benzo(a)pyrene in Mixture E. Pyrene also appears to be retained at relatively high levels which are independent of

exposure concentration. The benzofluoranthenes are retained to the least extent at each dosing concentration. As previously noted, these compounds were accumulated to the greatest extent, therefore any decreases due to depuration would be reflected in a proportionately lower ratio. Nonetheless, the recorded values are surprisingly low for a high molecular weight compound that is insoluble and lipophilic. Field concentrations of benzofluoranthenes rarely approached those recorded in the laboratory and therefore suggest them to be much less bioavailable in the field than in the laboratory trials.

TABLE 6.4

RETENTION OF PAHs IN *A. aquaticus*

mixture	*PAH (% retained)*						
	Fl	*Ph+A*	*Fa*	*Py*	*BA*	*BF*	*BP*
D	24.0	36.8	31.1	29.2	29.3	13.4	26.6
E	14.0	15.1	18.8	29.4	20.5	14.0	62.0
F	33.8	21.8	25.8	28.2	20.4	17.9	26.7

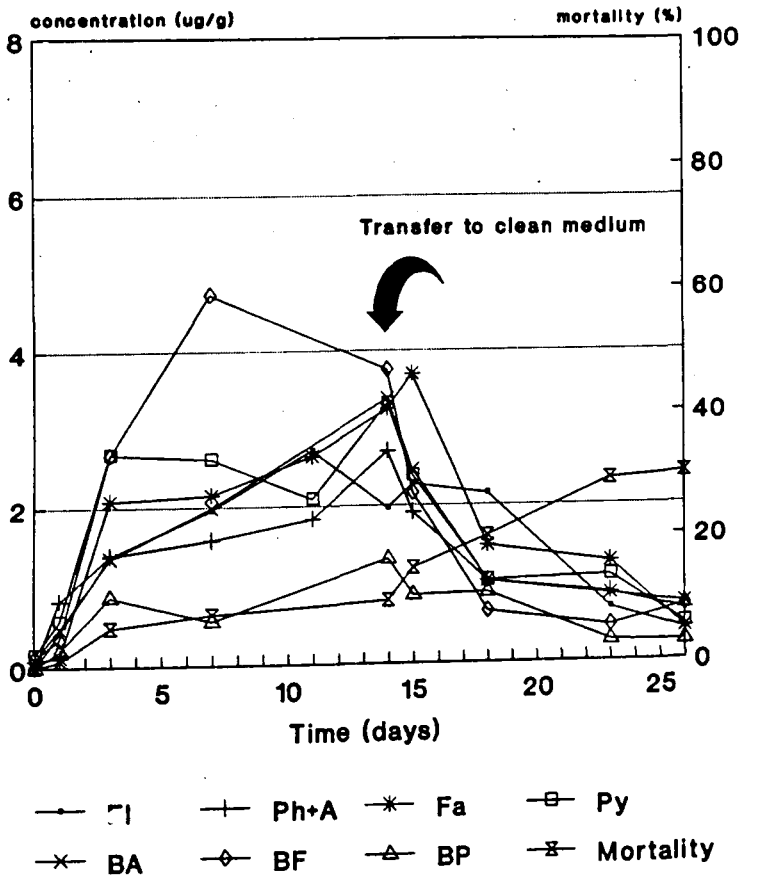


Fig. 6.4 Temporal variation of PAH in *A. aquaticus* exposed to Mixture D, followed by transfer to clean water.

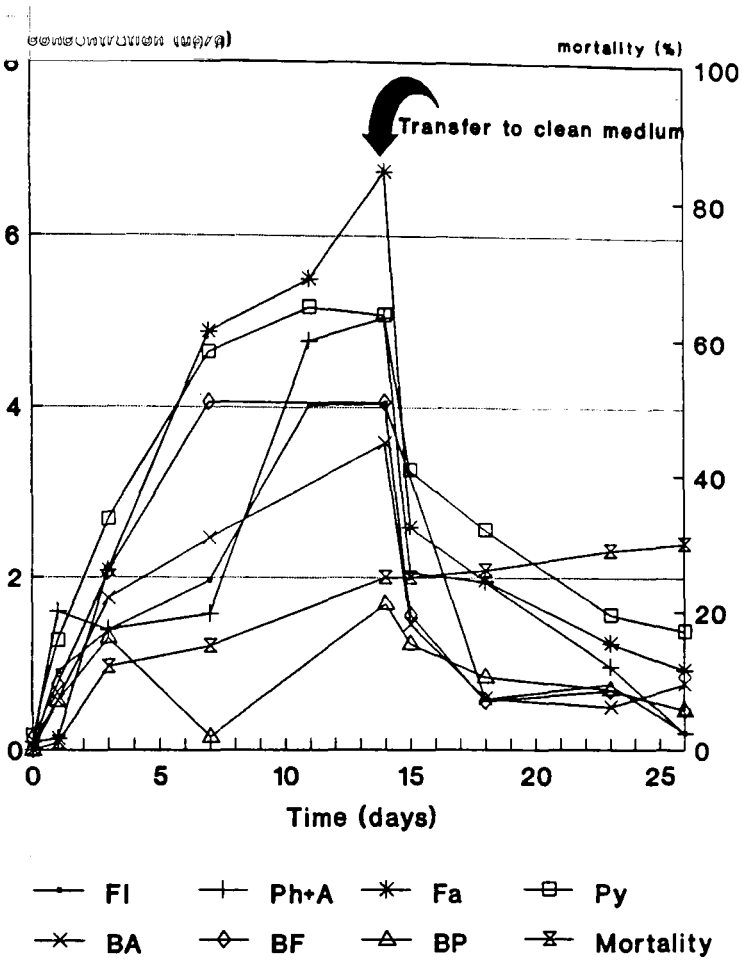


Fig. 6.5 Temporal variation of PAH in *A. aquaticus* exposed to Mixture E, followed by transfer to clean water.

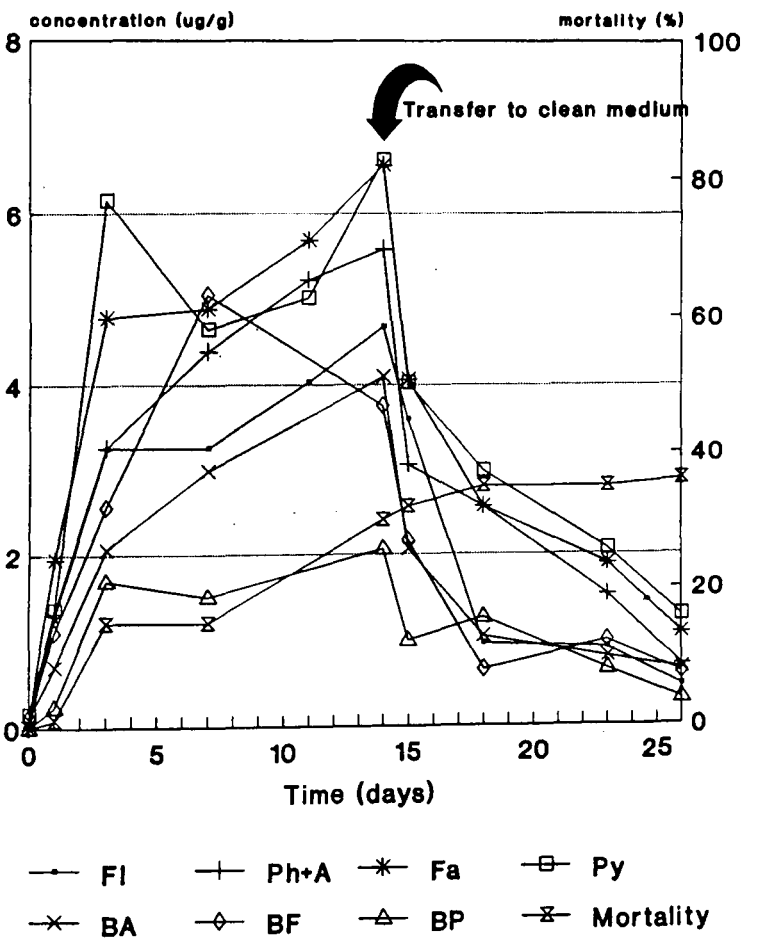


Fig. 6.6 Temporal variation of PAH in *A. aquaticus* exposed to Mixture F, followed by transfer to clean water.

6.5 BIOACCUMULATION AND DEPURATION OF ALKANES IN *L. PEREGRA*

L. peregra displays rapid accumulation of both alkanes and PAHs that are comparable to those previously described for *A. aquaticus*. The levels ultimately attained in *L. peregra* are generally, however, higher than those in *A. aquaticus* although in *A. aquaticus* the initial accumulation rate may be higher. Accumulation and depuration trials of *L. peregra* and *A. aquaticus* exposed to alkane Mixture A show that initially (2 days) the alkanes were accumulated more rapidly in *A. aquaticus* e.g. the C_{18}-C_{22} alkanes were present at 12 $\mu g\ g^{-1}$ in *A. aquaticus* compared with 8 $\mu g\ g^{-1}$ in *L. peregra*. After 3 days, however, higher levels were recorded in *L. peregra* for the three alkane classes. Before a stable tissue alkane level emerged, the rates of accumulation from Mixture A by *L. peregra* were 2.2 $\mu g\ g^{-1}\ day^{-1}$ for low range alkanes, 5.3 $\mu g\ g^{-1}\ day^{-1}$ for the mid-range alkanes and 3.2 $\mu g\ g^{-1}\ day^{-1}$ for the C_{23}-C_{30} alkanes. The highest levels attained were 6.5, 17 and 9 $\mu g\ g^{-1}$ for the three ranges, respectively.

On exposure to Mixture B, the alkane groups display highly differing accumulation patterns. The mid-range alkanes do not reach maximum tissue concentration until after 10 days exposure. In spite of the higher body concentration attained compared with Mixture A, the rate of accumulation is reduced to 3.5 $\mu g\ g^{-1}\ day^{-1}$. After 3 days exposure the high range alkanes follow a very similar path to the mid-range compounds, but then stabilize between 13 and 19 $\mu g\ g^{-1}$. The low range alkanes are variable in Mixture B with peak levels occurring after 7 days followed by a drop to 6.5 $\mu g\ g^{-1}$ which precedes the depuration phase.

Exposure of *L. peregra* to Mixture C results in an extremely rapid accumulation of the C_{18}-C_{22} alkanes reaching almost 40 $\mu g\ g^{-1}$ during the first 48 hrs of exposure. In contrast, the high and low range alkanes attain concentrations of 8 - 14 $\mu g\ g^{-1}$ which are very similar to those attained for Mixture B. As a result of this, the alkane assemblage has the form of a leptokurtic (highly peaked) distribution which centres around the C_{18}-C_{22} alkane range. It is interesting to note that this type of alkane distribution was commonly found in *L. peregra* collected from the *in situ* trials at the most highly contaminated Sites 6 and 7 in the Silk Stream (see Chapter 5). This highly peaked distribution was sometimes noted in *A. aquaticus* in the laboratory tests but very rarely

in the field trials. This feature would therefore suggest important species differences in terms of the enhanced ability of *L. peregra* to preferentially accumulate hydrocarbons in the relatively abundant C_{18}-C_{22} alkane range. Table 6.5 provides the bioconcentration factors for alkanes and shows that the mid-range alkanes possess, on average, the highest accumulation factor. It is also interesting to note the low ratio for the low range alkanes as previously noted with *A. aquaticus*. An experimental factor may exist here which is discussed further in Section 6.7.

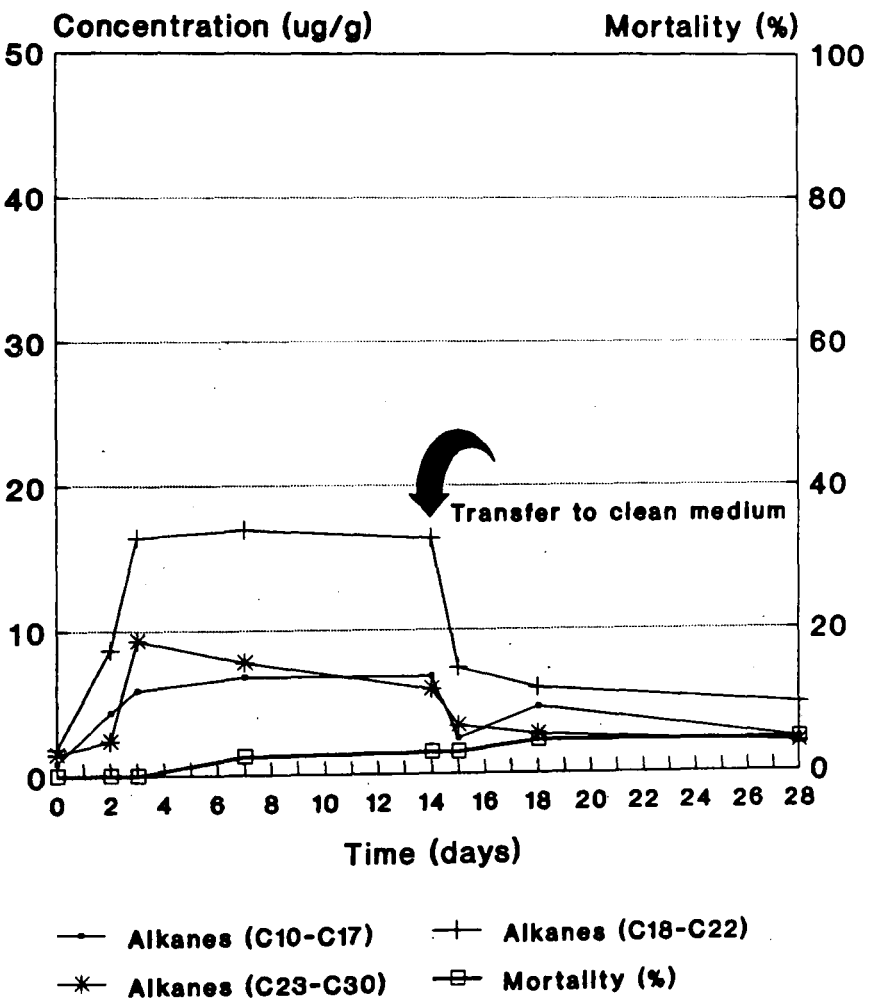


Fig. 6.7 Temporal variation of alkanes in *L. peregra* exposed to Mixture A, followed by transfer to clean water.

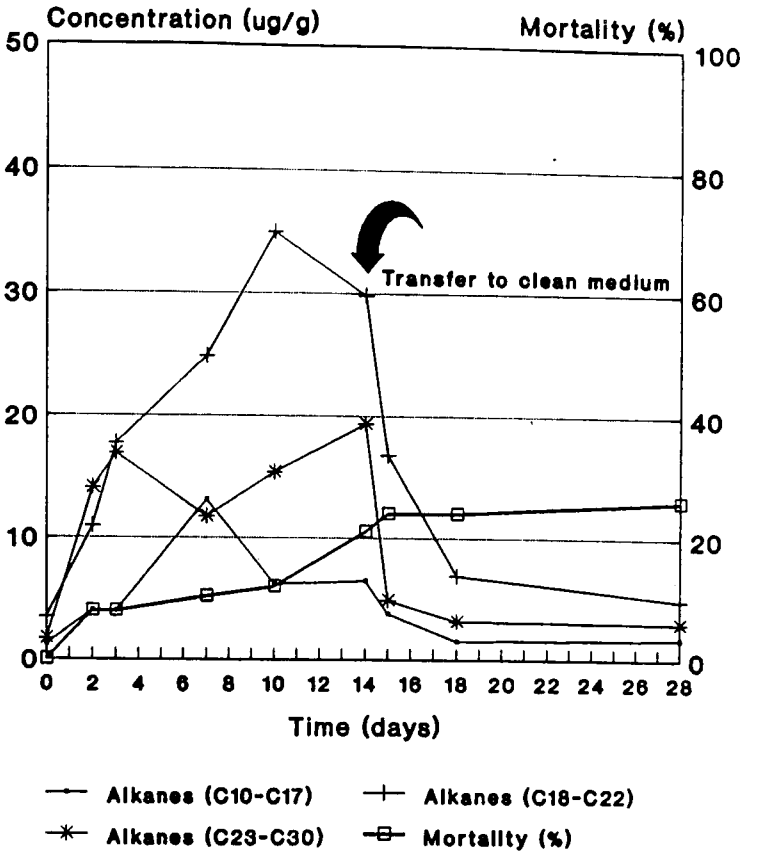


Fig.6.8 Temporal variation of alkanes in *L. peregra* exposed to Mixture B, followed by transfer to clean water.

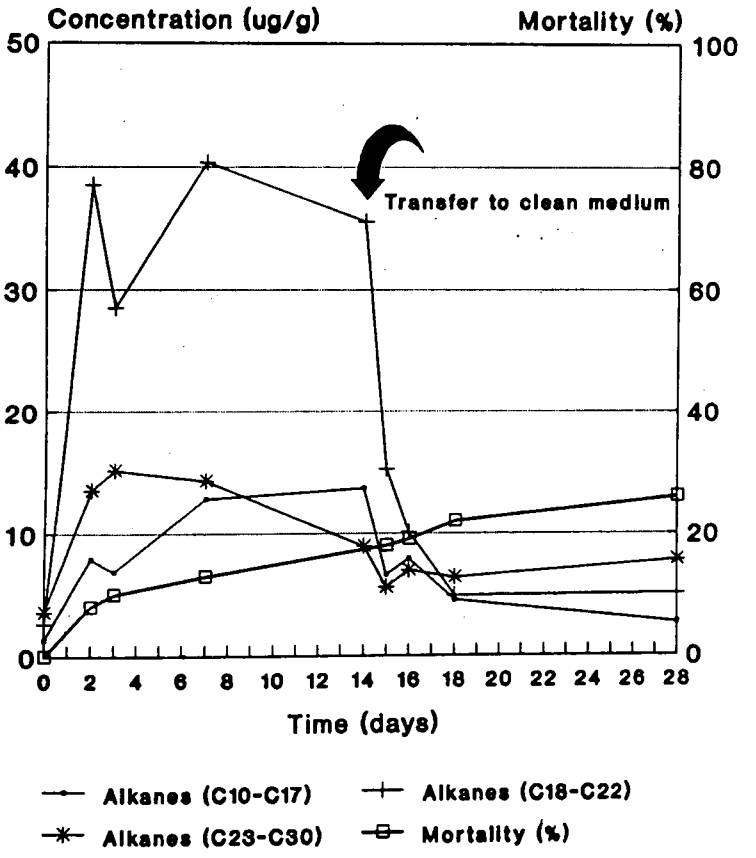


Fig 6.9 Temporal variation of alkanes in *L. peregra* exposed to Mixture C, followed by transfer to clean water.

TABLE 6.5

BIOCONCENTRATION FACTORS (l/g) FROM WATER IN *L. peregra*

mixture	*alkane group*		
	C10-C17	*C18-C22*	*C23-C30*
A	135	333	341
B	86	299	194
C	66	190	145

Examination of the depuration phases in *L. peregra* (Figs. 6.7 - 6.9) suggest that very little clearance occurs beyond 4 days after the transfer date. Initially, however, depuration is quite rapid and reaches a maximum rate of 6 $\mu g\ g^{-1}\ day^{-1}$ for the mid range alkanes following exposure of *L. peregra* to Mixture C. This high rate is reflected in the low retention figures for this class of alkanes as shown in Table 6.6. A further interesting feature is the high retention of the C_{23}-C_{30} alkanes at the top dosing concentration (Mixture C). A very similar result was previously observed for *A. aquaticus* (see Table 6.1) for the same group of alkanes and dosing concentration. The same argument used in Section 6.3 may be applied to explain this apparently anomalous result for *L. peregra* i.e. that the high concentrations lead to a possible higher level of hydrocarbon assimilation.

TABLE 6.6

RETENTION ALKANE RATIOS IN *L. Peregra*

mixture	*alkane group*		
	C10-C17	*C18-C22*	*C23-C30*
A	34.3	28.6	29.30
B	19.72	16.0	19.4
C	20.27	13.38	60.0

6.6 BIOACCUMULATION AND DEPURATION OF PAH IN *L. PEREGRA*

The patterns of accumulation and depuration of PAH in *L. peregra* (Figs. 6.10 - 6.12) illustrate further differences between the two test organisms. Initially PAH levels rise at very similar rates for the two organisms during the first few days of exposure. In fact, after 24 hrs, *A. aquaticus* body burdens tend to exceed those of *L. peregra*. Generally, however, after 3 days of exposure, PAH levels in *L. peregra* exceed those in *A. aquaticus*. There are two exceptions to this: fluorene which tends to fluctuate in concentration and benzo(a)pyrene which tends not to be accumulated excessively by either organism. Nevertheless, total PAH levels in *L. peregra* tend to stabilize at higher levels than in *A. aquaticus*. This is reflected in the higher tissue to water ratios presented in Table 6.7 where there is a clear general increase in values compared with those calculated for *A. aquaticus* (see Table 6.3).

TABLE 6.7

BIOCONCENTRATION FACTORS (l/g) FOR PAHs IN *L. peregra*

mixture	*PAH*						
	Fl	*Ph+A*	*Fa*	*Py*	*BA*	*BF*	*BP*
D	529	281	386	418	252	853	243
E	547	317	384	253	415	535	263
F	192	138	228	210	175	197	105

Following transfer to clean media, PAH concentrations, as previously observed for alkanes, fall rapidly within the first 4 days post-transfer reaching values of 1 to 2.5 $\mu g\ g^{-1}$ for individual compounds. Again, however, following the initial rapid clearance there is little continued depuration. This pattern, with the exception of benzofluoranthene, is best illustrated in Mixture E (Fig. 6.11). The retention ratios shown in Table 6.8 illustrate a consistent retention of PAHs with the exception of benzo(a)pyrene which again was the compound that was both accumulated and cleared to the least extent.

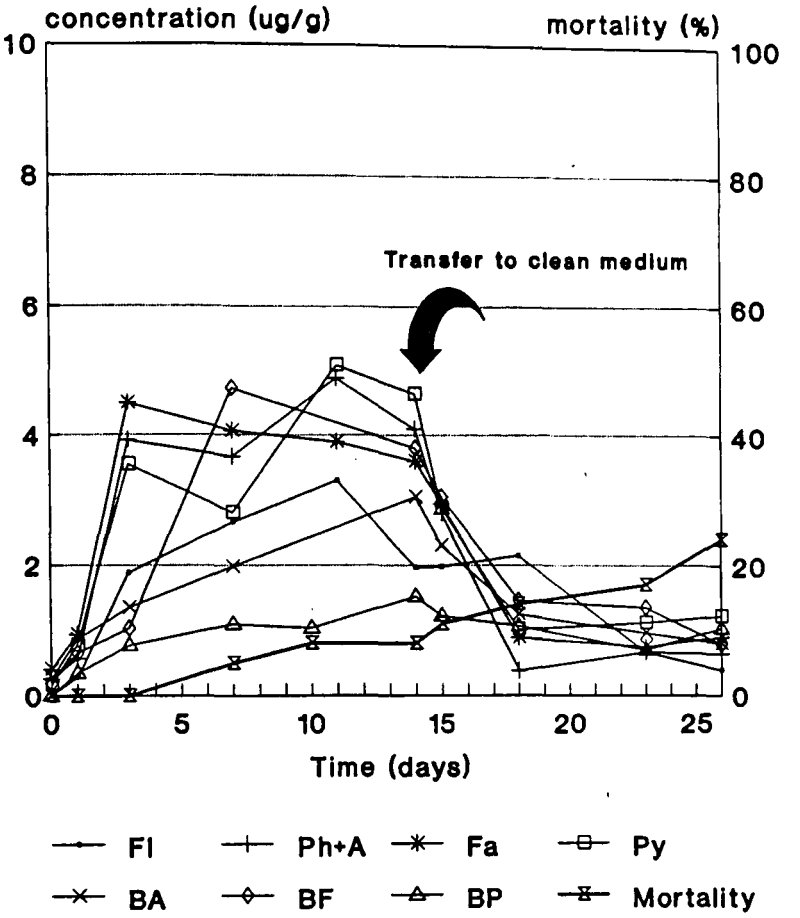


Fig.6.10 Temporal variation of alkanes in *L. peregra* exposed to Mixture D, followed by transfer to clean water.

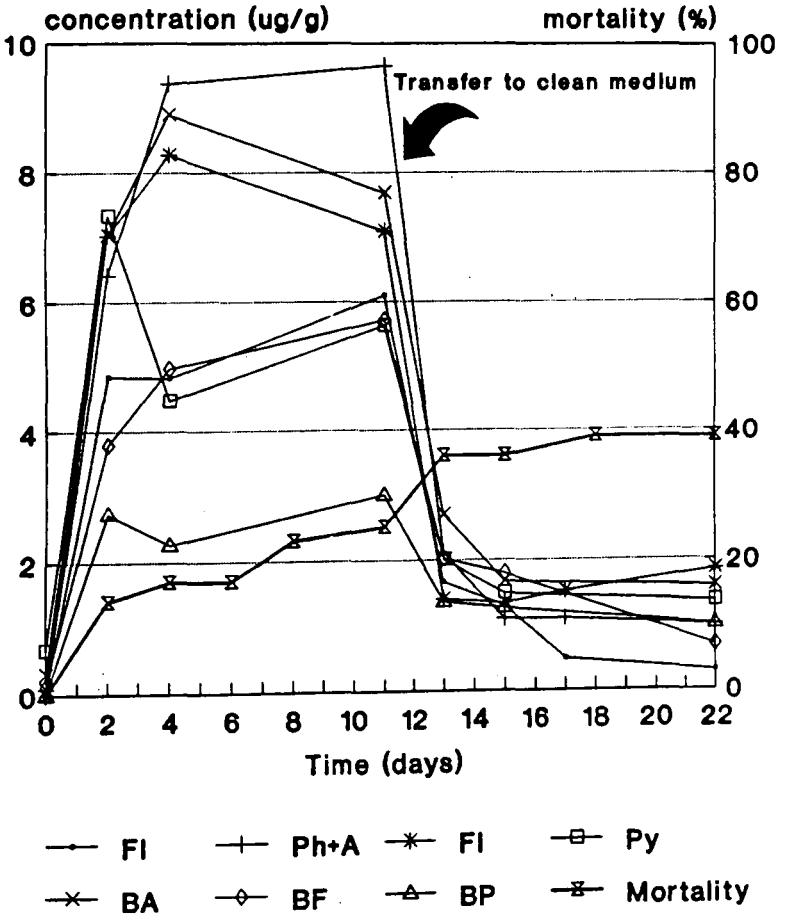


Fig.6.11 Temporal variation of alkanes in *L. peregra* exposed to Mixture E, followed by transfer to clean water.

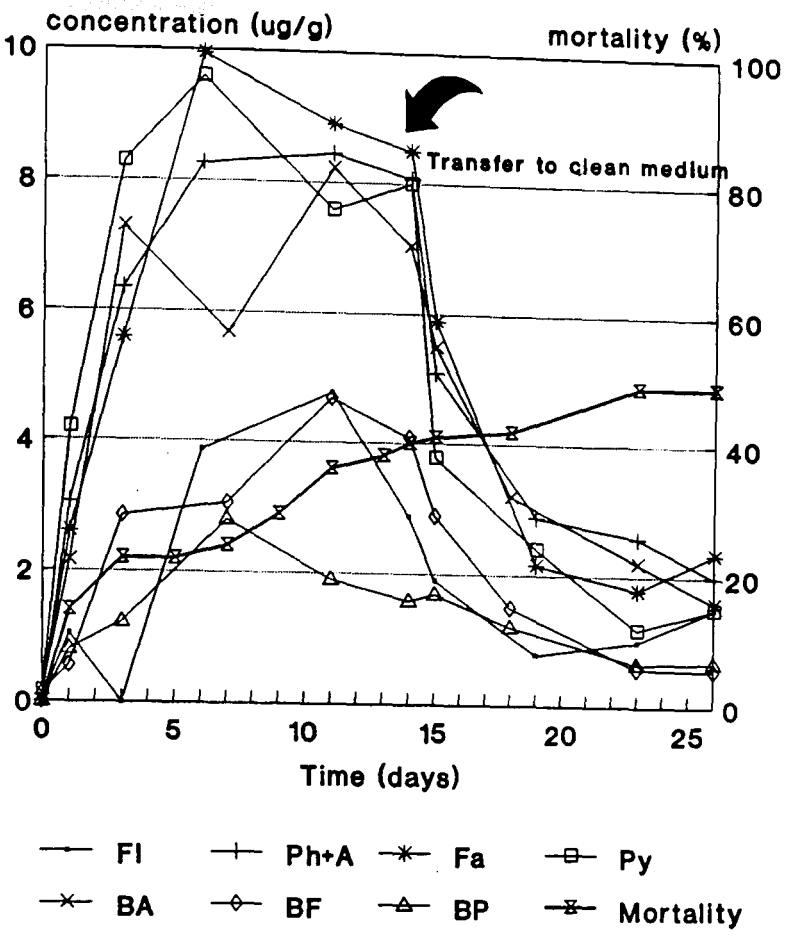


Fig 6.12 Temporal variation of alkanes in *L. peregra* exposed to Mixture F, followed by transfer to clean water.

TABLE 6.8

RETENTION RATIOS FOR PAHS IN *L. peregra*

mixture	*PAH*						
	Fl	*Ph+A*	*Fa*	*Py*	*BA*	*BF*	*BP*
D	20.0	15.4	31.1	28.1	34.9	25.1	71.5
E	5.3	10.4	12.0	26.9	19.3	12.9	38.0
F	32.4	27.7	17.2	16.0	27.2	14.5	31.7

In contrast to *L. peregra*, *A. aquaticus* displays a continued reduction in PAH levels throughout the depuration period. The initial elimination rate of both classes of compound is thus relatively rapid in both organisms but in *L. peregra* the clearance rate after approximately 5 days appears to slow down compared with *A. aquaticus*. These observations can be confirmed on examination of Table 6.9 in which *L. peregra* displays higher depuration half-lives compared with *A. aquaticus*. Molluscs are known to lack or possess in small concentrations the cytochrome P450 mediated Mixed

Function Oxidases (MFO) which effect the first and principal phase of xenobiotic removal. Lee *et al.* (1972) following studies on a mollusc, the mussel *Mytilus edulis* observed that benzo(a)pyrene initially showed elevated levels in the gill and that here absorption into the micellar layer was found to occur before subsequent distribution and assimilation into other tissues. These workers found rapid excretion of accumulated PAHs still occurred despite the absence of MFO in this organism. Similarly non-enzyme specific elimination may also occur with other organic compounds including alkanes. It would seem therefore, that in *L. peregra* as well as other molluscs, excreted compounds are likely to be released into clean media as a result of thermodynamically favourable equilibrium partition of non-assimilated compounds as opposed to active enzyme mediated removal. The initial first-phase rapid clearance of aliphatic hydrocarbons and PAHs by both *A.aquaticus* and *L. peregra* is likely to be the result of such diffusion reactions although a lower level and less rapid enzyme-driven response also probably occurs in *A. aquaticus*. The differences in longer term elimination of compounds, as is illustrated by the relatively persistent elevations of PAHs in *L. peregra*, would further support the argument that a biphasic depuration does occur in MFO-containing organisms such as *A. aquaticus*. The relative inability of *L. peregra* to eliminate presumably assimilated (those not removed initially) compounds, particularly the PAHs, represents a typical mollusc response to incorporation of xenobiotics in its soft tissue.

The issue of non-assimilated compounds has been addressed by a number of other workers who have also suggested that significant proportions of detected compounds in organism tissue may be in non-assimilated forms. Rossi (1977), for example, investigating the bioaccumulation of petroleum hydrocarbons from water, sediments and detritus in the marine annelid *Neanthes* sp., found naphthalene accumulated to a maximum of 6 $\mu g\ g^{-1}$ in 3-24 hrs. Within 300 hrs after return to a clean medium, naphthalene in biological tissues was not detected. One third of the excreted compound was unmetabolized and the remainder was in polar metabolized form. Thus, one third of the excreted compound could not be attributed to active biological elimination. A similar effect was circumstantially provided in Crustacea by Harris *et al.* (1977) who demonstrated that *Calanus* sp. accumulated naphthalene from sea water ($0.1\ \mu g\ l^{-1}$) and eliminated the compound in clean water with a half-life of 36 hrs. Elimination was

more rapid in organisms fed with algal cells than in starved animals. These data illustrate the importance of nutritional status in assessing possible bioaccumulation. The results, however, have been considered as further evidence of non-assimilation of the dosing compounds i.e. that the detected compounds may have been in the gut contents, and that feeding accelerated their excretion.

It must be again stressed, therefore, that accurate measurement of the rate of uptake of organics (as opposed to the amount accumulated) as well as the elucidation of the depuration pathway (including identifying unassimilated compounds) must involve the use of radiolabelling such that metabolite formation may be quantified .

In general, the patterns of hydrocarbon uptake in crustaceans are more varied than those in the more commonly used molluscs. These trends may be expected by the more active mode of life of Crustacea as well as their greater ability at a cellular level to metabolize PAHs. The higher initial accumulation rates sometimes observed in the currently reported data with *A. aquaticus* may also be explained by its more active feeding activity and metabolism.

6.7 ALKANE TISSUE PROFILES IN *L. PEREGRA* AND *A. AQUATICUS*

The alkane tissue distribution in dosed laboratory *A. aquaticus* and *L. peregra* have features which differ markedly from the *in situ* organism profiles (see Fig. 6.13). A prominent feature is the reduction in lower chain numbered alkanes C_{10}-C_{17}. Initial examination of the data suggests the organisms had accumulated the longer chained hydrocarbons in preference to the shorter ones as discussed in Sections 6.2 and 6.4. However, further tests involving the extraction and analyses of the dosed water indicated that some of the shorter chained alkanes were already reduced in concentration. Therefore, physical processes such as the loss of compounds by evaporation must be an important factor in excluding these compounds from *A. aquaticus* tissue in the laboratory experiments. It is likely that some of these losses came as a result of the driving-off of the more volatile fractions through aeration, an effect noted by Hedtke & Puglisi (1982). However, it was considered important to maintain good DO levels in spite of this possible shortcoming. Short carbon chain

length alkanes are known to demonstrate short-term toxicity to a variety of organisms but are also the most volatile and biodegradable (CONCAWE, 1992). This begs the question, as put by Betton (1994) of whether open system testing of such volatile compounds give invalid results or whether they more closely represent the real situation. While it is, of course, necessary to have sound baseline data on the toxicity of compounds it must be the case that laboratory tests which more closely resemble the real world will ultimately be more beneficial to our understanding of pollutants, especially volatile hydrocarbons in the aqueous environment.

It should be observed that longer chained alkanes (C_{22}-C_{28}) are also present in lower concentrations in the laboratory than might be expected if *A. aquaticus* levels mirrored dosed water levels. In the water phase, there was no significant reduction in C_{22}-C_{28} values as observed with the shorter chain length alkanes since these heavier compounds were consistently detected in similar proportions to the original dosing values. Therefore, the bioavailability of the long chained alkanes must be lower than that of the mid-range alkanes (C_{16}-C_{22}). This reduced bioavailability may be the result of the lower aqueous solubilities associated with the longer chained alkanes which would reduce their tendency to cross gill membranes and to be available for tissue accumulation. This however appears to conflict with the predicted bioaccumulation from n-octanol/water partition coefficient data which increases with increasing alkane chain length and would therefore predict increasing bioaccumulation with longer chained compounds (see Section 6.8). Since all the heavy alkanes are strongly lipophilic, however, it may be suggested that it is the increasing size of the molecule as opposed to the rather small decreases in, for example, water solubilities that govern whether the compound is likely to be accumulated by an organism.

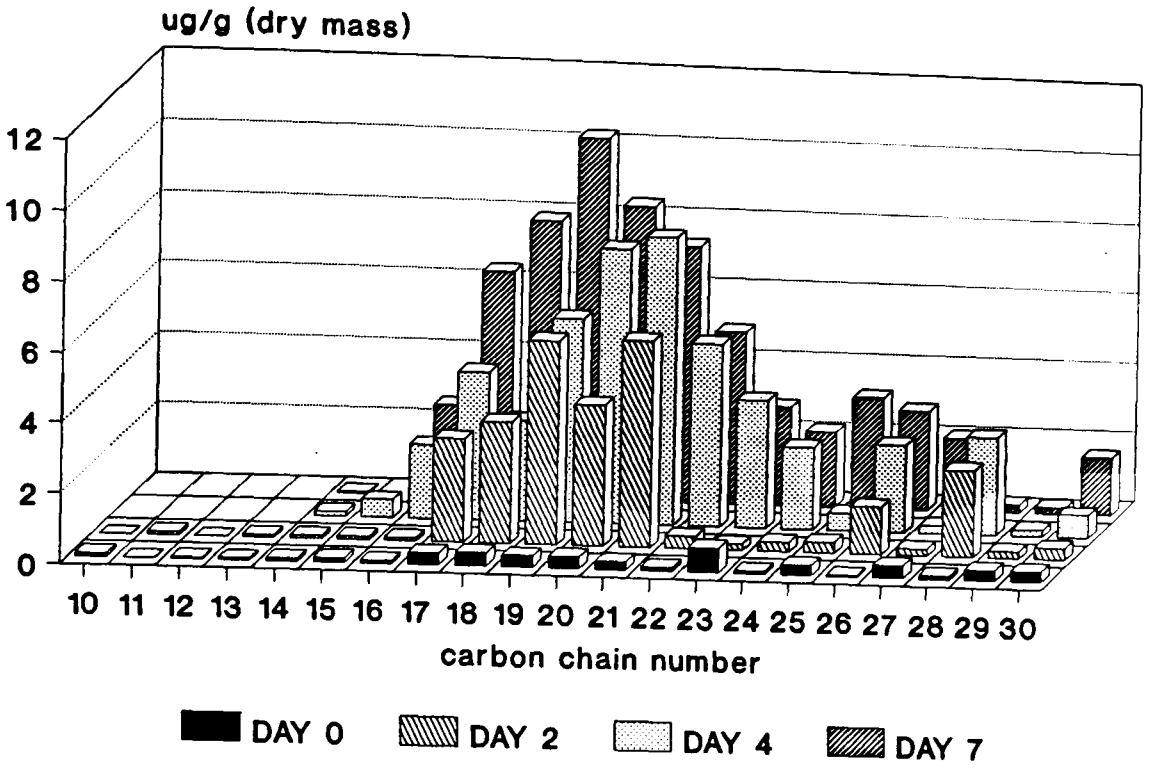


Fig 6.13 Alkane distribution in *A. aquaticus* exposed to Mixture B.

6.8 INVESTIGATION OF RELATIONSHIPS FOR BIOACCUMULATION DEPENDENCE

A number of workers have proposed physico-chemical properties such as water solubility and the n-octanol/water partition coefficient (K_{ow}) as useful indicators of the tendency of organic chemicals to bioaccumulate (e.g. Geyer *et al.*, 1982; Tichy, 1991; Dearden *et al.*, 1994) and this type of approach may be useful in explaining certain observed patterns of bioaccumulation. Many of the compounds analyzed in the laboratory have log K_{ow} values lying in the range 3 - 6 within which positive correlations with the log of bioconcentration factor (log BCF) have been found. A biological response such as toxicity, bioaccumulation or any other defined sublethal effect can, in principle, be expressed as a function of a physical attribute of which the K_{ow} is now in common use. Such expressions are in the form of mathematical equations known as Quantitative Structural Activity Relationships (QSARs). Compounds with log K_{ow} values of less than 3 are considered to have a low partitioning potential into octanol from water and therefore have a low affinity for lipids and, consequently, a

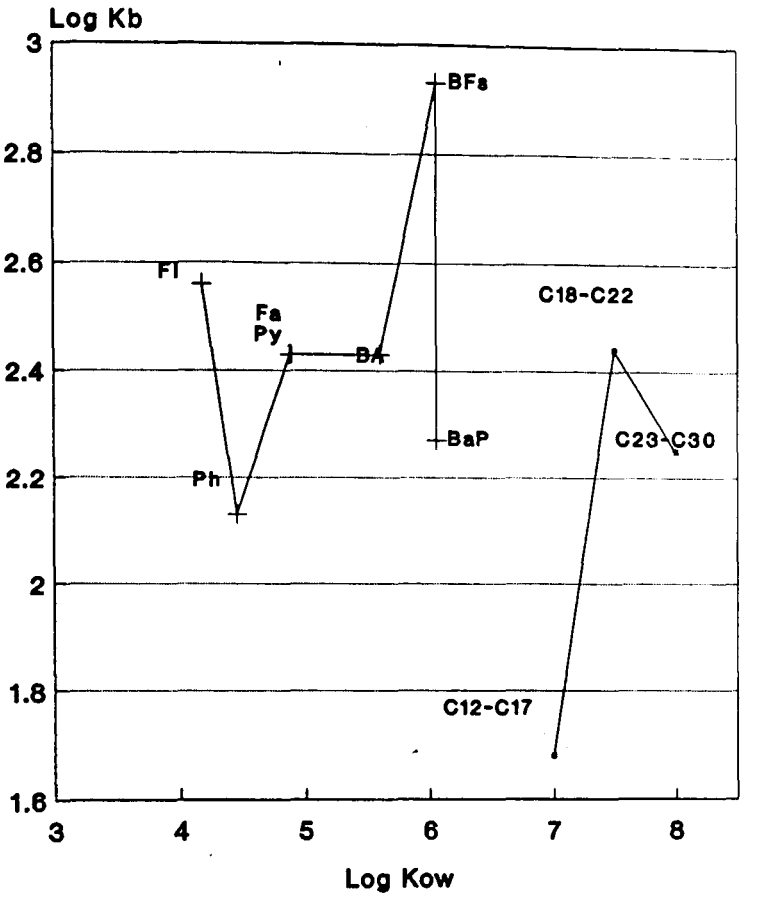


Fig. 6.14 Relationship between log K_{ow} and log BCF in *A. aquaticus*.

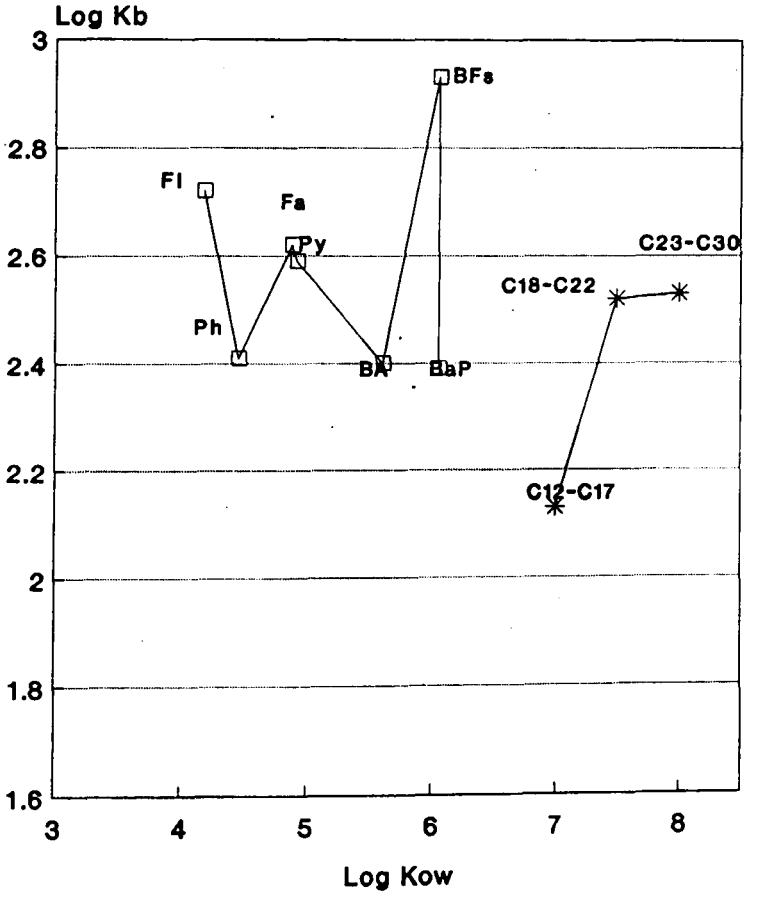


Fig. 6.15 Relationship between log K_{ow} and log BCF in *L. peregra*

low tendency to bioaccumulate. In the middle range (3 - 6),log K_{ow} and bioaccumulation are thought to be positively correlated. Above a log K_{ow} value of 6, with so-called superhydrophobic compounds, the relationship breaks down and bioaccumulation decreases.

The relationships between log K_{ow} (from Sangster, 1989) and log BCF are presented in Figs. 6.14 and 6.15 for *A. aquaticus* and *L.peregra* respectively. It can be seen, however, that there is no clear correlation (even within the predicted linear relationship band of log K_{ow} between 3 and 6) in the currently reported data between the octanol/water partition coefficient and the bioconcentration factor from water. Therefore, it has not been possible to develop any QSARs for these data. Inter-species differences, however, can be observed in which BCFs for *L. peregra* tend to exceed those for *A. aquaticus*. It is also interesting to observe the similarities in the relative extents of accumulation between the various compounds in the two organisms. The low molecular weight alkanes have lower BCFs than any of the PAHs. This would be expected since the relationship with K_{ow} breaks down in this range. However, the effect is probably the result of factors unrelated to the K_{ow} value. This is supported by the fact that for both organisms the mid and high molecular weight aliphatics display higher BCFs than the low molecular weight comounds, even though such highly hydrophobic compounds should display decreased accumulations. Both organisms can also be seen to accumulate the benzofluoranthenes to the greatest extent before a decline in accumulation with benzo(a)pyrene occurs. Again, these compounds, having log K_{ow} values of about 6 are at the limit of the documented range where significant correlations with log BCF have been demonstrated. The principal reasons for the breakdown in the relationship are because of the extremely low aqueous solubility which results in a low amount of material available for transfer. As mentioned for the high molecular weight alkanes (Section 6.7) the large molecular size also becomes an increasingly important factor in reducing membrane penetration of PAHs (Barron, 1990). In effect, these factors mean that a steady state may not obtained during the duration of a laboratory exposure.

Some workers e.g. Chiou (1985) and Hawker & Connell (1986) have considered the octanol/water partition coefficient as a poor model for partition into lipid membranes

since, although solubility of large molecular weight compounds decreases in octanol, the decline is not as great as that in lipid membranes. More recently, Chessels *et al.* (1993) have demonstrated that the solubilities of large organic compounds are reduced both in aqueous and lipid media which further reduces the tendency of highly hydrophobic compounds to bioaccumulate. Banerjee & Boheman (1991) have introduced a correction factor which allows for the anomalous behaviour of large organic compounds in lipids compared to octanol in their development of QSARs. QSARs based on partitioning are also considered accurate only if the chemicals of interest are not metabolized to any significant extent (Donkin, 1994). In view of the results reported in this chapter some doubt must exist over the extent of metabolism but it is suggested that given the relatively rapid depuration rates reported, significant turnover of organic compounds probably occurs in both organisms for all the measured compounds. As noted in Section 6.2 metabolite monitoring must be employed in order to quantify uptake rates and to distinguish between various extents of pollutant assimilation.

A further important point must be raised which questions the strength of the applicability of partition based QSARs: humic material in natural waters is known to bind hydrophobic organic compounds which severely reduce their bioavailability (Barron, 1990). It must be stressed that in the currently reported trials, natural unfiltered water was used in order to better simulate the in-stream locations. It may be argued, as in the possible losses of volatile organic compounds (see Section 6.3), that the results, because of the influences of such interfering factors, have reduced validities. Again, however, it should be the case that the test systems employed and the results that they produce are more valid and interesting if they include the same interfering factors that exist in the natural world. The principle of laboratory QSARs and their undoubted potential for prediction of environmental damage is acknowledged, particularly for large numbers of organic compounds, but it is how far the quantitative relationships stand in the natural world which remains the major issue in the use of laboratory tests. Thus, while utilizing established toxicity data, the use of open test systems, natural media and pulsed pollution simulations (e.g. Bascombe 1991; Mulliss 1994) must be the way forward in bridging the gap between field and laboratory data. A further important point regarding the failure to establish QSARs may be the synergistic and competitive effects of the use of hydrocarbon mixtures (e.g. Fortner &

Sick, 1985) compared with the more commonly used single compound dosing for the development of QSARs. The use of mixtures should again be defended on the grounds that they more closely represent the types of pollutant assemblages to which macroinvertebrates are exposed in urban streams.

QSARs involving K_{ow} values also assume that transfers exist from one dissolved phase to another. Where aqueous solubilities are extremely low, the amount of compound available for transfer from these phases must also be low. However, the field and laboratory results suggest that alkanes of high molecular weight are readily accumulated. Donkin *et al.* (1991) has proposed that uptake by organisms of highly hydrophobic hydrocarbons is from an emulsion phase and it must be the case that for such compounds both in the field and laboratory this is an important transfer route.

The issue of solubility was also raised by Neff (1979, 1985; see Section 2.6.2) who measured the uptake and release of four PAHs by the clam *Rangia cuneata*. Benzo(a)pyrene was found to be the most slowly accumulated compound but also the least rapidly depurated. Benzo(a)pyrene has a very low aqueous solubility (~ 1 ppb) and would therefore be mainly present in colloidal or particulate form which reduces its bioavailability. Once absorbed, however, BaP was thought to be relatively strongly associated and therefore would not easily partition back even to a very low aqueous concentration. Their arguments may be applied to the accumulation and subsequent depuration patterns of benzo(a)pyrene (for which dosing levels exceeded its aqueous solubility) reported both for *A. aquaticus* and *L. peregra* in the currently reported data: benzo(a)pyrene behaves in a similar fashion in that its accumulation rate is low yet, proportionately, its degree of retention following organism transfer to clean media is the highest of the compounds studied. By the same argument, the lipid/water partition coefficient of more water soluble compounds favours rapid release to water when compound concentrations in the medium are low. It may also be argued that such compounds with higher water solubilities would also be more immediately available for accumulation (though not necessarily in highly assimilated forms) i.e more rapid accumulation and depuration can be explained by increased water solubility which therefore results in greater bioavailablity since affinity for lipids of all hydrocarbons is relatively high. Lee *et al.* (1978) also observed these effects with studies on the oyster

Crassostrea virginica using a range of PAHs. The relatively soluble naphthalene and its alkyl derivatives were accumulated most rapidly. The rate of accumulation of other PAHs decreased with increasing molecular weight and with a general decrease in aqueous solubility. On transfer to a clean medium, the half lives varied from 2 days for naphthalene to 18 days for benzo(a)pyrene. Attempts to quantify the depuration half-lives in *L. peregra* and *A. aquaticus* showed that the linear decay coefficient (λ) could not readily be calculated for the alkanes (Figs. 6.16 and 6.17) because an estimate of the gradient could not be made. This further implies that the depuration of hydrocarbons from *A. aquaticus* and *L. peregra* is at least biphasic. However, estimates of the decay coefficients, and hence, the half-lives could be made for the PAHs (see examples in Figs. 6.18 and 6.19). The results of these calculations are presented in Table 6.9.

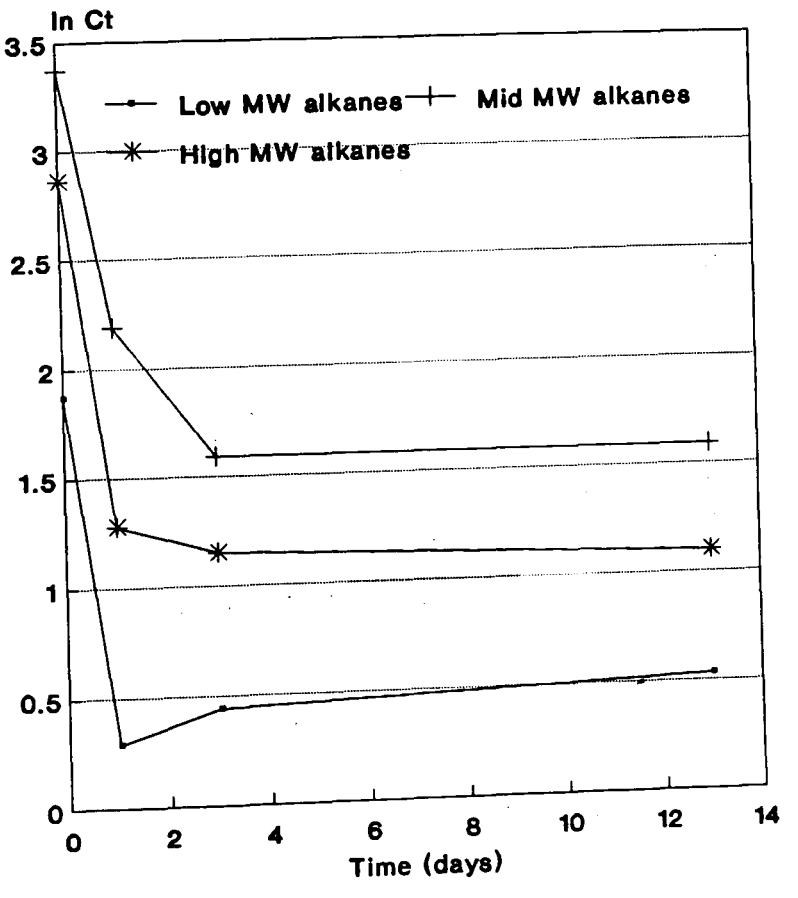


Fig. 6.16 Log alkane concentrations from Mixture B versus time for *A. aquaticus*.

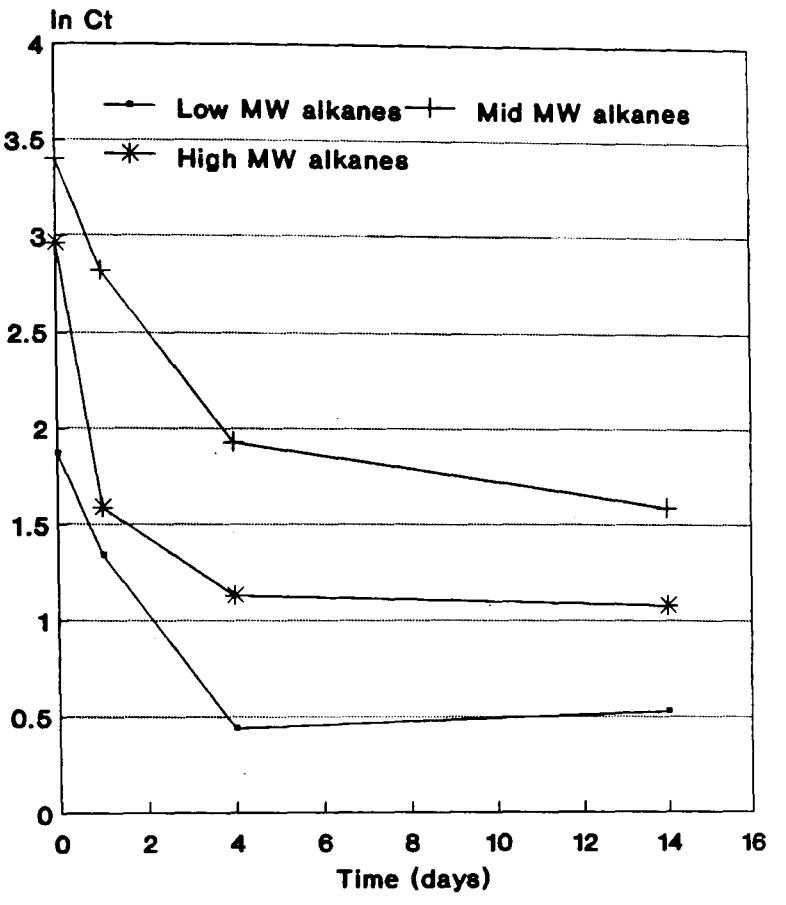


Fig. 6.17 Log alkane concentrations from Mixture B versus time for *L. peregra*

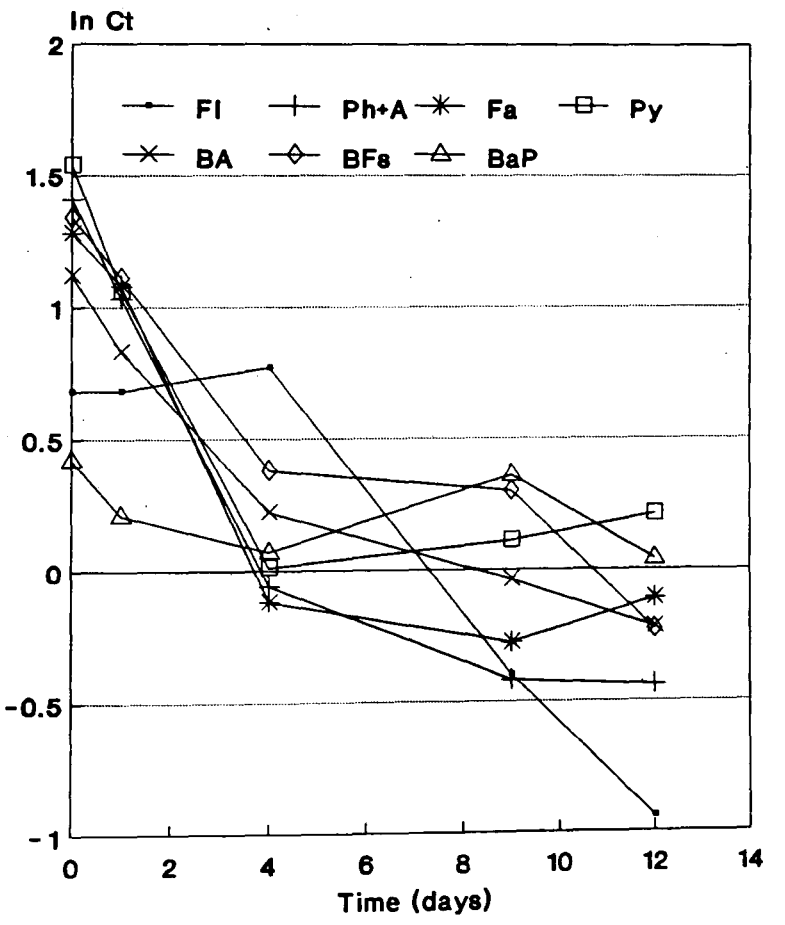


Fig. 6.18 Log PAH concentrations from Mixture E versus time for *L.peregra*

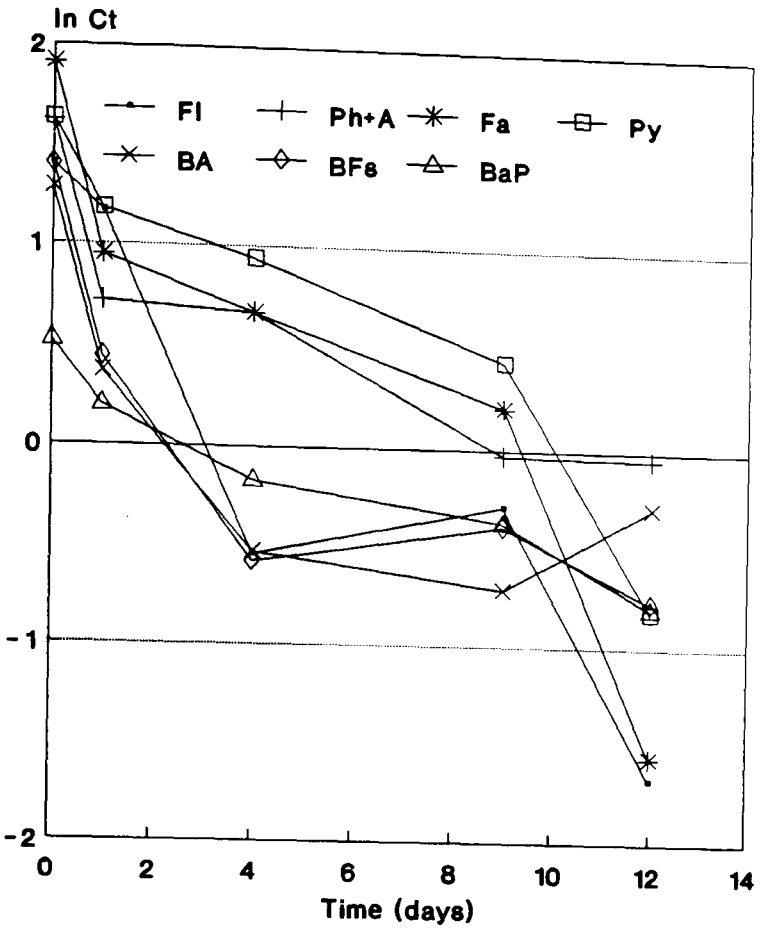


Fig. 6.19 Log PAH concentrations from Mixture D versus time for *A. aquaticus*.

TABLE 6.9

DEPURATION HALF-LIVES FOR *A. AQUATICUS* AND *L. PEREGRA*

Organism	*Half-life (days)*						
	Fl	*Ph+A*	*Fa*	*Py*	*BA*	*BFs*	*BP*
A. aquaticus	3.81	5.65	4.16	4.87	5.64	4.91	5.25
L. peregra	3.72	5.22	6.90	6.34	6.17	4.68	8.47

The PAH depuration half-lives as estimated by decay constant calculations do not show highly pronounced differences between compounds. Fluorene displays the shortest half-life in both organisms. In *A. aquaticus* no significant differences can be observed among the other compounds. *L. peregra* has generally higher half-lives with values for fluoranthene, pyrene benzo(a)anthracene and benzo(a)pyrene significantly exceeding those of *A. aquaticus*. The low depuration rate for benzo(a)pyrene in *L. peregra* is confirmed by the highest recorded value (8.47 days).

6.9 FACTORS INFLUENCING BIOAVAILABILITY IN THE FIELD AND LABORATORY

The sources of the hydrocarbons exposed to organisms in the laboratory and in environmental samples must also be considered when assessing bioavailability. The ratio of methylated groups to parent compounds in the abiotic PAH assemblages together with other indicators strongly suggested that in the Silk Stream catchment the hydrocarbons detected were chiefly of high temperature pyrogenic origins which masked a lower level unburnt oil signature. Such compounds of high formation temperatures lead to tight bonding to atmospheric particles and with the previously noted result of being subsequently less bioavailable to aquatic organisms.

Differing bioavailability theories have been proposed to explain other differences which have also been noted in this study between sediment hydrocarbon distributions and those of aquatic organisms which live in the sediment. In most coastal sediments, pyrolysis of fossil fuels has been proposed as the major source (Wakeham & Farrington, 1980) and this was equally the case for the Silk Stream sites. Farrington *et al.* (1983) compared the distributions of PAHs in mussels and polychaetes and the sediments in which they lived. The organism PAHs were found to be indicative of pyrogenic and petroleum sources while the PAHs in the sediments were chiefly of pyrogenic origin. The pyrogenic hydrocarbons were therefore considered less available for uptake than corresponding petrogenic compounds. Such results indicate that simple partition based transfers from the aqueous to lipid phases are less important than incorporation from particle-bound or emulsion phases.

The clear differences which exist between field and laboratory tested *A. aquaticus* in terms of alkane profiles (in comparison with ambient levels) would indicate that the mode of entry of the hydrocarbons must be an important consideration. Water and sediment extractions of field samples have revealed long chained alkanes as important constituents of the aliphatic profiles. The compounds are likely to be adsorbed onto sediment particles and would therefore be available to *A. aquaticus* via ingestion of detritus and other particles. In the laboratory this important mode of entry would be significantly reduced or eliminated leaving only the direct cross membrane route. In the

field, the biogenic portion of the long chained alkanes is also likely to be in a more bioavailable form than the corresponding anthropogenic compounds. Selective feeding on *Salix* sp. would result in ingestion of epicuticular waxes which may be in a more bioavailable form than pyrogenically introduced and tightly particulate bound alkanes.

6.10 MORTALITY RATES OF *A. AQUATICUS* AND *L. PEREGRA*

The mortality rates for *A. aquaticus* and *L.peregra* during the laboratory tests can be seen in Figs. 6.1 - 6.12. The recorded mortality rates for the dosed organisms show elevations above those recorded in control tanks which remained below 5% for both organisms during each trial. However, the mortalities were relatively low with 20% mortality being recorded in *A. aquaticus* after 10 and 20 days during exposure to alkane Mixtures C and B respectively. 20% mortality was not reached in *A. aquaticus* exposed to Mixture A. *L. peregra* displayed similar mortalities in response to alkane dosing but displayed slightly higher mortality rates during PAH exposure. In *A. aquaticus* a mortality rate of 35% was recorded after 15 days exposure to Mixture E and reached 40% at the end of the trial. *L. peregra* appeared more sensitive to the introduction of PAHs than *A. aquaticus*, displaying 50% mortality on exposure to Mixture E. If the total alkane and PAH concentrations are taken into account the lower total PAH concentration can be seen to produce a more toxic response from both organisms.

A further interesting feature of the mortality rates is the continued mortality commonly observed during the depuration phase. This may be seen for *A. aquaticus* in Mixture D (Fig 6.4) and in *L. peregra* in Mixture E (Fig. 6.8). Given that the mortalities observed are caused by the dosing it would appear that a substantial lag period must exist during which depuration can occur but in which the compounds still exert a toxic effect.

The mortality rates are generally substantially lower than those recorded in the field. This is particularly true in view of very much higher hydrocarbon rates of accumulation and levels recorded in laboratory organism tissue compared with field measurement as well as the presumed greater bioavailability of the artificially introduced compounds. The presence of the alkyl derivatives in the field which were not used in the laboratory

tests must also be considered.

The mechanism for hydrocarbon-caused mortality in invertebrates is not well understood and is generally referred to as a non-specific narcosis/anasthaesis action (Donkin *et al.* 1991). The site of such action is believed to be the lipid membrane. In view of the high total body concentrations attained in both organisms compared with the relatively low mortalities observed it must be the case that much of the bioaccumulated compounds do not reach the active sites where a narcotic action may take place. This is consistent with the previous discussions on the accumulation of hydrocarbons in which a substantial proportion of the accumulated compounds were considered only partly assimilated such as in the gut.

The laboratory trials must indicate therefore that hydrocarbons *per se* cannot be considered the primary cause of the observed mortalities in the field unless it is considered that the field hydrocarbons can, by some mechanism, more easily reach the active sites for narcotic action compared with those compounds introduced in the laboratory. This must be considered unlikely given that particulate bound hydrocarbons would be present in the field to a greater extent than in the laboratory. Other factors such as low DO at the sediment/water interface, the synergistic action of other toxins such as heavy metals and the physical stresses imposed by intermittent discharges must all be important considerations. The hydrocarbon compounds present however, do undoubtedly impose important additional stresses on organisms, particularly in the lower Silk Stream, where species already tolerate generally poor water quality. In the following chapter, the relationship between hydrocarbon tissue burden and mortalities in the field will be more closely examined.

6.11 SUMMARY

Both *A. aquaticus* and *L. peregra* accumulated alkanes and PAHs rapidly and an equilibrium was established after 3 - 7 days. The levels attained were higher than those recorded in the field. *L. peregra* tended to achieve higher total body concentrations for both sets of compounds compared with *A. aquaticus* which was attributed to its reduced ability to actively remove xenobiotic compounds. In both organisms, the mid-range

alkanes were accumulated to the greatest extents while the benzofluoranthenes were the most actively accumulated of the PAHs. Mortality increased with increased dosing and was generally higher for dosing by PAHs compared with alkanes in both organisms.

The rapid depuration phases observed indicated that some of the compounds were in non-assimilated forms eg as gut contents and that some evidence exists for a biphasic depuration response since it was not possible to calculate a decay constant for alkane depuration in both organisms. Depuration half-lives of between 3 and 8 days were calculated for the PAHs. The bioconcentration factors produced poor correlations with octanol/water partition coefficient data. The low mortality responses that resulted from relatively high tissue levels were considered the result of the hydrocarbons reaching the active sites necessary for organism narcosis to occur in reduced levels compared with the total body burden.

Chapter 7

APPLICATION OF PRINCIPAL COMPONENT ANALYSIS TO HYDROCARBON TISSUE CONCENTRATIONS IN *A. AQUATICUS* AND *L. PEREGRA*

7.1 INTRODUCTION

The results of work carried out during *in-situ* experiments (presented in Chapter 5) represent the measurement of 39 hydrocarbons in *A. aquaticus* tissue together with two further variables, rainfall and mortality. In this chapter, a statistical procedure is presented which:

- identifies the level of association between each measured variable, with the particular aim of identifying specific hydrocarbons or groups of hydrocarbons that are associated with measured invertebrate mortality.
- allows physical interpretations of the causes of the statistical relationships.

The procedure uses Principal Component Analysis (PCA) to reduce the dimensionality of the hydrocarbon tissue data matrices to provide interpretable results. This is achieved by maximising the total system variance in the form of sets of uncorrelated standardised variables that are linear combinations of the original parameters. The main objective of PCA is to identify through reduction of data, the recurring and independent modes of variation within a large, noisy data set so that meaningful and descriptive conclusions can be reached. The analysis sorts initially correlated data into a hierarchy of statistically independent modes of variation which explain successively less and less of the total variation. Hydrocarbon data are suited for this type of analysis because of the correlations between large numbers of individual components of homologous series such as the alkane groups and closely related compounds within the PAH group.

The issue in reduction of dimensionality in analyzing multivariate data is primarily concerned with the balance between simplicity and detail of the data. Simplicity must

be attained for understanding and interpretation of the data set but must be set against the retention of sufficient detail for adequate representation. Many problems exist in the general area of transformation of co-ordination and reduction of dimensionality. The fundamental question if one develops a transformed or derived set of reduced co-ordinates, is whether these can be given some meaning or interpretation that will facilitate the understanding of the actual problem (Gnandeskan, 1981). A number of workers have achieved this, particularly in the analysis of air pollution data through the use of PCA and related techniques (e.g. Thurston & Spengler, 1985; Henry 1987, Okomoto & Hayashi, 1990, Zeng & Hopke, 1992 Kessler *et al.* 1992). Such techniques have been used in the analysis of PAHs in airborne emissions (e.g. Westerholm & Li, 1994) and have been proposed by Aries *et al.*, 1991 as having broader applications in chemistry and related disciplines. The PCA procedure has been applied to water pollution data (e.g. Bomboi *et al.*, 1991) but it has rarely been applied to the analysis of macroinvertebrate tissue pollutant concentrations (Mulliss, 1994).

7.2 THEORY OF PRINCIPAL COMPONENT ANALYSIS (PCA)

The application of Principal Component Analysis produces a variable loading matrix containing the principal components and a factor score matrix by partitioning the standardized data matrix. The principal component model described by Equation 7.1 summarizes the procedure. Initially, the PCA analysis transforms the variable measurements into a dimensionless standardised form (Equation 7.2) in which variables have a standard deviation of one and a mean of zero. Linear combinations of the standardised variables represent the diagonalization of the correlation matrix of the variables, through the calculation of the eigenvalues and eigenvectors of the matrix.

<u>The PCA Model</u>

$$Z_{ik} = \sum A_{ij} P_{jk} \qquad \text{Equation 7.1}$$

or

$$_i[Z]^k = {}_i[A]^j \times {}_j[P]^k + E$$

Data Matrix	=	Variable Loading Matrix	x	Sample Scores Matrix	+	Residuals (Noise)

Data Standardization

$$Z_{ik} = \frac{(C_{ik} - C_i)}{r_i} \qquad \text{Equation 7.2}$$

Derivation of the Principal Components

$$[Q^{-1}]_{ixi}\,[R]_{ixi}\,[Q]_{ixi} = [/\backslash]_{ixi} \qquad \text{Equation 7.3}$$

Key

i = The total number of variables in the analysis
k = The total number of observations in the analysis
j = Significant sources of variation in the data
Z_{ik} = The standardised value for the variable i at observation k
C_{ik} = The concentration of that variable for that observation
C_i = The mean concentration for the i^{th} variable over all observations
r_i = The standard deviation of the distribution of concentrations of the i^{th} variable
A_{ij} = The loading of the i^{th} variable of the j^{th} principal component
P_{jk} = The k^{th} value of the j^{th} component
$[Q]_{ixi}$ = The diagonalization eigenvector matrix
$[R]_{ixi}$ = The correlation matrix
$[/\backslash]_{ixi}$ = The diagonal matrix of eigenvalues arranged in descending order

(adapted from Mulliss, 1994)

The first principal component (derived from the correlation matrix diagonalizing eigenvector; Equation 7.3) represents the linear combination of variables that accounts for the largest amount of total variance in the original variables. The second principal component is the linear combination that accounts for the next largest amount of variance in the data set not already accounted for by the first principal component. Decreasing eigenvalues represent progressively smaller proportions of the total sample variance and are explained by successive principal components. The variable loading eigenvector matrix represents both the regression coefficient and correlation coefficient between each variable and each principal component.

The PCA procedure assumes that the measured variables which form the data matrix, conform to a normal distribution before conversion to a standardised form. The distributions for the currently reported data range from normal to log-normal. It has

been suggested that non-normal distributions may result in some instability in the eigenvalue matrix during physical interpretation of the analysis. Despite this possible shortcoming, the data allowed natural interpretations and therefore it was considered important to attempt to identify the sources of variation. There are two modes of variation which may explain the principal components with regard to hydrocarbons in organism tissue.

1) Exogenous factors i.e. those which relate to the variations due to the original hydrocarbon source which incorporates levels of exposure from sediment and water.

2) Endogenous factors i.e. those related to variations due to the physiological characteristics of the organism.

The physical interpretation of the results should therefore be explained in terms of these two major factors.

7.3 INTERPRETATION OF PCA FOR HYDROCARBONS IN *A. AQUATICUS*

In this section the eigenvalues and rotated eigenvector matrices of the principal components are discussed for Sites 5, 6, 7 and 9. Many general points are made within the following section for Site 5, since the findings, with some exceptions are applicable to the three other sampling sites. Where significant inter-site differences exist, these are discussed under the sub-section on the particular site.

7.3.1 Site 5

Seven principal components, with eigenvalues of greater than 1.0, have been identified for Site 5 and the loadings on each are presented in Table 7.1. The third principal component can be seen to have the highest correlation with mortality (0.54) followed by PC6 (0.29) and PCs 1 and 2 (both 0.16). PCs 4, 5 and 7 are either very weakly or negatively correlated with mortality. For alkanes in the range C_{13} to C_{27}, PC1 consistently has a coefficient of greater than 0.40 and this rises to over 0.70 for the C_{15} - C_{25} alkanes. It is interesting to note that pristane and phytane possess higher loadings

for this principal component and can therefore be considered to have a common source of variation. This further supports the argument previously discussed in Chapter 4 in which pristane and phytane were considered inappropriate and unreliable source markers as a result of their highly localized and site-specific sources of variation. Previous work has considered these compounds to be of separate origins (Brassel *et al.*, 1978; see Chapter 4, Section 4.4.4)) but the currently reported findings at Site 5 do not support this assumption.

On examination of the data, PC1 suggests a vehicular source since the highest associations were found in the alkane range which is commonly associated with exhaust emissions of both petrol and diesel engines (Ball *et al.*, 1991). There exists a clear decline in the extent of association of the alkanes larger than C_{25} with PC1. This decline among the high molecular weight alkanes is paralleled by an increase in association with PC2 which appears to be partly related to odd-carbon chained alkanes. The alkanes C_{27}, C_{29} and C_{31} have associations of 0.82 0.90 and 0.90 respectively for PC2 compared with values for C_{26}, C_{28}, C_{30} and C_{32} of 0.29, 0.26, 0.59 and 0.83 respectively. Non-biogenic alkanes in this range are likely to be derived from a more mixed source including lubrication, fuel oil and exhaust sources (Vandermeulen & Hrudey, 1987) as opposed to the mid-range alkanes that are dominated by exhaust emissions. A vehicular source incorporating combustion products for PC1 is further supported on examination of the loading factors for PAHs. The two compounds with the highest associations with this component are fluoranthene and pyrene, both of which are almost exclusively synthesized by recent combustion (Laflamme & Hites, 1980). The PAHs as a group are recognized as important constituents of both petrol and diesel exhaust emissions (QUARG, 1993). However, it should be noted that mortality is weakly associated with this source of variation which suggests that a combustion source of pollution may not be the major influence on caged *A. aquaticus* mortality at this site.

As previously mentioned, PC2 appears to be influenced most strongly by high-molecular weight odd-numbered carbon chain length alkanes. This suggests a biogenic source of variation for PC2 (Eglinton *et al.*, 1962) which is further supported by the extremely low level of association of the measured PAHs (which have no known biogenic source; Neff, 1979) with PC2. Rainfall and mortality have a weak association

TABLE 7.1

THE EIGENVALUES AND VARIMAX ROTATED EIGENVECTOR MATRIX OF THE RETAINED PRINCIPAL COMPONENTS FOR *A. AQUATICUS* AT SITE 5.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Eigenvalue	17.37	7.69	2.99	1.88	1.51	1.32	1.07
Variance (%)	42.4	18.8	7.3	4.6	3.7	3.2	2.6
Variable							
Mortality	0.16	0.16	0.54	0.01	-0.15	0.29	0.03
Rainfall	0.11	0.03	0.29	0.25	0.06	-0.08	0.73
C_{11}	0.19	0.19	-0.06	0.69	-0.06	0.04	0.24
C_{12}	0.20	0.04	-0.11	0.83	-0.14	-0.03	0.12
C_{13}	0.45	-0.16	0.30	0.44	-0.51	-0.02	-0.25
C_{14}	0.49	-0.16	0.02	0.54	-0.47	-0.13	0.17
C_{15}	0.70	-0.01	0.02	0.48	-0.31	0.11	0.08
C_{16}	0.71	0.14	0.00	0.56	-0.16	0.01	0.13
C_{17}	0.77	0.21	0.08	0.52	-0.05	0.10	0.09
Pr	0.84	0.11	-0.03	-0.03	0.01	-0.04	0.27
C_{18}	0.70	0.24	0.17	0.47	-0.21	0.20	0.11
Ph	0.84	0.12	0.01	0.18	0.08	-0.06	0.29
C_{19}	0.72	0.13	0.17	0.38	0.03	0.31	0.10
C_{20}	0.81	0.11	0.31	0.33	0.03	0.05	-0.08
C_{21}	0.79	0.05	0.31	0.29	0.10	0.26	0.00
C_{22}	0.82	0.11	0.35	0.23	0.06	0.12	0.00
C_{23}	0.82	0.20	0.40	0.18	0.05	0.01	-0.02
C_{24}	0.84	0.26	0.19	0.19	0.15	0.05	-0.15
C_{25}	0.72	0.38	0.24	0.11	0.22	-0.04	-0.15
C_{26}	0.49	0.29	-0.01	0.62	0.31	-0.18	-0.26
C_{27}	0.41	0.82	0.15	0.07	0.11	-0.09	0.07
C_{28}	0.39	0.26	-0.07	0.71	0.13	-0.14	-0.19
C_{29}	0.26	0.90	-0.03	0.18	-0.15	-0.05	0.12
C_{30}	0.47	0.59	-0.23	0.44	0.20	-0.12	-0.11
C_{31}	0.14	0.90	-0.13	0.00	-0.01	-0.14	-0.04
C_{32}	-0.03	0.83	-0.10	0.01	0.08	0.10	-0.11
N	0.24	0.11	0.87	0.16	0.07	0.02	0.08
M-N	0.00	-0.15	0.90	-0.10	0.01	-0.15	0.15
F	0.48	0.53	0.07	0.47	-0.04	0.11	0.16
Ph+A	0.53	-0.58	0.34	-0.27	-0.04	-0.01	-0.21
M-Ph	0.14	-0.50	0.61	-0.17	0.37	-0.10	0.02
M-A	0.25	-0.41	0.65	-0.21	0.09	-0.05	-0.05
Fl	0.80	0.01	0.37	0.22	0.14	0.07	-0.14
Py	0.82	0.03	0.33	0.23	0.09	0.14	-0.08
M-Fl	0.31	-0.42	0.38	-0.08	0.56	0.00	0.10
M-Py	0.49	-0.28	0.50	-0.02	0.50	0.16	0.08
BA+Ch	0.65	-0.09	0.59	-0.09	-0.16	0.09	0.02
BFs	0.58	0.06	0.69	0.00	0.05	-0.21	0.08
BP	0.40	-0.17	0.65	-0.01	0.13	0.33	0.15
DBA	0.24	0.74	0.06	0.54	0.06	0.10	0.08
IPy	0.19	-0.07	-0.04	-0.07	0.04	0.84	0.08

with this source of variation. If PC2 does represent a biogenic component, it would be expected that a low association with mortality prevails, since biogenic components of hydrocarbon assemblages have lower toxicities and sub-lethal effects compared with shorter chain length aliphatics and the PAH class (Donkin *et al.* 1991; also see Chapter 2, Section 2.9). The variation in *A. aquaticus* mortality is predominantly explained (a coefficient of 0.54) by the third principal component. The same component accounts for low levels of variation in the alkanes with C_{23} representing the highest contribution to this PC with a correlation coefficient of 0.40. However, in the PAH group, PC3 has very high coefficients of 0.87 and 0.90 for naphthalene and methyl-naphthalene respectively. Methyl-phenanthrene, methyl-anthracene, methyl-pyrene, benzo (a) pyrene, benzo(a)anthracene and the benzofluoranthenes are also highly associated with this principal component. These results show an association between PAH tissue levels and mortality. It should be noted that four of the seven PC3-associated PAH are methylated homologues. As has previously been discussed, methylated compounds are known to be more commonly associated with non-combustion PAH sources such as lubricating oils as opposed to combustion sources which predominantly produce the parent compounds (Smith & Levy, 1990; Giger & Schaffner, 1975).

There may also be a biological factor involved with PC3 that relates to the postulated elevated bioavailability of methylated compounds (Farrington *et al.* 1983). This effect, discussed in Chapter 2, Section 2.5 may be related to physical factors that govern the bioavailability of lubricating compounds. Combustion derived compounds are more likely to be particle-associated than are lubricating compounds, and those lubricating oil derived compounds that <u>are</u> particle-bound will be less tightly bound than combustion ones. The mechanisms governing exposure rates to stream fauna of differently associated hydrocarbons are likely to be reflected in the tissue levels and by implication the principal components of those compounds in *A. aquaticus* tissue. This is discussed further in Section 7.3.5. The 5-ringed PAHs associated with PC3, although chiefly of combustion origin, are also likely to have a significant contribution from a lubricating source which is consistent with a non-combustion source for PC3. It may be inferred therefore that a non-combustion fraction of the hydrocarbon input has an association with toxicity in *A. aquaticus*. Although PC3 does not explain all the variation in mortality, the effect is important and demonstrates the higher toxic potential

of the PAHs compared with that of the alkanes for this species. The PAHs were also shown in the laboratory toxicity tests to cause greater mortality for *A. aquaticus* and *L. peregra* in comparison to the aliphatics (see Chapter 6, Section 6.10).

PC4 accounts for some variations in the lower molecular weight alkanes particularly up to C_{18} but excluding pristane and phytane. The level of association rises again in the higher molecular weight alkanes but is confined to the even carbon-numbered alkanes. In contrast, the odd carbon chain length alkanes display very low associations with this component. This component therefore is likely to be related to a long chained aliphatic, possibly lubricating or fuel oil source, unrelated to accumulation of biogenic compounds. It should be noted that there is also a small chained involvement in this PC implying that this contribution incorporates a very wide range of alkanes, and cannot therefore be attributed to lubricating or fuel oil contributions alone. Clearly, however the biogenic contribution to PC4 must be considered negligible.

PC5, which explains 3.7% of the total system variance, is strongly influenced by only two compounds. Interestingly, these are the methyl derivatives of fluoranthene and pyrene. These results provide further evidence of the quite different origins of parental PAHs and their methylated homologues. The parent compounds can be seen to be highly and exclusively associated with PC1 which is, as previously discussed, almost certainly associated with a combustion source. Methyl-pyrene and methyl-fluoranthene are also associated with PC1 and PC3 indicating that they have a more varied modes of variation. The association of methylated compounds with dripping sump oils in road 'black lines'compared with release through exhausts would result in different extents and types of particle association and as a consequence differing transport dynamics and behaviour in water.

PC6 with an eigenvalue of 1.32 accounts for 3.2% of the system variance. Only indeno(1,2,3-c,d)pyrene has a high association with this component which cannot readily be explained. This PC also has the second highest association (0.29) with mortality. PC7 has a high association with rainfall which is important to the overall findings as it should be noted that at this site neither hydrocarbon body burden nor mortality is influenced by rainfall. Previously it has been suggested (Chapter 5) that

hydrocarbon exposure due to storm conditions is likely to increase during periods of sediment resuspension. This may well remain the case, but clearly, short term (less than 5 days) increases in exposure cannot be seen to result in increases in body burdens in *A. aquaticus* at Site 5. Perhaps the most interesting finding is that mortality is not closely associated with this PC and therefore, at Site 5, rainfall and mortality have no common source of variation.

7.3.2 Site 6

Six principal components are identified (Table 7.2) at Site 6 on the lower Silk Stream. The alkanes in the range C_{16} - C_{24} have coefficients >0.80 and thus have a much higher association with PC1 (46.9% of total variance) compared to the equivalent PC at Site 5. There is a fall in the association with PC1 of the high molecular weight alkanes, as for Site 5, which is mirrored by an increase in association with PC2. A number of PAHs are also associated with PC1 with values of between 0.60 and 0.70 being exhibited by fluoranthene, pyrene, methyl-fluoranthene, methyl-pyrene, benzo(a)anthracene and chrysene and the benzofluoranthenes. As for Site 5, PC1 appears to be strongly related to a vehicular/combustion source, principally because of the high correlation with fluoranthene and pyrene. The association, however, is perhaps less well defined than at Site 5 because of the higher coefficients for methyl-fluoranthene and methyl-pyrene.

PC2 is primarily associated with the high molecular weight alkanes ($>C_{26}$) with coefficients of 0.58 - 0.89 but there appears to be no elevation in association with the biogenic alkanes as found at Site 5. It should be noted that at Site 6, the CPI for high molecular weight alkanes was very close to unity indicating a relatively low contribution of plant waxes i.e. this is masked by anthropogenic input. This may also account for the relatively high association of the high molecular weight anthropogenic dibenzo(a,h)anthracene and indeno(1,2,3-c,d)pyrene as well as fluorene. These results would also indicate that the biogenic and non-biogenic alkanes in this range have no major differences in terms of uptake pattern by *A. aquaticus* at this site.

4.6 % of total variance in the data is explained by PC3 which is most strongly associated

TABLE 7.2

THE EIGENVALUES AND VARIMAX ROTATED EIGENVECTOR MATRIX OF THE RETAINED PRINCIPAL COMPONENTS FOR *A. AQUATICUS* AT SITE 6.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7[a]
Eigenvalue	19.21	8.38	1.88	1.82	1.53	1.12	0.98
Variance (%)	46.9	20.5	4.6	4.4	3.7	2.7	2.4
Variable							
Mortality	0.22	0.16	0.03	0.08	0.03	0.84	-
Rainfall	0.14	0.05	0.82	0.01	-0.13	0.21	-
C_{11}	0.07	0.55	-0.04	0.65	-0.12	-0.18	-
C_{12}	0.18	0.08	0.06	0.75	0.29	0.28	-
C_{13}	0.45	0.37	0.26	0.63	-0.08	-0.03	-
C_{14}	0.60	0.33	0.40	0.23	0.00	0.04	-
C_{15}	0.77	0.40	0.1	0.13	-0.06	0.00	-
C_{16}	0.86	0.26	0.05	0.27	-0.10	0.03	-
C_{17}	0.89	0.20	0.16	0.04	-0.03	0.0	-
Pr	0.84	0.01	0.14	0.33	0.04	0.34	-
C_{18}	0.86	0.21	0.25	0.07	-0.05	0.09	-
Ph	0.77	0.01	0.14	0.33	0.04	0.35	-
C_{19}	0.89	0.08	0.32	0.12	0.12	0.11	-
C_{20}	0.82	0.04	0.41	0.10	0.25	0.07	-
C_{21}	0.81	0.00	0.46	0.05	0.18	0.01	-
C_{22}	0.84	0.12	0.37	-0.01	0.24	0.06	-
C_{23}	0.87	0.24	0.19	-0.04	0.18	-0.04	-
C_{24}	0.84	0.29	0.26	-0.04	0.19	-0.14	-
C_{25}	0.78	0.33	0.12	0.09	0.17	-0.03	-
C_{26}	0.60	0.58	-0.01	0.17	0.10	0.01	-
C_{27}	0.63	0.60	-0.28	-0.06	0.13	0.15	-
C_{28}	0.48	0.75	-0.10	0.19	0.08	-0.12	-
C_{29}	0.46	0.77	0.20	0.01	-0.10	0.00	-
C_{30}	0.33	0.86	-0.17	0.19	-0.01	0.30	-
C_{31}	0.15	0.89	-0.18	-0.12	-0.07	0.07	-
C_{32}	0.09	0.80	0.02	-0.30	0.04	0.29	-
N	0.59	0.46	0.19	0.16	0.05	0.31	-
M-N	0.46	-0.31	0.72	-0.06	-0.15	0.07	-
F	0.13	0.79	0.22	0.23	0.14	0.27	-
Ph+A	0.45	-0.41	0.71	-0.05	0.12	-0.13	-
M-Ph	0.29	-0.38	0.63	-0.05	0.39	-0.20	-
M-A	0.35	-0.46	0.68	-0.01	0.11	-0.04	-
Fl	0.65	0.21	0.43	0.05	0.40	0.08	-
Py	0.62	0.17	0.57	0.02	0.22	0.06	-
M-Fl	0.54	-0.23	0.21	0.21	0.62	0.12	-
M-Py	0.67	-0.27	0.35	-0.01	0.42	0.03	-
BA+Ch	0.64	0.27	-0.02	-0.01	0.37	0.39	-
BFs	0.69	0.26	0.41	0.21	0.11	0.09	-
BP	0.28	-0.07	0.69	0.18	0.43	-0.08	-
DBA	0.08	0.71	-0.11	0.30	-0.02	0.17	-
IPy	0.08	0.71	-0.11	0.30	-0.02	0.06	-

[a] Eigenvalue for PC7 <1 therefore not retained in eigenvector matrix

with rainfall and some PAHs. At Site 5 no similar relationship was found in that rainfall was highly associated with PC7 with which hydrocarbons had a very low association. Rainfall, methyl-naphthalene, phenanthrene/anthracene and their methyl derivatives, and benzo(a)pyrene appear to have a common source of variation. This phenomenon was not observed at other sites and it is hypothesized that it is the closeness of the outfall (see Chapter 3; Sections 3.1 & 3.2) that accounts for this effect. The outfall was known to discharge during baseflow, but importantly, also discharged storm runoff and dominated localized inputs near Site 6. This site should therefore be considered to have the most closely related hydrocarbon exposure/rainfall regime, based on direct inputs alone i.e. excluding sediment resuspension.

There exists, however, only a very low association between rainfall and mortality at this site, indicating that under storm conditions *A. aquaticus* was not affected. PC4, which explains 4.4% of the data is strongly related only to C_{11} - C_{13}. Clearly, some factor relating to the physical properties of these compounds such as their low boiling point must account for their grouping. An accurate interpretation, however, cannot readily be given although it is suggested that this factor relates to an experimental artifact caused by evaporation during extraction and build-up (see Chapter 3; Section 3.7.1 and Chapter 6; Section 6.7). For this possible reason, further physical interpretations of alkanes of carbon chain length less than 14 will not be made.

Methyl-fluoranthene (0.62), methyl-pyrene (0.42), benzo(a)pyrene (0.43) and fluoranthene (0.40) show the highest levels of association with PC5 which in total accounts for 3.7% of total variance. Mortality is virtually exclusively associated with PC6. Although this factor accounts only for 2.7% of the total variance, this is an important result since it demonstrates that, at Site 6, the hydrocarbons measured in *A. aquaticus* tissue have no common source of variation with that which causes mortality. The unmeasured variables causing mortality at this site may be linked to water column oxygen sags that are not related to storm events. The seventh principal component (eigenvalue 0.98) was not retained in the matrix, as this component can be considered as providing less contribution to the system variance as any one single variable did originally.

7.3.3 Site 7

Seven principal components (Table 7.3) have been identified at Site 7 which is located below the oil interceptor on the lower Silk Stream. The high association of PC1 with a broad range of alkanes as was observed at Sites 5 and 6 is repeated at Site 7 (Table 7.3). The range covers C_{13}-C_{30} (including pristane and phytane) with the highest associations (>0.70) occurring between C_{14} and C_{25}. Weaker associations occur with phenanthrene and anthracene, fluoranthene, pyrene and benzo(a)anthracene which again strongly implies a vehicular/combustion source, and which confirms the interpretations made at the other sites.

Examination of PCs 2 and 3, which account for a combined variance of 28.5% of the data set, indicates that the physical interpretation must be as described as Sites 5 and 6 i.e one component represents long-chained alkanes, including a biogenic component while the other is dominated by PAHs with a strong influence from methyl derivatives, representing some non-combustion product contributions to the *A. aquaticus* tissue burden. At Site 7, however the components are reversed with a strong PAH association with PC2 (particularly methyl-fluoranthene and methyl-pyrene) and high correlations of high MW alkanes with PC3. The probable explanation for these observations is that the PAHs simply explain more of the system variance than the high molecular weight alkanes as a result of greater PAH contamination which is mirrored in the PC output. Mortality also has a far higher association with PC2 compared with PC3 further indicating the non-toxic nature of the high molecular weight alkane fractions.

PCs 4 and 5 have low associations with rainfall and mortality and are related only to small groups of hydrocarbons which have no readily interpretable source.

PC6 shows a strong link between rainfall and mortality, but no high association with any of the measured hydrocarbons. This implies that hydrocarbons are not a primary contributor to *A. aquaticus* mortality at Site 7 and is in broad agreement with the interpretations of the laboratory toxicity tests (Chapter 6) in which *A. aquaticus* accumulated hydrocarbons to levels equal to or exceeding those measured in the field but which resulted in much lower mortalities than those recorded in the field. The link

TABLE 7.3

THE EIGENVALUES AND VARIMAX ROTATED EIGENVECTOR MATRIX OF THE RETAINED PRINCIPAL COMPONENTS FOR *A. AQUATICUS* AT SITE 7.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Eigenvalue	17.72	9.23	2.44	1.71	1.66	1.36	1.21
Variance (%)	43.2	22.5	6.0	4.2	4.0	3.3	3.0
Mortality	0.16	0.34	0.01	-0.19	0.23	0.69	0.18
Rain	0.25	0.01	0.12	0.19	-0.17	0.79	-0.15
C_{11}	0.11	-0.26	0.44	-0.09	0.67	0.02	0.24
C_{12}	0.19	-0.28	0.38	-0.15	0.63	-0.08	0.45
C_{13}	0.67	0.08	0.35	0.11	0.43	-0.17	-0.12
C_{14}	0.74	-0.11	0.46	0.02	0.16	-0.11	-0.06
C_{15}	0.77	-0.07	0.42	0.09	-0.04	-0.05	0.14
C_{16}	0.77	0.10	0.42	0.29	0.02	-0.06	-0.06
C_{17}	0.90	0.24	0.20	0.14	0.02	0.06	-0.07
Pr	0.86	0.18	0.26	0.18	0.09	-0.03	0.08
C_{18}	0.87	0.33	0.11	0.11	0.05	0.23	-0.08
Ph	0.84	0.30	0.15	0.15	0.07	0.17	-0.07
C_{19}	0.89	0.29	0.08	0.04	0.06	0.13	-0.20
C_{20}	0.87	0.36	0.06	0.05	0.06	0.12	-0.11
C_{21}	0.82	0.46	-0.02	-0.12	-0.03	0.13	0.04
C_{22}	0.83	0.46	0.01	0.05	-0.03	0.07	0.17
C_{23}	0.84	0.33	0.15	0.03	0.03	0.13	0.20
C_{24}	0.83	0.30	0.18	0.19	0.00	0.11	0.26
C_{25}	0.82	0.20	0.27	0.20	0.12	0.21	0.17
C_{26}	0.68	0.15	0.47	-0.02	0.13	0.24	0.32
C_{27}	0.57	-0.18	0.70	0.05	0.01	0.19	0.19
C_{28}	0.43	0.09	0.70	-0.05	0.07	0.29	0.25
C_{29}	0.47	-0.33	0.71	0.05	0.00	0.19	0.19
C_{30}	0.42	-0.29	0.67	-0.12	0.09	0.21	0.22
C_{31}	0.18	-0.27	0.88	-0.02	0.17	0.03	-0.01
C_{32}	0.21	-0.22	0.91	0.00	0.16	-0.04	-0.02
N	0.08	0.05	0.05	0.25	0.80	0.04	-0.16
M-N	0.19	0.20	-0.27	0.78	0.19	0.06	-0.09
F	0.27	0.37	0.65	0.12	0.08	-0.03	-0.03
Ph+A	0.45	0.67	-0.37	0.08	-0.16	0.00	-0.26
M-Ph	0.17	0.67	-0.24	0.43	0.19	-0.08	0.21
M-A	0.23	0.74	-0.43	0.11	0.15	-0.05	0.07
Fl	0.46	0.76	-0.14	0.06	0.07	0.11	-0.30
Py	0.43	0.69	-0.19	0.07	0.06	0.12	-0.39
M-Fl	0.16	0.85	-0.10	0.11	-0.02	-0.01	0.30
M-Py	0.25	0.85	0.16	0.14	0.08	0.08	0.08
BA+Ch	0.40	0.75	0.03	0.17	-0.07	0.31	-0.21
BFs	0.28	0.76	0.20	0.26	-0.12	0.10	-0.19
BP	0.24	0.48	0.11	0.71	0.05	-0.01	-0.10
DBA	0.05	0.07	0.88	0.09	0.18	-0.11	-0.17
IPy	0.14	0.14	0.21	0.91	0.00	0.01	0.07

between rainfall and mortality at this site is probably caused by sediment resuspension and resulting low DO concentrations during storm events which mask any possible effects of hydrocarbons. The results are also consistent with the findings at Site 6 where no link between hydrocarbon body burdens and *A. aquaticus* mortality could be established.

7.3.4 Site 9

At Site 9, situated within the receiving basin of the Welsh Harp reservoir, the data matrix was reduced to 10 PCs with eigenvalues >1 of which the first seven are shown in Table 7.4. The first PC with an eigenvalue of 11.78 explains 28.7% of the system variance which is by far the smallest proportion explained by the first PC at the four sites for which data were subjected to PCA. Thus, a greater spread of the distribution of the system variance occurs at Site 9. Such results indicate a 'noisy' data set in which there are a large number of influencing factors. Site 9 has previously been described, using a number of parameters, as the least polluted site and as such it might be expected to have the least noisy data set. These findings, however are not inconsistent with each other: the three stream sites are far more likely to be influenced by specific outfalls, sub-catchments or resuspended sediments than Site 9. The mixing and diluting of the various inputs at the lake site will result in *A. aquaticus* being exposed to a more diffuse assemblage of hydrocatbons. It is probable also, that in view of the lower localized aqueous inputs, longer distance, airborne hydrocarbon sources will be more influential on the data at Site 9. The larger and more exposed surface area of the Welsh Harp will also increase direct aerial deposition effects. The combined effect of these factors, therefore is to increase the noise of the data in terms of sources of variation, despite the lower total contaminant levels.

Despite these important differences, some general patterns which were observed among the major PCs at the other sites are still preserved at Site 9. A clear association (correlation coefficients of between 0.55 and 0.94) of the alkanes exists with PC1 although the range covered (C_{17} - C_{26}) is narrower. A strong association with PC2 for alkanes in the range C_{27} to C_{32} also occurs, which incorporates a biogenic element as observed at Site 5. The PAHs with the exceptions of fluorene and dibenzo(a,h)anthracene

TABLE 7.4

THE EIGENVALUES AND VARIMAX ROTATED EIGENVECTOR MATRIX OF THE RETAINED PRINCIPAL COMPONENTS FOR *A. AQUATICUS* AT SITE 9.

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Eigenvalue	11.78	8.68	2.99	2.12	1.80	1.71	1.57
Variance (%)	28.7	21.2	7.3	5.2	4.4	4.2	3.8
Mortality	0.05	0.06	-0.11	-0.07	-0.06	0.05	0.07
Rainfall	-0.17	0.11	0.23	0.24	0.11	0.59	-0.14
C_{11}	0.06	0.62	0.12	0.09	0.71	0.00	-0.06
C_{12}	0.21	0.55	0.00	0.14	0.57	-0.02	0.26
C_{13}	0.27	0.27	0.05	0.14	0.78	0.05	0.09
C_{14}	0.15	-0.05	-0.07	0.76	0.41	0.02	-0.01
C_{15}	0.01	0.15	0.23	0.82	0.10	0.20	0.01
C_{16}	0.30	0.23	0.38	0.74	-0.13	0.12	0.02
C_{17}	0.55	0.08	0.50	0.49	0.03	-0.13	0.15
Pr	0.47	0.22	0.57	0.24	-0.14	-0.14	0.16
C_{18}	0.57	0.05	0.59	0.36	0.16	-0.04	-0.07
Ph	0.26	0.01	0.65	0.34	-0.01	0.14	0.03
C_{19}	0.52	0.24	0.49	0.47	0.04	0.05	-0.07
C_{20}	0.70	0.08	0.47	0.30	0.17	0.00	0.05
C_{21}	0.84	-0.18	0.26	0.21	0.02	0.09	0.09
C_{22}	0.93	-0.05	0.13	0.11	0.06	0.09	0.06
C_{23}	0.91	0.00	0.17	0.08	0.10	0.05	0.05
C_{24}	0.94	0.07	0.14	0.07	0.15	0.03	-0.03
C_{25}	0.91	0.12	0.16	0.07	0.15	-0.04	-0.03
C_{26}	0.80	0.09	-0.01	-0.07	-0.08	0.18	0.22
C_{27}	0.06	0.91	0.08	0.14	-0.01	-0.05	-0.03
C_{28}	0.39	0.71	-0.14	-0.01	0.03	-0.16	0.16
C_{29}	-0.02	0.95	-0.09	0.04	0.04	0.10	-0.03
C_{30}	0.09	0.83	-0.16	0.02	0.08	0.10	-0.02
C_{31}	-0.14	0.91	-0.07	0.07	0.17	-0.09	0.03
C_{32}	-0.02	0.72	-0.07	0.04	0.56	-0.08	-0.13
N	0.27	0.16	0.05	0.67	-0.06	-0.24	0.21
M-N	0.48	-0.14	0.07	0.20	0.05	0.11	0.22
F	0.38	0.77	0.00	0.07	0.09	-0.18	0.09
Ph+A	0.08	-0.59	0.65	0.04	-0.12	0.19	0.12
M-Ph	0.47	-0.51	0.39	-0.11	-0.14	-0.13	0.15
M-A	0.11	-0.33	0.07	0.06	-0.04	-0.01	0.10
Fl	0.26	-0.13	0.84	0.12	0.04	0.16	0.00
Py	0.20	-0.15	0.81	0.02	0.08	0.04	0.13
M-Fl	0.15	-0.16	0.07	0.03	-0.01	0.82	0.04
M-Py	0.27	0.08	0.13	-0.01	-0.07	0.60	0.31
BA+Ch	0.31	-0.28	0.54	-0.03	0.10	0.36	-0.02
BFs	0.07	0.27	0.36	-0.09	0.15	0.00	0.77
BP	0.19	-0.20	-0.02	0.24	-0.02	0.07	0.75
DBA	0.11	0.89	0.14	0.08	0.09	-0.03	0.07
IPy	-0.01	0.14	-0.17	0.04	0.14	-0.20	-0.07

have low or negative associations with this component which further support a biogenic origin for PC2. Fluoranthene and pyrene have the strongest associations with PC3 together with the C_{17}-C_{20} alkanes. A combustion origin is therefore likely to be associated with PC3. Generally, the PAHs are more highly partitioned among the PCs at Site 9, further demonstrating differing modes of variation and sources and less contributions from any single, localized source. Further low molecular weight alkane associations with PCs 4 and 5 can also be seen at Site 9 which have no influence on mortality. Naphthalene also has an association with PC4 suggesting that the low boiling point of these compounds, in some way, accounts for their grouping. A similar grouping was noted for PC4 at Site 6 but as previously noted no physical source could readily be attributed to it.

Methyl-fluoranthene, methyl-pyrene and rainfall have an association with PC6 but have a negligible effect on mortality. A possible explanation for the relationship between rainfall and the methylated homologues is that the methylated compounds, as components of the lubricating fraction will form readily transportable slicks which, as non-particle associated fractions, will be more bioavailable than corresponding combustion derived, particle associated groups. Since localized inputs at Site 9 are considered the least important for all the sites (compared with airborne transport) a lubricating component would also be expected to be the least important. Thus if the methyl derivatives of fluoranthene and pyrene represent a lubricating component it is reasonable that they should appear with a PC that represents the lowest total system variance for that component of the four test sites and which, as a consequence, has a negligible effect on mortality. In fact, mortality was associated with the ninth PC (0.82) and had no association with any hydrocarbons at this site. This would be expected since mortality was consistently lowest at Site 9 where a recovery in water quality occurs and in which no qualitative associations between tissue hydrocarbon levels and mortality have been made.

7.3.5 Overview of PCA analysis for *A. aquaticus*

If *A. aquaticus* is considered as a pollutant target or receptor alone, then we would expect the hydrocarbon distributions to reflect those of the urban pollution inputs to

which it is exposed. There is no doubt that this is the case (see Chapter 5.1) as the *A. aquaticus* tissue levels mirror those recorded in the abiotic environment, both in terms of the absolute extent of contamination and the relative distributions of individual compounds. In this chapter, natural interpretations of the first three principal components have also shown the mode of variation of hydrocarbons in the body tissues readily explainable in terms of the abiotic assemblages and particularly in terms of the sources of hydrocarbons. Generally, therefore *A. aquaticus* appears to act simply as a receptor, reflecting the hydrocarbon composition in the abiotic environment and exerting little effect on the distributions and hence the interpretations of the derived principal components. This statement does not, of course, take into account the ability of *A. aquaticus* to transform the incorporated compounds to excretable metabolites, but rather the lack of change of distribution of the original compounds, and as a consequence, low biological activity. It is suggested therefore that when principal components for the hydrocarbons are readily explainable in terms of abiotic factors, their biological activities are reduced. Thus, the top three PCs can be viewed in this study at each site, as combustion (principally characterized by pyrene and fluoranthene), biogenic (high MW odd-carbon chained alkanes) and lubricating (methyl-derivatives of PAHs) sources. Similar conclusions were made by Bomboi *et al.* (1991) in their PCA analysis (with VARIMAX rotation) of 15 hydrocarbon parameters from artificial (hosed) urban runoff from Madrid. The top three principal components accounted for 81.9 % of the total variance as a result of the use of fewer parameters but probably also due to the fact that these data were less noisy than those in the Welsh Harp. In the Madrid study, PCs 1, 2 and 3 were also identified as combustion, lubrication and biogenic components, respectively. It must be stressed, however, that these results were for runoff composition and did not consider bioavailability.

As has previously been discussed there are two notable exceptions in which tissue concentrations differ from those in the abiotic environment.

1) Elevation in methyl-group concentrations.
2) Elevations in high molecular weight odd carbon numbered alkanes.

Given that the major sources of variation in *A. aquaticus* hydrocarbons tissue levels

appear to be the original sources of hydrocarbons, it might therefore be expected that any biologically-influenced modes of variation should appear in PCs other than the first three and which would explain smaller proportions of the total variance. Such factors may explain PC6 (with methyl-fluoranthene and methyl-pyrene at Site 9), PC7 (the benzofluoranthenes and benzo(a)pyrene at Site 9) and PC5 (methyl-pyrene and methyl-fluoranthene at Site 5). It is suggested that such a component may be related to an organism 'lipophilic factor', i.e. related to variations in the composition of the target organism itself rather than the variations in compound exposure. Such a factor would also be related to sex, age, life-cycle stage and general stress of invertebrates. Further work involving tissue analysis incorporating lipid measurement and the above factors would provide interesting comparative data which could be incorporated into a PCA model and which would aid the identification of the organism's bioaccumulation and mortality controlling factors.

Elevations in high molecular weight alkane accumulation do not appear in any PCs other than those in the top three at any sites. Given that the top three PCs can be strongly related to the original source and that lower PCs may be related to the organism, it may be implied that odd-alkane accumulation is not related to any intrinsic characteristic of the target organism such as those that may influence methyl-PAH accumulation.

7.4 INTERPRETATION OF PCA FOR HYDROCARBONS IN *L. PEREGRA*

The data for the second biomonitor species, *L. peregra*, were subjected to the PCA procedure. *L. peregra* displays some similar patterns to those previously described for *A. aquaticus*. The data in general, however, have a greater scatter among the PCs and tend therefore not to be so readily explainable in terms of the original sources of hydrocarbons.

7.4.1 Site 5

PC1, which explains 34.3% of the total system variance, correlates with a broad range of alkanes (C_{13}-C_{25}) but has a generally low association with the PAHs with only pyrene

TABLE 7.5

THE EIGENVALUES AND VARIMAX ROTATED EIGENVECTOR MATRIX OF THE RETAINED PRINCIPAL COMPONENTS FOR *L. PEREGRA* AT SITE 5.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Eigenvalue	13.02	8.69	4.21	2.92	2.37	2.31	1.40
Variance (%)	34.3	22.9	11.1	7.7	6.2	6.1	3.7
Variable							
Mortality	0.01	0.82	0.07	-0.06	0.31	0.22	-0.04
Rainfall	-0.36	0.68	0.06	-0.52	-0.11	0.23	-0.17
C_{12}	-0.12	-0.26	-0.02	0.88	-0.04	-0.17	0.30
C_{13}	0.43	-0.23	-0.07	0.24	-0.35	-0.46	0.56
C_{14}	0.51	-0.14	-0.13	0.68	-0.30	-0.03	0.32
C_{15}	0.49	-0.18	-0.05	0.46	-0.65	-0.09	0.09
C_{16}	0.90	-0.01	-0.06	0.46	-0.66	0.15	-0.13
C_{17}	0.95	0.02	-0.17	0.07	-0.04	0.04	0.02
Pr	0.68	-0.16	-0.15	0.57	-0.13	-0.23	-0.21
C_{18}	0.93	0.03	0.10	-0.01	0.20	0.11	0.20
Ph	0.75	0.18	0.23	0.05	-0.17	-0.14	-0.34
C_{19}	0.84	-0.05	0.04	0.17	0.10	-0.42	0.03
C_{20}	0.70	0.01	0.13	0.03	0.07	-0.55	0.29
C_{21}	0.86	0.16	0.28	-0.14	0.17	0.00	-0.03
C_{22}	0.81	0.16	0.48	-0.13	0.11	0.02	0.00
C_{23}	0.84	0.32	0.13	0.04	0.40	0.03	0.00
C_{24}	0.68	0.43	0.03	0.22	0.39	-0.04	0.26
C_{25}	0.60	0.19	0.28	0.12	0.59	-0.29	0.11
C_{26}	0.16	0.40	0.09	0.24	0.62	-0.01	0.59
C_{27}	0.29	0.10	0.02	-0.23	0.90	0.00	0.03
C_{28}	0.08	0.29	0.05	0.89	-0.10	0.13	-0.14
C_{29}	-0.02	0.17	0.04	-0.01	-0.10	0.97	0.17
C_{30}	-0.10	-0.17	0.05	0.06	0.08	0.14	0.93
C_{31}	-0.02	-0.14	-0.72	-0.40	-0.20	0.29	0.09
N	-0.01	0.51	0.13	0.43	0.07	-0.54	0.03
M-N	-0.06	0.82	0.47	-0.07	-0.01	-0.10	-0.16
F	-0.25	0.41	0.76	0.36	-0.07	0.02	-0.01
Ph+A	0.38	0.55	0.34	0.10	0.11	0.23	0.22
M-Ph	0.30	0.76	0.28	0.01	0.10	0.17	-0.10
M-A	0.12	0.93	0.20	0.02	-0.04	0.04	-0.12
Fl	0.32	0.47	0.74	-0.19	0.11	0.01	-0.05
Py	0.42	0.24	0.68	-0.23	0.30	0.30	0.10
M-Fl	0.13	0.78	0.52	-0.05	0.19	-0.06	-0.10
M-Py	0.20	0.86	0.15	0.14	0.22	-0.15	0.21
BA+Ch	0.37	0.30	0.59	0.57	0.08	-0.09	0.14
BFs	0.06	0.43	0.71	-0.30	-0.14	0.01	0.22
BP	0.18	0.57	0.51	-0.37	0.12	0.34	-0.16
DBA	-0.17	0.23	0.32	-0.43	0.09	-0.02	0.05

exceeding 0.40 (Table 7.5). There is no relationship between mortality and rainfall with any of the measured alkanes or PAHs in this principal component. In contrast to the *A. aquaticus* results, mortality and rainfall appear strongly associated (0.82 and 0.68) with the second principal component. Interestingly, there is generally a very low association with the alkanes but an increasing association with C_{24} and C_{26}. Nine of the 14 measured PAHs have high associations with this PC with the methylated homologues of naphthalene, phenanthrene, anthracene, fluoranthene and pyrene having particularly high coefficients. This strongly suggests that PC2 has a lubricating oil source which is supported by the (albeit smaller) association with the high molecular weight (even) alkanes. Significantly, this lubricating-oil source of variation was also independently identified in *A. aquaticus* at the same location and was also found to be associated with the highest coefficient of mortality for that site. These combined observations therefore strongly implicate the methylated PAHs (which are well known source markers for lubricating oils) as contributors to mortalities at Site 5. For *A. aquaticus* (see Section 7.3.1), rainfall and tissue hydrocarbon levels were shown to have a very low association. This is clearly not the case for *L. peregra* where oil releases during storm events appear to be reflected in its tissue levels.

PC3 has a high association with fluorene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, the benzofluoranthenes and benzo(a)pyrene. All of these compounds are commonly produced by combustion but is interesting to note that mortality has a very low association with this component, further suggesting that it is the lubricating hydrocarbons and not the combustion source hydrocarbons that are most toxic to stream fauna. PCs 4 and 5 show evidence of correlation to low and high molecular weight alkane fractions, respectively and mortality has a low to medium association with PC5. As was found for *A. aquaticus*, however, physical interpretation of components beyond the first three PCs are extremely difficult to make.

7.4.2 Site 6

Five principal components have been identified at Site 6 (Table 7.6) with the most predominant (PC1) explaining 58.6% of the data variance. A broad mixture of compounds are associated with this component which make any possible interpretation

TABLE 7.6

THE EIGENVALUES AND VARIMAX ROTATED EIGENVECTOR MATRIX OF THE RETAINED PRINCIPAL COMPONENTS FOR *L. PEREGRA* AT SITE 6.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Eigenvalue	22.27	5.29	4.61	2.96	2.2	-	-
Variance (%)	58.6	13.9	12.1	7.8	5.8	-	-
Variable							
Mortality	0.34	0.23	0.10	-0.13	0.89	-	-
Rainfall	0.11	0.03	-0.30	0.05	0.94	-	-
C_{12}	0.32	0.08	0.40	-0.17	0.17	-	-
C_{13}	-0.31	0.76	0.40	-0.17	0.17	-	-
C_{14}	0.28	-0.05	0.29	0.77	-0.37	-	-
C_{15}	0.02	0.27	0.01	0.93	-0.15	-	-
C_{16}	-0.04	0.66	0.14	0.73	0.07	-	-
C_{17}	0.20	0.87	0.03	0.44	0.04	-	-
Pr	0.22	0.87	-0.03	0.43	0.12	-	-
C_{18}	0.17	0.67	0.24	0.60	0.23	-	-
Ph	0.57	0.57	0.01	0.55	0.21	-	-
C_{19}	0.42	0.82	0.24	0.27	0.11	-	-
C_{20}	0.21	0.90	0.20	0.32	0.12	-	-
C_{21}	0.56	0.70	0.30	0.31	-0.10	-	-
C_{22}	0.50	0.67	0.45	0.23	-0.03	-	-
C_{23}	0.40	0.89	0.14	0.04	0.09	-	-
C_{24}	0.45	0.59	0.57	0.32	0.04	-	-
C_{25}	0.76	0.51	0.29	0.13	-0.23	-	-
C_{26}	0.63	0.61	0.41	0.01	-0.21	-	-
C_{27}	0.58	0.57	0.37	-0.08	-0.31	-	-
C_{28}	0.08	0.53	0.78	-0.07	-0.27	-	-
C_{29}	0.14	0.16	0.93	0.10	-0.30	-	-
C_{30}	0.37	-	0.81	0.40	0.05	-	-
C_{31}	-0.88	-0.03	0.33	0.32	0.05	-	-
N	0.47	0.86	-0.08	-0.01	-0.15	-	-
M-N	0.88	0.41	0.16	0.16	0.05	-	-
F	0.14	0.73	0.38	0.27	0.43	-	-
Ph+A	0.63	0.41	0.43	0.45	0.22	-	-
M-Ph	0.85	0.17	0.39	0.19	0.23	-	-
M-A	0.81	0.07	0.44	0.36	0.11	-	-
Fl	0.78	0.54	0.23	0.21	0.06	-	-
Py	0.65	0.45	0.36	0.41	0.25	-	-
M-Fl	0.89	0.38	0.11	0.18	-0.11	-	-
M-Py	0.88	0.13	0.39	0.22	-0.08	-	-
BA+Ch	0.71	0.61	0.14	0.03	0.31	-	-
BFs	0.98	0.15	0.02	0.10	-0.11	-	-
BP	0.93	-0.03	-0.11	-0.09	0.29	-	-
DBA	0.09	0.96	-0.19	-0.11	-0.17	-	-

TABLE 7.7

THE EIGENVALUES AND VARIMAX ROTATED EIGENVECTOR MATRIX OF THE RETAINED PRINCIPAL COMPONENTS FOR *L. PEREGRA* AT SITE 7.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Eigenvalue	21.5	3.73	2.64	2.17	1.75	1.33	-
Variance (%)	62.4	10.7	7.6	6.2	5.0	3.8	-
Variable							
Mortality	0.64	0.07	0.62	0.33	-0.19	0.09	-
Rainfall	-0.02	-0.14	0.71	0.31	0.21	-0.26	-
C_{13}	-0.08	-0.02	0.10	0.15	0.08	0.96	-
C_{14}	0.23	0.08	0.22	0.22	0.89	0.11	-
C_{15}	0.29	0.22	0.18	0.32	0.82	0.14	-
C_{16}	0.10	0.63	0.14	-0.10	0.70	-0.08	-
C_{17}	0.44	0.43	0.54	0.36	0.35	0.11	-
Pr	0.34	0.58	0.28	0.36	0.23	0.37	-
C_{18}	0.58	0.38	0.38	0.50	0.21	0.29	-
Ph	0.55	0.28	0.26	0.57	0.30	0.33	-
C_{19}	0.58	0.55	0.20	0.51	0.03	0.18	-
C_{20}	0.65	0.55	0.36	0.11	0.12	0.31	-
C_{21}	0.61	0.67	0.32	-0.02	0.05	0.24	-
C_{22}	0.75	0.35	0.43	-0.06	0.23	0.24	-
C_{23}	0.61	0.41	0.45	-0.28	0.41	-0.05	-
C_{24}	0.70	0.49	0.34	0.16	0.30	0.13	-
C_{25}	0.33	0.90	0.13	-0.03	0.14	0.10	-
C_{26}	0.17	0.69	0.11	0.26	0.52	-0.12	-
C_{27}	0.11	0.89	0.06	0.37	0.13	-0.02	-
C_{28}	0.22	0.30	0.12	0.86	0.20	0.17	-
C_{29}	0.35	0.36	0.33	0.67	0.04	-0.06	-
C_{30}	0.46	0.49	0.63	0.03	0.25	-0.10	-
N	0.95	0.07	0.18	0.13	0.09	-0.03	-
M-N	0.95	0.07	0.18	0.13	0.09	-0.04	-
F	0.89	0.18	0.21	0.06	0.23	-0.17	-
Ph+A	0.83	0.35	-0.04	0.25	0.15	-0.06	-
M-Ph	0.88	0.19	0.39	0.18	0.16	-0.02	-
M-A	0.16	0.09	0.93	0.13	0.11	0.17	-
Fl	0.28	0.59	0.67	0.05	0.28	0.11	-
Py	0.46	0.49	0.63	0.03	0.25	-0.01	-
M-Fl	0.26	0.44	0.81	0.04	0.16	0.16	-
M-Py	0.69	0.13	0.58	0.30	0.19	0.09	-
BA+Ch	0.82	0.36	-0.09	0.27	0.07	0.04	-
BFs	0.47	0.09	0.86	-0.02	0.10	0.07	-
BP	0.70	-0.09	0.61	0.27	0.09	0.14	-

unclear. The five ringed PAHs have extremely high associations with this component (e.g. benzo(a)pyrene, 0.98) but it can also be seen that a range of mid to high range alkanes as well as 3 and 4 ringed PAHs together with their methyl derivatives are also represented. Surprisingly, perhaps, in view of the Site 5 results (which show links between hydrocarbons and mortality at a less contaminated site), the association of the hydrocarbons at this highly contaminated site with mortality is only moderate (0.34) and there is only a low association with rainfall (0.11). The second principal component also contains a wide range of compounds which are highly associated and which in terms of an interpretation of the source of variation cannot be readily distinguished from PC1. Again, however, it can be seen that mortality is not strongly linked with these compounds. Further alkane associations among the high molecular weight fractions as well as for methyl-phenanthrene and methyl-pyrene suggest a lubricating origin for PC3 but, unlike the Site 5 results, no association with mortality occurs.

PC5 has the highest association with mortality and is strongly related to rainfall at this site. This supports the qualitative observations made in Chapter 5 (Section 5.6) in which rainfall was linked with mortality and is in partial agreement with the findings at Site 5 where a rainfall, hydrocarbon and mortality association was established. At Site 6, however no hydrocarbons (with the exception of fluorene) were associated with PC5. These results imply that at this most polluted site, hydrocarbons cannot be viewed as strong contributors to toxicity in *L. peregra*. This may be considered surprising in view of the reported links at Site 5 and the slightly elevated laboratory toxicities of the PAHs in *L. peregra* compared with *A. aquaticus*. It has been previously suggested, however, that other factors such as the extremely low DO and high SODs at this site are possibly masking the effects of other toxins such as metals and hydrocarbons. The effect of the nearby outfall at this site also cannot be ignored. It may well be the case that *L. peregra* is more sensitive than *A. aquaticus* to other, unmeasured variables, that are influenced by rainfall and the nearby outfall.

7.4.3 Site 7

A further illustration of the association between rainfall and mortality can be seen at Site 7 (Table 7.7) where coefficients of 0.62 and 0.71 respectively for these parameters

can be observed with PC3. This PC also provides further evidence of an association between mortality and the PAHs. Here, methyl-anthracene, methyl-fluoranthene and the benzofluoranthenes are most highly associated with mortality and rainfall. A wide range of hydrocarbons are also associated with mortality and with PC1 (which explains 62.4% of total variance). Some moderately high coefficients with the alkanes (C_{18}-C_{24}) were recorded but the highest associations were confined to the PAH group. These results again demonstrate the *in-situ* higher toxicities associated with the PAH class of hydrocarbons compared with the aliphatics.

It is probable that PC2 has some biogenic source in view of the high coefficients for C_{25} and C_{27} of 0.90 and 0.89, respectively, as well as the expected low mortality association with such a source. The remaining three PCs which account for 15% of the system variance have extremely scattered associations among the measured compounds and cannot be accurately interpreted.

The high association of rainfall with PC3 and not PC1 may be explained if PC3 is assumed to have a lubricating oil source component. As such, it is likely to form slicks that are highly mobile during storm events, as well as being non-particulate associated, and, consequently more bioavailable.

7.4.4 Site 9

The results for *L. peregra* at Site 9 (Table 7.8) have some similarities with those recorded for *A. aquaticus* at the same site in that the scatter of the PCs is greatest at this site, indicating a noisy data set with a large number of sources of variation. However, PC1 accounts for a substantial proportion of the system variance (41.5). For *L. peregra* the first PC at each site has explained more of the system variance than for *A. aquaticus* at the same sites. This component is most strongly related to the high-molecular weight alkanes, but, unlike previous results for *A. aquaticus* at Sites 5 and 9, there is no clear biogenic element. It is possible, however, that in view of the generally low association of the PAHs with this PC, a biogenic component is likely.

The mortality response is highly associated with the 5th Principal Component and the

TABLE 7.8

THE EIGENVALUES AND VARIMAX ROTATED EIGENVECTOR MATRIX OF THE RETAINED PRINCIPAL COMPONENTS FOR *L. PEREGRA* AT SITE 9.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Eigenvalue	15.37	6.45	4.04	2.83	1.90	1.74	1.55
Variance (%)	41.5	17.4	10.9	7.7	5.1	4.7	4.2
Variable							
Mortality	0.05	0.04	0.18	0.11	0.93	-0.08	0.09
Rainfall	-0.14	-0.15	0.18	0.07	-0.17	0.87	-0.06
C_{13}	-0.14	0.36	-0.21	0.51	0.61	0.35	-0.05
C_{14}	-0.23	0.45	0.61	0.34	0.26	0.26	0.17
C_{15}	-0.07	0.53	0.44	0.18	0.14	0.62	0.17
C_{16}	-0.20	0.68	0.06	-0.01	0.24	0.50	0.36
C_{17}	0.14	0.80	0.18	0.14	0.19	0.32	0.03
Pr	0.45	0.80	0.03	0.15	0.04	0.08	0.20
C_{18}	0.28	0.87	-0.01	0.27	-0.16	-0.19	-0.05
Ph	0.49	0.77	0.23	0.15	-0.07	-0.19	-0.01
C_{19}	0.37	0.85	-0.21	-0.01	0.20	-0.04	-0.05
C_{20}	0.39	0.87	0.04	-0.17	0.19	0.01	-0.07
C_{21}	0.47	0.81	0.03	0.16	-0.07	-0.09	0.14
C_{22}	0.77	0.62	0.08	0.03	-0.04	-0.06	-0.01
C_{23}	0.91	0.33	-0.02	0.07	0.04	0.06	0.06
C_{24}	0.93	0.26	0.04	0.08	0.05	0.02	0.04
C_{25}	0.90	0.25	0.14	0.08	-0.04	-0.08	-0.10
C_{26}	0.90	0.30	0.04	0.25	0.00	-0.10	0.02
C_{27}	0.89	0.12	-0.05	-0.11	-0.18	-0.09	-0.17
C_{28}	0.85	0.16	0.15	0.23	0.12	-0.02	0.37
C_{29}	0.90	0.13	-0.25	-0.01	-0.11	0.06	-0.01
C_{30}	0.91	0.22	0.16	-0.00	0.05	-0.23	0.13
C_{31}	0.77	0.19	0.18	0.07	0.47	-0.26	0.15
N	0.27	0.59	0.39	0.16	-0.08	-0.30	0.31
M-N	0.29	0.10	0.90	0.17	0.14	0.04	0.11
F	0.21	0.09	0.33	0.88	-0.19	0.07	0.05
Ph+A	0.10	0.06	0.24	0.64	0.14	0.23	-0.52
M-Ph	0.11	0.19	0.04	0.00	-0.08	0.06	0.88
M-A	0.15	0.27	0.28	0.66	0.26	-0.16	0.28
Fl	0.16	0.10	0.45	0.66	0.41	0.03	-0.13
Py	0.60	0.16	-0.15	0.56	-0.04	-0.07	0.20
M-Fl	0.34	0.10	-0.74	0.06	-0.32	-0.13	0.41
M-Py	0.44	-0.02	0.78	0.29	-0.08	0.14	0.03
BA+Ch	0.43	0.30	0.04	0.04	-0.41	-0.15	0.03
BFs	-0.01	0.10	-0.15	0.11	-0.89	0.08	0.27
BP	-0.01	-0.10	-0.15	0.10	0.13	0.12	-0.07
DBA	-0.28	0.09	0.69	0.54	0.04	0.14	-0.05

association with hydrocarbons is very low. The very low mortality response at this site suggest that pollution effects other than those caused by hydrocarbons also have a negligible impact on mortality at this site. Rainfall and mortality are also unrelated at this site, a feature clearly related to the buffering capacity of the lake site as well as the generally reduced contamination both directly from outfalls and from resuspended sediments. It should be restated that, in contrast, a strong relationship between rainfall and mortality was demonstrated at the three stream sites for *L. peregra*. The results at Site 9 are consistent with those for *A. aquaticus* in that associations between tissue hydrocarbons, rainfall and mortality are consistently low.

7.4.5 Overview of PCA analysis for *L. peregra*

Mortality has a high association with rainfall at the three stream sites (Sites 5, 6 and 7) and a very low association at the lake site (Site 9). These results are in agreement with those suggested in Chapter 5 in which qualitative links between these variables were made. Sites 5 and 7 also display important correlations of hydrocarbons, notably the PAHs and their methylated derivatives, with rainfall and organism mortality. The data are generally difficult to interpret in terms of the original source of the compound. This suggests significant changes in the relative compositions and modes of variations of the original compounds occur following biaccumulation by *L. peregra*. This would also imply that a smaller proportion of the measured hydrocarbons were in the form of partially or non-assimilated compounds such as in the gut content compared with *A. aquaticus*. It is also suggested that a lipophilic factor may also be detected in *L. peregra* and may be more important in this organism. The comments made in Section 7.3.5 regarding lipid measurenent and other physiological factors also apply strongly here and should be incorporated in future work.

Some important agreements with the *A. aquaticus* results can also be seen: at Site 5 a lubricating oil source was also identified as an important contributor to mortality. Also, hydrocarbon tissue burden and mortality were not linked at Site 6 suggesting that non-hydrocarbon pollution (e.g. low DO) related to the nearby outfall were masking potential hydrocarbon effects. The lubricating component also displayed high coefficients with mortality at Site 7 and at Site 9 the low recorded mortalities could not

7.5 SUMMARY

In general, mortality in *L. peregra* is more readily explainable in terms of the measured variables than in *A. aquaticus*. Conversely, the tissue distributions are less interpretable in *L. peregra* in terms of the original source of the compound. For *A. aquaticus*, natural interpretations were possible because of the limited extent to which the original modes of variation of hydrocarbon were altered after incorporation into the body tissues. In Section 6.6 it was demonstrated that in the laboratory *L. peregra* had a longer hydrocarbon retention time compared with *A. aquaticus*. It was also demonstrated that compounds could be accumulated to a greater extent. The greater lipid content of molluscs as well as lack of enzyme activity for xenobiotic removal will inevitably result in changes in the mode of variation of measured hydrocarbons (as expressed in the principal component analyses). The fact that *A. aquaticus* hydrocarbon distribution is naturally interpretable in terms of original hydrocarbon source suggests that hydrocarbons play a rather passive role in *A. aquaticus*, neither being altered significantly in composition nor exerting a highly toxic impact. It may be implied therefore, that a proportion of the measured hydrocarbons in *A. aquaticus* tissue is in an unassimilated form such as in the gut contents. The depuration experiments conducted in the laboratory (Chapter 6) also concluded that substantial proportions of the measured hydrocarbons could be in such partial or non-assimilated forms. Thus, some of the compounds are not biologically active and *A. aquaticus* may indeed be considered as a generally passive target or receptor for such compounds. Nevertheless, it is a suitable organism for hydrocarbon biomonitoring, because of its ability to tolerate high contaminant levels and to provide an example of a mixed function oxidase system-containing invertebrate response to pollutant exposure. In contrast, hydrocarbons in *L. peregra* must be considered more biologically active because of the demonstrated toxic impacts and the more difficult interpretation of the original source of variation of the hydrocarbons.

Both of these observations are consistent with a typical mollusc response in that the lack or scarcity of a MFO system allows toxic compounds to accumulate and that more bioavailable compounds e.g. methylated groups were able to accumulate to a much greater extent than represented in the abiotic surroundings. *L. peregra* can also be

bioavailable compounds e.g. methylated groups were able to accumulate to a much greater extent than represented in the abiotic surroundings. *L. peregra* can also be considered successful as a biomonitoring tool in urban rivers, in that it greatly aids the identification of the fraction of the hydrocarbon assemblage that is primarily associated with mortality in the Silk Stream catchment. The application of PCA to organism tissue data must therefore be considered an accurate and useful analytical technique for the identification of pollutant groups that may cause ecological damage. The findings of these analyses strongly implicate the lubricating fraction of the hydrocarbon assemblage as the source most consistently associated with organism mortality. In *L. peregra*, this fraction was identified at the three stream locations where an association with rainfall was also shown. For *A. aquaticus* a similar association with the lubricating fraction was established at Site 5. Mortality, however must be related to other unmeasured variables at the three other sites for *A. aquaticus*.

The laboratory tests presented in Chapter 6 suggested that the measured hydrocarbons could not be the primary cause of the mortalities in the field, and indeed greater body burdens with lower toxicities were attained in the laboratory studies compared to field studies. However, consistency can be found in the results that are common to the field and the laboratory: the parental PAHs in both organisms exerted a greater toxic effect than the alkanes. *L. peregra* also accumulated more hydrocarbons and exhibited greater mortality than *A. aquaticus*. Both the field and laboratory trials also suggest that substantial proportions of the measured hydrocarbons may be in unassimilated forms. Two further important points should be considered when comparing the laboratory data of Chapter 6 with the field data of Chapters 5 and 7.

1) methylated groups, primarily implicated in field mortalities were not measured in the laboratory toxicity studies: inclusion of these compounds in future laboratory tests may produce closer correlations with field mortalities.

2) other compounds also related to a lubricating oil fraction, displaying the same source of variation as the methyl PAHs may be influencing the *in situ* mortality.

It must also be stated that no statistical procedure can fully implicate any compound in

physically interpretable source of variation that has been shown to associate with invertebrate mortality. PCA also indicates where mortality is caused by parameters or impacts other than those specifically measured. These findings may allow further more focused investigations, that result in more targeted water pollution management strategies that are directly related to the effect on the stream ecology.

Chapter 8

CONCLUSIONS AND DIRECTIONS FOR FURTHER RESEARCH

8.1 BIOLOGICAL WATER QUALITY

A clear deterioration in the water quality of the Silk Stream from the headwater sites (Sites 2 and 3) through Sites 4 and 5 and to the downstream locations (Sites 6 and 7) is evident from the chemical and biological data presented in Chapters 4 and 5 and may be related to the increasing urbanization that occurs with downstream progression. At the downstream Silk Stream sites the biological water quality is of very poor quality as indicated by the presence of only two or three pollution-tolerant organisms. At Site 9, in the Welsh Harp receiving basin, a substantial recovery in biological water quality was shown.

In terms of community diversity and equating BMWP values to the NWC classification, Site 3 would fall across the Class II/III boundary and Sites 4 and 5 would be Class III. Sites 6 and 7 represent areas of poor river quality being on the boundary of Class III and IV or within Class IV.

8.2 HYDROCARBON CONTAMINATION OF THE ABIOTIC AQUATIC ENVIRONMENT

Concentrations of hydrocarbons in abiotic samples were consistently found to mirror the general decline in water quality in the Silk Stream catchment. The extent of hydrocarbon contamination of sediment and water was very high in the lowest reaches of the Silk Stream and comparable to the highest levels reported in the literature. The change from the headwater to lower Silk Stream sites represented an approximate ten fold increase in hydrocarbon contamination.

The analysis of aliphatic distributions showed that substantial inputs of biogenic hydrocarbons were present throughout the sampling locations. Carbon Preference

Indices (CPI) of greater than one in the high molecular weight range (C_{21}-C_{32}) suggested that contributions from epicuticular waxes of higher plants were important and could be identified even at the most highly polluted sites. In contrast, the low molecular weight CPIs (C_{11}-C_{20}) used to identify biogenic aliphatic inputs from algal sources were close to unity which strongly suggests that such sources were negligible. Levels of the branched isoprenoids pristane and phytane, suggested that the hydrocarbon suite was not highly weathered or biologically degraded, indicating low bacterial activity and the continuing input of fresh hydrocarbon assemblages.

The analysis of the aromatic fraction showed that pyrogenic sources provided the greatest contributions to the assemblage as indicated by the consistently abundant compounds pyrene and fluoranthene as well as other primarily combustion derived compounds such as benzo(a)pyrene. The most highly polluted Silk Stream sites displayed important contributions from lubricating oil components, as indicated by increasing concentrations of methylated homologues of the parental PAHs. The increasing concentrations were both in absolute terms and in relation to the amount of parent compound present. Thus, although pyrogenic compounds dominated the abiotic hydrocarbon suites throughout the Silk Stream catchment a lubricating oil-source signature was increasingly prominent at the downstream sites. The recovery in biological and chemical water quality at Site 9 was reflected in a reduction in hydrocarbon contamination and particularly in the reduction in lubricating oil inputs.

8.3 HYDROCARBON CONTAMINATION AND EFFECTS ON MACROINVEREBRATES

The freshwater shrimp, *Gammarus pulex* and the stickleback, *Gasterosteus aculeatus*, were considered as potential biomonitor species but were found unsuitable for the intended type of cage design and because of their sensitivity to the polluted test sites. Following initial tests, *A. aquaticus* and *L. peregra* were considered suitable biomonitors and were found, following transfer from relatively uncontaminated rural sites, to accumulate hydrocarbons rapidly and to high levels. Relatively unstable maxima of tissue hydrocarbon concentrations were reached within 5 - 10 days of transfer to the contaminated sites. The degree of bioaccumulation was found to mirror

the extent of contamination at the various test sites.

Mortality rates for both species were found to be highest at the downstream sites (Sites 6 and 7) and lowest at Site 9. Site 5 had mortality rates that were intermediate between these sites. *A. aquaticus* generally displayed lower mortality rates compared to *L. peregra*.

The application of principal component analysis (PCA) to the tissue hydrocarbon data showed that for *A. aquaticus* at Site 5, the PAH group and in particular the methylated compounds, had the same source of variation as mortality. Similar relationships were demonstrated for *L. peregra* at Sites 5 and 7 which strongly implicate the lubricating oil source as an important contributor to invertebrate mortality at the downstream Silk Stream sites. Negligible associations between mortality and biogenically derived sources were also demonstrated. Associations of other non-biogenic aliphatics with mortality were generally low, except when linked to the methylated PAHs. Links between rainfall and mortality were also established, indicating the importance of sediment resuspension and hydraulic stress effects. At Site 6 the influence of the adjacent outfall may have masked the effects described at the other Silk Stream sites. At the receiving basin site the low recorded mortality rates had no common source of variation with any measured hydrocarbons or with rainfall, emphasising the substantial recovery that occurred at this site.

In the laboratory, bioaccumulation of hydrocarbons was more rapid than in the field and both organisms were able to accumulate hydrocarbons to higher levels. Although the laboratory hydrocarbons would therefore appear to be in a more bioavailable form, lower mortalities from similar or higher body burdens occur in the laboratory compared with the *in situ* tests. The aromatic fraction was found to exert a greater toxic impact than the aliphatic fraction and generally *L. peregra* was more sensitive than *A. aquaticus* to both classes of hydrocarbon. The depuration phases in both compounds were rapid and probably biphasic since calculation of a linear decay constant was approximate in the PAHs and not possible for the alkanes. It is suggested that the elimination of hydrocarbons was the result of a combination of thermodynamically favourable partition effects, the excretion of gut contents, and the active enzyme-driven

response of the organism. Highly assimilated compounds may therefore only have represented a small part of the total body burdens measured which therefore resulted in reduced hydrocarbon levels at the sites necessary for organism narcosis.

8.4 POSSIBLE DIRECTIONS FOR FUTURE RESEARCH

The PCA approach in particular may provide a highly targeted and effective tool for the assessment of the role of a wide range of aquatic pollutants in freshwaters. The following suggested directions for future research may provide further interesting insights into the nature of the ecological effects of hydrocarbon contamination in urban watercourses.

- Since the PCA approach to tissue levels is a novel application it should be tested by applying it to other urban and rural locations that represent wide ranges of hydrocarbon contamination.

- Although hydrocarbon exposure can be related to mortality at some Silk Stream locations, it is evident that it is not the sole cause of the mortality at these sites and at other sites explains very little of the recorded mortalities. Future studies should ideally incorporate analysis of heavy metal and other urban pollution components both in organism tissues and in sediments and water. PCA analysis of such comprehensive sets of water quality measurements may then more clearly identify the causes of any observed *in situ* mortalities.

- The work may also be expanded to higher trophic levels or other common pollution-tolerant invertebrates such as leeches and bloodworms. While it is unlikely that biomagnification effects occur to any significant extent, the inclusion of other organisms will enhance our understanding of the local ecosystem cycling of hydrocarbons.

- While this work considers some established physiological characteristics of the studied organisms in explaining patterns of bioaccumulation, future studies may incorporate measurement of variables such as tissue lipid levels, age, sex, life-

cycle stage and feeding behaviour.

- The laboratory experiments indicated that substantial proportions of the measured hydrocarbons were unassimilated in the test organisms. Further work that tracks metabolite production and establishes turnover rates would provide important information on the extent of assimilation of hydrocarbons. Thus, the proportion of measured hydrocarbons that contaminate at a cellular level and disrupt biological function at this level may be identified. A related but broader direction for future work would be to investigate the potential for environmental stress caused by the excreted metabolites.

- A simple relationship between bioconcentration factors and the K_{ow} values of specific hydrocarbons could not be established in this work. There would appear to be a need to develop QSARs for macroinvertebrates that take into account competitive effects and interference of other compounds under field and laboratory conditions. Such experiments should involve the measurement of bioaccumulation of single hydrocarbons in the absence and presence of other, competing compounds. Controlled dosing of humic acids or other dissolved organic matter should also be included.

- The reported associations between the lubricating oil source of variation and *in situ* macroinverebrate mortality represent important and interesting findings which require further investigation. A more focused investigation incorporating the analysis of other non-combustion derived hydrocarbons may pinpoint components of the lubricating oil fraction that are responsible for *in situ* mortalities. Laboratory toxicity trials incorporating series of methylated derivatives would be an essential part of this investigation i.e. can the methylated PAHs demonstrate a high laboratory mortality response or do other, unmeasured variables, having the same source of variation as the methylated PAHs exert the toxic effect?

- A further approach is to examine mechanisms of hydrocarbon toxicity: can a more specific mode of death other than general narcosis by hydrocarbons be

established? The possibility of long term effects such as mutagenesis or carcinogenesis in invertebrates at sites heavily contaminated with PAHs may also warrant investigation.

- This work has attempted to examine the effects of hydrocarbon contamination on macroinvertebrate mortality. Clearly, other, sublethal responses to urban pollution must also occur such as weight or fecundity changes. Behavioural changes may also be investigated. The use of video or infra red sensors *in situ* could provide interesting on-line data in such investigations .

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