ENVIRNONMENTAL RISK MANAGEMENT OF CONTAMINATION OF MARINE BIOTA BY HYDROCARBONS SPECIFICALLY THOSE ARISING FOLLOWING AN OIL SPILL

RITA ENWERE

A thesis submitted in partial fulfillment of the requirement of
The Robert Gordon University
for the degree of Doctor of Philosophy

This research programme was carried out in collaboration with the Fisheries Research Services (FRS) Marine Laboratory, Aberdeen.

March 2009

Declaration

I hereby declare that the work or any portion of it, referred to in this thesis has not been submitted in support of an application for another degree or qualification of this, or any other university or institute of learning. This is an original piece of work undertaken by myself. All results and work other than my own are clearly cited and acknowledged.

Rita Enwere

Abstract

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Marine pollution resulting from oil spillage has received much attention mostly due to the damaging effects it has on fisheries and aquacultures. One component of oil that is widely studied due to its toxic and carcinogenic properties is the polycyclic aromatic hydrocarbons. The physical and chemical properties of these compounds control their distribution into the various phases of the environment. The rates of elimination of these compounds from impacted organisms were investigated in laboratory and field experiments using selected marine organisms (*Mytilus edulis* and *Salmo salar*).

The elimination of individual PAH compounds followed first order kinetics. Elimination rate varied among compounds and generally decreased with increase in molecular weight and degree of alkylation. Elimination rate constants (k_2) and biological half-lives ($t_{1/2}$) evaluated from chronically exposed mussels (collected from Aberdeen harbour) in separate laboratory and field studies were comparable but differed from those evaluated from acutely exposed mussels. Shorter $t_{1/2}$ were obtained from acutely exposed mussels. The $t_{1/2}$ ranged between 0.5- 22 d (acute exposure) and 3.8- 31.5 d (chronic exposure).The longer apparent $t_{1/2}$ calculated for the chronically impacted mussels was attributed to the retention of the compounds in a stable compartment due to long period of exposure that limited exchange with the surrounding water.

Contrary to expectation, $t_{1/2}$ for similar compounds was higher in salmon than in mussels. The reason for this was unknown but attributed to the route of elimination. A good correlation ($r^2 > 0.72$) was found between PAHs tissue concentration and taint intensity in salmon.

Comparison of the results from this study with literature data showed that tank water replacement time and exposure duration affects rate of PAHs elimination. The data generated in this study and some of the reviewed studies will find application in different oil spill scenarios.

The usefulness and limitations of the *n*-alkanes profile, PAH distribution and concentration ratios, and specific biomarker ratios from organisms in oil spill source identification was also demonstrated.

Acknowledgements

You answer us by giving us victory O LORD, and you do wonderful things to save us! Psalm 65: 5

My profound gratitude goes to my supervisors: Prof. Pat Pollard (RGU), Dr. Ian Davies (FRS), Dr. Lynda Webster (FRS) and Prof. Colin Moffat (FRS) for their time, support, patience, advice and diligent review of my work progress. I have really learnt a lot from them during this short period of study. Ian, your prompt responses to my mails fanned the flame for me.

I would also like to thank all the staff of the Analytical Investigation and Environmental Impact groups of FRS Marine Laboratory, Aberdeen, for their support, guidance, suggestions and friendliness. I would like to thank you Marie and Ines for the analysis of my samples; Gillian and Judith for the training on PAHs and lipid content analysis respectively; Anne for your assistance with sensory analysis; Craig for helping out with the risk assessment of my experimental protocols; Alistair, for your assistance and advice with the project licence; Margaret, for your help with the EROD analysis; Phillip, for helping out with the logistics for my sampling trips; and particularly Eric, for your timely assistance whenever needed. Sandya, Lesley, and Pam, it has been great working with you at one time or the other during my stay at FRS.

To my husband; Cajetan, thanks for the patience, compassion and love throughout my research work, and to my daughter; Jessica for the understanding during the busy periods.

I am indebted to my Mother; Victoria, sisters; Euchay and Buchi, brother in-law; Linus; sisters' in-law Bibian and Ugochi…word cannot appropriately express how your persistent prayers and encouragement have helped to me surmount the difficult moments. I owe my success to you all!
To all my friends and colleagues; especially, Getrude, Ogoo and Kyari who encouraged and supported me throughout my studies, thank you.

To my sponsors Petroleum Technology Development Fund, Nigeria, and Univation Ltd, Thanks for providing the fund and making my dreams a reality.

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Glossary

ASE	Accelerated Solvent Extraction
BCF	Bioconcentration Factor
BHL	Biological half-life
BSAF	Biota Sediment Accumulation Factor
CPI	Carbon Preference Index
CV	Coefficient of variation
DCM	Dichloromethane
DNA	Deoxyribonucleic Acid
DR	Diagnostic Ratio
EA	Environmental Agency
EC	European Commission
EPT	Equilibrium Partition Theory
ERA	Environmental Risk Assessment
EROD	Ethoxyresorufin-*0*-deethylase
EU	European Union
FEPA	Food and Environment Protection Act
FRS ML	Fisheries Research Services, Marine Laboratory
FSA	Food Standard Agency
GC-ECD	Gas Chromatography Electron Capture Detection
GC-FID	Gas Chromatography Flame Ionization Detection
GC-MSD	Gas Chromatography Mass Selective Detection
HPLC	High Performance Liquid Chromatography
IARC	International Agency for Research on Cancer
ISO	International Standards organization
K_{ow}	Octanol-water partition coefficient
LLE	Liquid-liquid extraction
LOD	Limit of Detection
LRM	Laboratory Reference Material
MCA	Maritime and Coastal Agency
MFO	Mixed Function Oxidase

NCP	National Contingency Plan
NOAA	National Oceanic and Atmospheric Administration
NRC	National Research Council
NRCC	National Research Council Canada
OCPs	Organochlorinated Pesticides
OSPAR	Oslo and Paris Commission
PAHs	Polycyclic Aromatic Hydrocarbons
PCA	Principal Component Analysis
PCBs	Polychlorinated biphenyls
PNA	Polynuclear Aromatic Hydrocarbon
POPs	Persistent Organic Pollutants
QUASIMEME	Quality Assurance of Information for Marine Environmental Monitoring in Europe
SBSE	Stir bar Sorptive Extraction
SCA	Standing Committee of Analysts
SEERAD	Scottish Executive Environment and Rural Affairs Department
SFPA	Scottish Fisheries Protection Agency
SIM	Selected Ion Monitoring
SOP	Standard Operating Procedures
SPMD	Semi permeable membrane devices
SPME	Solid Phase micro-extraction
TEF	Toxicity Equivalence Factor
UCM	Unresolved Complex Mixture
UKAS	United Kingdom Accreditation Scheme
UNEP	United Nations Environmental Programme
USA	United States of America
USEPA	United Sates Environmental Protection Agency
WFD	Water Framework Directive
WHO	World Health Organization

CHAPTER ONE

Introduction

1.1 Background

In recent years, the distribution of polycyclic aromatic hydrocarbons (PAHs) in the environment has been widely studied due to their well known toxic, mutagenic and carcinogenic characteristics (IARC 1983; WHO 2005). PAHs present in crude oils and derived products enter the terrestrial and aquatic environment through petroleum production and consumption related activities such as offshore exploration, petroleum refining, petroleum transportation and consumption. PAHs are also produced during combustion of wood and other organic materials. In the event of oil pollution, say an accidental oil spill, a primary concern is the contamination of marine biota, including commercial fish and shellfish species. Contamination can be so severe as to give rise directly to concern for human health, for example from high concentrations of carcinogenic PAHs including benz[*a*]anthracene, benzo[*a*]pyrene, diben[*a,h*]anthracene (UK Committee on Toxicology and Chemicals, EU Standing Committee on Food SCF/Sc/CNTM/PAH/29, Commission Recommendation 2005/108/EC, Commission Regulation (EC) No 208/2005), to lighter PAHs (e.g. naphthalene and substituted naphthalenes) which can taint the edible tissues of fish and shellfish, thereby rendering these produce unsuitable for the market.

Following a recent review of the European Union Commission Regulation setting the maximum limit for contaminants in food as regards PAHs [Commission Regulation (EC) No 466/2001], a number of additional compounds was incorporated into the list of priority PAHs for reporting in environmental assessment due to their perceived carcinogenic properties. These include cyclopenta[*c, d*] pyrene and various isomeric forms of dibenzopyrene: dibenzo[*a,e*]pyrene, dibenzo[*a,h*]pyrene, dibenzo[*a,i*]pyrene, and dibenzo[*a,l*]pyrene.

Current approaches to oil spill mitigation and management (closure of fisheries and harvesting areas accompanied by chemical analysis and taint assessment) provide the

security that potentially toxic products (bivalve molluscs – benzo[*a*]pyrene 10 ng g^{-1} wet weight; crustaceans – benzo[*a*]pyrene 5 ng g^{-1} wet weight; muscle meat of fish – benzo[*a*]pyrene 2 ng g^{-1} wet weight) or tainted products, do not enter the human food chain [Commission Regulation (EC) No 208/2005]. However, if harvesting and/or marketing are prevented, the question is immediately raised by producers as to when normal commercial activity could be resumed.

At present, the ability to predict the rate of loss of PAH compounds from fish and shellfish (and consequently the likely persistent of taint) is limited, and at times the rates of loss have been considered in relation to "total" PAHs rather than individual compounds of particular tainting or toxicological significance.

In the past, several studies have examined the dynamics of uptake and depuration of PAHs in aquatic organisms (for example, Pruell *et al*., 1986; Guilherme 1998; Sericano *et al*., 1996; McIntosh *et al*., 2004; Richardson *et al*., 2005). However, the result is that, a range of conflicting reports have been published on the rates of elimination of PAHs from marine organisms; clams, oysters and mussels (Table **1.1**).

Pruell *et al*. (1986) monitored the elimination of PAHs from blue mussels exposed to PAH-contaminated sediment in aquaria for 40 days and observed elimination of PAHs with biological half life between 14-30 days. Richardson *et al*. (2005) reported almost complete elimination of PAHs in 2 days from mussels contaminated with specific PAH standards, while McIntosh *et al*. (2004) reported ~ 50 % reduction in PAHs concentration over 122 days from mussels chronically contaminated from PAHs originating from an aluminium smelter. Boehm *et al*. (1977) reported only slight elimination after 120 days in chronically contaminated Clams while Tenacredi & Cardenas (1991) observed no elimination after 45 days in the same specie of organism exposed to specific PAH standards for 2 days. Blumer *et al*. (1970) and Stegeman and Teal (1973) both exposed Oysters to No. 2 fuel oil for 60 days and 49 days respectively. However, while Blumer reported little elimination of PAHs in 180 days, Stegeman and Teal reported almost complete elimination in 28 days.

Table **1.1**: Summary of experimental results reported by different authors on depuration rates of PAHs from organisms (especially bivalves) over the last 4 decades

Bivalve	**Exposure Medium/Time**	**Observation**	**Reference**	**Details**
Oysters	N^{o} 2 Fuel Oil (60days)	Little depuration after 180 days	Blumer *et al*. (1970)	Mar. Biol. 5, 195-202
Oysters	N^{o} 2 Fuel Oil (49days)	Nearly complete depuration in 28days	Stegeman and Teal (1973)	Mar. Biol. 22, 37-44
Clams	Chronically polluted	Slight depuration after 120days	Boehm and Quinn (1977)	Mar. Biol. 44, 227-233
Oysters	Chronically polluted	Nearly complete depuration with BHLs = 4.4days	Wormell (1979)	PhD Dissertation, Rutgers University, NJ
Oysters	PAHs (15 day)	Analytes below detection limits after 4 days	Pittinger *et al*. (1985)	Environ. Toxicol. Chem. 4, 379-387
Mussels	PAHs (40 days)	Depuration with BHL between 14-30days	Pruell *et al*. (1986)	Mar. Biol. 91, 497-507
Clams	PAHs (2 days)	No depuration in 45 days	Tanacredi and Cardenas (1991)	Environmental Science Tech. 25, 1453 -1461
Oysters	Chronic pollution (48days)	Significant depuration in 50 days	Sericano *et al*. 1996	Sci. Total. Environ. 179, 149- 160.
Mussels	PAHs (20 days)	Depuration very rapid in 2days and then reduced to minimum thereafter	Richardson B.J *et al*. (2005)	Marine pollution Bulletin, 51, 975-993
Mussels	Chronically polluted	Total PAHs fall - 50% in 122days	McIntosh *et al*. (2004)	J. Environ. Monit., 6, 209 - 218

The reasons for the variations in the reports are not well known but may be attributed to the differences in the experimental designs and PAH compounds studied. It has been noted that the rate of elimination of hydrophobic compounds decreases with increase in hydrophobicity (Gewurtz *et al*., 2002). Other factors such as seasonal changes, the animal specie used, physical factors, and the presence of other contaminants may well affect the elimination rates. Webster *et al*. (2003) has noted that apart from the concentration of PAH in the organisms' surrounding environment and the duration of exposure of an organism to it, seasonal changes can also affect the observed tissue burden in organisms. Moreover, while some authors acutely exposed organisms to specific PAHs standards or petroleum products, others depurated chronically contaminated organisms.

1.2 Hydrocarbon input to the marine environment.

Hydrocarbons found in the environment originate from diverse sources. Hydrocarbons are naturally present in fossil fuels, woods and organic materials; However, anthropogenic activities such as combustion of organic matter, industrial activity, natural fires and petroleum consumption are mainly responsible for the hydrocarbon contamination reported in many areas around the world (e.g. see; Bence *et al*., 1996; Boehm *et al*., 1997 Burns *et al*., 1997; and Page *et al*., 1999). Hydrocarbons enter into the marine environment by several different pathways. These compounds are major constituents of unburned petroleum and can be released directly into the environment either by natural seepage or through series of petroleum production activities ranging from offshore exploration to transportation. About 43, 000 metric tons of petroleum is discharged into the atmosphere each year, and another 230, 000 tons enter aquatic environments (Eisler, 1987). Petroleum hydrocarbons can also find their way into the aquatic environment through discharges from human activities such as industrial and domestic sewage effluents: run-offs from paved roads and parking lots. Although petroleum substances occur in relatively low concentrations in urban run-off, but total amount discharged is relatively high due to the large volumes involved (UNEP, 2003).

NRC (2003) recognized that petroleum hydrocarbons enter the environment through four different routes; natural seeps, petroleum extraction, petroleum transportation, and petroleum consumption.

Natural oil seepage is a purely natural phenomenon that occurs when crude oil seeps from the geological strata beneath the seafloor to the overlying water column. These seeps release vast amount of crude oil annually, yet these volumes are released at the rate low enough that the surrounding ecosystem can adapt and even thrive in their presence (NRC 2003). According to Wang (2006), natural oil seeps are a worldwide phenomenon that contributes petroleum to the environment more than all other sources combined.

Contamination from petroleum extraction results from the activities of man associated with the effort to explore and produce petroleum such as seen in produce water (e. g. Jacobs *et al.,* 1992). Petroleum transportation can result in the release of varying amount of petroleum product; ranging from major spills during vessel accident [Torrey Canyon (Cornwall) UK, 1967; Amoco Cadiz 1978; Qualida 1986; Exxon Valdez, 1989; Persian Gulf, 1991; Shetland Island, UK, 1993; Prestige (Spanish Coast), 2002; etc.] to minor operational discharges. Annual worldwide spills exceed 1,300,000 metric tons (NRC 2002). Petroleum consumption can result in the release of components of petroleum and their oxidative components in the environment and therefore contributes to the greater percentage that enters the marine environment (Blumer *et al.,* 1975).

1.3 Composition of petroleum products

Crude oil is a natural occurring complex mixture of thousands of different organic compounds formed from a variety of organic materials that are chemically converted under differing geological conditions over long period of time (Peters and Moldowan, 1993). The physical properties and exact chemical composition of crude oil varies from one locality to another. In general, crude oil components are classified in bulk group as saturates, olefins, aromatics, resin and asphaltenes (Wang *et al*., 2006). Saturates are the predominant class of hydrocarbons in most crude oils. They include straight chain, branched chain and cyclo-alkanes. Biomarker terpanes and steranes are branched cyclo-alkanes consisting of multiple

condensed five- or six- carbon rings. Sterane and terpanes bear intrinsic fingerprints of their source rocks (Peters and Moldowan 1993) and have played an important role in the fingerprinting of spilled oils e.g. in the Nordtest system (Nordtest Method NT CHEM 001)

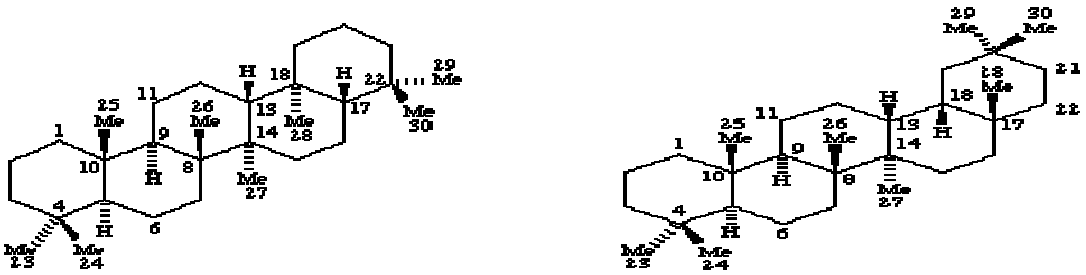
Aromatic hydrocarbons are cyclic planar compounds that resemble benzene in electronic configuration and chemical behaviour. Aromatics in petroleum include the more volatile mono-aromatic hydrocarbons BTEX; benzene, toluene, ethyl benzene and xylene (NRC, 1985; Speight 1993), alkyl substituted benzene compounds, and the polycyclic aromatic hydrocarbons (PAHs). PAHs are also known as polynuclear aromatic hydrocarbons (PNA).

PAHs are ubiquitous in the environment and persist even when its input has ceased. Some PAHs has been implicated to be toxic and carcinogenic (IARC 1983; WHO 2003). It is not surprising that over the years, PAHs has been the chemical species within petroleum that are most frequently discussed in terms of toxicity (Aas *et al*., 2000; Aas *et al*., 2001; Cavalieri and Rogan 1992). Subsequent sections will be dedicated to the discussion of the environmental fate of PAHs in the marine environment and the relevance of geochemical biomarkers in PAH source identification.

1.3.1 Geochemical Biomarkers.

Geochemical biomarkers are non-toxic hydrocarbon components of crude oil that bears fingerprints of the source rock from which the oil originates (Peters *et al*., 2005). Biomarker fingerprinting has historically been used by petroleum geochemists in characterisation of marine oils in terms of source rock, genetic family, migration and maturation properties, and in identification of petroleum deposits (Peters and Moldowan 1993). Terpanes and steranes biomarker are composed of six monomer units called isoprene [2-methyl-1, 3-butadiene, [CH2 = $C(CH_3)$ – CH = CH_2] (Wang *et al*., 1999).

The structures of some biomarkers are shown below.



Gammacerane Oleanane

Fig.**1.1**: Chemical structures of gammacerane and oleanane

1.3.2 Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons constitute a wide class of compounds composed of two or more aromatic rings in a planar configuration. Within the group, the compound range from semi-volatile molecules to molecules with high boiling points. The compound may exist with a great number of structures and depending on the complexity of the PAH, in a large number of isomers e.g. dibenzopyrene. Wise and Sander (1997) published the chemical structures and nomenclature of 660 PAHs.

The abundance of PAHs in petroleum usually decreases with increasing molecular weight. In most cases one ring through to three-ring aromatic hydrocarbons, and related heterocyclic aromatic hydrocarbons such as dibenzothiophene, account for at least 90 percent of the aromatic hydrocarbons that can be resolved in crude petroleum by conventional analytical methods (Neff, 1990). These compounds are generally hydrophobic, meaning that they have higher tendency to associate with particles than to dissolve in water; a property that increases with increase in water-octanol partition coefficient, K_{ow}. This property of PAHs is further discussed in details in section **1.4**.

PAHs can be divided into two groups based on their physical, chemical, and biological characteristics. The lower molecular weight PAHs (e.g., 2- to 3- ring PAHs such as naphthalenes, fluorenes, phenanthrenes, and anthracenes) which have significant acute toxicity to aquatic organisms; and the high molecular weight PAHs, 4- to 7- ring (from chrysenes to coronenes) which do not but many have been known to be carcinogenic.

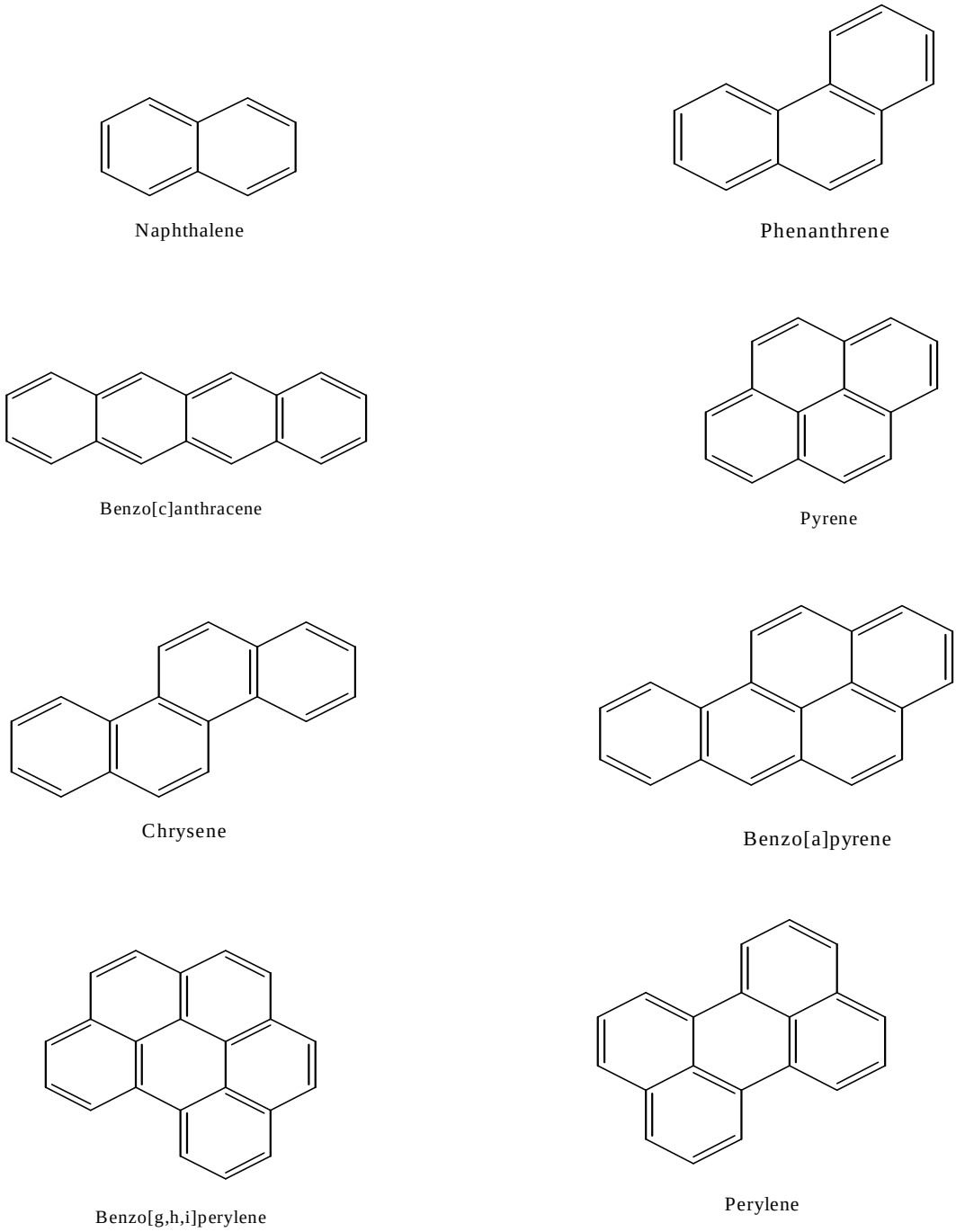


Fig. **1.2**: Chemical names and sructures of some common PAH compounds

The most commonly studied PAHs are the 2- to 6-ring compounds with their alkylated homologues. The structures of some common unsubstituted PAHs are shown in Fig. **1.2**

1.3.2.1 PAHs Source Recognition

There are three major types of sources of hydrocarbons found in the environment; petrogenic, biogenic and pyrolytic sources (Blumer and Youngblood, 1997; Lima *et al*., 2005; Wang *et al*., 1999).

Hydrocarbons released from un-burnt petroleum are generally referred to as petrogenic hydrocarbons. PAHs occur naturally in bituminous fossil fuels, such as coal and crude oil deposits, as a result of diagenesis (i.e. the low temperature, 100-150 °C, combustion of organic material over a significant span of time). This process favours the formation of alkylated PAHs; the un-substituted (or the parent) compounds being relatively low in abundance in these sources (NRCC, 1983).

Hydrocarbons generated from the incomplete combustion of fossil fuels (coal and oil) and organic materials such as wood are generally termed pyrolytic hydrocarbons (Page *et al.*, 1999). In a review of the formation of polycyclic aromatic hydrocarbons combustion processes, Lima *et al*. (2005) indicated that during combustion, the organic compounds present in the fuel are fragmented into free radicals that can react in a number of different chemical pathways to produce the first aromatic ring. Further reaction of this aromatic ring with small compounds (2- to 3- carbons; e.g. C_2H_2 – ethyne) leads to formation of larger and more stable rings. The type of fuel and the combustion conditions both affects the quantitative composition of hydrocarbons produced during combustion (Blumer, 1976; Mastral *et al*., 1998) although qualitative mixtures may be similar.

Biogenic hydrocarbons are generated by biological processes or by early stages of digenesis in recent marine sediments (Wang *et al*., 1999). Biological sources include land plants, phytoplankton, animals, bacteria, macroalgae and microalgae. It has been reported (Cretney *et al*., 1987; Barrick *et al*., 1980; Requejo *et al*., 1983; Wang *et al*., 1995; Wang *et al*., 1998) that the biogenic hydrocarbons have the following chemical composition characteristics: (1) *n*-alkanes show a distribution pattern of odd carbon-numbered alkanes

being much abundant than even carbon-numbered alkanes in the range of *n*-C_{21}–*n*-C_{33}, resulting in unusually high carbon preference index (CPI) values, which is defined as the sum of the odd carbon-numbered alkanes to the sum of the even carbon-numbered alkanes (2) notable absence of the 'unresolved complex mixture (UCM)' hump in the chromatograms; (3) pristane is often more abundant than phytane, suggesting a phytoplankton input (Blumer *et al*., 1971; Peters and Moldowan 1983; Kennicutt II *et al*., 1994) and resulting in abnormally high pristane/phytane ratio values; (4) wide distribution of the biogenic PAH perylene, an unsubstituted PAH produced in subtidal sediments by a process known as early diagenesis (Peters and Moldowan 1983). Biogenic source is one of the potential sources contributing to background hydrocarbons in the environment (Wang *et al*., 1999).

With increased enforcement of oil spill and other pollution laws, it has become extremely important that measures are in place to identify the sources of oil pollution, for legal and liability purposes. Several methods have been in use for the tracing of PAHs found in the environment to the sources. Some of these methods include the matching of the *n*-alkane profile of the samples, (Yunker *et al*., 1999; Stella *et al*., 2002, Webster *et al*., 2003), use of PAH distribution profile and PAH source diagnostic ratios (Budzinski *et al*., 1997; Wang *et al*., 1999; Yunker *et al*., 2002; Webster *et al*., 2003) and most recently, geochemical biomarker fingerprinting (e.g. Boehm *et al*., 1997 and Barakat *et al*., 2002; Faksness *et al*., 2002; Dahlmann, 2003).

The chemical composition of organic matter and the operating temperature affect the yield and distribution of PAHs formed during incomplete combustion of organic matter (Lima *et al*., 2005). As was explained by Budzinski *et al*. (1999), thermal PAH formation can occur over a wide range of temperatures. At low temperatures, the compound distribution is governed by thermal stability and the most stable isomers are formed, while at high temperatures, PAHs of higher formation enthalpy can be generated.

In general, pyrolytic PAHs are characterised by the dominance of a wide range of un-substituted (parent) compounds over their corresponding alkylated homologues and the dominance of the high molecular mass (4- to 6-ringss) compounds over the low molecular mass (2- and 3-ringss) compounds. In contrast, petrogenic patterns, such as those obtained

during the slow maturation of petroleum are characterised by dominance of the alkylated compounds over their corresponding un-substituted homologues and the high abundance of the 2- and 3-rings PAHs relative to the 4- to 6-ringss. For the petrogenic PAHs, their distribution profile in the environment are readily modified to $C_0 < C_1 < C_2 < C_3$ by weathering or degradation processes (Wang *et al*., 1999; Yunker *et al*., 2002).

In addition to this qualitative characteristic, source diagnostic indices; a quantitative approach; such as ratio of phenanthrene to anthracene (P/A), fluoranthene to pyrene (Fl/Py), sum of methyl-phenanthrene and methyl-anthracene to phenanthrene (∑MP/P) have also been developed in the past and is being used extensively in discriminating between PAH sources in sediment samples (e.g. Budzinski *et al*., 1997; Baumard *et al*., 1998; Dahle *et al*., 2003; and Webster *et al*., 2004). These indices which are based on thermodynamic stability of specific PAH compounds are used to characterise compounds distribution according to the process underlying their generation. Considering the ratio P/A, Phenanthrene (P) is more thermodynamically stable than anthracene (A). Pyrolysis of organic matter at very high temperature generates PAH compounds of low P/A ratio, while the slow maturation of petroleum at lower temperatures produces PAHs with large P/A ratio (Raoux, 1991). The same is applicable to fluoranthene and pyrene index, fluoranthene is less thermodynamically stable than pyrene and dominates if source is petrogenic (Budzinski *et al*., 1997).
Webster *et al*. (2003) extended the use of these indices to the study and assignment of sources to PAHs determined in mussels from various locations in Scotland. A summary of the definitions of some commonly applied ratios in PAHs studies are shown in Table **1.2**.

Table **1.2**: PAH diagnostic ratios used for source discrimination (A = Anthracene, P = Phenanthrene, MP = methyl phenanthrene, Fl = Fluoranthene and Py = Pyrene).

Diagnostic ratio	Pyrolytic	Petrogenic
Phenanthrene/Anthracene (P/A)	<10	>10
Fluoranthene/Pyrene (Fl/Py)	>1	<1
[Methylphenanthrenes]/Phenanthrene	<2	>2
Fluoranthene + pyrene/ methyls [(Fl+Py)/(MFl+MPy)]	3	<3

However, according to Yunker *et al*. (2002), the use of these ratios for source characterizations requires an understanding of the relative discrimination ability (relative thermodynamic stability) of the different parent PAHs, the characteristics of the different PAH sources and the relative stability of different PAH isomers.

Geochemical biomarker fingerprinting has also found unique application in distinguishing petrogenic PAH sources from other sources. Geochemical biomarkers are organic compounds characterised by their source specificity and molecular stability that maintain the fingerprint of their origins (Medeiros and Bicego, 2004). In field samples, it is often difficult to absolutely identify which PAHs have been introduced from petrogenic or pyrolytic source using PAH distribution ratios alone (Webster *et al*., 2003). This is because the many ways in which PAHs are introduced into the environment may result in PAH signature from one source being masked by PAHs from other sources (Yunker *et al*., 2002). Therefore, since PAHs are commonly found in the environment as complex mixtures deriving from multiple sources, the confident discrimination of petrogenic PAHs from other PAH sources requires use of various geochemical biomarkers (Yunker *et al*., 2002). Peters and Moldowan (1993) noted that petroleum biomarkers are resistant to degradation by weathering and therefore remains relatively unchanged over time, in contrast to the losses during weathering of *n*-alkanes, PAHs and eventually their alkyl homologues. Analysis of samples for geochemical biomarkers, such as the pentacyclic triterpanes (hopanes) and the tetracyclic steranes, has proved useful in oil pollution source identification (e.g. Wang *et al*., 1998). Biomarkers are distinctive features of oil and vary from oil to oil, hence have also been successfully applied to discriminate against oil sources (e.g. Boehm *et al*., 1997; Barakat *et al*., 2002; Abboud et *al*., 2005). Crude oils differ from one another depending on where they were formed (source rock) and how this process occurred. Oil from terrestrial materials is thus different from oil from e.g. marine materials. The composition of the oil is also affected by how rapidly it had formed, the geological processes after formation, the oxygen content of the atmosphere, the age of the oil, and so on (Faksness, 2002). A gas chromatogram of oil from a certain specific deposit has its own identifiable fingerprint. Therefore the criteria used in sample correlations are based on matching the distribution pattern of steranes and hopanes and similarities of the molecular parameters of these biomarker compounds. Correlation can be a matter of the presence of

certain compounds in a certain type of oil or a specific concentration ratio of two compounds (Wang *et al*., 1999).

Specific biomarker diagnostic ratios which are in use (e.g. Nordtest method) in oil source identification are summarized in the table below.

Table **1.3**: Some diagnostic ratios used oil source correlations

Diagnostic ratio	**Definition**
DR-27Ts	27Ts/27Tm
DR-28ab	28ab/30ab
DR-29Ts	29Ts/30ab
DR-30O	30O/30ab
DR-30G	30G/30ab
DR-29ab	29ab/30ab
DR-30d	30d/30ab
DR-29aaS	29aaS/29aaR
DR-29bb	29bb(S+R)/29aa(S+R)

where 27Ts = 18α(H)-22,29,30-trinorhopane, 27Tm = 17α(H)-22,29,30-trisnorhopane, 29ab =17α(H),21β(H)-30-norhopane, 28ab = 17α(H), 21β–28,30-bisnorhopane, 29Ts = 18 α(H)-30 -norneohopane, 30d = diahopane, 30O = 18α(H) oleanane, 30G = gammacerane, 30ab = 17 α(H), 21β(H)-hopane, 31abS = 17α(H), 21β(H)-22S-homohopane, 31abR = 17α(H), 21β(H)-22R-homohopane, 32abS = 17α(H), 21β(H)-22S-bishomohopane, 32abR = 17α(H), 21β(H)-22R-bishomohopane, 33abS = 17α(H), 21β(H)-22S-trishomohopane, 33abR = 17α(H), 21β(H)-22R-trishomohopane, 34abS = 17α(H), 21β(H)-22S-tetrakishomohopane, 34abR = 17α(H), 21β(H)-22R-tetrakishomohopane, 35abS = 17α(H), 21β(H)-22S-pentakishomohopane, 35abR = 17α(H), 21β(H)-22R-pentakishomohopane, 29aaS = 5α(H), 14α(H), 17α(H)-24-ethylcholestane (20S), 29aaR = 5α(H), 14α(H), 17α(H)-24-ethylcholestane (20R), 29bbS = 5α(H), 14β(H), 17β(H)-24-ethylcholestane (20S), 29bbR = 5α(H), 14β(H), 17β(H)-24-ethylcholestane (20R).

1.4 Distribution and fate of PAHs in the marine environment

The behaviour and distribution of PAHs in the marine environment is fundamental in the control of their movement and impact on marine organisms (King *et al*., 2004). Due to their varying degrees of resistant to oxidation, reduction, and vaporization which increases with increasing molecular weight and aqueous solubility which decreases with increase in molecular weight, PAHs differ in their behaviour, distribution in the environment, and their effects on biological systems (Eisler, 1987).

Oil spilled into the marine environment is susceptible to spreading, evaporation, dissolution, microbial degradation, photo oxidation, and interaction between oil and sediment. The combination of these processes termed "weathering" reduces the concentration of hydrocarbons (and hence PAHs) in sediment and water and at the same time alters the composition of the spilled oil (Payne *et al.*, 2003). The alteration in the composition of spilled oil (e.g. by biodegradation) has profound effect in the oil's toxicity and biological impact over time. Before formation of non-toxic and harmless end products by various enzymatic and nonenzymatic reactions, PAHs are converted to arene oxide intermediates followed by formation of derivatives of *trans*-dihydrodiols, phenols, and quinones. These intermediate products are known to be toxic, carcinogenic, and/or mutagenic (Lima *et al*., 2005)

In the study of water and sediment samples from Brighton marina to quantify the intensity, spatial and temporal variation of PAH contamination, King *et al*. (2004) reported that the PAH behaviour in the marine systems is highly complex, and controlled by the interplay of PAH sources, field conditions, compound's physicochemical properties and biological factors. The structure and physical properties of PAHs can greatly impact their volatility, solubility, sorption, and decomposition behaviours (Lima *et al*., 2005). The more volatile compounds (e.g. naphthalene) are lost through evaporation which is one of the short-term weathering processes.

Solubility is another property that governs and defines the fate of PAHs released from spills. In the marine environment, PAHs are distributed into the water and sediment phases due to the partial dissolution of selected lower molecular weight 2- to 3-ring compounds

with log K_{ow} values between 3.7 and 4.8 (Payne *et al.*, 2003). Physical transport and mechanical factors accounts for the PAHs distribution observed in sediments. As was reported by Moore and Ramamoorthy (1984), due to their hydrophobic nature, PAHs entering the aquatic environment exhibit a high affinity for suspended particulates in the water column. As PAHs tend to sorb to these particles, they are eventually settled out of the water column onto the bottom sediments. Thus, the PAH concentrations in water are usually quite low relative to the concentrations in the bottom sediments.

Parameters such as octanol-water partition coefficient (K_{ow}), the sorption coefficient (K_{oc}), equilibrium partition theory (EPT), bioconcentration factor and biota-sediment accumulation factor (BSAF) have been used by different authors to explain the fate and distribution of organic trace pollutants in the marine environment.

The octanol-water partition coefficient is defined simply by;

$$K_{ow} = C_{oc\tan ol} \Big/ C_{Water} \quad \text{..... (1.1)}$$

where $C_{oc\tan ol}$ is the molar concentration of the organic compound in the octanol phase, and C_{water} is the molar concentration of the organic compound in water when the system is at equilibrium.

The logarithm of water/octanol partition coefficient (log K_{ow}) gives a measure of a compounds' hydrophobicity, with large values reflecting relative insolubility in aqueous solutions. This model has been used by many authors (for example, Pruell *et al.*, 1986; Baumard *et al.*, 1999; King *et al.*, 2004; Gourlay *et al.*, 2005; Oen *et al.*, 2006) to describe the interaction of organic contaminants with the marine environment assuming equilibration.

Equilibrium partition theory; the most relevant hypothesis was initially proposed by Shea in 1988, and further developed by DiToro *et al*.(1991), who proposed that organic chemicals

which sorbed to soil or sediment are in equilibrium with the aqueous phase or pore water, the same aqueous phase to which benthic and terrestrial organisms are exposed.
According to Mitra *et al*. (1999), in a two-phase aqueous system at equilibrium, the concentration of a chemical in a freely dissolved phase (Cw, mg/l) relative to that in a sorbed phase (Cs, mg/kg), is described by a linear equation –

$$K_D = C_S / C_W \qquad \text{(1.2)}$$

where K_D is the sediment to pore water distribution coefficient.

However, it has been established that the partition behaviour of hydrophobic chemicals, Polychlorinated biphenyls (PCBs), Organochloro pesticides (OCPs), and PAHs, in sediment, water and biota is mainly determined by the lipid and organic carbon content; the more hydrophobic a compound, the greater the partition to the lipid and organic carbon content (Van der Oost *et al*., 2003). Hence, the use of bioconcentration factor (BCF) to explain the relationship between organism and pore water. BCF of a chemical has been defined as the ratio of its concentrations in the organism to that in water during steady state equilibrium.

$$BCF = K_W / K_B = C_B / C_W \qquad \text{(1.3)}$$

If sorption of hydrophobic chemicals is considered as a partitioning between water and the organic fraction of the sediment, then equilibrium sorption coefficient (K_{oc}) can be expressed as:

$$K_{OC} = k_W / k_S = C_s / C_w \qquad \text{(1.4)}$$

where w and s refer to water and sediment respectively.

If the processes of bioconcentration (1.3) and sorption on sediment (1.4) have both reached equilibrium, then biota to sediment accumulation factor (BSAF) is described which is the relationship between a chemical sorption to sediment and organism (Van der Oost *et al*., 2003).

$$BSAF = {C_B}/{C_S} = {BCF}/{K_{OC}} \quad \text{..... (1.5)}$$

Hence, once available in the water phase or adsorbed on particulate matter, filter feeding organisms like mussels can accumulate PAHs to concentrations higher than are present in the water phase due their relatively poor ability to rapidly metabolize PAHs. As a general rule, water is the dominant source of exposure for organic compounds with low log K_{ow} (<5) while sediment particles (as food) can contribute substantially to bioaccumulation for those with high log K_{ow} (>5) (Belfoid *et al*., 1996). Since these compounds are biologically available, PAH retained in sediment can also adversely affect biota or result in high concentrations in the tissues of indigenous organisms (Yim *et al.*, 2002).

1.4.1 PAHs in marine organisms

The presence of a xenobiotic compound in a segment of an aquatic ecosystem does not, by itself, indicate injurious effects. Connections must be established between external levels of exposure, internal levels of tissue contamination and adverse effects (Van der Oost *et al.*, 2003). Over the years, researchers have used living organisms to monitor the concentration and effect of pollutants in the environment. PAHs are lipophilic and therefore are preferentially accumulated in the lipids of organisms where they cause a variety of sublethal effects. The interest in using organisms to monitor marine pollution relies on the fact that analyses of the tissues give an indication of the bioavailable fraction of the environmental contaminant (Gourlay *et al*., 2005) and the biological effect (e.g. Kennicutt *et al.*, 1994; Aas *et al.*, 2000; Aas *et al*., 2001). For example, the mussel watch project in USA and the Roseau National d' Observation (RNO) in France have been developed using mussel and oysters to monitor spatial and temporal trends of contaminant concentrations in coastal and estuarine regions (Cantilo, 1991).

In marine organisms, the resultant tissue burden is controlled primarily by the uptake and elimination kinetics (Davies *et al*., 1997). While uptake is governed primarily by bioavailability, elimination of PAH is governed by its metabolism and biotransformation processes. These concepts are discussed in details in subsequent sections.

1.4.1.1 Bioavailability

The concept of bioavailability is extremely important in understanding and describing the environmental fates and biological effects of PAHs in the marine environment. In a review by Belfroid *et al*. (1996), bioavailability was defined as the fraction of the bulk amount of the chemical present in sediment and water that can potentially be taken up during an organism's lifetime into the organism's tissues (excluding the digestive tract). Also, in aquatic toxicology, bioavailability is defined as the extent to which a chemical can be absorbed or adsorbed by a living organism by active (biological) or passive (physical or chemical) processes. A chemical is said to be bioavailable if it is in a form that can move through or bind to the surface coating (e.g., skin, gill epithelium, gut lining, cell membrane) of an aquatic organism (Kleinow *et al.,* 2000).When measuring bioaccumulation behaviour, the bioavailability of the substance is considered a crucial parameter for valid results.

Various methods have been used to measure the bioavailable concentrations of PAH in environmental studies and these have included the direct measurement of aqueous PAH concentration in water (e.g. Neff, 1991); measurements of tissue PAH concentrations in sentinel organisms such as mussels and oysters in the context of routine monitoring (Sericano *et al.,* 1996; Bender *et al.,* 1987; Grundy *et al.,* 1996; Baumard *et al.,* 1999; King *et al.,* 2004) and in oil spill assessments (Page *et al*., 1999; Page *et al*., 2005; Johansson *et al.,* 1980); and the use of semi-permeable membrane devices (SPMDs) (Gourlay *et al.,* 2005; Boehm *et al.,* 2005; Richardson *et al.,* 2005) and silicone rubbers (Yates *et al*., 2007) as passive abiotic samplers. The concept of bioavailability his has been a topic for scientific debate in recent times. Organisms respond only to the bioavailable fraction of contaminants. However, most extraction methods used to analyse soil and sediment samples for PAHs are exhaustive. This means that, these methods measure the total concentration of the contaminant in a given environmental sample even when their

availability to living organisms has been limited by ageing. According to Harmsen (2007), the bioavailable fractions of contaminants are dependent on soil properties and various processes varying with time and on the behaviour of the target organism. Bioavailability is a promising tool in risk assessment when viewed as the determination and assessment of exposure and measuring and assessment of effects. The working group 'Bioavailability' of ISO/TC190-Soil Quality has developed a guidance document for development and selection of methods to assess bioavailability for different target species with regard to several classes of contaminants.

1.4.1.2 Bioaccumulation

The concentration of trace pollutants in the tissue burden of an organism is dependent on the biological availability of the compound, the length of exposure of an organism to it, and the organism's capacity for metabolic transformations (Van der Oost *et al*., 2003). Apart from bioavailability, another key factor that affects organisms' tissue burden is the uptake route (feeding mode). Elaborating on the feeding mode, Van der Oost *et al*. (2003) and Guilherme (1998) reported that persistent hydrophobic chemicals may accumulate in aquatic organisms through different mechanisms: via the direct uptake from water by the gills or skin (bioconcentration), via uptake of suspended particles (ingestion) and via consumption of contaminated food (biomagnifications). When organisms are exposed to only the dissolved phase, they tend to accumulate more of the soluble fraction of contaminant available in the water column. Whereas, organisms exposed to high turbidity water column or located close to the sediment tend to accumulate high molecular weight PAHs and less soluble compounds from the sediment and particulate matters (King *et al*., 2004).

Different models have been used to study the bioaccumulation of contaminants. Baumard *et al*. (1999) used the bioaccumulation factor (BAF) model; which is the ratio of organism concentration of a compound to the sediment concentration of the compound, to access the PAH tissue burden of mussels from different marine environments. They concluded that the location (habitat) of an organism within an aquatic environment govern the major

uptake route of a contaminant and therefore should be a determinant factor in the selection of appropriate model in estimating bioaccumulation.

1.4.1.3 Metabolism and Biotransformation of PAHs

Invertebrates have less ability to metabolise xenobiotics than vertebrates and this can result in tissue burden of invertebrates been relatively high even when the contaminant has virtually disappeared from the environment (Gobas *et al.,* 1988). However, Wiel *et al (*1989); Stegeman (2000); and Pearce (1997) have reported that vertebrates have a high capacity for metabolizing aromatic hydrocarbons including PAHs through cytochrome P450 1A mediated oxidation. Therefore, elevation of cytochrome P450 1A levels in fish may indicate exposure to some aromatic hydrocarbons, even though tissue levels may not show elevated concentrations. Measurement of hydrocarbon metabolites in tissues where elevated cytochrome P450 1A is observed has provided evidence of the relationship between hydrocarbon exposure, metabolism and cytochrome P450 1A activity (Aas *et al.*, 2001; Ferreira *et al*., 2006; Wirgin *et al.*, 1995). Metabolism of hydrocarbon mixtures may result in excretion of some compounds but also activation of other compounds to toxic metabolites including DNA adducts (Wirgin *et al.,* 1995). According to Van der Oost *et al*. (2003), an organism has two major ways of eliminating a chemical, which is either; excretion of the chemical in its original form or biotransforming the chemical into a metabolite (Fig. **1.3**).

Biotransformation is another important factor in examining tissue burdens and biological effects. An organism's capacity for biotransformation of hydrocarbons has been used in many instances as an estimate of exposure in the absence of measurable hydrocarbon concentrations. Biotransformation generally leads to the formation of a more hydrophilic compound which is more easily excreted than the original compound (Vermeulen, 1996). However, biotransformation may also alter the toxicity of the compound, which may be either beneficial or harmful to the organism. In case of a detoxification reaction, the toxicity of the compound is reduced while excretion rate is generally elevated (Fig. **1.4**)

In case of bioactivation, however, the compound is transformed into a reactive metabolite, which is more toxic than the parent compound (Cavalieri and Rogan, 1995).

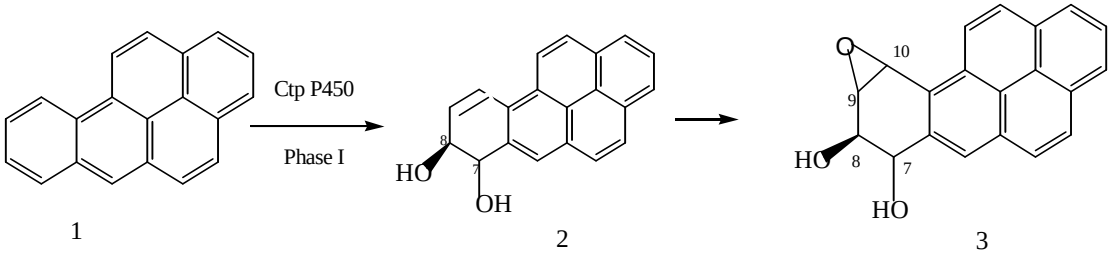


Fig. **1.3**: Pathway involved in the activation of benzo[*a*]pyrene to a reactive intermediate; B[*a*]P-7,8-dihyrodiol, an ultimate carcinogen. (1) benzo[*a*]pyrene; B[*a*]P, (2) B[*a*]P-7,8-dihyrodiol, (3) B(a)P-7,8-dihydrodiol-9,10 epoxide

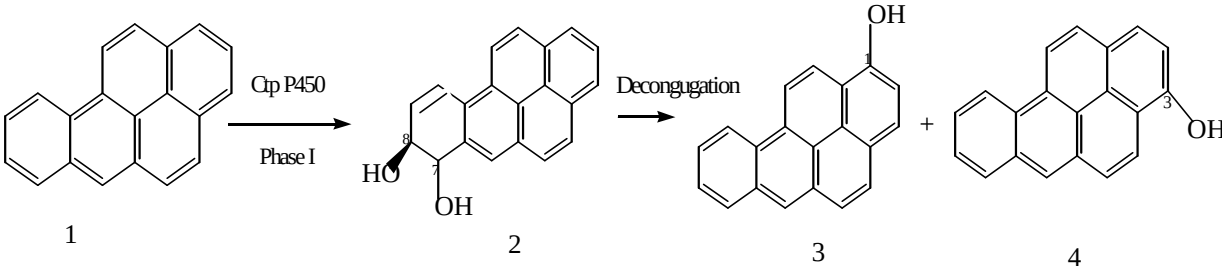


Fig. **1.4**: Pathway involved in the phase 1 metabolism (detoxification) of benzo[*a*]pyrene. (1) benzo[*a*]pyrene, B[*a*]P; (2) B[*a*]P-7,8-dihyrodiol, (3) 1-hydroxyl B[*a*]P, (4) 3-hydroxyl B[*a*]P.

However, not all PAHs are metabolically activated and relatively a few have been shown to be carcinogenic, e.g. Benzo[*a*]pyrene, benzo[*a*]anthracene, dibenz[*a,h*]anthracene (WHO 2000). Moreover not all organisms have sufficiently well developed mixed function oxidase (MFO) capable of activating such compounds. Biotransformation process is thus important in determining the biological activity of a compound, the effect and duration of that activity, and the half-life of the compound in the body.

Biota sediment accumulation factor (see section **1.3**) one of the partitioning models used in monitoring uptake of PAHs in the environment may be distorted by biotransformation (see section **1.3**). Increased clearance of contaminants through effective biotransformation may cause severe deviations in BSAFs from the expected values. However, when uptake rates are significantly higher than metabolic clearance rates bioaccumulation can still occur even

though the substance is readily biodegradable (Van der Oost *et al.*, 2003). Pollutants concentrations in tissues and differences in excretion of metabolites can be a function of tissues and conditions controlling the activity of biotransformation enzymes. Measurements of metabolites in bile and liver tissues of fish are also being used as biomarkers to assess exposure of these organisms to PAHs (e.g. Gagnon and Holdway, 2002)

1.4.2 Theory of kinetics of elimination of PAHs

Different kinetic models have been applied to investigate the kinetics of elimination PAHs from marine organisms (Pruell *et al.*, 1986, McIntosh *et al.*, 2004, Richardson *et al.*, 2005). Rate constant models relate the concentration of a compound in one compartment with that in another. Generally, one compartment models, where the organism is treated as a homogenous compartment, and two compartment models, which assumed a central compartment and a slowly exchanging compartment, have been used. However, several authors have reported the adequacy of first order one-compartment model in estimating the kinetic of elimination of hydrophobic contaminants (PAHs, PCBs and OCPs) from organisms (Sericano *et al.*, 1996, Gewurtz *et al.*, 2002). In his study, Sericano used first order kinetics to describe the accumulation and elimination of PAHs from American oyster as follows-

$$\frac{dC_t}{dt} = k_u C_w - k_d C_t \quad \text{..... (1.6)}$$

where C_t is the concentration (ng g^{-1}) in the mussel at time *t*, K_u and K_d are the uptake and depuration rate constants respectively, and C_w is the concentration (ng ml^{-1}) in the seawater.

If concentration in the seawater (C_w) is regarded as zero during depuration, then equation one reduces to

$$\frac{dCt}{dt} = k_2 C_t \quad \text{..... (1.7)}$$

Integration of equation (1.7) gives

$\ln C_t = -k_2 t + \ln C_0$ …….. (1.8), or the log transformed equivalent

$\log C_t = \log C_0 - (k_2 / 2.303)t$ ……. (1.9),

where C_0 is the PAH concentration in mussels prior to depuration.

The depuration constant (k_2) of individual PAH can be evaluated from equation (1.8), and half-life from equation 1.10

$$halflife \left(t_{1/2}\right) = \frac{\ln 2}{k_2} = \frac{0.693}{k_2} \text{ ….. (1. 10)}$$

Note: $K_d = k_2$

1.5 Regulation, toxicity and effects of PAHs

PAHs are amongst the compounds present on the Oslo and Paris (OSPAR) Commission List of Chemicals for Priority Action (OSPAR- 02/21/1-E, Annex 5) and the EU Water Framework Directive List of Hazardous Substances (Directive - 2000/60/EC). Also the United States Environmental Protection Agency (US EPA) identified 16 priority PAHs, some of which are considered possible or probable human carcinogens.

PAHs have been implicated in various plants, animals and human tissue disorders and this has raised awareness on the need for monitoring and regulation of these chemicals in the environment. In order to safeguard public health, a number of national and international bodies have also set guidelines to monitor the level of PAHs especially benzo[*a*]pyrene in consumer goods.

In 1974, United States Congress (US) passed the Safe Drinking Water Act; this prompted the United States Environmental Protection Agency (USEPA) to set the maximum contaminant level (MCL) for benzo[a]pyrene to 0.2 ppb in potable water which became effective in 1994. And, in December 2000, the European Union adopted the Water Framework Directive (WFD) which purpose was to protect the inland surface waters, coastal and groundwater, and estuaries from hazardous contaminants (Directive 2000/60/EC).

In addition, European Commission Regulation (EC) 466/2001, which sets maximum limits for certain contaminants in foodstuffs, has applied across the European Union since April 2002. The aim of the Regulation is to keep contaminants at levels that are toxicologically acceptable and to exclude grossly contaminated food from entering the food chain. On 4 February 2005, the European Commission amended this regulation as regards PAHs in Commission Regulation (EC) No 208/2005. The later regulation sets the maximum limit for benzo[a]pyrene in fish and shellfish as follows – muscle meat of fish (2.0 ng g^{-1}), muscle meat of smoked fish (5.0 ng g^{-1}) and bivalves molluscs (10.0 ng g^{-1}). The commission also advised the measurement of the following compounds – benz[*a*]anthracene, benzo[*a*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*g,h,i*]-perylene, chrysene, cyclopenta[*c,d*]pyrene, dibenz[*a,h*]anthracene, dibenzo[*a,e*]pyrene, dibenzo[*a,h*]pyrene, dibenzo[*a,i*]pyrene, dibenzo[*a,l*]pyrene, indeno[1,2,3-*c, d*]pyrene and 5-methylchrysene in consumer products as a necessity which will inform a future review of the suitability of maintaining benzo[*a*]pyrene as a marker.

The 2- and 3-rings PAHs (naphthalenes and phenanthrenes) are acutely toxic and have been implicated in tainting of fish and shellfish (Heras *et al*., 1992), while the 4- to 6-rings PAHs have been reported as probable human carcinogens e.g. benz[*a*]anthracene, dibenz[*a,h*]anthracene and Benzo[*a*]pyrene which is an established human carcinogen (Cavalieri and Rogan 1992; IARC 1983). In fish and mammals, the immunotoxic effects of PAHs have been widely demonstrated (Galvan *et al*., 2005; Roos *et al*., 2004; Platt *et al*., 2004; and Hu *et al*., 2006). It has been estimated that exposure to environmental chemical carcinogens may contribute significantly to the causation of a sizable fraction, perhaps a majority, of human cancers, when exposures are related to "life-style" factors such as diet, tobacco use, etc (Wogan *et al*., 2004). In most cases, the polar bio-chemically reactive

electrophilic species (ultimate carcinogenic metabolites) interacts with cellular macromolecules, particularly nucleic acids and proteins (Xue and Warshawsky, 2005).

According to the EU Scientific Committee on Food Directive (Directive 2005/108/EC), benzo[*a*]pyrene (B[*a*]P) can be used as a marker for the occurrence and effect of carcinogenic PAH in food. The frequent use of B[*a*]P as a model compound to assess the potential risk of PAHs stems from the fact that it has been established beyond doubt to be a carcinogen (Cavalieri and Rogan, 1992; IARC, 1983). Based on this, the B[*a*]P toxic equivalency approach was developed to relate the capacity of priority PAHs to induce cancer to that of B[*a*]P. The potencies relative to B[*a*]P of other PAHs are based primarily on animal bioassay studies. The potencies suggested by different authors are summarised in Table **1.4**.

Table **1.4** Relative PAH potency estimate derived from various sources (adapted from Yender *et al*., 2002).

Relative PAH Potency					
Compound	ICF/EPA[a]	USEPA[b]	FDA[c]	CA EPA[d]	Nisbet & Lagoy[e]
Benzo[*a*]pyrene	1.0	1.0	1.00	1.00	1
Dibenzo[*a,h*]anthracene	1.11	1.0	1.05	0.36	5
Indeno[1,2,3-*c,d*]pyrene	0.232	0.1	0.25	0.10	0.1
Pyrene	0.081		0.13*		0.001
Benzo[*b*]fluoranthene	0.140	0.1	0.11	0.10	0.1
Benzo[*k*]fluoranthene	0.066	0.01	0.07	0.10	0.1
Benzo[*g,h,i*]perylene	0.022		0.03		0.01
Fluoranthene			0.02*		0.001
Benz[*a*]anthracene	0.145	0.1	0.014	0.10	0.1
Chrysene	0.0044	0.001	0.013	0.01	0.01
Anthanthrene	0.320**				
Benzo[*j*]fluoranthene	0.061				
Benzo[*e*]pyrene	0.004				
Cyclopentadieno[*c,d*]-pyrene	0.023				
Anthracene					0.01
Acenaphthene					0.001
Acenaphthylene					0.001
Fluorene					0.001
2-Methylnaphthalene					0.001
Naphthalene					0.001
Phenanthrene					0.001

[a] ICF-Clements Associates (1988).

** Identified in Nisbet and LaGoy (1992) as anthracene.

[b] U.S. Environmental Protection Agency (1993).

[c] U.S. Food and Drug Administration, Contaminants Standards Monitoring and Programs Branch, Centre for Food Safety and Applied Nutrition (Bolger et al. 1996)

* Division of Mathematics, Centre for Food Safety and Applied Nutrition.

[d] California Environmental Protection Agency (1997).

[e] Nisbet and LaGoy (1992).

1.6 Managing risks from petrogenic PAHs.

As was mentioned in section **1.2**, PAHs are the major components of crude oils and derived products that have been found to constitute serious environmental hazard. The challenges and complexities of trying to assess and then manage the environmental risks associated with PAHs in the environment should not be underestimated. From the outset, assessing the risks posed by a single compound and identifying what can be readily achieved in terms of controls is a complex task. One of the challenges with PAHs is that they occur naturally and also result from anthropogenic activities; consequently PAHs have numerous sources other than those that can be directly attributable to the oil industry. This leads to problems when attempting to identify and prioritize all the relevant sources and assess their potential environmental risk. To further complicate the issue, the range of species of PAH is vast and there is a lack of clarity as to how long each PAH lingers in the environment and the extent of risk individual PAH poses to human health and the environment.

In a publication by the European Environmental Agency, risk assessment was defined as the procedure by which the risks posed by inherent hazards involved in processes or situations are estimated either quantitatively or qualitatively (Fireman *et al.*, 1998). Van der Oost *et al*. (2003), in a review of PAHs biomarkers also defined Environmental Risk Assessment (ERA) as the procedure by which the likely or actual adverse effects of pollutants and other anthropogenic activities on the ecosystem and their components are estimated with known degree of certainty using scientific methodologies.

Risk assessments vary widely in scope and application. Some look at single risk in a range of exposure scenarios while others look at the range of risks. However, a serious shortcoming of most environmental risk assessment processes is the absence of baseline data. According to (Fireman *et al*., 1998), environmental monitoring seeks to generate relevant information and accomplishes the following:

i. Determine the indicators to be used in monitoring activities,
ii. Collection of meaningful and relevant information,
iii. Application of measurable criteria in relation to chosen indicators,
iv. Reviewing objective judgments on the information collected,

v. Draw tangible conclusions based on the processing of information,
vi. Making rational decision based on the conclusion drawn, and
vii. Recommendation of improved mitigation measures to be undertaken

One problem beginning to face the regulatory agencies is differentiating between perceived risks as opposed to actual risk of environmental contaminants. Both are important and need to be managed. This problem has occurred more frequently in recent years as a consequence of the increased focus on environmental issues and the demands to reduce the release of hazardous chemicals into the environment. Therefore, in any environmental risk assessment, it is necessary to establish whether any potential exposure routes exist (i.e. essentially establishing a source–pathway–receptor). The concept of bioavailability is therefore a relevant approach in risk assessment of PAH contamination in the marine environment.

1.8 Research Application

Effective determination of the fate of spilled oil in the environment and the successful identification of source(s) of spilled oil and petroleum products is extremely important in oil related environmental studies and liability cases (Wang *et al.,* 1999). Samples collected from the suspected spill sites are analysed to:

i. Assess both the short term and long term environmental impacts on the biological communities within the area.
ii. To evaluate the chemical changes that occurs during weathering, as these processes can alter the distribution of the oil components
iii. Trace the source(s) of the spilled oil.

It is the responsibility of FRS to provide expert scientific and technical advice and information on marine and freshwater fisheries, on aquaculture and on the protection of the aquatic environment and its wildlife. This is to ensure as far as possible, that Government policy and its regulatory and statutory activities are informed by a full and up-to-date knowledge of marine and freshwater fisheries, of aquaculture and of aquatic environment.

One of the functions of the laboratory is to protect the marine environment from pollution through routine monitoring and measuring of priority contaminants and then proffer advice on their control.

The ability to predict the fate of individual PAH compound after spills and to trace spilled oil to its source would be invaluable in monitoring exercises and environmental risk management. The data generated from this study and similar studies at FRS will form the basis for the development of procedures for emergency responses during oil spill and accidental discharge incidents.

1.9 Project Aim

The purpose of this research work is to improve the underlying knowledge base of the occurrence and behaviour of PAHs in the marine environment and thereby enhance the reliability of management responses to oil spills and related incidents. The current study focused on the evaluation of the depuration kinetics of selected priority PAH compounds by monitoring the elimination rates of these compounds from both naturally incurred and artificially exposed mussel. In addition, the changes in geochemical biomarker profile (steranes and triterpaes) were also investigated to ascertain the suitability of using the biomarkers in living organisms for oil spill source identification.

Project Milestones

1 Review of works relating to sources, behaviour and fate of PAHs in the environment to gain background knowledge on the subject of study.

2 Evaluate procedures and methods used in PAHs studies to select an appropriate method for the present study, and develop new methods or procedures where necessary, to suit the study objective.

3 Develop and validate methodologies to allow measurement of the additional PAHs: cyclopenta[*c, d*] pyrene and isomeric forms of dibenzopyrene for environmental monitoring.

4 Investigate and compare the rates of elimination of individual PAHs from naturally contaminated blue lipid mussels (*Mytilus edulis*) depurated in a laboratory flow-through systems with that depurated in the field.

5 Investigate the depuration rates of individual priority PAHs and geochemical biomarkers from salmon (*Salmo salar*) and blue lipid mussels artificially exposed to crude oil.

6 Investigate the biomarker profiles in farmed mussels artificially exposed to crude oils from different geological source rocks and also compare the changes in biomarker profiles in mussels resulting from the laboratory and field depuration experiments.

7 Calculate the depuration rate of each PAH studied and therefore develop recommendations for improving oil spill response based on results obtained and literature data.

CHAPTER TWO

General Analytical Methods

Several analytical procedures involving extraction, clean up, fractionation and quantification, have been described in literature for the determination of PAHs in different matrices. These are reviewed below and specific FRS methods used for the thesis given in subsequent paragraphs.

2.1 Extraction

The first step in analytical procedure is always the isolation of the interest analyte from its matrix and hence from interferences. Several methods of extraction have been developed and reported in the literature for the isolation of priority PAHs for determination depending on the matrix involved. Common extraction methods used for PAHs analysis have ranged from liquid–liquid extraction (LLE) as described by Lin *et al*. (2005), solid-phase extraction (SPE) as reported by Garcia-Falcon *et al.* (2005), solid-phase micro-extraction (SPME) (Cortazar *et al.*, 2002), and stir bar sorptive extraction (SBSE) (Zuin *et al*., 2005) in wastewater samples; Microwave assisted extraction (Baumard *et al.*, 1999a and 1999b), Soxhlet extraction (Martinez *et al*., 2004; Medeiros and Bicego 2004) and sonication (Martinez *et al*., 2004) in sediment and biota samples; while saponification followed by two steps solvent extraction has been predominantly used for biota analysis (Webster *et al*., 1997; Hyotylainen *et al*., 2002; McIntosh *et al*., 2004; Martinez *et al*., 2004).

2.2 Determination

Common methods to analyze PAHs in solid samples (sediments, soils, biota) are based on US Environmental Agency (EPA) methods 8310 (HPLC-UV or HPLC-F) and 8270C (GC-MS). Liquid chromatography has been widely applied to the analysis of PAHs, mainly due to the specificity and low detection limits of fluorescence detection (HPLC-F).

However, the analysis by GC-MS has the advantages of its selectivity, high chromatographic resolution and sufficiently low detection limits for trace analysis purposes. Along with the ability to make qualitative determinations, the method is also an invaluable tool for providing quantitative results (US EPA). GC-MS can be used to analyze the parent as well as the alkylated PAH compounds, and because its selectivity enables positive identification of compounds without additional sample processing, GC-MS in the selective ion monitoring mode (SIM) has been widely used in environmental analyses. Therefore this method is used in the present study.

2.3 FRS standard methods

The sections below briefly describe the standard analytical methods used in this thesis. These methods are well established at FRS Marine Laboratory (FRS ML) and are accredited by the United Kingdom Accreditation Service (UKAS) to ISO 17025. Detailed outline of the methods are available in the FRS ML standard operating procedures given as Appendix **1**.

2.3.1 Quality assurance

Environmental trace analysis of persistent organic pollutants (POPs) requires consideration of quality control and assurance. Consequently, particular care was taken in cleaning glassware, checks on contamination of solvents and correct handling of the samples and equipment to avoid problems of contamination. Where necessary, work was carried out using dedicated laboratory space (environment) and equipment. The procedures described in this chapter are UKAS accredited under ISO 17025 (Webster *et al*., 2005). Samples were kept in designated freezers and properly logged into the FRS quality system for traceability and also to maintain their integrity. Procedural, field blanks and laboratory reference materials (LRM) were included in analyses and where necessary, monitored using control charts as a check for recovery and contamination, as well as the use of

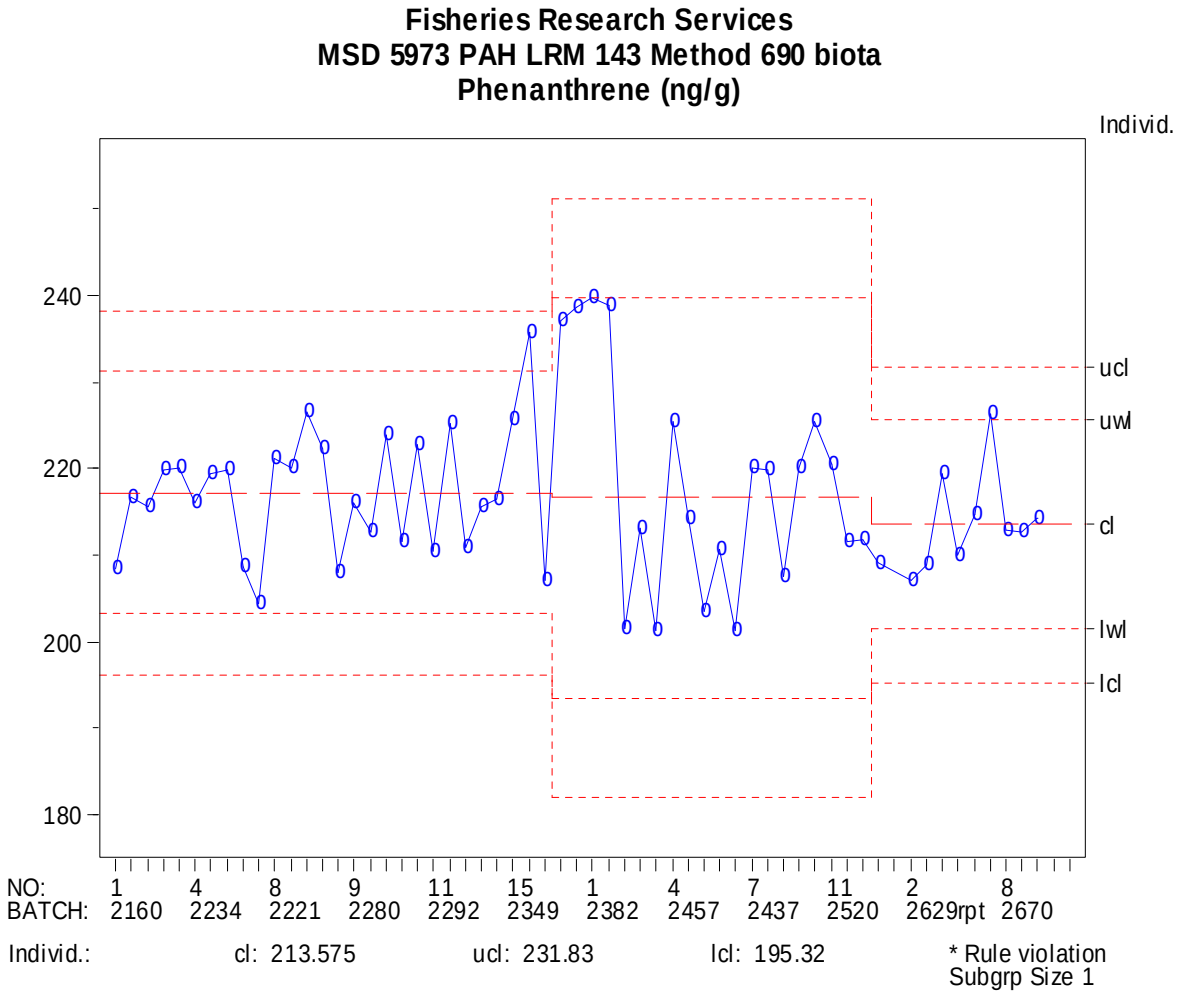


Fig. **2.1**: Example of a Shewhart control chart for phenanthrene showing warning and action control limits, based on ± 2 × and ± 3 × the standard deviation of results obtained. Each data point on the plot represents a value from a single analysis of an LRM in a batch.

internal standards. Shewhart control charts (Fig**. 2.1**) are used to monitor the performance of a method (LRM or a blank) for individual compounds by updating the data after each analysis with warning and action limits drawn at ± 2 × and ± 3 × the standard deviation of results obtained respectively. As part of the quality assurance, the laboratory participates successfully in the external, Quality Assurance of Information for Marine Environmental Monitoring in Europe (QUASIMEME) laboratory performance study scheme for PAHs (Law *et al*., 1997).

2.3.2 PAH analysis of biota

This method is used to analyse tissue samples collected from living organisms for PAHs. The tissues analysed in the study include, mussel muscle tissue and salmon muscle tissue. The method involves the saponification of a known weight of the tissue with 40 ml of 10 % methanolic sodium hydroxide followed by liquid-liquid extraction of the organic portion with iso-hexane and methanol-water. The extract is concentrated and HPLC separated into aliphatic and aromatic fractions before GC analysis and determination.

2.3.2.1 Treatment of Glassware

Hydrocarbon analysis was carried out in a clean environment avoiding contamination of samples and reagents. To avoid carry over of contamination from previous samples, all glassware used were either washed in Decon® 180 solution and rinsed with distilled water or washed in a CAMLAB GW 4050 glassware washer and dried in an oven at 100 ± 5 ^{o}C. Before use, the glassware were rinsed twice each with dichloromethane (DCM) and *iso*-hexane, and the latter was allowed to evaporate to dryness prior to use.

2.3.2.2 Solvent and PAH standard purity checks

HPLC grade solvents (dichloromethane, ethyl acetate, acetone, methanol and *iso*-hexane) are purchased from Rathburn Chemicals Ltd, Scotland, UK. An aliquot of each new batch of dichloromethane or *iso*-hexane (100 ± 5 ml) are measured using a measuring cylinder and transferred to a round bottom flask. To this is added the aliphatic standard (100 ± 5 µl) containing heptamethylnonane and squalane. The DCM or *iso*-hexane solution is reduced to a small volume (~0.5 ml) by rotary evaporation. In the case of *iso*-hexane this solution is transferred to a gas chromatography vial insert, with washings, using a Socerex pipettor and reduced further to ± 50 µl under a steam of scrubbed nitrogen prior to GC-FID analysis. The DCM has to be replaced with *iso*-hexane. This is carried out by addition of the solvent (25 ± 2 ml) followed by rotary evaporation to a small volume (~0.5 ml). This is then

transferred to a GC vial, with washings and reduced further to ~50 µl under a steam of scrubbed nitrogen.

The GC-FID chromatogram is qualitatively and quantitatively assessed to ensure it does not contain more than 100 ng of individual hydrocarbon components. If there are unexpected peaks, or individual hydrocarbons are at levels higher than 100 ng that particular batch of solvent is rejected.

Certified solid standards for PAHs (including deuterated PAHs) were obtained from QMX Laboratories, Essex, UK. Chemical standards used in the preparation of calibration solutions are of high quality and dissolved in *iso*-hexane to obtain required concentrations of standard and spiking solutions. Concentrations were adjusted for purity where necessary.

2.3.2.3 Anhydrous sodium sulphate

The anhydrous sodium sulphate used for drying organic extracts of biota was prepared by washing sodium sulphate in dichloromethane and *i*so-hexane. A 500 ml conical flask is filled to ¾ with the anhydrous sodium sulphate (Na_2SO_4) powder and DCM added to cover the sodium sulphate and the flask covered with aluminium foil and ultra sonicated for 15 min. The DCM is then decanted to waste and the washing procedure repeated using *iso*-hexane covering the sodium sulphate and the washings also decanted to waste. The washed anhydrous sodium sulphate is dried for 16 ± 2 h in an oven set at 60 ^{o}C. The solvent washed Na_2SO_4 is stoppered, stored at room temperature and marked with expiry date of one month from the day of washing.

2.3.2.4 Methanolic sodium hydroxide

Methanolic sodium hydroxide (10 %) used for the saponification of all biota samples was prepared in a fume cupboard by dissolving 50 g ± 1 g of analytical grade anhydrous sodium hydroxide (NaOH) pellets in 50 ml ± 5 ml of distilled water in a Duran bottle. The bottle content was continuously stirred using a magnetic stirrer until all the pellets have dissolved.

450 ml ± 10 ml of analytical grade methanol was slowly added to the resulting solution with stirring until completely mixed. This solution is stable for 3 months.

2.3.2.5 Extraction of biota for PAHs and biomarkers

Extractions of biota for organic contaminants analysis was carried out using UKAS accredited method (ML M 690). This method describes the determination of polycyclic aromatic hydrocarbons (PAHs) in biota. The analysis incorporates two- to six-ring compounds, both parent and alkylated PAHs but does not cover all of the many PAH compounds that exist.

2.3.2.5.1 Saponification of biota samples

The isolation of hydrocarbons from biota (mussel and salmon) was as described in Webster, *et al*. (1997). Biota (~ 10 g for mussels and 4.5 g for salmon) was accurately weighed into a 250 ml round bottom flask and 200 µl ± 10 µl of aliphatic internal standard (containing approximately 3.2 µg each of heptamethylnonane and squalane) and 100 µl ± 10 µl deuterated PAH aromatic internal standard containing D_8-naphthalene, D_{10}-biphenyl, D_8-dibenzothiophene, D_{10}-anthracene, D_{10}-pyrene and D_{12}-benzo[*a*]pyrene (100 µl; approximately 1 µg ml^{-1} each) were then added to the sample. Sodium hydroxide solution [10 %, 40 ml ± 4 ml; 50 g ± 1 g $NaOH_{(s)}$ in methanol/H_2O (90:10 $^{v}/_{v}$)] and a few anti-bumping granules were also added to the flask and a cleaned reflux condenser fitted to the flask and lowered onto a heated sand bath (maintained at 75 ± 5 ^{o}C, monitored using a calibrated thermometer in a beaker of water). Each sample was saponified for 225 min and distilled water (10 ml ± 0.1 ml) then added and further heated for 15 min.

2.3.2.5.2 Liquid - liquid extraction

The hot solution was transferred to a 250 ml separating funnel containing *iso*-hexane (80 ml ± 5 ml) and methanol: water (4:1 $^{v}/_{v;}$ 40 ± 4 ml) was used to rinse the round bottom flask

and added to the separating funnel. The mixture was thoroughly shaken and the lower aqueous layer transferred to a second separating funnel containing *iso*-hexane (80 ml ± 5 ml) and the solution thoroughly mixed. The first *iso*-hexane extract was washed with 40 ml ± 4 ml methanol: water (1:1 $^{v}/_{v}$) by shaking vigorously and allowed to separate. The aqueous layer from the second *iso*-hexane extraction was run-off to waste and the methanol/water layer from the first separating funnel added to the second separating funnel. This was shaken, allowed to settle and the aqueous layer was drained to waste. The extracts from the two separating funnels were then re-combined and washed three times with 40 ml ± 4 ml distilled water, each time draining the bottom aqueous layer to waste. The washed extract was then passed through anhydrous sodium sulphate columns to remove any trace of water (drying) and collected in a 250 ml round bottomed flask, and the column rinsed with 50 ml ± 5 ml of *iso*-hexane. The eluate from the Na_2SO_4 column was concentrated by rotary evaporation at a water bath temperature of 30 ºC followed by nitrogen blow down to 500 ± 10 µl.

2.3.2.5.3 Clean- up and isocratic HPLC separation of extract

The reduced extract was fractionated into the aliphatic and aromatic hydrocarbons by isocratic normal-phase high performance liquid chromatography (HPLC). HPLC fractionation is performed on an aliquot (150 µl ± 10 µl) measured using a calibrated 250 µl syringe on a Genesis metal free HPLC column (25 cm x 4.6 mm). Elution is by *iso*-hexane at a flow rate of 2 ml ± 0.1 ml min^{-1}. The aliphatic fraction was collected from injection to the split time and the aromatic fraction collected from the split time to 20 min. of injection. The HPLC is cleaned after every 7 samples. The split time used (2 min 30 sec – 2 min 45 sec.) is determined after every 84 samples using 150 ± 10 µl of a mixture containing 200 µl ± 10 µl each of concentrated deuterated standard, aliphatic standard and PAH internal standard. The aromatic and aliphatic fractions were concentrated separately in 25 ml round bottom flasks using the rotary evaporator, transferred to GC vials and subsequently reduced further to 50 µl ± 10 µl and 25 µl ± 5 µl respectively, under a stream of scrubbed nitrogen gas. The aromatic fractions were analysed for PAHs using Gas Chromatography-Mass Selective Detection (GC-MSD) and the aliphatic fractions were analyses for geochemical biomarkers and *n*-alkanes using GC-MSD and Gas Chromatography-Flame Ionisation

Detection respectively. Recoveries of ≥ 82 % with precision ≤ 9 % were obtained for mussel samples spiked with 1, 10 and 100 ng g^{-1} PAH solution, for individual PAHs.

2.3.2.6 Determination and quantification

Prior to using the GC-FID, the aliphatic reference sample are analysed and the retention times, peak areas and peak shapes assessed. Analysis of samples are continued only if there is no peak tailing, the retention times are within the time windows of the data system and peak areas are within ± 3 SD of the "correct" figure. The GC-MSD is calibrated biannually and results are calculated using the HP data analysis software. A check is made on the continuing validity of the calibration by running two calibration check solutions with each batch of samples.

2.3.2.6.1 Gas chromatography- flame ionization detection (GC-FID) of *n*-alkanes

The aliphatic portions were analysed for *n*-alkanes by GC-FID using an HP 5890 series II gas chromatograph equipped with an HP 7673 automated on-column injector and fitted with a non-polar, ultra column (25 x 0.2 mm i.d., film thickness 0.33 µm. The carrier gas was helium (16 psi), injections were made at 60 ^{o}C and the oven temperature held constant for 3 minutes. Thereafter the temperature was raised at 4 ^{o}C min^{-1} up to 280 ^{o}C and held at this temperature until the end of the run. Data were processed using Turbochrom Navigator software.

2.3.2.6.2 Gas chromatography-mass selective detection (GC-MSD) of PAHs

The concentrations and composition of the PAHs were determined by GC-MSD using an HP6890 Series Gas Chromatograph interfaced with an HP5973 MSD fitted with a cool on-column injector (Webster *et al.*, 2005). Briefly, a non-polar HP5 (30 m × 0.25 mm id, 0.25 µm film thickness; Agilent Technologies, Stockport, England) column was used for the analyses with helium as the carrier gas, controlled using the constant flow mode at 0.7 ml

min^{-1}. The MSD was set for selective ion monitoring (SIM) with a dwell time of 50 min. Injections was made at 50 ^{o}C and the oven temperature held constant for 3 min. Thereafter, the temperature was raised at 20 ^{o}C min^{-1} up to 100 ^{o}C, followed by a slower ramp of 4 ^{o}C min^{-1} up to a final temperature of 270 ^{o}C. A total of 31 ion (46 compounds) (Table **2.1**) plus the six internal standard ions were measured over the analysis period, thus incorporating 2- to 6- ring, parent and branched PAHs. Limits of detection based on multiplying the standard deviation of the mean of the lowest standard (0.005 ng ml^{-1}) by 4.65 were found to be < 0.2 ng g^{-1} for chrysene and < 0.1 ng g^{-1} for benzo[*a*]pyrene. The GC-MSD is calibrated using seven different concentrations of a solution containing 33 PAHs.

Table **2.1**: List of ions measured using the GC-MSD in SIM mode

PAH	Molecular Weight/ Da				
	Parent PAH	Alkylated PAH			
		C1	C2	C3	C4
Naphthalene	128	142	156	170	184
Phenanthrene	178	192	206	220	
Dibenzothiophene	184	198**	212**	226**	
Fluoranthene/ Pyrene	202	216	230	244**	
Benzo[*c*]phenanthrene/Benz[*a*]anthracene/ Benz[*b*]anthracene**/Chrysene+Triphenylene	228	242	256		
Benzofluoranthene/Benzo[*e*]pyrene/ Benzo[a]pyrene/ Perylene	252	266	280**		
Benzo[*g,h,i*]perylene/ Indeno[*1,2,3-c,d*]pyrene	276	290**	304**		
Acenaphthylene	152				
Acenaphthene	154				
Fluorene	166				
Dibenz[*a,h*]anthracene	278				
Cyclopenta[*c,d*]pyrene (226)**	226				
Dibenz[*a,l*]pyrene (302)**	302				
Dibenz[*a,e*]pyrene (302)**	302				
Naphtho[2,1-*a*]pyrene (302)**	302				
Dibenz[*a,i*]pyrene (302)**	302				
Dibenz[*a,h*]pyrene (302)**	302				
D_8- Naphthalene*	136				
D_{10}- Biphenyl*	164				
D_{10}- Anthracene*	188				
D_8- Dibenzothiophene*	192				
D_{10}- Pyrene*	212				
D_{12}-Benzo[*a*]pyrene*	264				

* Deuterated PAHs used as internal standards

** Determination not UKAS accredited to the Laboratory

2.3.2.6.3 Gas chromatography-mass selective detection (GC-MSD) geochemical biomarkers

The aliphatic fractions were analysed for steranes and triterpanes by GC – MS in selective ion monitoring mode using an HP6890 Series gas chromatograph interfaced with an HP5973 MS and fitted with an on-column injector. Injections were made at 60 ^{o}C and the

oven temperature held constant for 0.5 minutes after which it was increased at 40 ^{o}C min^{-1} up to 150 ^{o}C. This was followed by a slower ramp at 5 ^{o}C min^{-1} up to a final temperature of 300 ^{o}C and held at this temperature for 22 minutes. The carrier gas was helium set at a constant flow of 0.7 ml min^{-1}. Geochemical biomarker analysis was carried out using the selected ion monitoring mode (SIM). Triterpanes were monitored at *m/z* 191 while steranes were monitored at *m/z* 217 and 218 (Webster *et al*., 2004).

Table **2.2**: List of triterpane and sterane geochemical biomarkers measured using the GC-MSD in SIM mode (α and β signify geometrical isomers)

ID	Name	QIon	Molecular Formula
Ts	18α(H)-22,29,30-trinorhopane	191	$C_{27}H_{46}$
Tm	17α(H)-22,29,30-trisnorhopane	191	$C_{27}H_{46}$
28αβ	17α(H), 21β–28,30-bisnorhopane	191	$C_{27}H_{46}$
29αβ	17α(H),21β(H)-30-norhopane	191	$C_{29}H_{50}$
29Ts	18α(H)-norneohopane	191	$C_{29}H_{50}$
30d	Diahopane	191	$C_{30}H_{52}$
30O	Oleanane	191	$C_{30}H_{52}$
30αβ	17α(H), 21β(H)-hopane	191	$C_{30}H_{52}$
30βα	17β(H), 21α -(H)-hopane	191	$C_{30}H_{52}$
31αβS	17α(H), 21β(H)-22S-homohopane	191	$C_{31}H_{52}$
31αβR	17α(H), 21β(H)-22R-homohopane	191	$C_{31}H_{54}$
30G	Gammacerane	191	$C_{30}H_{52}$
Diploptene	17β (H),21 β H)-hop-22(29)-ene	191	$C_{30}H_{50}$
32αβS	17α(H), 21β(H)-22S-bishomohopane	191	$C_{32}H_{56}$
32αβR	17α(H), 21β(H)-22R-bishomohopane	191	$C_{32}H_{56}$
33αβS	17α(H), 21β(H)-22S-trishomohopane	191	$C_{33}H_{58}$
33αβR	17α(H), 21β(H)-22R-trishomohopane	191	$C_{33}H_{58}$
34αβS	17α(H), 21β(H)-22S-tetrakishomohopane	191	$C_{34}H_{60}$
34αβR	17α(H), 21β(H)-22R-tetrakishomohopane	191	$C_{34}H_{60}$
35αβS	17α(H), 21β(H)-22S-pentakishomohopane	191	$C_{35}H_{62}$
35αβR	17α(H), 21β(H)-22R-pentakishomohopane	191	$C_{35}H_{62}$
C29ααS	5α(H), 14α(H), 17α(H)-24-ethylcholestane (20S)	217	$C_{29}H_{52}$
C29ββS	5α(H), 14β(H), 17β(H)-24-ethylcholestane (20S)	217	$C_{29}H_{52}$
C29ββR	5α(H), 14β(H), 17β(H)-24-ethylcholestane (20R)	217	$C_{29}H_{52}$
C29ααR	5α(H), 14α(H), 17α(H)-24-ethylcholestane (20R)	217	$C_{29}H_{52}$

2.3.3 Method re-validation

Method validation is the process to confirm that the analytical procedure employed for a specific test is reproducible and rugged over the specified range that an analyte will be analyzed and is suitable for its intended use.

Methods need to be validated or revalidated

- before their introduction into routine use
- whenever the conditions change for which the method has been validated, e.g., instrument with different characteristics
- whenever the method is changed, and the change is outside the original scope of the method.

The introduction of cyclopenta[*c,d*]pyrene, dibenzo[*a,e*]pyrene, naphtho[2,1-*a*]pyrene, dibenzo[*a, h*]pyrene, dibenzo[*a, i*]pyrene, and dibenzo[*a, l*]pyrene, to the PAH suite analysed by FRS necessitated the re-validation of the method to allow measurement of these PAHs. The parameters re-validated for are as follows –

- Method Recovery
- Method limit of detection
- Instrument limit of detection
- Methods reproducibility

2.3.3.1 Preparation of the stock solution

Stock solution (20µg ml^{-1}) used for the re-validation exercise was prepared by weighing out approximately 5 mg each of the PAH compound given in Table 2.3 and dissolving them in 250 ml of *iso*-hexane.

Table **2.3**: The PAH compounds used for the preparation of the stock solution

Compound	**Purity**	**Wt. (mg)**	**Conc. (ug/ml)**
Naphthalene	99+	5.24	21.0
2-Methylnaphthalene	99.5	4.90	19.6
1-Methylnaphthalene (Liquid)	99.5	5.67	22.7
2,6-Dimethylnaphthalene	99.8	4.44	17.8
2,3,5-Trimethylnaphthalene (Liquid)	99.4	6.00	24.0
1,4,6,7-Tetramethylnaphthalene	99.0	6.43	25.7
Phenanthrene	99.5	5.85	23.4
Anthracene	99.9	6.38	25.5
2-Methylphenanthrene (C1-178)	**98.4**	**4.53**	**17.8**
3,6-Dimethylphenanthrene (C2-178)	99.8	5.91	23.6
2,6,9-Trimethylphenanthrene (C3-178)	99.3	6.58	26.3
Dibenzothiophene	99+	7.70	30.8
Fluoranthene	99.8	5.12	20.5
Pyrene	99.9	5.60	22.4
1-Methylfluoranthene (C1-202)	99.7	4.74	19.0
2,7-Dimethylpyrene (C2-202)	**97.2**	**4.52**	**17.6**
Benzo[*c*]phenanthrene	99.9	4.48	17.9
1,2-Benz[*a*]anthracene	99.4	4.97	19.9
Chrysene	99.9	4.70	18.8
2-Methylchrysene (C1-228)	99.5	4.37	17.5
Dimethylbenz[*a*]anthracene (C2-228)	99.8	4.61	18.4
Benzo[*b*]fluoranthene	99.9	6.10	24.4
Benzo[*k*]fluoranthene	99.9	4.61	18.4
Benzo[*e*]pyrene	99.4	6.53	26.1
Benzo*[a*]pyrene	99.8	6.84	27.4
Perylene	99.6	5.66	22.6
7-Methylbenzo[*a*]pyrene (C1-252)	99.0	5.08	20.3
Indenopyrene	99.6	4.44	17.8
Benzo[*g,h,i*]perylene	99.7	5.73	22.9
Acenaphthylene	99.5	5.17	20.7
Acenaphthene	99.5	6.63	26.5
Fluorene	99.8	5.93	23.7
Dibenz[ah]anthracene	99.4	5.76	23.0
4-methyldibenzothiophene*	**96.0**	**10.45**	**40.1**
5-methylchrysene	99.5	4.72	18.9
Dibenz[*a,e*]pyrene	99.8	4.70	18.8
Dibenz[*a,i*]pyrene	99.9	4.30	17.2

Dibenz[*a,h*]pyrene	99.8	5.05	20.2
Dibenz[*a,l*]pyrene	99.8	4.74	19.0
Cyclopenta[*c,d*]pyrene	99.5	4.45	17.8
Naphtho-[2,1-*a*]pyrene	**98.9**	**4.35**	**17.2**
Benz[*b*]anthracene	**98.0**	**5.56**	**21.8**
D12-chrysene	99.5	4.47	17.9
D10-fluoranthene	99.5	4.84	19.4
D12-benzo[*e*]pyrene	99.9	5.14	20.6
D10-fluorene	99.0	4.75	19.0

Compound in bold were corrected for purity.

100 ml of the stock solution was transferred to 200 ml flask with addition of 10 ml of toluene and ultra-sonicated for 5 minutes to completely dissolve the PAH compounds. The dissolved solution was made up to the 200 ml mark with *iso*-hexane. From the resulting solution (10µg/ml^{-1}), the working solutions were made.

Table **2.4**: Volumes of solvent (*iso*-hexane) used in preparing the working solutions

Levels	**Volume of stock solution (ml)**	**Final volume (ml)**	**Concentration (ng/ml)**
A	2 ± 0.1	20 ± 0.1	1000
B	2 ± 0.1 (A)	20 ± 0.1	100

2.3.3.2. Analysis for method recovery

Low matrix mussels from Loch Etive shellfish farm was used to analyze for the method recovery. Approximately 10 g of the homogenized mussel tissues in 250 ml round bottom flasks were spiked with the PAH standard solutions to achieve a nominal concentrations of 1ng g^{-1}, 10 ng g^{-1} and 100 ng g^{-1}. The extraction was carried out as detailed in section **2.3.2.5**.

Table **2.5**: Methods recovery of PAHs determined from triplicate analysis of spiked low matrix biota samples.

Compounds	**% Recovery**		
	100 ng g^{-1}	**10 ng g^{-1}**	**1 ng g^{-1}**
Naphthalene	97.5	96.0	102.4
2-Methyl Naphthalene	119.9	115.6	119.4
1-Methyl Naphthalene	117.9	114.7	116.1
C2 Naphthalenes	108.7	123.5	76.7
C3 Naphthalenes	95.6	90.3	72.0
C4 Naphthalenes	103.1	99.2	98.7
Phenanthrene (178)	98.9	99.4	95.0
Anthracene (178)	98.4	98.3	97.8
C1-Phenan/anthracene	114.4	110.5	45.1
C2-Phenan/anthracene	101.5	91.4	30.1
C3-Phenan/anthracene	106.3	98.4	27.6
Dibenzothiophene	93.9	94.2	93.4
C1-Dibenzothiophenes	106.4	112.3	99.7
Fluoranthene (202)	92.1	90.7	92.8
Pyrene (202)	89.4	89.6	98.2
C1-Flouranthene/Pyrene	102.0	85.2	0.0
C2-Flouranthene/Pyrene	114.5	96.9	0.0
Benzo[*c*]phenanthrene (228)	102.6	106.1	112.1
Benz[*a*]anthracene (228)	106.0	104.7	94.2
Chrysene/Triphenylene (228)	98.4	99.5	88.9
Benz[*b*]anthracene (228)	62.9	26.6	5.4
C1-228	117.9	103.7	0.0
C2-228	95.6	85.1	0.0
Benzo[*b*]fluoranthene (252)	100.8	99.1	55.5
Benzo[*k*]fluoranthene (252)	88.2	96.3	113.1
Benzo[*e*]pyrene (252)	88.7	94.7	95.7
Benzo[*a*]pyrene (252)	89.3	90.5	84.6
Perylene (252)	95.4	95.9	98.8
C1-252	105.5	97.6	35.1
Indenopyrene (276)	111.4	106.7	110.7
Benzoperylene (276)	97.0	96.5	97.1
Acenaphthylene (152)	110.7	112.6	100.9
Acenaphthene (154)	110.1	117.6	104.1
Fluorene (166)	89.7	84.7	81.8
Dibenz[*a,h*]anthracene (278)	110.5	105.4	90.5
Cyclopenta[*c,d*]pyrene (226)	193.7	155.7	162.1
Dibenz[*a,l*]pyrene (302)	97.5	88.9	85.3
Dibenz[*a,e*]pyrene (302)	107.4	73.4	60.0
Naphtho[*2,1-a*]pyrene (302)	90.1	142.5	83.5
Dibenz[*a,i*]pyrene (302)	99.5	85.3	69.5
Dibenz[*a,h*]pyrene (302)	71.6	55.0	58.3

2.3.3.3 Instrument limit of detection

The instrument limit of detection was determined by running 7 replicate determinations of a low standard on the same day and the standard deviation expressed as a concentration.

LOD = 4.65 x the standard deviation of the replicate standards.

2.3.3.4 Method limit of detection

The method limit of detection was determined by running 7 replicated determinations of a low matrix sample over several days. The extraction procedure was the same used for the recovery determination.

2.3.3.5 Reproducibility

This was determined by the GC- MSD analysis of 7 replicate determinations of standards at the upper end (90 %) and lower end (10 %) of the calibration range. The analysis was carried out on separate days and expressed as concentrations.

The results obtained from the validation process gave good recoveries for most of the PAH compounds except for the additional suite of PAHs (cyclopenta[*c, d*] pyrene, dibenzo[*a,e*]pyrene, dibenzo[*a,h*]pyrene, dibenzo[*a,i*]pyrene, and dibenzo[*a,l*]pyrene). The method and instrument limits of detection obtained were greater than 1 ng g^{-1} for most compounds. The reason for the poor results was traced to the batch of PAH standards used for the preparation of the stock solution. Therefore, the validation procedure was repeated with a new batch of PAH standards but however, not as part of this project.

CHAPTER THREE

Investigation of PAH depuration kinetics in blue mussels (*Mytilus edulis*)

3.1 Introduction

Shellfish farming in Scottish coastal waters is almost exclusively concerned with mollusc production. The main species are blue lipid mussels *(Mytilus edulis),* native oysters *(Ostrea edulis),* pacific oysters *(Crassostrea gigas),* and king and queen scallops *(Pecten maximus and Aequipecten opercularis).* There were approximately 183 producing shellfish farming companies in Scotland (FRS, 2005). Most suitable sites are found in the West Coast of Scotland; the Hebrides, Orkney and Shetland islands.
In this study, wild mussels collected from Aberdeen harbour were used as indicator organism in experiments devised to determine the rate of elimination of polycyclic aromatic hydrocarbons (PAHs) from aquatic organisms. It has been shown that invertebrates (especially bivalves) have less developed mechanisms to metabolize xenobiotics than vertebrates (Suteau and Narbonne, 1988; McElroy *et al*., 2000; Meador, 2003). Vertebrates (e.g. fish), which have strongly developed mechanism for PAH metabolism, may assimilate large amounts of the compounds, for example through ingestion or exchange across external surfaces, show biological responses, but still not contain large concentrations of the parent compounds. These species of organisms are therefore best suited for monitoring effects of contaminants as the mutagenic metabolites often formed are more toxic than the parent compounds.

The study was carried out in two separate environments. A laboratory experiment in flow-through tank systems, and a field study in an open sea loch. The rationale for the field experiment was to compare the elimination rates obtained in the laboratory with that obtained in the field. Although generally, kinetic data measured in the laboratory are directly applied to the field environment (Thorsen *et al*., 2004), the presence of different variables in the field may influence results. For example, the effect of temperature and presence of other xenobiotics on the filtering rate of mussels have been reported (Gossiaux *et al*., 1996).

Farmed mussels are usually rope-grown in open waters; areas with potential to be affected by accidental oil spill incidents. Their sedentary state compared to mobile organisms like fish and birds makes them more vulnerable to point contaminations and hence applicable to spatial surveys. Knowledge of the fate and elimination rate of hazardous components of oils (e.g. PAHs) from affected organisms will be very useful in protecting consumer safety and in formulating oil spill mitigation policies. The data obtained with mussels can be appropriately extended to other shellfish. As was discussed in Chapter **1**, the concentration of an environmental contaminant in the tissues (tissue burden) of an organism is a good indication of the bioavailable fraction of that contaminant. Therefore, as well as being susceptible to oil pollution as a result of accidental discharges, mussels are ideal organisms for monitoring the concentration and fate of trace organic pollutants and have been widely used in environmental risk assessments and monitoring of trace contaminants (Blumer *et al*., 1970; Webster *et al.*, 2004; McIntosh *et al.*, 2004; Richardson *et al.*, 2005; Page *et al*., 2005). The characteristics of mussel that makes it suitable for monitoring spatial contamination are summarized below.

- It accumulates pollutants in sufficient amount without being killed by the concentrations encountered in the environment
- It is representative of the study area because it is sedentary
- It is available all year round and hence enable continuation of survey
- It is of reasonable size therefore gives adequate tissue for analysis
- It is very easy to sample and adaptive and hence survives in a laboratory environment

3.2 Experimental design

The test and control mussels for the laboratory and the field experiments were collected from areas within Scotland. For the laboratory experiment, wild mussels from Aberdeen harbour and rope-grown mussels from a shellfish farm in Loch Etive were used as the test and control samples respectively. Aberdeen harbour is a world class port situated in the heart of Aberdeen city and handling around 5 million tonnes of cargo, for a wide range of industries, including the offshore oil and gas industry. Loch Etive is located in the West

Coast of Scotland; a site remote from industrial activities. The rationale behind using Loch Etive mussels as control relies on the fact that previous analysis carried out by Fisheries Research Services (FRS), Marine laboratory, Aberdeen, have shown that Loch Etive mussels have very low PAH concentrations (typically < 50.0 ng g^{-1} wet weight); values which are regarded as background levels in environmental samples (Webster *et al*., 2003, and McIntosh, *et al.*, 2004).

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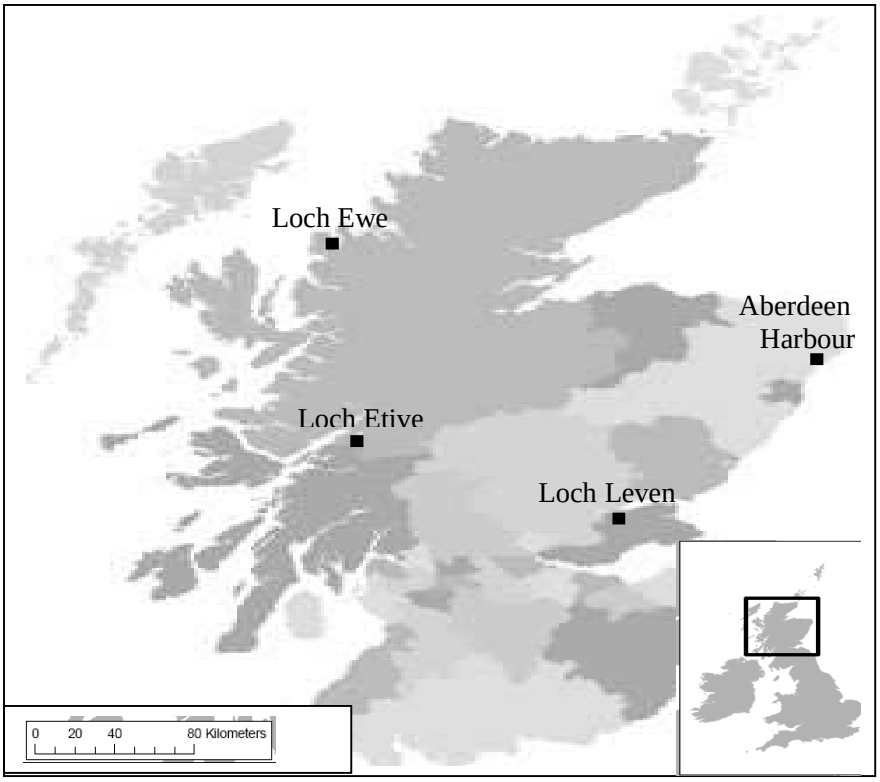


Fig. **3.1**: Map of Scotland showing the approximate locations of the sampling sites.

For the field experiment, wild mussels from Aberdeen harbour and rope-grown mussels from a shellfish farm in Loch Leven were used as the test mussels while native mussels from Loch Ewe were used as the control sample. In the past, mussels sampled from areas in Loch Leven have shown elevated concentrations of the high molecular weight PAHs (4- to 6-rings) dominated by the five ring compounds (McIntosh *et al*., 2004). The source of these compounds has been linked to the effluent resulting from an aluminum smelter which was sited close to the shellfish farm. Loch Ewe is also a relatively clean site also in the West Coast as shown in Fig. **3.1**.

3.2.1 Sample transportation and deployment

The mussels for the laboratory and field experiments were transported in insulated boxes to FRS Aberdeen and FRS Autbea respectively. Cool packs were placed at the bottom of the containers and seaweed placed over the mussels. The mussels were delivered to FRS within 8 h of being removed from the water. Sub-samples were removed from both sample sites before deployment to analyse for the initial concentration (t = 0) of analytes prior to depuration. The remaining mussels were placed in net bags of 25-30 mussels and deployed for depuration. The samples for the laboratory study were placed in glass fibre tanks (360 l) equipped with a flow-through supply (flow rate: 0.6 ± 0.05 l s^{-1}) of filtered seawater from Nigg Bay; a tank each for the test and control mussels. The temperature of the seawater ($13.0 \pm 6^{\circ}$C), salinity (35 parts per thousand) and pH (8.3 ± 0.25) were measured throughout the depuration period. The mussels were fed with *Isocrysis* and *Pavlova lutheri* (from Ardtoe Marine laboratory) on alternate days at a density of 500 ml of culture *per* day. The experiment lasted for 56 days.

Upon delivery to Loch Ewe Aultbea, the samples for the field experiment were placed in holding tanks overnight. The mussels were subsequently deployed on the body of water, suspended from net bags. Each sample site was marked with a colour coded buoy and anchored to prevent the sample being swept away by waves/currents. The native mussels from Aultbea (control samples) were also deployed the same way as the test samples.

3.2.2 Sub – Sampling ad sample preparation

Mussels were removed from each sample site prior to depuration (time = 0) and subsequently after 7, 21, 35 and 56 days of depuration for the laboratory experiment, and after 5, 12, 19, 26, 33, 47 and 68 days for the field experiment. The mussels were opened, the entrained water drained off onto a tissue paper and the total soft tissue excised into solvent washed aluminum cans, homogenized by Ultraturrax$^{(TM)}$ and frozen at -18 to - 20 ^{o}C in a dedicated freezer until required for analysis.

3.3 Analytical methods

The extraction, clean-up and determination methods are detailed in chapter 2. Briefly, the defrosted mussel tissues (5 -10 g) were saponified for 4 h in methanolic sodium hydroxide (NaOH) and liquid-liquid extracted in *iso*-hexane. The extracts were dried with solvent washed sodium sulphate (Na_2SO_4) powder and fractionated into the aliphatic and aromatic portions by isocratic normal phase HPLC. The aliphatic fractions collected between after 150 s were analyzed for *n*-alkanes and geochemical biomarkers using GC-FID and GC-MSD respectively. The aromatic fractions, collected in the second fraction were analyzed for PAHs using GC-MSD.

3.4 Results and Discussion

The polycyclic aromatic hydrocarbons measured in both studies consist primarily of the 2- to 6-ring compounds and include the following families of compounds; naphthalene, phenanthrene/anthracene, fluoranthene/pyrene, fluorene, dibenzothiophene, chrysene/triphenylene, benzofluoranthenes/benzopyrenes, perylene, indonenopyrene/ benzoperylene, and their alkylated compounds. The sum of PAHs as used in this thesis is the total concentrations of the individual compounds determined (see Table **2.2** for details). The PAH concentrations and elimination kinetics determined in both studies are discussed separately in sections **3.4.1** and **3.4.2** and the results compared in section **3.4.3**.

3.4.1 Laboratory experiment

3.4.1.1 PAH concentration and distribution profile

Fig. **3.2** shows the graphical representation of the total PAH concentrations determined for the Harbour and Loch Etive mussels. As mentioned earlier, total PAH as used in this study relates to the sum of the 2- to 6-rings parent compounds and their alkylated compounds as given in Table **2.2**. The harbour mussels returned a total PAH concentration of 1492.8 ng g^{-1} wet weight, prior to depuration (t = 0). This site showed an initial PAH profile

dominated by the 2- and 3-rings PAHs compounds (naphthalenes and alkylated compounds (C1-C4):11.8 %, phenanthrene/ anthracene and alkylated compounds (C1-C3): 48.2 %, and dibenzothiophene and alkylated compound (C1-C3): 17.6 %) with phenanthrene/anthracene and their alkylated compounds accounting for ~ 50 % of the total PAHs determined (Table **3.1**). The total PAH concentration determined for the reference mussels at time (t = 0) was 17.2 ng g^{-1} wet weight. This is likely expected considering the time of the year the samples were collected. It has been shown that seasonal variations affect the PAH tissue burden of mussels and that concentrations are highest during the winter season and lowest during the summer season. The result obtained for the reference mussels is in within the values determined by McIntosh *et al*. (2004) between April 1999 and March 2002 for this site which ranged from 17.0 ng g^{-1} to 150.6 ng g^{-1} wet weight with the highest concentrations found between January and March 2001 during the winter season.

The total PAH concentration (1492.8 ng g^{-1}) of the harbour mussels is considerably higher than those determined for mussels in other locations in Scottish coastal waters by Webster *et al*. (2003) in a 3 months survey carried out between October and December in 1999. The survey showed concentrations ranging from 91.1 ng g^{-1} (Shuna Sound) to 344 ng g^{-1} wet weight (Granton East) , for wild mussel beds, and 8.4 ng g^{-1} (Loch Kentra) to 138 ng g^{-1} wet weight (Olna Firth - Shetland) for cultivated rope grown mussels, respectively (Table 3.2). The PAH concentration found in the Aberdeen harbour mussels was also higher than the maximum (1450 ng g^{-1} wet weight) determined for mussels within the designated FEPA exclusion zone following the *Braer* oil spill in January 1993 (Davies and Topping, 1997). Although most of these sites are remote areas from the cities and hence expected to have low contaminant burden arising from industries, sites like Eden estuary, Granton East, and Fairlie are closer to industries. Therefore, the concentration observed for the harbour mussels is higher than is naturally found in Scottish coastal waters and must have been introduced through anthropogenic sources.

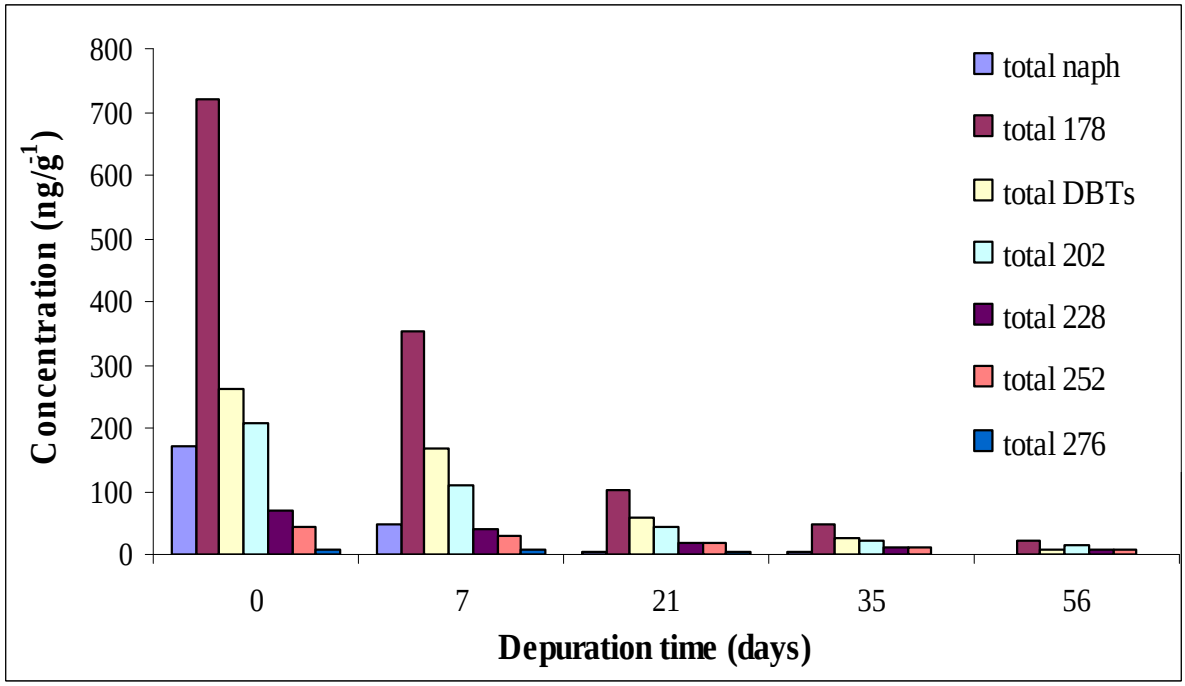


Fig**. 3.2**: Distribution of PAHs sub-groups in Aberdeen Harbour mussels depurated over a period of 56 days in laboratory flow-through tank system.

Note: [∑Naph = naphthalenes (parent and C1-C4); ∑178 = Phenanthrene/anthracene (parent and C1-C3); ∑DBT = Dibenzothiophene (parent and C1-C3); ∑202 = Fluoranthene/pyrene (parent and C1-C3); ∑228 = Benzanthracenes/benzophenanthrene /chrysene/triphenylene (parent and C1-C2); ∑252 = Benzofluoranthenes/benzopyrenes/perylene (parent and C1-C2); ∑276 = Indenopyrene/benzoperylene (parent and C1-C2)].

Table **3.1**: % PAH subgroups, % of parent PAHs, and sum of parent PAHs in Aberdeen harbour and Loch Etive mussels.

PAH compound	**Depuration time (days)**					
	Aberdeen Harbour					**Loch Etive**
	0	**7**	**21**	**35**	**56**	**0**
% 128	11.6	6.4	1.1	1.0	1.0	11.1
% 178	48.2	47.2	41.8	38.7	34.2	33.1
% DBTs	17.6	22.1	23.2	21.1	10.0	6.4
% 202	13.8	14.2	17.4	20.2	27.2	21.5
% 228	4.5	5.2	8.0	9.4	12.0	8.7
% 252	3.0	3.9	7.1	8.2	13.0	15.1
% 276	0.5	0.8	1.2	1.4	2.5	2.9
% Acenaphthylene (152)	0.0	-	-	-	-	-
% Acenaphthene (154)	0.5	-	-	-	-	-
% Fluorene (166)	0.30	0.04	0.08	-	-	1.2
% Dibenz[a,h]anthracene (278)	0.02	0.04	0.08	-	-	-
∑ Parent	174.1	71.7	30.9	18.5	13.8	6.5
% Parent	11.7	9.5	12.5	16.0	23.0	37.8
% 2- and 3-ring	78.1	75.6	66.4	61.3	45.2	51.7
% 4- to 6-ring	21.9	24.4	33.6	38.7	54.8	48.3

Table **3.2**: Total PAH concentrations (ng g^{-1} wet weight) in mussels collected around Scotland over a three month period in 1999

Location	Total [PAH]/ng g^{-1} wet weight
*Fairlie	300.3
Loch Drovinish	13.8
*Scapa Flow (Orkney)	140.5
*Shuna Sound	91.1
*Granton East	344.1
Loch Eishort	45.2
Loch Caroy	27.3
Olna Firth (Shetland)	138.8
*Eden Estuary	178.8
Bracadale	33.3
Loch Greshornish	18.6
Loch Torranish	47.5
Glenuig Bay	16.8
Loch Kentra	8.4
Milovaig	28.6
Barraglom	44.2
Loch Beag	22.5

*denotes cultivated rope-grown mussels beds (Webster *et al*., 2003).

However, higher PAH concentrations have been reported in some areas around Scotland and these have been traced successfully to their input sources. McIntosh *et al*. (2004) reported a PAH concentration of 8256 ng g^{-1} wet weight in mussels collected from a shellfish farm in Kinlochleven in September 1999 before the closure of an aluminum smelter located near the commercial mussel farm. According to their report, this high concentration was as a result of effluent discharged from the aluminum smelter and the PAH profile was dominated by the 5-ring compounds. In another instance, Webster *et al*., (2003) reported a PAH concentration of 1537 ng g^{-1} and 7177 ng g^{-1} wet weight

respectively in mussels collected from Long Hope (Orkney) and Dury Voe (Grunna) in Shetland island in 1998. According to the report, the aliphatic, PAHs and geochemical biomarker profile of the samples suggest petrogenic contamination of these sites.

3.4.1.2 Kinetics of loss of PAHs

Upon transfer of the harbour mussels to the flow through system, PAHs exhibited a rapid decrease in concentrations over the first 3 weeks after which the decrease becomes gradual and then reduced to a minimum (Fig. **3.2**), except for the DBTs which maintained a faster and steady rate of elimination throughout the depuration period compared to other PAH subgroups. Over 70 % reduction in concentration was observed for the naphthalenes (98 %), phenanthrenes (85.7 %), and the DBTs (78.2 %) within 21 days of depuration. The total PAHs concentration decreased from 1492.8 ng g^{-1} wet weight to 59.9 ng g^{-1} wet weight during the 56 days of depuration (Appendix **2**).

Various rate constant models as described in chapter **1** have been used to evaluate the kinetics of elimination of hydrophobic contaminants in invertebrates (Sericano *et al*., 1996; Gewurtz *et al*., 2002). In one compartment model, the organism is treated as a homogenous compartment, and many authors have reported the adequacy this model in estimating the kinetic of elimination of hydrophobic contaminants (PAHs, PCBs and OCPs) from organisms, for example Pruell *et al*., 1986, McIntosh *et al.*, 2004 and Richardson *et al.*, 2005). In this study first order kinetics as described by Sericano and Richardson was used and assuming no uptake from the seawater during depuration.

In a two component model described by Zitko (1980), and modified in Richardson *et al.*, (2005), the uptake (k_1) and elimination (k_2) rate constants were given as

$$\textit{Water (dissolved phase)} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \textit{mussel}$$

The equation for the exposure is given as:

$$C_m = C_w . K_{bc} 1 - e^{-k_2 t} \quad \text{..... (a)}$$

where C_m and C_w are contaminant concentrations in mussels (ng g^{-1} wet weight) and in dissolved phase (ng mL^{-1}), respectively, and t is the exposure time (day). From this the equilibrium bioconcentration factor was defined as

$$K_{bc} = C_m / C_w = k_1 / k_2 \quad \text{..... (b)}$$

Combining equations (a) and (b) gives

$$C_m = C_w . k_1 / k_2 . (1 - e^{-k_2 t}) \quad \text{..... ..... (c)}$$

The elimination rate constant (k_2) is estimated from the depuration period, during which C_w = 0 and equation (a) is altered to an exponential decay equation.

$$C_m = C_{m0} . e^{-k_2 . t} \quad \text{..... (d)}$$

where C_{m0} is the initial analyte concentration in mussel prior to depuration. This curvilinear relationship was simplified by transformation into a logarithmic linear equation to give

$$\ln C_m = -k_2 t + \ln C_{m0} \quad \text{..... (e)}$$

The depuration rate constants for individual PAH compound was evaluated from a plot of *ln* C_m against depuration time, t (days) (see example graph in Fig. **3.3**)

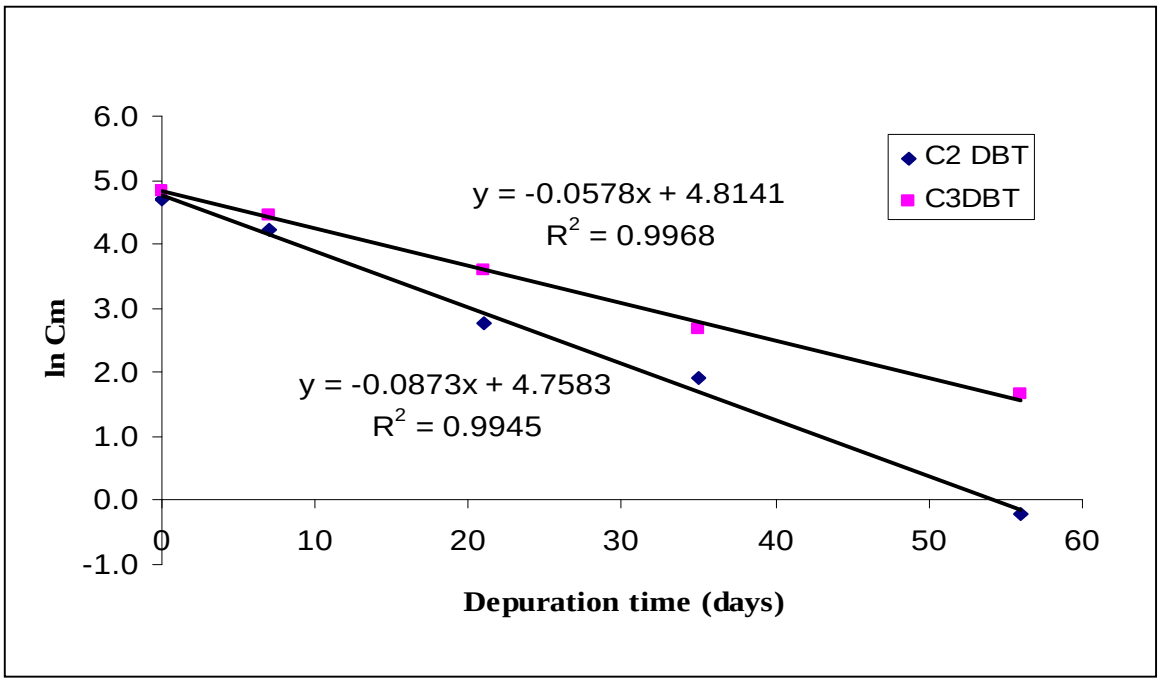


Fig. **3.3**: Logarithmic graph representing the elimination kinetics of the C2 and C3 dibenzothiophenes (where C_m = PAH concentration in ng g^{-1} wet weight).

The elimination rate constants (k_2), correlation coefficients (r^2) and biological half-lives ($t_{1/2}$) of 28 PAH are presented in (Table **3.3**). The k_2 for naphthalene, 1-methyl naphthalene, 2-methyl naphthalene, dibenzothiophene, acenaphthalene, and acenaphthylene could not be evaluated because they were rapidly loss within the first two weeks of depuration.

Table **3.3**: Elimination rate constant (k_2), correlation coefficient (r^2) and biological half-lives ($t_{1/2}$) determined for individual PAH assuming first order depuration.

PAH Compounds	**[a]log K_{ow}**	**r^2**	**k_2**	***p*-values (n=5)**	**($t_{1/2}$)**
C2-Naphthalenes	4.37	0.71	0.056	0.071	12.4
C3-Naphthalenes	5.00	0.80	0.093	0.040	7.5
C4-Naphthalenes	5.55	0.99	0.184	0.006	3.8
Phenanthrene (178)	4.57	0.59	0.051	0.127	13.6
Anthracene (178)	4.54	0.89	0.050	0.056[c]	13.9
C1-Phenan/anthracene	5.14	0.79	0.062	0.044	11.2
C2-Phenan/anthracene	5.51	0.94	0.068	0.007	10.2
C3-Phenan/anthracene	6.00	0.99	0.061	0.000	11.3
C1-Dibenzothiophenes	4.86	0.95	0.059	0.024 [c]	11.7
C2-Dibenzothiophenes	5.50	0.99	0.087	0.000	7.9
C3-Dibenzothiophenes	5.73	1.00	0.058	0.000	12.0
Fluoranthene (202)	5.22	0.85	0.067	0.027	10.4
Pyrene (202)	5.18	0.79	0.044	0.044	15.8
C1-Fluoranthene/Pyrene	5.72	0.90	0.049	0.014	14.2
C2- Fluoranthene/Pyrene	6.03	0.96	0.043	0.003	16.2
C3- Fluoranthene/Pyrene*	-	0.97	0.034	0.003	20.3
Benzo[c]phenanthrene (228)	5.76	0.96	0.043	0.003	16.2
Benz[*a*]anthracene (228)	5.91	0.81	0.034	0.038	20.6
Chrysene/Triphenylene (228)	5.86	0.91	0.048	0.011	14.5
Benz[*b*]anthracene (228) *	-	0.90	0.059	0.013 [c]	11.7
C1-228	6.42	0.98	0.040	0.002	17.4
C2-228	6.88	0.93	0.032	0.007	22.0
Benzofluoranthenes (252)	-	0.94	0.041	0.006	16.9
Benzo[*e*]pyrene (252)	6.20	0.96	0.022	0.003	31.0
Benzo[*a*]pyrene (252)	6.04	0.75	0.037	0.057	18.7
Perylene (252)	6.30	0.93	0.030	0.008	23.1
C1-252	-	0.92	0.029	0.010	23.5
C2-252*	-	0.87	0.029	0.022	24.1
Indeno[*123, cd*]pyrene (276)	7.00	0.89	0.039	0.019	17.7
Benzo[*ghi*]perylene (276)	6.50	0.88	0.024	0.017	29.5
C1-276*	-	0.81	0.034	0.037	20.3

* Compounds not UKAS accredited to FRS, [a] Log K_{ow} of PAHs are from Sangster (2005), [b]from Neff and Burns (1996), n = number of data points making up plot, ([c] n = 4).

Elimination rate constants of other compounds ranged from 0.02 day^{-1} for benzo[*e*]pyrene to 0.18 day^{-1} for C4- naphthalene. The correlation coefficient of all compounds were good (≥ 0.71, p-values ≤ 0.07) with the exception of phenanthrene ($r^2 = 0.59$, p-value 0.127). The high r^2 values implied that the actual elimination kinetics is in good agreement with the mathematical model [equation (d)] applied. The rate of loss was found to decreases with increase in molecular weight and hence hydrophobicity e.g. from naphthalenes (2-rings) to benzo[*e*]pyrene (5-rings) and this is in agreement with literature reports (e.g. McIntosh *et al*., 2004, Gewurtz *et al*., 2002). However, the k_2 values are different from those reported by Gewurtz *et al.* (2002) and Thorsen *et al.* (2004) for the individual compounds in freshwater mussels (*Elliptio Complanata)*. While Gewurtz *et al*. (2002) reported k_2 range of 0.10 to 0.22 day^{-1}, Thorsen *et al*., (2004) reported values of 0.04 to 0.26 $day^{-1.}$ The reason for the differences observed between the values obtained in this study with the values obtained in the studies mentioned may be due to the differences in the specie of mussels used and the length of exposure of the organism to the contaminant. Thorsen *et al*. (2002) depurated mussels exposed for 10 days to creosote contaminated sediment and while Gewurtz *et al*. (2002), depurated mussels exposed to PAH compounds for 5 days. Jackim and Wilson (1977), and Sericano *et al*. (1996) reported lower depuration rates of PAHs from chronically exposed bivalves than those from acute exposure. The mussels from Aberdeen harbour have been exposed to chronic contamination, and this may account for the lower depuration rates observed.

The biological half-lives ($t_{1/2}$) evaluated for individual PAH compound in this study ranged from 3.8 days for C4-naphthalene to 31 days for benzo[*e*]pyrene. Gewurtz *et al*. (2002), reported $t_{1/2}$ of 3.2 days for flourene to 18.7 days for benzo[*k*]fluoranthrene (benzo[*e*]pyrene was not measured in that study). However, the half-lives measured in this study are close to those reported by Pruell *et al*. (1986) and Sericano *et al*. (1996). While Pruell *et al*. (1986) reported PAH half-lives range of 14 – 30 days for benzo[*e*]pyrene, chrysene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrent, benz[*a*]anthracene and Fluoranthene in blue mussels (*Mytilus edulis*) exposed for 40 days to contaminated sediment, Sericano *et al*. (1996), reported half-lives of 9 to 26 days and 10-32 days for the same group of compounds in transplanted (exposed for 48 days) and chronically exposed indigenous American Oyster *(Crassostrea virginica)* .

The implication of the variations in the reported results may be that in addition to the effect of the compounds hydrophobicity, other factors such as the specie of organism used, the PAH sources, and the length of exposure of organism to the contaminant may effect the depuration of these compounds.

To assess the role of chemical hydrophobicity on elimination kinetics of PAHs, k_2 values for 24 compounds except C4-naphthalene (value obtained not very reliable) obtained in this study were plotted as a function of the octanol/water partition coefficient water (K_{ow}) (Fig **3.5**). The linear regression equation for this plot gives the relationship; $k_2 = 0.1305 - 0.0143(\log K_{ow})$. The plot shows a declining trend of k_2 values with increasing values of *log* K_{ow} and therefore the dependency of PAH elimination on hydrophobicity (p-value = 0.001, $r^2 = 0.37$). This trend is similar to the trend observed in most literature reports as presented in Table **3.4.**

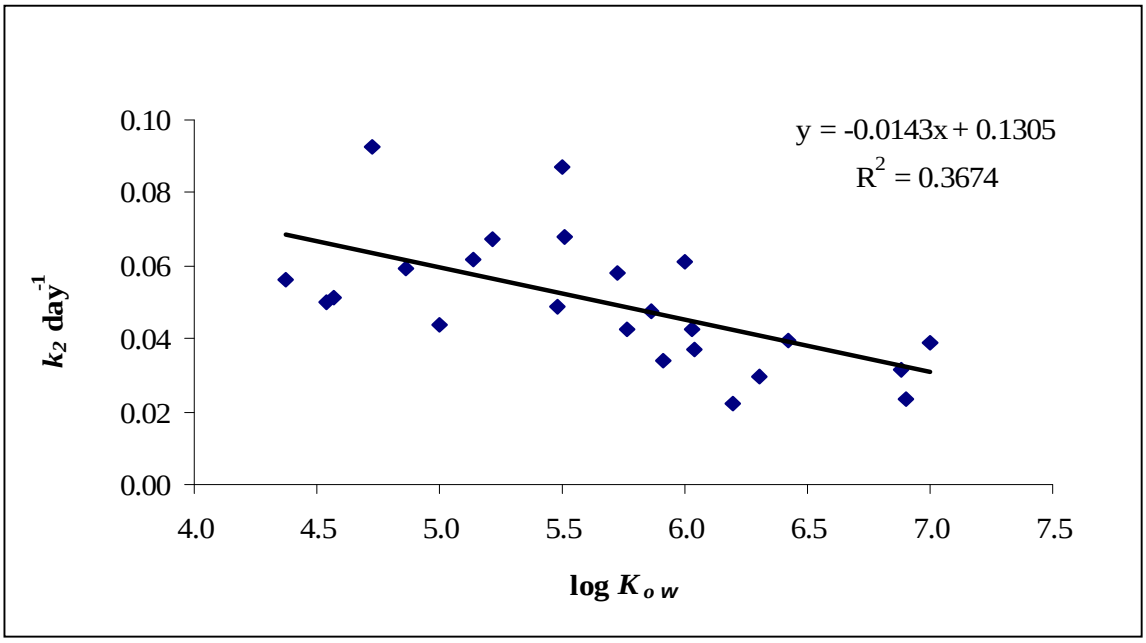


Fig. **3.4**: A regression plot of elimination rate constants (k_2) against log K_{ow} of 25 PAH compounds for the harbour mussels. The mussels were depurated for 56 days in a flow-through tank system in the laboratory.

Table **3.4**: Relationship between elimination rate constants (k_2) and log K_{ow} of hydrophobic organic contaminants in different species of invertebrates. Table adopted from Gewurtz *et al*. (2002).

Species	Com	n	Regression equation	r^2	Experimental conditions	Ref.
Eastern Elliptio (*Elliptio complanata*)	PAHs, PCBs, OCs	11	$k_2 = 0.34 - 0.04\log K_{ow}$	0.44	Lab studies, exposure through water for 5 and 11 days, temperature = 17 ºC and 20 ºC	a,b
Zebra mussel (*Dreissena polymorpha)*	PCB	35	$k_2 = 0.39 - 0.05\log K_{ow}$	0.59	Lab study, exposed in field for 2 days, temperature = 13 ºC	C
Green lipid mussel (*Perna viridis*)	PCB	72	$k_2 = 0.39 - 0.05\log K_{ow}$	0.50	Field study, 17 days exposure	D
Asian clam (*Corbicula fluminea*)	PAH	4	$k_2 = 0.50 - 0.08\log K_{ow}$	0.46	Lab study, exposure via sediment for 30 days, temperature = 20 ºC	E
Eastern oysters (*Crassostrea virginica*)	PAH	7	$k_2 = 0.60 - 0.09\log K_{ow}$	0.75	Lab study, exposure via sediment for 28 days temperature = 25º C	F
Hard clam (*Merceneria mercerneria*)	PAH	7	$k_2 = 0.60 - 0.09\log K_{ow}$	0.01	Lab study, exposure via sediment for 28 days temperature = 25 ºC	F
Eastern oysters (*Crassostrea virginica*)	PAH	7	$k_2 = -0.05 + 0.02\log K_{ow}$	0.26	Field study, 48 days exposure	G
Eastern oysters (*Crassostrea virginica*)	PAH	7	$k_2 = -0.06 + 0.02\log K_{ow}$	0.32	Field study, chronic exposure	G
Blue mussels (*M .edulis*)	PAH	9	$k_2 = -0.04 + 0.01\log K_{ow}$	0.33	40 days exposure in lab through sediment, temperature = 15 ºC	H
*Unionid mussel (*Elliptio complanata*)	PAH		$k_2 = -0.056 + 0.44\log K_{ow}$	0.83	Lab exposure to creosote contaminated sediment for 10 days	I
*Blue mussels (*Mytilius edulis*)	PAH		$k_2 = 0.13 - 0.02\ (\log K_{ow})$	0.37	Lab depuration of Chronically exposed organisms	J

[a]Gewurtz *et al*.(2002), [b]Russell and Gobas, (1989), [c]Morrisson *et al*.(1995), [d]Tanabe *et al.(*1987), [e]Narbonne *et al*.(1999), [f]Bender *et al*.(1988), [g]Sericano *et al*.(1996), [h]Pruell *et al*. (1986), [i]Thorsen *et al*. (2004), [j]this study, *not in the original table.

3.4.2 The Field study

3.4.2.1 PAH concentration and distribution profile

The total PAH concentration determined in mussels from the three sites studied; Aberdeen harbour, Loch Leven and Loch Ewe are summarized in Table **3.4**. When this study was initiated in October 2006, the total PAH concentration found in mussels collected from Aberdeen harbour, Loch Leven shellfish farm and Loch Ewe were 3734.8 ± 216.0 ng g^{-1}, 124.1 ± 3.6 ng g^{-1} and 23.9 ± 2.0 (n = 3) respectively. The harbour mussels' initial (t = 0) PAH profile showed dominance of the 2- and 3-ringss compounds (naphthalenes 8.6 %, phenanthrenes 54.4 %, dibenzothiophenes 14.8 %) over the 4- to 6-rings compounds, with phenanthrene/anthracene and their alkylated compounds accounting for over 50 % of the total PAHs determined (Table **3.5**). In contrast, a totally different PAH distribution was observed for the Loch Leven mussels (t = 0). The initial profile of these mussels showed dominance of the 4- to 6-ring compounds (95.5 %), with the 5-ring compounds accounting for ~ 60 % of the total PAH determined (Table **3.5**). The proportion of the 5-ring compound found in the Loch Leven mussels in this study is in agreement with former studies carried out around Loch Leven pre-and post closure of the aluminium smelter sited close to the sample sites (McIntosh *et al*., 2004).

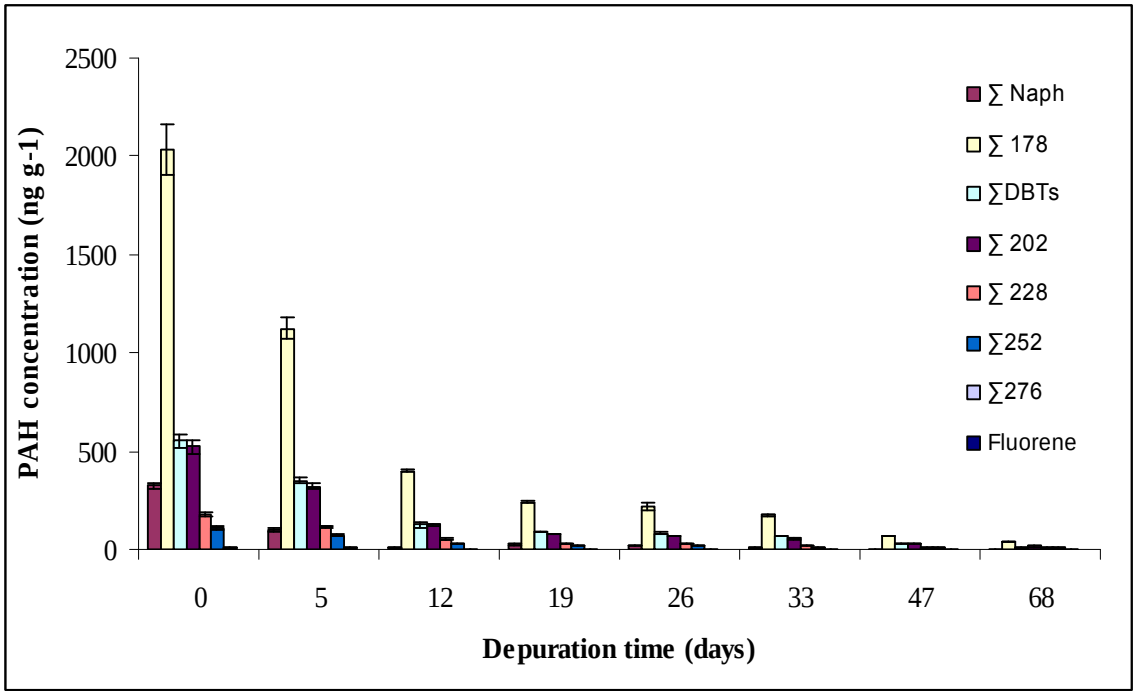


Fig**. 3.5**: Distribution of PAHs subgroups in Aberdeen Harbour mussels depurated over a period of 68 days in Loch Ewe.

The total PAH concentration determined for the reference mussels at time (t = 0) was 23.9 ± 2.0 ng g^{-1} wet weight. This is typical for Loch Etive mussels, collected at this time of year. Loch Etive has been used as reference site by FRS for environmental monitoring since 1999 (McIntosh *et al*., 2004)

Upon deployment of the mussels to Loch Ewe, a rapid loss of PAHs from the harbour (> 79 %) and Loch Leven mussels (> 56 %) was observed within 12 days of depuration (Fig. **3.5** and **3.6**), and subsequently, a slower reduction in total PAHs.

Table 3.5: % by concentration of PAH sub-groups, % 2-and 3-ring PAHs, % 4-to 6-ring PAHs for Aberdeen harbour and Loch Leven mussels.

PAHs groups/ Dep. time (days)	**Aberdeen Harbour**								**Loch Leven**							
	0	5	12	19	26	33	47	68	0	5	12	19	26	33	47	68
% 128	8.6	4.9	1.5	5.3	4.9	3.9	2.9	2.7	1.0	2.1	2.6	6.8	8.3	7.5	8.7	3.7
% 178	54.4	53.7	53.0	49.5	49.5	49.3	47.7	45.4	3.1	4.6	8.1	11.8	18.9	17.4	18.3	12.8
% DBT	14.8	16.7	16.6	18.7	18.9	19.8	18.2	16.1	0.6	1.5	2.2	3.7	6.9	5.9	6.0	5.9
% 202	14.0	15.3	17.0	16.0	15.9	16.1	18.5	18.1	9.9	7.5	8.3	9.0	13.0	15.1	21.1	20.2
% 228	4.7	5.5	7.1	6.3	6.3	6.5	8.0	10.0	12.0	11.2	11.8	11.0	12.5	16.0	13.7	18.2
% 252	2.9	3.5	4.3	3.8	4.0	4.1	4.4	7.2	59.9	61.1	58.7	48.4	34.3	33.1	30.6	35.2
% 276	0.4	0.3	0.4	0.3	0.4	0.3	0.1	0.5	11.3	10.4	7.7	8.4	5.4	4.2	1.6	4.0
% Acenaphthylene	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
% Acenaphthene	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
% Fluorene	0.1	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total PAHs	3734.8	2097.0	752.2	485.3	437.8	352.7	146.0	83.0	89.8	53.8	35.8	30.8	14.9	12.7	10.5	8.6
% parent	9.3	8.8	8.8	8.1	8.1	8.3	10.1	12.4	72.4	70.4	66.0	56.7	43.8	44.1	49.8	47.4
% 2- and 3-rings	78.0	75.3	71.2	73.5	73.3	73.1	68.9	64.2	4.6	8.2	12.8	22.3	34.1	30.9	33.0	22.4
% 4 to 6 rings	22.0	24.7	28.9	26.5	26.7	26.9	31.1	35.8	95.5	91.9	87.2	77.7	65.9	69.1	67.0	77.6

However, an overall gradual decrease in total PAHs concentration was observed throughout the depuration period for the test mussels. The total PAHs concentration of the harbour mussels decreased from 3734.8 ± 216.05 ng g^{-1} to 83.03 ± 0.5 ng g^{-1} wet weight and Loch Leven mussels from 124.1 ± 3.6 ng g^{-1} to 18.17 ± 0.68 ng g^{-1} wet weight after 68 days of depuration.

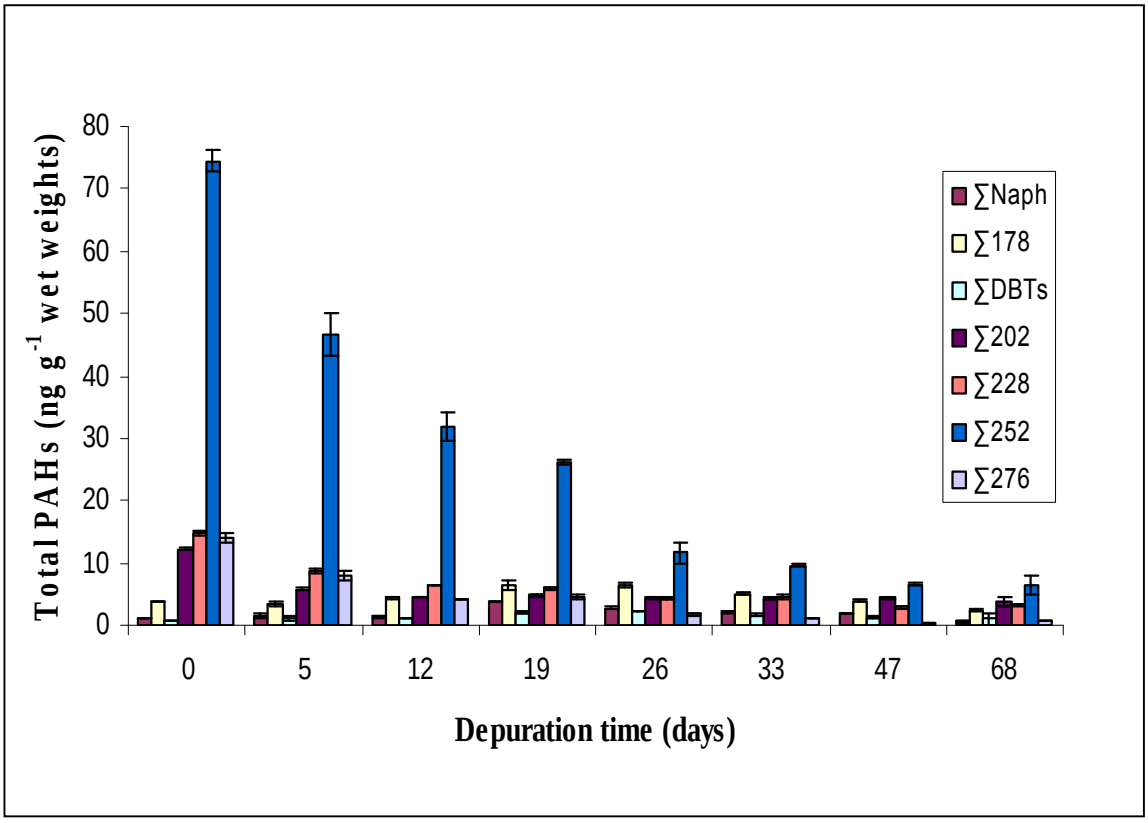


Fig**. 3.6**: Distribution of PAHs subgroups in Loch Leven mussels depurated over a period of 68 days

3.4.2.2 Kinetics of loss of PAHs

As with the laboratory experiment, first order kinetics was applied to calculate the rate of depuration of individual PAH compound (see the details of the theory and any assumptions in section **3.4.1.2**).

The elimination rate constants (k_2), correlation coefficients (r^2) and biological half-lives *($t_{1/2}$)* of 30 PAH investigated are presented in (Table **3.6**). Elimination rate constants calculated from the harbour mussels ranged from 0.06 day^{-1} for pyrene to 0.04 day^{-1} for Benzo[*a*]pyrene. The k_2 for naphthalene, 1-methyl naphthalene, 2-methyl naphthalene, phenanthrene, dibenzothiophene, acenaphthalene, and acenaphthylene could not be evaluated because they were rapidly loss within the first two weeks of depuration. The correlation coefficients of all compounds were good (≥ 0.63). As with the laboratory experiment, the high r^2 values implied that the actual elimination kinetics is in good agreement with the mathematical model applied. The biological half-lives ($t_{1/2}$) evaluated for individual PAH compound from the harbour mussels ranged from 11.1 days for pyrene to 19.6 days for benzo[*a*]pyrene.

The elimination rate constants of individual PAHs obtained from the Loch Leven mussels are also presented in Table **3.2**. Apart from the 5-ring and 6-ring compounds which returned half life values close to those obtained from the harbour mussels, a number of the compounds returned values that are unreliable. The $t_{1/2}$ (shown in italics) are unreliable and may be as a result of the initial concentration of these compounds prior to depuration which were already close to the background concentrations found in mussels ($\sum 202 - 12.5$ ng g^{-1}, $\sum 228 - 15.4$ ng g^{-1} wet weight, etc).

The $t_{1/2}$ for some of the compounds are close to the values obtained by McIntosh *et al*. (2004) from 122 days depuration of mussels from two Loch Leven shellfish farms in the laboratory aquaria, e.g. chrysene, benzo[*c*]phenanthrene, C2-Naphthalenes and C4-Naphthalenes (Table **3.7**)

Table **3.6**: PAHs eimination rate constant (k_2), correlation coefficient (r^2) and biological half-lives ($t_{1/2}$) determined fro Aberdeen mussels and mussels collected fro Loch Leven ad depurated in clean environment in Loch Ewe.

PAH Compounds	**[a]Log K_{ow}**	**Aberdeen harbour**				**Loch Leven**			
		r^2	**k_2**	**$t_{(1/2)}$**	**p-values**	**r^2**	**k_2**	**$t_{(1/2)}$**	**p-values**
Naphthalene	3.37	ND	ND	ND	ND	ND	ND	ND	ND
2-methynaphthalene	4.00	ND	ND	ND	ND	ND	ND	ND	ND
1-methylnaphthalene	3.87	ND	ND	ND	ND	ND	ND	ND	ND
C2-Naphthalenes	4.37	0.63	0.045	15.3	0.019	0.38	0.012	*57.0*	0.104
C3-Naphthalenes	4.73 [b]	0.76	0.062	11.2	0.005	0.02	0.004	*171.5*	0.716
C4-Naphthalenes	5.55	0.79	0.065	10.6	0.003	0.12	0.010	*71.8*	0.399
Phenanthrene (178)	4.57	0.67	0.046	15.1	0.013	0.74	0.009	*76.1*	0.006
Anthracene	4.54	ND	ND	ND	ND	ND	ND	ND	ND
C1-Phenan/anthracene	5.14	0.83	0.062	11.2	0.002	0.27	0.009	*76.2*	0.190
C2 -Phenan/anthracene	5.51	0.91	0.060	11.5	0.000	0.02	0.004	*188.3*	0.719
C3-Phenan/anthracene	6.00	0.95	0.050	13.7	0.000	0.04	0.003	*270.0*	0.657
Fluorene	4.18	0.67	0.059	11.7	0.046	ND	ND	ND	ND
Dibenzothiophene	4.49	0.634	0.058	11.9	0.058	ND	ND	ND	ND
C1 Dibenzothiophenes	4.86	0.88	0.065	10.7	0.001	0.18	0.005	*147.9*	0.296
C2 Dibenzothiophenes	5.50	0.94	0.057	12.1	0.000	0.04	0.003	*204.1*	0.605
C3 Dibenzothiophenes	5.73	0.95	0.047	14.7	0.000	0.21	0.010	*69.5*	0.255
Fluoranthene (202)	5.22	0.78	0.054	12.9	0.004	0.23	0.008	*85.9*	0.234

Pyrene (202)	5.18	0.86	0.062	11.2	0.001	0.44	0.014	*49.9*	0.072
C1-Flouranthene/Pyrene	5.72	0.95	0.056	12.3	0.000	0.40	0.011	*66.0*	0.092
C2-Flouranthene/Pyrene	-	0.93	0.044	15.8	0.000	0.45	0.012	*56.3*	0.069
C3-Flouranthene/Pyrene	-	0.93	0.043	16.2	0.000	0.60	0.015	*46.1*	0.024
Benzo[*c*]phenanthrene (228)	5.76	0.94	0.045	15.4	0.000	0.59	0.017	*41.1*	0.027
Benz[*a*]anthracene (228)	5.91	0.87	0.049	14.3	0.001	0.60	0.017	*40.4*	0.025
Chrysene/Triphenylene (228)	5.86	0.88	0.049	14.1	0.001	0.64	0.018	*38.2*	0.017
Benz[*b*]anthracene (228)	-	0.91	0.046	15.1	0.001	ND	ND	ND	ND
C1-228	6.42	0.94	0.046	15.2	0.000	0.83	0.027	25.9	0.002
C2-228	6.88	0.86	0.037	18.6	0.001	0.55	0.020	34.1	0.035
Benzofluoranthenes (252)	-	0.86	0.046	15.2	0.001	0.83	0.043	16.0	0.002
Benzo[*e*]pyrene (252)	6.20	0.91	0.038	18.4	0.000	0.89	0.033	21.1	0.000
Benzo[*a*]pyrene (252)	6.04	0.68	0.035	19.6	0.012	0.70	0.030	22.8	0.010
Perylene (252)	6.30	0.85	0.036	19.4	0.001	0.73	0.024	28.7	0.015
C1-252	-	0.84	0.047	14.7	0.001	0.86	0.042	16.5	0.001
C2-252	-	0.88	0.055	12.5	0.002	0.29	0.005	*139.4*	0.216
Indenopyrene (276)	7.00	0.83	0.046	15.1	0.005	0.84	0.044	15.8	0.001
Benzoperylene (276)	6.90[b]	0.87	0.048	14.5	0.001	0.79	0.049	14.1	0.003
C1-276	ND	ND	ND	ND	ND	0.60	0.053	13.1	0.125

[a] From Neff and Burns (1996), [b] Sangster, 2005, ND -not determined

Table **3.7**: Comparison of the $t_{1/2}$ values of individual PAH compounds obtained for Loch Leven in this study with values by McIntosh *et al*. (2004).

PAH compound	This study	Kinlochleven	Ballachulish
Naphthalene	NC	35	44
2-Methynaphthalene	NC	276	210
1-Methylnaphthalene	NC	83	141
C2-Naphthalenes	57.0	68	67
C3-Naphthalenes	171.5	46	36
C4-Naphthalenes	71.8	78	102
Phenanthrene (178)	76.1	14	16
Anthracene	NC	10	13
C1-Phenan/anthracene	76.2	14	21
C2-Phenan/anthracene	188.3	26	40
C3-Phenan/anthracene	270.0	47	50
Fluorene	NC	39	42
Dibenzothiophene	147.9	21	27
C1-Dibenzothiophenes	204.1	26	70
C2-Dibenzothiophenes	69.5	43	139
C3-Dibenzothiophenes	85.9	116	159
Fluoranthene (202)	49.9	13	20
Pyrene (202)	66.0	19	28
C1-Flouranthene/Pyrene	56.3	29	37
C2- Flouranthene /Pyrene	46.1	34	43
Benzo[*c*]phenanthrene (228)	41.1	40	63
Benz[*a*]anthracene (228)	40.4	62	59
Chrysene/Triphenylene (228)	38.2	45	41
Benz[*b*]anthracene (228)	NC	28	49
C1-228	25.9	48	45
C2-228	34.1	68	66
Benzo[*b*]fluoranthenes (252)	16.0*	130	22
Benzo[*k*]fluoranthenes (252)		62	57
Benzo[*e*]pyrene (252)	21.1	54	67
Benzo[*a*]pyrene (252)	22.8	48	41

Perylene (252)	28.7	91	141
C1-252	16.5	90	94
C2-252	139.4	66	161
Indenopyrene (276)	15.8	36	46
Benzoperylene (276)	14.1	47	87
C1-276	13.1	79	153

*measured as benzofluoranthenes, NC; no value calculated

To assess the role of chemical hydrophobicity on elimination kinetics of the PAHs, k_2 values of 26 compounds of known log K_{ow} obtained in this study (from the harbour mussels) were plotted as a function of chemical K_{ow} (Fig. **3.3**). The plot shows a declining trend of k_2 values with increasing values of *log* K_{ow} and the linear regression analysis of k_2 versus log K_{ow} shows a dependency of PAH elimination on hydrophobicity ($r^2 = 0.35$, p-value 0.001) with a regression equation: $k_2 = 0.0899\text{-}0.007(\log K_{ow})$. This trend is similar to that reported by Pruell *et al*. (1986) who found that $k_2 = 0.011(\log K_{ow})$ -0.04 from the depuration of *Mytilus edulis* exposed to contaminated sediment for 40 days.

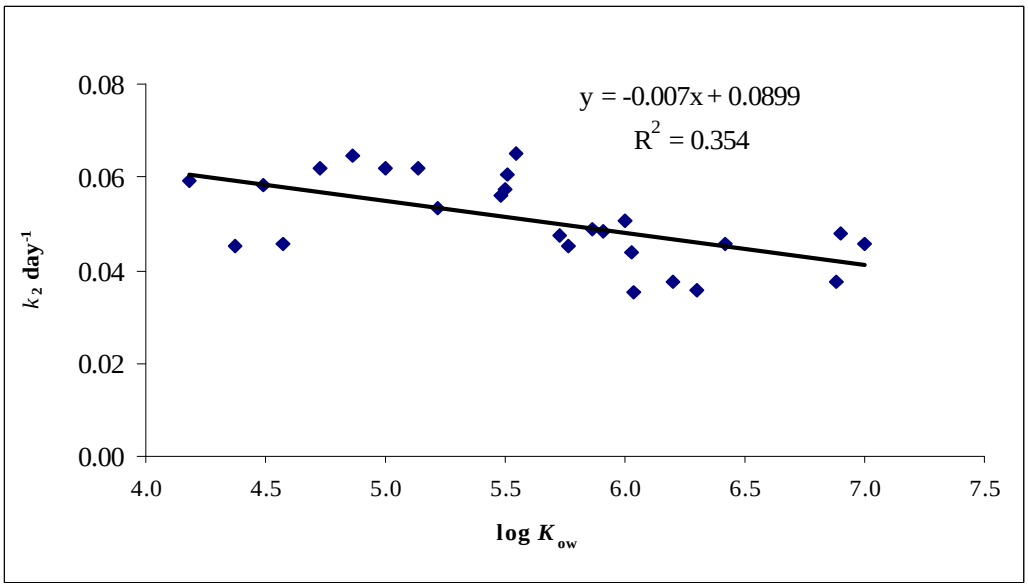


Fig**. 3.7**: A regression plot of elimination rate constants (k_2) and log k_{ow} of 26 PAH compounds for the harbour mussels. The mussels were depurated for 68 days in the field.

3.4.3 Comparison of the field result with the laboratory depuration result

The following sections compare the result obtained in this field study with that obtained in the laboratory study. The comparison will concentrate on the PAH distribution pattern and depuration kinetics.

3.4.3.1 PAH concentration and distribution profile

Aberdeen harbour mussels used in the laboratory and field studies showed different total PAH concentrations; 1492.8 ng g^{-1} and 3734.8 ± 216.0 ng g^{-1} respectively. However, the initial PAH distribution profiles observed in these mussels were similar, and characterized by dominance of the 2- and 3-rings compounds. These groups of compounds accounted for 78.1 % (laboratory) and 78.0 % (field) of the total PAHs determined. There was also dominance of the alkylated homologues over the parent compounds. The contribution of the parents compounds to the total PAH concentration was very low; between 11.7- 23.0 %(laboratory) and 8.1 -12.4% (field) throughout the depuration period (Table **3.2** and **3.5**). The similarity in the PAH profile suggest a similar but persistent input source.

The reason for the observed increase in mussels' PAHs tissue burden between June and October could be attributed to the different time of the year the mussels were sampled. Seasonal variation in mussels' PAHs tissue burden has been reported, with generally higher concentrations in the winter, early spring and late autumn (Webster *et al.*, 1997, McIntosh *et al.*, 2001). For example, McIntosh *et al*. (2004) reported an increase in PAHs concentrations in mussels from Kinlochleven shellfish farm during October 1999 to February 2000, which decreased after the mussels' spawning. Mussels are known to accumulate PAHs in their lipid tissues which are laid down prior to spawning.

3.4.4 The elimination kinetics

A reduction of 43.8 % and 49.7 % in total PAH concentrations were observed after 5 and and 7 days of depuration from the laboratory and field studies respectively. At the end of the depuration studies, mussels depurated in the laboratory for 56 days lost 95.5% of the initial PAH tissue burden while those depurated in the field lost a total of 97.7% within the 68 days. The trend in PAH loss from both studies suggest a similar PAH elimination pattern.

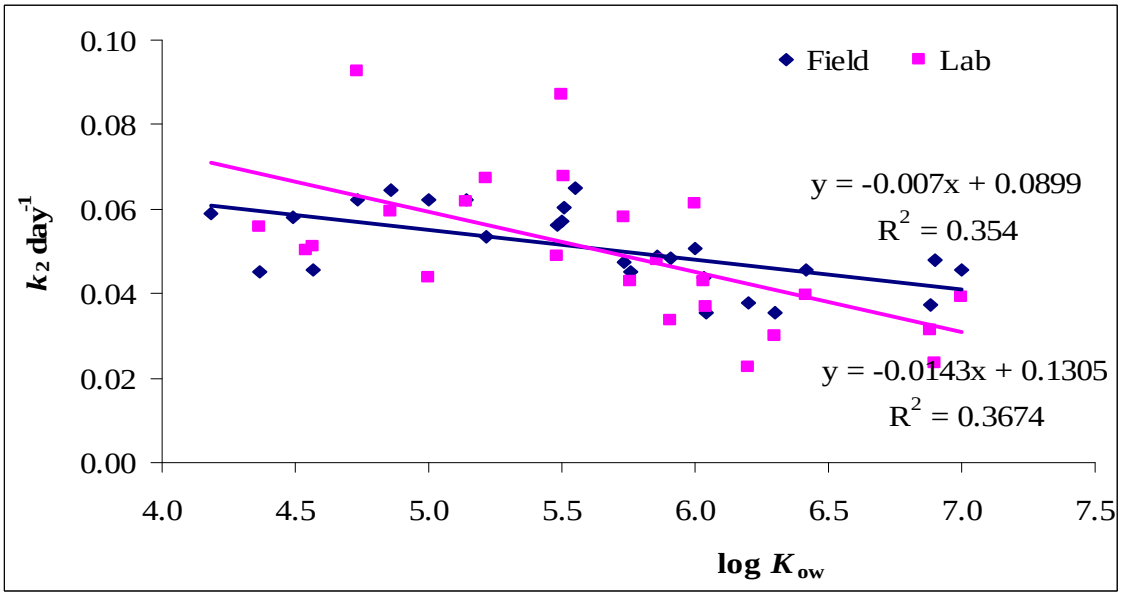


Fig. **3.8**: Comparison of the plots of linear regression analysis of k_2 versus log K_{ow} obtained from depuration of Aberdeen harbour mussels in a laboratory and the field experiments (n = 24 and 27 respectively)

In line with the pattern of reduction in total PAHs discussed above, the elimination rate constants (k_2) determined in the field study were similar to those determined from the laboratory study. The k_2 values carried through to the values of the biological half-lifves obtained (Fig **3.9**). For example, biological half-lives were 15.1/13.6 (phenanthrene), 11.2/11.2 d (C1-phenanthrene/anthracenes), 10.7/11.7 d (C1-dibenzothiophenes), 15.8/16.2 d (C2-flouranthene/pyrene), 19.6/18.7 d (benzo[*a*]pyrene), 15.4/16.2 d (benzo[*c*]penanthrene, 14.1/14.5 d (chrysene/triphenylene), etc., for the field and laboratory

studies respectively. However, some of the vaules varied; e.g. 18.4/31 d (benzo[*e*]pyrene), 12.1/7.9 d (C2-DBT) and 10.6/3.8 d (C4-naphthalenes), respectively for the field and laboratory studies (Table **3.8**).

Table **3.8**: Comparison of the elimination rate constant (k_2), correlation coefficient (r^2) and biological half-lives ($t_{1/2}$) obtained for the laboratory and field depuration studies.

PAH Compound	Field			Lab		
	r^2	k_2	$t_{1/2}$	R^2	K_2	$t_{1/2}$
C2-Naphthalenes	0.63	0.045	15.3	0.71	0.056	12.4
C3-Naphthalenes	0.76	0.062	11.2	0.8	0.093	7.5
C4-Naphthalenes	0.79	0.065	10.6	0.99	0.184	3.8
Phenanthrene (178)	0.67	0.046	15.1	0.59	0.051	13.6
Anthracene	ND	ND	ND	0.89	0.050	13.9
C1-Phenan/anthracene	0.83	0.062	11.2	0.79	0.062	11.2
C2-Phenan/anthracene	0.91	0.060	11.5	0.94	0.068	10.2
C3-Phenan/anthracene	0.95	0.050	13.7	1.00	0.061	11.3
Fluorene (166)	0.67	0.059	11.7	ND	ND	ND
Dibenzothiophene	0.63	0.058	11.9	ND	ND	ND
C1-Dibenzothiophenes	0.88	0.065	10.7	0.95	0.059	11.7
C2-Dibenzothiophenes	0.94	0.057	12.1	1.00	0.087	7.9
C3-Dibenzothiophenes	0.95	0.047	14.7	1.00	0.058	12
Fluoranthene (202)	0.78	0.054	12.9	0.85	0.067	10.3
Pyrene (202)	0.86	0.062	11.2	0.79	0.044	15.9
C1- Flouranthene/Pyrene	0.95	0.056	12.3	0.9	0.049	14.2
C2- Flouranthene/Pyrene	0.93	0.044	15.8	0.96	0.043	16.2
C3- Flouranthene/Pyrene	0.93	0.043	16.2	0.97	0.034	20.4
Benzo[*c*]phenanthrene (228)	0.94	0.045	15.4	0.96	0.043	16.2
Benz[*a*]anthracene (228)	0.87	0.049	14.3	0.81	0.034	20.6
Chrysene/Triphenylene (228)	0.88	0.049	14.1	0.91	0.048	14.5

Benz[*b*]anthracene (228)	0.91	0.046	15.1	0.97	0.038	11.7
C1-228	0.94	0.046	15.2	0.98	0.040	17.4
C2-228	0.86	0.037	18.6	0.93	0.031	22
Benzofluoranthenes (252)	0.86	0.046	15.2	0.94	0.041	16.9
Benzo[*e*]pyrene (252)	0.91	0.038	18.4	0.96	0.022	31
Benzo[*a*]pyrene (252)	0.68	0.035	19.6	0.75	0.037	18.7
Perylene (252)	0.85	0.036	19.4	0.93	0.030	23.2
C1-252	0.84	0.047	14.7	0.92	0.030	23.5
C2-252	0.88	0.055	12.5	0.87	0.029	24
Indenopyrene (276)	0.83	0.046	15.1	0.88	0.039	17.7
Benzoperylene (276)	0.87	0.048	14.5	0.89	0.024	29.5
C1-276	ND	ND	ND	0.81	0.034	20.3

The half lives determined for individual PAHs in both experiments ranged from 3.8 days to 31 days. A close look at Fig. **3.8** indicates that the heavier compounds (5- and 6-rings) were eliminated at a slower rate in the laboratory experiment, while the converse is the case for the lower rings.

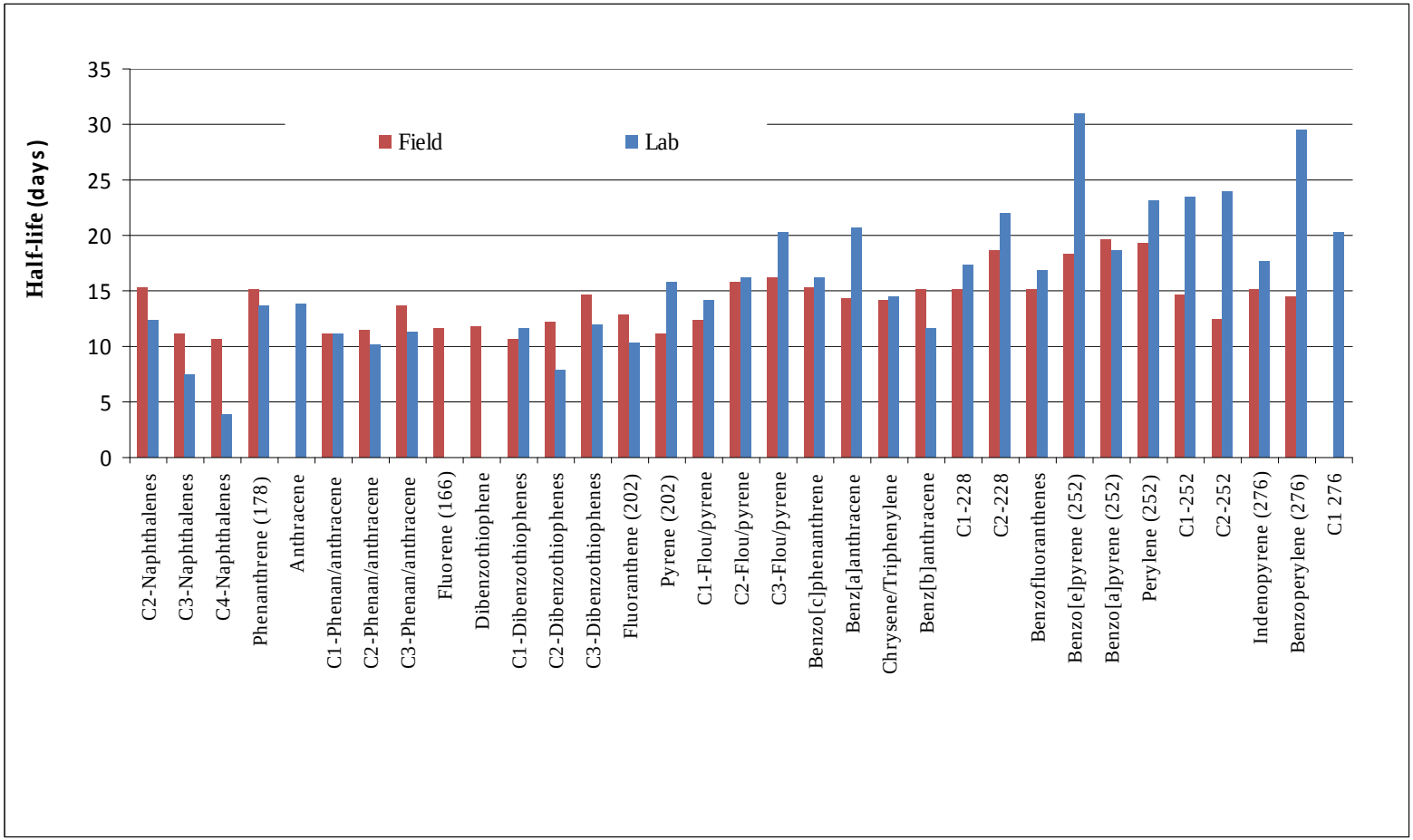


Fig. **3.9**: Comparison of the biological half-lives determined in mussels depurated in the laboratory and field studie

Conclusion

The rates of elimination of individual polycyclic aromatic hydrocarbon compounds in marine bivalves were investigated in a separate laboratory and field studies using naturally impacted blue mussels (*Mytilius edulis*). The elimination rates determined varied among individual PAH compounds, but were similar for the laboratory and field studies. The similarity between the kinetic data obtained from the two studies shows that laboratory data can be directly applied to field situations. Elimination pattern generally followed first order kinetics and estimated biological half-lives ranged between 3.8 and 31 days. This shows that mussels can eliminate PAHs and impacted mussels can return to safe concentrations within a relatively short period of time. The higher molecular weight compounds were eliminated at a much slower rate than the lower molecular weight compounds, suggesting that hydrophobicity has effect on rate of elimination. A regression analysis of k_2 against log K_{ow} shows up to 35 % dependency of elimination rate on compound's hydrophobicity. However, the low percentage obtained in this study indicates that k_2 cannot be accurately estimated from the log K_{ow} assuming a perfect linear relationship. This further suggests that, apart from the compound's hydrophobicity, other factors could be responsible for the variations in the elimination rate of individual compounds obtained in this study.

CHAPTER FOUR

Source of PAH input to Aberdeen harbour and investigation of changes in geochemical biomarkers in blue mussels (*Mytilus edulis*)

4.1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are introduced into the environment mainly from human related activities. Over the years, the PAH compounds found in the environment have been commonly classified as pyrolytic or petrogenic. As has been elaborately discussed in chapter **1**, some other sources of PAHs which occur naturally in the environment are classified as being of biogenic origin. However, the chronic release of pyrolytic PAHs into the environment (from combustion of fossils fuels) can result in PAH profiles being dominated by these pyrolytic PAHs, and any petrogenic input in the environment may be masked (Yunker *et al*., 2002). Some methods have been devised to distinguish PAHs of petrogenic origin from pyrolytic sources. These methods use information from the *n*-alkanes distribution, PAHs distribution profile, specific PAH concentration ratios and the presence or absence of geochemical biomarkers (geochemical biomarkers are present if source is petrogenic) in the environmental matrix being analysed to characterise the PAH source. The use of this approach has proved very successful in the characterization of PAHs determined in sediment and water samples (Baumard *et al*., 1998; Wang *et al*., 1998; Webster *et al*., 2004).

However, petroleum products when released into the environment are susceptible to changes due to degradation which can in effect alter the chemical composition (Payne *et al*., 2003). In such situations, the alteration can render PAH source identification using only the *n*-alkanes and PAH distribution profiles alone unreliable. Therefore where the *n*-alkanes and PAHs profiles have been severely weathered, the presence or absence of geochemical biomarkers can further provide useful information. The use of geochemical biomarkers in tracing petrogenic contamination to their input source is well documented. Geochemical biomarkers are source specific compounds which bear fingerprints of the contributing materials in oil source rocks (Peters and Moldowan 1993). Also, in oil spill investigation,

oil correlations have been made by either the presence or absence of specific biomarker compounds and the relative abundances of specific biomarker compounds to C_{30}-hopane in the suspect sample compared to the spilled sample. These ratios are generally referred to as Diagnostic ratios or indices (DR). Geochemical biomarker diagnostic ratios (DRs); which originate from geochemistry are currently in use for oil spill studies and environmental forensics. These ratios widely used by geochemists for oil-oil correlation, determination of organic input, assessment of thermal maturity and evaluation of in-reservoir oil biodegradation (Peters *et al*., 2005). The merit of comparing diagnostic ratios of spilled oil and suspected oil sources is that concentration effects are minimized. In addition, the use of ratio tends to induce a self-normalizing effect on the data because, the influence of operational differences, instrumental fluctuations and matrix effects are minimized. Previous reports have shown that these ratios are stable over time in oil and sediment samples (e.g. Wang *et al.*, 1995, Wang *et al*., 1998, Wang *et al*., 1999; Webster *et al*., 2003, Webster *et al*., 2004) collected from spill sites. However, no research to date has looked into the stability of these ratios in living organisms affected by oil spill incidents.

The current study therefore evaluates the hydrocarbon composition of Aberdeen harbour mussels in order to classify the PAH contamination source. The relevant components studied were the *n*-alkanes, PAH distributions and concentration ratios, and the biomarkers (steranes and triterpanes) profiles. The results presented in this chapter were obtained from analysis of mussels used for laboratory and field PAH kinetic studies reported in Chapter **3**.

4.2 The *n*-alkanes profile of the harbour mussels.

The *n*-alkanes distribution and concentrations (ng g^{-1} wet weight) in Aberdeen mussels, Loch Etive mussels (used for the laboratory experiment), and Hutton crude oil (North Sea oil) are presented in Fig. **4.1**. The figure shows the relative distribution of the odd and even numbered normal alkanes ranging from C11 to C33. The digits represent the number of carbon atoms in one molecule of the compounds. Carbon preference index (CPI) is a measure of the relative abundance of odd verses even carbon numbered *n*-alkanes and can be used to assess whether a hydrocarbon input source is predominantly biogenic or petrogenic (Eganhouse and Kaplan, 1982). It has been established that *n*-alkanes from

petrogenic sources have a CPI approximately equal to 1.0 while those from biogenic sources have CPI greater than 1.0 (Eganhouse and Kaplan, 1982; Peters and Moldowan 1993; Webster *et al*., 2004).

CPI is calculated from:

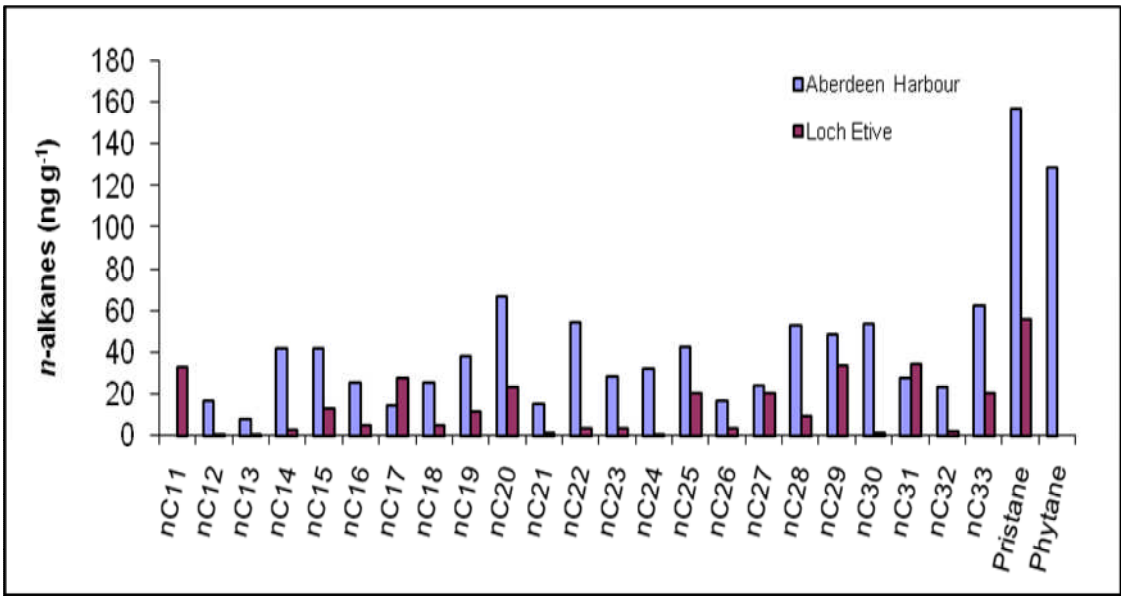
$$CPI = \frac{(C_{23} + C_{33}) + 2(C_{25} + C_{27} + C_{29} + C_{31})}{2(C_{24} + C_{26} + C_{28} + C_{30} + C_{32})}$$ ((Bray and Evans, 1961))

As expected the Hutton crude oil profile is an even distribution of *n*-alkanes and returned a CPI value of 1.0. Aberdeen harbour mussels show dominance of the even numbered *n*-alkanes over the odd numbered alkanes and with a CPI of 1.0. Loch Etive mussels returned a CPI of 7.2 (Table **3.2**). A CPI value greater than 1.0 from the Loch Etive mussels and dominance of the odd numbered carbon alkanes is typical of a terrestrial or vascular plant input.

Petroleum also contains a substantial proportion of hydrocarbons which are not properly resolved by conventional gas chromatography. These components are often referred to as the unresolved complex mixture (UCM), and are especially pronounced for biodegraded petroleum and certain refined fractions such as lubricating oils (Gough and Rowland, 1990). According to Wang *et al*. (1999), UCM is a common feature of weathered petroleum products. This feature is also present in the harbour mussels' *n*-alkane chromatogram but absent in the control mussels' chromatogram and virtually not noticeable in the fresh Hutton crude oil.

Some other interesting deductions can be made from the pattern revealed in the GC-FID chromatography profile of the mussels analyzed (Fig. **4.1** (a)). The largest peaks in this plot are the more recalcitrant iso-alkanes of which the two most abundant, pristane and phytane are identified.

(a)



(b)

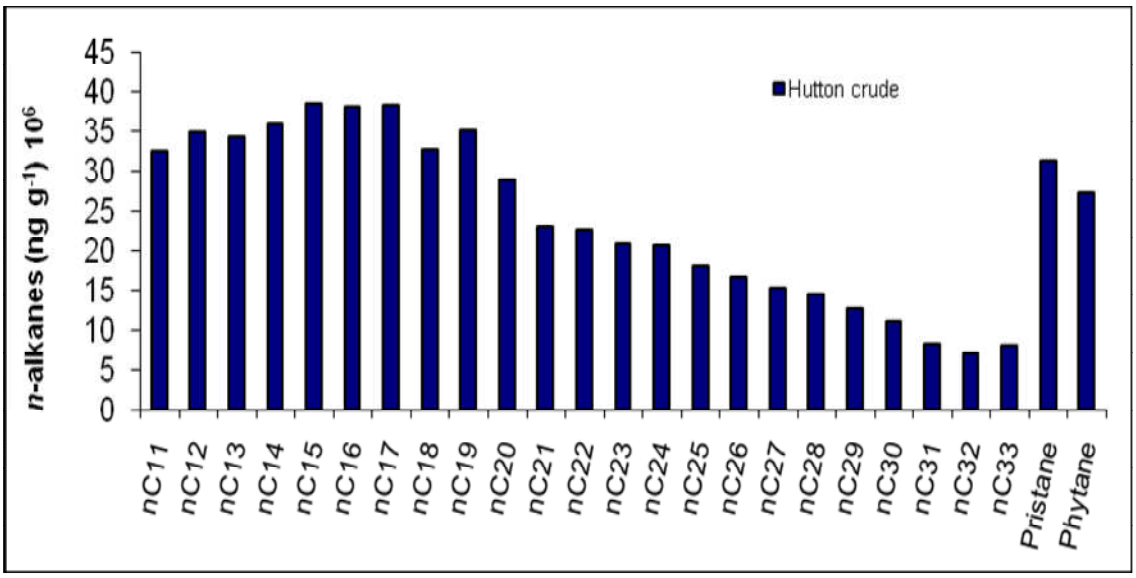


Fig. **4.1**: The distribution and concentration (ng g^{-1} wet weight) of *n*-alkanes in (a) Aberdeen harbour and Loch Etive mussels and (b) Hutton crude oil (a North Sea oil).

According to Peters *et al*. (2005), pristane/phytane ratio reflects the nature of the contributing organic matter in crude oils and increases with thermal maturation. The ratio of pristane/phytane (Table **4.1**) determined in Aberdeen harbour mussels is 1.2. This value is comparable to that calculated for the Hutton crude (1.1). The close value for this ratio is indicative of a similar contributing organic matter for the oil and the source of the harbour mussels' contaminant.

However, there are differences in the pristane/n-C_{17} (0.10 and 1.23) and phytane/n-C_{18} (0.20 and 1.19) ratios observed for the harbour mussels and Hutton crude oil respectively. The huge difference in these ratios; known as the biodegradation indicators may be as a result of microbial degradation of the petroleum content in the harbour mussels and other factors relating to weathering which has been shown to alter the composition of aliphatic hydrocarbon compounds in oils (e.g. Boehm *et al*., 1997; Boehm *et al*., 2001; Nordtest 2001; Wang *et al*., 2004). Considering the similarities in the CPI values and the pristine/phytane ratios of the Hutton crude oil and the harbour mussels and the differences in the biodegradation indices (pristane/n-C_{17} and phytane/n-C_{18}), it will be logical to suggest that the contributing oil component in the harbour mussels have undergone a fair degree of weathering or biological degradation.

Table 4.1: *n*-alkanes ratios determined for Aberdeen harbour mussels, Loch Etive mussels and Hutton crude oil.

Indicators	Loch Etive mussels	Harbour mussels	Hutton Crude oil
CPI	7.20	1.00	1.00
Pristane/phytane	-	1.20	1.10
n-C17/pristane	0.49	0.10	1.23
n-C18/phytane	-	0.20	1.19

A serious limitation of using the *n*-alkane profile alone in PAH source classification or oil source correlation is the ease with which *n*-alkane profiles are altered in the environment. The weathering processes that petroleum products are subjected to upon release to the environment include evaporation, water washing and dissolution, and biodegradation. These processes cause the chemical composition of the product to change, as it weathers in some cases quickly and drastically. *n*-alkanes are among the most biodegradable hydrocarbons, so they are readily broken down and preferentially depleted from the environment. Therefore, the uncertainties surrounding the compositional changes of the *n*-alkane pattern due to weathering renders this approach inadequate and requires consideration of the more refractory classes of hydrocarbons such as the PAHs and geochemical biomarkers (Galperin and Camp, 2002).

4.3 PAH distribution profile and concentration ratios

As was explained in Section **1.3.2.1**, the PAH distribution profile is a useful qualitative tool in distinguishing between PAH sources. In general, pyrolytic PAHs are characterized by the dominance of the un-substituted (parent) compounds over their corresponding alkylated homologues and the dominance of the high molecular mass (4- to 6-ring) compounds over the low molecular mass (2- and 3-ring) compounds. In contrast, petrogenic patterns are characterized by dominance of the alkylated compounds over their corresponding parent homologues and the high abundance of the 2- and 3-ring PAHs over the 4- to 6-rings (Dahle *et al*., 2003 and Budzinsky *et al*., 1997). Webster *et al*. (2003) also noted that phenanthrene and pyrene are more thermodynamically stable than anthracene and fluoranthene, and dominate if the source is petrogenic.

Table 4.2: % PAH subgroups, % of parent PAHs, Total parent PAHs, and % of 2- and 3-rings and 4- to 6-rings PAHs in Loch Leven, Loch Etive, Loch Ewe and Aberdeen harbour mussels used for the laboratory and field experiments.

PAH sub-groups	Aberdeen Harbour (lab)	Aberdeen Harbour (field)	Loch Leven	Loch Etive	Loch Ewe
% 128	11.6	8.6	1.0	11.1	17.6
% 178	48.2	54.4	3.1	33.1	20..8
% DBTs	17.6	14.8	0.6	6.4	6.9
% 202	13.8	14.0	9.9	21.5	30.7
% 228	4.5	4.7	12.0	8.7	9.4
% 252	3.0	2.9	59.9	15.1	10.3
% 276	0.5	0.4	11.3	2.9	3.5
% Acenaphthene (154)	0.5	0.0	0.0	0.0	0.0
% Fluorene (166)	0.30	0.1	0.0	1.2	0.8
% Dibenz[a,h]anthracene (278)	0.02	0.0	0.0	0.0	0.0
∑ Parent	174.1	345.8	89.8	6.5	8.6
% Parent	11.7	9.3	72.4	37.8	35.8
% 2- and 3-rings	78.1	78.0	4.6	51.7	46.3
% 4- to 6-rings	21.9	22.0	95.5	48.3	53.8

The initial PAH profiles (Table **4.2**) of the harbour mussels used for both the laboratory and the field experiments showed a high abundance of the 2- and 3-rings PAHs (~ 78 %) and low proportions of parent PAHs (< 12 %) relative to their alkylated homologues. For the samples used in the laboratory study, the profile observed contained (naphthalenes and alkylated compounds (C1-C4):11.8 %, phenanthrene/ anthracene and alkylated compounds (C1-C3): 48.2 %, and dibenzothiophene and alkylated compound (C1-C3): 17.6 %), while that for the field experiment showed (naphthalenes and alkylated compounds (C1-C4): 8.6 %, phenanthrene/ anthracene and alkylated compounds (C1-C3): 54.4 %, and

dibenzothiophene and alkylated compound (C1-C3): 14.8 % (Table **4.2**). The PAH profile obtained for the Loch Leven mussels is different from that of the harbour mussels. Loch Leven profile showed dominance of the parent PAH compounds (72.4 %) over their alkylated homologues and a high abundance (95.5 %) of the 4- to 6-rings compounds (Table **4.2**) relative to the 2- and 3-rings compounds (4.5 %). The control samples show an even spread of the lighter and heavier PAH compounds (51.7/46.3 % of the 2- and 3-rings and 48.3/53.8 % of the 4- to 6-rings) for the Loch Etive and Loch Ewe mussels respectively.

Specific PAH ratios; P/A, Fl/Py, ∑MP/P and (Fl+Py)/MFl+MPy described in Chapter **1** Section **1.3.2.1** and given in Table **4.3** were also evaluated. These PAH source diagnostic indices have been used extensively to differentiate between PAH sources, especially in sediment samples (Budzinki *et al*., 1997; Baumard *et al*., 1998; Baumard *et al*., 1999; Yunker *et al*., 2002; Webster *et al*., 2004). Webster *et al*. (2003) extended the use of this ratio to the source classification in mussels' samples from the Scottish sea loch. However, the stability of these ratios in organisms (especially mussels) and hence the reliability of use has not properly been investigated.
The values of these ratios calculated for the harbour and the Loch Leven mussels are presented in Table **4.4**. The plots of the values of the MP/P vs. Fl/Py ratios calculated for each sample point during the depuration experiment are also presented in Fig. **4.2**.

Table **4.3**: PAH diagnostic ratios used for source discrimination (A = Anthracene, P Phenanthrene, MP = methyl phenanthrene, Fl = Fluoranthene and Py = Pyrene).

Diagnostic ratio	Pyrolytic	Petrogenic
P/A	<10	>10
Fl/Py	>1	<1
MP/P	<2	>2
(Fl+Py)/(MFl+MPy)	3	<3

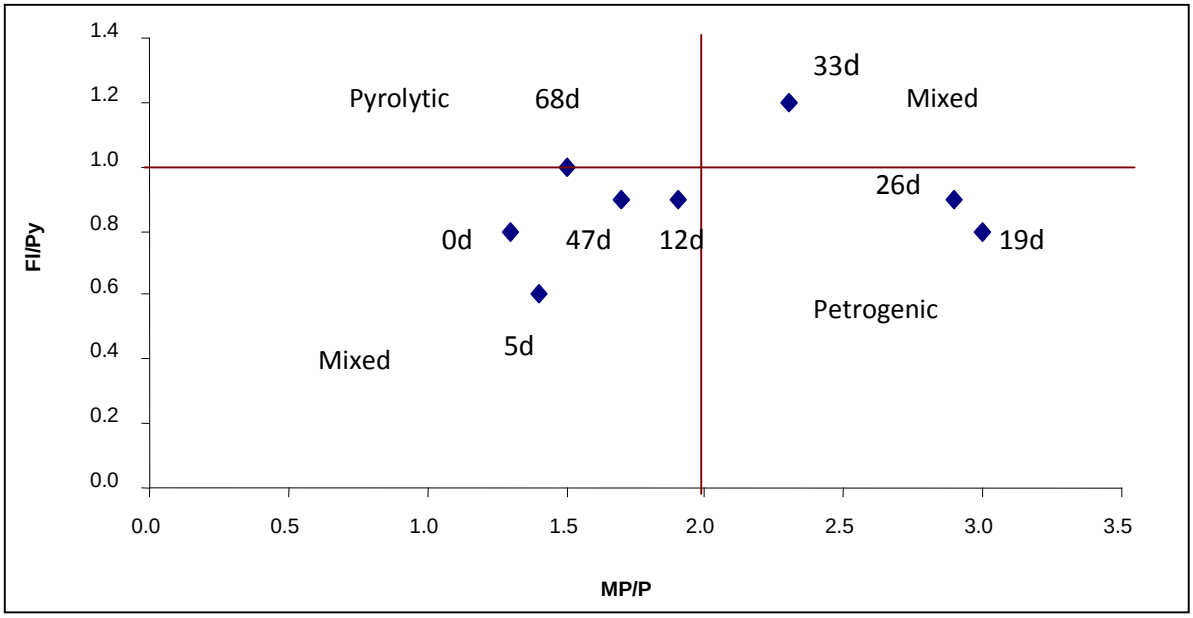
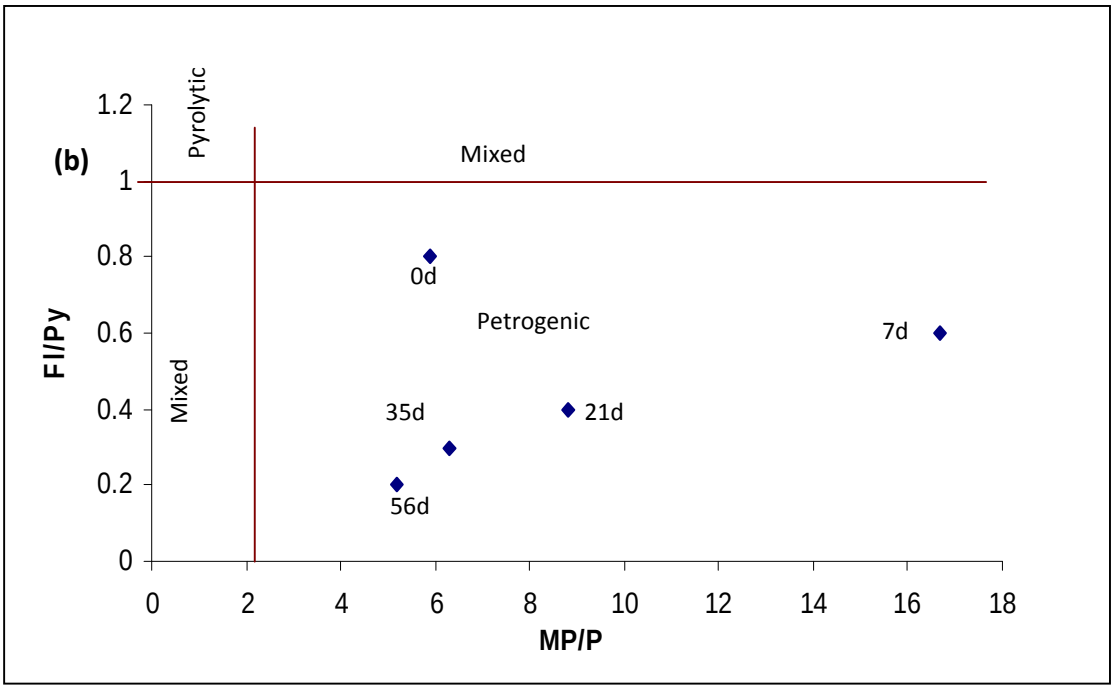


Fig. **4.2**(**a**): The Fl/Py vs. MP/P ratio of Loch Leven mussels depurated in an open sea in Loch Ewe.

If the plots are considered as quadrants, the area with MP/P >2 and Fl/Py <1 defines petrogenic character while the converse is true for pyrolytic character. The areas opposite these quadrants define probable mixed sources (Wang *et al*., 1999; Yunker *et al*., 2002; Webster *et al*., 2003).

Aberdeen harbour mussels used in the laboratory study returned all five points on the petrogenic quadrant (Fig **4.2b**), and the field study (Fig. **4.2c**) returned seven points on the petrogenic quadrant and a point on the mixed input source quadrant. Therefore the two plots suggest a predominantly petrogenic PAH input to the harbour. Fig. **4.2 (a**), which is an equivalent plot for the Loch Leven mussels showed five points on the mixed input quadrant, one point on the pyrolytic quadrant, and two points on the petrogenic quadrant; suggesting a mixed input source.



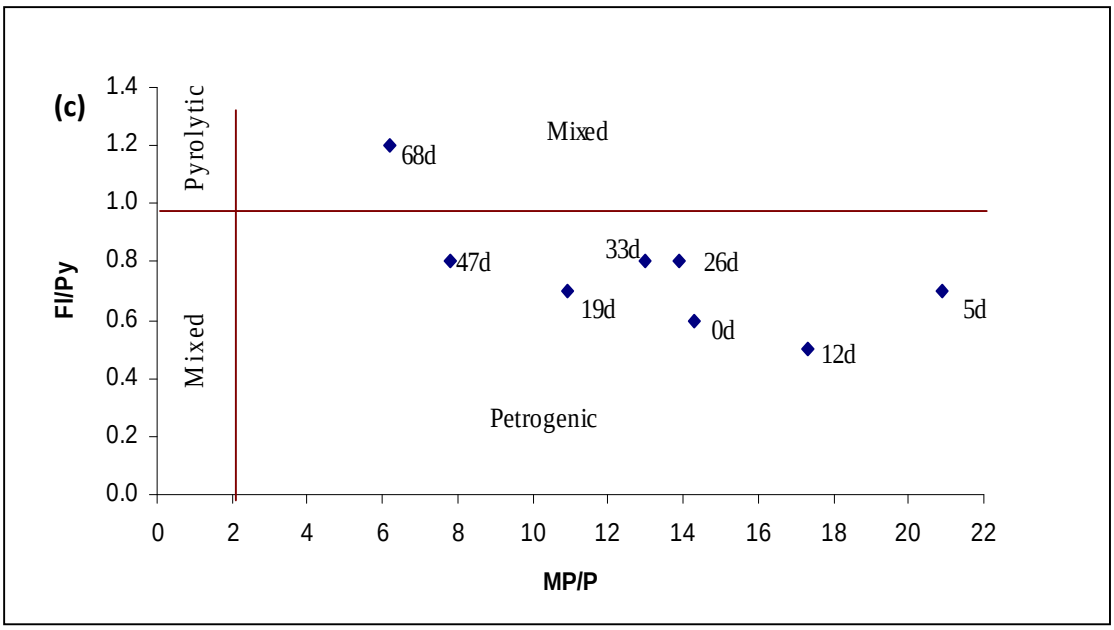


Fig. **4.2**: The Fl/Py vs. MP/P ratio determined for of Aberdeen harbour mussels depurated in (**b**) the laboratory for 56 days and (**c**) in the field for 68 days.

The plots also reveal the susceptibility of these ratios to changes in specific PAH compound concentration (hence with depuration time). The trends in variation of the indices with time seem inconsistent (Table **4.4**). The P/A ratio varied from about 9.9 at the beginning of depuration to 2.3 at 35 days of depuration and thereafter could not be evaluated because anthracene (A) concentration reduced to values below the detection limit. From Table **4.4**, it can be seen that the value of this ratio determined within 5 days of depuration (field study) indicate petrogenic contamination but subsequent values do not. The values of Fl/Py and MP/P for the Loch Leven mussels also suggest different sources at different time points (Table **4.4**). As Lima *et al*. (2005) pointed out; source diagnostic ratios should be used with care and in the context of the study area. As is evident from the current study, isolated values from different sample time points can suggest different sources and therefore result in misleading inferences. The observed variations suggest that the ratios can be affected by the rate of elimination or degradation of the compounds by organisms.

Table **4.4**: Ratios [phenanthrene/anthracene (P/A), methylphenanthrene/phenanthrene (MP/P), Fluoranthene/pyrene (Fl/Py) and [Fluoranthene + Pyrene / methylfluoranthene + methylpyrene) (Fl + Py)/ MFl + MPy)]] of PAH compounds found in the test mussels from Aberdeen harbour and Loch Leven.

Sample site	Diagnostic ratio	Depuration time (days)							
		0	7	21	35	56			
Aberdeen harbour mussel in laboratory study	P/A	9.9	2.9	1.5	2.3	NV			
	Fl/Py	0.8	0.6	0.4	0.3	0.2			
	MP/P	5.9	16.7	8.8	6.3	5.2			
	(Fl+Py)/MFl+MPy)	0.8	0.5	0.4	0.5	0.6			
		0	5	12	19	26	33	47	68
Aberdeen harbour mussel in field study	P/A	10.4 ± 0.2	24.7 ±6.8	3.8 ± 0.4	NV	NV	NV	NV	NV
	Fl/Py	0.6	0.7	0.5 ± 0.1	0.7	0.8	0.8	0.8 ± 0.1	1.2 ±0.1
	MP/P	14.3 ± 0.3	20.9 ± 0.4	17.3 ± 0.2	10.9 ±1.6	13.9 ± 0.9	13.0 ± 2.8	7.8 ±1.6	6.2 ± 1.8
	(Fl+Py)/MFl+MPy)	0.7	0.5	0.2	0.3	0.3	0.3	0.5	0.4 ± 1.0
Loch Leven mussel in field study	P/A	NV	NV	NV	NV	NV	NV	NV	NV
	Fl/Py	0.8	0.6 ±0.1	0.9	0.8 ± 0.1	0.9	1.2	0.9	1.0 ± 0.2
	MP/P	1.3 ± 0.1	1.4 ± 0.3	1.9 ± 0.1	3.0 ± 0.2	2.9 ± 0.5	2.3 ± 0.2	1.7 ± 0.1	1.5 ± 0.2
	(Fl+Py)/MFl+MPy)	1.0	0.9 ± 0.1	1.0	0.8	0.9	1.0	1.7 ± 0.1	0.7 ± 0.3

4.4 Geochemical Biomarkers (Steranes and Triterpanes)

4.4.1 Triterpane

Fig. **4.3** (**a**), and (**b**) show the distribution/abundance of triterpanes (*m/z* = 191) geochemical biomarkers of the Aberdeen harbour mussels (t = 0) in the laboratory experiment and Hutton crude oil, respectively (see Table **4.5** for peak full identities). The *m/z* 191 chromatogram of Hutton crude oil showed the five doublet peaks due to the C_{31} - C_{35} homohopane diastereoisomers (22S and 22R). It has been widely recognized (Peters and Moldowan 1993; Boehm *et al*., 1997; Barakat *et al*., 2002; Webster *et al*., 2004) that the five doublet peaks which decrease in size with increasing carbon number are characteristic features of most crude oils. These hopane peaks are also present in the harbour mussels' chromatogram. There is also the presence of bisnorhopane (28ab) peak in the Hutton crude oil and harbour chromatograms. According to Dahlmann (2003), the broader platform area of the North Sea seems to be especially characterised by relatively high concentrations of the C_{28}- bisnorhopane, a compound, which is not a member of the regular hopane series. This triterpane compound (28ab) is therefore a unique characteristic of the North Sea oils and the presence in the harbour mussels suggest an oil input from this source. The homohopane doublet peaks and the bisnorhopane peak are completely absent in the reference mussels from Loch Etive (Fig. **4.3** (**c**)). It has also been established that Middle Eastern oils do not contain bisnorhopane. The ratio of norhopane to hopane (30ab) normally found in Middle Eastern oils is > 1 whereas those of the North Sea oils are ≤ 0.5 (Dahlmann, 2003; Webster *et al*., 2004, Wang *et al*., 2006). The ratio of bisnorhopane to norhopane (29ab) ratio found in the harbour mussels was 0.13 and the norhopane to hopane ratio was 0.81. These values are not typical either for the North Sea oils or the Middle Eastern oils. A most probable explanation why the norhopane to hopane ratio (0.81) found in the harbour mussels is greater than normally found in the North Sea oils (≤ 0.5) but less than that characteristic of Middle Eastern oils (> 1) is mixed contamination from both sources. In addition, a bisnorhopane to hopane ratio of < 0.20 for the harbour mussels also suggest a greater contribution from oil which does not contain this specific biomarker.

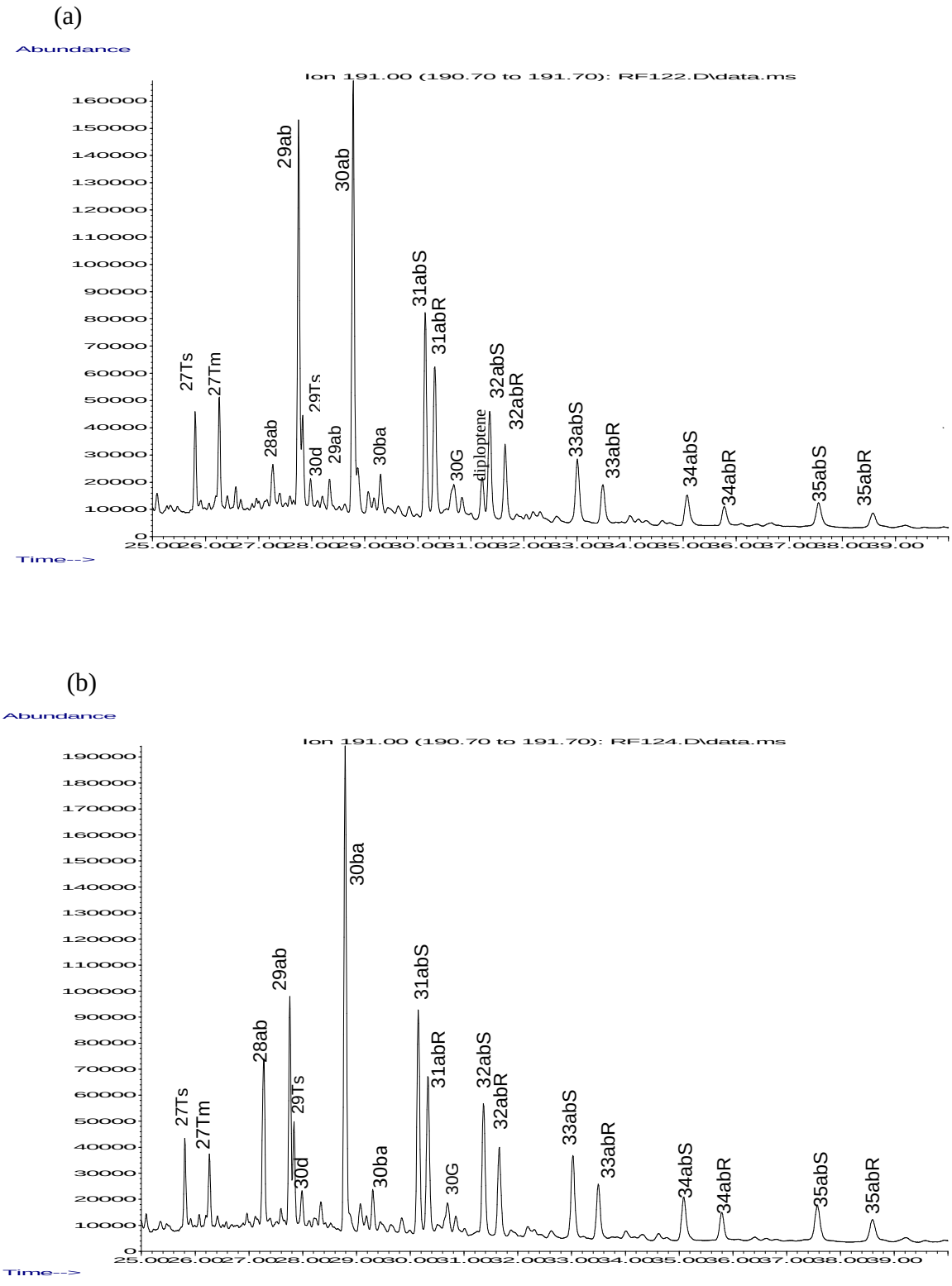


Fig. **4.3**: Triterpane (*m/z* 191) profile of (a) Aberdeen harbour mussel (b) Hutton crude oil

Table **4.4**: Peak identifications for chromatograms in figures **4.5** and **4.6,** and diagnostic ratio descriptions.

Peak label	Compound	*m/z*
Ts	18α(H) -22, 29, 30-trinorhopane	191
Tm	17α(H)-22, 29,30-trisnorhopane	191
28ab	17α(H),21β(H)-28,30-bisnorhopane	191
29ab	17α(H), 21β(H)-30-norhopane	191
29Ts	18α(H)-norneohopane	191
30d	15α-methyl-17 α (H)-27-norhopane (diahopane)	191
29ba	17 β(H)-21α(H)-30norhopane (normoretane)	191
30G	Gammacerane	191
30ab	17 α(H), 21β(H)-hopane	191
31ab (S & R)	17 α(H), 21β(H)-homohopane (22S & 22R)	191
32ab (S & R)	17 α(H), 21β(H)-bishomohopane (22S & 22R)	191
33ab (S & R)	17 α(H), 21β(H)-trishomohopane (22S & 22R)	191
34ab (S & R)	17 α(H), 21β(H)-tetrahomohopane (22S & 22R)	191
35ab (S & R)	17α(H), 21β(H)-pentakishomohopane (22S & 22R)	191
27dbS (a)	13β (H), 17α(H) diacholestane (20S)	217
27dbR (b)	13β (H), 17α(H) diacholestane(20R)	217
28aaS (c)	5α(H), 14α(H), 17α(H)-24-methylcholestane (20S)	217
28bbR (d)	5α(H), 14β(H), 17β(H)-24-methylcholestane (20R)	217
28bbS (e)	5α(H), 14β(H), 17β(H)-24-methylcholestane (20S)	217
28aaR (f)	5α(H), 14α(H), 17α(H)-24-methylcholestane (20R)	217
29aaS (g)	5α(H), 14α(H), 17α(H)-24-ethylcholestane (20S)	217
29bbR (h)	5α(H), 14β(H), 17β(H)-24-ethylcholestane (20R)	217
29bbS (i)	5α(H), 14β(H), 17β(H)-24-ethylcholestane (20S)	217
29aaR (j)	5α(H), 14α(H), 17α(H)-24-ethylcholestane (20R)	217
%DR-27Ts	[27Ts/ (27Ts +27Tm)]*100	
%DR-28ab	[28ab/ (28ab +30ab)]*100	
%DR-29Ts	[29Ts/ (29Ts +30ab)]*100	
%DR-30G	[30G/ (30G +30ab)]*100	
%DR-29ab	[29ab/ (29ab +30ab)]*100	
%DR-30d	[30d/ (30d+30ab)]*100	
%DR-32abS	[32abS/32ab (S+R)]*100	
%DR-29aaS	[29aaS/ (29aaS + 29aaR)]*100	
%DR-29bb	[29bb(S+R)/[29bb (S+R) + 29aa(S+R)]]*100	

The profile of the control mussels (Loch Etive) is also dominated by diploptene. Diploptene is a natural triterpane, and is also present in the harbour mussels' *m/z* 191 chromatogram [Fig. **3.6** (**a**)] but somehow concealed by the presence of the more pronounced peaks arising from petroleum contamination. This peak is totally absent in the Hutton *m/z* 191 chromatogram.

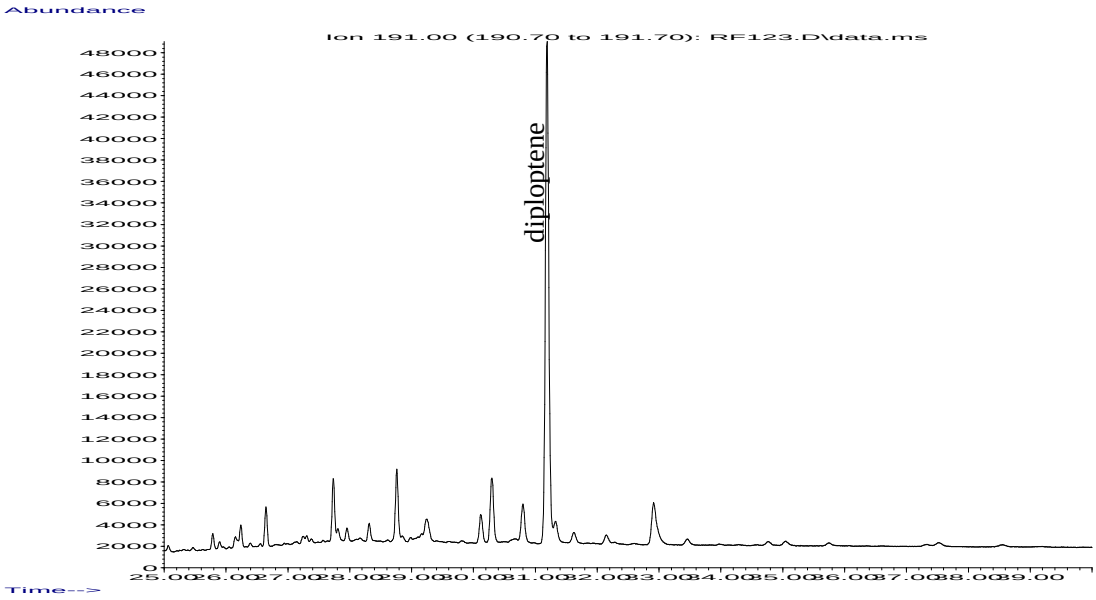


Fig**. 4.3(c)**: Triterpane (*m/z* 191) profile of Loch Etive mussel.

The *m/z* chromatograms of the test and control mussels used in the field experiment are also presented. Fig. **4.5** (**a**), (**b**), and (**c**) show the triterpane biomarker distribution/abundance of Aberdeen harbour, Loch Leven, and the Loch Ewe mussels are respectively. The peak identities are as given in Table **4.5**.

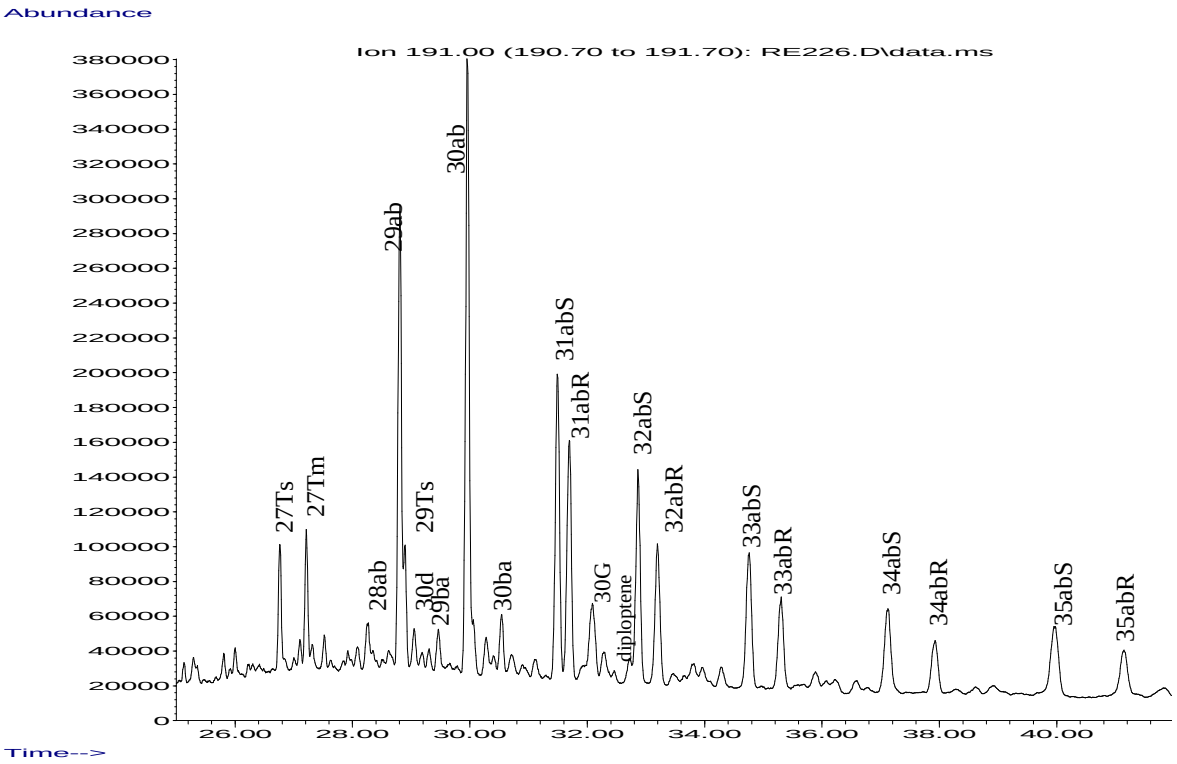


Fig**. 4.4(a)**: Triterpane (*m/z* 191) profile of Aberdeen harbour mussel

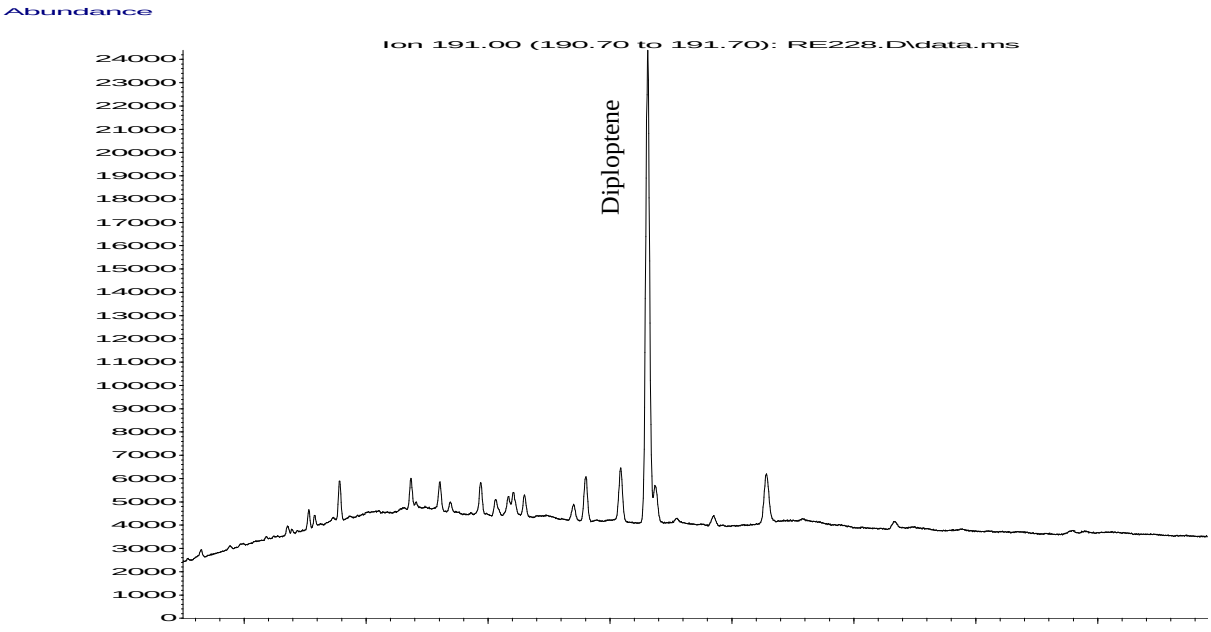


Fig. **4.4 (b)**: Triterpane (*m/z* 191) profile of Loch Leven

The *m/z* 191 chromatogram of Aberdeen harbour mussels used for the field experiment (**Fig. 4.4(a))** showed similar compounds identified in the mussels used for the laboratory experiment; the only difference been the relative intensities of individual compound which is higher in the former. This was also reflected in the PAH tissue burden of the mussels. The total PAH concentration of the mussels used in the laboratory experiment was lower (1492.8 ng g^{-1}) than that used in the field experiment (3734.8 ± 216.0 ng g^{-1}). However, the bisnorhopane/norhopane and norhopane (C_{29})/hopane (C_{30}) ratios wre less than determined in the laboratory experiment; 0.07 and (0.71 ± 0.05) respectively but still suggests mixed input from the North Sea and Middle Eastern oils.

The *m/z* 191 profile of the Loch Leven mussels (Fig. **4.4b**) showed no identifiable petroleum related triterpane peak. The chromatogram was dominated by the diploptene peak which is a natural triterpane also available in all mussel samples analysed. Therefore, the profile of these mussels portrays that the PAH contamination may be predominantly from a pyrolytic source.

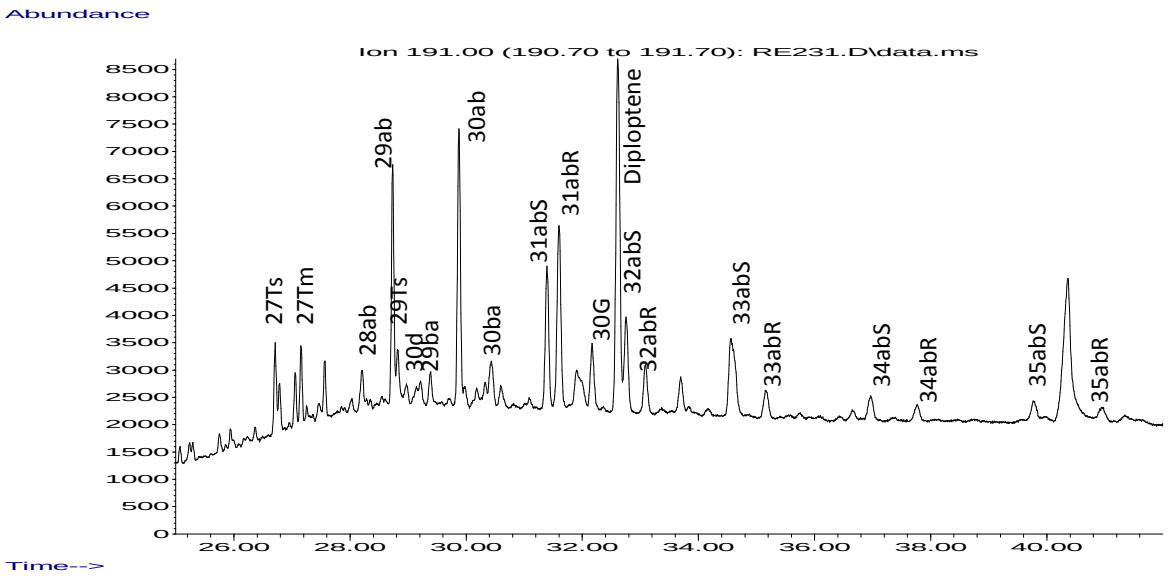


Fig. **4.4** (**c**): Triterpane (*m/z* 191) profile of Loch Ewe mussel

The chromatogram of the Loch Ewe mussels used as the control mussels (Fig. **4.4** (**c**)) showed traces of all the petroleum biomarkers determined in the harbour mussels, including the homohopane doublet peaks. This is not expected but not surprising as Loch Ewe is very

famous for its salmon fishing and the loch is always busy with sailing activities especially during the summer when the area attracts large pools of tourists. Therefore, oil leakages from boats or washings may have contributed to the trace petroleum contamination observed in this area.

4.4.2 Steranes

The sterane (*m/z* 217) chromatogram of Aberdeen harbour mussel and Hutton crude oil are presented in Fig. **4.5** (**a**) and (**b**) respectively. The figures show that all the labelled peaks (a – j) present in the Hutton oil chromatogram are also present in the harbour mussels chromatogram. However, the relative abundance of the compounds in Aberdeen harbour mussels differs from those of the pure oil sample. The Loch Leven mussels' sterane profiles presented in (Fig. **4.6** (**a**)) like the *m/z* 191 profile, shows no identifiable petroleum related sterane compound. Therefore, the absence of sterane peaks in the Loch Leven mussels is a good indication that the differences in the relative abundances of the sterance compounds between the Hutton and harbour mussels' *m/z* 217 did not arise from the mussels, but rather may be as a result of additional oil input source. Like its *m/z* 191 profile, the sterane profile of the Loch Ewe mussels (Fig. **4.6** (**b**)), also showed peaks; C_{27}, C_{28} and C_{29} that are characteristics of petroleum products. However, the percentage abundances of these compounds are relatively small compared to the abundances found in the harbour mussels. The probable reasons why there exist trace biomarker compounds in the reference mussels profile has been discussed in the preceding section.

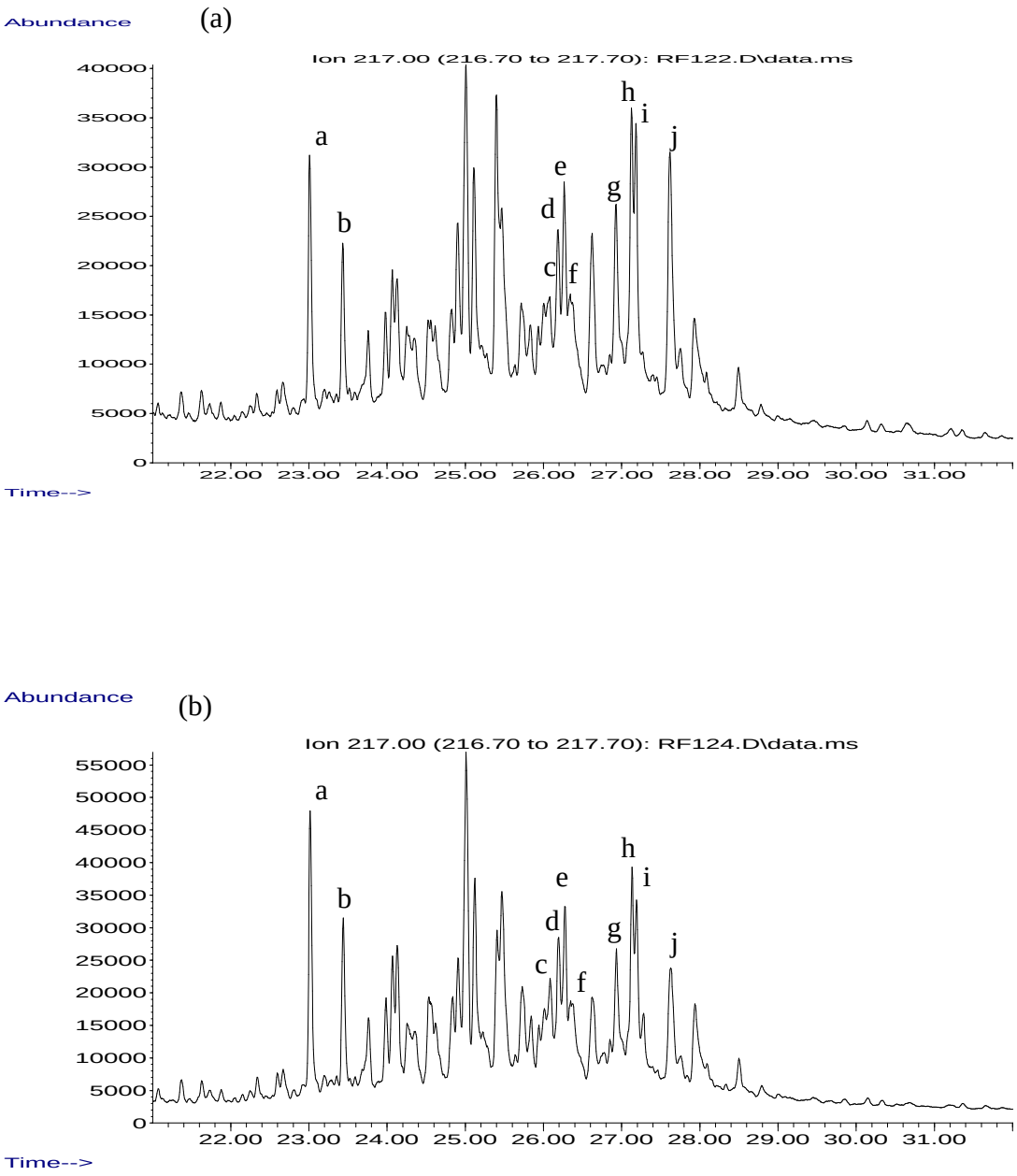
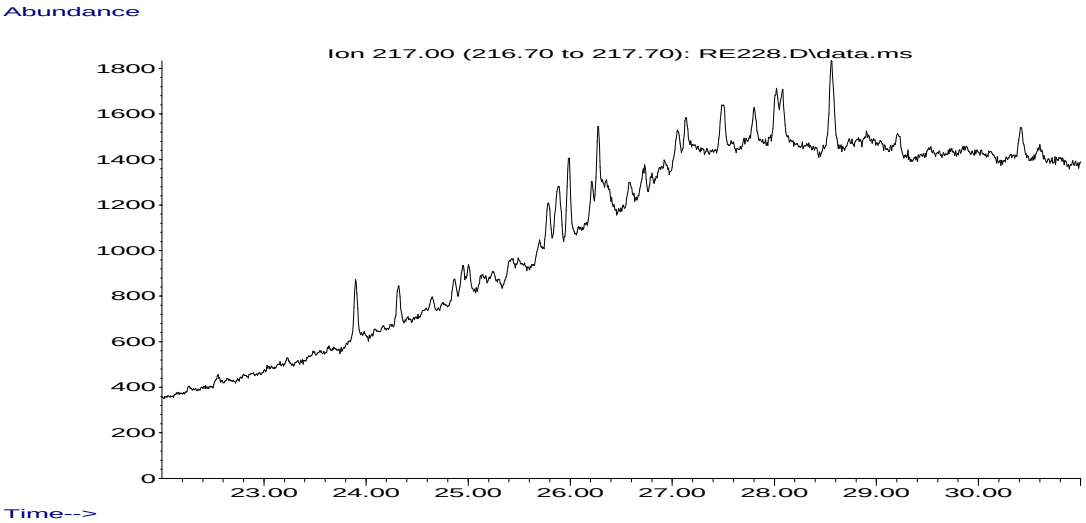


Fig. **4.5**: Sterane (*m/z* 217) profiles of (a) Aberdeen harbour mussels and (b) Hutton crude oil. Where a = 27dbS, b = 27dbR, c = 28aaS, d = 28bbR, e = 28bbS, f = 28aaR, g = 29aaS, h = 29bbR, i = 29bbS and j = 29aaR

(a)



(b)

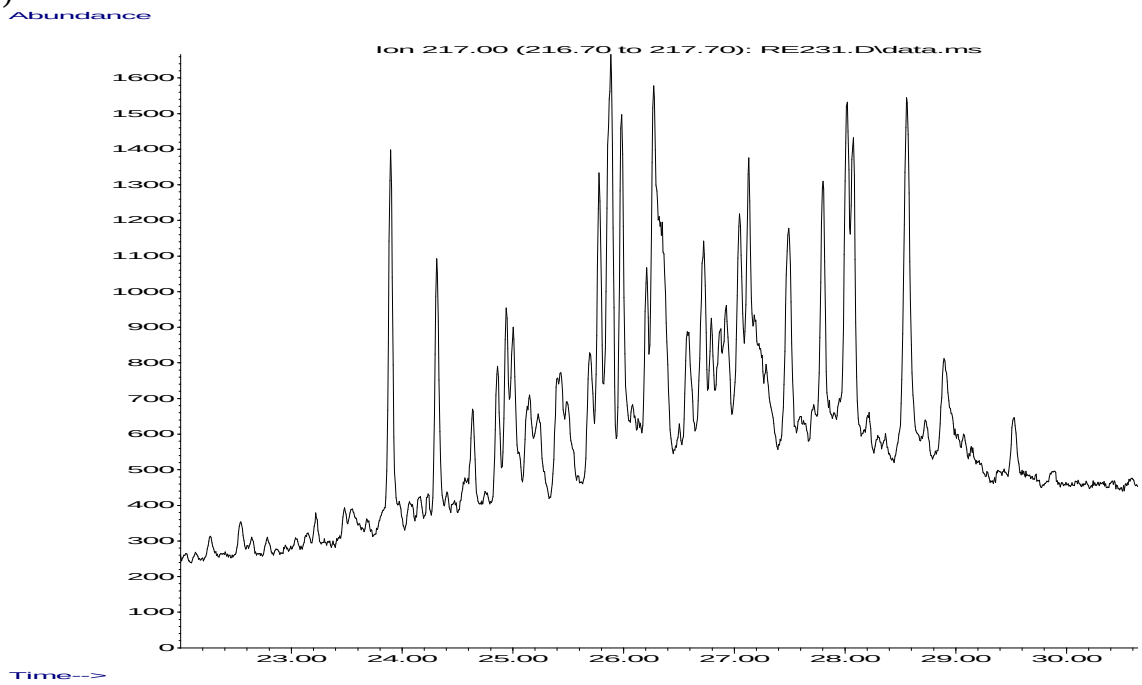


Fig. **4.6**: Sterane (*m/z* 217) profile of (a) Loch Leven mussels (b) Loch Ewe and (a = 27dbS, b = 27dbR, c = 28aaS, d = 28bbR, e = 28bbS, f = 28aaR, g = 29aaS, h = 29bbR, i = 29bbS and j = 29aaR)

4.5 Changes in biomarker profile with depuration time.

The relative abundance (intensity) of individual biomarker was found to decrease with depuration time for a given weight of sample (see Appendix **3**). Although the biomarkers were not absolutely quantified, a decrease in intensity (abundance) of individual compounds relative to diploptene (a non crude oil biomarker) was observed. The intensity of diploptene increased progressively until it became the dominant peak in the *m/z* 191 chromatograms of the mussels as intensities of the compounds approach that of the control samples (see Appenxdix **3**). Therefore to better characterize the changes in the biomarker profile, biomarker diagnostic ratios were used. The ratios reported are calculated using the relative abundances of the peak areas for the triterpanes and the peak heights for the steranes. Measuring diagnostic ratios based on peak heights and peak areas (semi-quantitative) within the same molecular ion has been recommended and is being used by Nordtest (Faksness *et al*., 2002) in environmental forensics. The ratios are of the type

$$\left[a \middle/ (a+b) \right] * 100$$

where a and b are peak heights or peak areas within the same molecular ion.

The accuracy of the instrumental analysis and diagnostic ratio calculation relies on the measured variability among triplicate analysis (Nordtest, 2002). For the field experiment, the relative variation at a 95 % confidence interval was calculated for the first triplicate samples using the "student's t" test. The confidence interval is an expression stating that the true mean μ, is likely to lie within a certain distance from the measured mean, *x*. The confidence interval of μ is given by

$$\mu = \chi \pm \frac{t.s}{\sqrt{N}}$$

where *s* is the measured standard deviation between triplicate samples, *x* is the mean value, *t* is the Student's t and N is the number of observations. For the t' table, the number of degrees of freedom equals N-1. The DR ratios obtained in both the laboratory and field

studies are presented in Table **4.5** and correlation plots of the time zero sample and subsequent samples using the Nordtest methodology are presented in Fig**. 4.7**.

Table **4.5** shows that, over the study period, the value of the triterpane DRs remains relatively stable with only a little enhancement except for DR-30G which ranged from 6.4 to 12.4. The compound Gammacerene; which is the numerator in this ratio co-elutes with an unknown compound and therefore the values obtained for this DR are not very reliable. The result from the current study suggests that although the studied triterpane biomarkers are eliminated by organisms (absolute abundance decreases with depuration time), the ratio of specific compounds remains relatively stable over time.

The studied sterane diagnostic ratios % DR29aaS and %DR29bb showed continuous decrease with depuration time (Table **4.5**). A regression analysis of the sterane DRs and depuration time showed a strong positive correlation ($p \leq 0.001$), with r^2 for each diagnostic ratio approaching unity. This casts doubt on the reliability of using specific sterane ratios in oil pollution assessments.

However, Fig. **4.7** shows that the according to the Nordtest method of spill identification, the DRs are stable over 12 days during the depuration. Such a plot cannot be given for the laboratory experiment as the initial samples were not analyzed in triplicate.

Table 4.5: Hopane and sterane diagnostic ratios evaluated from Aberdeen harbour mussels

Dep time	**% DR-27Ts**	**% DR-28ab**	**% DR-29Ts**	**% DR-30G**	**% DR-29ab**	**% DR-30d**	**%DR32abS**	**% DR-29aaS**	**% DR-29bb**
Lab									
0	49	10	15	7.1	45	4.7	59	41	53
7	48	9.2	15	12	45	5.1	59	39	52
21	49	8.4	16	12	45	7.8	59	31	47
35	51	13	18	12	48	5.7	60	24	40
56	52	14	18	6.4	47	5.8	57	17	34
Field									
0	44	6.7	12	16	41	5.0	58	38	53
5	43	7.6	14	13	41	8.9	59	40	51
12	41	8.0	15	14	41	5.9	59	32	46
19	46	14	17	11	44	6.9	60	26	42
26	47	14	18	11	46	6.5	61	25	41
33	45	16	17	13	46	6.9	62	22	39
47	47	14	17	10	45	5.9	66	17	33
68	47	11	18	10	45	5.9	68	21	39
89	41	14	18	8.7	48	10	68	18	35

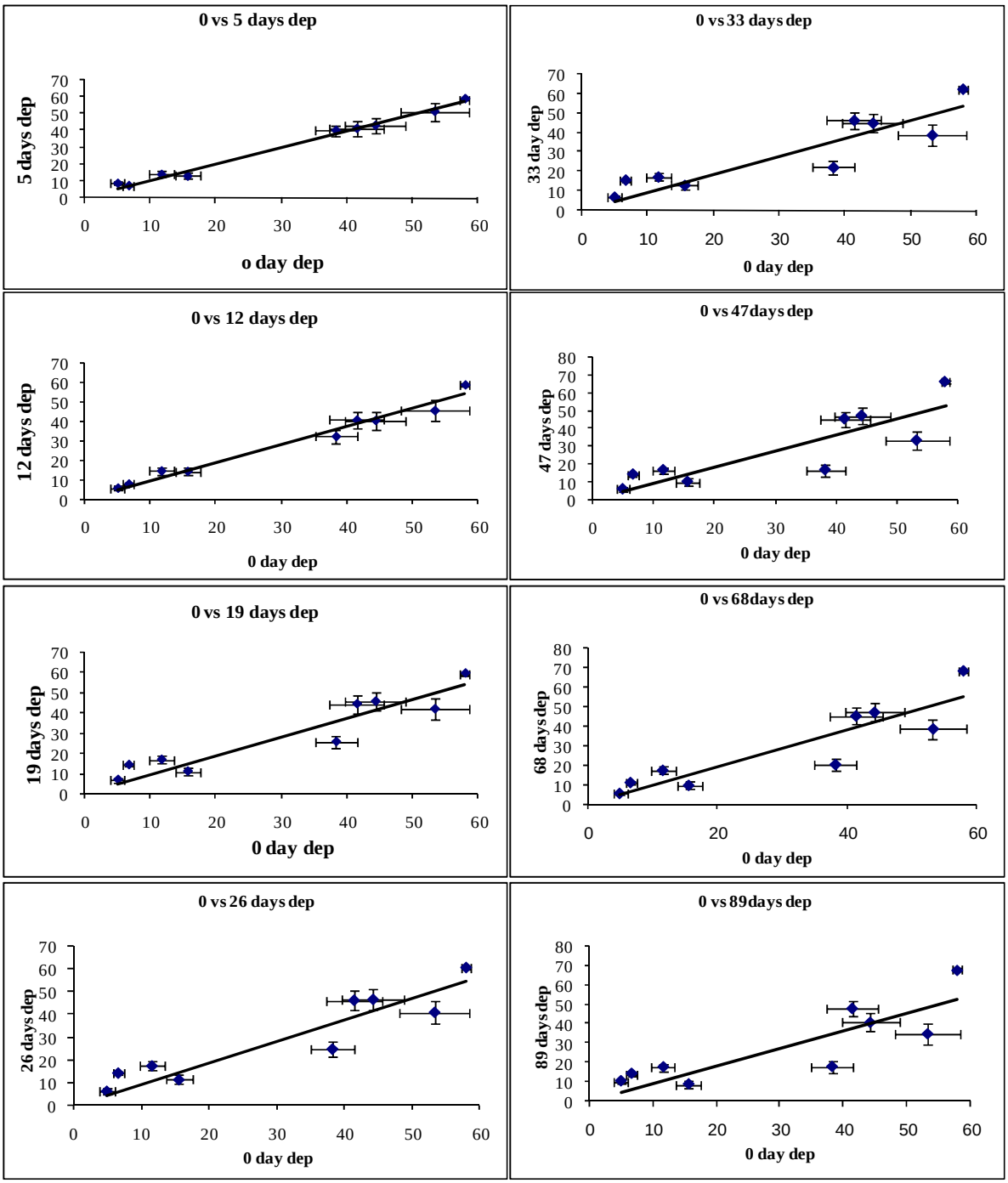


Fig. **4.7**: Correlation between biomarker DRs of Aberdeen harbour mussels collected at time 0 and subsequent samples collected at the given time points, using 95% confidence interval.

4.6 Conclusion

The *n*-alkane profile of Aberdeen harbour mussels contains unresolved complex mixture, gave a CPI of 1 and showed alkane distribution with no dominance of either the odd or the even numbered carbon atoms; therefore has a chemical fingerprints similar to those of weathered crude oils. The PAHs distribution and concentration ratios were similar to that described by petrogenic sources. The presence of geochemical biomarkers (sterane and triterpanes) in the mussels'biomarker fingerprint further indicates that the PAH contamination is from a petrogenic source. Geochemical biomarker compounds are unique features of crude oils and derived products and have been used to discriminate agaist oil sources. The presence of specific biomarker compounds (bishnorhopane) and biomarker ratios characteristic features of the Norths Sea and Middle Eastern oils indicate contribution from both sources. A decrease in the intensities of triterpanes with depuration time relative to diploptene was also observed and this suggests that similar to PAHs, these compounds are eliminated by the organisms. The change in biomarker profile monitored using specific biomarker diagnostic indices showed a little enhancement of the triterpane ratios with time while specific sterane ratios studied showed continuous decrease with depuration time. However, a good correlation was obtained between the DR profile of the time zero sample and samples collected within 12 days of depuration using the Nordtest approach. The retention of the initial biomarker fingerprint by the mussels for a period of time outside of the contamination zone indicates that mussels collected from oil spill sites can provide useful information regarding the oil source. However, this study shows that the biomarker information from mussels may not be very reliable after a long period of spill incident.

CHAPTER FIVE

Chemical fingerprinting of different oil samples (Gullfaks, Forties, Hutton and Arabian light crude oils) to aid classification of Aberdeen harbour PAH input source.

5.1 Introduction

Chemical fingerprinting is the application of chemistry to identify the sources of complex environmental pollutants, including petroleum. This practice has advanced into a science where the original source(s) of complex mixtures (e.g. crude oil) can often be identified by the relative abundance of major individual compounds (e.g. *n*-alkanes) forming a chemical pattern by ratio of specific constituents or by identifying source-specific compounds or markers (e.g. triterpanes) in the environmental sample being investigated. In oil spill chemical fingerprinting, saturated hydrocarbons (*n*-alkanes and isoprenoids), PAH distributions, and geochemical biomarkers (steranes and triterpanes) are usually the important parameters considered (e.g. Faksness *et al*., 2002; Dahlmann, 2003; Peters *et al*., 2005).

The geochemical biomarker approach has been used for the exploration of fossil fuels and reservoir geochemistry (Peters and Moldowan 2003; Peters *et al*., 2005). In recent times, this approach has been carried over to oil spill investigations (environmental forensics); where extensive weathering of the more labile compounds (*n*-alkanes and PAHs) often leave biomarkers as primary analytical chemistry alternative for use in fingerprinting of spilled oils (Boehm *et al*., 1997; Requejo and Boehm, 1985; Barakat *et al*., 2001). To unambiguously identify spilled oils and petroleum products and to link them to the known sources is extremely important in settling questions of environmental impact and legal liability (Wang *et al*., 1999). The chemical fingerprinting of petroleum is made possible by the numerous individual hydrocarbons present and the great variability in the relative abundances of these compounds in different crude oils, and between crude oils and their refined products (as described by Faksness *et al*., 2002). The variability is as a result of the differing geological environment and conditions under which the oils are formed and the

biological makeup of the oils. According to Faksness *et al*. (2002), defensible chemical fingerprinting is a critical part of oil spill investigation and site assessments. Forensic identification of marine oil spills in Scandinavia and in many other countries has in the last 10 years followed the Nordtest methodology (1991), using the biomarkers for the characterization of spilled oils and the identification of their source by qualitative comparisons of isomer patterns in GC-MS chromatograms of the spilled oil and those of the suspect sources. This is normally done by comparing the patterns in specific biomarker diagnostic ratios (Table **5.1**). These biomarker diagnostic ratios have been shown to be stable (show little or no change) over time in studies relating to; spilled oil samples, slightly weathered oils, and in sediment samples collected from spill sites (Poulsen *et al*., 2002, Wang *et al*., 1999, Faksness *et al*., 2002; Peters *et al*., 2005). However, the stability of these diagnostic ratios in organisms exposed to oil contamination has not been explored.

The complex geochemical biomarker profile obtained from the Aberdeen harbour mussels led to further analysis of common crude oil samples to attempt to classify the pollution source. The sources considered were restricted to crude oils due to the presence of pronounced $C_{31} - C_{35}$ homohopane doublet peaks (characteristic feature of all crude oils) in the mussels' triterpane profile. However, the presence of BNH (28ab) peak; a unique triterpane feature of the North Sea oils and a C_{29}/C_{30}- hopane ratio approximating those of the Middle Eastern oils as found in pure samples of these crude oils reduced the possible suspect sources to the North Sea and Middle Eastern oils. Therefore, the following oil samples were analyzed for the geochemical biomarkers; Gulfaks crude oil (Norwegian Sector, North Sea); Forties crude oil, Hutton crude oil (UK Sector, North Sea); and Arabian light crude oil (Middle Eastern oil).

5.2. Analytical methods.

The oil samples were prepared, separated into the aliphatic and aromatic components and analyzed with GC-MS using the same method as samples as detailed in FRS laboratory manual for biomarker determination (SOPs, 1265, 1625, 1640, and 1660, see appendix **1**).

5.2.1. Oil sample preparation

The crude oil samples used for the analysis were prepared by adding 2 ml of *iso*-hexane to approximately 0.5 mg of each crude oil sample in a vial and shaking vigorously to homogenize the solution. Various combinations of the oils were also prepared by mixing 1:1, 1:3 and 3:1 (v/v) of specific oils using a dedicated syringe. The oil mixtures prepared were Forties/Arabian light oils and Gulfaks/Hutton oils in the volumetric ratios described above.

5.2.2. HPLC clean-up and fractionation

Approximately 150 µl of the oil in *iso*-hexane (and also their mixtures - see Table **5.1**) was injected into the HPLC and eluted with *iso*- hexane at a flow rate of 2 ml min^{-1}. The aliphatic fraction was collected within 2 min 30 seconds of injection and reduced to 500 µl using the rotary evaporator with a water bath temperature set at 30 ºC. The reduced eluate was transferred to a vial and further reduced to 25 µl under stream of purified nitrogen gas before analysis by GC – MS.

5.2.3. GC-MSD determination of biomarkers

The sterane and triterpanes compositions were determined by GC-MS fitted with a cool on-column injector. Geochemical biomarker analysis was carried out using the selected ion-monitoring mode (SIM). Triterpanes were monitored using *m/z* 191 and steranes using *m/z* 217. Identification of compounds was carried out by matching the retention time and peaks of compounds in the test samples with those in reference oil analyzed with each batch of 12 samples.

5.3. Results and discussion

The results obtained from the chemical fingerprinting of the individual crude oil and their mixtures are presented in Section **5.3.1** and the result from this study is compared with the biomarker profile of Aberdeen harbour mussels in Section **5.3.2.**

5.3.1. Individual oil biomarker profiles

5.3.1.1. Triterpanes

The *m/z* 191 chromatograms of the analysed crude oil samples; Forties, Gulfaks, Hutton and Arabian light crude oils are presented in Fig. **5.1** to **5.4** respectively. The chromatograms showed the five doublet peaks (C_{31}-C_{35} homohopane peaks) identified as (31abS - 35abR), which are characteristic features of crude oils (Peters *et al*., 2005). The North Sea oils: Hutton, Gulfaks and Forties crude oils also show 17α (H), 21β (H)-28, 30-bisnorhopane peak (28ab, BNH). As expected, BNH is a unique characteristic of the North Sea oils (Faksness *et al*., 2002; Dahlmann, 2003; Russell *et al*., 2005). In contrast, this compound is absent in the *m/z* 191 chromatogram of the Arabian light oil. BNH absent in Middle Eastern oils is well documented (Dahlmann, 2003; Russell *et al*., 2005). Another distinguishing feature of the North Sea oils is the relative abundance of 17α(H), 21β (H)-30-norhopane (C_{29}-hopane; 29ab) to 17 α(H), 21β(H)-hopane (C_{30}-hopane; 30ab). The C_{29}-hopane to C_{30}-hopane ratio of the North Sea oils reported in the literature is always < 50 %. The C_{29}-hopane to C_{30}-hopane values determined in this study for the North Sea oils are 0.41, 0.42 and 0.39 for Forties, Hutton and Gulfaks respectively, this is in agreement with literature values (Russell *et al*., 2005). These values (0.41, 0.42 and 0.39) are comparable but obviously different from that determined for the Arabian light oil (1.16). The value of C_{29}/C_{30} obtained for the Arabian light oil collaborated reports that C_{29} hopane is more abundant than C_{30} hopane in Middle Eastern oils (Webster *et al*., 2003; Dahlmann, 2003; Russell *et al*., 2005).

Table **5.1**: Biomaker peak identifications and diagnostic ratio description

Peak label	**Compound**	***m/z***
27Ts	18α(H) -22, 29, 30-trisnorneohopane	191
27Tm	17α(H)-22, 29,30-trisnorhopane	191
28ab	17α(H),21β(H)-28,30-bisnorhopane	191
29ab	17α(H), 21β(H)-30-norhopane	191
29Ts	18α(H)-norneohopane	191
30d	15α-methyl-17 α (H)-27-norhopane (diahopane)	191
29ba	17 β(H)-21α(H)-30norhopane (normoretane)	191
30G	Gammacerane	191
30ab	17α(H), 21β(H)-hopane	191
31ab (S & R)	17α(H), 21β(H)-homohopane (22S & 22R)	191
32ab (S & R)	17α(H), 21β(H)-bishomohopane (22S & 22R)	191
33ab (S & R)	17α(H), 21β(H)-trishomohopane (22S & 22R)	191
34ab (S & R)	17α(H), 21β(H)-tetrahomohopane (22S & 22R)	191
35ab (S & R)	17α(H), 21β(H)-pentakishomohopane (22S & 22R)	191
27dbS (a)	13β (H), 17α(H) diacholestane (20S)	217
27dbR (b)	13β (H), 17α(H) diacholestane(20R)	217
28aaS (c)	5α(H), 14α(H), 17α(H)-24-methylcholestane (20S)	217
28bbR (d)	5α(H), 14β(H), 17β(H)-24-methylcholestane (20R)	217
28bbS (e)	5α(H), 14β(H), 17β(H)-24-methylcholestane (20S)	217
28aaR (f)	5α(H), 14α(H), 17α(H)-24-methylcholestane (20R)	217
29aaS (g)	5α(H), 14α(H), 17α(H)-24-ethylcholestane (20S)	217
29bbR (h)	5α(H), 14β(H), 17β(H)-24-ethylcholestane (20R)	217
29bbS (i)	5α(H), 14β(H), 17β(H)-24-ethylcholestane (20S)	217
29aaR (j)	5α(H), 14α(H), 17α(H)-24-ethylcholestane (20R)	217

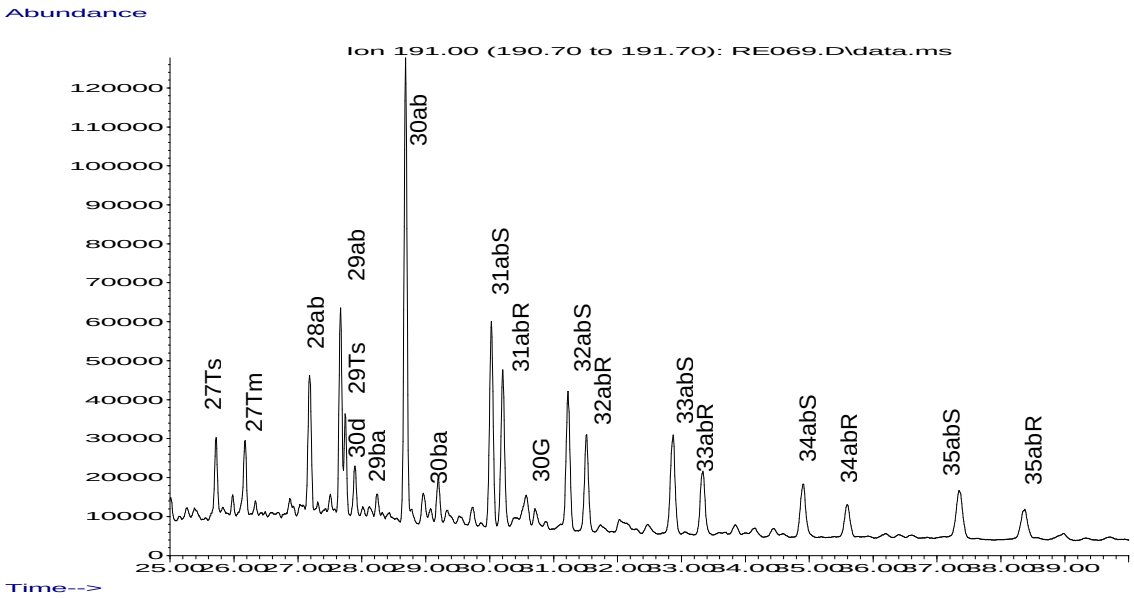


Fig. **5.1**: Capillary column gas chromatogram the triterpane profile of Forties crude oil.

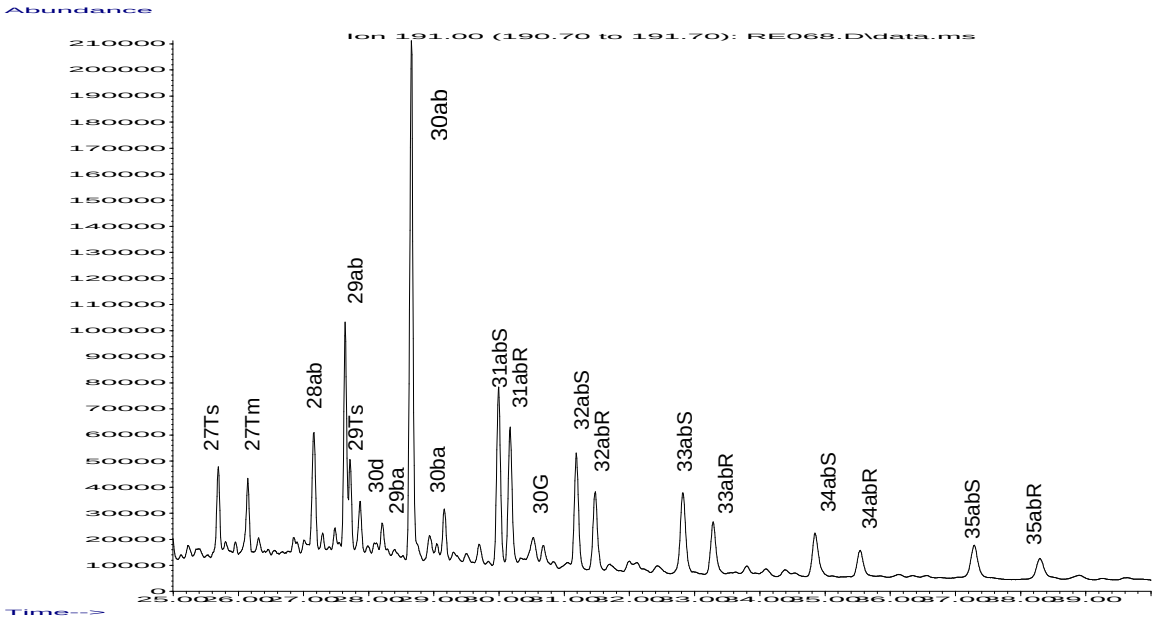


Fig. **5.2**: Capillary column gas chromatogram the triterpane profile of Gulfaks crude oil.

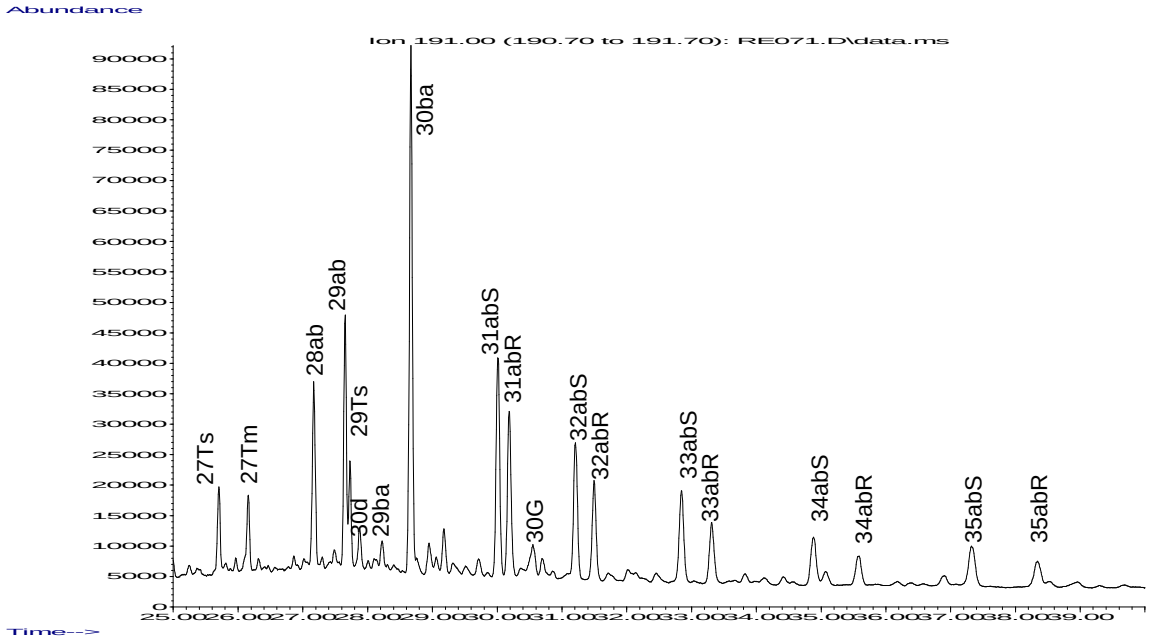


Fig. **5.3**: Capillary column gas chromatogram of the triterpane profile of Hutton crude oil.

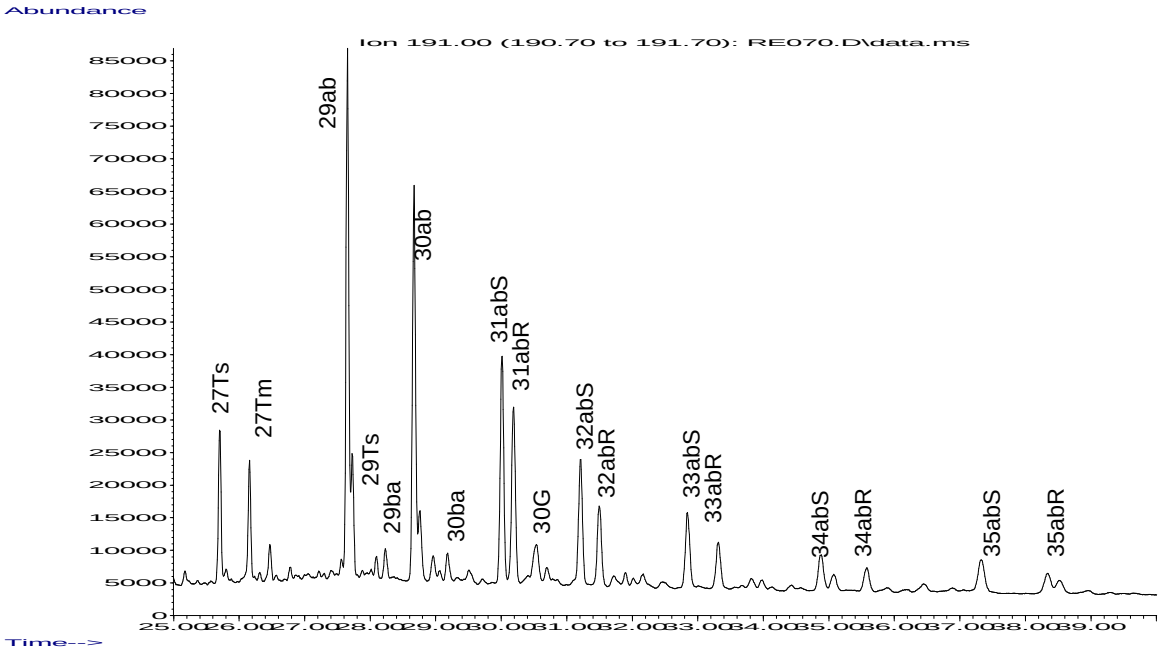


Fig. **5.4**: Capillary column gas chromatogram of the triterpane profile of Arabian light crude oil.

The variations in the relative abundances of biomarkers observed in the analyzed oil samples support the fact that they are controlled by the make-up of the contributing source rock, and hence their usefulness in oil source investigation. The value of C_{29}/C_{30} for the Arabian light oil reported in this study is similar with the values from Poulsen, *et al*. (2006) who reported a C_{29}/C_{30} hopane average of 1.18 from triplicate analysis o Arabian light oil standards (1.22, 1.15, and 1.22), and 1.30 for Kuwait oil standards (1.30, 1.32, and 1.27). Therefore, the absence of BNH in the *m/z* 191 chromatogram of the Arabian light oil and the relative abundances of C_{29}/C_{30} hopane in these chromatograms clearly distinguishes the North Sea oils from the Arabian light oil (and probably the Middle Eastern oils)

5.3.1.2.Steranes

The sterane profiles of the individual oil samples are presented in Fig. **5.5** (**a-d**). The labeled peaks are the C_{27}, C_{28} and C_{29} steranes (Table **5.1**). The North Sea oils; Forties, Gulfaks and Hutton crude oils show similar sterane fingerprint; a pronounced C_{27} steranes peaks [identified as (**a)** and (**b**] in addition to the dominant C_{29} steranes (**g-j**). The Arabian light oil's sterane profile on the other hand, showed dominance of the C_{29} steranes over the C_{27} and C_{28} steranes. The C_{27}, C_{28} and C_{29} steranes are maturity indicators and their specific ratios have been widely applied in petroleum geochemistry to investigate the state of maturity of oils, especially the epimer ratio of 20S/(20S+20R).

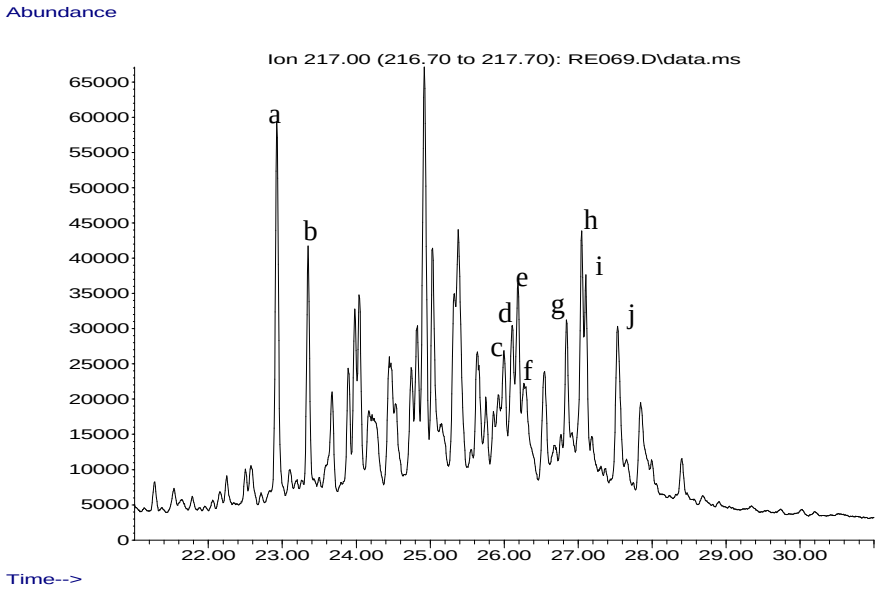


Fig. **5.5(a)**: Capillary column gas chromatogram of the sterane (*m/z* 217) profile of Forties crude oil

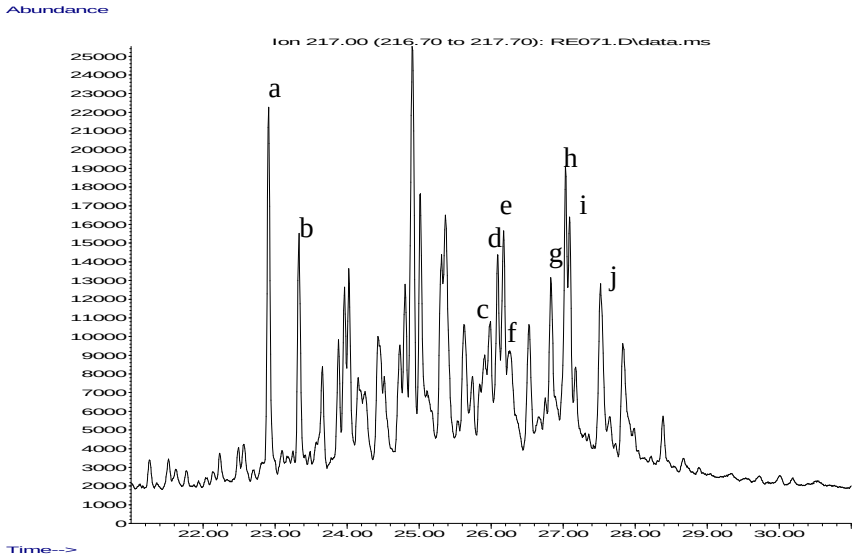


Fig. **5.5(b)**: Capillary column gas chromatogram of the sterane (*m/z* 217) profile of Hutton crude oil

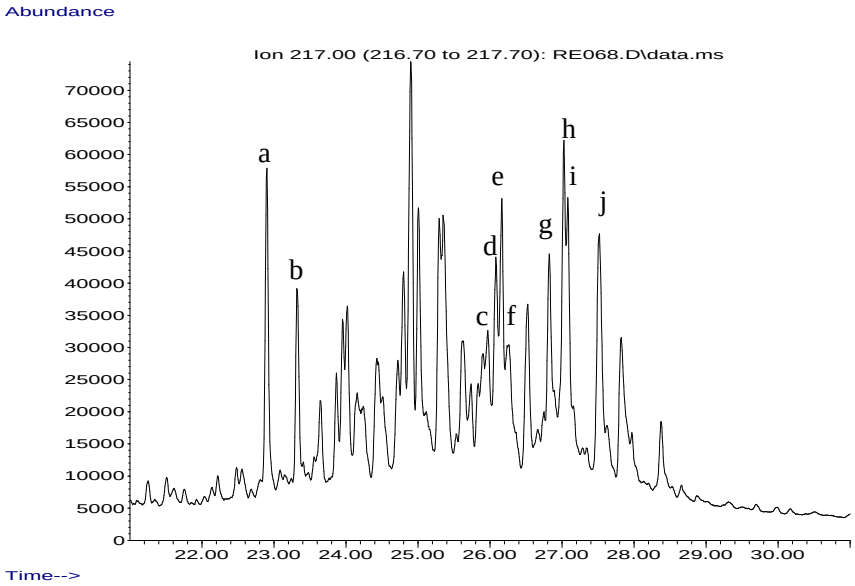


Fig. **5.5**(**c**): Capillary column gas chromatogram of the sterane (*m/z* 217) profile of Gulfaks crude oil

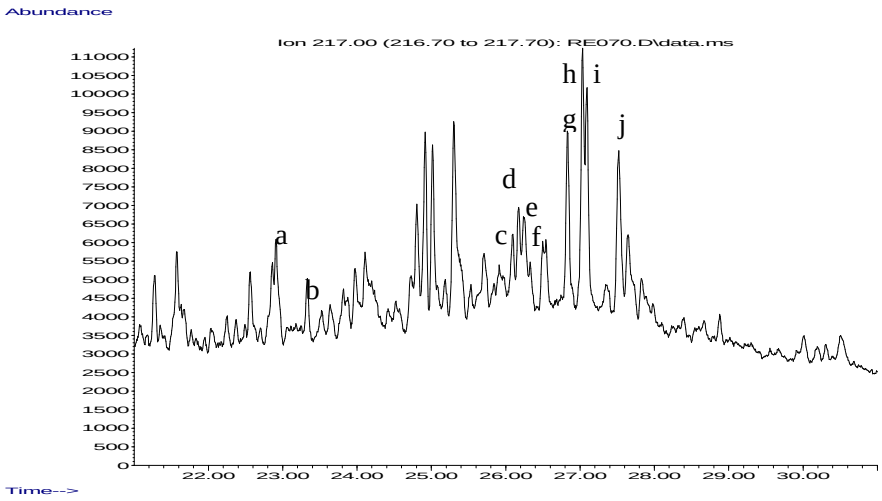


Fig. **5.5**(**d**): Capillary column gas chromatogram of the sterane (*m/z* 217) profile of Arabian light crude oil

5.3.2. Oil mixtures profiles

Figures **5.6** to **5.11** show the *m/z* 191 chromatograms of the different mixtures of the studied crude oils.

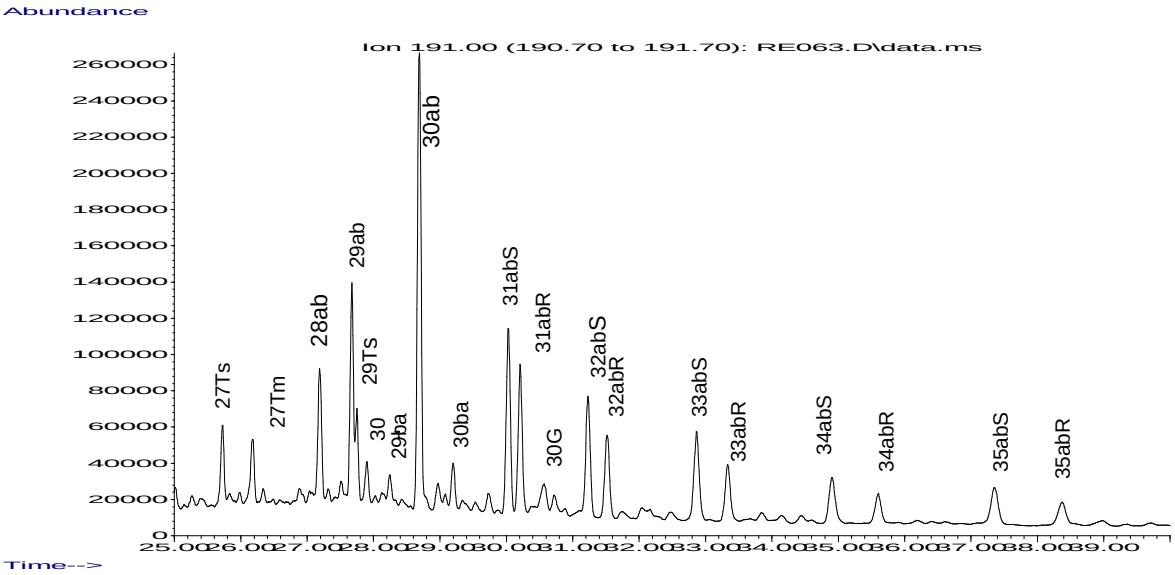


Fig. **5.6**: Triterpane (*m/z* 191) profile of Gullfaks crude and Hutton crude oil mixture in the ratio 1:1.

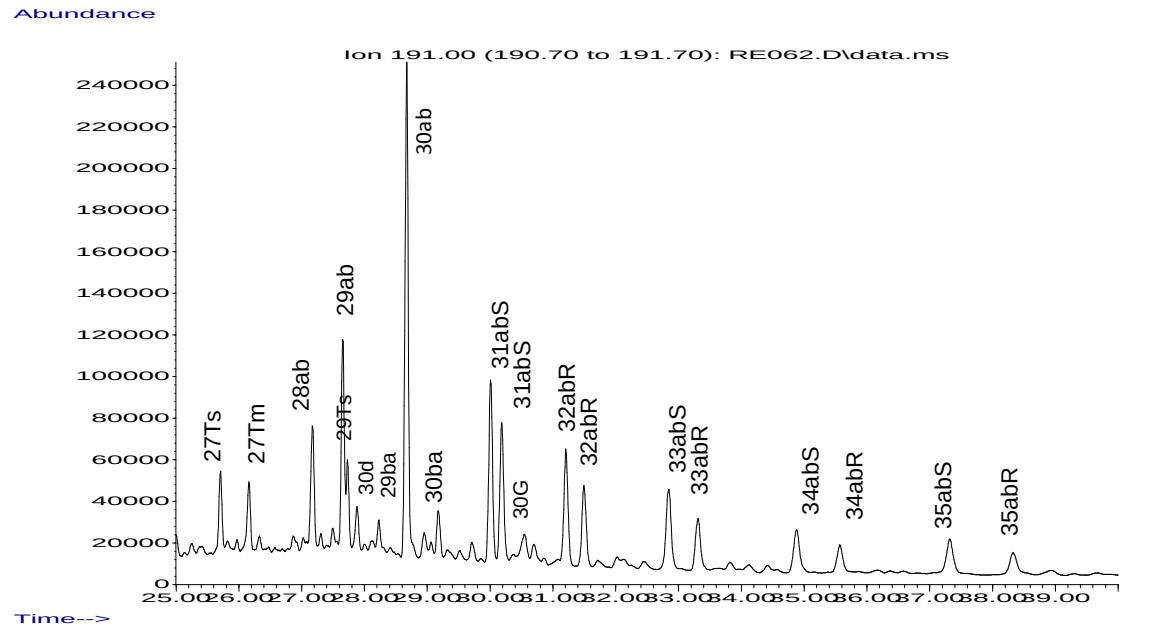


Fig. **5.7**: Triterpane (*m/z* 191) profile of Gullfaks crude and Hutton crude oil mixture in the ratio 3:1.respectively.

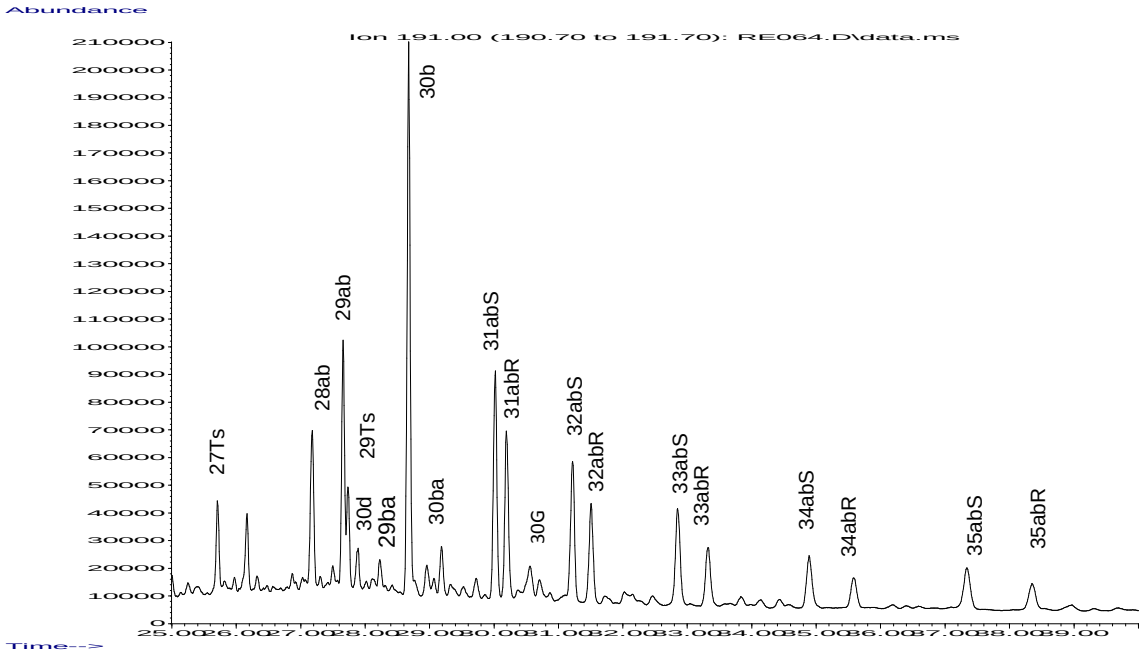


Fig. **5.8**: Terpane (*m/z* 191) profile of Gullfaks crude and Hutton crude oil mixture in the ratio 1:3 respectively

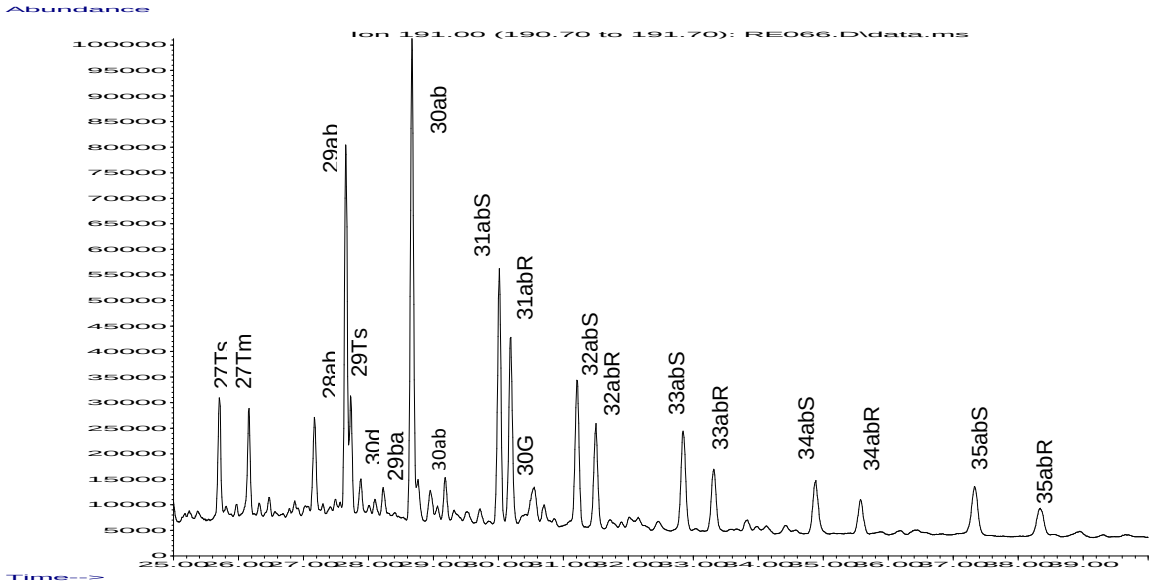


Fig. **5.9**: Terpane (*m/z* 191) profile of Forties crude and Arabian light crude oil mixture in the ratio 1:1.

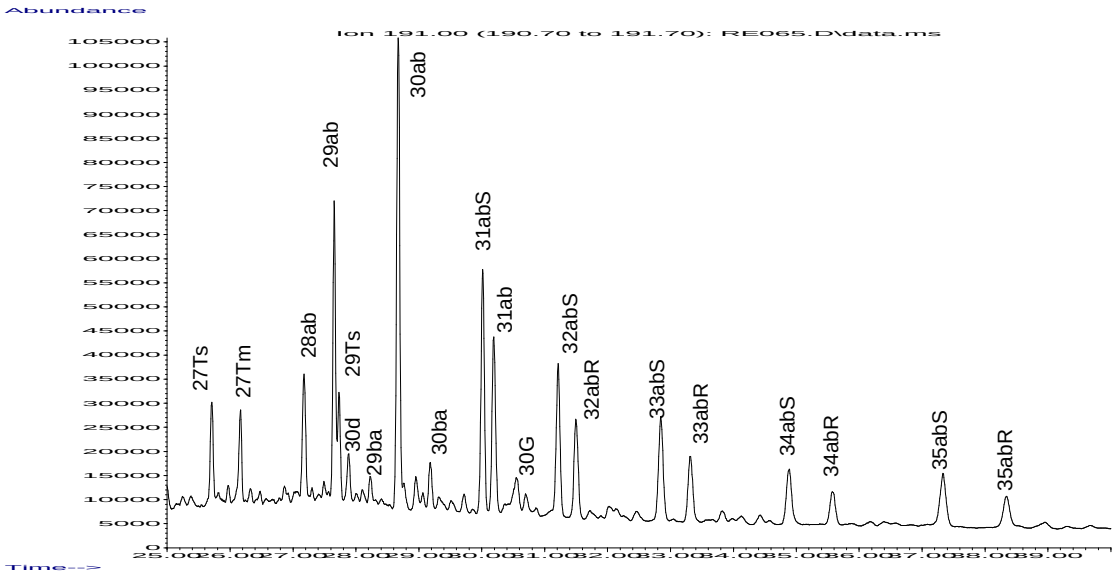


Fig. **5.10:** Terpane (*m/z* 191) profile of Forties crude and Arabian light crude oil mixture in the ratio 3:1 respectively.

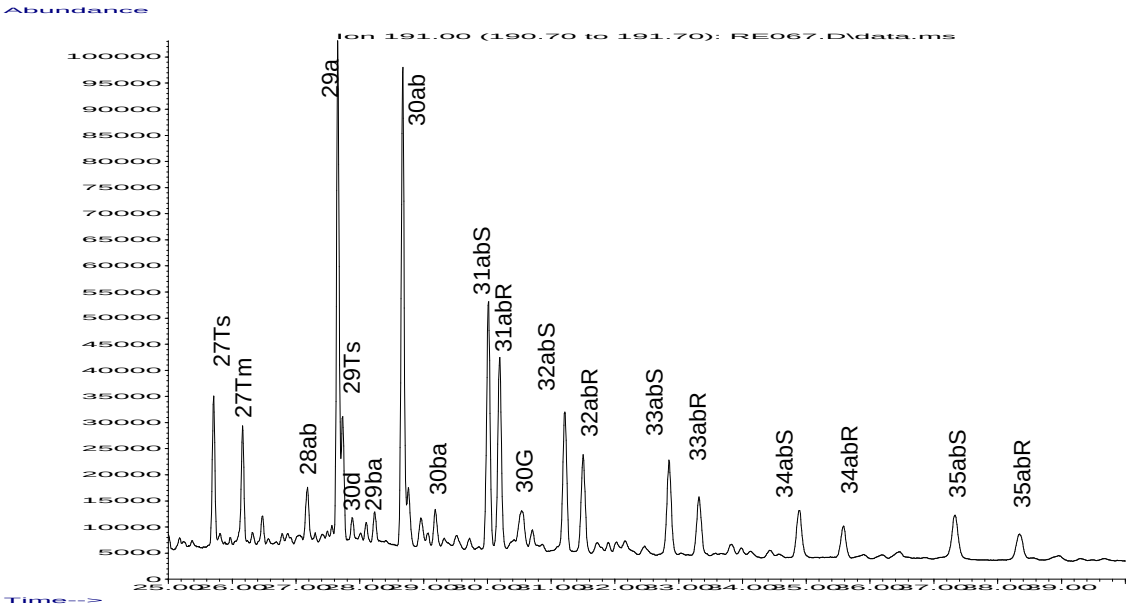


Fig. **5.11**: Triterpane (*m/z* 191) profile of Forties crude and Arabian light crude oil mixture in the ratio 1:3 respectively

5.3.3. Comparison of the geochemical biomarker profile of Aberdeen harbour with those of the crude oils

It was established in previous chapters using the *n*-alkanes, PAHs distribution and the biomarker profile that the mussels collected from Aberdeen harbour and used for the laboratory and field PAH kinetics studies showed petrogenic contamination. The sections below compare the geochemical biomarker profiles of the harbour mussels with those measured in the different crude oils and their various mixtures

5.3.3.1. Harbour mussels versus the crude oil triterpane and sterane biomarkers

The harbour mussels (Fig. **5.12**) showed all the triterpanes identified in the various crude oil samples but in different percentage abundances (Fig. **5.1-5.4**). As with the North Sea oils (Fig. **5.1-5.3**), the harbour mussels showed a bisnorhopane (BNH) peak. The presence of this unique triterpane in the mussels' chromatogram is indicative of a contribution from the North Sea oils, however, the relative abundance of BNH to hopane (30ab) present in the harbour mussels (0.11) is less than is normally found in the North Sea oils (Faksness *et al*., 2002, Dahlmann, 2003) typified by the values determined in this study for the individual North Sea oils and their mixtures (0.24 – 0.34). The mussels also showed C_{29}/C_{30} – hopane ratio which is not close to what is characteristics of neither the North Sea oils nor the Arabian light oil (Middle Eastern oil). Therefore, based on these specific biomarkers, the mussels' profile is not an exact match to any of the individual oils analyzed.

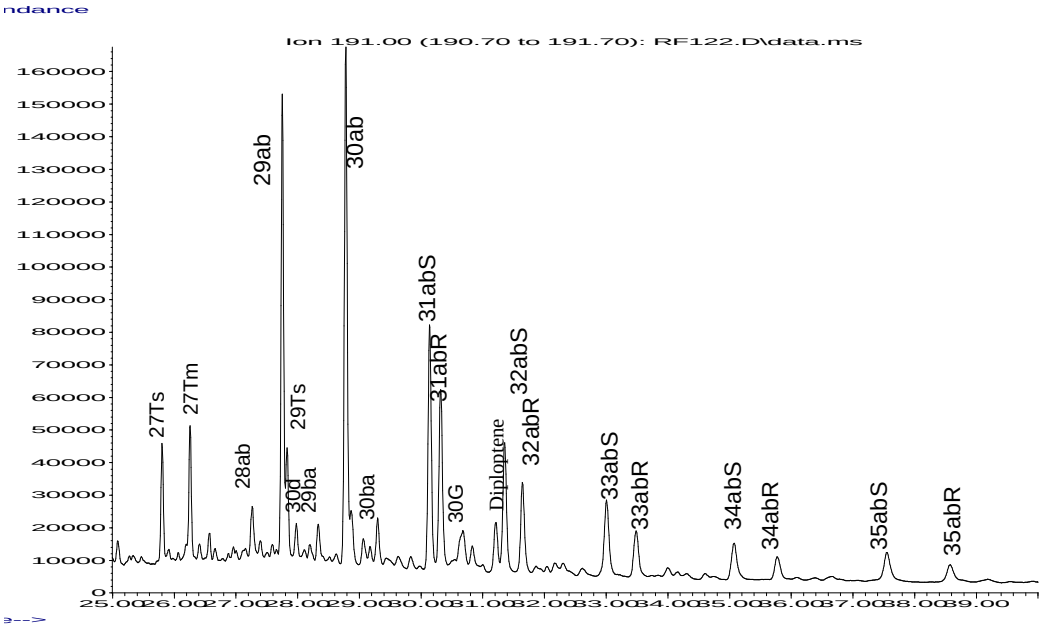


Fig. **5.12**: Triterpane (*m/z* 191) profile Aberdeen harbour mussel

5.3.3.2. Aberdeen harbour mussels versus crude oil mixtures

All the biomarker triterpane and sterane compounds identified in the habour mussels' chromatogram are also present in the oil mixtures chromatogram except diploptene. Diplotene is a natural triterpene component of most plants and animal and therefore its absence in the crude oils samples is expected. Fig. 5.6 -5.11 show that the oil mixtures have similar biomarker profile but in varying compositions. The uptake and accumulation of contaminants by organisms is dependent on a range of complex biological processes affecting absorption, distribution, metabolism and elimination of contaminants. Research has shown that factors like partition coefficient, route of uptake, etc, affects contaminant tissue burden. It follows that due to these reasons, some compounds may be preferentially accumulated over others. Compared to the habour mussels' profile, some of the similarities observed in the oil mixtures profiles include;

(a) %DR 28ab which is 10 for the habour mussels and the 10 for the 1:3 mixture of Arabian light oil and Forties crude oil (Table 5.2).

(b) % DR 29ab which is 45 for the harbour mussels and 47 and 41 respectively for the 1:3 and 1:1 mixtures of Arabian light oil and Forties crude oil.

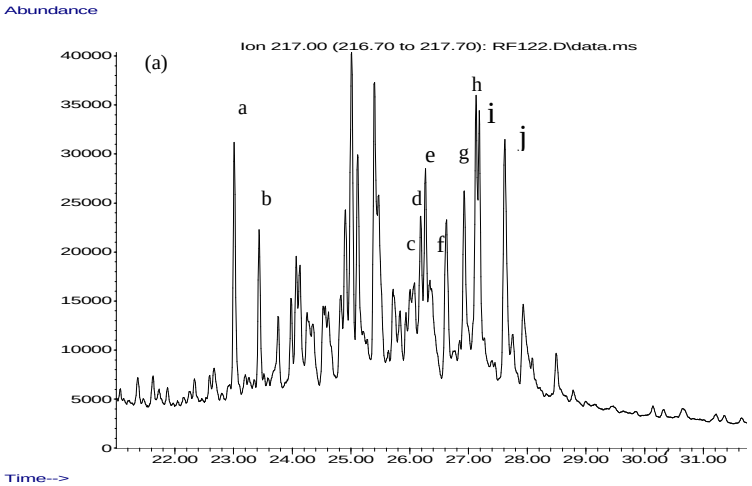
(c) %DR 30d which is 4.7 for the habour mussels and 3.7 and 5.91:3 and 1:1 mixtures of Arabian light oil and Forties crude oil.

(d) %DR29aaS which is 41for the habour mussels and 40 for the 1:1mixture of of Arabian light oil and Forties crude oil

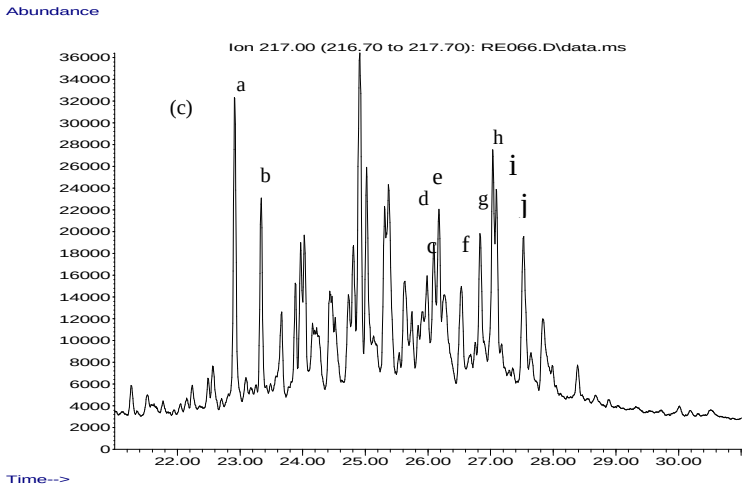
(e) % DR29bb which is 53 for the harbor mussels and 53 for both the 3:1 and 1:1 mixtures of Arabian light oil and Forties crude oil.

The sterane chromatograms of Aberdeen harbour mussels and those of the various mixtures of Forties crude oil and the Arabian light oil are also presented in Fig. **5.13**. The individual oil sterane chromatograms are presented in Section **5.3.1.2** (Fig. **5.5**). A close visual examination of the chromatograms portrays similarity in the sterane fingerprint of the harnour mussels and the mixtures of Forties and Arabian light crude oils. Further qualitative deductions could not be made from the sterane profiles because of their complexities and poor GC-MS resolution. It is noteworthy that, while n-alkanes and the PAHs are typically well resolved by low resolution GC-MS, steranes are not well resolved and often yield chromatograms with numerous co-eluting peaks.

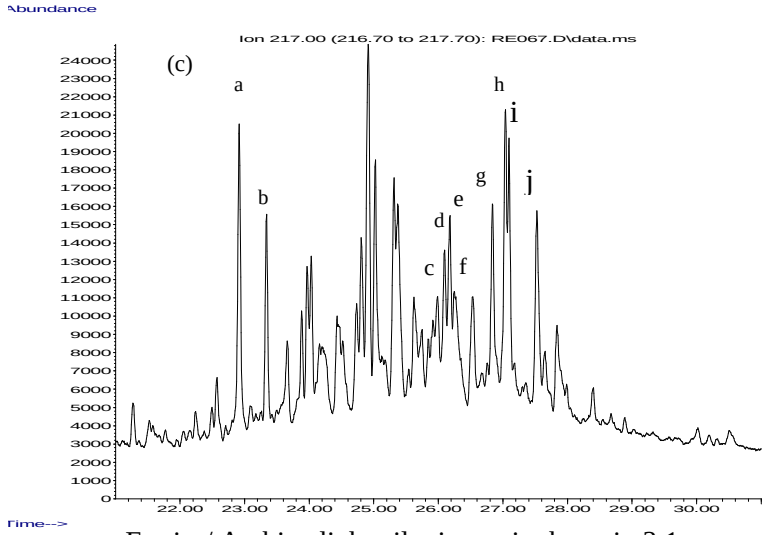
From the triterpane and the sterane fingerprints, the crude oil mixtures that bear close resemblance in profile to the profile of Aberdeen harbour mussels' are the 1:1 and 1:3 mixtures of Forties and Arabian light oil respectively. The similarities in profile between the harbour mussels' profile and the 1:1 and 1:3 mixtures F/A crude oils support the proposition of a possible contribution from the Middle Eastern oil made in Chapter **4**. As was discussed in section **5.3.1.1**, the presence of specific North Sea and Middle Eastern biomarker characteristics and the deviation from pure North Sea crude oil compositions suggest a mixed petrogenic contamination from these sources.



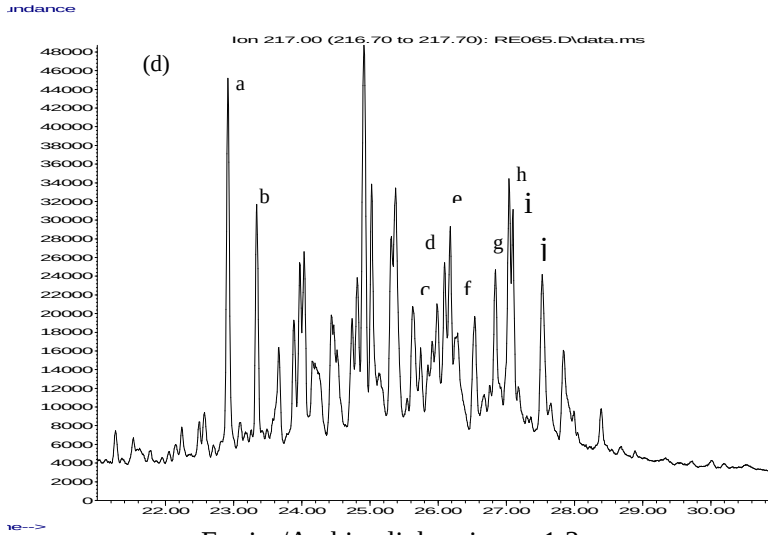
Aberdeen harbour mussel



Forties/ Arabian light oil mixture in the ratio 1:1



Forties/ Arabian light oil mixture in the ratio 3:1



Forties/Arabian light mixture 1:3

Fig. **5.13**: Sterane profiles of Aberdeen harbour mussels and various mixtures of Forties and Arabian crude oil.

5.3.3.3. Evaluation of the biomarker diagnostic ratios

To further classify the harbour contaminant input source, biomarker diagnostic ratios of the harbour mussels, individual crude oils samples, and their mixtures were calculated. The results obtained are presented in Table **5.2.** According to Wang *et al*. (2007), genetic oil – oil correlations are based on the concept that the composition of biomarkers in spill samples does not differ from those of the candidate source oils. The seven hopane and two sterane diagnostic ratios used in this study are among the ratios currently in use by Nordtest in oil spill investigations.

The ratios reported are calculated using the relative peak areas for the triterpanes and the peak heights for the steranes. Measuring diagnostic ratios based on peak heights and peak areas (semi-quantitative) within the same molecular ion has been recommended and is being used by Nordtest in environmental forensics (Faksness *et al*., 2002). The ratios are of the type

$$\left[a \middle/ (a+b) \right] * 100$$

where *a* and *b* are peak heights or areas within the same molecular ion (see Table **5.1**).

Table **5.2** shows the values of the diagnostic ratios (DR) obtained for the Aberdeen harbour mussels and the various oil sample combinations analyzed. The ratio of %DR27Ts calculated for individual oils were 53 for Hutton (H) crude oil, 54 for Gullfaks (G)and Forties (F) crude oil and 57 for the Arabian light oil (A), and 53 -55 for the oil mixtures. This ratio is an oil maturity ratio which increases from 0 at immaturity to 50 % in the oil window, and tend to increase to 80-90% at late maturity. The ratio is influenced by source type (Faksness *et al*., 2002). This ratio distinguishes the North Sea oils from the Arabian light oils but not the oil mixtures. The %DR27Ts values obtained for all the oil mixtures followed unexpected trend, especially 1:1 G/H (55) and 3:1 F/A (53) crude oils. The former is higher while the later is lower than the values obtained from individual oil samples. Conventionally, it is expected that the values will lie between those of the constituting pure oil samples. The value of %DR 27Ts for Aberdeen harbour mussels is lower than that determined for the oil samples. This may be connected to the abnormal trend observed for this DR. %DR 28ab, which is the ratio of 28,30-bisnorhopane /C30-hopane is different for

the North Sea oils; ranging from 19 for Gulfaks crude to 26 for Hutton crude. As 28,30-bisnorhopane is completely absent in Arabian light oil, this ratio is 0. This ratio is a good reflection of the changes in oil composition among the oil mixtures; with increase in the proportion of the Arabian light oil decreasing the value of %DR28ab.

Table **5.2**: Summary of selected *m/z* 191 and *m/z* 217 diagnostic ratios measured for Forties crude oil, Hutton crude oil, Gulfaks crude oil, Arabian light oil and Aberdeen harbour mussels.

Sample Id	**%DR-27Ts**	**%DR-28ab**	**%DR-29Ts**	**%DR-30G**	**%DR-29ab**	**%DR-30d**	**%DR-32abS**	**%DR-29aaS**	**%DR-29bb**
Forties Crude	54	24	15	10.5	29	9.5	57	39	54
Hutton Crude	53	26	14	9.2	30	7.3	57	48	60
Gullfaks Crude	54	19	12	7.0	28	8.3	58	39	52
Arabian Light Crude	57	0	21	13.9	54	1.0	57	52	59
G/H 3:1	54	20	12	7.5	28	7.6	58	38	51
G/H 1:1	55	22	13	8.3	29	7.1	58	39	50
G/H 1:3	54	24	14	9.1	29	6.9	59	38	52
F/A 3:1	53	21	16	12.1	35	8.7	57	39	53
F/A 1:1	54	16	17	11.7	41	5.9	57	40	53
F/A 1:3	55	10	18	12.8	47	3.7	57	50	59
Aberdeen Harbour mussel	49	10	15	7.1	45	4.7	59	41	53

G (Gullfaks crude), H (Hutton crude), F (Forties crude), A (Arabian light crude)

The %DR 29ab for the North Sea oils (Gullfaks, Hutton, and Forties) and mixtures of Gulfaks and Hutton oils (G/H) ranged between 28 and 30 while those for mixtures containing the Arabian light oil ranged between 35 and 54 with the highest value being that of the pure sample of Arabian crude oil. The ratio separates the samples into two distinct groups: the group with ≥ 50 % Arabian light oil (% DR-29ab range 41 -54) and the group

with < 50 % Arabian light oil (% DR-29ab range 28-30). According to this classification, Aberdeen Harbour mussels can be said to contain up to 50 % of the Arabian light oil.

The DR results highlighted a close similarity between the biomarker profiles of the Aberdeen harbour mussels and the 1:1 ratio of the Forties crude and Arabian light oil (F/A 1:1).

5.3.3.4. Comparison of the DRs of the oils with A.H mussels using the Nordtest approach.

The diagnostic ratio approach is the method used by Nordtest to compare spill samples with the suspect sources. The accuracy of the instrumental analysis and diagnostic ratio calculation relies on the measured variability among triplicate analysis (Nordtest, 2002). Nordtest recommends that a triplicate analysis, preferably that of the spill source be used to establish the confidence interval of the mean. The confidence interval is an expression stating that the true mean μ, is likely to lie within a certain distance from the measured mean, *x*. The confidence interval of μ is given by

$$\mu = \chi \pm \frac{t.s}{\sqrt{N}}$$

where *s* is the measured standard deviation between triplicate samples, *x* is the mean value, *t* is the Student's *t* and N is the number of observations. For the *t*-table, the number of degrees of freedom equals N-1for triplicate analysis.

A regression analysis of the profiles of the spill versus the suspect sample should give a straight line with x = y for a 'perfect match' or have the error bars of the DRs overlapping the line for a positive match within the analytical variations. The classification in use in the Nordtest system is summarized below:

Table **5.3**: suggested criteria for classification of spill samples from correlation studies of diagnostic ratios

Classification	Definition
Positive match	All DR with the CL 95 %
Possible match	All DR with the CL 98 %
No match	Any key DR outside of CL 98 %

Figs. **5.14** (**a-j**) below show the correlation between the DRs of the harbour mussels with each of the oil sample and mixtures analyzed using 95 % confidence interval. According to the Nordtest classification, all the DRs of the spill sample and the suspect sample should be in a straight line for a perfect match or have the error bars overlapping the line for a positive match. The plots of pure samples of the North Sea oils (Forties, Gullfaks, and Hutton) and all mixtures of Gullfaks and Hutton (G/H) have some of the DRs outside of the x=y line. Also pure sample of the Arabian light oil and its various mixtures with Forties crude oil show no positive match with the harbour mussels. However, the 1:1 mixture of Forties and Arabian light oil show a possible match with the harbour mussels [Fig. **5.14(i)**].

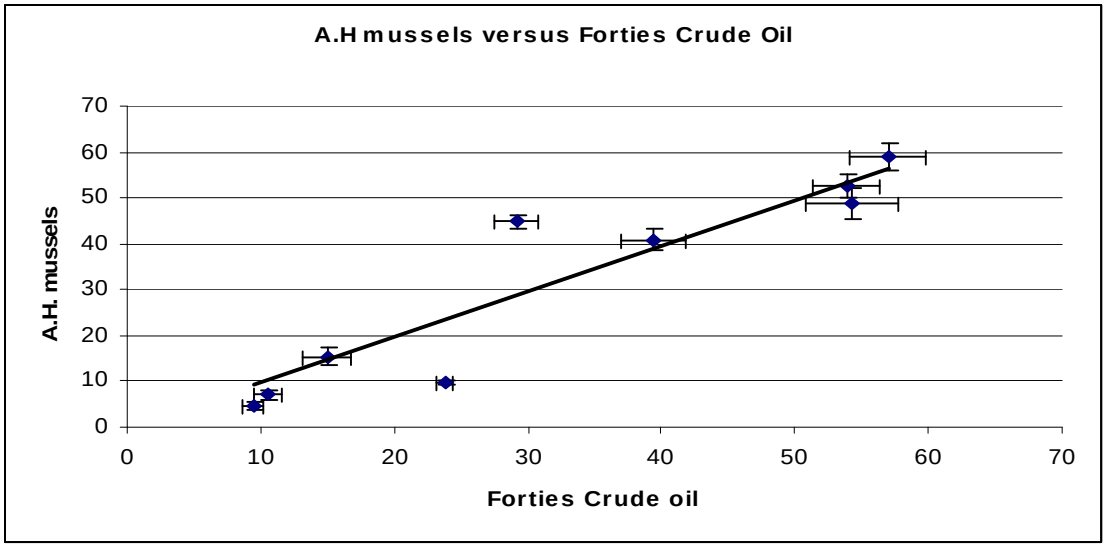


Fig. **5.14** (**a**): Correlation between Aberdeen harbour mussels and Forties crude oil.

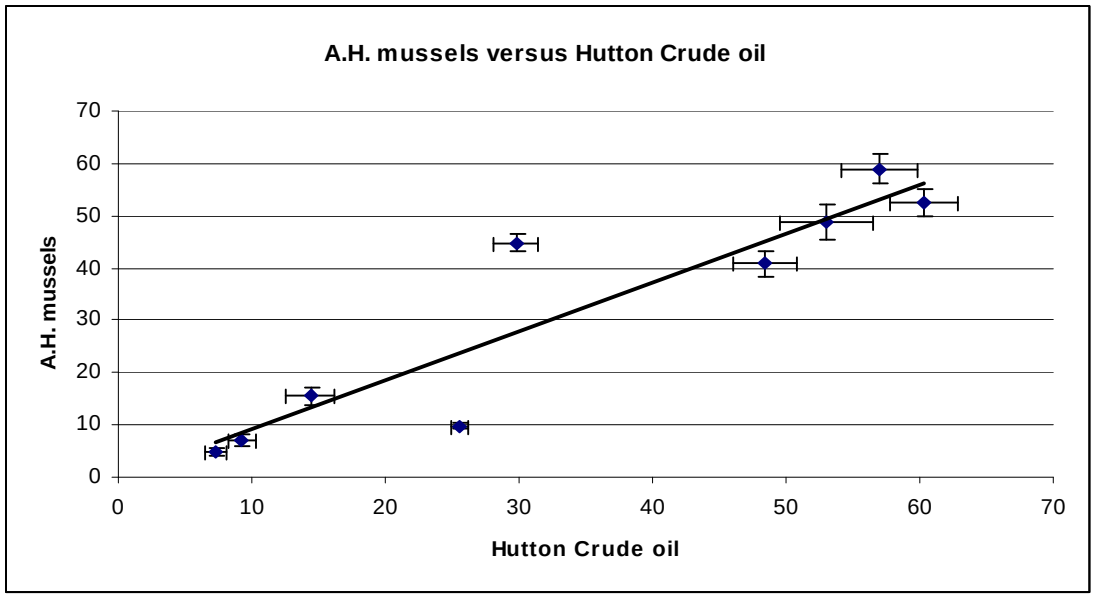


Fig. **5.14** (**b**): Correlation between Aberdeen harbour mussels and Hutton crude oil

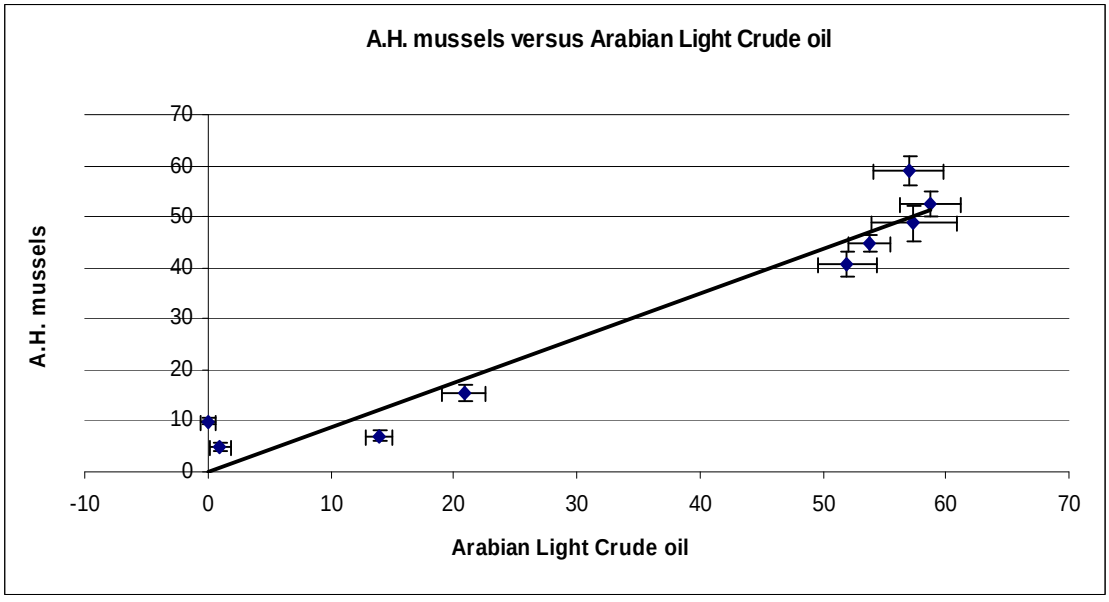


Fig. **5.14** (**c**): Correlation between Aberdeen harbour mussels and Arabian light crude oil

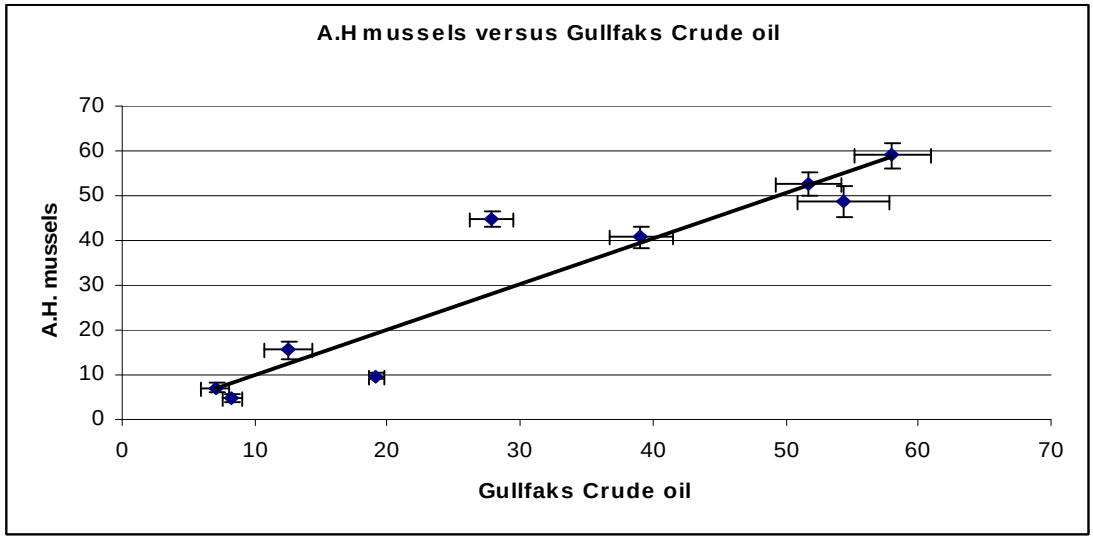


Fig. **5.14** (**d**): Correlation between Aberdeen harbour mussels and Gullfaks crude oil

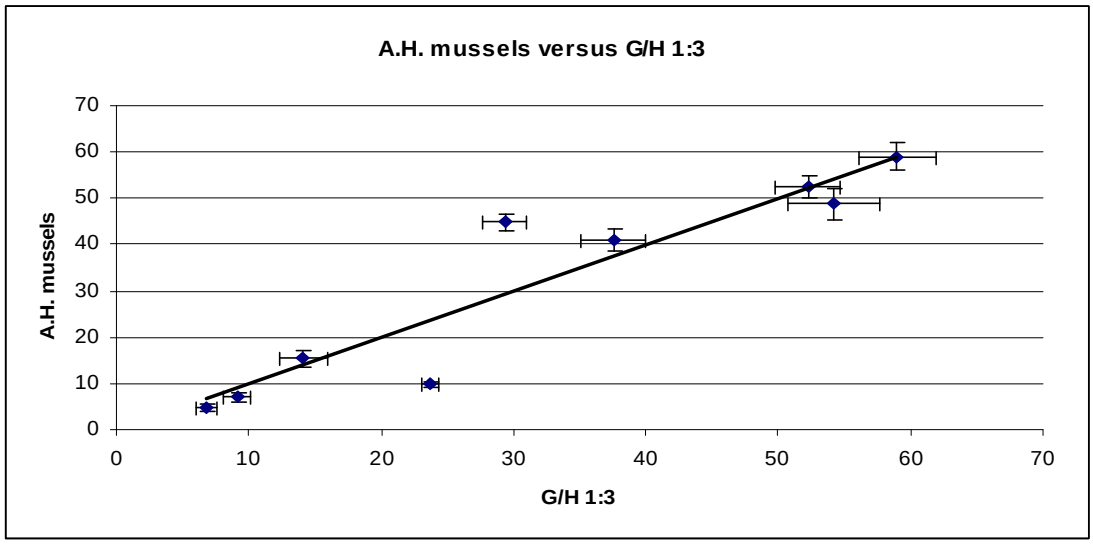


Fig. **5.14** (**e**): Correlation between Aberdeen harbour mussels and 1:3 mixture of Gullfaks and Hutton crude oils.

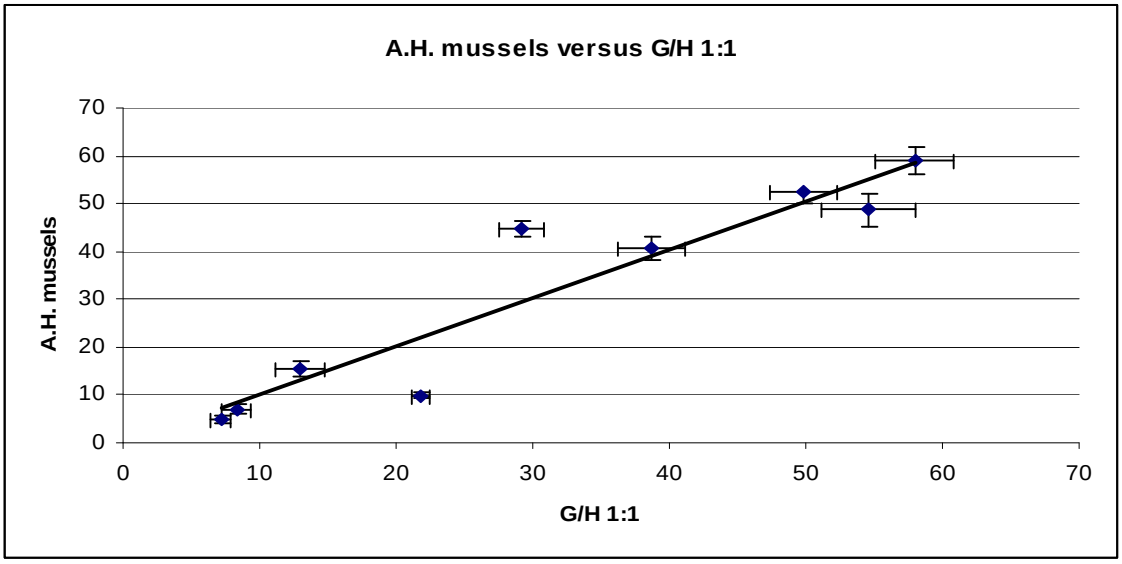


Fig. **5.14** (**f**): Correlation between Aberdeen harbour mussels and 1:1 mixture of Gullfaks and Hutton crude oils.

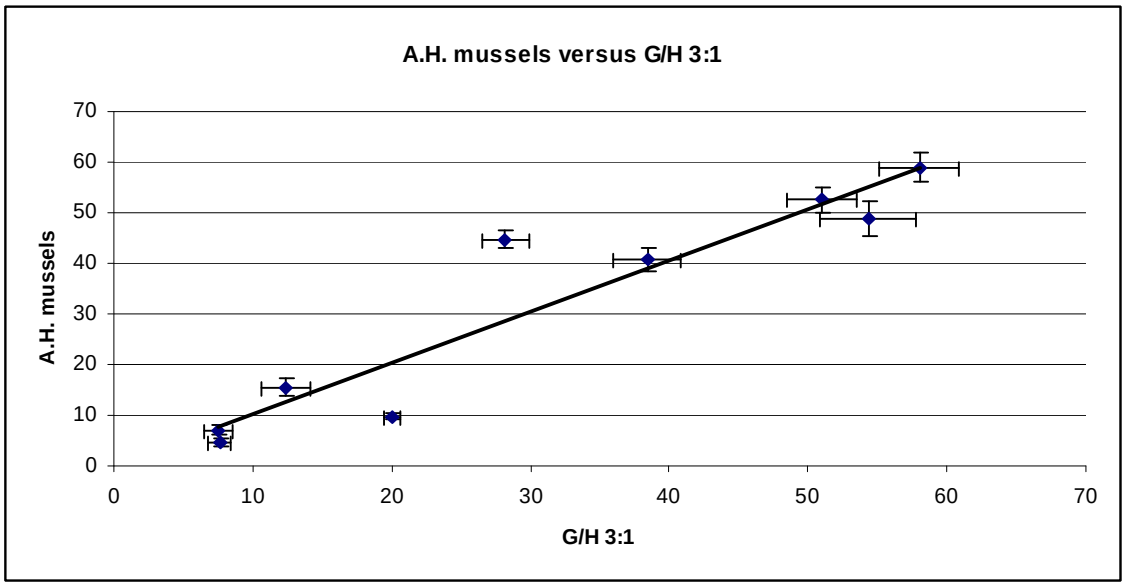


Fig. **5.14** (**g**): Correlation between Aberdeen harbour mussels and 3:1 mixture of Gullfaks and Hutton crude oils.

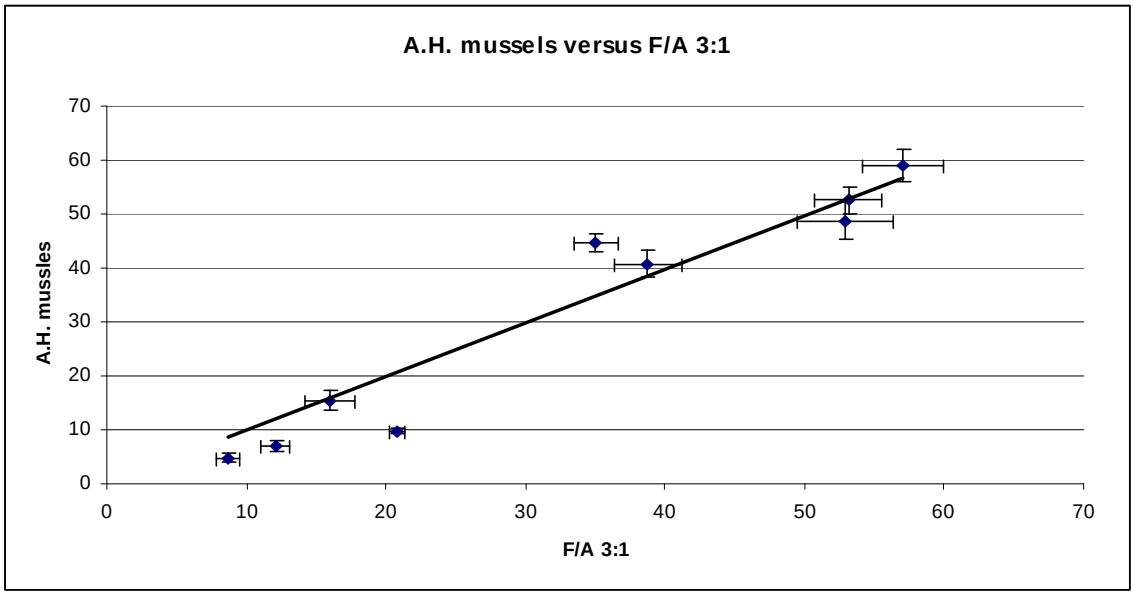


Fig. **5.14** (**h**): Correlation between Aberdeen harbour mussels and 3:1 mixture of Forties and Arabian light crude oils

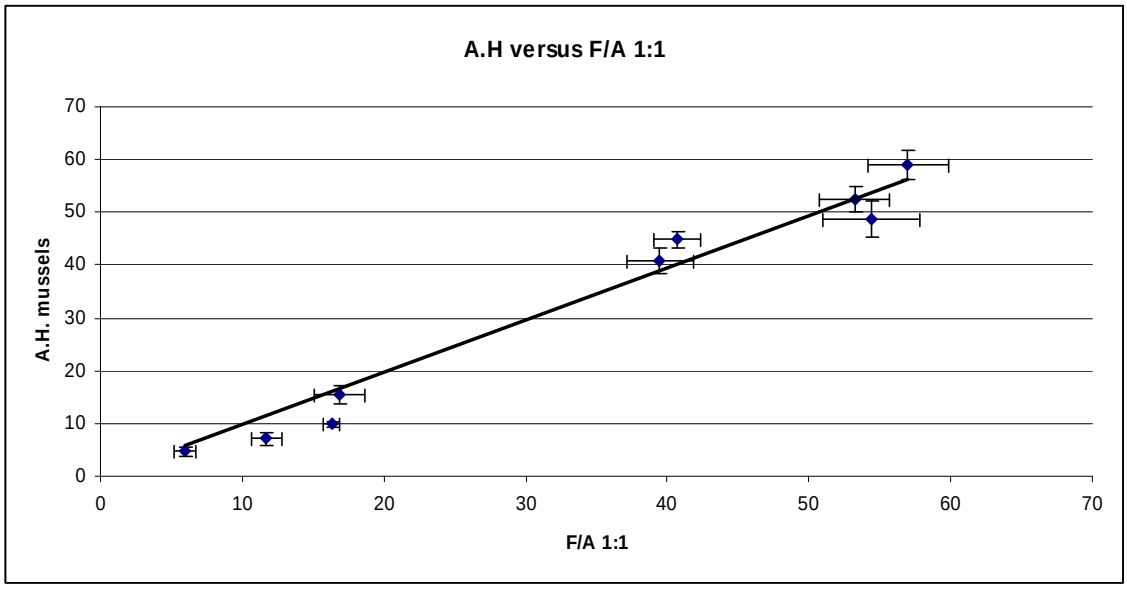


Fig. **5.14** (**i**): Correlation between Aberdeen harbour mussels and 1:1 mixture of Forties and Arabian light crude oils

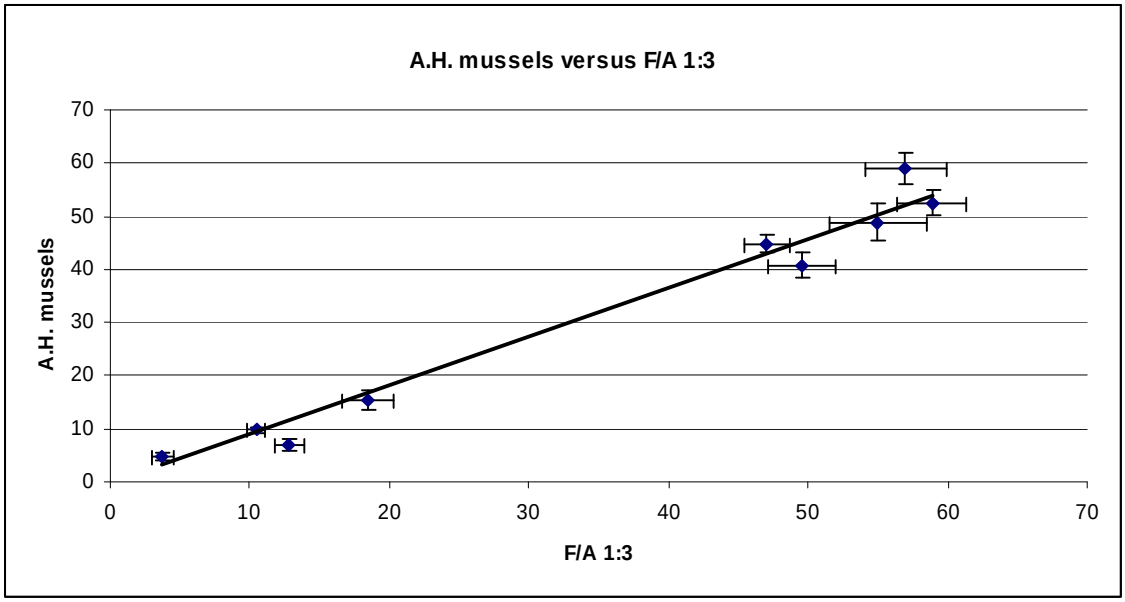


Fig. **5.14 (j)**: Correlation between Aberdeen harbour mussels and 1:3 mixture of Forties and Arabian light crude oils.

However, in the Nordtest system, some specific PAH indices are also used together with the geochemical biomarker indices for oil spill source classification.

Although the results from this study indicate that the harbour mussels were contaminated from a combination of North Sea oil and of Middle Eastern oil, it does not clearly indicate the exact percentage contributions of each suspect oil to the contamination reported. The lack of a perfect match between Aberdeen harbour mussel and the mixtures of Arabian light and Forties (especially F/A 1.1) suggests that either the % contribution from each oil less or more proposed or that the components of the oil has undergone some alteration within the spill environment and/or the organism or both.

5.4. Conclusion.

Geochemical biomarker fingerprints of the harbour mussels has proved very useful in the classification of the source of PAHs determined in the mussels. The presence of specific geochemical biomarker compounds (steranes and triterpanes) characteristics of crude oils in the harbour mussel indicates that the PAHs found in these mussels has some petrogenic origin. The sterane and triterpane distributions show specific fingerprints characteristics of both the North Sea and Middle Eastern crude oils, indicating possible contribution of oils from both areas to the harbour contamination. Specific biomarker diagnostic ratios studied (%DR-27Ts, %DR-29Ts, %DR-28ab, %DR-29ab, %DR-30G, %DR-30d, and %DR-32abS) showed variability among pure oil samples, their mixtures and the harbour mussels. However, a possible match (at a 95% confidence interval of the mean) was established between the test samples and the 1:1 (v/v) mixture of Forties and Arabian light crude oils. The slight deviations observed between these two samples may be due to weathering effects or differences in the actual and proposed oil % compositions. Although there was no documented evidence of a spill involving these crude oils around the harbour in recent times, contributors of petrogenic contamination to the harbour environment may include operational discharges from tankers, leaky tanks, minor accidental spills, etc. This kind of situation is not unexpected of the harbour area considering the density of cargo using the harbour. Being at the centre of activity for the offshore oil and gas industry's marine support operations in the North-west Europe; Aberdeen harbour is characterized by high shipping traffic.

Chapter Six

Investigation of PAH elimination kinetics and the changes in the geochemical biomarker profile in mussels exposed to crude oils

6.1 Introduction

Elimination kinetics of specific PAHs were estimated from wild mussels collected from Aberdeen harbour and depurated in relatively clean sea water in the laboratory. The depuration kinetics calculated from these mussels differed from values reported in the literature for acutely exposed mussels but were close to literature values for chronically exposed mussels. The reason for the differences in the k_2 values calculated from chronically and acutely exposed organisms could be attributed to the PAH contamination source and duration of exposure of the organisms to it. The present study, therefore, uses mussels exposed to acute artificial oil contamination and depurated in the same laboratory condition as the Aberdeen harbour mussels to investigate the elimination kinetics of specific PAH compound from acutely exposed mussels in order to compare the rates obtained in both experimental conditions with literature values. In addition, the changes in geochemical biomarkers (steranes and triterpanes) with PAHs elimination were also investigated.

6.1.1 Experimental design

Mussels accumulate hydrophobic contaminants readily due to their ability to filter high volumes of water (high water filtration rate). Mussels filter, on average, 7.5 litres of sea water/hour (Reed, 2002). As a consequence of this, they accumulate and concentrate pollutants from sea water, particularly those associated with particles. This ability to accumulate materials facilitates the detection and measurement of pollutants that are sparingly soluble in water and may be in the water column at very low concentrations. The experiment was designed to expose mussels to artificial oil contamination and measure the rate of clearance of PAHs and changes in geochemical biomarker profile with clearance

of PAHs. In this experiment, the mussels were either exposed to Arabian light oil, Gullfaks crude oil or Brent crude oil mechanically dissolved in water for 2 days.

6.1.2 Sample collection

The mussels used for the experiment were collected from Loch Etive shellfish farm. Loch Etive is a relatively clean site located on the west coast of Scotland, where industrial activities are minimal. The mussels were transported to FRS within six hours of being removed from water and kept in a holding tank supplied with continuous flow of filtered sea water overnight before exposure to oil contamination.

6.1.3 Oil exposure

The mussels were divided into 3 groups and transferred to 3 separate 100 litre glass fibre tanks containing 100 litres of filtered seawater. Using a calibrated pipette, 2.5 ml of either Gulfaks crude, Brent crude or Arabian light crude oils was added to the exposure tanks and mechanically mixed with the water. The mussels were shielded from direct sunlight by covering the tanks with opaque materials, and supplied with continuous aeration. The exposure period lasted for two days and no mussel died during this period.

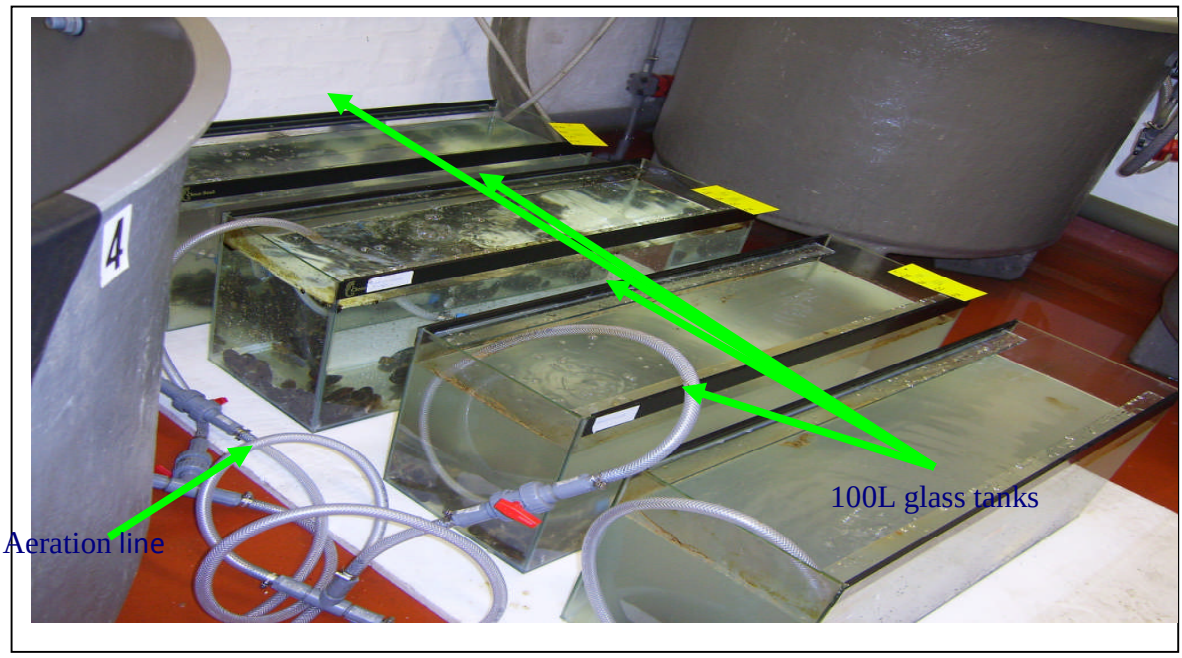


Figure 6.1: Experimental set-up showing the exposure of mussels to crude oils

6.1.4 Sample depuration

At the end of the two days exposure, the mussels were removed from the exposure medium and transferred to clean tanks for depuration; a separate tank for mussels exposed to each of the crude oils. The depuration tanks (360 litres) were continuously supplied with filtered seawater (0.6 ± 0.05 l/s). The depuration phase lasted for 11 days. An aliquot of 25-30 mussels was removed to measure the background PAH and biomarker concentration in the mussels prior to exposure to the crude oils. Sub-samples were also removed at the end of the exposure period, prior to depuration and at 0.25, 1, 2, 4, 7, and 11days of depuration. The mussels were opened, the entrained water drained off onto a tissue paper and the total soft tissue excised into solvent washed aluminum cans, homogenized by Ultraturrax$^{(TM)}$ and frozen at -18 to - 20 ºC in a dedicated freezer until required for analysis.

6.2 Analytical methods

The extraction, isocratic HPLC fractionation and GC-MS determination were as detailed in Chapter **2**.

6.3 Results and discussion

6.3.1 Polycyclic aromatic hydrocarbons

The background total PAH (2- to 6- ring parent compounds and their alkylated homologues) concentrations found in the mussels prior to exposure was 15 ± 0.3 ng g^{-1} wet weight. Mussels accumulated different concentrations of PAH from the three crude oils used. The total PAH concentrations were 26932.3 ± 1376.1 ng g^{-1} for mussels exposed to Gullfaks crude oil, 16124.6 ± 113.1 ng g^{-1} for Arabian light oil and 9109.9 ± 105.0 ng g^{-1} for Brent crude oil (Table **6.1**). The naphthalenes (parent and alkylated compounds) contributed 77.3 %, 48.1 % and 78.3 respectively of these totals.

The similarity of the North Sea crude oils (Gullfaks and Brent) is reflected in the percentages of specific PAH sub-groups accumulated by the mussels (Table **6.1**). For example, total naphthalenes (77.3 % and 78.3%); total phenanthrene/anthracenes (12.9% and 12.9%), total DBTs (6.0% and 5.3%), etc, for Gullfaks and Brent respectively. The percentage concentration of the 2- and 3-rings compounds relative to the 4- to 6-rings compounds found in mussels exposed to either of the two crude oils are also similar.

The PAH pattern in the mussels exposed to the Arabian light oil was different to those of the North Sea crudes. The naphthalenes accounted for only 48 % of the total concentration determined compared to >75 % found in samples exposed to the North Sea oils. The percentage of DBTs (38.4 %) in mussels exposed to Arabian light oil is also more than six times greater than found in mussels exposed to the North Sea oils.
Surprisingly, the cumulative percentages of the 2- and 3-rings compounds found in all the mussels are similar; 97.4%, 97.1% and 97.7% for mussels exposed to Arabian light oil, Gullfaks and Brent crude oils respectively. It also follows that the percentage compositions

of the heavier (4- to 6-ring) compounds in the three sample sets are similar (2.7%, 2.9% and 2.3%) respectively, irrespective of the source (Fig**. 6.2**).

Table **6.1**: The sum and % composition of various PAH subgroups determined in control mussels exposed to 2.5 ml of PAH of either Arabian light crude oil, Gullfaks crude oil or Brent crude oil for 2 days (details of all PAH compounds measured are given in Table **2.2** in Chapter **2**)

PAH subgroups	PAH concentration (ng g^{-1}) n = 3			% PAH concentration		
	Arabian Light	Gullfaks	Brent	Arabian light	Gullfaks	Brent
Total 128	7763.9 ± 45.7	20816.7 ± 1084.3	7137.1 ± 75.8	48.1	77.3	78.3
Total 178	1683.9 ± 32.9	3474.2 ± 160.7	1177.4 ± 22.2	10.4	12.9	12.9
Total DBTs	6196.4 ± 113.8	1608.7 ± 83.2	485.7 ± 16.3	38.4	6.0	5.3
Total 202	248.5 ± 7.4	480.4 ± 23.8	123.5 ± 5.5	1.5	1.8	1.4
Total 228	134.3 ± 2.7	234.2 ± 12.0	71.6 ± 2.0	0.8	0.9	0.8
Total 252	41.3 ± 0.9	53.1 ± 3.8	14.9 ± 0.7	0.3	0.2	0.2
Total 276	2.7 ± 0.2	5.0 ± 0.1	1.0 ± 0.2	0.02	0.02	0.01
Acenaphthylene	-	0.5 ± 0.0	-	-	0.002	-
Acenaphthene	7.6 ± 0.2	34.7 ± 2.4	9.1 ± 0.3	0.05	0.13	0.10
Fluorene	44.63 ± 0.4	224.0 ± 9.0	89.3 ± 1.7	0.28	0.83	0.98
Dibenz[*a*,*h*]anthracene	0.2 ± 0.1	0.6 ± 0.0	0.2 ±0.0	0.001	0.002	0.002
Total PAH	16124.6 ± 113.1	26932.3 ± 1376.1	9109.9 ± 105.0			
% parent PAH				4.1	6.1	9.2
% 2- and 3-rings				97.3	97.1	97.7
% 4- to 6-rings				2.7	2.9	2.3

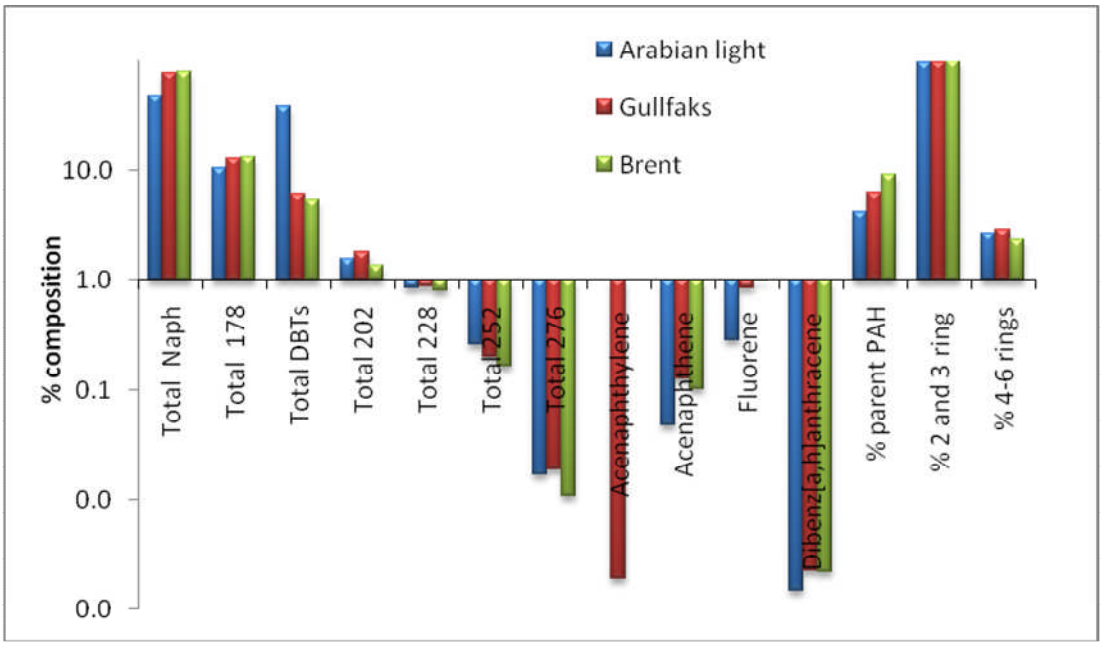


Fig.**6.2**: A plot showing the relative % composition of PAH sub-groups and % composition of the lighter and heavier PAH compounds determined in mussels exposed to Arabian light oil, Gullfaks crude oil and Brent crude oil.

The 2- and 3-rings PAHs are acutely toxic and are often associated with taint in fish and shellfish species (Heras *et al*., 1992 and Davies *et al*., 2002). These compounds were accumulated in concentrations exceeding values that have been reported to induce taint in shellfish (Topping *et al*., 1997)). The concentration of benzo[*a*]pyrene (an established carcinogen) and some of the priority PAHs benz[*a*]anthracene, dibenz[*a,h*]anthracene, and benzofluoranthenes found in the mussels were lower than the limits set by the European Union for these compounds in shellfish. Other priority compounds; pyrene and their alkylated compounds (C1-C3) and chrysene and (C1-C2) were accumulated in concentrations 30-100 times higher than found in the control mussels. The new suite of PAHs (cyclopenta[*c, d*] pyrene, dibenzo[*a,e*]pyrene, dibenzo[*a,h*]pyrene, dibenzo[*a,i*]pyrene, and dibenzo[*a,l*]pyrene) recently included as priority chemicals by the EU [Commission Regulation (EC) No 466/2001] were rarely detected in all samples.

6.3.1.1 Investigation of PAHs elimination kinetics

PAHs were lost quickly from the mussels during the depuration period. After 6 hours, decreases of 5.9 %, 17.0 % and 10.1 % in total PAH concentration were observed for mussels exposed to Arabian light oil (AL), Gullfaks crude (GU) and Brent crude oils (BR) respectively.. Concentrations continued to fall, such that after 2 days of depuration, 43.9 % (AL), 55.9 % (GU) and 53.0 % (BR) had been lost, and after 11 days depuration period 75.2 % (AL), 89.7 % (GU) and 95.0 % (BR) had been lost.

Elimination rate constants were evaluated by fitting the depuration data into first order kinetics. The background PAH concentrations in the mussels (prior to oil exposure) were subtracted from the measured concentration at each sample point. The elimination rate constants obtained ranged between 0.003 and 1.923 day^{-1} (Appendix 4). The 2- to 4- ring compounds exhibited a perfect linear relationship between ln-transformed concentration and time (Fig **6.3**), This can be seen in the values of the correlation coefficient ($r^2 > 0.95$) and *p*-values (<0.001) recorded for these compounds (Appendix 4). The good regression lines obtained show that the first order kinetics model used in this study is appropriate for describing the elimination rates of these compounds from the test organisms.

Generally, the estimated elimination rate constants (k_2) were similar among the three sets of test samples. Elimination was faster for the lighter compounds; naphthalenes, phenathrenes/anthracenes, and dibenzothiophenes (Table **6.2**), giving half-lives of 0.6/0.5/0.4 d (naphthalene), 1.0/1.0 /0.9 d (2-methyl naphthalene), 1.0/1.0 /0.8 d (1-methly naphthalene) 5.3/5.5/3.5 d (C4 naphthalenes), 2.6/2.7/2.0 d (phenanthrene), etc, in mussels exposed to Gullfaks, Arabian light and Brent crude oils respectively. Although these compounds were accumulated in higher concentrations, they were also readily eliminated from the organisms. Elimination rate constant was found to decrease with increase in alkylation (Table **6.2**). It follows that the biological half-lives ($t_{1/2}$) of a homologues series increases with increase in alkylation, for examples, 0.6 days for naphthalene and 5.3 days for C4-naphthalene.

Some of the 4-, 5- and 6-ring compounds e.g. benzo[*e*]pyrene, benz[*a*]anthracene, perylene, indeno[1,2,3,*c*,*d*]pyrene, benzo[*g*,*h*,*i*]perylene returned inconsistent depuration pattern where the initial concentration was low (< 4 ng g^{-1} wet weight), and generally exhibited biological half-lives higher than the lower rings.

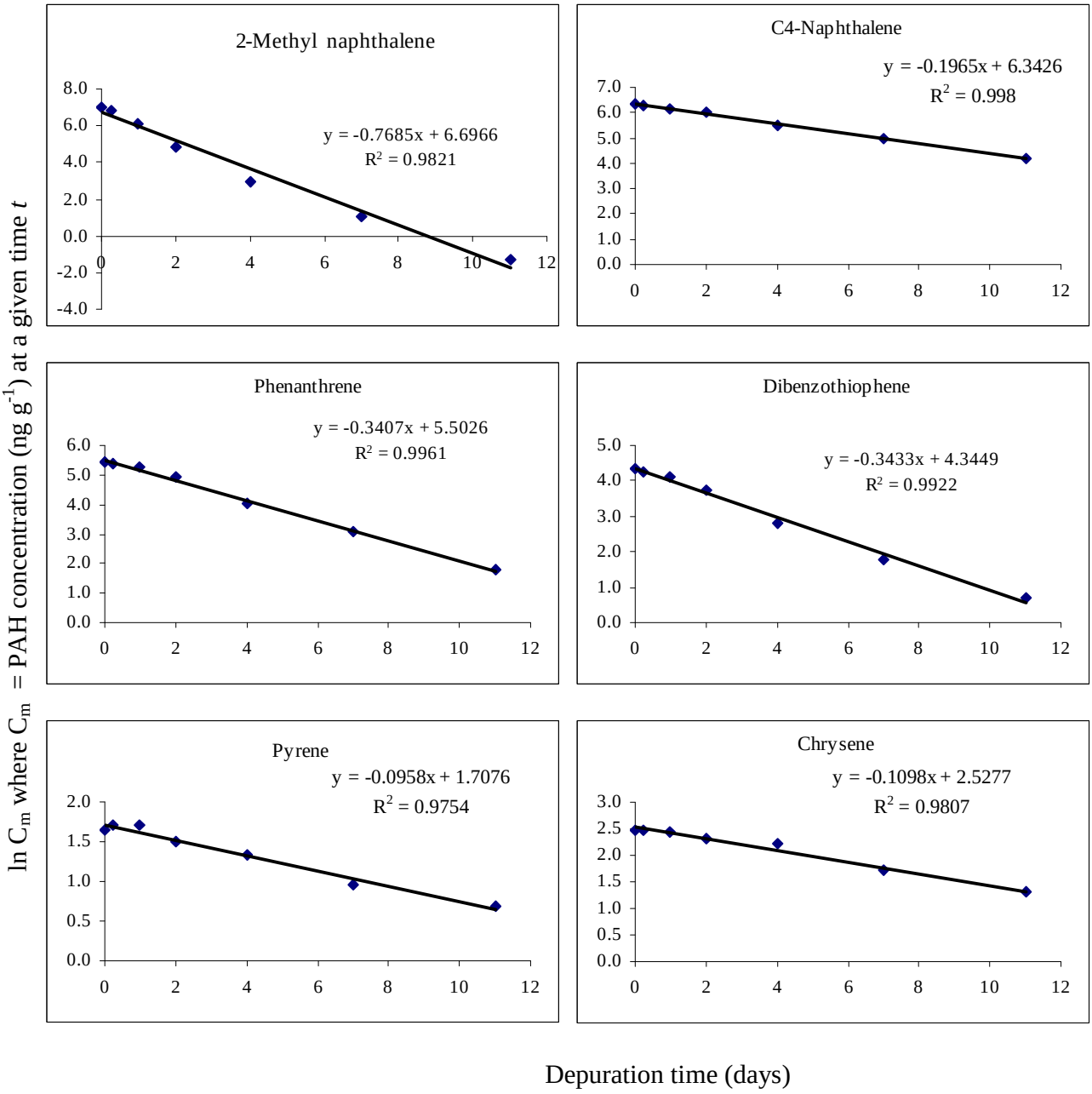


Fig. **6.3**: Example elimination plots (lnCm versus time) of individual PAH compound. Plots taken from the data of mussels exposed to Brent crude oil.

Table **6.2**: Median elimination rate constants (k_2) and biological half-lives ($t_{1/2}$) determined for individual PAH from mussels exposed to each of Arabian light, Gullfaks and Brent crude oils assuming first order depuration.

Compounds	AL	GU	BR	Median k_2	Median $t_{1/2}$
Naphthalene	0.5	0.6	0.4	1.4167	0.5
2-Methyl Naphthalene	1.0	1.0	0.9	0.6993	1.0
1-Methyl Naphthalene	1.0	1.0	0.8	0.6873	1.0
C2-Naphthalenes	1.9	1.7	1.5	0.3980	1.7
C3-Naphthalenes	3.4	3.1	2.5	0.2217	3.1
C4-Naphthalenes	5.5	5.3	3.5	0.1310	5.3
Acenaphthene	1.8	2.0	1.7	0.3853	1.8
Fluorene	2.1	1.8	1.6	0.3910	1.8
Phenanthrene	2.6	2.5	2.0	0.2783	2.5
Anthracene	96.3	2.7	3.9	0.2168	3.2
C1-Phenan/Anthracenes	5.0	4.6	3.1	0.1500	4.6
C2-Phenan/Anthracenes	9.3	8.6	4.9	0.0803	8.6
C3-Phenan/Anthracenes	13.8	11.1	5.9	0.0622	11.1
Dibenzothiophene	2.4	2.3	1.9	0.2967	2.3
C1-Dibenzothiophenes	4.4	4.2	3.0	0.1633	4.2
C2-Dibenzothiophenes	8.4	8.7	4.6	0.0828	8.4
C3-Dibenzothiophenes	12.8	13.5	6.3	0.0542	12.8
Fluoranthene (202)	4.1	18.1	12.4	0.0557	12.4
Pyrene (202)	17.1	8.1	7.2	0.0853	8.1
C1-Flouranthene/Pyrene	12.2	9.5	5.6	0.0730	9.5
C2-Flouranthene/Pyrene	18.8	12.2	6.1	0.0568	12.2
C3-Flouranthene/Pyrene	17.0	10.4	6.5	0.0667	10.4
Benz[*a*]anthracene	15.0	6.2	4.1	0.1120	6.2
Chrysene/Triphenylene	13.6	10.9	6.4	0.0638	10.9
Benz[*b*]anthracene				0.0564	12.3
C1-Chrysenes	16.6	11.6	6.9	0.0595	11.6
C2-Chrysenes	9.7	7.4	4.7	0.0932	7.4
Benzofluoranthenes (252)	22.3	12.8	9.1	0.0540	12.8
Benzo[*e*]pyrene (252)	232.3	49.3	22.0	0.0315	22.0
Benzo[*a*]pyrene (252)	10.3	5.4	2.1	0.1287	5.4
Perylene (252)	ND	19.8	ND	0.0350	19.8
C1-252	2.7	7.1	5.7	0.1223	5.7
C2-252	11.6	6.1	5.5	0.1137	6.1
Indeno[123*cd*]pyrene	103.4	8.5	15.7	0.0442	15.7
Benzo[*ghi*]perylene	43.0	10.3	18.0	0.0385	18.0
C1-276	6.2	4.2	18.3	0.1110	6.2
Dibenz[*a,h]*anthracene		6.4		0.1076	6.4

6.3.1.2 Relationship between k_2 and log K_{ow}

The k_2 values obtained in this study were plotted against log K_{ow} to assess the role of hydrophobicity on the elimination rate of PAH compounds. The plot shows that the relationship between k_2 and log K_{ow} is not best described by a linear relationship (Fig.**6.4**). There was an initial decrease in k_2 with increasing log K_{ow} until log K_{ow} of about 5.5, and thereafter a plateau effect causing a deviation from a linear relationship.

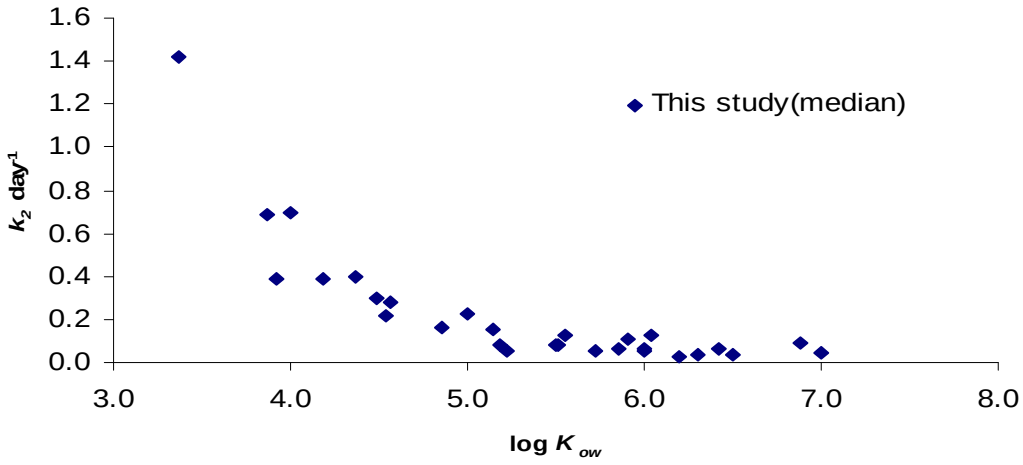


Fig.**6.4**: Trend in k_2 versus log K_{ow} deterined for mussels acutely exposed to crude oil contamination and depurated in flow-through tank systems in the laboratory.

Gewurtz *et al*. (2002) and Thorsen *et al*. (2004) observed similar plateau effect for PAHs with log K_{ow} values ≥ 5.5 and 6.0 respectively (Fig. 6.5b). Kayal and Connell (1990) also found that a regression analysis of log K_{oc} versus log K_{ow} gave a maximum value at log K_{ow} value of about 5.5, and thereafter yielded a parabola. This apparent plateau in k_2 values for PAHs of log K_{ow} > 5.5 may be as a result of steric hindrance of the larger cross-sectional diameter of higher molecular PAHs as explained by Luellen and Shea (2002).

Therefore, to accurately describe the relationship between k_2 and log K_{ow} in this study, a simple regression was performed using the logarithm of the median values of the combined k_2 data. The regression yielded a relationship which is best described by a logarithmic function as shown in Fig. **6.5** below.

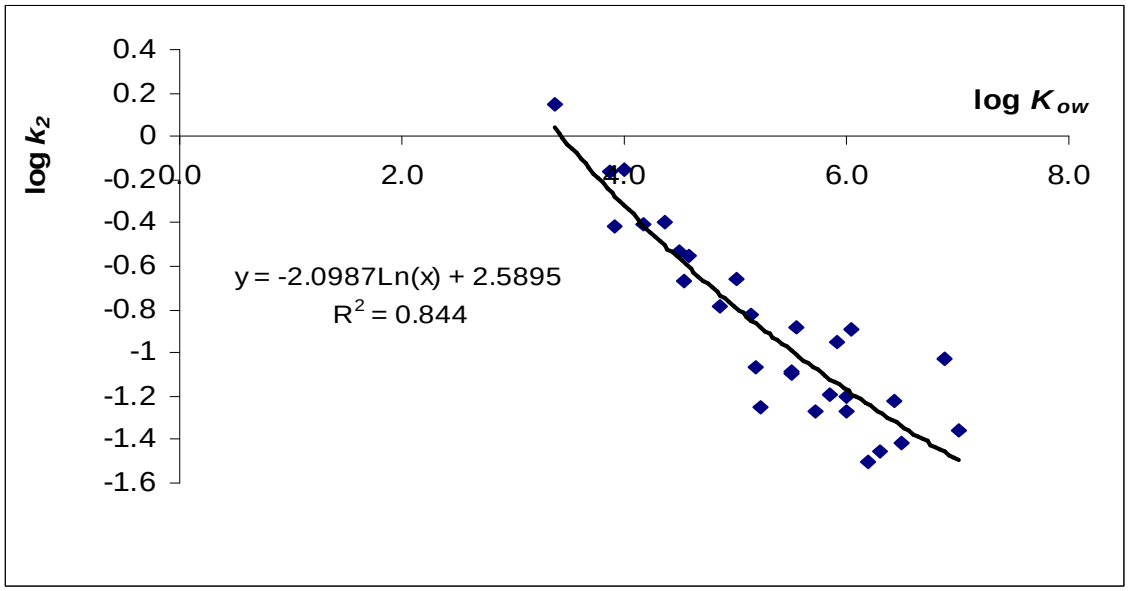


Fig. **6.5**: Log k_2 versus log K_{ow}, where log k_2 = log of median k_2 value obtained in this study; the equation of the plot is log k_2 = 2.5895 – 4.833 [log (log K_{ow})]

6.3.2 Steranes and triterpane biomarker distribution

The triterpane (*m/z* 191) and sterane (*m/z* 217) fingerprints of the mussels before and after the oil exposure are shown in Fig. **6.6** and **6.7** respectively. The chromatogram of the test samples were derived from the GC-MS analysis of ~ 10 g of samples diluted threefold, while that of the control sample was undiluted. The biomarker abundances in the test mussels followed the pattern Gullfaks > Arabian light > Brent. This trend is similar to that reported for the total PAHs (Section **6.3.1**), indicating the same mode of accumulation of PAHs and biomarkers by the mussels.

The triterpane fragmentogram of the control mussels showed dominance of diploptene, a natural terpane present in most animal and plant species. There were also traces of norhopane (29ab) and hopane (30ab). The homohopane doublets peaks were however absent but present in the test samples. These compounds labeled as 31abS - 35abR [Fig. **6.6** (**a-c**)]; are characteristic features of crude oils.

All test mussels contains the same range of biomarker compounds (Fig. **6.6** (**a-c**)) except for bisnorhopane (28ab) which is completely absent in the mussels exposed to Arabian light oil. This is expected as Middle Eastern oils have been characterized by the absence of this specific triterpane (Dahlmann, 2003). However, the relative abundance (intensity) of individual triterpane compound varied among samples. Mussels exposed to the North Sea oils showed a higher abundance of C30-hopane (30ab) over 28,30-norhopane (29ab). A difference in pattern is seen in the mussels exposed to Arabian light oil, with norhopane dominating. It can be seen from the biomarker fingerprints that the ratio of 29ab/30ab is < 1 in mussels exposed to the North Sea oils and > 1 in those exposed to Arabian light oil. This specific triterpane index has been used to distinguish between pure samples of North Sea oils and Middle Eastern oils (Dahlmann, 2003; Webster *et al*., 2004)

The sterane distributions (Fig. **6.7**) of all test mussels show the C27 and C29 regular steranes. Those exposed to Gullfaks and Brent crude oils show profiles dominated by the C27 steranes while the C29 steranes dominated the profile of the mussels exposed to the Arabian light oil. The control mussels show sterane fingerprint close to the patterns observed in mussels exposed to the North Sea oil but in percentage abundances that could be referred to as background, typified by total PAHs determined in these mussels.

Although the biomarkers were not absolutely quantified, a decrease in intensity (abundance) of individual compounds relative to diploptene (a non crude oil biomarker) was observed. The observed trend suggest that the geochemical biomarker compounds are either eliminated (actively or passively) or degraded by the mussels.

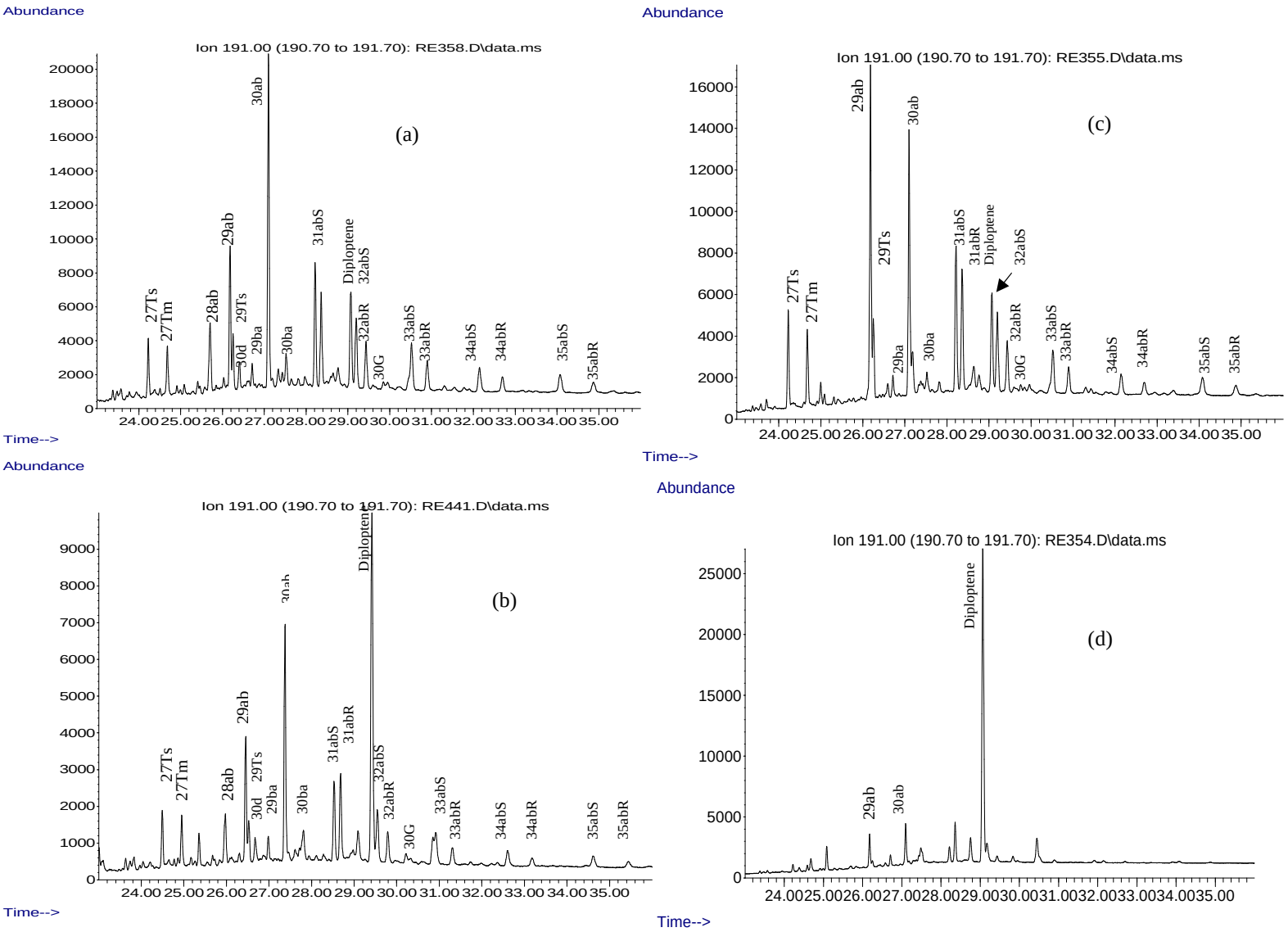


Fig. **6.6**: The triterpane (*m/z* 191) of mussels exposed to (a) Gullfaks crude oil, (b) Brent crude oil (c) Arabian light oil (d) control mussels. The extract from ~ 10g of sample was diluted 3x for samples a, b and c.

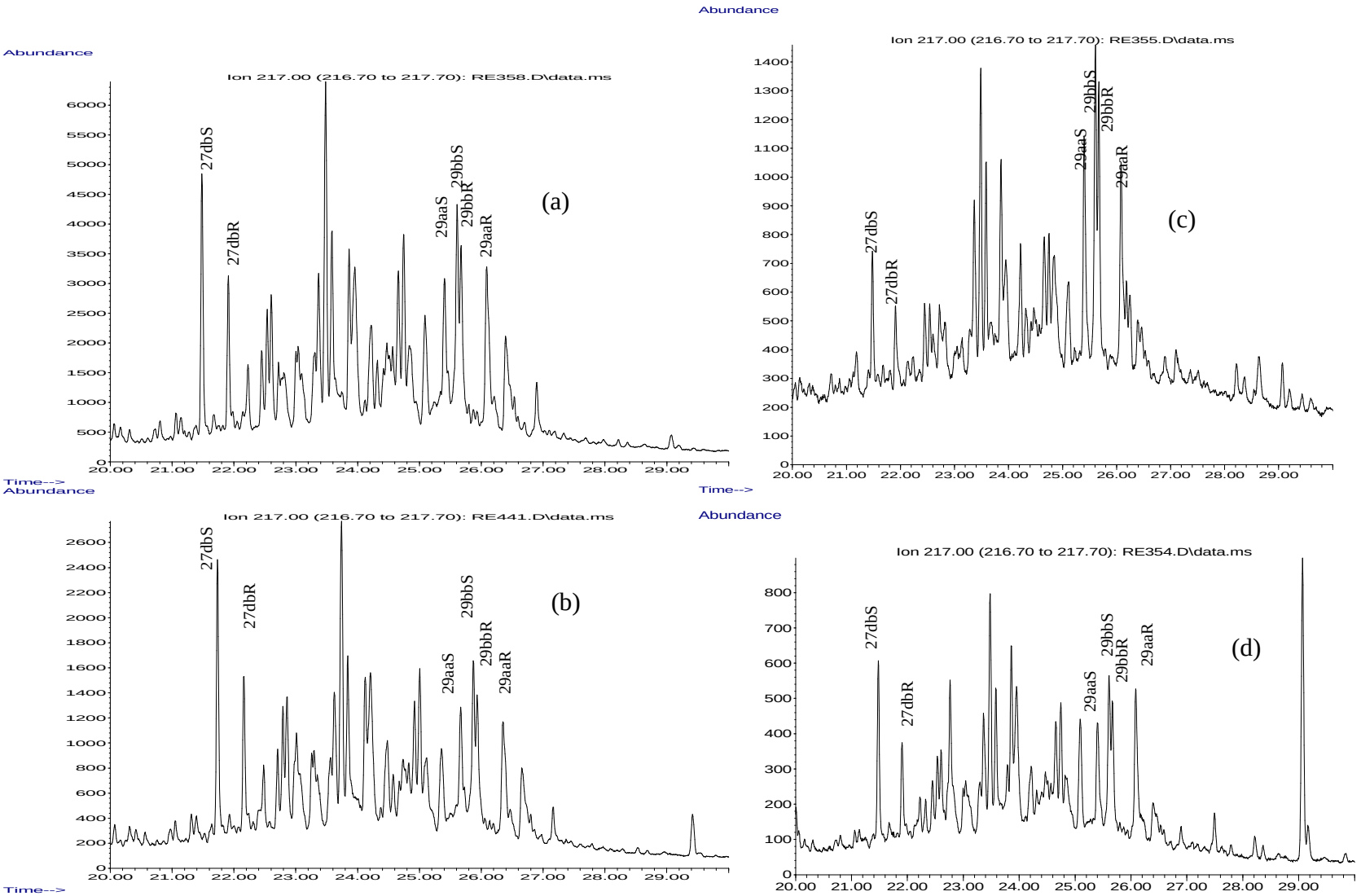


Fig. **6.7**: The sterane (*m/z* 217) of mussels exposed to (a) Gullfaks crude oil, (b) Brent crude oil (c) Arabian light oil (d) control mussels. The extract from ~ 10g of sample was diluted 3x for samples a, b and c.

6.3.3 Biomarker diagnostic ratios

In the absence of absolute biomarker quantification, the trend in specific biomarker ratios with depuration time in the mussels was monitored to investigate the link between clearances of PAH compounds and changes in geochemical biomarker profile. As already explained in chapter 4, these biomarker diagnostic indices have been found to be stable over time in oil and sediment samples and are used by Nordtest in oil spill source identification by matching the relative peak areas or peak heights in the test sample with those of the suspect samples.

Table **6.3**: Hopane and sterane biomarker diagnostic ratios of Loch Etive mussels artificially exposed to a specific crude oil crude oil (Arabian light, Gullfaks and Brent crude for 48 hours)

Exposure oil	Dep. Time	%DR-27Ts	%DR-28ab	%DR-29Ts	%DR-32abS	%DR-30G	%DR-29ab	%DR-30d	DR-29aaS	DR-29bb	Total PAHs (ng/g)
Arabian light	0	52	0.0	23	60	12	54	3.3	54	60	16108.8
	0.25	51	0.0	26	61	17	58	2.0	54	60	15157.7
	1	51	0.0	27	63	11	57	4.5	53	62	13159.0
	2	50	0.0	24	61	13	56	2.1	52	61	9029.1
	4	51	0.0	28	60	18	60	1.6	53	61	ND
	7	57	0.0	25	71	11	57	2.6	50	60	4352.0
	11	50	0.0	24	68	12	56	2.4	44	58	3987.0
Gullfaks	0	51	21	15	60	5.3	31	8.3	48	57	26916.5
	0.25	51	20	14	62	11.1	31	7.6	50	58	22329.4
	1	51	20	14	61	5.3	30	7.5	50	57	18936.6
	2	52	22	14	60	8.0	31	7.8	49	58	11872.7
	4	52	21	14	61	8.2	30	7.3	48	58	7749.7
	7	59	22	15	69	5.1	33	7.4	49	60	4892.6
	11	53	21	15	67	5.1	33	6.9	46	57	2753.8
Brent	0	49	24	15	66	15.9	34	11.9	51	54	9094.1
	0.25	49	22	16	69	20.2	34	8.4	48	56	8178.3
	1	51	21	14	69	16.0	33	7.6	49	57	6139.0
	2	49	21	15	63	9.3	34	9.0	50	57	4263.5
	4	51	22	15	64	11.5	29	8.1	48	57	2008.3
	7	58	19	16	77	5.9	36	7.0	51	59	1010.7
	11	56	18	15	76	6.5	37	6.3	47	58	437.8

Similar information was obtained from Aberdeen harbour mussels depurated in the laboratory and field studies reported in Chapter **4** sections **4.5.** The hopane indices showed enhancements with loss of PAHs while the sterane indices showed continuous decrease with loss of PAHs and hence depuration time. A direct stepwise comparison between the two studies is difficult due to the sampling intervals. However, the decrease in the sterane DR for the harbour mussels was small within the first 7 days (laboratory) and 12 days (field) depuration experiments. Therefore, the shorter depuration period, coupled with the high initial concentration of the compounds may be a factor contributing to the observed trend in the present study.

In applying the Nordtest method, the values of the DRs obtained from the time zero samples was plotted against the subsequent sample points to investigate whether the profiles of the latter samples are different from the initial sample. The plots for the mussels exposed to Arabian light oil, Gullfaks and Brent crude oils are presented in Fig. **6.8**, **6.9**, and **6.10** respectively, allowing for the 95 % confidence interval of mean. The plots show that all the DRs (with the error bars) are overlapping the line (x = y), and therefore gave positive matches to the time zero sample. This indicates that the profiles of the mussels sampled at other times were not very different from that of the initial sample. The illustrations show that the observed changes in the DRs were not significant as they do not affect the final inference using the Nordtest methodology. A similar trend was also observed with the harbour mussels used in the field studies. The DRs of subsequent samples correlated well with the time zero samples until after 12 days of depuration. It is worth mentioning, however, that for oil spill source correlation studies, Nordtest method uses samples collected on site from the area affected by the spill and not samples from continuous monitoring program. In contrast, results reported in this study were obtained from organisms removed from the contaminant source and subjected to depuration in a clean environment. The retention of the specific biomarker indices by the mussels outside of the contaminant zone for a period of time shows that mussels collected *in-situ* can be a good indicator of the contaminant source, but only for about two weeks.

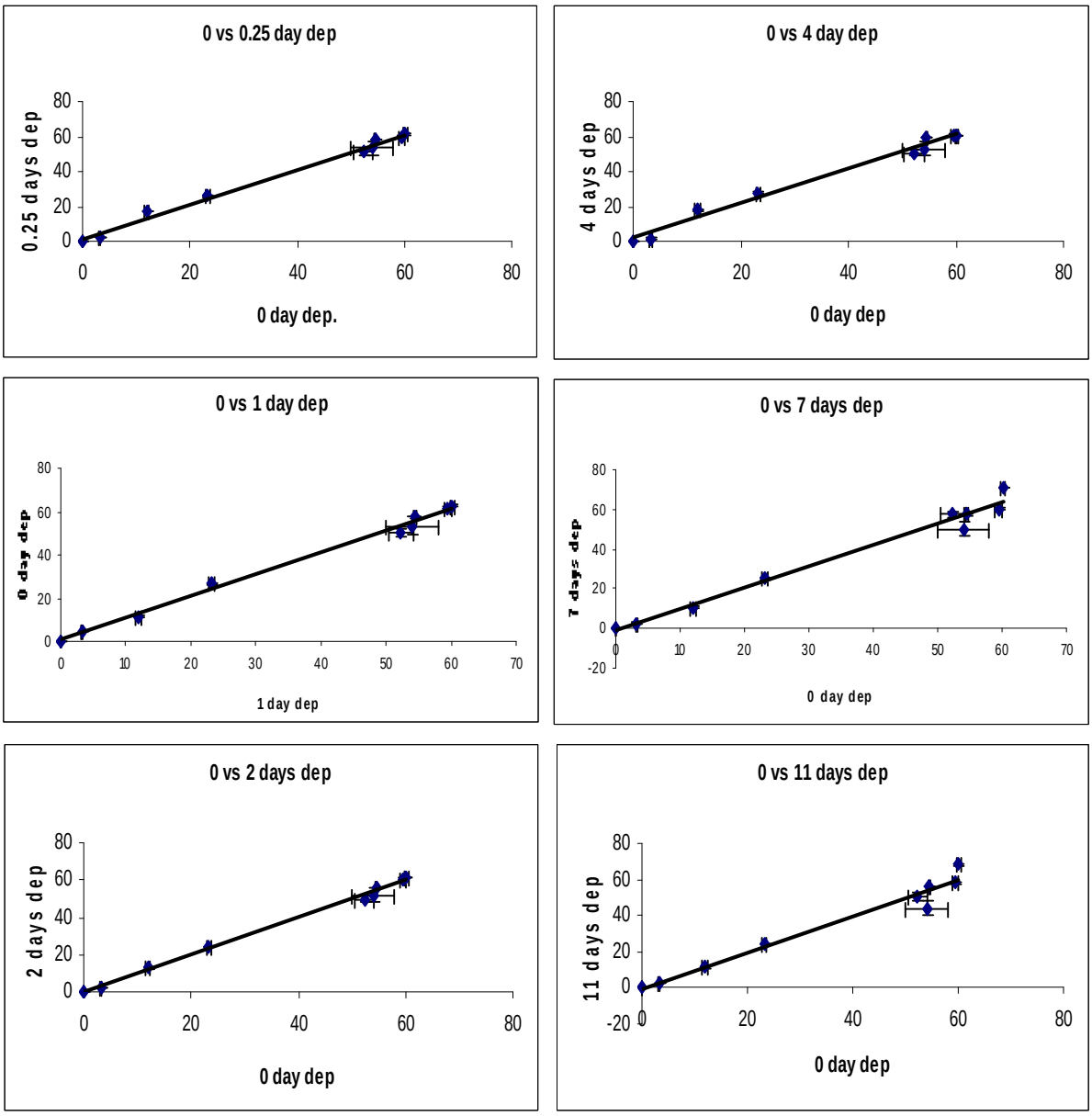


Fig.**6.8**: Correlation between time zero depuration sample and subsequent samples collected at different time intervals during depuration of mussels expose to Arabian light crude oil.

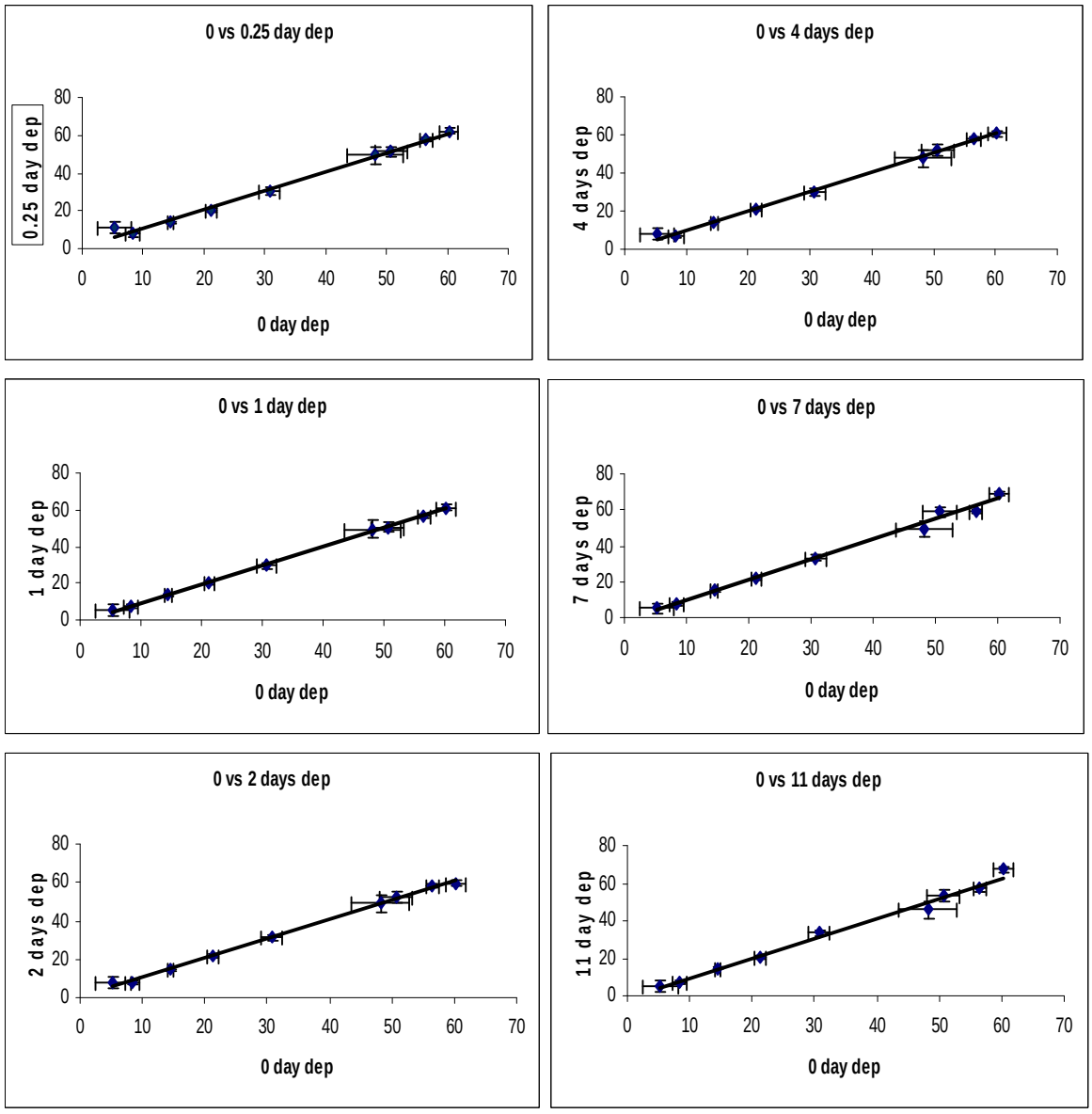


Fig.**6.9:** Correlation between time zero depuration sample and subsequent samples collected at different time intervals during depuration of mussels expose to Gullfaks crude oil

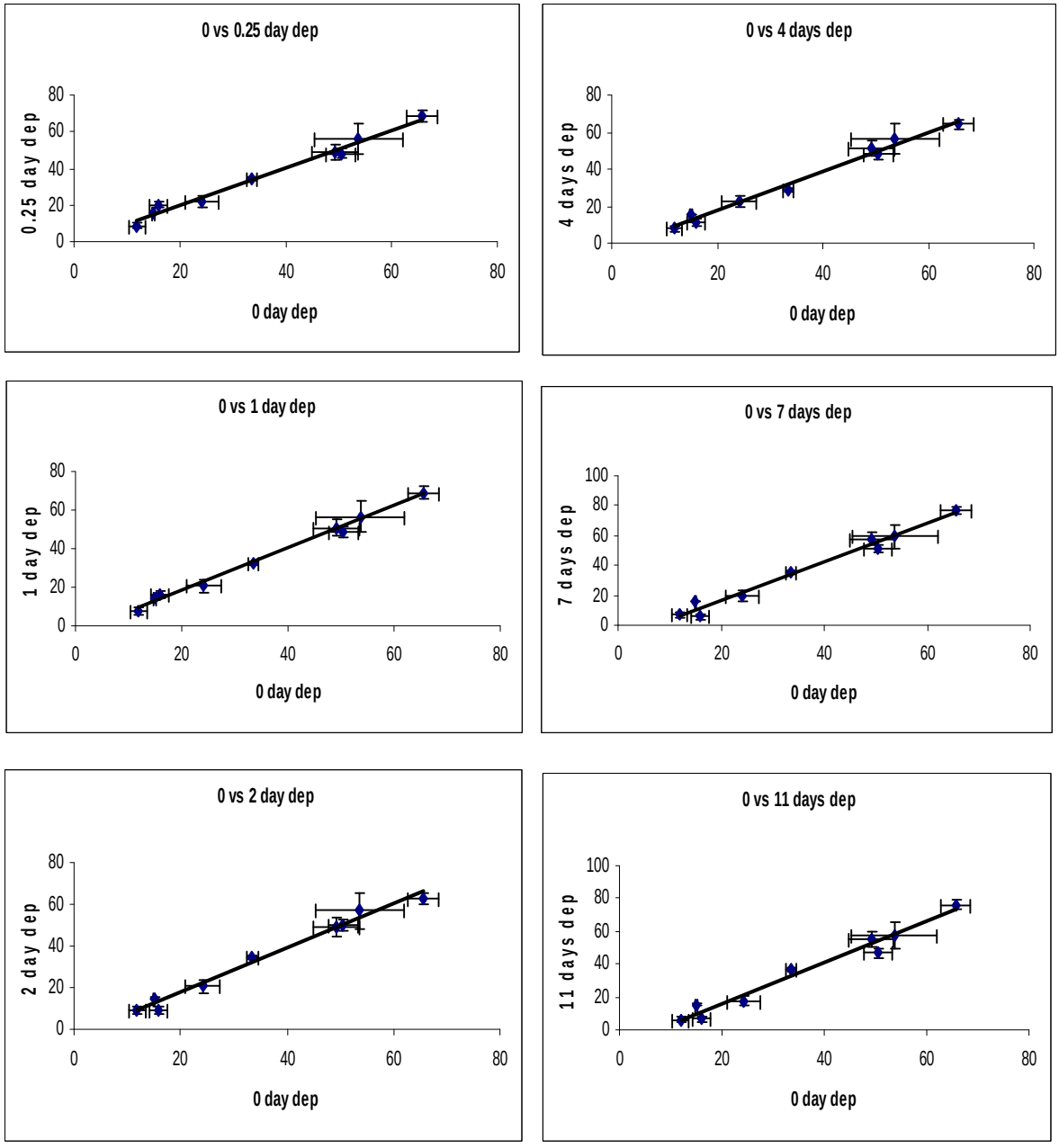


Fig.**6.10**: Correlation between time zero depuration sample and subsequent samples collected at different time intervals during depuration of mussels expose to Brent crude oil

6.4 Conclusion

The elimination rates of polycyclic aromatic hydrocarbons (PAHs) were investigated in *Mytilus edulis* experimentally exposed to crude oil contamination for 48 h. The water- only exposure study has shown that mussels readily accumulate a wide range of PAH compounds from the dissolved phase within short exposure duration. This is an indication that the dissolved phase is an important PAH uptake route for this species. The 2-and 3-ring compounds dominated the PAH profile found in the mussels. These compounds are acutely toxic and often associated with taint. Although the concentration of the EU regulated compound; benzo[*a*]pyrene did not exceed the maximum limit in shellfish, other priority compounds such as chrysene and pyrene (and their alkylated homologues, C1-C3) were accumulated in high concentrations.

Elimination of the PAH compounds upon transfer of the organisms to a clean environment was rapid and followed first order kinetics. Elimination rate constants were comparable among the three crude oils used in the exposures, but varied among PAH compound. The estimated biological half-lives decreased with increase in molecular weight and degree of alkylation for the 2- to 4- ring compounds. Biological half-lives of naphthalenes (parent and C1-C4) were generally lower (0.5 – 5.3 d) than literature values (> 3.5 d) but followed the same trend as literature reports. A regression analysis of k_2 and log K_{ow} show a non linear relationship but high dependency of k_2 on hydrophobicity ($r^2 > 0.75$). The kinetic data obtained in this study can be directly applied to field conditions provided the source of contamination is not persistent and there is no re-suspension from the underlying sediment.

The biomarker patterns found in the mussels exposed to the North Sea crude oils (Gullfaks and Brent) differed from that of the mussels exposed to the Arabian light oil; but reflected major characteristics of the exposure oils. Specific biomarker ratios studied were relatively stable over the depuration period except %DR-30d and 29aaS that showed slight decreases with time. However, the Nordtest approach adopted in this study indicates that the biomarker profile of the mussels sampled at different intervals within the 11 days depuration period correlated well with the time zero samples.

This study therefore indicates that biomarker information of mussels collected within a short period from spill sites can provide useful information on the source (s) of the spill.

CHAPTER SEVEN

Investigation of PAH depuration kinetics and changes in geochemical biomarkers in Atlantic salmon (*Salmo Salar*) artificially exposed to PAH compound and petroleum contamination

7.1 Introduction

The definition of salmon under the "Salmon Act 1986" includes the Atlantic salmon *(Salmo Salar)* and Sea trout *(Salmo trutta)*; a migratory form of brown trout. Both species of fish are economically important and also form a central part of Scotland's natural heritage. As migratory organisms, salmon and sea trout spend part of their life in freshwater and part in the sea. In both species, adults migrate from the sea to freshwater to spawn. Salmon fishing is vital to the Scottish economy. Atlantic salmon is widely regarded as Scotland's most iconic freshwater fish species (Scottish Government, 2008).

According to a survey report published by the Scottish Executive Environment and Rural Affairs Department, coarse and game angling in Scotland results in the Scottish economy producing (after displacement) over £100 million worth of annual output, which supports around 2,800 jobs and generates close to £50 million in wages and self employment into Scottish households, many of which are in rural areas (SEERAD, 2004).

Consequently, the effect of large quantities of oil on commercial salmon farms can have a devastating effect on farm owners as well as significance for the national economy. A typical example is during the *Braer* oil spill in Shetland where a vessel ran aground spilling up to 85 000 t of Norwegian Gullfaks crude and some bunker fuel oil. The incident led to the destruction of two year classes (1991 &1992) of salmon within the affected zone (Whittle *et al*., 1997). Chemical and sensory analyses (taint analyses) have been jointly used to make informed decision regarding the lifting of bans on harvesting of fish and shellfish in areas affected by oil spills. According to the International Standards

Organisation (ISO), taint is described as a flavour or odour foreign to the product. In a food product, taint may cause consumers to believe that the food is possibly contaminated and therefore loss of confidence in the product. Taint is a sensory experience and can only be detected and measured by sensory procedures, i.e. by smelling or tasting the fish or shellfish suspected of being tainted. Chemical tests can be useful for monitoring but, ultimately, a sensory test is needed because chemical data can only predict that a food might be tainted (Davis *et al*., 2002). During the *Braer* oil spill, salmon due to be harvested in 1992 were destroyed after testing positive to taint assessment, even though the PAH concentrations had declined (Whittle *et al*., 1997).

Reported adverse effects of petroleum hydrocarbons on farmed and wild salmon communities include:

i. Tainting of the edible tissue (e.g. Heras *et al*., 1992; Davis *et al*., 2002; Whittle *et al*., 2005).
ii. Induction of hepatic 7-ethoxyresorufin O-deethylase (EROD) activity due to increased tissue PAH concentration (e.g. Stagg *et al*., 1995, Wiedmer, *et al*, 1996).
iii. Reduction in reproductive capability of the specie affected (e.g. Truscott *et al*., 1983), etc.

The above effects have been linked directly to the polycyclic aromatic hydrocarbon (PAH) tissue burden of the affected organisms. PAHs are the major component of petroleum extensively studied because of their toxicity and carcinogenicity. In some cases, sensory assessment is used as a screening tool to eliminate or prioritize samples for more sophisticated, costly and time consuming chemical analysis where a wide area is being assessed (Saxby, 1996; Whittle *et al*., 1997). It is therefore important to always compare chemical data with sensory data to safeguard consumer confidence. FRS Marine Laboratory is the UK centre of expertise for organoleptic assessment. Taste testing of fish products in FRS is carried out by a group of panelists trained to detect taint in edible parts of animals that have been deliberately contaminated by hydrocarbons in carefully controlled environment.

FRS sensory assessment panel got accreditation from the United Kingdom Accreditation Service (UKAS) under international standard ISO 17025 in 2003.

This study investigates the kinetics of elimination of PAH compounds accumulated by salmon acutely exposed to petroleum contamination as a reflection of field conditions during oil spill incidents. The half-life of individual PAH compound and the relationship between loss of taint and PAH tissue burden are investigated. The geochemical fingerprints in the organisms were also assessed.

7.2 Ethical requirement.

As part of the requirements of the protected animal right law, permission to use live animals for experimental purposes was obtained from the Home Office before this study commenced. The appropriate training courses on the planned procedures were received. The project licence was received as an amendment on the existing licence held by FRS under the animals (Scientific Procedures) Act 1986. The Animals (Scientific Procedures) Act 1986 came into force on 1 January 1987 and makes provision for the protection of animals used for experimental or other scientific purposes in the United Kingdom. It replaced the Cruelty to Animals Act 1876 and implements the requirements of the European Directive 86/609/EEC on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental or other scientific purposes. According to the details of the act, a “protected animal means any living vertebrate other than man and any invertebrate of the species *Octopus vulgaris* from the stage of its development when it becomes capable of independent feeding’. Details of the act can be found on the Home Office website under [(Animals Scientific Procedures) Act 1986].

During the cause of this study, strict adherence to the provisions of the project licence was maintained.

7.3 Experimental design

This section describes the steps taken from sample collection to the completion of the experiment. The experiment was carried out in the laboratory. The organisms were exposed to PAH contaminated feed for 150 h, and subsequently to Forties crude oil for 6 h. The PAH extract used for the pre-treatment of the salmon feed was obtained from sediment samples collected from Loch Leven. These samples showed elevated PAHs concentrations with predominantly the 4- to 6-rings compounds. The use of PAH treated feed was to simulate the uptake of the heavier compounds which are not normally accumulated by organisms within a short period of exposure to petroleum products. In fact, exposing fish to concentrations of crude oil for an extended period of time to allow weathering of the oil components and hence accumulation of heavier compounds is neither practicable nor desirable. Concentration of crude oil used for the exposure reflects concentrations detected during the oil incidents such as the *Braer* grounding in Shetland.

7.3.1 Sample collection and transportation.

All salmon used for the experiment were collected from a relatively uncontaminated environment. Salmon were bred in Aultbea, Gairloch in the West Coast of Scotland, from stock collected from the River Don in Aberdeen and ranged in length from 295.0 to 435.0 mm and weight from 239.5 to 887.7 g. They were transported to FRS Aberdeen by road in insulated tanks provided with aeration and oxygen.

7.3.2 Sample acclimation

The salmon were acclimatized for 3 weeks in a holding tank in the laboratory before the experiment.

7.3.2.1 Extraction of PAHs for salmon feed pre-treatment

Sediment sub samples ~ 10 g were weighed into a centrifuge tube. Deuterated PAH aromatic internal standard containing D_8-naphthalene, D_{10}-biphenyl, D_8-dibenzothiophene, D_{10}-anthracene, D_{10}-pyrene and D_{12}-benzo[*a*]pyrene (100 or 200 µl; approximately 1 µg ml^{-1} each) was added dependent on the estimated concentration of hydrocarbons. 200 ± 10 µl of aliphatic standard (containing approximately 3.2 µg each of heptamethylnonane and squalane) was then added to the sample. 20 ± 2 ml of DCM and methanol respectively were then added to the centrifuge tube and the solution thoroughly mixed by swirling to break up the sediment. The sample was then sonicated for 5 min, followed by centrifugation at 1800 rev/s for 10 min at 5 ± 0.5 ^{o}C. The liquid layer was the decanted into a separating funnel containing 18 ± 2 ml of water and thoroughly shaken. The bottom DCM layer was consequently transferred to a 100 ml flask containing 10 ± 1 g of anhydrous sodium sulphate. The sediment was re-extracted by sonication for 5 min with fresh 20 ± 2 ml of DCM, centrifuged and the solvent layer decanted into the separating funnel. This was thoroughly mixed, allowed to separate and the DCM layer combined with the first DCM extract in the 100 ml conical flask. The extract was then dried over the anhydrous sodium sulphate for ~ 10 min, concentrated by rotary evaporation and exchanged into *iso*-hexane by the addition *iso*-hexane and further reduced by rotary evaporation to 100 ml.

7.3.2.2 Exposure of salmon to PAH pre-treated feed

The resulting extract was mixed with the weighed salmon feed (1530 g) using a spraying bottle in a fume cupboard. The feed was allowed to dry at room temperature and stored in a Teflon container and fed to the salmon as required. Analysis of the treatment feed showed concentration ranging from 1623.7 to 2017.1 ng g^{-1} feed.

7.3.3 Exposure of salmon to Forties crude oil

After 150 h of feeding with contaminated feed, the remaining 44 salmon were transferred to four different rectangular Teflon tanks in buckets filled with water. The exposure tanks containing 206 litres of freshwater and holding 11 fish *per* tank were supplied with continuous aeration. The salmon were exposed to Forties crude oil by mechanically mixing 4.0 ± 0.05 ml of the crude oil with the exposure water. Exposure of fish to the crude oil in water lasted for 6 hours. Six salmon died during the exposure period probably due to stress but none died during the depuration phase.

7.3.4 Transfer of salmon to the depuration tank

At the end of six hours, salmon were transferred as above to a clean tank supplied with freshwater to depurate the accumulated PAH compounds.

7.3.5 Sub- sampling

Four control fish were removed prior to exposure of the organisms to contaminated feed and crude oil. Also, sub-samples of four salmon each were removed at 54 h and 150 h during exposure to feed and at the end of 6 hours oil exposure before transferring to the depuration tank, and subsequently at 2, 4, 7, 12, 18, 25, and 32 during depuration.

7.3.6 Gutting, filleting and storage of samples

Salmon were killed by concussion of the brain according to “Schedule 1” provision of the project licence by a blow to the head. The dead fish was then dissected to remove the gut and then preserved in ice in the fridge (setting 3.5 - 4.0 ºC) overnight to allow rigor mortis. Then fish was filleted and flesh samples collected for chemical and sensory analysis.

The samples for the sensory analysis were wrapped in aluminum foil and frozen within a maximum of 1 hour from preparation. To optimize freezing conditions, the fillets were frozen in a single layer thickness by air blast freezing for 2 hours, and then stored in the dedicated freezer until required for analysis. The samples for the chemical analysis were stored in solvent washed aluminum cans and kept in a designated freezer at -20 ºC until required for analysis.

7.4 Analytical methods

7.4.1 Chemical Analysis

The samples were analyzed for PAHs and total lipid content using the methods described below.

7.4.1.1 PAH Analysis

The saponification, liquid-liquid extraction, HPLC isocratic fractionation /clean up and GC-MS determination for the PAHs were the same as has already been described for mussels in Chapter **2**.

7.4.1.2 Lipid content determination

The total lipid content of the sample was determinated using a method developed by Smedes (1999). This method is capable of detecting lipid content up to 100%. Briefly, 3.0 ± 0.5 g of the homogenized salmon sample was weighed into a solvent washed 100 ml centrifuge tube. To this were added 20.0 ± 1.0 ml and 18 .0 ± 1.0 ml of cyclohexane and isopropanol respectively with a measuring cylinder. Using an Ultra Turrax (13500 rpm), the contents of the tube was mixed over ice for 2 minutes monitored by a calibrated timer. De-ionised water (18 ml) was added and also mixed for 1 minute with the Ultra Turrax.

The resulting mixture was centrifuged for 10 minutes at 1800 rpm and 10 ± 0.5 ml of the organic layer removed into a cleaned 100ml flask of known weight. The remaining portion of the organic layer was removed by suction to waste. Further 20.0 ± 1 ml of 13 % isopropanol:cyclohexane mixture was then added to the aqueous layer in the centrifuge and mixed with the Ultra Turrax for 1 minute and centrifuged as above. Another, 10.0 ± 1 ml of the second organic layer was removed and mixed with the first extract in the 100 ml flask. This was rotary evaporated (75 ºC ± 2 ºC) to remove all solvent and then transferred to dry in an oven (80 ºC ± 5 ºC) for 1 hr. The flask was then cooled to room temperature in a dessicator and re-weighed to calculate the weight of the residue.

% lipid = [(weight of residue (g) /aliquot factor)/ weight of sample extracted (g)]*100
Aliquot factor = (A+B) /(C+D)

Where A – Volume of organic layer removed from first extract
B - Volume of organic layer removed from second extract
C – Volume of solvent used in first extraction
D – Volume of solvent used in second extraction

7.4.2 Sensory Analysis

The frozen fish were thawed by leaving for 24 hours in a fridge at 4 ºC. Fish were cooked in a casserole using a microwave for the required duration (probe temperature ≥ 65 ºC but not > 80 ºC). Fish were flaked with a fork at the end of cooking before presenting to the panel. A reference fish was assessed alongside the test samples at each assessment session. The reference is a sample of salmon supplied with the test samples and treated to the same conditions as the test samples except for exposure to pre-treated feed or crude oil.

Samples were tasted (smell and taste) by individual member of the panel and the taint intensity rated using the scale given below.

0 = absence	3 = strong
1 = slight	4 = very strong
2 = moderate	5 = extremely strong

7.5 Results and discussion

7.5.1 Polycyclic aromatic hydrocarbons

The mean total PAH concentrations (2- to 6- rings) found in the salmon after the feed exposure (2.4 ± 1.4 ng g^{-1}) was not very different from those of the control samples (1.1 ± 0.6 ng g^{-1}). This suggests no accumulation from the pre-treated feed. However, chemical analysis of the pre-treated feed showed total PAHs in excess of 1600 ng g^{-1} constituting the whole range of the 2- to 6- ring compounds (up to 9.3 % of the 2- and 3-ring and 90.7 % of the 4- to 6-ring PAH compounds). The reasons for this unexpected result was uncertain but could possibly be due to the following-

i. The method used in the extraction of the compound from the sediment was an exhaustive method; therefore the PAHs may have associated with the feed as in the sediment and be in the form that is not bioavailable such that uptake from feed was extremely slow or non-existent.

ii. The test fish did not feed during the duration of the experiment – this is unlikely as the presence of undigested feed in the guts of test samples during sample preparations suggest otherwise.

iii. The PAHs have been washed off the feed when introduced into the experimental tanks; the probability of this happening is also very low as extraction using homogenized (ground) feed yielded higher total PAH concentration (2017.1 ng g^{-1}) than lump feeds (1623.7 ng g^{-1}); indicating

that the PAH compounds were strongly bound to the feed.

Four salmon sampled immediately after the oil exposure were analyzed individually. The total PAH found in the fish varied considerably and were 485.4, 717.0, 752.1 and 1018.0 ng g^{-1} wet weight; giving a mean of 743.1ng g^{-1} and a standard deviation of 218.1 ng g^{-1}. The variations show heterogeneity among organisms and followed no particular trend with the organism's weight or lipid content (Table **7.1**). Of the total PAHs determined, the naphthalenes (parent and alkylated compounds; C1-C4) accounted for >93 %, phenanthrene/anthracene and (C1-C3) accounted for 2.71 to 3.75 %, while the dibenzothiophenes (parent and C1-C3) ranged between 1.44 and 2.08 % in the samples analyzed. The naphthalenes have relatively high aqueous solubility (log K_{ow} 3.37- 5.55) and are readily available in the water phase. The % distribution of the naphthalenes found in the salmon is represented in Fig. **7.1**. The C1-naphthalenes (1-methyl naphthalene and 2-methyl naphthalene) were 43.3 - 46.2 % of the total PAHs, and the C2-naphthalene about 27.8-29.1%. This relative distribution is similar to that reported by Whittle *et al*., (1997) in caged salmon sampled following the *Braer* oil incident. The latter reported over 90 % of total naphthalenes, of which C1-naphthalenes accounted for up to 41% of total PAH determined. The heavier and more persistent compounds (4- to 6-rings) were below detection limits and some were probably not accumulated within the short exposure duration. Dominance of the naphthalenes over phenanthrenes/anthracene and the dibenzothiophenes, and very low concentrations of the heavier ring compounds has also been reported in salmon samples collected from Scalloway in Shetland after an emergency towing vessel (*Anglian Sovereign*) struck rock and spilt ~ 80 tonnes of marine diesel on 3rd September 2005. The profiles of PAH observed in this study and Whittle *et al*. (1997) suggest similar route and mode of PAH accumulation by salmon.

Table **7.1**: Total naphthalenes concentration and sensory data obtained from salmon exposed to crude oil contamination.

Field ID	Dep. Time (days)	Lipid (g^{-1})	Total naphthalenes (ng g^{-1})	Taint rating	Mean Taint Scores (n=4)	Taint SD
Day 6.25 feed exposure 1	-	0.031	1.5	12.5	0.25	0.46
Day 6.25 feed exposure 2	-	0.030	1.6	0.0	0.00	0.00
Day 6.25 feed exposure 3	-	0.023	1.7	11.1	0.13	0.35
Day 6.25 feed exposure 4	-	0.023	1.3	44.4	0.67	0.87
6 hour oil exp. (0 Dep.) 1	0	0.005	667.3	100.0	3.75	1.16
6 hour oil exp.(0 Dep.) 2	0	0.020	451.9	80.0	3.00	1.87
6 hour oil exp. (0 Dep.) 3	0	0.021	962.6	100.0	2.61	1.50
6 hour oil exp. (0 Dep.) 4	0	0.035	711.5	100.0	2.94	1.27
Day 2 depuration 1	2	0.020	332.6	100.0	2.22	1.20
Day 2 depuration 2	2	0.015	513.9	100.0	1.56	1.12
Day 2 depuration 3	2	0.021	512.9	80.0	1.90	2.07
Day 2 depuration 4	2	0.015	136.1	100.0	1.81	0.13
Day 4 depuration 1	4	0.007	101.5	44.4	0.50	0.61
Day 4 depuration 2	4	0.010	105.5	87.5	1.25	0.71
Day 4 depuration 3	4	0.020	101.1	80.0	1.20	1.10
Day 4 depuration 4	4	0.017	269.7	33.3	0.50	0.76
Day 7 depuration 1	7	0.017	196.3	44.4	1.11	1.54
Day 7 depuration 2	7	0.024	164.0	37.5	0.38	0.52
Day 7 depuration 3	7	0.074	210.3	25.0	0.40	0.89
Day 7 depuration 4	7	0.021	158.8	22.2	0.25	0.46
Day 12 depuration 1	12	0.030	81.2	12.5	0.22	0.67
Day 12 depuration 2	12	0.033	26.1	25.0	0.25	0.46
Day 12 depuration 3	12	0.031	53.0	40.0	0.40	0.55
Day 12 depuration 4	12	0.021	222.1	11.1	0.25	0.71
Day 18 depuration 1	18	0.046	25.1	22.2	0.19	0.37
Day 18 depuration 2	18	0.033	38.6	33.3	0.44	0.73
Day 18 depuration 3	18	0.038	98.1	12.5	0.13	0.35
Day 18 depuration 4	18	0.028	27.0	0.0	0.00	0.00
Day 25 depuration 1	25	0.014	23.5	0.0	0.00	0.00
Day 25 depuration 2	25	0.027	33.1	11.1	0.11	0.33
Day 25 depuration 3	25	0.026	10.3	25.0	0.25	0.46
Day 25 depuration 4	25	0.021	18.0	0.0	0.00	0.00
Day 32 depuration 1	32	0.022	12.3	40.0	0.30	0.45
Day 32 depuration 2	32	0.033	11.6	12.5	0.13	0.35
Day 32 depuration 3	32	0.016	7.4	22.2	0.56	1.13
Day 32 depuration 4	32	0.021	8.1	11.1	0.25	0.71

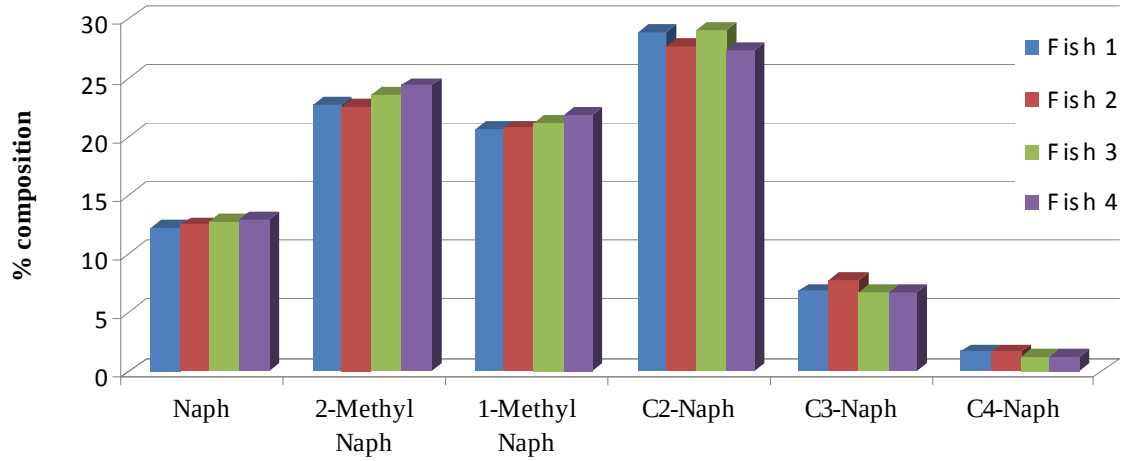


Fig.**7.1**: The naphthalenes (parent and C1-C4) distribution in salmon (*Salmo Solar*) experimentally exposed to crude oil for 6 h.

7.5.2 Assessment of PAHs concentrations and implications for food safety

Some PAHs are known to cause taint; others have recognized carcinogenic and mutagenic potential. The European commission, in 2005 established a standard guideline for PAHs in food products to ensure consumer safety. The commission regulation (EC) No 208/2005 set the maximum limit of benzo[*a*]pyrene in muscle meat of un-smoked fish and bivalve mollusks, at 2.0 ng g^{-1} and 10.0 ng g^{-1} wet weights respectively. Prior to this limit, the UK Food Standard Agency did adopt an interim pragmatic guideline based on advice from UK committee on Toxicity of Chemical in Food, Consumer Products and Environment (COT), for benzo[*a*]pyrene, benz[*a*]anthracene and benzo[*a*,*h*]anthracene. This limit was 15 ng g^{-1} for each of the indicated PAHs and concentration above this renders the product unfit for human consumption. These compounds were below detection limits in the test samples, and therefore pose no food safety concern. Another quality of fish and shellfish product that impacts its desirability and fit for purpose is 'taint'. This is discussed in the next section.

7.5.2.1 Sensory assessment

The test samples were also subjected to taint analysis and the result is also presented in Table 7.1. The sensory panel in this study constituted 5 to 9 analysts. Where up to 50% of the panel scores are 1 or above, the sample is deemed to be tainted; between 20% and 50% the sample is regarded as suspect; and less than or equal to 20%, not tainted. Table 7.1 shows that the salmon developed strong petrogenic taint (80-100%) after the oil exposure. The percentage of taint positives were 100% for three of the four samples collected immediately after the oil exposure, and 80% for the remaining one. These salmon were rated very strongly tainted by most of the panelist ($3 \geq$ score ≤ 5), giving an average taint intensity score of 3.75 (Table **7.1**). It is very surprising that such low petrogenic PAH concentration (717-1018 ng g^{-1}) as determined in this study can render commercial fish product undesirable, while field spill incidents have recorded as high as 14 000 ng g^{-1} in caged salmon (Whittle *et al*., 1997).

7.5.2.2 Depuration of taint

Upon transfer of the salmon to the depuration tank, a decline in taint was observed. Taint decreased progressively with increased depuration time (Fig. **7.2**). The salmon moved from "tainted" (between 50-100 %) to "suspect" (between 20-50 %) in 7 days and thereafter returned to "not tainted" (between 0-20%) in about 18 days of depuration. An exceptional result was observed for samples collected on the 32 days of depuration. These samples were classified as "suspect" by the analysts. Although the mean PAH concentrations were lowest in these samples, the average taint score result still show presence of slight taint. This shows that taint is not lost as rapidly as it develops, and further suggests that the return to PAH background concentration in the aftermath of oil spill incident may not necessarily mean the disappearance of taint. A typical example is the report of Whittle *et al*. (1997) where a batch of salmon was destroyed after testing positive to taint irrespective of PAH concentration that is close to background concentrations. Therefore, the result from this study emphasizes the relevance of both chemical and sensory analysis in making informed decisions concerning oil spill management.

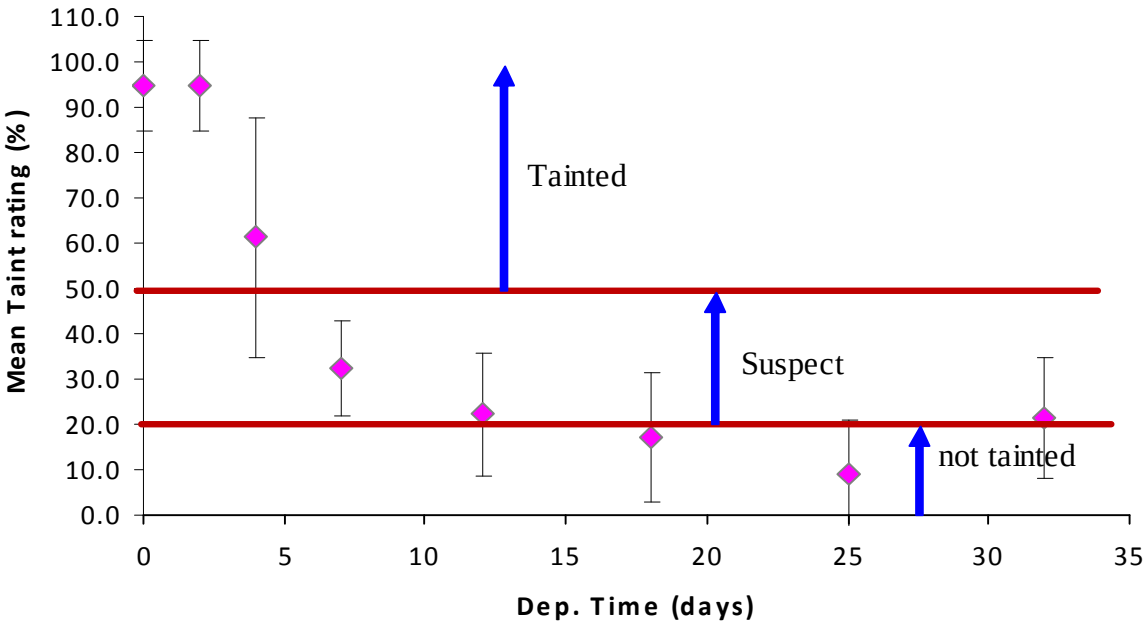


Fig. **7.2**: Depuration of taint in salmon exposed to crude oil contamination. Error bars = 1 standard deviation from the mean.

7.5.3 Relationship between the chemical and sensory data

A simple regression analysis of the total PAH concentration of a sample against the assigned taint intensity is shown in Fig **7.3**. The plot yielded a linear relationship with increase in PAH concentration generally resulting in increased petrogenic taint intensity. The plot shows good correlation between the sensory and the chemical data. Naphthalene and the alkylated compounds; the group of hydrocarbons often associated with taint accounted for over 93% of the PAH total in each case. The equation describing the relationship between taint intensity and total PAH concentration is - total PAHs = 211.13 (taint intensity) + 16.83 with r^2 of 0.72. The equation can be rearranged to give

$$\text{Taint intensity } (\chi) = {}^{TotalPAH\,(y) - 16.831}\!\!\diagup\!\!_{211.130}$$

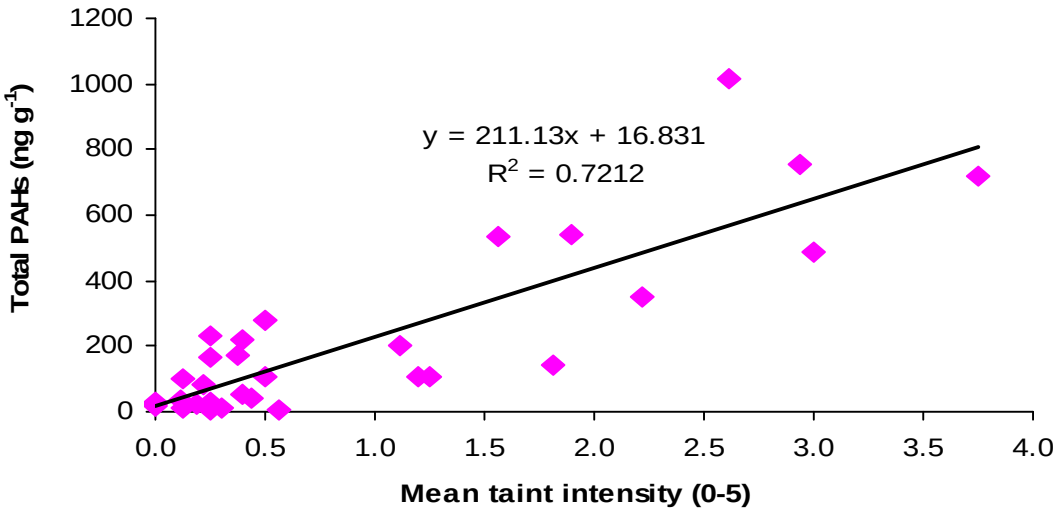


Fig. **7.3**: A regression plot showing the relationship between taint intensity and total PAH concentration in salmon exposed to acute crude oil contamination for 6 hours.

7.5.4 Kinetics of loss of PAHs

The PAH profile of the salmon as seen in section **7.5.1** contains only the 2- and 3-ring compounds. Naphthalene was lost relatively faster than any other member of the homologues series, with an average of 90.5% and 99.8 % decrease after 4 and 25 days of depuration respectively. C2, C3 and C4-naphthalenes showed slower rate of decrease with average loss of 62.4%, 55.0%, 73.9% and 94.7%, 89.8 %, 93.6% after 4 and 25 days respectively. Phenanthrene/anthracene and dibenzothiophene together with their alkylated compounds were almost lost within the first 18 days of depuration.

The PAH concentrations were normalized for lipid content. The rate of loss of individual compound was fitted into first order kinetics equation. A plot of the natural logarithm of the lipid normalized concentration against time gave a graph with elimination rate constant (k_2)

as the slope. Sample plots obtained from 1-methyl naphthalene and 2-methyl naphthalenes are presented in Fig. **7.4** and the k_2 and the biological half-life for individual compounds given in Table **7.2**. The depuration rate constant decreases with an increase in degree of alkylation; with the parent compounds having shorter biological half-lives than the alkylated compounds.

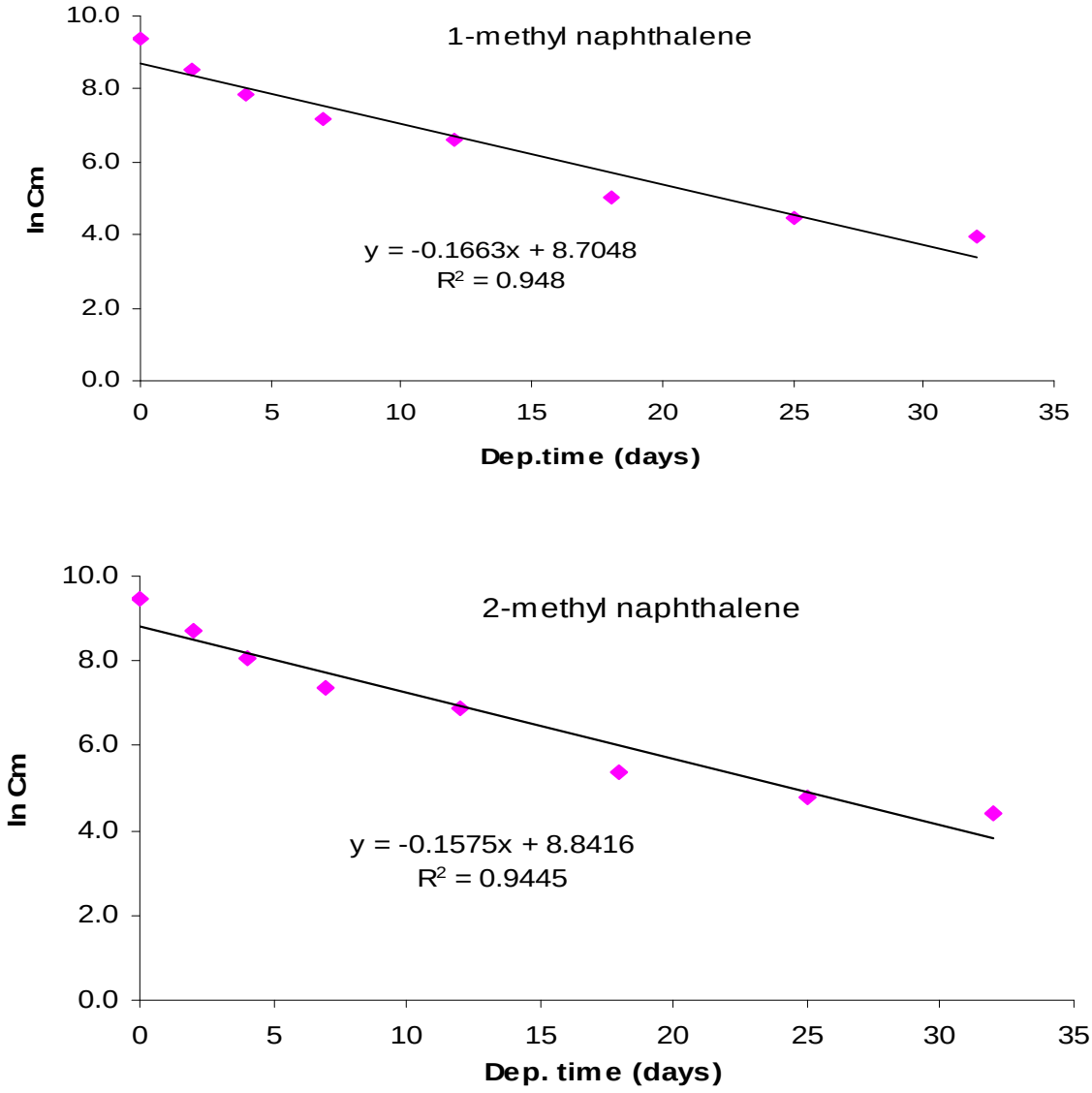


Fig. **7.4**: Elimination plot of PAH compounds (lnCm versus *t*) where Cm is the lipid normalized concentration in ng g^{-1} lipid in salmon and *t* is the depuration time in days.

Table **7.2**: The depuration constant (k_2), correlation co-efficient (r^2), and biological half-lives ($t_{1/2}$) determined in salmon exposed to Forties crude oil contamination.

Compounds	**log K_{ow}**	**r^2**	**k_2**	**$t_{1/2}$**	***p*-value**
Naphthalene	3.37	0.76	0.186	3.7	0.005
2-Methyl Naphthalene	4.00	0.94	0.157	4.4	0.000
1-Methyl Naphthalene	3.87	0.95	0.166	4.2	0.000
C2-Naphthalenes	4.37	0.91	0.115	6.0	0.000
C3-Naphthalenes	5.00	0.91	0.102	6.8	0.001
C4-Naphthalenes	5.55	0.84	0.119	5.8	0.004
Acenaphthene	3.92	0.79	0.078	8.9	0.003
Fluorene	4.18	0.92	0.124	5.6	0.000
Phenanthrene	4.57	0.89	0.161	4.3	0.002
Anthracene	4.54	ND	ND	ND	ND
C1-Phenanthrene/anthracene	5.14	0.69	0.137	5.1	0.020
C2-Phenanthrene/anthracene	5.51	*0.42*	*0.092*	*7.7*	*0.165*
C3-Phenanthrene/anthracene	6.00	*0.06*	*0.022*	*31.5*	*0.646*
Dibenzothiophene	4.49	0.89	0.150	4.6	0.001
C1-Dibenzothiophenes	4.86	0.70	0.247	2.8	0.078
C2-Dibenzothiophenes	5.50	*0.36*	*0.078*	*8.9*	*0.205*
C3-Dibenzothiophenes	5.73	*0.29*	*0.059*	*11.8*	*0.346*

7.5.5 Comparison of the profiles in salmon and mussels

7.5.5.1 PAH Accumulation

Control mussels (*Mytilius edulis*) exposed to acute PAH concentration showed PAH profile quite different from that observed in salmon. Mussels exposed to 2.5 ml of crude oil in 100 ml of water for 2 days accumulated the whole range (2- to 6-ring) of PAH compounds and in higher concentrations (> 9000 ng g^{-1}) than found in the salmon (485.4 - 1018.0 ng g^{-1}). Although the PAH profiles of mussels were also dominated by the 2- and 3-ring compounds as observed in salmon (Table 7.3), the 4- to 6-rings compounds were accumulated to ~ 2.3 %; ranging in concentration from as low as 0.5 ng g^{-1} for benzo[*a*]pyrene to ~ 126 ng g^{-1} for C2-chrysene/triphenylene. The differences in the suite of PAHs accumulated in these two species of organisms may be specifically due to the uptake route controlled by the organism's feeding mechanisms (Bjork, 1995; Meador *et al.*, 1995), however, this is not covered in this study. Vertebrates are known to rapidly metabolize

PAHs, especially the higher molecular weight compounds. This was not the case why lower PAh concentrations were found in the salmon as ethoxyresorufin-*O*-deethylase (EROD) activity measured using the liver samples collected from the salmon showed no significant positive effect (Fig.**7.5**)

Table **7.3**: Differences and similarities in PAH profile of mussels and salmon exposed to crude oil contamination.

PAH subgroups	Mussels exposed to crude oils			Salmon
	Arabian light	Gullfaks	Brent	
% Naphthalenes	48.1	77.3	78.3	94.0
% 178	10.4	12.9	12.9	3.1
% DBTs	38.4	6.0	5.3	1.7
% 202	1.5	1.8	1.4	-
% 228	0.8	0.9	0.8	-
% 252	0.3	0.2	0.2	-
% 276	0.0	0.0	0.0	-
% Acenaphthylene	-	0.0	-	-
% Acenaphthene	0.1	0.1	0.1	0.1
% Fluorene	0.3	0.8	1.0	0.6
% Dibenz[*a*,*h*]anthracene	0.001	0.002	0.002	-
Total PAH	16124.6 ± 113.1	26932.3 ± 1376.1	9109.9 ± 105.0	743.1 ± 218
% parent PAH	4.1	6.1	9.2	14.7
% 2- and 3-rings	97.4	97.1	97.7	>99.0
% 4- to 6-rings	2.7	2.9	2.3	<0.001

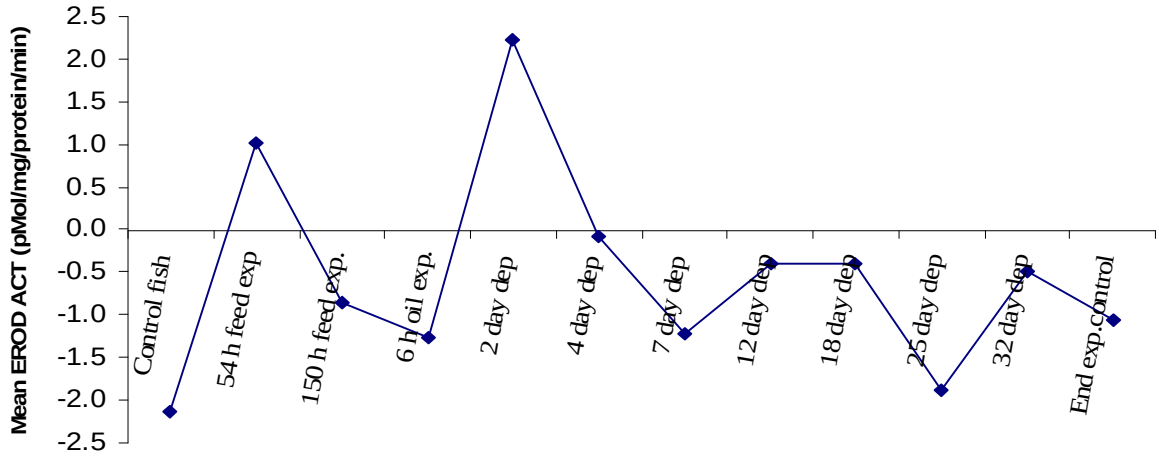


Fig. **7.5**: Induction of ethoxyresorufin-*O*-deethylase (EROD) activity measured in salmon exposed to contaminated feed and Forties crude oil.

7.5.5.2 Elimination kinetics.

The elimination kinetics (k_2) of specific PAH compounds in both studies followed first order kinetics. The k_2 and the biological half-lives obtained in both studies are presented in Table **7.4**. Elimination of the compounds followed similar trend with increase in alkylation resulting in increased biological half-life. The biological half-life ranged between 0.5 – 12.8 and 2.8-31.5 days for the mussels and salmon respectively. Generally, elimination was slower in salmon than in mussels; for example, the half-lives for naphthalene and 1-methyl naphthalene/2-methyl naphthalene were up to 6 and 4 times lower respectively in mussels than in salmon. This is contrary to expectation.

Vertebrates have been known to possess developed mixed-function oxidase (MFO) system. This system enables fast and efficient metabolism and excretion of PAH compounds from the latter than invertebrates (SGC, 2001). However, literature report has it that the low molecular weights PAHs are eliminated much slower in fish (Meador *et al.*, 1995).

This is because, these groups of compounds are not biotransformed but eliminated through the gills, and to a lesser extent through the skin of the organism (Varanasi, 1987). This possibly explains the result obtained in these studies.

Table **7.4**: Comparison of PAH elimination kinetics measured in mussels and salmon experimentally exposed differently to acute crude oil contamination

PAH compound	Mussels(median value)		Salmon (n=4)	
	k_2	$t_{1/2}$	k_2	$t_{1/2}$
Naphthalene	1.417	0.5	0.186	3.7
2-Methyl Naphthalene	0.699	1.0	0.157	4.4
1-Methyl Naphthalene	0.687	1.0	0.166	4.2
C2-Naphthalenes	0.398	1.7	0.115	6.0
C3-Naphthalenes	0.222	3.1	0.102	6.8
C4-Naphthalenes	0.131	5.3	0.119	5.8
Acenaphthene	0.278	2.5	0.078	8.9
Fluorene	0.385	1.8	0.124	5.6
Phenanthrene	0.391	1.8	0.161	4.3
Anthracene	0.217	3.2	ND	ND
C1-Phen/Anthracenes	0.150	4.6	0.137	5.1
C2-Phen/Anthracenes	0.080	8.6	0.092	7.7
C3-Phen/Anthracenes	0.062	11.1	0.022	31.5
Dibenzothiophene	0.297	2.3	0.150	4.6
C1-Dibenzothiophenes	0.163	4.2	0.247	2.8
C2-Dibenzothiophenes	0.083	8.4	0.078	8.9
C3-Dibenzothiophenes	0.054	12.8	0.059	11.8

7.5.5.3 Relationship between k_2 and log K_{ow}

The trend in k_2 values with increase in compound's hydrophobicity (log K_{ow}) was plotted for the mussels and salmon. The relationship presented in Fig 7.7 shows a declining trend in k_2 with increase in log K_{ow} in both species of organisms. However, the dependency of elimination rate constant on log K_{ow} is more pronounced for the mussels than for salmon. The equations of the plots are: log k_2 = -0.0498 log K_{ow} +1.717 and log k_2 = -0.202 log K_{ow} -0.021 with correlation coefficients (r^2) of 0.90 and 0.48 for mussels and salmon respectively.

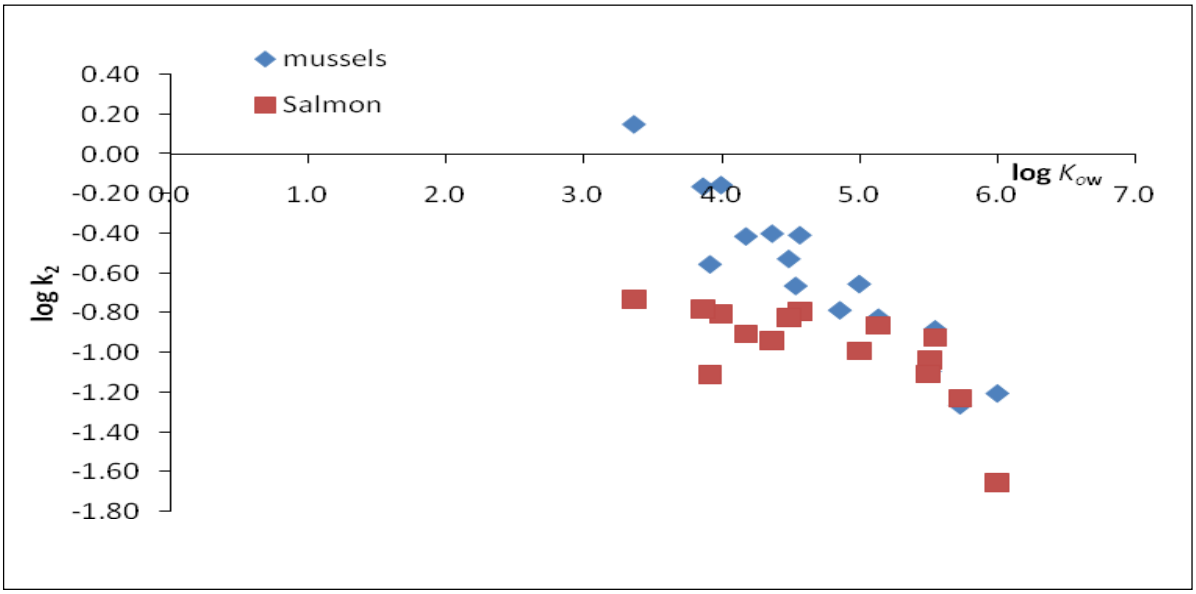


Fig. **7.6**: Relationship between elimination constant (k_2) and partition coefficient (log K_{ow}) determined in mussels (*Mytilius edulis*) and salmon (*Salmo salar)* exposed to artificial crude oil contamination.

7.5.5.4 Geochemical biomarker Profile (Steranes and Triterpanes)

The sterane and triterpane fingerprints of the salmon before and after oil exposure are presented in Fig **7.7** (**a-e**) and the peak identities are presented in Table **7.5**. The biomarker profile of the control fish (Fig **7.7** (**a**)) show no distinct petroleum related biomarker compound. The fish sampled after the oil exposure showed specific petroleum biomarker compounds (Fig **7.7** (**b-e**). For example, all the fish showed the 17α(H)-22, 29,30-trisnorhopane, 17α(H)-22, 29,30-trisnorhopane, 17α(H), 21β(H)-30-norhopane, 18α(H)-norneohopane, 17 α(H), 21β(H)-hopane peaks labelled as 1, 2, 3, 4, and 5 respectively. There is also the presence of the homohopane doublet peaks due to the C31 to C35 homohopane diastereoisomers (22S and 22R) labelled as peaks (6-15), which are distinct characteristic features of all crude oils.

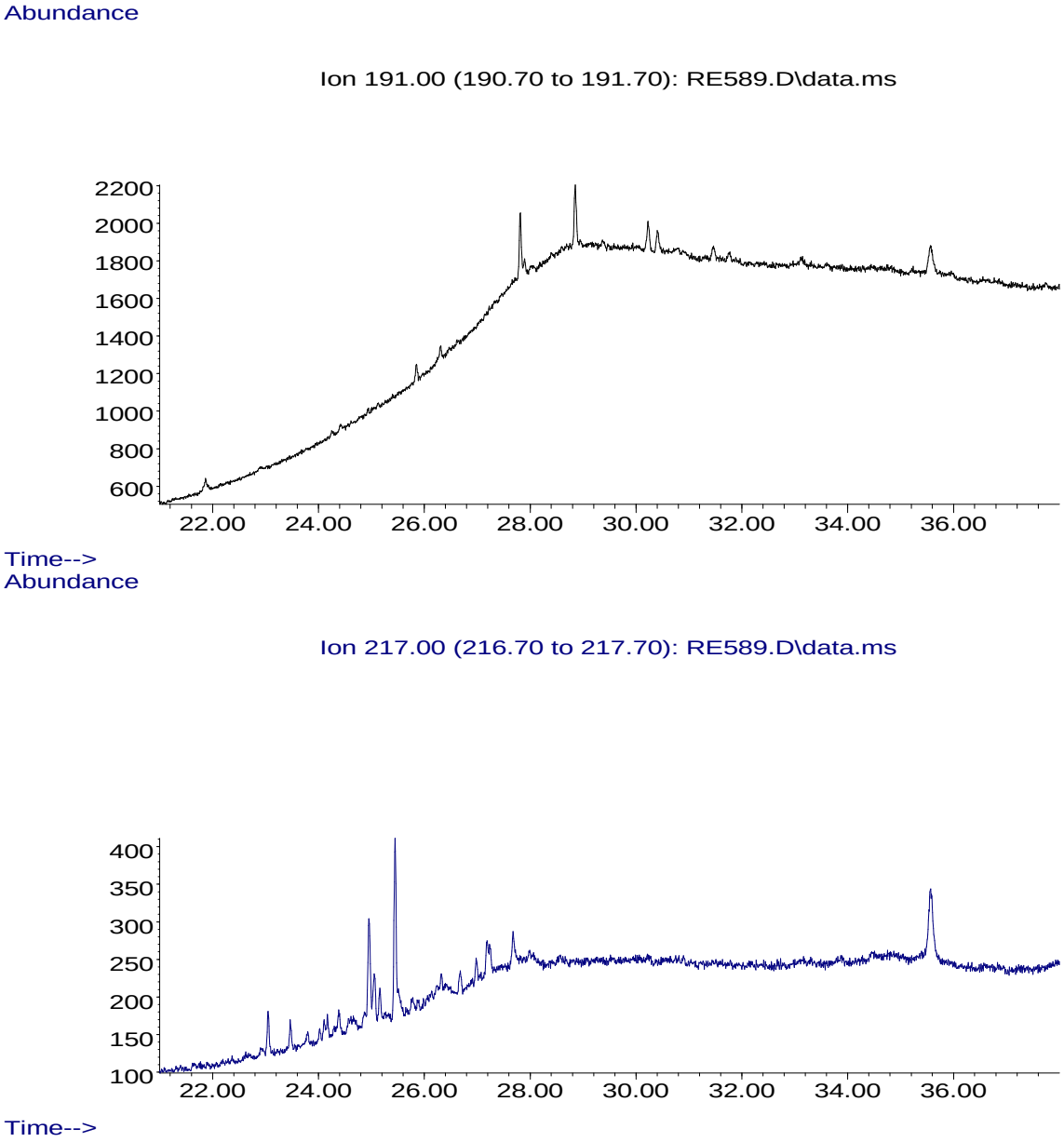
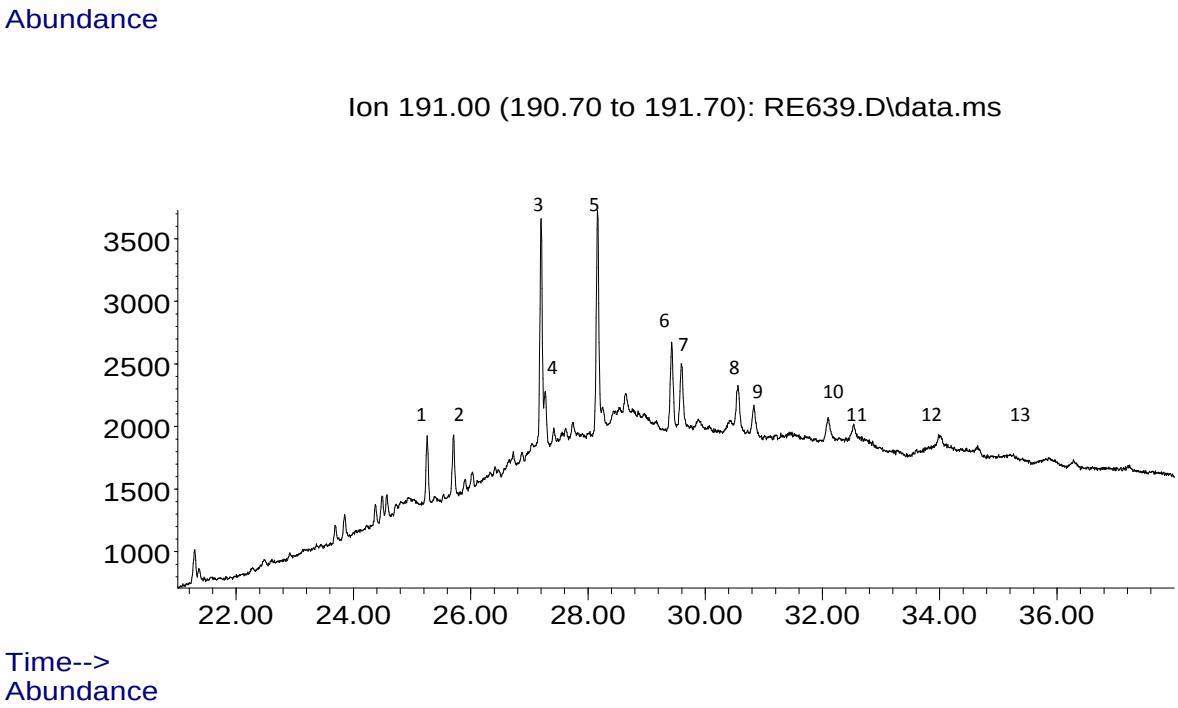


Fig.**7.7** (**a**): The triterpane (*m/z* 191) and sterane (*m/z* 217) chromatograms of control salmon (prior to oil exposure)



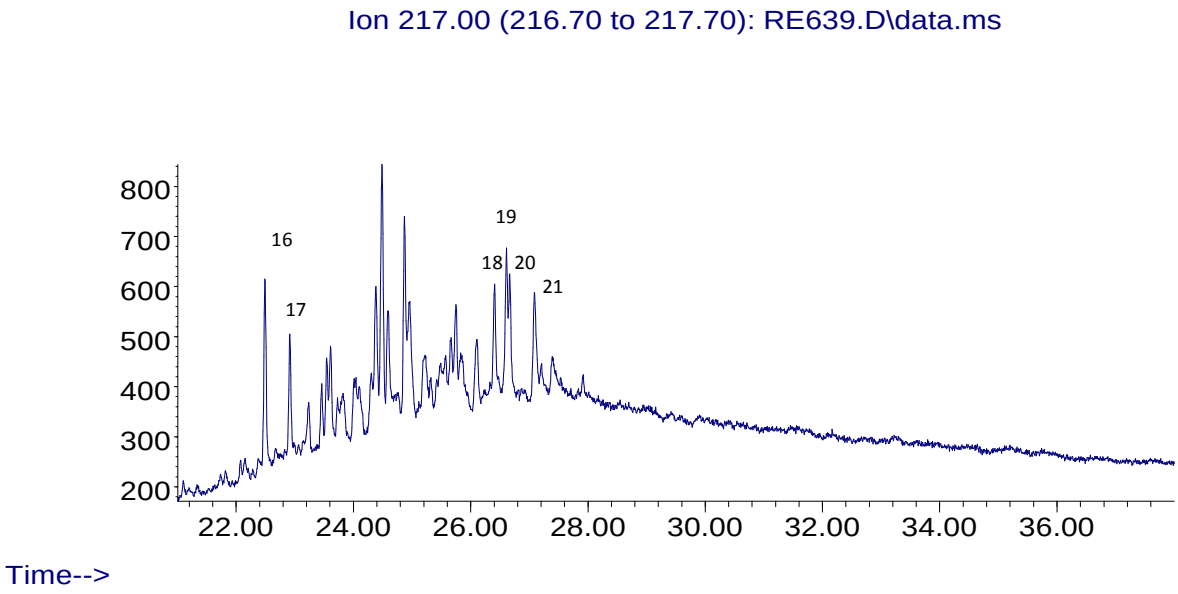


Fig. **7.7** (**b**): The triterpane (*m/z* 191) and sterane (*m/z* 217) chromatograms of salmon (Fish 1) exposed to Forties crude oil for 6 hours.

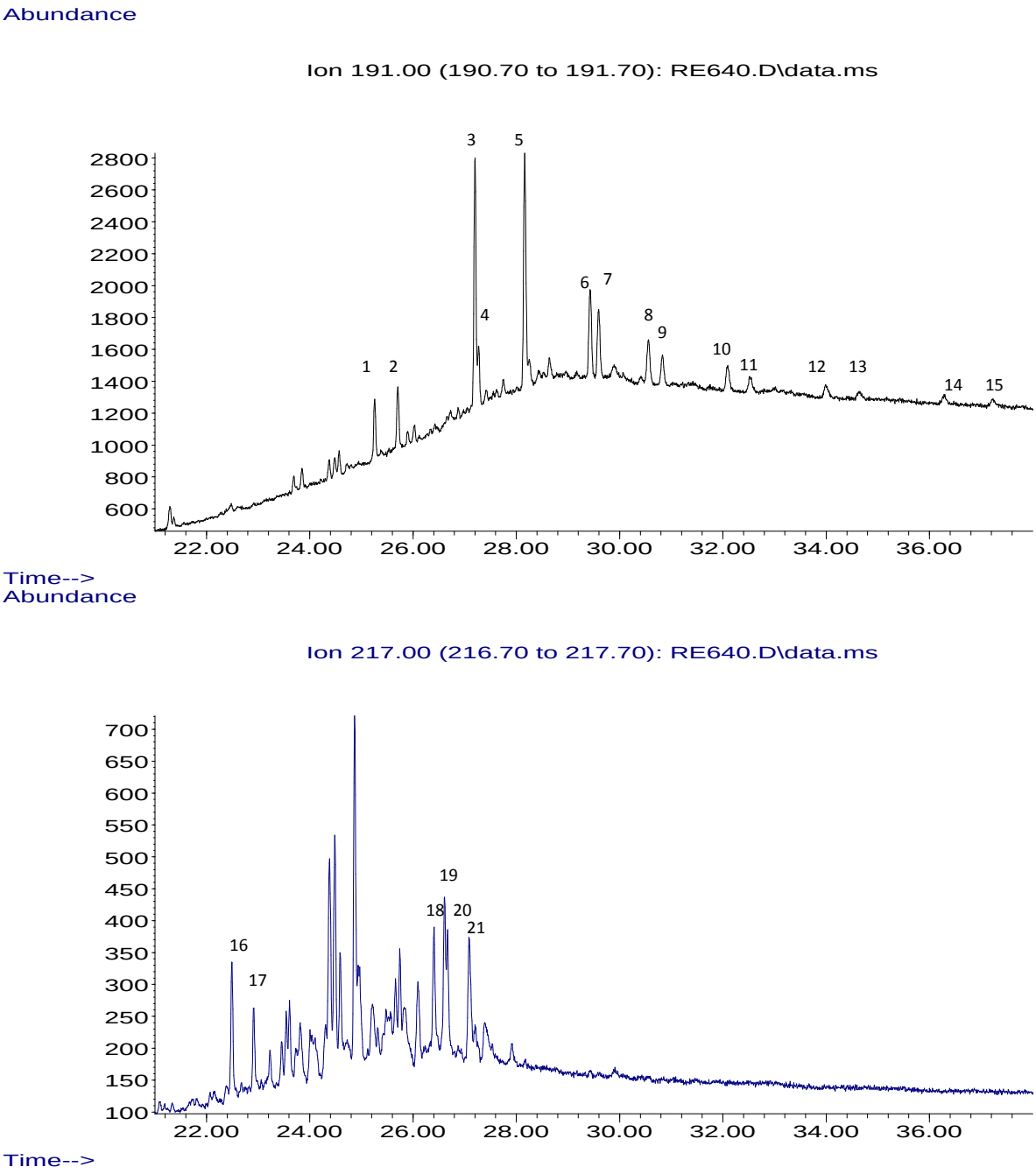


Fig. **7.7** (**c**): The triterpane (*m/z* 191) and sterane (*m/z* 217) chromatograms of salmon (Fish 2) exposed to Forties crude oil for 6 hours.

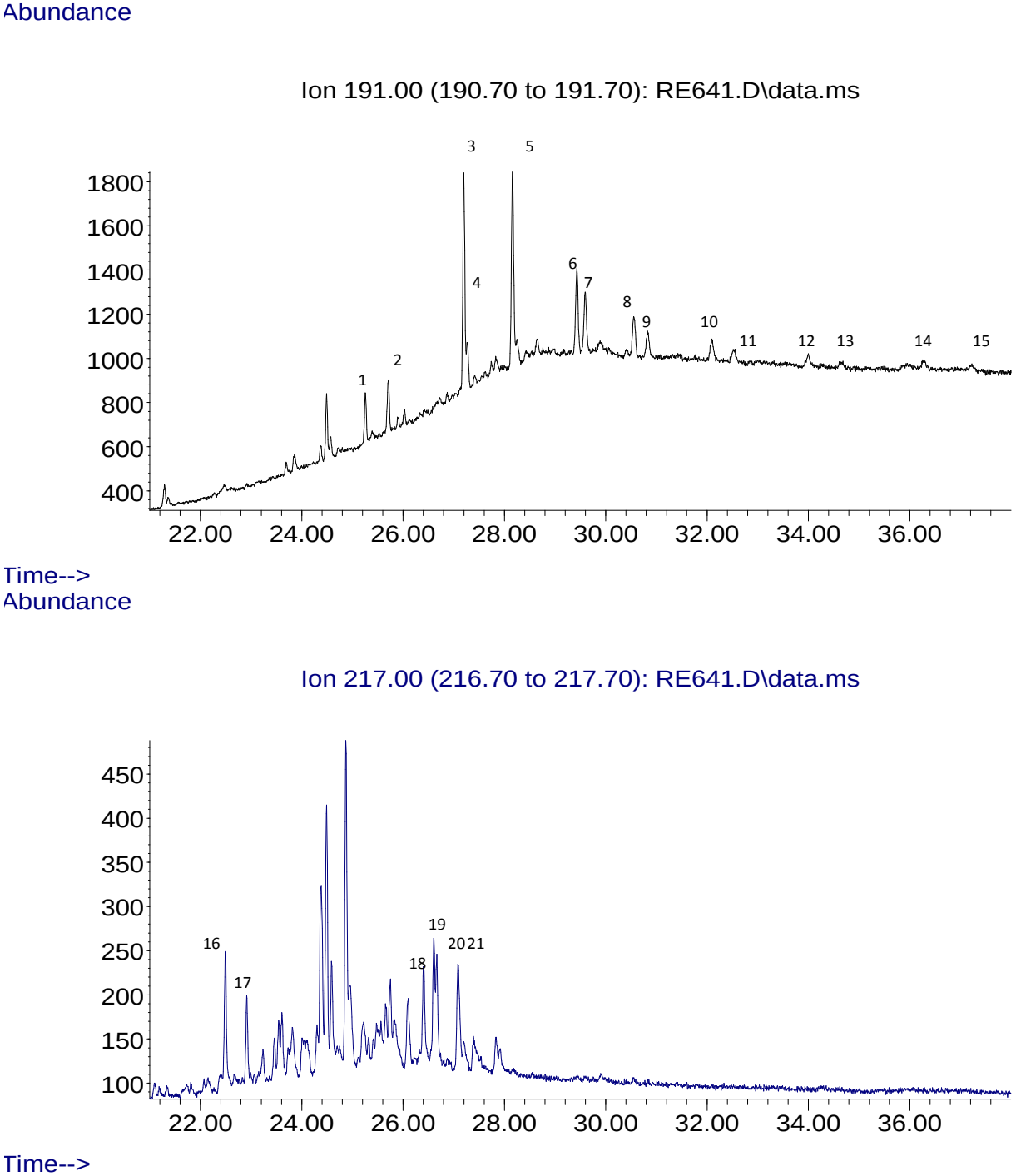


Fig. **7.7** (**d**): The triterpane (*m/z* 191) and sterane (*m/z* 217) chromatograms of salmon (Fish 3) exposed to Forties crude oil for 6 hours.

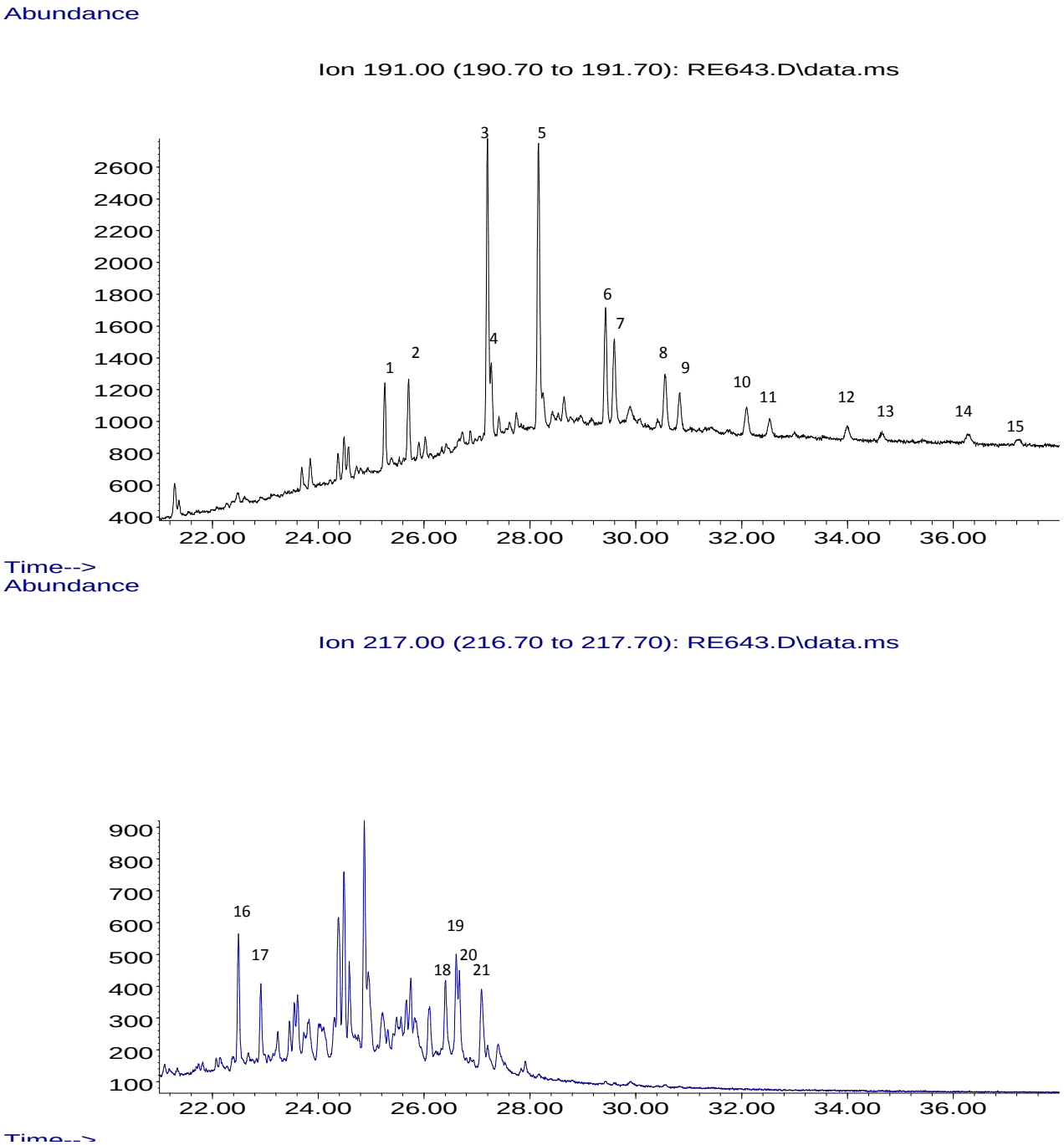


Fig. **7.7** (**e**): The triterpane (*m/z* 191) and sterane (*m/z* 217) chromatograms of salmon (Fish 4) exposed to Forties crude oil for 6 hours.

Table **7.5**: Peak identifications for chromatograms in figures 7.7, and diagnostic ratio descriptions

Peak label	**Abbrev.**	**Compound**	***m/z***
1	27Ts	18α(H) -22, 29, 30-trsinorhopane	191
2	27Tm	17α(H)-22, 29,30-trisnorhopane	191
	28ab	17α(H),21β(H)-28,30-bisnorhopane	191
3	29ab	17α(H), 21β(H)-30-norhopane	191
4	29Ts	18α(H)-norneohopane	191
5	30ab	17 α(H), 21β(H)-hopane	191
6 & 7	31ab (S & R)	17 α(H), 21β(H)-homohopane (22S & 22R)	191
8 & 9	32ab (S & R)	17 α(H), 21β(H)-bishomohopane (22S & 22R)	191
10 & 11	33ab (S & R)	17 α(H), 21β(H)-trishomohopane (22S & 22R)	191
12 &13	34ab (S & R)	17 α(H), 21β(H)-tetrahomohopane (22S & 22R)	191
14 &15	35ab (S & R)	17α(H), 21β(H)-pentakishomohopane (22S & 22R)	191
16	27dbS	13β (H), 17α(H) diacholestane (20S)	217
17	27dbR	13β (H), 17α(H) diacholestane(20R)	217
18	29aaS	5α(H), 14α(H), 17α(H)-24-ethylcholestane (20S)	217
19	29bbR	5α(H), 14β(H), 17β(H)-24-ethylcholestane (20R)	217
20	29bbS	5α(H), 14β(H), 17β(H)-24-ethylcholestane (20S)	217
21	29aaR	5α(H), 14α(H), 17α(H)-24-ethylcholestane (20R)	217

However, the 17α(H), 21β(H)-30-norhopane(3) to 17 α(H), 21β(H)-hopane (5) ratio observed in the fish (>0.9) is higher than normally found in pure samples of North Sea oils. Also, the absence of a pronounced bisnorhopane peak in the *m/z* 191 chromatograms of the fish is surprising. Bisnorhopane is a distinct characteristics of the North Sea oils and are normally found in ~ ≥ 0.5 ratio to norhopane. This means that salmon preferentially accumulated some biomarker compounds over others within the given exposure duration. In oil spill source investigations, use is made of the relative abundances, presence and/or absence of specific biomarker compounds in tracing spill oil sample to the source oil. It is therefore expected that the spill sample will contain identical biomarker compounds as the source oil and in relative abundances similar to that of the source oil. This is because geochemical biomarker compounds are resistant to degradation and bears fingerprints of their origin. The triterpane fingerprint observed in the fish is therefore not a true reflection

of the known characteristic features of Forties crude oil as one of the North Sea oils and could lead to misjudgement in oil spill source classification.

7.5.5.5 Comparison of biomarker profile with that found in mussels

The triterpane biomarker profile of the control mussels (Fig **6.7**(**d**)) differed from that of the control fish (e.g. Fig.**7.7** (**a**)). The former showed *m/z* 191 chromatogram dominated by diploptene; a natural triterpane, which according to Naraoka *et al*., (2000) are derived from cyanobacteria and chemotrophic bacteria. Diploptene was absent in the control salmon *m/z* 191 chromatogram. This distinct difference could be linked to the feeding habit of both species of organisms. The test mussels (Fig. **6.7** [**a-c**]) showed relative biomarker composition similar to those of the exposure oils. For example, the mussels exposed to the Arabian light oil showed norhopane to hopane ratio typical of the Middle Eastern oils and no bisnorhopane peak, while those exposed to the North Sea oils showed distinct bisnorhopane peaks and a norhopane to hopane relative abundance typical of the North Sea oils. The test salmon showed biomarker fingerprint that is different from the profile of the exposure oil. The pronounced differences between the biomarker compositions of the crude oil and the salmon observed in this study suggest that salmon may not accurately indicate environmental contaminant loading and therefore not a reliable indicator in oil source assessment.

7.6 Conclusion.

Salmon exposed to PAH pre-treat feed and crude oil dissolved in water for 150 h and 6 h respectively showed no evidence of PAH accumulation from the feed. Although analysis of the pre-treated feed showed PAH concentration in excess of 1600 ng g^{-1} and comprising of the whole range of the 2- to 6- ring compounds; no difference was observed in the PAH tissue burden of the test samples after the feed exposure. This suggests that either the PAHs are probably not in the form readily available for accumulation by the salmon or that the exposure duration was not sufficient to impact on the PAH tissue burden of the organisms. Exposure of the organisms to crude oil in the dissolved phase enabled the accumulation of

only the lighter PAH compounds; naphthalenes, phenanthrene/anthracene, dibenzothiophene and their alkylated compounds within the exposure duration. Preferential accumulation of lighter PAHs from the dissolved phase is typical and in agreement with literature reports from the immediate aftermath of oil spill incidents. These compounds, especially naphthalene and alkylated compounds have high aqueous solubility and are therefore readily available in the dissolved phase. Although individual fish showed heterogeneity in total PAH tissue burden, similar naphthalene % composition was found in all samples analyzed immediately after the oil exposure. This finding indicates that similar factors control the accumulation of this group of PAH compounds in salmon. All fish sampled immediately after oil exposure tested positive to taint, this also may be due to the high naphthalene concentration.

Elimination of PAHs was rapid upon transfer of the fish to relatively clean environment and followed first order kinetics. Biological half-lives of individual PAH compounds increased with increase in alkylation and were less than 32 days for the lighter PAH compounds. Taint was not lost as rapidly as it developed, however, a relatively good correlation was found between the taint intensity and PAH tissue burden of organisms.

Salmon also showed unique crude oil geochemical biomarker compounds (steranes and triterpanes) after exposure to the crude oil. However, the composition and relative abundances of some of the compounds in salmon differed greatly from the known profile in the exposure oil. The absence of 17α(H),21β(H)-28,30-bisnorhopane; a unique feature of the North Sea oils and a 17α(H), 21β(H)-30-norhopane to 17 α(H), 21β(H)-hopane ratio of >0.9 show that salmon preferentially accumulated some of the biomarker compounds over others. The biomarker information derived from salmon in this study is misleading and suggest that salmon is not a good indicator of comtaminant loading and are therefore not a reliable matrix in oil spill source investigation.

CHAPTER EIGHT

Comparison of depuration constants (k_2) and biological half-life ($t_{1/2}$) obtained in this study with literature data and the development of recommendations for improving marine oil spill response

8.1 Introduction

In an event of oil spill, concern is always raised regarding seafood safety. The potential and actual contamination of seafood by toxic and carcinogenic petroleum components can effect harvesting of seafood for commercial, recreational or subsistence use. In the last 3 decades, several studies have investigated the dynamics of uptake and depuration of PAHs from marine organisms. PAHs are one component of oils that is of major toxicological significance. These studies have used various species of marine organisms, PAH sources, length and route of exposure of the organism to the PAH source, variable environmental conditions and other experimental designs. Most of the reported studies used bivalve molluscs (oysters, clams, mussels) as sentinel organisms. While some studies monitored organisms chronically exposed to contaminants, others experimentally exposed organisms to acute PAH contamination using various PAH sources. However, the result is that diverse kinetic information has been reported regarding the elimination of PAHs from bivalves. A brief summary of the results from some of the studies is given in Table **8.1.**

A major limitation of most of the studies listed in Table **8.1** is that the change in PAH tissue burden of the organisms over time were reported in terms of 'total PAHs' instead of individual compounds of toxicological significance. This generalisation can be misleading. As the current studies have shown, the rate of elimination of PAH compounds from organisms vary even among isomers of the same compound. Consequently, a study investigating the elimination kinetics of a suite of PAHs dominated by only the 2- to 3- ring compounds is likely to observe a more rapid reduction in total PAH concentration over time than a study using a higher proportion of 4- to 6-rings compounds. This is because factors which have been reported to affect PAH elimination rate include partition co-efficient and molecular weight amongst others, and these parameters increase from the 2- to 6- ring

compounds. Therefore, it is expected that the lower ring compounds would be eliminated faster than the higher ring compounds. Hence in comparing PAH elimination results quoted as 'total PAHs' consideration should be given to the suite of PAHs making up the total compounds studied.

In the current research study, the elimination kinetics of individual PAH compounds were investigated in mussels (*Mytilius edulis*) and Atlantic salmon (*Salmo salar*). Four separate studies were carried out and these include:

I. Laboratory depuration of chronically contaminated indigenous mussels collected from Aberdeen harbour.
II. Field depuration study using mussels collected from the same site as above
III. Laboratory depuration of mussels experimentally exposed to petroleum products; Arabian light, Gulfaks or Brent crude oils, for 2 days.
IV. Laboratory depuration of Atlantic salmon experimentally exposed to PAH contaminated feed and Forties crude oil.

Therefore, for clarity, in this chapter, only the studies which reported elimination kinetics of individual PAH compounds are compared with the results from the current studies. However, reference is also made to real spill incidents where data are available.

Table **8.1**: Summary of literature reports on the rate of elimination of PAHs from bivalves, and data obtained from current studies (BHL = biological half-life)

Bivalve	Exposure medium	Exposure route/time	Tank water replacement time	Observation	Reference
Oysters	No. 2 fuel oil spill	Water and sediment (60 d)	In-situ	Little depuration after 180 days	Blumer *et al*. (1970)
Oysters	No. 2 fuel oil in laboratory	Water- only (49 d)	Every 2 days	Nearly complete depuration in 28 days	Stegeman and Teal (1973)
Clams	Chronically polluted	Water and particulate	37 times per day	Slight depuration after 120 days	Boehm and Quinn (1977)
Mussels	PAH contaminated sediment	Sediment in water (40 d)	-	Half-lives between 14-30 days	Pruell *et al*. (1986)
Clams	PAH standards	Water-only (2 d)	Used carbon filters	No depuration in 45 days	Tanacredi and Cardenas (1991)
Oysters	Chronic pollution	(48d)		Significant depuration in 50 days	Sericano *et al*. (1996)
Mussels	PAHs	Water – only (20 days)	Daily	Depuration very rapid in 2 d and then reduced to minimum thereafter	Richardson *et al*. (2005)
Mussels	Chronic pollution	Water /particulate	Once every 30 hours	Total PAHs fall 50% in 122 d	McIntosh *et al*. (2004)
Mussels	PAH standards	Water – only. (5 days)	Used carbon filters	Good depuration with BHL of 3.2 -18.7 d	Gewurtz *et al*. (2002)
Mussels	Creosote contaminated sediment	Sediment – water (10 d)	6 times a day	Rapid depuration with BHL of 2.9 -16.5 d	Thorsen *et al*. (2004).
Mussels	Chronic pollution	Water /particulate	Every 0.2h	Good depuration with BHL 3.8-31 d	This study I
Mussels	Chronic contamination	Water /particulate	Field	Good depuration with BHL 10.6-19.6 d	This study II
Mussels	Crude oil	Water-only	Every 0.2 h	Rapid depuration with BHL 0.5-22 d	This study III

8.2 Comparison of the rate constants and half-times derived from mussels with literature values.

8.2.1 Depuration rate constants

Table **8.2** gives the k_2 values obtained from the present studies; studies I, II & III and other literature reports. k_2 varied among the PAH compounds and amng the studies. These variations are likely due to the variations in the experimental designs; the PAH sources, route and length of exposure, and water replacement rates which varied among the studies. To enhance understanding of subsequent sections, a brief summary of the experimental conditions used in each study is given below.

The experimental designs used for studies I & II, and study III have already been given in Chapters **3** and **6** respectively.

Thorsen *et al*. (2004)
Relatively clean mussels (*Elliptio complanata*) were exposed to creosote contaminated sediment for 10 days. 6-7 mussels were placed in 4 L jars containing 1 kg sediment and 3 L water. The mussels were depurated in 70 L tanks with the depuration water replaced 6 times per day. Mussels were not fed during the experiment. Single mussels were analyzed as sample.

Gewurtz *et al*. (2002)
Mussels (*Elliptio complanata*) were exposed to solution of PAH standards (Table **8.2**) in DCM evaporated onto glass wool for 5 days. The mussels were depurated in 30 L tanks filled with tap water. Carbon and power filters were placed in the tank to prevent recycling of eliminated compounds by the organisms. Temperature ranged from 16.5 ºC to 18 ºC during the experiment. Single mussels were analyzed as sample.

McIntosh *et al*. (2004)
Mussels (*Mytilus edulis*) were collected from a contaminated area in Loch Leven and depurated in the laboratory (150 L tanks) for 122 days. The flow rate of the depuration

water was 5 l per hour giving a replacement time of 30 hours. The temperature of the water was 10 ± 2 ºC. Mussels were analyzed as aliquots of 20 mussels.

The main similarity in the reported values is that k_2 generally decrease with increase in molecular weight and alkylation (Table **8.2**). The k_2 values obtained from the harbour mussels depurated in the laboratory (study I) are comparable to the values obtained in the field (study II) irrespective of the initial concentration of the contaminants. For example, biological half-lives were 15.1/13.6 (phenanthrene), 11.2/11.2 (C1-phenanthrene/anthracenes), 10.7/11.7 (C1-dibenzothiophenes), 15.8/16.2 (C2-flouranthene/pyrene), 19.6/18.7 (benzo[*a*]pyrene), 15.4/16.2 (benzo[*c*]penanthrene, 14.1/14.5 (chrysene/triphenylene), etc., for the field and laboratory studies respectively (see Chapter **3,** Table **3.8**). This indicates that the laboratory study can be directly applied to field situations. The harbour mussels *n*-alkane profile, PAH concentration ratios and geochemical biomarker profile suggest petrogenic PAH contamination. However, the k_2 values determined from these mussels differed from those obtained in study III and Thorsen *et al*. (2004) that used similar (petrogenic) PAH source of contamination and similar water replacement times. k_2 values obtained in studies I & II are consistently lower than those of study III and Thorsen *et al*. (2004). The differences may be due to the length of exposure of the organisms to the contaminant source.

Table **8.2**: Comparison of the depuration rate constants (k_2 day^{-1})obtained in the present studies with those obtained by Thorsen *et al*. (2004), Getwurtz *et al*. (2002) and McIntosh *et al*. (2004) (note water replace decreases fro right to left except for study II which is a field depuration study).

Compounds	Log K_{ow}	Study I	Study II	Study III	Thorsen *et al.* (2004)	Gerwurtz *et al* (2002)	McIntosh *et al,* (2004)a	McIntosh *et al.* (2004)b
Naphthalene	3.37			1.4167	0.2217		0.0198	0.0158
2-Methyl Naphthalene	4.00			0.6993	0.2059			
1-Methyl Naphthalene	3.87			0.6873	0.2486			
C2-Naphthalenes	4.37	0.0560	0.0453	0.3980	0.2124			
C3-Naphthalenes	5.00	0.0926	0.0620	0.2217	0.1793		0.0151	0.0193
C4-Naphthalenes	5.55		0.0652	0.1310	0.1543			
Acenapthylene					0.1847	0.0460		
Acenaphthene	3.92			0.2783	0.2372	0.0950		
Fluorene	4.18		0.0592	0.3853	0.1902	0.2170	0.0178	0.0165
Phenanthrene	4.57	0.0510	0.0458	0.3910	0.1707	0.1170	0.0495	0.0433
Anthracene	4.54	0.0500		0.2168	0.1792	0.1630	0.0693	0.0533
C1-Phenan/Anthracenes	5.14	0.0619	0.0620	0.1500	0.1656		0.0495	0.0330
C2-Phenan/Anthracenes	5.51	0.0678	0.0605	0.0803	0.1318		0.0267	0.0173
C3-Phenan/Anthracenes	6.00	0.0612	0.0505	0.0622	0.0939		0.0147	0.0139
Dibenzothiophene	4.49		0.0582	0.2967	0.1611		0.0347	0.0257
C1-Dibenzothiophenes	4.86	0.0594	0.0647	0.1633	0.1473		0.0267	
C2-Dibenzothiophenes	5.50	0.0873	0.0573	0.0828	0.0821		0.0161	
C3-Dibenzothiophenes	5.73	0.0578	0.0473	0.0542	0.0687		0.0060	
Fluoranthene (202)	5.22	0.0675	0.0536	0.0557	0.1257	0.1300	0.0533	0.0347
Pyrene (202)	5.18	0.0437	0.0621	0.0853	0.1635	0.1440	0.0365	0.0248
C1-Flouranthene/Pyrene	5.72	0.0487	0.0563	0.0730	0.0919		0.0239	0.0187
C2-Flouranthene/Pyrene		0.0428	0.0439	0.0568			0.0204	0.0161
C3-Flouranthene/Pyrene				0.0667			0.0131	0.0108
Benzo[*c*]phenanthrene (228)	5.76	0.0428	0.0450				0.0173	0.0110
Benz[*a*]anthracene	5.91	0.0337	0.0485	0.1120	0.0924	0.1480	0.0112	0.0117

Chrysene/Triphenylene	5.86	0.0477	0.0490	0.0638	0.0836	0.1050	0.0154	0.0169
Benz[b]anthracene (228)							0.0248	
C1-Chrysenes	6.42	0.0397	0.0455	0.0564	0.0838		0.0144	0.0154
C2-Chrysene	6.88	0.0315	0.0373	0.0595	0.0697		0.0102	0.0105
Benzo[b]fluoranthene	5.8				0.0827	0.0370		0.0315
Benzo[k]fluoranthene	6.00				0.0589	0.1030	0.0112	0.0122
Benzofluoranthenes (252)	6.00			0.0932				
Benzo[e]pyrene (252)	6.20	0.0224	0.0377	0.0540	0.0727		0.0128	0.0103
Benzo[a]pyrene (252)	6.04	0.0371	0.0353	0.0315	0.0755		0.0144	0.0169
Perylene (252)	6.30	0.0299	0.0357	0.1287	0.0421		0.0076	0.0049
C1 252				0.0350			0.0077	0.0074
C2 252				0.1223			0.0105	
Indeno[123cd]pyrene	7.00	0.0391	0.0458	0.1137	0.0471	0.1620	0.0193	0.0151
Benzo[ghi]perylene	6.50	0.0235	0.0478	0.0442	0.0599	0.0800	0.0147	0.0080
C1 276				0.0385				
Dibenz[a,h]anthracene (278)	6.75			0.1110	0.0687	0.0480	0.0136	0.0128

The mussels collected from Aberdeen harbour had been chronically exposed to the contaminant source for an unknown (but long) period of time. Decrease in k_2 with increased exposure duration have been reported in American oysters. Sericano *et al*. (1996) reported higher k_2 values for indigenous oysters than oysters transplanted to the same location even when their tissue burden were similar. This, according to Stegeman and Teal (1973), may be due to the retention of the hydrocarbon in a stable compartment within the organism that prevents loss of these compounds, hence increasing half-lives. In the study with Aberdeen harbour mussels, initial rapid elimination of the PAHs was followed by a slower elimination as concentrations approached background levels (Chapter **3**)

The k_2 values reported by McIntosh *et al*. (2004) are consistently higher than those obtained in all the other studies. A major difference which exists in the experimental designs used in McIntosh *et al*. (2004) and the other studies is the replacement time of the depuration water. McIntosh *et al*. (2004) used a flow-rate (5 L h^{-1} in 150 L tank); a replacement time of 30 hours. Mussels filter high volumes of water and hence are capable of re-absorbing PAH compounds available in the surrounding water. If the flow rate of the depuration water is not fast enough to enable efficient flushing of the contaminants away from the mussels as soon as they are eliminated from the organisms, opportunity will exist for the compounds to be recycled back into the organisms, resulting in increased apparent half-lives. By contrast, the flow rates used in studies I and III (36 L min^{-1}) and in Thorsen *et al*. 2004 (0.3 L min^{-1}) replaced the depuration water in 0.2 h and 5.6 h respectively, and these may have been high enough to prevent recycling of the eliminated PAH compounds by the organisms. Gewurtz *et al*. (2002) also used a carbon filter to capture the PAH compounds as they are eliminated, making them unavailable in the water phase for possible re-absorption by the mussels. Study II was a field depuration study, with high water flow rate. Therefore, the replacement time used by McIntosh *et al*. (2004) was very long compared to other studies and could have led to re-cycling of the eliminated compounds by the mussels and this may be why the half-lives reported by the latter are consistently higher than those reported in the other studies.

In addition, the mussels monitored by McIntosh *et al*. (2004) had been chronically exposed to PAH of pyrolytic origin arising from the effluent discharge of an aluminum smelter situated close to the sample site. The longer duration of exposure also contributed to the

extended half-lives observed for individual PAH compounds relative to similar compounds in other studies.

8.2.1.1 Relationship between k_2 and log K_{ow}

Previous studies have shown that the rate of elimination of hydrophobic contaminants from organisms is influenced by the hydrophobicity of the compounds. Therefore, the k_2 values obtained in these studies are plotted against the log K_{ow} values of the compounds to compare the influence of hydrophobicity on elimination constants for each study. The plot shows high dependency of k_2 on hydrophobicity, with increase in log K_{ow} leading to decrease in k_2 value.

In study III, between log K_{ow} of about 3.3 and 5.5, k_2 decreases strongly with increasing log K_{ow}. There is no clear trend in k_2 at higher values of log K_{ow} (5.5 – 7.0). The observed plateau in k_2 values from log $K_{ow} \geq 5.5$ is also common among the other studies, but less pronounced for some. In Thorsen *et al*. (2004) and Gewurtz *et al*. (2002), only small changes in k_2 were observed for PAHs with log K_{ow} values ≥ 5.5 and 6.0 respectively. The same effect is seen in studies I & II for log $K_{ow} \geq 6.0$. In McIntosh *et al*. (2004), this effect is also seen but less pronounced than the others because of the variability in the k_2 values for compounds of lower K_{ow}. A similar trend has also been reported by Kayal *et al*. (1990) where a regression analysis of log K_{oc} versus log K_{ow} also gave a maximum value at log K_{ow} value of about 5.5, and thereafter yielded a parabola. Therefore, the observed relative constancy of k_2 values for PAHs of log $K_{ow} > 5.5$ is common observation in many studies and may be as a result of steric hindrance of the larger cross-sectional diameter of higher molecular PAHs, as described by Lullen and Shea (2002).

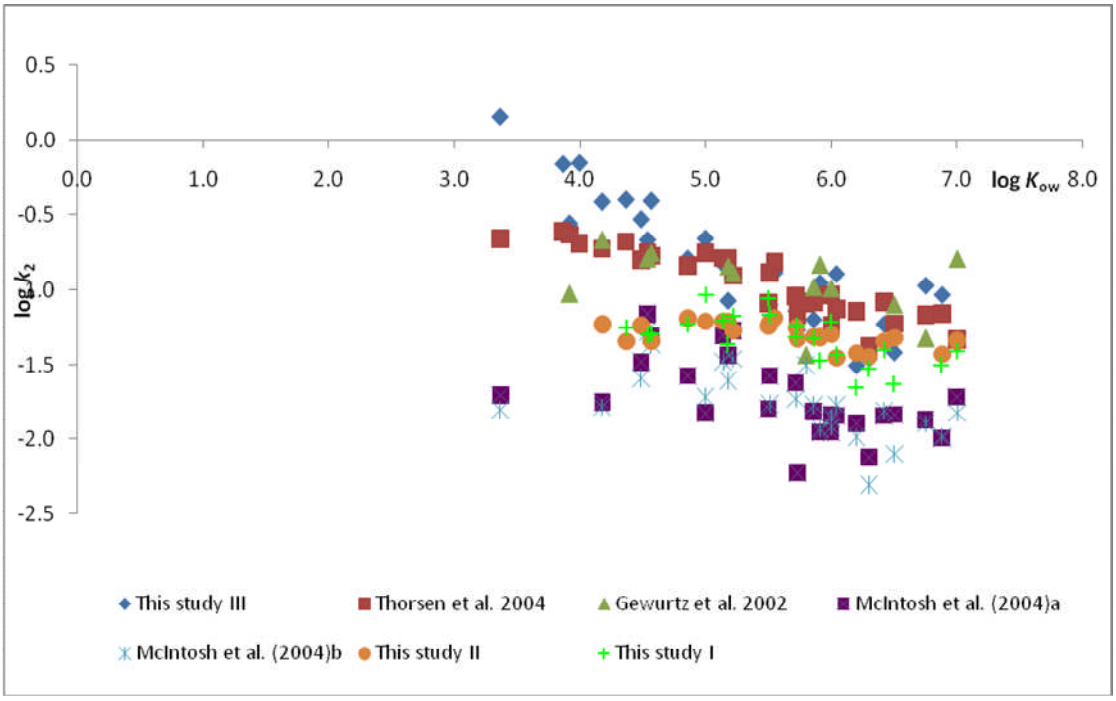


Fig. **8.1:** log k_2 versus log K_{ow} obtained in studies I, II and III, and those calculated for Thorsen *et al*. (2004), Gewurtz *et al*. (2002), and McIntosh *et al*. (2004). [a and b are samples collected from Kinlochleven and Ballachulish respectively]

8.2.2 Biological half-lives ($t_{1/2}$)

The similarities and differences between the k_2 values from the current and previous studies are very clear in the estimated biological half-lives ($t_{1/2}$). For example, $t_{1/2}$ for study III and Thorsen *et al.* (2004) respectively were 3.1/3.9 d (C3-napthalene), 2.5/2.9 d (acenaphthene), 3.2/3.9 d (anthracene), 4.6/4.2 d (C1-phenanthrene/anthracene), 4.2/4.7d (C1-dibenzothiophenes), 8.4/8.4 d (C2- dibenzothiophenes) and 15.7/14.7 d [benzo(*g,h,i*)perylene], etc, (Table **8.3**). However, some of the values were higher than Thorsen *et al*. (2004). For example, $t_{1/2}$ for naphthalene and 2-methyl naphthalene/1-methyl naphthalene are, respectively, more than 6 and 2 times greater than those of Thorsen *et al*. (2004).

Table **8.3**: Comparison of the biological half-lives ($t_{1/2}$, days) obtained in the present studies with those obtained by Thorsen *et al*. (2004), Getwurtz *et al*. (2002) and McIntosh *et al*. (2004).

Compounds	This study I	This study II	This study III	Thorsen et al. (2004)	Gewurtz et al. (2002)	McIntosh et al. (2004)a	McIntosh et al. (2004)b
Naphthalene			0.5	3.1		35	44
2-Methyl Naphthalene			1.0	3.4			
1-Methyl Naphthalene			1.0	2.9			
C2-Naphthalenes	12.4	15.3	1.7	3.3			
C3-Naphthalenes	7.5	11.2	3.1	3.9		46	36
C4-Naphthalenes	3.8	10.6	5.3	4.5			
Acenapthylene				3.8	15.1		
Acenaphthene			1.8	2.9	7.3		
Fluorene		11.7	1.8	3.6	3.2	39	42
Phenanthrene	13.6	15.1	2.5	4.1	5.9	14	16
Anthracene	13.9		3.2	3.9	4.3	10	13
C1-Phen/Anthracenes	11.2	11.2	4.6	4.2		14	21
C2-Phen/Anthracenes	10.2	11.5	8.6	5.3		26	40
C3-Phen/Anthracenes	11.3	13.7	11.1	7.4		47	50
Dibenzothiophene		11.9	2.3	4.3		20	27
C1-Dibenzothiophenes	11.7	10.7	4.2	4.7		26	
C2-Dibenzothiophenes	7.9	12.1	8.4	8.4		43	
C3-Dibenzothiophenes	12.0	14.7	12.8	10.1		116	
Fluoranthene (202)	10.3	12.9	12.4	5.5	5.3	13	20
Pyrene (202)	15.9	11.2	8.1	4.2	4.8	19	28
C1-Flouranthene/Pyrene	14.2	12.3	9.5	7.5		29	37
C2-Flouranthene/Pyrene	16.2	15.8	12.2			34	43

C3-Flouranthene/Pyrene			10.4			53	64
Benzo[c]phenanthrene (228)	16.2	15.4				40	63
Benz[*a*]anthracene	20.6	14.3	6.2	7.5	4.7	62	59
Chrysene/Triphenylene	14.5	14.1	10.9	8.3	6.6	45	41
Benz[*b*]anthracene (228)						28	
C1-Chrysenes	17.4	15.2	11.6	8.3		48	45
C2-Chrysenes	22.0	18.6	7.4	9.9		68	66
Benzo[*b*]fluoranthene				11.8	18.7		22
Benzo[*k*]fluoranthene				8.4	6.7	62	57
Benzofluoranthenes (252)			12.8				
Benzo[*e*]pyrene (252)	31.0	18.4	22.0	9.5		54	67
Benzo[*a*]pyrene (252)	18.7	19.6	5.4	9.2		48	41
Perylene (252)	23.2	19.4	19.8	16.5		91	141
C1-252			5.7			90	94
C2-252			6.1			66	
Indeno[123,*cd*]pyrene	17.7	15.1	15.7	4.3	4.3	36	46
Benzo[*ghi*]perylene	29.5	14.5	18.0	14.7	8.7	47	87
C1-276			6.2				
Dibenz[*a,h*]anthracene (278)			6.4	10.1	14.4	51	54

The $t_{1/2}$ data from study III also differed from those of Gewurtz *et al*. (2002). Apart from anthracene (3.2/4.3 d), benz[*a*]anthracene (6.2/4.7d), and chrysene/triphenylene (8.3/6.6 d) which returned values that are similar in study III and Gewurtz *et al*. (2002) respectively, the values obtained for other compounds varied considerably (Table **8.3**). Again, the $t_{1/2}$ values reported by McIntosh *et al*. (2004) are consistently higher than obtained in all other studies. The different data generated from the mussels affected by petrogenic contamination can be applied in different spill scenarios. This is discussed in section **8.5**.

8.3 Comparison of the salmon data with literature values.

The profile of PAHs found in salmon in this study (Chapter **7**) compares well with literature reports from real spill situations. Whittle *et al.* (1997) and Law and Kelly (2004) reported dominance of the 2- and 3-ring PAH compounds in caged and wild salmon affected by two major oil spills; the *Braer* and the Sea Empress respectively. The 2- and 3-rings compounds (naphthalenes and C1-C4, phenanthrene/anthracene and C1-C3, and dibenzothiophene and C1-C3) have higher aqueous solubility than the higher molecular weight compounds and are available in the water phase. This indicates that the dissolved phase is the major route of uptake by this specie of organisms in the short term.

Study VI shows half-life range of 2.8 to 31.5 for the 2- and 3-ring compound with C3-phenanthrene/anthracene persisting longer (Table **7.2**). The PAH tissue burden of salmon reduced from 743.13 ± 218.13 ng g^{-1} to 22.7± 10.68 ng g^{-1} wet weight (Chapter **7**) and 14,000 ng g^{-1} to 1000 ng g^{-1} (Whittle *et al*., 1997) within 25 days; giving a reduction in total PAHs of 97 % and 93 % respectively. Although the reduction in PAH tissue burden of salmon were reported as total PAHs (∑PAHs = sum of compounds in the first paragraph of this section) by Whittle *et al*. (1997) and Law and Kelly (2004) (Table **8.4**), the patterns of decrease in total PAH concentration were similar to that observed in this study.

In the current study (study IV), it was observed that samples were generally free of taint and suspicion of taint when the total PAH tissue burden declined below 200 ng g^{-1} (**Fig. 8.2**). Likewise, all salmon sampled from the affected area during the Sea Empress grounding in 1996 were free of taint, and total PAH tissue burden ranged between 12-186

ng g^{-1} (Law and Kelly, 2004). However, some of the caged salmon affected by the *Braer* spill incident still tested positive to taint even when the PAH tissue burden had reduced to less than 20ng g^{-1}. This suggests that taint is not lost as rapidly as it develops, probably due to its conversion and retention in a form different from the contributing compounds The absence of taint in all samples analyzed by Law and Kelly (2004) further suggests that the compounds responsible for inducing taint must reach a certain threshold in an organism before taint can be detected and this is governed not by the PAHs tissue burden of organism at a given time point but by the initial concentration in an impacted organism. Davies *et al*. (2002) reported that increase in oil concentration and duration of exposure increases the persistence of taint. The statement above is further supported by the fact that salmon samples analyzed after 32 days of depuration in the current study came out as suspect to taint while the samples collected at 18 and 25 days with higher PAH tissue burden were free of taint. The reason for this could have been as a result of the initial PAH concentrations in these salmon. The report of Whittle *et al*. (2004) has shown that the higher the intensity of taint, the longer it takes to depurate.

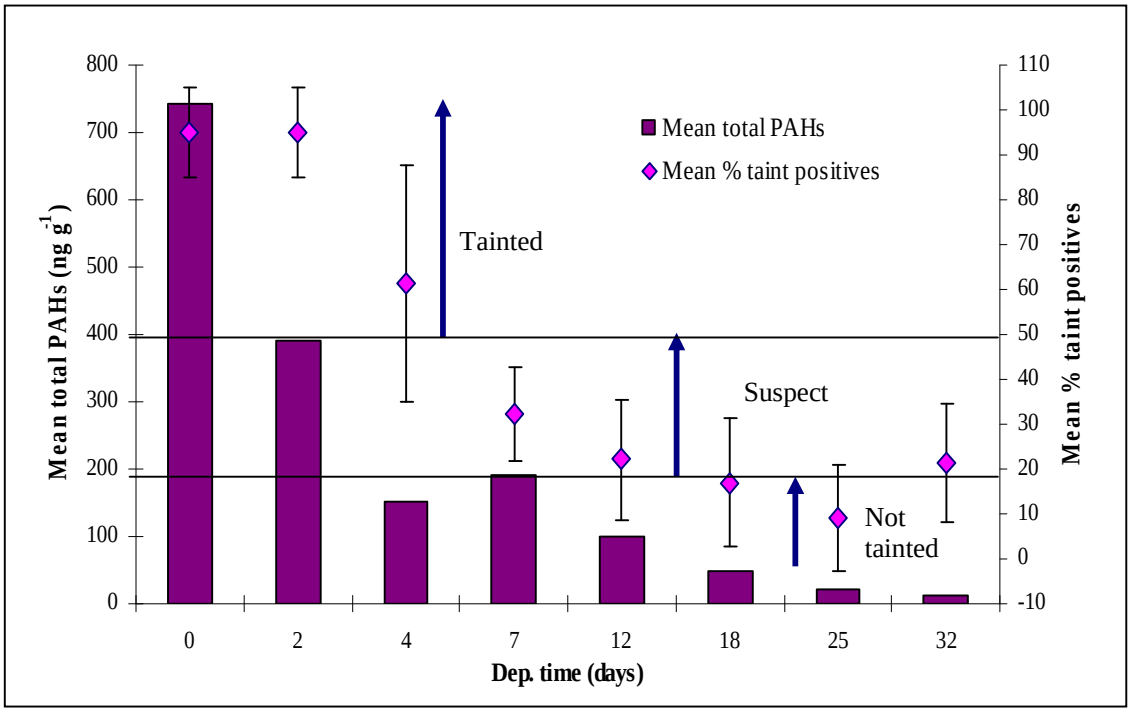


Fig.**8.2**: Depuration of taint in salmon exposed to crude oil contamination. Error bars = 1 standard deviation from the mean.

Table **8.4**: PAHs tissue burden and persistence of taint in organisms affected by some major spill incidents (Adopted from Yender *et al*., 2002)

Spill Name	**Tissue PAH Concentration (ng/g or ppb wet weight) and Persistence**	**Taint Persistence**
Finfish		
T/V Sea Empress	Wild salmon: 12-186 Declined "rapidly"	Wild salmon: No taint
T/V *Braer*	Cod: 1.3-74 Haddock: 8-262 Plaice: 15-184 Whiting: 9-2,650 Lemon sole: 6-1,240 Dab: 25-2,160 All but dab reached background in 1 month; dab in 2 months Caged salmon: up to 14,000; rapid loss to 1,000 in 25 days, reached background in 5 months	Cod: No taint Haddock: 1 month Plaice: Suspect taint 2 months Whiting: No data Dab: 1 month, Lemon sole: No taint Caged salmon: 7 months
T/B North Cape	Finfish: 5-1,100; 0 months because no increase over background was observed	All finfish: No taint in 416 samples
Crustaceans		
M/V Kure	Rock crab: 5-350; 0.5 months	Crab: No taint
M/V New Carissa	Dungeness crab: < 15	No sensory testing conducted
T/V Braer	Lobster: 112-1,060; 1 month Velvet crab: 94-308; 2 months Edible crab white meat: 19-281; brown meat: 104-1,390; 12 months for crabs	Lobster: 1 month Edible crab: No taint
T/B North Cape	Lobster: 0-33,150; 2.5-5 months	Lobster: 2.5-5 months
Bivalves		
M/V Kure	Oyster: 264-4,467; 0.5 months	Oyster: No taint
M/V New Carissa	Oyster: 70-1,200; 3 weeks	Oyster: No taint
T/V Sea Empress	Whelk: 50-3,800; 4 months Mussel: up to 19,500; 2.5-5 months Cockle: similar to mussels	Whelk: No taint Mussel: No data
T/V Braer	Whelk: 45-1,130; 12 months Scallop: 223-3,580; 17 months	Whelk: No data Scallop: Suspect taint 2 months
T/B North Cape	Steamer clam: 8,500-18,400; 3 months Oyster: 1,400-13,500; 3 months Mussel: 4,200-24,300; 3 months	Steamer clam: No taint Oyster: No taint Mussel: No taint
Refinery Spill, El Salvador	Oysters: 30,000; <1 month	Oysters: No data
T/V Exxon Valdez	Bivalves from four small areas were above 100; 1 year All other areas < 100	Bivalves: No data

8.4 Review of current oil spill response procedure

In the United Kingdom, the Maritime and Coastal Agency (MCA) is the lead agency in oil spill response. MCA is responsible for activating the national contingency plan in cases requiring a national response and resources. Other organisations are also involved in the response to oil or chemical spill but at the regional levels. The chain of different groups and agencies involved in oil spill response therefore depends on the category of the spill. In the UK, spills are categorised by the internationally adopted Tier system as:

Tier 1: A small operational spill employing local resources during any clean-up
Tier 2: A medium sized spill, requiring regional assistance and resources
Tier 3: Large oil spill, requiring a national response and resources; the National Contingency Plan (NCP) is activated in this case (EA, 2004).

The roles of the many agencies and organizations at all levels involved in oil spill mitigation are specified in the oil spill national contingency plan. For an oil spill requiring regional attention, various regional environmental groups are contacted. For example, The Department for Environment Food and Rural Affairs regulates the use of dispersants in England and Wales, and this function is carried out by the Scottish Executive Environment and Rural Affairs Department in Scotland. The responsibilities and functions of the Environment Agency (in England and Wales) are similar to those of the Scottish Environment Protection Agency. However for the purpose of this research, attention will only be given to the role and practices of the environmental monitoring agencies responsible for advising on fisheries and aquaculture.

Aquaculture (fish and shellfish farming) is an important sector in many countries of the world. According to a report published by the Scottish Executive's Environment and Rural Affairs Department (SEERAD) in 2006, the United Kingdom is the third highest producer of farmed salmon after Norway and Chile and Scotland is responsible for 80 per cent of UK’s aquaculture production.

In Scotland, the responsibility of advising Ministers on policy relating to fisheries (in both inshore waters and in EU waters) falls on SEERAD. In relation to fisheries, SEERAD is assisted by the Fisheries Research Services (FRS) and the Scottish Fisheries Protection Agency (SFPA). The Fisheries Research Services monitors and advises on fish stocks and on issues which may affect the marine environment, while the Scottish Fisheries Protection Agency has the remit of enforcing the UK and EU sea fisheries regulations in ports and at sea within British Fishery Limits around Scotland. They also monitor compliance by the industry.

According to the National Contingency Plan published by the Maritime and Coastal Agency, "Under Part I of the Food and Environment Protection Act 1985 (FEPA), Departments or Agencies with food safety responsibilities can prohibit the taking of fish and edible plants from a designated sea area. They may do this when the consumption of contaminated food from that area could present a health risk to consumers. They may therefore restrict fishing, on a precautionary basis, if resources are, or are likely to become, contaminated" (MCA, 2007)

This approach is aimed at protecting consumers' interests and the ban is normally initiated by the Food Standard Agency (FSA) according to the Food and Environment Protection Act, 1985, based on substantial information on the nature and extent of the spill. Typical examples where temporary bans have been imposed on harvesting of fish and shellfish were the *Braer* oil spill incident in 1993 and the Sea Empress grounding in 1996 (Whittle *et al*., 1997; Law and Kelly, 2004).

The guide to practices, procedures and methodologies following oil spill contamination incidents was published by the Standing Committee of Analysts (SCA) for the Environmental Agency in 2004. This ensures that adequate and representative samples are collected from the affected area and analyzed with standard methods. The documented guide published as 'Blue book' contains guidelines for sampling and methodologies for analysis of different environmental matrices relevant for making informed decision regarding imposition and lifting of harvesting bans and other spill mitigating actions.

8.5 Recommendation for improving oil spill response

Credible decision-making regarding oil spill response strategies should be based on sound scientific principles. In marine oil spill response regarding aquaculture, knowledge of fisheries management is essential. The understanding of the composition and fate of different oil components is also important in managing the aftermath of oil spill. Also important is background information on different important species and matrices that may be used as relevant indicators of spill loadings and effects, e.g. indigenous biota, sediment, water, etc., while not neglecting the impact of seasonal variations on these matrices. The sections below discuss how these factors can be properly managed to ensure efficient response to spill in the marine environment.

8.5.1 Background information

Baseline data is vital in advising response to and monitoring recovery from spill incidents, as mentioned in the bluebook (EA, 2004). Lack of baseline data was among the setbacks suffered in coordinating the scope of sampling during the *Braer* incident in 1993 (Whittle *et al*., 1997). As this was not available for the wild species within the FEPA exclusion zone, it was difficult to know when the PAH concentration returned to background levels. However, the Fisheries Research Services (FRS) currently undertakes monitoring programs that covers the relevant matrices in most Scottish Coastal waters and relevant aquaculture farming areas in Scotland (Webster *et al*., 2003; Webster *et al*., 2004; Russell *et al*., 2005). The information from such monitoring excercises will be of crucial value when a spill or other damaging event occurs. Additionally, there is need for the continuous monitoring of several commercial species (e.g. mussels, oysters, crustaceans, and finfish) in areas affected by oil spill to determine the long-term impact of the contamination. Whilst some of these data may seem to be only primarily of local relevance, the results could be of wider significance in relation to understanding the effects of oil spills on fisheries, given the quality of the baseline data generated by FRS for some of these populations.

8.5.2 Developing comprehensive sampling plan

The blue book (EA, 2004; section **6**) explicitly explained the merits of a timely, comprehensive and representative sample in assessing the impact of spills. However, it did not discuss the statistical aspects of sampling plan. A key factor to be considered in selecting appropriate number of samples is the heterogeneity of contaminant tissue burden that occurs within same species of organisms. In this study, where Atlantic salmon were exposed to Forties crude oil, four fish sampled immediately after the oil exposure showed variable tissue concentrations (485.4, 717.0, 752.1 and 1018.0 ng g^{-1} wet weight), giving a mean of 743.1ng g^{-1} and a standard deviation of 218.1 ng g^{-1}. The difference between the lower and the upper range was very wide (532.6 ng g^{-1}). Such variations in tissue burden among species should be documented and made available to the appropriate authorities so that such variations can be taken into consideration in designing sampling plans to suit specific incidents.

Another limitation to developing an appropriate sampling plan is the knowledge of when exposure has ceased so that the kinetic data obtained in this study can be applied. For finfish that can move away from the contaminant zone and at the same time readily metabolize PAHs, exposure to such contaminants is short lived and depuration is fast. In their report on the effect of the *Braer* oil incident on caged salmon, Whittle *et al*. (1997) reported that the salmon reached the maximum PAHs concentration within 10 days of the incident and subsequently rapidly depurated the accumulated compounds. However, a report by Topping et al., on the impact of the same incident on wild finfish showed consistent reduction in the tissue burden of the fish. This suggests that for penned finfish and sessile molluscs (e.g. mussels), exposure to the oil components can last longer so far as the source of contaminant persists.
Therefore, the modelled kinetic equations (section 8.5.4) can only be used with confidence when it has been established through analysis that exposed has ceased.

8.5.3 The understanding of the composition of various oil fractions.

Vast oil database exist that gives the various physical and chemical properties of the different fractions of petroleum products. However, the information therein seems to be of little relevance to the sort of information required in managing spill response. Crude oils and derived products differ in composition according to their sources and refining processes. Knowledge of the composition of the spilled oil and the physical and chemical properties of its constituents will help in the prediction of the expected short-term and long-term effects of the oil on the marine environment. For example, according to Dahlmann 2003), very light fuel oil contains compounds that are highly volatile, toxic and readily weathered. The composition of this oil suggests that impacted species will be more susceptible to taint than pollution from heavy PAH compounds. On the other hand, heavy oils (Heavy crude oils, No. 6 fuel oils, Bunker C) show little or no dissolution over time, have high aromatic contents, weathers slowly and have the possibility of long-term contamination of underlying sediment (NOAA, 2005). Therefore, organisms affected by heavy oils will likely suffer risk of contamination from heavy PAH compounds which can persist for longer duration. Diesel is regarded as the most toxic petroleum fraction but grouped together with the No. 2 fuel oil and light crude oils (NOAA, 1994). These groups of oils are moderately volatile, contins moderate concentrations of the toxic (soluble) compounds and will leave residue for some days. Contamination of intertidal resource can be long-term especially in turbulent waters.

The influence of oil composition and prevailing atmospheric conditions on the impact and persistence of oil spill is probably reflected by the *Braer* and the Sea Empress incidents. The *Braer* spilled 84, 700 t of light Gulfaks crude oil and up to 1,500 t of bunker oil (Ritchie 1997), while the Sea Empress spilled 72, 000 t of Forties blend crude oil and 480 t of heavy fuel oil (Law and Kelly, 2004).

This issue should be well discussed in the blue book and case studies properly documented as the information are very vital in spill management.

8.5.4 Understanding the fate of oil components in different marine organisms and matrices.

Understanding the fate of specific oil components that are of toxicological significant (e.g. PAHs) in different environmental matrices is vital in predicting the effect and persistence of these contaminants. According to Payne *et al*. (2003), oil released into the marine environment is immediately subject to short-term weathering processes including spreading, evaporation, dispersion of whole oil droplets into the water column, and partial dissolution of selected lower molecular weight 2- to 3- ring polycyclic aromatic hydrocarbons with log K_{ow} values between 3.7 and 4.8. The partition co-efficient of the various compounds to some extent govern their distribution into the various phases of the environment; water, sediment, biota, and air.

The current studies and previous studies (Table **8.3**) have shown that these compounds are preferentially accumulated and depurated by living organisms. The real spill case studies (Table **8.4**) show that different species of organisms exposed to similar concentration of specific contaminants show variable tissue burdens. This is supported by the result from the current studies. For example, *Mytilus edulis* exposed to crude oil accumulated the whole range of PAH compounds while *Salmo salar* exposed to similar concentration of crude oil accumulated only the 2- and 3-rings PAH compounds and in lower concentrations than found in mussels. Similar trend in PAH accumulation was reported by Topping *et al*. (1997) for samples collected after the *Braer* incident. The mean concentrations of PAHs found in wild fish (cod, lemon sole, dab, plaice, etc.) which ranged between 384 and 794 ng g^{-1} generally contained less than 5% of the 4- to 6-rings compounds, while higher mean PAH concentrations were found in bivalve molluscs with % compositions of the 4- to 6-rings compounds reaching up to 60%. Therefore, finfish rarely accumulate the high molecular weight carcinogenic compounds during short term acute oil exposures such as occurred during vessel accidents.

From the past and present studies being discussed, it is evident that shellfish (particularly the bivalve molluscs) are the species of organisms that are mostly at risks of severe contamination by both the acutely toxic (2- and 3-rings) and the carcinogenic (4- to 6-rings) PAH compounds. Concentrations of PAH compounds found in mussels, oysters, lobster, scallops, whelks, etc., during vessel accidents have always posed food safety concerns (Topping *et al*., 1997; Law and Hellou, 1999)

The results of the current and previous studies presented inTable **8.3** will find application in the management of different spill scenarios. Therefore, it is recommended that the kinetic data obtained in studies III and Thorsen *et al*. (2004) (for mussels), and IV (for salmon) is used for the prediction of the duration of persistence of PAHs in the mentioned species if contamination is from:

- A small and non-persistent operational spill in low turbulent weather condition.
- Medium oil spill affecting only wild species in low turbulent waters.

For large spills, like the *Braer* incident where the oil was still present in the zone for over one month, resulting in simultaneous accumulation and elimination, and severe weather conditions prevailed during the period, the kinetic data from studies I and II can be applied with caution for shellfish species, being weary of the possibility of re-suspension from the underlying sediment.

Table **8.5** presents a guideline which can be used to predict the duration of persistence of individual PAH compound in mussels (bivalve molluscs) affected by oil spill. The figures were generated by fitting the k_2 values into first order decay equation. The k_2 values used were a combination of the values obtained from the different studies as explained in Table **8.5**. The critical compounds presented in italics, these compounds seem to persist longer than others in the environment and have been implicated as probable carcinogens.

Table **8.5**: Modelled kinetic equations for the estimation of the duration of persistence of individual PAH compounds in mussels in oil spill management.

Compounds	Spill type I	Spill type II
Naphthalene	$t = (\ln C_0 - \ln C_t)/0.2217$ [a]	
2-Methyl Naphthalene	$t = (\ln C_0 - \ln C_t)/0.2059$ [a]	
1-Methyl Naphthalene	$t = (\ln C_0 - \ln C_t)/0.2486$ [a]	
C2-Naphthalenes	$t = (\ln C_0 - \ln C_t)/0.2124$ [a]	$t = (\ln C_0 - \ln C_t)/0.0560$ [c]
C3-Naphthalenes	$t = (\ln C_0 - \ln C_t)/0.1793$ [a]	$t = (\ln C_0 - \ln C_t)/0.0620$ [d]
C4-Naphthalenes	$t = (\ln C_0 - \ln C_t)/0.1543$ [a]	$t = (\ln C_0 - \ln C_t)/0.0652$ [d]
Acenapthylene	$t = (\ln C_0 - \ln C_t)/0.1847$ [a]	
Acenaphthene	$t = (\ln C_0 - \ln C_t)/0.2372$ [a]	
Fluorene	$t = (\ln C_0 - \ln C_t)/0.1902$ [a]	$t = (\ln C_0 - \ln C_t)/0.0592$ [d]
Phenanthrene	$t = (\ln C_0 - \ln C_t)/0.1707$ [a]	$t = (\ln C_0 - \ln C_t)/0.0510$ [c]
Anthracene	$t = (\ln C_0 - \ln C_t)/0.1792$ [a]	$t = (\ln C_0 - \ln C_t)/0.0500$ [c]
C1-Phenan/Anthracenes	$t = (\ln C_0 - \ln C_t)/0.1500$ [b]	$t = (\ln C_0 - \ln C_t)/0.0620$ [d]
C2-Phenan/Anthracenes	$t = (\ln C_0 - \ln C_t)/0.0803$ [b]	$t = (\ln C_0 - \ln C_t)/0.0605$ [d]
C3-Phenan/Anthracenes	$t = (\ln C_0 - \ln C_t)/0.0622$ [b]	$t = (\ln C_0 - \ln C_t)/0.0505$ [d]
Dibenzothiophene	$t = (\ln C_0 - \ln C_t)/0.1611$ [a]	$t = (\ln C_0 - \ln C_t)/0.0582$ [d]
C1-Dibenzothiophenes	$t = (\ln C_0 - \ln C_t)/0.1473$ [a]	$t = (\ln C_0 - \ln C_t)/0.0647$ [d]
C2-Dibenzothiophenes	$t = (\ln C_0 - \ln C_t)/0.0828$ [b]	$t = (\ln C_0 - \ln C_t)/0.0573$ [d]
C3-Dibenzothiophenes	$t = (\ln C_0 - \ln C_t)/0.0542$ [b]	$t = (\ln C_0 - \ln C_t)/0.0473$ [d]
Fluoranthene (202)	$t = (\ln C_0 - \ln C_t)/0.0557$ [b]	$t = (\ln C_0 - \ln C_t)/0.0536$ [d]
Pyrene (202)	$t = (\ln C_0 - \ln C_t)/0.0853$ [b]	$t = (\ln C_0 - \ln C_t)/0.0437$ [c]
C1-Fluoranthene/Pyrenes	$t = (\ln C_0 - \ln C_t)/0.0730$ [b]	$t = (\ln C_0 - \ln C_t)/0.0487$ [c]
C2-Fluoranthene/Pyrenes	$t = (\ln C_0 - \ln C_t)/0.0568$ [b]	$t = (\ln C_0 - \ln C_t)/0.0428$ [c]
C3-Fluoranthene/Pyrenes	$t = (\ln C_0 - \ln C_t)/0.0667$ [b]	
Benzo[*c*]phenanthrene (228)		$t = (\ln C_0 - \ln C_t)/0.0428$ [c]
Benz[*a*]anthracene	$t = (\ln C_0 - \ln C_t)/0.0924$ [a]	$t = (\ln C_0 - \ln C_t)/0.0337$ [c]
Chrysene/Triphenylene	$t = (\ln C_0 - \ln C_t)/0.0638$ [b]	$t = (\ln C_0 - \ln C_t)/0.0477$ [c]
Benz[*b*]anthracene (228)	$t = (\ln C_0 - \ln C_t)/0.0564$ [b]	
C1-Chrysenes	$t = (\ln C_0 - \ln C_t)/0.0595$ [b]	$t = (\ln C_0 - \ln C_t)/0.0397$ [c]
C2-Chrysenes	$t = (\ln C_0 - \ln C_t)/0.0697$ [a]	$t = (\ln C_0 - \ln C_t)/0.0315$ [c]
Benzo[*b]*fluoranthene	$t = (\ln C_0 - \ln C_t)/0.0827$ [a]	
Benzo[*k*]fluoranthene	$t = (\ln C_0 - \ln C_t)/0.0589$ [a]	
Benzofluoranthenes (252)	$t = (\ln C_0 - \ln C_t)/0.0540$ [b]	
Benzo[e]pyrene (252)	$t = (\ln C_0 - \ln C_t)/0.0315$ [b]	$t = (\ln C_0 - \ln C_t)/0.0224$ [c]
Benzo[a]pyrene (252)	$t = (\ln C_0 - \ln C_t)/0.0755$ [a]	$t = (\ln C_0 - \ln C_t)/0.0353$ [c]
Perylene (252)	$t = (\ln C_0 - \ln C_t)/0.0350$ [b]	$t = (\ln C_0 - \ln C_t)/0.0299$ [c]
C1-252	$t = (\ln C_0 - \ln C_t)/0.1223$ [b]	

C2-252	$t = (\ln C_0 - \ln C_t)/0.1137$ [b]	
Indeno[1,2,3-cd]pyrene	$t = (\ln C_0 - \ln C_t)/0.0442$ [b]	$t = (\ln C_0 - \ln C_t)/0.0391$ [c]
Benzo[ghi]perylene	$t = (\ln C_0 - \ln C_t)/0.0385$ [b]	$t = (\ln C_0 - \ln C_t)/0.0234$ [c]
C1-276	$t = (\ln C_0 - \ln C_t)/0.1110$ [b]	
Dibenz[a,h]anthracene (278)	$t = (\ln C_0 - \ln C_t)/0.0687$ [a]	

Where C_t = desired PAHs concentration, C_0 = initial concentration of the PAH compound in the organism, and *t* = time required to reach the desired PAH concentration.

[a]k_2 value obtained from Thorsen *et al*. (2004)

[b]k_2 value obtained from study III (Chapter **6**)

[c]k_2 value obtained from study I (Chapter **3**)

[d]k_2 value obtained from study II (Chapter **3**)

Spill type I: Non- persistent operational spills involving small amounts of oil and medium spills in low turbulent waters, where contamination of the underlying sediment is minimal.

Spill type II: Medium spills in turbulent waters resulting in severe contamination of the underlying sediment, large spills with oil persisting for longer duration like the *Braer* incident (Davies *et al*, 1997).

8.5.5 Understanding the relationship between taint and PAH tissue burden.

The knowledge of the relationship between taint and PAH tissue burden is vital when taint is to be used as a screening tool to prioritize samples for more comprehensive chemical analysis. The present study (Chapter **4**), Heras *et al*. (1992), Davies *et al*. (2002), Topping *et al*. (1997) and Whittle *et al*. (1997) have shown that petrogenic taint has a direct relationship with petrogenic PAH concentration in the immediate aftermath of oil exposure. However, taint threshold was also found to vary between species and different oil fractions (Davies *et al*., 2002). The same authors also reported that while the lowest taint threshold was found in mussels (0.032 mg/l) exposed to Forties crude oil, crab exposed to similar concentration of oil was resistant to taint (> 7.7 mg/l). This suggests that petrogenic taint data for crab cannot be accurately applied as an indicator of the PAH tissue burden.

The naphthalenes, and in some instances phenanthrenes/anthracenes, and dibenzothiophens have been implicated in petrogenic tainting of fish and shellfish (Heras *et al*., 1992; Davies *et al*., 2002; Whittle *et al*., 1997). These groups of compounds dominate the profile of PAHs found in organisms exposed to oil contamination. While shellfish are capable of accumulation the whole range of PAH compounds within a short period of time, finfish profiles are normally dominated by the 2- and 3-rings compounds and rarely contains the 4- to 6-rings compounds (Topping *et al*., 1997; Whittle *et al*., 1997; Law and Kelly, 2004, and this study Chapters **3, 6** and **7)**). Therefore, it is right to state that in the immediate aftermath of oil spill incident; sensory analysis is the easiest, cost effective and a reliable approach of safeguarding consumer confidence with regards to finfish. Chemical analysis of the affected organisms can be carried out later to access the PAH tissue burden when taint declines.

Moreover, taint intensity can be used as an estimate of the PAH tissue burden if the relationship between taint intensity and PAH tissue burden is known for an organism.
Equation **8.1** can be used to estimate the PAH tissue burden of salmon recovering from oil spill incident from the sensory data. A sample pattern is shown in Table **8.6**. The figures used were generated from the exposure experiment (study IV) reported in Chapter **7**. A major limitation of this approach in oil spill management will be the absence of baseline data and the variability that exist among species of the same organisms. Since taint is known to result from petrogenic PAH contamination, organisms with (unknown) high background PAHs will generate grossly inaccurate results. This is because, PAHs of pyrolytic origin normally comprise the 4- to -6-ring compounds which do not contribute to taint. For example, during the T/B North Cape oil spill, all 416 finfish samples analyzed tasted negative to taint although the PAH tissue burden ranged between 5 and 1,100 ng g^{-1} wet weight. This concentration according to the Mauseth *et al*. (1997) was however within the background concentration determined before the spill and therefore not a direct impact from the spill. Consequently, in the absence of background PAH data before an oil spill incident, relying on sensory data alone can lead to misjudgment of the actual PAH tissue burden of organisms.

Table **8.6**: Sample table showing the relationship between average taint intensity and mean PAH concentration calculated using equation **8.1**

Mean Taint intensity	*Mean Total PAHs (ng g^{-1})
5.0	≥ 1072.3
4.9	1051.2
4.8	1030.1
3.5	755.7
3.2	692.3
3.0	650.1
2.8	607.9
2.6	565.7
2.2	481.2
2.0	439.0
1.8	396.8
1.6	354.6
1.4	312.3
1.2	270.1
1.0	227.9
0.8	185.7
0.6	143.5
0.5	122.4
0.4	101.2
0.3	80.1
0.2	59.0
0.1	37.9

* = Sum of naphthalenes (parent and C1-C4), phenanthrene/anthracene (parent and C1-C3) and dibenzothiophenes (parent and C1-C3)

Mean total PAHs concentration (MTPC) was calculated from the relationship:

MTPC = 211.1mean taint intensity (MTI) + 16.2 …….. (8.1)

(See Chapter **7** section **7.5.3**)

Equation **8.1** is just a guide, as previous reports have shown that the persistence of taint increases with increase in oil concentration and duration of exposure (Davies *et al*., 2002).

8.6 Conclusion

Every action taken in responding to oil spill is geared towards protecting the natural environment from the long-term effects of the spill and safeguarding consumers' interests. Spilled oil affects living organisms within its pathway. The current and previous studies show that the degree to which an organism is contaminated is not a measure of the amount of oil in its pathway but the extent to which it can absorb and retain oil components. Therefore, the duration of persistence of PAHs in organisms is affected by the following factors:

i. The duration of exposure of organism to the contaminant (point or continuous exposure). Elimination rate decreases with increase in exposure duration.
ii. The rate at which an organism can eliminate the compounds.
iii. The exposure pathway; this controls the suite of compounds accumulated by organisms. Individual PAHs are eliminated at different rates.
iv. The oil fraction spilled; this affects the partitioning behaviour.

Although each oil spill is unique and affected by the prevailing weather and physical factors, the current research has provided an insight into the fate of toxic hydrocarbons in selected marine organisms. The kinetic data generated from this study will answer some of the important questions normally raised by farmers and consumers as to when a temporary ban can be lifted.The modelled kinetic equations (Table 8.5) will find application in the estmation of the duration of persistence of PAH compounds in mussels if spill is:

- A non- persistent operational spills involving small amounts of oil or medium spills in low turbulent waters, where contamination of the underlying sediment is minimal (Spill type I).
- Medium spill in turbulent waters resulting in severe contamination of the underlying sediment, large spills with oil persisting for longer duration (spill type II).

A table is also presented (Table **8.6**) which relates the sensory data from Atlantic salmon to the PAH tissue burden. In the immediate aftermath of spills, the information therein will be a useful guide in the estimation of the PAH tissue burden of an impacted salmon community from the sensory data. However, this cannot be accurately applied in long monitoring programs.

Chapter Nine
Overall Conclusions and Future Work

9.1 Background

The fate and effect of polycyclic aromatic hydrocarbons (PAHs) in the marine environment have been widely studied in the last 3 decades (Blumer *et al*., 1970; Baumard *et al*., 1999b; Dahle *et al*., 2003). This is due to their persistence and the perceived environmental health risks (IARC 1983; Heras *et al*., 1992; Aas *et al*., 2000; Aas *et al*., 2001). PAHs are introduced into the marine environment through diverse sources which include natural (forest fires, volcanic eruptions, etc.) as well as anthropogenic sources (automobile exhaust, combustion of fossil fuels and exploration and transportation of petroleum products). Petroleum exploration and consumption release varying amounts and composition of hydrocarbons into the marine environment in the form of operational discharges, leaks or vessel accidents. Oil spill arising from vessel accidents have always received international attention due to the volumes of oil discharged at a time (Huijer, 2005) and the increased risks to commercial fisheries and aquaculture (Topping *et al*., 1997, Whittle *et al*., 1997, Law and Hellou, 1999). Oil spills present the potential for enormous harm to deep Ocean and coastal fishing and fisheries. The immediate effects of toxic oil components may be mass mortality and contamination of fish and other food species, but long-term ecological effects may be worse (Embach, 2008).

The potential costs of oil spill on commercial fisheries can be economically damaging. This may include loss of consumer confidence in fish and shellfish products as a result of detectable petrogenic taint, economic losses due to imposition of fishing bans, compensation costs to tanker owners and their insurers, and the concern for public health due to carcinogenic PAH compounds in food.

The elimination rates of selected priority PAH compounds investigated in naturally incurred and experimentally exposed *Mytilus edulis* and also in experimentally exposed *Salmo salar* in separate laboratory and field studies showed that these organisms depurate PAH compounds. The data generated will be useful in future prediction of the duration of

persistence of these compounds in commercial species impacted by oil spills. The relationship between PAH tissue burden, taint and changes in geochemical biomarkers were also studied.

9.2 Conclusion on PAH depuration studies

The contaminated mussels when transferred to clean environments depurated the PAHs within a relatively short period of time. The pattern in the loss of PAH compounds with time observed for mussels depurated in separate laboratory and field studies (Chapter **3**) were similar. The elimination kinetics (depuration constant and biological half-lives) calculated for individual compounds varied among the homologues series but was comparable between the two studies. This is an indication that laboratory data can be directly applied to the field provided the source of contamination is not persistent.

Similarly, farmed mussels experimentally exposed to equal concentrations of Arabian light oil, Gullfaks crude oil or Brent crude oil (Chapter **6**) showed similar PAH percentage compositions (2.3-2.9% of the 2- aand 3- ring compounds and 97.1-97.7% of the 4- to 6-rings compounds) and depuration patterns. Elimination of PAHs from the mussels in all the studies (Chapters **3** & **6**) followed first order kinetics. Generally, elimination was faster for the lower molecular weight compounds than the higher molecular weight compounds. Biological half-lives increased with increase in molecular weight and degree of alkylation and ranged between 3.8 d (C4-naphthalene) to 31 d (Benzo[*e*]pyrene) for the Aberdeen harbour mussels (chronic exposure) and 0.5 d to 22 d for the mussels exposed to acute crude oil contamination. The k_2 values determined from the harbour mussels (Chapter **3**) were consistently higher than the values obtained from the mussels exposed to acute crude oil contamination (Chapter **6**), suggesting that increase in exposure duration increases the apparent half-lives. Similar observation was reported by Sericano *et al*. (1996), from the depuration of chronically (indigenous) and acutely (transplanted) exposed American oysters. At the end of 50 days of depuration in a clean environment, the authors reported that the tissue burden of the indigenous oysters was 40% higher than found in the transplanted oysters, therefore returning longer apparent half-lives for the similar

compounds. This again suggests that the duration of exposure is a critical factors affecting the rate of PAH elimination from organisms.

Atlantic salmon exposed to crude oil contamination only accumulated the 2- and 3-rings PAH compounds [the naphthalenes (parent and C1-C4), phenanthrenes/anthracenes (parent and C1-C3) and dibenzothiophenes (parent and C1-C3), acenaphthene and fluorene] within the exposure period (Chapter **7**). The naphthalenes accounted for over 93% of the total PAHs found in salmon (743.1± 218.1 ng g^{-1}). Four salmon sampled immediately after exposure showed similar PAH % composition suggesting similar mode of uptake. Similar observation was reported by Topping *et al*. (1997) in finfish affected by the *Braer* oil spill incident. The PAH distribution and % compositions found in the salmon were also similar to other literature reports from spill incidents (Whittle *et al*., 1997; Law and Kelly, 2004).

Similar to mussels, salmon eliminated the accumulated PAHs upon transfer to a clean water flow-through system but with longer biological half-lives for similar compounds than mussels exposed to acute crude oil contamination. This was unexpected, but may be as a result of the route of elimination in both species. Elimination followed first order kinetics, and biological half-lives ranged between 2.8 and 31.5 d. The k_2 value was also found to decrease with increased hydrophobicity and degree of alkylation.

The kinetic data obtained from this study and previous studies were modelled into kinetic equations (Table **8.5**). These equations will be a useful guide for predicting the duration of persistence of PAHs compounds in the management of specific oil spills incidents.

9.3 Conclusion on the links between PAH concentration and taint

Salmon samples collected immediately after the oil exposure showed strong petrogenic taint (80-100% taint positives). This is possibly due to the concentration of naphthalenes (> 93 %) accumulated by the salmon (Chapter **7**). These compounds have been implicated in petrogenic tainting of edible parts of fish and shellfish (Heras *et al*., 1992; Davies *et al*., 2002). Taint decreased progressively with loss of PAH upon transfer to the clean flow-through system. A regression analysis of the lipid normalized PAH concentration and taint

rating gave a relatively good correlation ($r^2 = 0.72$). Suggesting that, if contamination is sorely petrogenic, non tainted salmon species need not be analysed for PAH tissue concentration.

Sensory data is good indicator of PAH tissue burden of salmon in the immediate aftermath of oil spill and can be used in screening and prioritizing samples for further costly and time consuming chemical analysis. However, PAH tissue burden of salmon cannot be accurately estimated from taint data in a long monitoring program as studies have shown that taint is not lost as rapidly as it develops.

9.3.1 Conclusion on the use of *n*-alkanes, PAH distribution profile and concentration ratios and geochemical biomarkers in oil spill source identification

The profiles of PAH compounds can be diagnostic of the sources of hydrocarbon contamination, as can the patterns of geochemical biomarkers; steranes and triterpenes (Bence *et al*., 1996; Yunker *et al*., 2002; Webster *et al*., 2004). The *n*-alkane profile of the mussels collected from Aberdeen showed a profile similar to that characteristics of weathered crude oil and returned a CPI of 1(Chapter **4**). The PAH distribution was also similar to that described for petrogenic sources (Wang *et al*., 1999; Yunker *et al*., 2002), and the PAH concentration ratios; Fl/Py and MP/P indicate predominantly petrogenic input source (Chapter **4**). Although fractionation, through the differential uptake that occurs as hydrocarbons are transferred from water/sediment phases into organisms, metabolism and excretion processes can alter the original composition of the more labile *n*-alkanes and PAHs, the presence of geochemical biomarkers in the mussels' profile indicate that the PAH contamination originated from petrogenic sources (Chapter **4**). The geochemical biomarker (triterpane and sterane) profile of the harbour mussels suggests a contribution from both the North Sea and the Middle Eastern oils. Further analysis of crude oils from these areas and their mixtures (Chapter **5**) suggest that the harbour mussels were possibly contaminated by mixture of a North Sea and Middle Eastern crude oils.

Similarly farmed mussels experimentally exposed to Gullfaks, Arabian light or Brent crude oils reflected the geochemical characteristics of the exposure oils (Chapter **6**). This is an indication that mussels are good indicators of contaminant loadings and that the result obtained from the harbour mussels was not by chance.

Contrary to the result observed with the mussels, Atlantic salmon exposed to Forties crude oil showed biomarker fingerprint quite different from that of the exposure oil (Chapter **7**). This suggests that salmon are not good indicators of spill source and geochemical biomarker information from this source can give misleading information on spill source.

9.4 Conclusion on changes in geochemical biomarker profile in mussels with loss of PAHs

Geochemical biomarker compounds (e.g. steranes and triterpanes) are used in geochemistry and environmental forensics to correlate oil and spilled samples to their sources. The stability of specific ratios of these compounds over time in spill samples is well documented (Wang *et al*., 1995, Wang *et al*., 1996; Barakat *et al*., 2002, Wang *et al*., 2005). Mussels used in the current studies retained their original biomarker fingerprint when transferred to depurate in clean environment for only a short period of time (Chapter **4** and **6**).The relative abundances (intensity) of the biomarker compounds was found to decrease relative to diploptene; a non oil biomarker (Appendix 2). This suggests that these compounds like PAHs are also eliminated by mussels.
The changes in biomarker profile monitored using specific triterpane (%DR27Tm, %DR28ab, %DR30d, %DR29ab,%DR30G, %DR32abS) and sterane (%DRs29aaS and %DR29bb) diagnostic indices, showed a change from the initial profile after about 12 days of depuration, especially in the %DR32abS, %DR29aaS and %DR29bb indices. This is an indication that biomarker information from mussels may not be very reliable after a long period of time after spill. However, given the consideration that samples used in oil spill correlations are collected from the vicinity of the spill, it follows that mussels collected at spill sites can provide useful information on the source of the spilled oil due to continuous exposure.

9.5 Recommendations for future work.

PAHs have been identified as a component of petroleum products that is of major food safety concern (NRC, 1983; NRCC, 1985, Davies and Topping, 1997). The problem posed by these compounds in oil spill management is the different environmental, physical and biological factors that affect their distribution in various phases of the marine environment.

Several studies have shown that marine organisms depurate PAHs at different rates depending on their metabolic capacities and other environmental factors. Further studies are needed to investigate the rates of PAHs elimination from various species of commercial relevance. Such studies can be carried out simultaneously using different species of organisms and subjecting them to similar experimental conditions, the outcome of such studies will enable accurate comparison of the rates of depuration in the species used. Interpolations can be made using such data in cases where the data relating to some species are not readily available.

The rate of reaction in chemical and biological processes generally increases 2- to 4-fold for a 10°C increase in temperature (Kennedy *et al*., 1989; French 2000). The elimination curves from which the kinetic data was modelled was obtained at a given temperature. This follows that a deviation from the calculated kinetics may be observed if the organisms were monitored at a different temperature. Therefore, it will be important to investigate and measure the effect of changes in water temperature on the uptake and elimination kinetics of PAHs by the specific species monitored.

Sensory data can be used as a good indicator of PAH loading in finfish (Davis *et al*., 2002, study IV), however this does not hold in a long monitoring program as taint is not lost in direct proportion with PAHs concentration. The relationship between loss of petrogenic hydrocarbons and taint in finfish should be further investigated to determine if the compounds responsible for taint are transformed by organisms.
In addition, as taint threshold differs among organisms, similar studies need to be carried out with other species of organisms; mussels, oysters, crustaceans, etc., to generate a database which can aid prediction of when taint intensity in impacted organism has disappeared.

Several studies have reported the stability of geochemical biomarker compounds (sterane and triterpane) in spill samples (Peters and Moldowan, 1993; Wang *et al*., 1995; Wang *et al*., 1998; Barakat *et al*., 2002). The current study also demonstrated the relative stability over 12 days of specific biomarker diagnostic ratios in mussels exposed to crude oils (see Chapters **4** and **6**). However, as the relative abundance of all petroleum related biomarkers were found to decrease with depuration time relative to diploptene; a non- oil biomarker compound, further studies which will measure the absolute concentrations of individual biomarker compound in both mussels and sediment samples are required to accurately establish how absolute individual biomarker concentrations change with changes in PAH concentration.

In addition, samples for scientific investigation should be representative of the sample area. Therefore, the stability of the specific biomarker compounds in mussels will need to be monitored on site to verify if the change in the biomarker profile was due to the isolation of the organism from the contaminant zone.

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APPENDIX 1

FISHRIES RESEARCH SERVICE, MARINE LABORATORY STANDARD OPERATING PROCEDURES (SOPs) (SEE THE ATTAHED CD)

APPENDIX 2

TABLE FROM CHAPTER THREE

Appendix 2

PAH concentrations (ng g^{-1}) found in Aberdeen harbour depurated in the laboratory for 56 days

PAH Compounds	0 day	7 days	21 days	35 days	56 days
Naphthalene	2.2	2.0	0.2	TR	TR
2-Methyl Naphthalene	4.3	0.5	TR	TR	TR
1-Methyl Naphthalene	2.6	0.3	TR	TR	TR
C2 Naphthalenes	14.9	2.1	0.7	0.5	0.4
C3 Naphthalenes	46.2	8.3	0.5	0.4	0.2
C4 Naphthalenes	102.2	35.4	1.4	0.2	ND
Total Naphthalenes	**172.4**	**48.6**	**2.8**	**1.1**	**0.6**
Phenanthrene (178)	18.9	2.3	0.6	0.7	0.6
Anthracene (178)	1.9	0.8	0.4	0.3	TR
C1 Phenan/anthracene	111.1	38.3	5.3	4.4	3.1
C2 Phenan/anthracene	293.5	138.8	31.2	13.6	6.8
C3 Phenan/anthracene	294.4	177.4	65.5	25.7	10.0
Total 178	**719.8**	**357.6**	**103.0**	**44.7**	**20.5**
Dibenzothiophene	2.2	0.6	0.2	TR	ND
C1 Dibenzothiophenes	26.3	12.0	5.1	3.1	ND
C2 Dibenzothiophenes	109.3	68.6	16.2	6.8	0.8
C3 Dibenzothiophenes	125.1	86.2	35.8	14.5	5.2
Total DBTs	**262.9**	**167.4**	**57.3**	**24.4**	**6.0**
Fluoranthene (202)	25.0	7.6	1.3	0.8	0.5
Pyrene (202)	31.5	12.6	3.7	2.9	2.4
C1 Flouran/pyrene	73.2	39.5	12.5	6.8	4.9
C2 Flouran/pyrene	51.0	31.8	15.5	7.4	4.8
C3 Flouran/pyrene	25.1	16.2	10.0	5.5	3.7
Total 202	**205.8**	**107.7**	**43.0**	**23.4**	**16.3**
Benzo[c]phenanthrene (228)	3.1	2.2	0.9	0.5	0.3
Benz[a]anthracene (228)	7.4	2.9	1.5	1.2	0.9
Chrysene/Triphenylene (228)	24.8	10.3	4.2	2.5	1.5
Benz[b]anthracene (228)	1.6	1.0	0.6	0.4	ND
C1 228	18.2	11.9	6.5	3.3	2.0
C2 228	12.3	11.2	6.1	3.0	2.5
Total 228	**67.4**	**39.5**	**19.8**	**10.9**	**7.2**
Benzofluoranthenes (252)	19.9	11.9	6.4	2.8	2.1
Benzo[e]pyrene (252)	10.6	8.8	6.2	3.9	3.2
Benzo[a]pyrene (252)	4.0	1.2	0.7	0.4	0.4
Perylene (252)	2.8	2.4	1.3	0.7	0.6

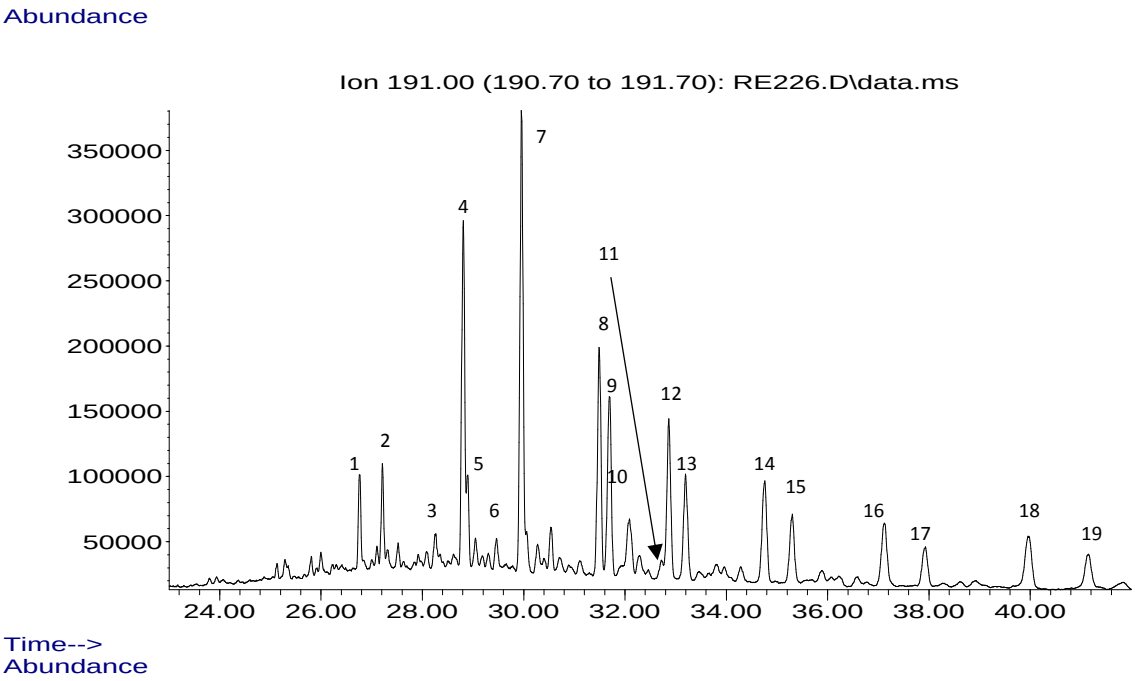
C1 252	5.6	3.8	2.2	1.3	1.1
C2 252	1.7	1.5	0.7	0.4	0.4
Total 252	**44.6**	**29.6**	**17.5**	**9.5**	**7.8**
Indenopyrene (276)	3.1	2.1	0.9	0.4	0.4
Benzoperylene (276)	3.3	2.4	1.4	1.0	0.9
C1 276	1.3	0.8	0.3	0.2	0.2
C2 276	0.4	0.9	0.3	TR	ND
Total 276	**8.1**	**6.2**	**2.9**	**1.6**	**1.5**
Acenaphthylene (152)	0.3	TR	ND	ND	ND
Acenaphthene (154)	6.7	TR	ND	ND	ND
Fluorene (166)	4.5	0.3	0.2	TR	TR
Dibenz[a,h]anthracene (278)	0.3	0.3	0.2	TR	TR
Cyclopenta[c,d]pyrene (226)	NM	ND	ND	ND	ND
Dibenz[a,l]pyrene (302)	NM	0.6	0.2	ND	ND
Dibenz[a,e]pyrene (302)	NM	TR	0.2	TR	ND
Naphtho[2,1-a]pyrene (302)	NM	0.2	TR	ND	ND
Dibenz[a,i]pyrene (302)	NM	ND	ND	ND	ND
Dibenz[a,h]pyrene (302)	NM	ND	ND	ND	ND
Total PAHs	**1492.8**	**758**	**247.1**	**115.6**	**59.9**

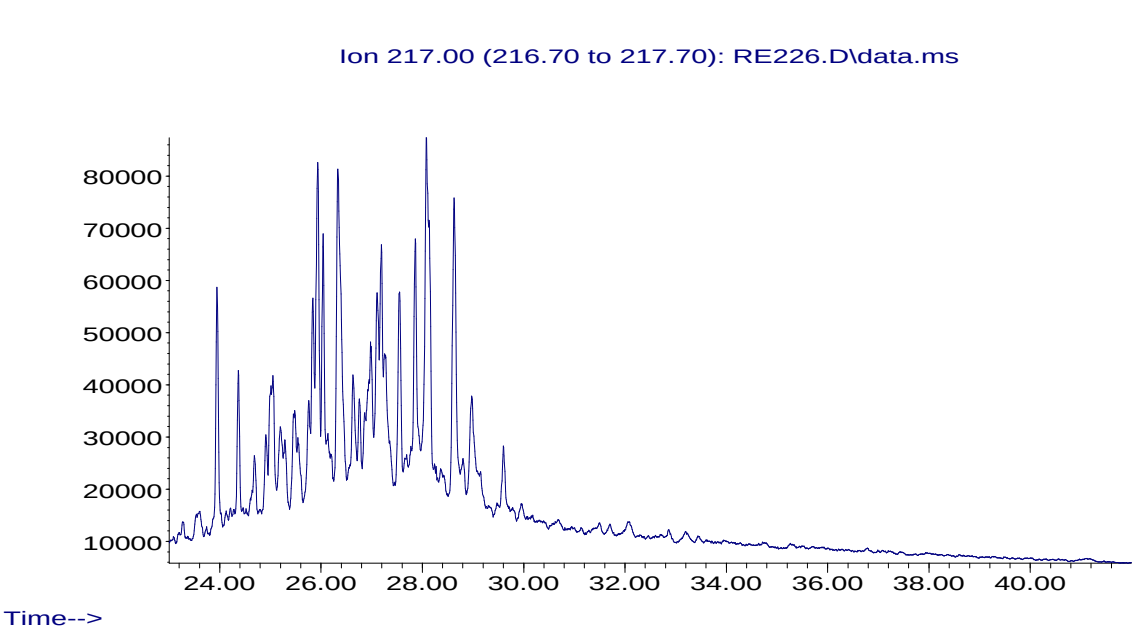
NM = not measured, ND = not detected, TR = trace

APPENDIX 3

CHROMATOGRAMS FROM CHAPTER FOUR

Appendix 3





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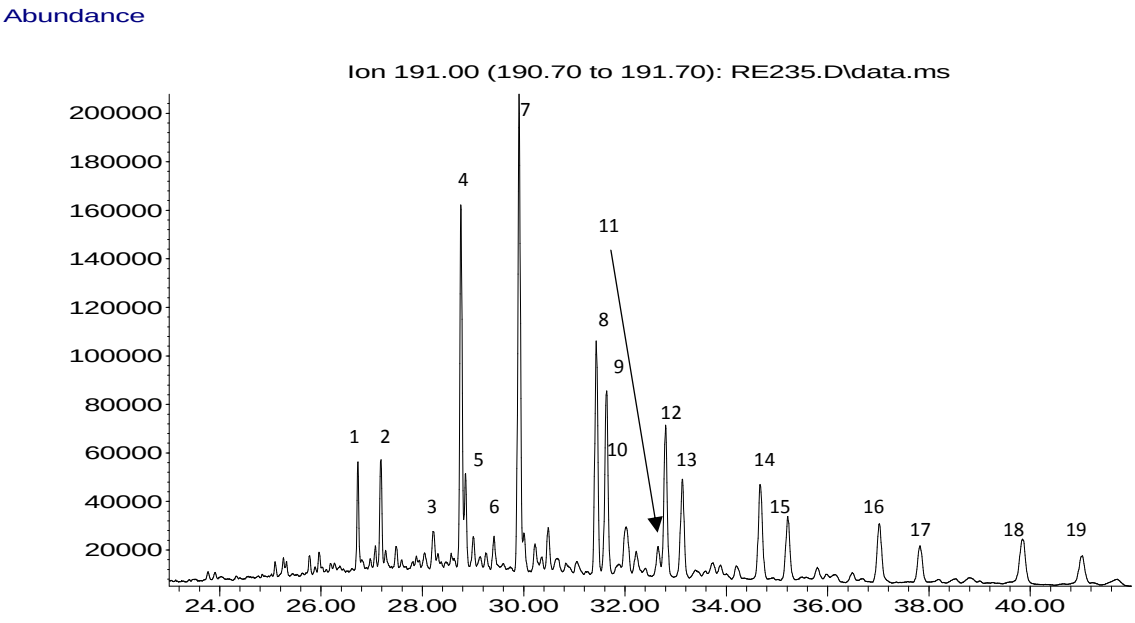

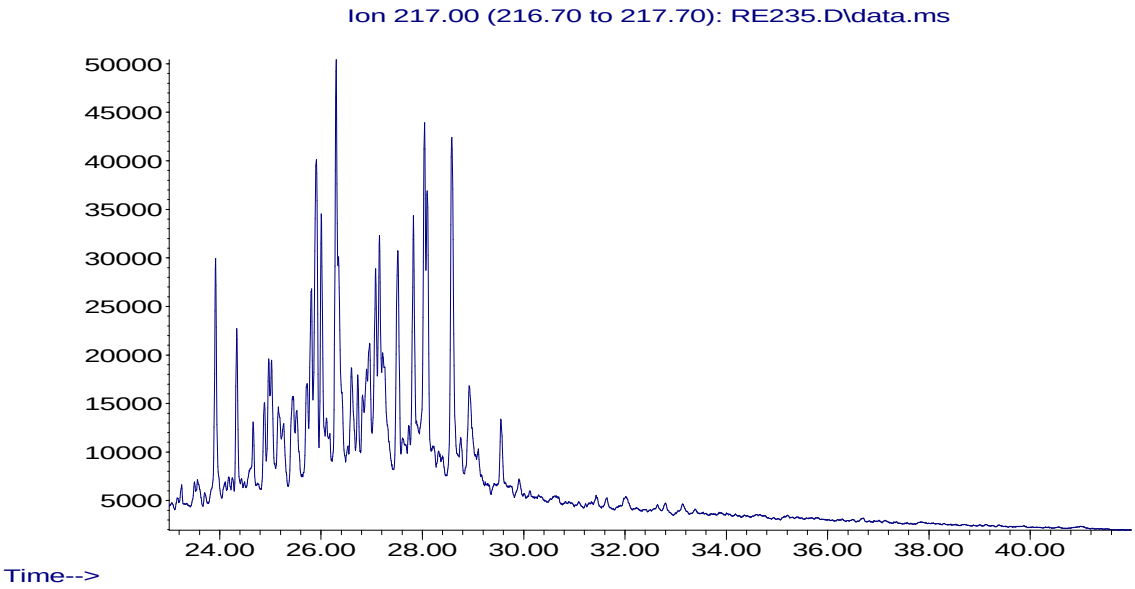

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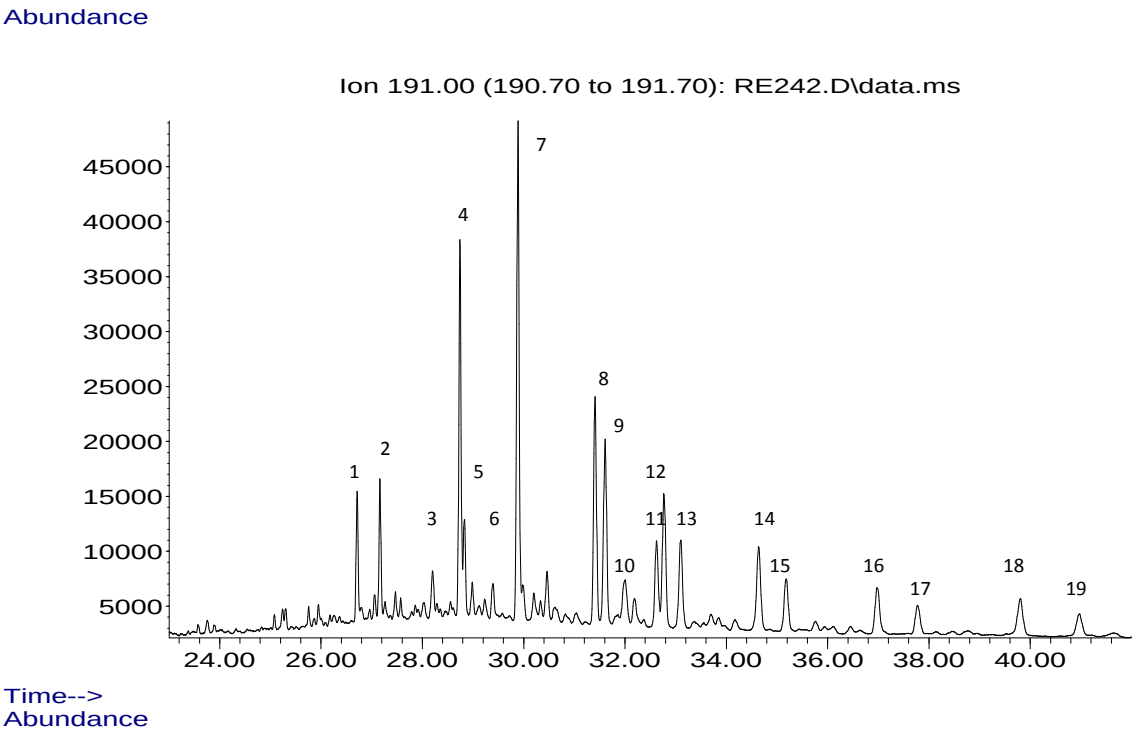

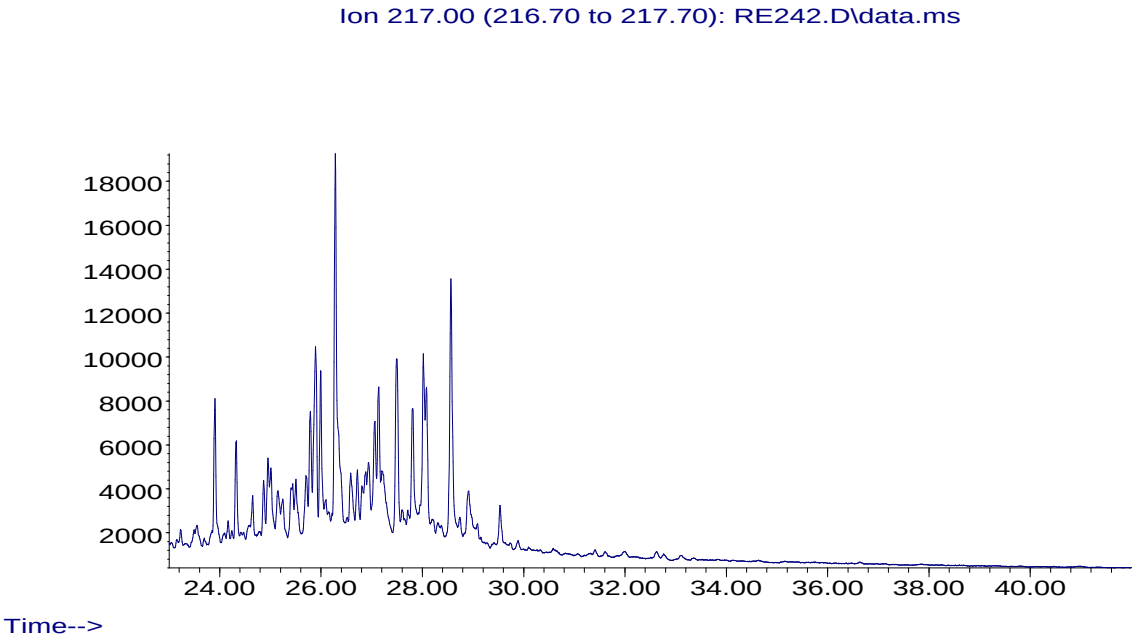

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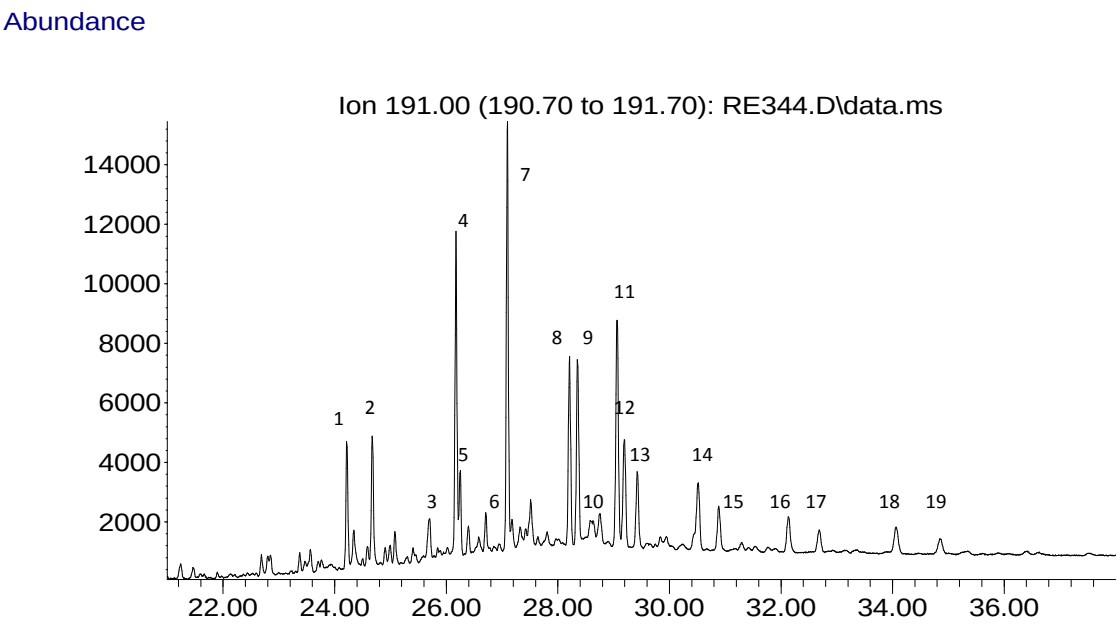

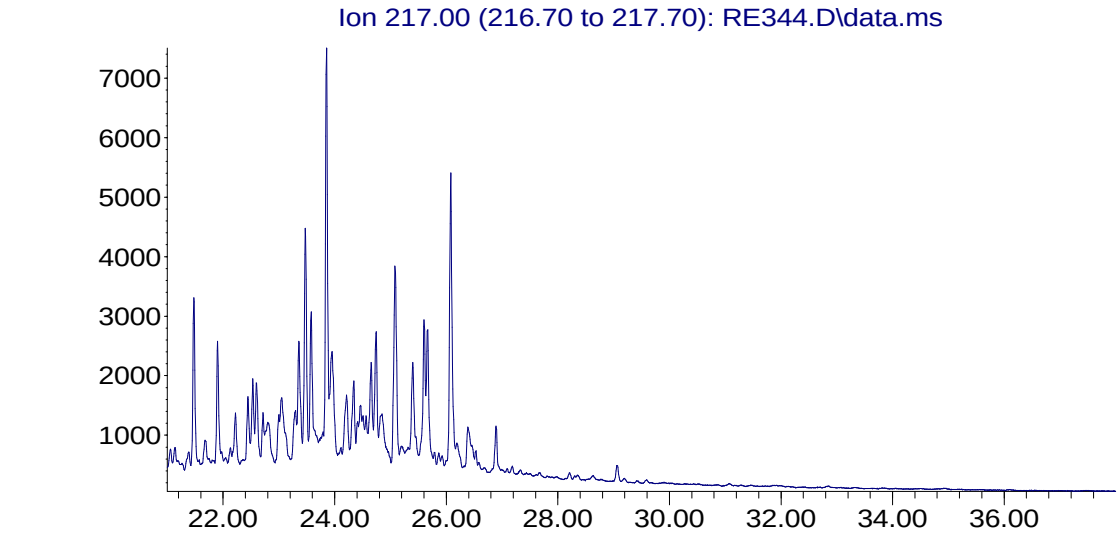

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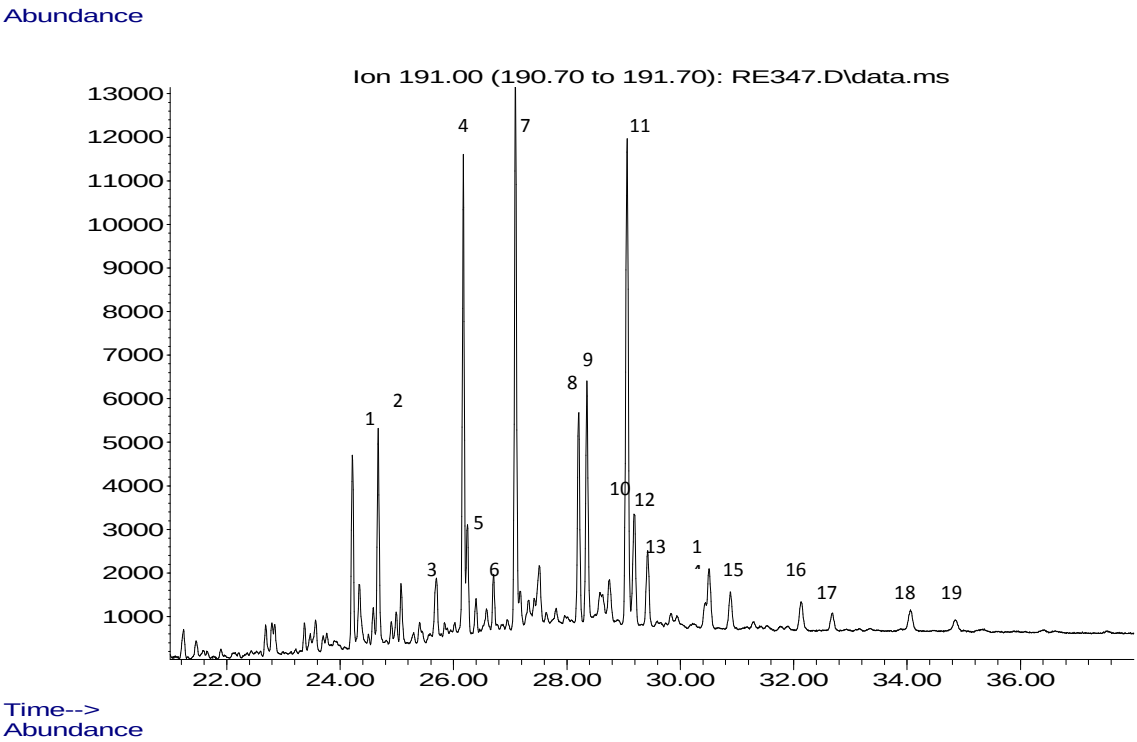

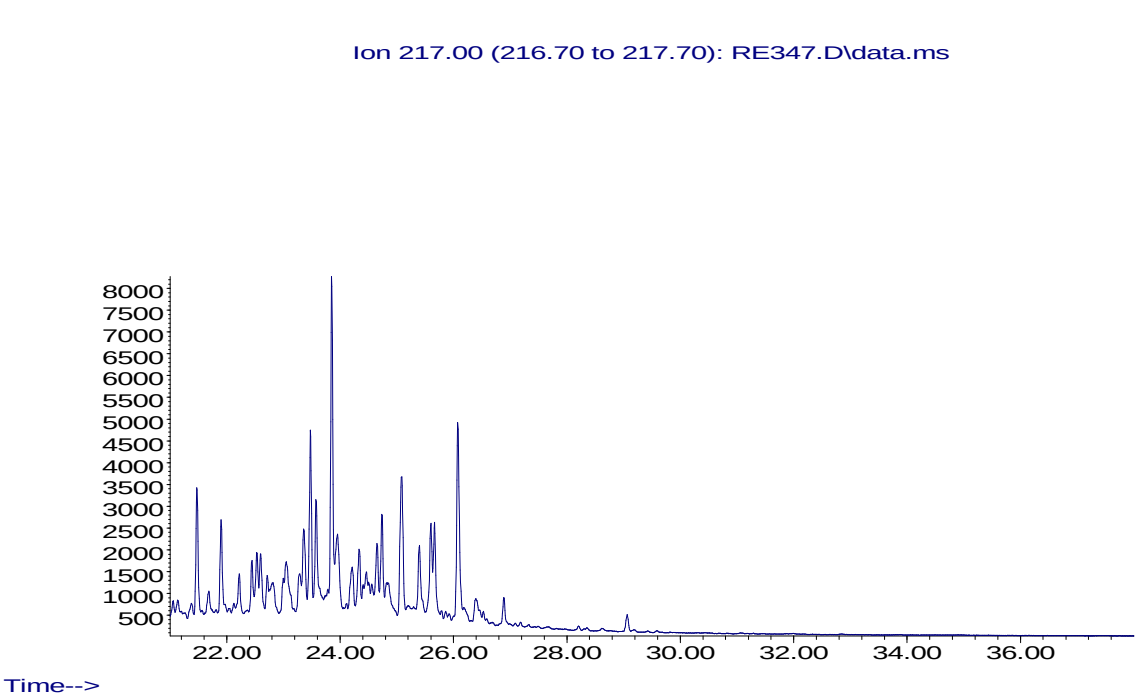

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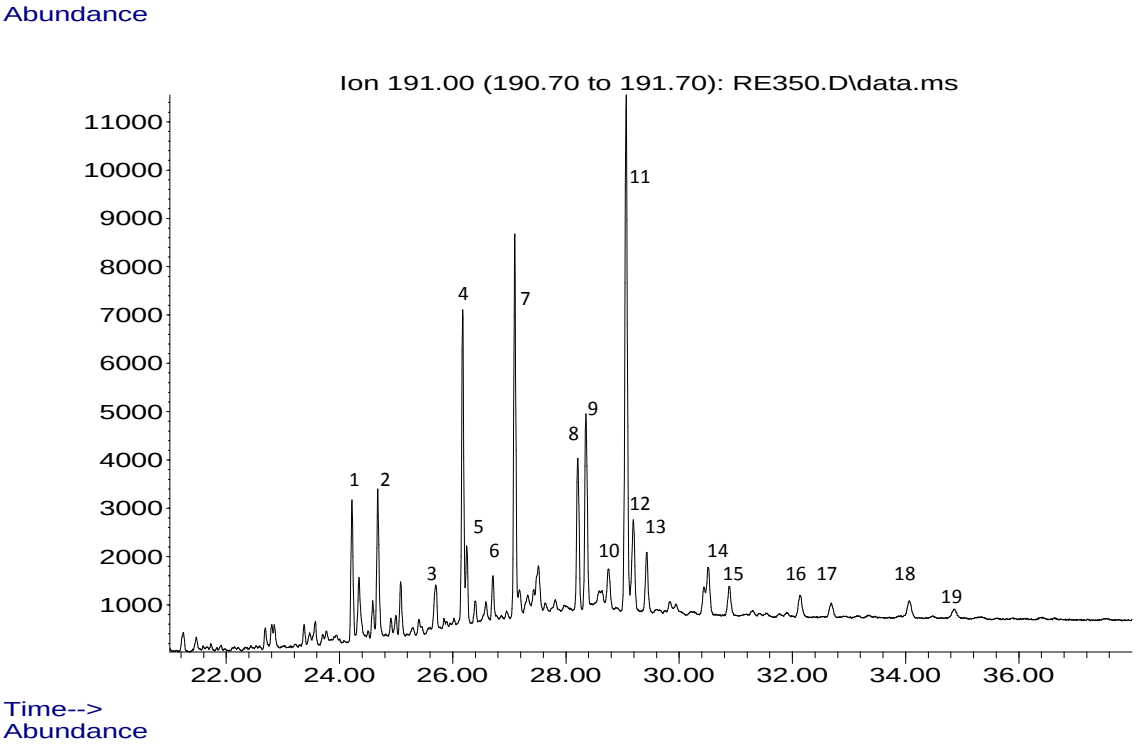

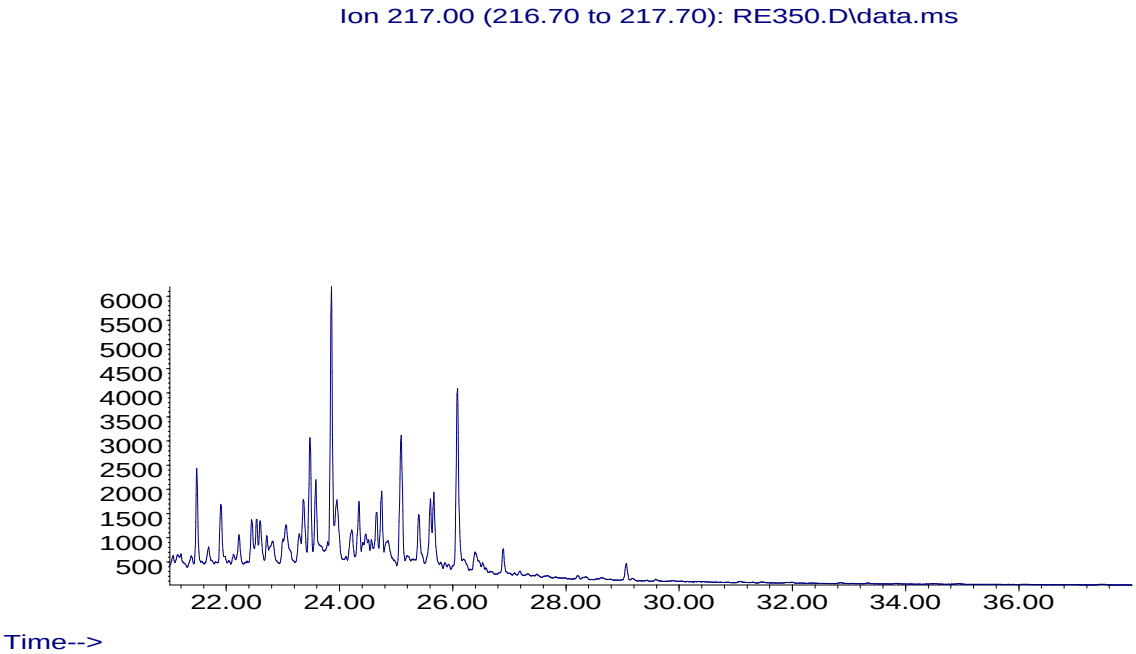

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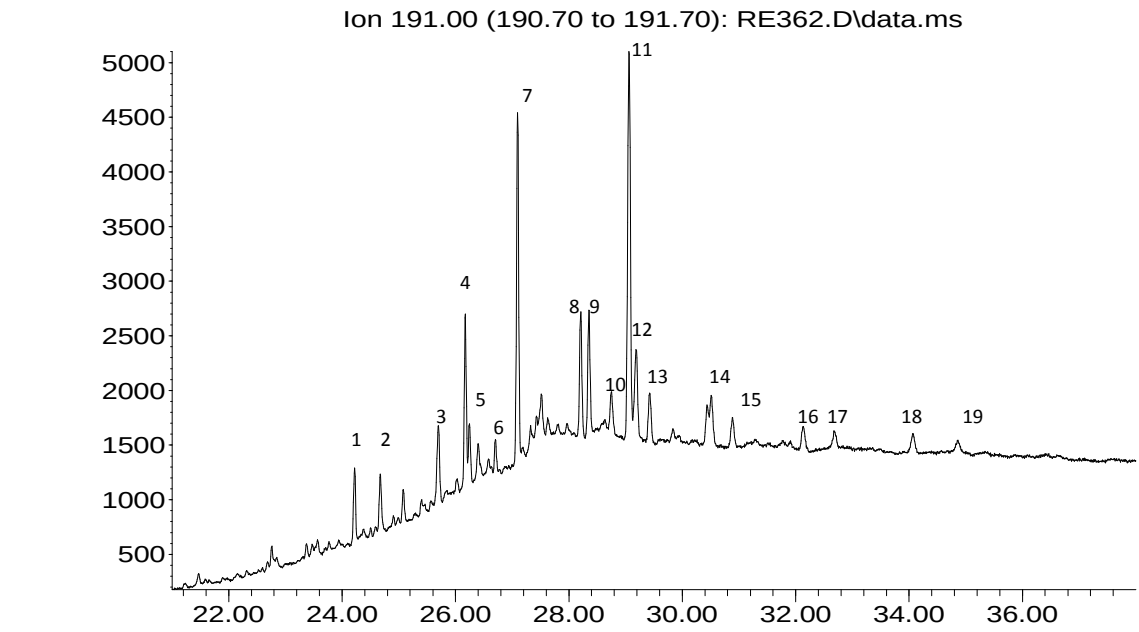

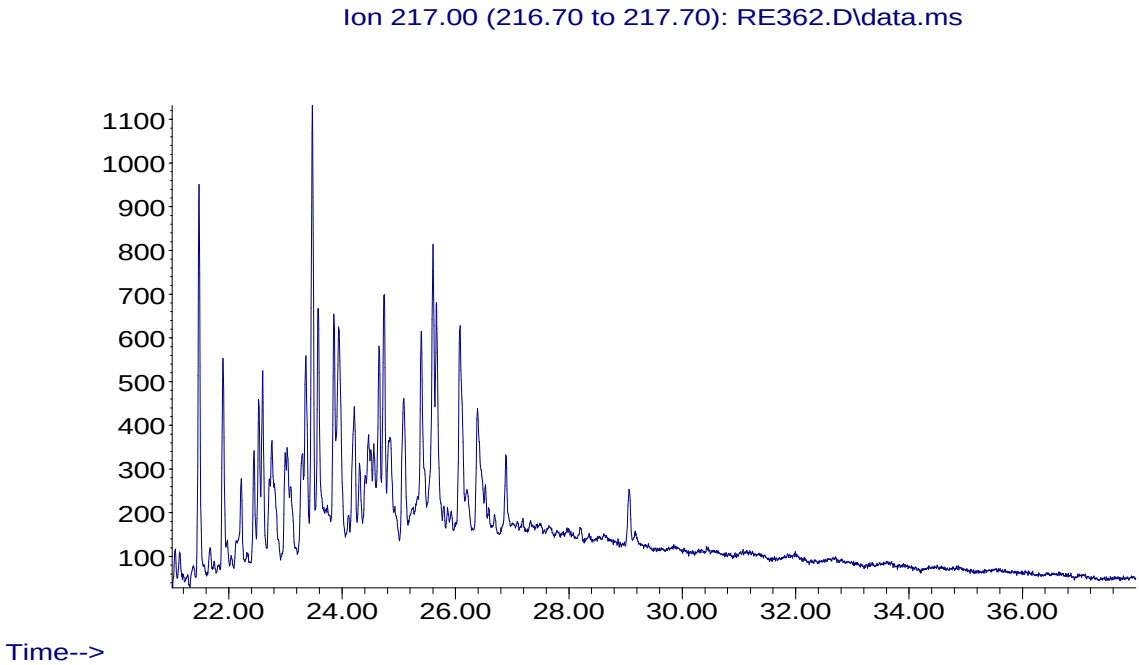

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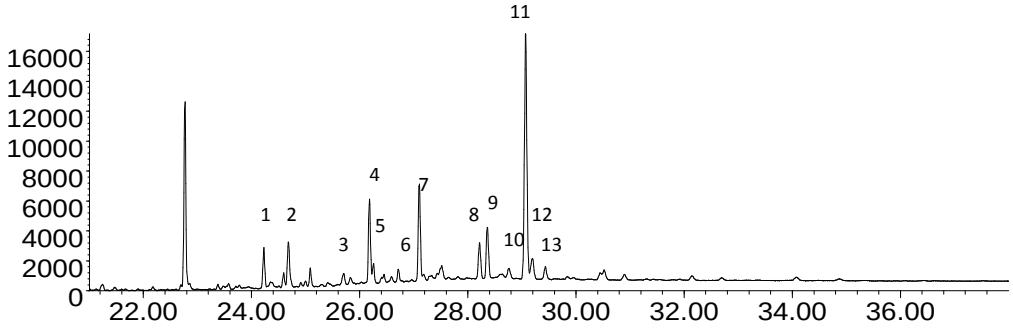
Abundance



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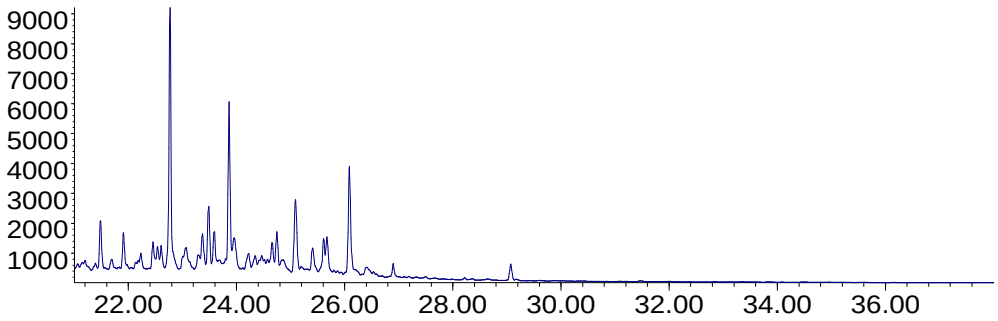

Time-->

89 days depuration

Key to peak labels

Peak label	Peak id	Compound	*m/z*
1	27Ts	18α(H) -22, 29, 30-trinorhopane	191
2	27Tm	17α(H)-22, 29,30-trisnorhopane	191
3	28ab	17α(H),21β(H)-28,30-bisnorhopane	191
4	29ab	17α(H), 21β(H)-30-norhopane	191
5	29Ts	18α(H)-norneohopane	191
6	30d	15α-methyl-17 α (H)-27-norhopane (diahopane)	191
7	30ab	17 α(H), 21β(H)-hopane	191
8 & 9	31ab (S & R)	17 α(H), 21β(H)-homohopane (22S & 22R)	191
10	30G	Gammacerane	191
11		diploptene	191
12 & 13	32ab (S & R)	17 α(H), 21β(H)-bishomohopane (22S & 22R)	191
14 & 15	33ab (S & R)	17 α(H), 21β(H)-trishomohopane (22S & 22R)	191
16 & 17	34ab (S & R)	17 α(H), 21β(H)-tetrahomohopane (22S & 22R)	191
18 & 19	35ab (S & R)	17α(H), 21β(H)-pentakishomohopane (22S & 22R)	191

APPENDIX 4

TABLE FROM CHAPTER SIX

Appendix 4: Elimination rate constants (k_2 day^{-1}), biological half-lives ($t_{1/2}$ days) and correlation co-efficients (r^2) determined from mussels exposed to Arabian light, Gullfaks and Brent crude oils

PAH compounds	Mussels exposed to Arabian light oil				Mussels exposed to Gullfaks crude oil				Mussels exposed to Brent crude oil			
	k_2	r^2	$t_{1/2}$	*p*-value	k_2	r^2	$t_{1/2}$	*p*-value	k_2	r^2	$t_{1/2}$	*p*-value
Naphthalene	1.417± 0.015	0.99	0.5	0.005	1.240± 0.012	0.94	0.6	0.002	1.923±0.101	1.00	0.4	0.000
2-Methyl Naphthalene	0.699± 0.006	0.99	1.0	0.000	0.660± 0.010	0.97	1.0	0.000	0.761±0.038	0.98	0.9	0.000
1-Methyl Naphthalene	0.687± 0.006	0.99	1.0	0.000	0.665± 0.011	0.97	1.0	0.000	0.847±0.002	0.90	0.8	0.000
C2-Naphthalenes	0.370± 0.001	0.98	1.9	0.000	0.398± 0.008	0.99	1.7	0.000	0.454±0.002	0.99	1.5	0.000
C3-Naphthalenes	0.203± 0.002	0.98	3.4	0.000	0.222± 0.011	0.99	3.1	0.000	0.279±0.002	1.00	2.5	0.000
C4-Naphthalenes	0.125± 0.003	0.97	5.5	0.000	0.131± 0.010	0.99	5.3	0.000	0.196±0.002	1.00	3.5	0.000
Acenaphthene	0.385± 0.062	0.95	1.8	0.001	0.346± 0.010	0.99	2.0	0.000	0.415±0.002	0.98	1.7	0.000
Fluorene	0.336± 0.004	0.97	2.1	0.000	0.391± 0.011	0.98	1.8	0.000	0.445±0.001	0.99	1.6	0.000
Phenanthrene	0.269± 0.003	0.98	2.6	0.000	0.278± 0.007	0.99	2.5	0.000	0.345±0.001	1.00	2.0	0.000
Anthracene	0.007±0.004	0.30	96.3	0.911	0.256± 0.293	0.36	2.7	0.156	0.177±0.008	0.66	3.9	0.027
C1-Phenan/Anhracene	0.138± 0.003	0.97	5.0	0.000	0.150± 0.007	0.99	4.6	0.000	0.222±0.002	1.00	3.1	0.000
C2-Phenan/Anhracene	0.075± 0.004	0.55	9.3	0.091	0.080± 0.007	0.97	8.6	0.000	0.141±0.003	0.96	4.9	0.000
C3-Phenan/Anhracene	0.050± 0.004	0.32	13.8	0.244	0.062± 0.008	0.94	11.1	0.000	0.117±0.002	0.98	5.9	0.000
Dibenzothiophene	0.285± 0.002	0.98	2.4	0.000	0.297± 0.007	0.98	2.3	0.000	0.365±0.003	0.99	1.9	0.000
C1-Dibenzothiophenes	0.158± 0.002	0.97	4.4	0.000	0.163± 0.007	0.98	4.2	0.000	0.232±0.001	1.00	3.0	0.000
C2-Dibenzothiophenes	0.083± 0.003	0.95	8.4	0.001	0.080± 0.007	0.97	8.7	0.000	0.152±0.002	0.99	4.6	0.000
C3- Dibenzothiophenes	0.054± 0.004	0.36	12.8	0.212	0.051± 0.007	0.96	13.5	0.000	0.110±0.003	1.00	6.3	0.000
Fluoranthene (202)	0.171± 0.036	0.56	4.1	0.086	0.038± 0.010	0.32	18.1	0.185	0.056±0.002	0.29	12.4	0.211
Pyrene (202)	0.041± 0.009	0.20	17.1	0.373	0.085± 0.005	0.90	8.1	0.001	0.096 ±0.086	0.98	7.2	0.000
C1-Flouranthene/Pyrene	0.057± 0.004	0.37	12.2	0.198	0.073± 0.006	0.96	9.5	0.000	0.125±0.003	0.99	5.6	0.000
C2 -Flouranthene/Pyrene	0.037± 0.005	0.26	18.8	0.301	0.0568± 0.007	0.92	12.2	0.001	0.113±0.003	0.97	6.1	0.000
C3-Flouranthene/Pyrene	0.041± 0.003	0.22	17.0	0.349	0.067± 0.007	0.92	10.4	0.001	0.106±0.003	0.99	6.5	0.001
Benz[*a*]anthracene	0.046± 0.014	0.03	15.0	0.737	0.112± 0.006	0.91	6.2	0.001	0.170±0.008	0.91	4.1	0.001
Chrysene/Triphenylene	0.051± 0.005	0.28	13.6	0.285	0.064± 0.007	0.90	10.9	0.001	0.108±0.002	0.98	6.4	0.000
C1-Chrysenes	0.042± 0.004	0.21	16.6	0.362	0.060± 0.006	0.90	11.6	0.001	0.100±0.004	0.98	6.9	0.000

C2-Chrysenes	0.072± 0.002	0.94	9.7	0.001	0.093± 0.008	0.96	7.4	0.000	0.146±0.004	0.99	4.7	0.000
Benzofluoranthenes (252)	0.031± 0.005	0.31	22.3	0.254	0.054± 0.012	0.95	12.8	0.000	0.076±0.003	0.93	9.1	0.000
Benzo[*e*]pyrene (252)	0.003± 0.002	0.10	232.3	0.955	0.014± 0.008	0.44	49.3	0.103	0.032±0.002	0.77	22.0	0.010
Benzo[*a*]pyrene (252)	0.067± 0.037	0.48	10.3	0.128	0.129± 0.021	0.96	5.4	0.000	0.336±0.180	0.82	2.1	0.005
Perylene (252)	ND	ND	ND	ND	0.035± 0.013	0.82	19.8	0.005	ND	ND	ND	ND
C1-252	0.256± 0.330	0.88	2.7	0.005	0.097± 0.008	0.94	7.1	0.000	0.122±0.009	0.96	5.7	0.000
C2-252	0.060± 0.046	0.97	11.6	0.000	0.114± 0.007	0.97	6.1	0.000	0.126±0.005	0.97	5.5	0.000
Indeno[1,2,3-*cd*]pyrene	0.007± 0.012	0.04	103.4	0.739	0.082± 0.008	0.84	8.5	0.004	ND	ND	ND	ND
Benzo[*g,h,i*]perylene	0.016± 0.005	0.18	43.0	0.399	0.068± 0.006	0.96	10.3	0.000	0.039±0.003	0.77	18.0	0.010
C1-276	0.111± 0.008	0.53	6.2	0.101	0.166± 0.020	0.98	4.2	0.000	0.038±0.020	0.10	18.3	0.549
Dibenz[*a,h*]anthracene	ND	ND	ND	ND	0.108± 0.012	0.88	6.4	0.062	ND	ND	ND	ND