CENTRE FOR ENVIRONMENT, FISHERIES AND
AQUACULTURE SCIENCE

AQUATIC ENVIRONMENT MONITORING REPORT
Number 54

Monitoring of the quality of the marine environment, 1999-2000

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FOREWORD

Aquatic Environment Monitoring Report No.54 collects together work carried out in 1999-2000 by CEFAS scientists in support of our monitoring and surveillance duties (see overleaf). The information covers both general quality monitoring at offshore and coastal sites and site-specific work carried out in support of risk assessments and regulatory work. Some of the science reported here forms part of wider efforts to integrate data from Departments and Agencies in the UK to provide a comprehensive picture of the quality of the marine environment via the National Marine Monitoring Programme (NMMP). Other components are unique to CEFAS due to our requirement to understand ecosystem response resulting from potential pressures by disposal and discharge activities.

The strategy for the NMMP programme is described in publications commissioned by the Marine Pollution Monitoring Management Group (MPMMG) - Green Book which is available in downloadable format from the Fisheries Research Services, Aberdeen web site: www.marlab.ac.uk The programme seeks to develop trend data for a small number of sites around the UK and the work is augmented by special surveys of compounds likely to pose specific risks. Determination of the fate of booster biocides used in antifouling paints and the uptake of polycyclic aromatic hydrocarbons in bivalves are two examples of these special surveys and the results are reported in Chapters 2 and 6.

The growing list of contaminants of concern dictate that it is impossible to measure all contaminants in marine waters and, even if we did, the combined effects of mixtures of contaminants would be difficult to predict. In order to achieve better protection of the marine environment an "ecosystem approach" to monitoring is being developed. In summary this approach seeks to see how the observable aspects of marine ecosystems are changing outside normal limits of variability and to understand the causal factors. To this end we have developed a cascade of methods to describe early warning signs and effects at an individual level (Chapters 8 to 11) and changes at a community level (Chapter 13). We are increasingly using acoustic methods to describe large-scale changes to habitats and the results of one such study is reported for Roughs Tower disposal site in Chapter 17.

This report and earlier reports in the series are available in downloadable format from the CEFAS web site: www.cefas.co.uk

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BACKGROUND TO THE WORK

As an Executive Agency of the Department for Environment, Food and Rural Affairs (Defra), CEFAS carries out work in support of the Government aim to enhance the quality of life through promoting:

- a better environment;
- thriving rural economies and communities;
- diversity and abundance of wildlife resources;
- a countryside for all to enjoy; and
- sustainable and diverse farming and food industries that work together to meet the needs of consumers.

Within these overarching objectives, environment work at CEFAS is directed at research, monitoring and assessment of the impact of potentially harmful substances or activities on the quality of the marine, coastal and estuarine environments. We are involved directly in advising on UK and international legislation and in developing policy relating to management of the aquatic environment. We provide advice to Governments, enforcement agencies and policymakers throughout the world on the development and implementation of monitoring and assessment programmes and control measures.

An important component of our work is to provide advice to Defra Ministers and other Government Departments on all aspects of non-radioactive contamination of the aquatic environment. Specifically under Part II of the Food and Environment Protection Act (1985) (FEPA) (Great Britain-Parliament, 1985), Defra has the responsibility to licence and control the deposit of material to the sea. Following the cessation of disposal of sewage sludge to sea, licensed materials are predominantly sediment, derived from maintenance and capital dredging activities. Disposal at sea is also regulated internationally by OSPAR, and our work enables the UK to carry out its obligations as a Contracting Party.

The CEFAS Inspectorate evaluates scientific and technical aspects of licence applications and makes regular visits to licence holders to ensure any stipulated conditions are being met. Conducting monitoring programmes in support of risk assessments enables Defra to ensure the effectiveness of the assessment process and provides a basis for decisions on future policy for management of marine resources. Environmental scientists at CEFAS monitor environmental conditions at marine disposal sites and compare the results with those from more general environmental quality monitoring, allowing suitable action to be taken if unexpected or unacceptable impacts should occur.

Under the Water Resources Act (1991) (Great Britain-Parliament, 1991), Defra is a statutory consultee for all discharges to controlled (tidal) waters. CEFAS scientists assess the fishery implications of applications for consent to discharge permits. Consideration is given to resources in the area, toxicity of the effluent, local hydrographic conditions and any standards set out in national policy or EU Directives.

We also provide advice to the Department of Trade and Industry (DTI) and the Department of Land, Transport and the Regions (DLTR) concerning the control of pollution in other areas of industrial activity affecting the marine environment including the offshore oil and gas industry and marine aggregate extraction. The Offshore Chemical Notification Scheme and Government View on the winning of aggregates respectively control these activities, but the regulatory regimes for both are presently changing to statutory schemes.

On Defra's behalf, CEFAS is responsible for monitoring intermediate and offshore stations in the UK National Marine Monitoring Programme (NMMP), which seeks to integrate national and international monitoring programmes for all UK agencies. Each year we collect samples of seawater, sediment and biota for chemical analyses and deploy a number of biological effects techniques, including water and sediment bioassays and fish disease surveys. The first phase of spatial surveys evaluated the pattern of marine quality around the UK providing a picture of generally healthy conditions in UK coastal waters. Phase II, which began in 1999, is focused on the detection of long-term temporal trends and the introduction of new biological effects studies. The NMMP allows us to ascertain the effectiveness of regulatory measures to reduce the inputs of hazardous substances to UK seas. In addition, it contributes to the UK's international monitoring obligations to demonstrate UK compliance with various EC Directives: Dangerous Substances Directive (76/464/EEC); Shellfish Waters Directive (79/923/EEC); Shellfish Hygiene Directive (91/492/EEC); Fishery Products Directive (91/493/EEC); Commission Decision 93/351/EEC concerning maximum mercury limits in fishery products, and similar requirements under OSPAR.

In order to ensure that the advice provided to Defra and other Regulators is always based on the most up-to-date knowledge and techniques, CEFAS carries out a wide range of research and development to provide for future needs of monitoring and surveillance programmes. For example, we have developed new and more sensitive bioassay techniques, analytical methods, unattended sampling and monitoring devices and we are currently leading on a Europe-wide collaborative research project on the quality assurance in biological effects testing methods.

Environment Science at CEFAS has a track record of more than 50 years experience in aquatic studies. During this period we have made a number of significant contributions to environmental protection and as a consequence of our work have established a worldwide reputation in the field of aquatic environmental research. More information on our research programmes is listed on the CEFAS web site (www.cefas.co.uk).

GLOSSARY OF TERMS

AEMR	Annual Environmental Monitoring Report
ANOSIM	Analysis of similarities
AP	Alcohols
APEC	Carboxylic Acids
APEOs	Alkylphenol Polyethoxylates
As	Arsenic
B*a*P	Benzo(*a*)pyrene
B*b*F	Benzo(*b*)fluoranthene
B*a*A	Benzo(*a*)anthracene
BDE	Brominated Diphenyl Ether
BEQUALM	Biological Effects Quality Assurance in Monitoring programme
BNF	British Nuclear Fuels
CB	Chlorinated Biphenyl/Chlorobiphenyl
CEFAS	Centre for Environment, Fisheries and Aquaculture Science
CF	Condition Factor
CPDU	1-(3-chlorophenyl)-3,1-dimethylurea
DCPMU	1-(3,4-dichlorophenyl)-3-methylurea
DCPU	1-(3,4-dichlorophenyl)urea
DDE	Dichlorodiethylene
Defra	Department for Environment, Food & Rural Affairs
DETR	Department of Environment, Transport and the Regions
DMSO	Dimethyl Sulphoxide
DNA	Deoxyribose Nucleic Acid
DO	Dissolved Oxygen
DRZ	Diagonal Radioactive Zones
EC	European Community
EHS	Environmental Heritage Service (Northern Ireland)
EQS	Environmental Quality Standard
EROD	Ethoxyresorufin-O-deethylase
EU	European Union
FCA	Foci of Cellular Alteration
FEPA	Food and Environmental Protection Act 1985
FRS	Fisheries Research Services
GC-MS	Gas Chromatography–Mass Spectrometry
GC-MS (SIM)	Gas Chromatography–Mass Spectrometry Single Ion Monitoring
HPLC-MS	High Performance Liquid Chromatography Coupled to Mass Spectrometry
HSE	Health and Safety Executive
IUPAC	International Union of Pure and Applied Chemistry
ICES	International Council for the Exploration of the Sea
JAMP	Joint Assessment and Monitoring Programme
LAT	Lowest Astronomical Tide
LOD	Limits of Detection
MACs	Maximum Allowable Concentrations
MAFF	Ministry of Agriculture, Fisheries and Food
MDS	Multi-Dimensional Scaling
MFO	Mixed Function Oxygenase
NBF	Neutral Buffered Formalin
NOAA	National Oceanic & Atmospheric Administration (USA)
NMAQC	National Marine Analytical Quality Scheme
NMMP	National Marine Monitoring Programme
NMP	National Monitoring Programme
NP	Nonylphenols
OSPAR	Oslo and Paris Commission
PAH	Polycyclic Aromatic Hydrocarbon
PBDE	Polybrominated Diphenyl Ether
PCB	Polychlorinated Biphenyl
pGSI	Pseudo Gonad Somatic Index

PMTDI	Permitted Maximum Tolerable Daily Intake
PSA	Particle Size Analysis
QSAR	Quantitative Structure-Activity Relationship
RAP	Registry of Aquatic Pathology
RV	Research Vessel
SERAD	Scottish Executive Rural Affairs Department
SIMPER	Similarities Percentages Programme
SEPA	Scottish Fisheries Protection Agency
SD	Standard Deviation
SFI	Sea Fisheries Inspectorate
SPE	Solid Phase Extraction
TCMTB	Thiocyanomethylthio Benzothiazole
TOC	Total Organic Carbon
ToXN	Total Oxidised Nitrogen
UVF	Fluorescence Spectrometry
UK	United Kingdom

SEA WATER

1. ALKYLPHENOLS IN SEAWATER AND MARINE SEDIMENTS SAMPLED IN COASTAL AND OFFSHORE WATERS AROUND ENGLAND AND WALES IN 1999

1.1 Alkylphenols in seawater

1.1.1 Introduction

Alkylphenol polyethoxylates (APEOs) are non-ionic surfactants used extensively in both commercial and domestic applications. As well as being one of the most widely used substrates in industrial detergents, they are also used in such diverse applications as paint additives, wetting agents and contraceptives (Department of the Environment, 1993) although their use in many areas is being phased out. Ten major manufacturers of polyacrylamide emulsion compounds have agreed a Europe-wide exclusion of APEOs by the year 2001 (ENDS Report, 1999). However, this only covers an estimated 1-2% of total APEO consumption within the European Community (EC), and chemical companies in the United States and Japan have formed an alliance to protect APEOs from environmental legislation. Nonylphenol ethoxylates have been banned from cleaning products in Germany and Switzerland (ENDS Report, 1999) due to the growing concern over their environmental effects, and a voluntary ban is in force within Europe on the household use of these products.

Alkylphenol surfactants have been suggested as remediators for contaminated soils and aquifers, without proper investigation into the effect that the surfactant itself will have on the surrounding environment. Their extensive use has lead to investigations into their fate and effects in the environment, particularly since their biodegradation metabolites are relatively stable. Unlike many surfactants, APEOs are degraded starting from the hydrophilic component of the molecule (Ahel *et al*., 1996; Swisher, 1987), resulting in more hydrophobic, stable and potentially more toxic metabolites. Since surfactants are usually used in aqueous solutions, these metabolites are often formed during the breakdown of products during waste water treatment. Alkylphenol polyethoxylates break down to give short chain ethoxylates, carboxylic acids (APEC) and alcohols (AP), namely nonylphenol (NP) and octylphenol derivatives. These breakdown products have been identified as having the ability to mimic natural hormones by interacting with the oestrogen receptor, and have the potential to bioaccumulate (Ahel *et al*., 1996). Exposure to endocrine disrupting chemicals such as NP has been found to increase the production of oestrogen-responsive proteins such as vitellogen and zona radiata protein (Arukwe *et al*., 1997) in fish. This oestrogenicity has also been implicated in such toxic effects as the retardation of testicular development in rainbow trout (Jobling *et al*., 1996), the development of testis-ova in male Japanese medaka (Gray and Metcalfe, 1997) and the reduced testicular size and sperm production in pre/neonatally exposed male rats (Sharpe *et al*., 1995). Several studies of APEOs have been carried out on environmental samples, including river water, sewage effluent, sewage sludge, sediments and biota (e.g. Blackburn and Waldock, 1995; Rudel *et al.*, 1998; Marcomoni and Giger, 1987; Ahel *et al*., 1996). Bisphenol A, which is used in plasticisers, has also been identified as a potential endocrine disrupter. Since it can be extracted using similar methods to those which we use for alkylphenols, our study has been extended to include bisphenol A as one of our determinands.

1.1.2 Methods

Water sampling, using National Marine Monitoring Programme (NMMP) guidelines, was conducted at a total of 48 sites during two research vessel cruises, *RV CIROLANA* 3a/99 and *RV CIROLANA* 3b/99. Filtered (dissolved) and unfiltered (total) samples were analysed for nonylphenol, nonylphenol mono- and diethoxylate, octylphenol and bisphenol A.

Methods have been fully described elsewhere (Blackburn and Waldock, 1995). In brief, alkylphenols were isolated by means of solid phase extraction and eluted with ethyl acetate and dichloromethane. Analysis was performed by Gas Chromatography-Mass Spectrometry (GC-MS) using selected ion monitoring.

1.1.3 Results

Results are presented in Table 1 and Figure 1.

Nonylphenol was detected in all samples. In the unfiltered samples (total nonylphenol), nonylphenol had a range of 0.03–6.6 µg l^{-1} and a mean of 0.41 µg l^{-1}. Dissolved nonylphenol was found to have a range of 0.03-2.0 µg l^{-1} and a mean of 0.18 µg l^{-1}. The highest concentration was found at Tees Dabholm Gut, and only in the Tees were concentrations of greater than 1 µg l^{-1} found. Average values have fallen compared to 1998 data, although the number and location of sites is not identical.

Table 1. Concentrations of alkylphenol ethoxylates (µg l^{-1}) in sea water. Sites within the Tees Estuary are shown in bold

NMMP Site	Location	Position (Latitude/ Longitude)	Nonylphenol		Ethoxylates*		Octylphenol		Bisphenol A	
			Total	Dissolved	Total	Dissolved	Total	Dissolved	Total	Dissolved
	Bremerhaven 9	55° 29.721'N 04° 08.093'E	0.59	0.41	4.2	1.0	0.12	0.06	0.05	0.02
	Tyne sewage Disposal Site N Ref.	55°11.161'N 01° 22.341'W	0.05	0.03	0.20	0.18	0.50	0.34	<0.01	<0.01
	Tyne sewage Disposal Site	55° 03.246'N 01° 17.078'W	0.29	0.14	0.61	0.41	0.19	0.07	0.05	0.03
	Tyne Dredge Disposal Site D3	55 02.536'N 01° 19.392'W	0.05	0.04	0.26	0.23	0.05	0.04	<0.01	<0.01
	Tyne Dredge Disposal Site D5	55° 01.163'N 01° 16.948'W	0.05	0.03	0.28	0.14	0.04	0.02	0.04	<0.01
	Tyne Dredge Disposal Site D23	54° 56.031'N 01° 13.959'W	0.04	0.04	0.15	0.16	0.27	0.05	<0.01	<0.01
	Tyne Dredge Disposal Site D27	54° 58.088'N 01° 15.000'W	0.04	0.03	0.14	0.14	0.13	0.03	<0.01	<0.01
	Tyne Dredge Disposal Site D20	54° 58.874'N 01° 15.928'W	0.10	0.05	0.36	0.33	1.3	0.68	<0.01	<0.01
245	Off Tyne	55° 00.460'N 01° 08.039'W	0.10	0.10	0.69	0.62	0.03	0.03	0.12	0.12
285	West Dogger	54° 45.538'N 01° 18.389'E	0.47	0.22	0.98	0.62	0.07	0.03	0.02	0.03
	Tees Bay	54° 39.380'N 01° 09.800'W	0.04	0.04	0.75	0.56	0.01	0.01	0.10	0.05
	Tees Bay	54° 39.800'N 01° 10.300'W	0.11	0.08	0.97	0.49	0.02	0.01	<0.01	<0.01
	Tees Bay	54° 37.750'N 01° 05.580'W	0.06	0.05	0.75	0.54	0.01	0.01	<0.01	<0.01
	Tees No. 5 Buoy	54° 38.910'N 01° 08.450'W	0.04	0.03	0.58	0.36	0.01	0.01	0.05	0.02
	Tees No.6 Buoy	54° 38.720'N 01° 08.350'W	0.17	0.07	0.99	0.60	0.02	0.01	<0.01	0.02
	Tees Dabholm Gut	54° 36.760'N 01° 08.600'W	6.6	2.0	13	5.9	1.2	0.74	1.3	0.86
	Tees No. 15 Buoy	54° 37.170'N 01° 09.200'W	1.4	0.81	2.5	1.7	0.46	0.39	0.19	0.19
	Tees No. 12 Buoy	54° 37.900'N 01° 09.300'W	0.37	0.12	0.73	0.23	0.05	0.02	0.11	0.03
	Tees No. 19 Buoy	54° 36.600'N 01° 09.300'W	1.1	0.82	2.0	1.8	0.46	0.39	0.14	0.15
	Tees Ramp Outfall(ICI 4)	54° 36.280'N 01° 09.800'W	0.29	0.11	1.0	0.56	0.04	0.02	0.05	0.03
	Tees ICI 2	54° 35.840'N 01° 10.400'W	0.09	0.08	1.7	0.71	0.03	0.02	0.06	0.04
	Tees No 27 Buoy NW	54° 35.350'N 01° 11.380'W	0.14	0.09	0.70	0.47	0.03	0.02	0.07	0.03
	Tees Cargo Fleet Wharf	54° 35.030'N 01° 11.700'W	0.17	0.07	0.69	0.3	0.03	0.01	<0.01	0.03
	Tees Transporter Bridge shallow	54° 35.080'N 01° 13.560'W	0.23	0.15	0.59	0.41	0.10	0.04	0.08	0.03
	Tees No. 29 Buoy mid	54° 35.250'N 01° 14.200'W	0.23	0.13	0.56	0.33	0.05	0.04	0.06	0.04
	Tees Bamletts Wharf	54° 35.440'N 01° 14.600'W	0.16	0.13	0.61	0.43	0.04	0.03	0.04	0.05
	Bremerhaven 1	54° 04.385'N 08° 08.129'E	0.21	0.12	0.8	0.26	0.03	0.03	0.03	0.02
	NSTE 16	53° 32.114'N 00° 19.852'E	1.7	0.15	4.3	0.43	1.1	0.04	1.6	0.04

Table 1. continued: Concentrations of alkylphenol ethoxylates (µg l^{-1}) in sea water

NMMP Site	Location	Position (Latitude/ Longitude)	Nonylphenol		Ethoxylates*		Octylphenol		Bisphenol A	
			Total	Dissolved	Total	Dissolved	Total	Dissolved	Total	Dissolved
375	Humber	53° 19.100'N 00° 25.284'E	0.18	0.12	0.51	0.51	0.05	0.03	0.05	0.02
385	Wash	53° 08.572'N 00° 33.132'E	0.09	0.10	0.74	0.49	0.04	0.02	0.07	0.01
	Thames (central Disposal) G9	51° 40.759'N 01° 18.724'E	0.21	0.21	0.78	0.54	0.14	0.06	0.08	0.07
345 (E)	Off Humber/Wash	53° 00.043'N 01° 59.992'E	0.03	0.05	0.45	0.21	0.04	0.01	<0.01	<0.01
465	Thames Wharf	51° 31.298'N 00° 58.386'E	0.76	0.37	0.85	0.38	0.06	0.02	0.06	0.03
	South Varne	50° 56.786'N 01° 17.887'E	0.17	0.12	0.44	0.23	0.02	0.01	0.03	0.01
	Rye Bay	50° 51.429'N 00° 47.476'E	0.15	0.09	2.7	2.32	0.01	0.02	0.02	0.01
536	Lyme Bay	50° 25.810'N 03° 07.312'W	0.32	0.12	0.67	0.31	0.18	0.08	0.03	0.02
	Plymouth	50° 20.936'N 04° 07.798'W	0.15	0.12	0.45	0.23	0.11	0.07	0.03	0.02
	Falmouth	50° 07.001'N 05° 02.424'W	0.22	0.14	0.58	0.30	0.15	0.12	0.09	0.04
605	Celtic Deep	51° 14.968'N 05° 59.921'W	0.28	0.13	3.0	1.1	0.04	0.02	0.02	0.01
	Swansea Bay Transect	51° 31.775'N 03° 54.802'W	0.27	0.19	0.37	0.36	0.18	0.15	0.18	0.10
656	Inner Cardigan Bay	52 16.452'N 04° 20.083'W	0.12	0.04	0.55	0.21	0.01	0.01	0.02	0.01
665	Off Cardigan Bay	52° 22.071'N 04° 53.909'W	0.31	0.2	0.87	0.46	0.26	0.02	0.02	0.01
776	Red Wharf Bay	53° 21.973'N 04° 11.317'W	0.17	0.05	0.98	0.24	0.03	0.01	0.02	0.01
	TREND	53° 23.618'N 03° 36.560'W	0.17	0.09	0.47	0.29	0.03	0.01	0.02	<0.01
705	Liverpool Bay	53° 28.288'N 03° 21.054'W	0.09	0.03	0.97	0.30	0.04	0.01	0.07	0.01
	Mersey	53° 53.281'N 03° 25.545'W	0.27	0.05	2.13	0.45	0.07	0.02	0.11	0.02
805	SE Isle of Man	53° 59.909'N 03° 50.266'W	0.22	0.21	2.3	2.3	0.38	0.04	0.58	0.07
815	Dundrum Bay	54° 03.848'N 05° 30.222'W	0.43	0.10	5.3	0.76	0.03	0.01	0.02	0.03

**Sum of mono- and diethoxylate*

Total nonylphenol mono- and diethoxylate was detected at all sites. Concentrations range from 0.14 µg l^{-1} to 13 µg l^{-1} at Tyne Dredge Disposal Site D27 and Tees Dabholm Gut respectively. The mean value for total ethoxylates is 1.4 µg l^{-1}. The range of concentrations for dissolved mono- and diethoxylates is from 0.14 µg l^{-1} to 5.9 µg l^{-1}. The mean for these values is 0.66 µg l^{-1}. This shows a marked difference from 1998 data, where at many offshore sites, no ethoxylates at all could be detected. The mean concentration has decreased compared to 1998 data despite more intensive sampling within the Tees estuary, where ethoxylate levels are relatively high.

Octylphenol was detected at all sites in the total (unfiltered) samples. Total octylphenol ranged from 0.01–1.3 µg l^{-1} and had a mean value of 0.17 µg l^{-1}, with only three sites having concentration greater than 1 µg l^{-1}. Those are Tyne Dredge Disposal D20, Tees Dabholm Gut, and NSTE 16 (Humber). Most sites were found to have relatively low concentrations of octylphenol (below 0.2 µg l^{-1}). Dissolved octylphenol ranges from 0.01 µg l^{-1} to 0.74 µg l^{-1}, the maximum again being at Tees Dabholm Gut. The only other site with a concentration in excess of 0.5 µg l^{-1} is Tyne Dredge Disposal D20. The mean value for dissolved octylphenol is 0.08 µg l^{-1}.

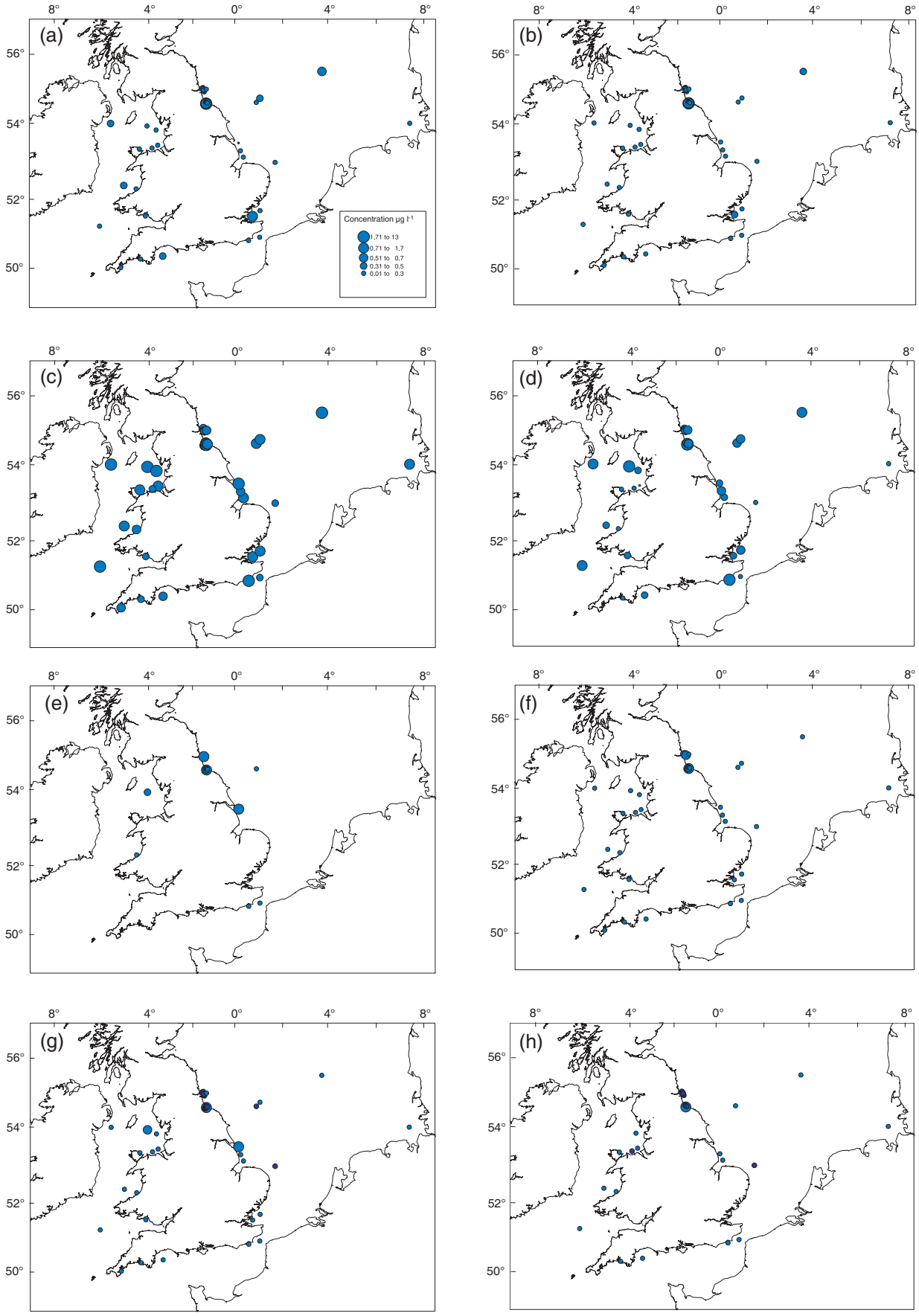

Figure 1. ***Spatial distribution of alkylphenol ethoxylate concentrations in marine and coastal waters around England and Wales, 1999 of (a) total nonylphenol, (b) dissolved nonylphenol, (c) total mono- and diethoxylates, (d) dissolved mono- and diethoxylates, (e) total octylphenol, (f) dissolved octylphenol, (g) total bisphenol and (h) dissolved bisphenol***

Total bisphenol A concentrations ranged from below limits of detection (0.1 µg l^{-1}) to 1.6 µg l^{-1} at NSTE 16. The mean concentration was 0.14 µg l^{-1}. The only other site with a concentration greater than 1 µg l^{-1} was Tees Dabholm Gut. Most sites were found to have relatively low concentrations of bisphenol A (below 0.2 µg l^{-1}). Dissolved bisphenol A concentrations ranged from below limits of detection to 0.86 µg l^{-1}.

Concentrations in dissolved phase samples are invariably lower than in the corresponding unfiltered samples, within experimental error. In areas of high turbidity (e.g. within the Tees estuary), the difference is more marked than in those less turbid areas, as more particulates (and the contamination that may be associated with them) are removed by filtration.

1.1.4 Discussion

Discharge limits for alkylphenol ethoxylates have been set by the environmental quality standards steering group (Matthiessen, *pers. comm.*) on the basis of QSAR (Quantitative Structure-Activity Relationship). Maximum Allowable Concentrations (MACs) for nonylphenol and octylphenol are 2.5 µg l^{-1}. In our study, nonylphenol exceeds this limit at just one site, Tees Dabholm Gut. This data cannot be compared to 1998 data since the sites that were above the MAC in 1998 were not sampled in 1999. Octylphenol concentrations do not exceed the MAC at any of the sites sampled in this study. This is in contrast to 1998 data, where there were four sites above the MAC. Three of those sites; NMMP 245, NMMP 345 and Tyne Sewage Disposal Site, were sampled in both 1998 and 1999. Of those three sites, all now have octylphenol concentrations below 0.5 µg l^{-1}.

MACs for mono- and diethoxylates are 3.3 µg l^{-1} and 4.3 µg l^{-1} respectively, which tentatively leads to an allowable concentration of 7.7 µg l^{-1} for the sum of the mono- and diethoxylates. This limit is exceeded at only one site, Tees Dabholm Gut. The 1998 data showed that Amble had the highest ethoxylate concentration. This site was not sampled in 1999, and so no comparison can be made. No discharge limits have been set as yet for bisphenol A.

Of the sites that were monitored in both years, all nonylphenol concentrations have decreased. Ethoxylate concentrations have increased at offshore sites, but decreased elsewhere. Octylphenol concentrations have decreased at the majority of sites.

The majority of the highly contaminated samples in this study come from coastal or estuarine sources, in areas of high industrial activity, leading to the conclusion that the contamination could originate from industrial or domestic discharges.

Continuation of this monitoring scheme is required to compare results and verify their consistency. In achieving validity in the results found, we can then move on to assessing their significance in the aquatic environment.

1.2 Alkylphenols in sediment

1.2.1 Methods

Sampling was carried out using NMMP guidelines at a total of 89 sites during two research cruises, *RV CIROLANA* 3a/99 and *RV CIROLANA* 3b/99. Samples were taken using a Day Grab. A core sample was also taken at a dredge disposal site using a Rieneck Box Corer during research cruise *RV CIROLANA* 3b/99.

Samples were extracted with 3 x 25 ml ethyl acetate after addition of butylphenol internal standard. Extracts were reduced to approximately 1 ml, added to the top of a 10% deactivated alumina column and eluted with dichloromethane. Analysis was carried out by GC-MS.

1.2.2 Results

Results are presented in Table 2 and Figure 2.

Nonylphenol concentrations ranged from below limits of detection (0.19 µg g^{-1}) to 30 µg g^{-1} at Tees Outfalls (not an NMMP site). In almost all samples, nonylphenol was found to be below the Limits of Detection (LOD), except for those taken in or around the Tees. Within the Tees estuary itself, the concentration exceeded 1 µg g^{-1} in 19 of 24 sites. The average nonylphenol concentration over all sites was 2.6 µg g^{-1}, but if only the values within the Tees estuary were averaged, the value was 9.4 µg g^{-1}. The concentrations found in the core samples remained above 2 µg g^{-1} to a depth of 16 cm.

Nonylphenol mono- and diethoxylates ranged from below limits of detection (1 µg g^{-1}) to 20 at Tees Outfalls. As in the case of nonylphenol, the only samples that had ethoxylate concentrations above the detection limit were within the Tees estuary. The concentrations found in the core samples remain above 1 µg g^{-1} to a depth of 16 cm.

Octylphenol was found to be above the limits of detection (0.01 µg g^{-1}) at very few sites, and the concentration of octylphenol found at those sites was generally below 0.02 µg g^{-1}. Within the Tees estuary, all sites had concentrations above the limit of detection, although only 10 exceeded 0.5 µg g^{-1}.

Bisphenol A was found to be below limits of detection (0.03 µg g^{-1}) at all sites.

Table 2. Concentrations of alkylphenol ethoxylates (µg g^{-1}) in sediment. Sites within the Tees Estuary are shown in bold

NMMP site	Location	Latitude	Longitude	Nonylphenol	Ethoxylates*	Octylphenol	Bisphenol A
	Bremerhaven 9	55° 29.721'N	04° 08.093'E	<0.19	<1.00	<0.01	<0.03
	Tyne sewage Disposal Site N Ref.	55° 11.161'N	01° 22.341'W	<0.19	<1.00	0.01	<0.03
	Tyne sewage Disposal Site	55° 03.246'N	01° 17.078'W	<0.19	<1.00	<0.01	<0.03
	Tyne Dredge Disposal Site D3	55° 02.536'N	01° 19.392'W	<0.19	<1.00	<0.01	<0.03
	Tyne Dredge Disposal Site D5	55° 01.163'N	01° 16.948'W	<0.19	<1.00	<0.01	<0.03
	Tyne Dredge Disposal Site D23	54° 56.031'N	01° 13.959'W	<0.19	<1.00	0.02	<0.03
245(A)	Off Tyne	55° 00.556'N	01° 08.017'W	<0.19	<1.00	<0.01	<0.03
245(B)	Off Tyne	55° 00.527'N	01° 07.873'W	<0.19	<1.00	<0.01	<0.03
245(C)	Off Tyne	55° 00.497'N	01° 08.031'W	<0.19	<1.00	<0.01	<0.03
245(D)	Off Tyne	55° 00.504'N	01° 08.024'W	<0.19	<1.00	<0.01	<0.03
245(E)	Off Tyne	55° 00.510'N	01° 08.002'W	<0.19	<1.00	<0.01	<0.03
285	West Dogger	54° 45.538'N	01° 18.389'E	<0.19	<1.00	0.01	<0.03
295	Off Tees	54° 43.991'N	00° 52.883'W	<0.19	<1.00	0.01	<0.03
	Tees Bay G1	54° 40.926'N	01° 05.010'W	<0.19	<1.00	0.02	<0.03
	Tees Bay G2	54° 39.696'N	01° 05.009'W	0.27	<1.00	0.03	<0.03
	Tees Bay G3	54° 39.408'N	01° 06.840'W	<0.19	<1.00	0.01	<0.03
	Tees Bay G4	54° 41.643'N	01° 07.218'W	2.33	2.2	0.69	<0.03
	Tees Bay G5	54° 39.916'N	01° 08.893'W	<0.19	<1.00	0.01	<0.03
	Tees Bay G6	54° 41.179'N	01° 09.004'W	<0.19	<1.00	0.02	<0.03
	Tees Bay	54° 39.800'N	01° 10.300'W	<0.19	<1.00	0.01	<0.03
	Tees Bay	54° 39.380'N	01° 09.800'W	<0.19	<1.00	<0.01	<0.03
	Tees Bay	54° 38.180'N	01° 06.640'W	0.47	2.4	0.11	<0.03
	Tees Bay	54° 37.750'N	01° 05.580'W	<0.19	<1.00	0.01	<0.03
	Tees Estuary (seal sands)	54° 37.570'N	01° 10.850'W	0.25	<1.00	0.02	<0.03
	Tees Estuary (seal sands)	54° 37.870'N	01° 10.450'W	9.3	4.7	0.47	<0.03
	Tees Bay Inshore Dredge Disposal C1	54° 41.376'N	01° 02.094'W	0.36	<1.00	0.02	<0.03
	Tees Bay Inshore Dredge Disposal C2	54° 40.995'N	01° 00.797'W	0.46	<1.00	0.03	<0.03
	Tees Bay Inshore Dredge Disposal C3	54° 40.886'N	01° 01.816'W	1.5	<1.00	0.07	<0.03
	Tees Bay Inshore Dredge Disposal C4	54° 40 806'N	01° 02.989'W	3.0	1.4	0.14	<0.03
	Tees Bay Inshore Dredge Disposal C5	54° 40.589'N	01° 02.328'W	1.3	<1.00	0.04	<0.03
	Tees Bay Inshore Dredge Disposal C6	54° 40.414'N	01° 01.639'W	2.1	<1.00	0.06	<0.03
	Tees Bay Inshore Dredge Disposal 0-2	54° 41.378'N	01° 02.106'W	0.57	<1.00	0.01	<0.03
	Tees Bay Inshore Dredge Disposal 2-4	54° 41.378'N	01° 02.106'W	1.5	1.1	0.13	<0.03
	Tees Bay Inshore Dredge Disposal 4-6	54° 41.378'N	01° 02.106'W	2.7	1.7	0.23	<0.03
	Tees Bay Inshore Dredge Disposal 6-8	54° 41.378'N	01° 02.106'W	2.4	1.2	0.13	<0.03
	Tees Bay Inshore Dredge Disposal 8-10	54° 41.378'N	01° 02.106'W	1.3	1.1	0.06	<0.03
	Tees Bay Inshore Dredge Disposal 12-14	54° 41.378'N	01° 02.106'W	1.9	1.6	0.11	<0.03
	Tees Bay Inshore Dredge Disposal 14-16	54° 41.378'N	01° 02.106'W	2.1	1.4	0.12	<0.03
	Tees Bay Inshore Dredge Disposal 16-18	54° 41.378'N	01° 02.106'W	0.90	<1.00	0.05	<0.03
	Tees Bay Inshore Dredge Disposal 0-2	54° 40.822'N	01° 02.987'W	2.90	1.10	0.11	<0.03
	Tees No. 5 Buoy	54° 38.910'N	01° 08.450'W	0.64	1.18	0.03	<0.03
	Tees Dabholm Gut (Radar Channel)	54° 36.760'N	01° 08.600'W	26	15	2.2	<0.03
	Tees No. 12 Buoy	54° 37.900'N	01° 09.300'W	2.3	<1.00	0.06	<0.03
	Tees No. 15 Buoy	54° 37.170'N	01° 09.200'W	13	9.9	0.40	<0.03
	Tees Outfalls	54° 37.190'N	01° 09.000'W	30	20	0.73	<0.03
	Tees No. 19 shallow	54° 34.630'N	01° 09.550'W	2.8	1.2	0.09	<0.03
	Tees No. 19 Buoy	54° 36.600'N	01° 09.300'W	1.2	<1.00	0.04	<0.03
	Tees Ramp Outfall(ICI 4)	54° 36.280'N	01° 09.800'W	1.9	1.5	0.08	<0.03
	Tees ICI 2	54° 35.840'N	01° 10.400'W	2.2	1.6	0.10	<0.03
	Tees No 27 Buoy NW	54° 35.350'N	01° 11.380'W	29	3.9	3.8	<0.03
	Tees No. 27 Buoy mid	54° 35.290'N	01° 11.260'W	12	5.2	3.1	<0.03
	Tees No. 27 Buoy East	54° 35.250'N	01° 11.200'W	9.0	12	0.99	<0.03
	Tees No. 27 Buoy	54° 35.080'N	01° 11.770'W	10	6.5	1.0	<0.03
	Tees Cargo Fleet Wharf	54° 35.030'N	01° 11.700'W	3.6	2.0	0.26	<0.03
	Tees Storage Co.	54° 34.880'N	01° 12.630'W	5.3	13	0.34	<0.03
	Tees Transporter Bridge	54° 35.100'N	01° 13.450'W	8.8	14	0.80	<0.03
	Tees Transporter Bridge shallow	54° 35.080'N	01° 13.560'W	5.3	4.8	0.36	<0.03
	Tees No. 29 Buoy shallow	54° 35.280'N	01° 14.160'W	23	<1.00	3.6	<0.03

Table 2. continued: Concentrations of alkylphenol ethoxylates (µg g^{-1}) in sediment. Sites within the Tees Estuary are shown in bold.

NMMP site	Location	Latitude	Longitude	Nonylphenol	Ethoxylates*	Octylphenol	Bisphenol A
	Tees No. 31 Buoy	54° 35.440'N	01° 14.560'W	11	<1.00	1.9	<0.03
	Tees Bamletts Wharf	54° 35.440'N	01° 14.600'W	2.3	2.0	0.17	<0.03
	Tees Bamletts Bight	54° 35.400'N	01° 15.200'W	0.82	<1.00	0.05	<0.03
	Bremerhaven 1	54° 04.385'N	08° 08.129'E	<0.19	<1.00	<0.01	<0.03
375	Humber	53° 19.100'N	00° 25.284'E	<0.19	<1.00	<0.01	<0.03
385	Wash	53° 08.572'N	00° 33.132'E	<0.19	<1.00	0.01	<0.03
345	Off Humber/Wash	53° 00.043'N	01° 59.992'E	<0.19	<1.00	0.01	<0.03
	New Roughs Disposal site G3	51° 52.273'N	01° 45.827'E	<0.19	<1.00	<0.01	<0.03
	Roughs Tower Capital Disposal	51° 50.110'N	01° 28.020'E	<0.19	<1.00	<0.01	<0.03
	Thames (north of disposal)	51° 43.659'N	01° 23.341'E	<0.19	<1.00	0.01	<0.03
	Thames (central Disposal) G9	51° 40.759'N	01° 18.724'E	<0.19	<1.00	<0.01	<0.03
	Thames (south of Disposal) G9	51° 35.540'N	01° 12.079'E	<0.19	<1.00	<0.01	<0.03
	Roughs Tower Capital Disposal	51° 33.445'N	01° 31.315'E	<0.19	<1.00	0.01	<0.03
465	Thames Wharf	51° 31.298'N	00° 58.386'E	<0.19	<1.00	0.01	<0.03
486	Rye Bay	50° 51.429'N	00° 47.476'E	<0.19	<1.00	0.02	<0.03
	I.o.W. South Disposal Site N4	50° 36.044'N	00° 55.615'W	<0.19	<1.00	0.01	<0.03
	I.o.W. Outer Disposal Site N3	50° 35.118'N	00° 58.869'W	<0.19	<1.00	<0.01	<0.03
536	Lyme Bay	50° 25.810'N	03° 07.312'W	<0.19	<1.00	<0.01	<0.03
	Plymouth	50° 20.936'N	04° 07.798'W	<0.19	<1.00	<0.01	<0.03
	Falmouth	50° 06.226'N	04° 56.272'W	<0.19	<1.00	<0.01	<0.03
	Falmouth	50° 07.001'N	05° 02.424'W	<0.19	<1.00	<0.01	<0.03
605	Celtic Deep	51° 15.119'N	06° 00.186'W	<0.19	<1.00	0.03	<0.03
	Swansea Bay	51° 33.057'N	03° 52.486'W	<0.19	<1.00	<0.01	<0.03
	Swansea Bay	51° 32.681'N	03° 53.225'W	<0.19	<1.00	<0.01	<0.03
	Swansea Bay Transect	51° 32.290'N	03° 54.116'W	<0.19	<1.00	0.01	<0.03
	Swansea Bay Transect	51° 31.775'N	03° 54.802'W	<0.19	<1.00	0.01	<0.03
	Swansea Bay	51° 31.559'N	03° 55.434'W	<0.19	<1.00	0.01	<0.03
	Swansea Bay Transect	51° 31.146'N	03° 56.383'W	<0.19	<1.00	0.01	<0.03
655	Inner Cardigan Bay	52° 16.452'N	04° 20.083'W	<0.19	<1.00	0.01	<0.03
665	Outer Cardigan Bay	52° 22.071'N	04° 53.909'W	<0.19	<1.00	0.01	<0.03
776	Red Wharf Bay	53° 21.973'N	04° 11.317'W	<0.19	<1.00	0.02	<0.03
	TREND	53° 23.618'N	03° 36.560'W	<0.19	<1.00	0.01	<0.03
705	Liverpool Bay	53° 28.288'N	03° 21.054'W	<0.19	<1.00	<0.01	<0.03
795	Off Morecambe Bay	53° 53.371'N	03° 25.380'W	<0.19	<1.00	0.01	<0.03
805	SE Isle of Man	54° 00.000'N	03° 48.787'W	<0.19	<1.00	0.01	<0.03
815	Dundrum Bay	54° 03.976'N	05° 30.063'W	<0.19	<1.00	0.01	<0.03

* *Sum of mono- and diethoxylate*

1.2.3 Discussion

Concentrations are low or undetectable at intermediate or offshore sites. This concurs with 1998, and no further work need be undertaken in these areas. Currently there is no data on 'safe' levels of alkylphenols and their ethoxylates in sediments. Significant concentrations of alkylphenols have been found in the Tees estuary and at dredge disposal sites outside the mouth of the Tees. A sediment core sample was taken at the dredge disposal site and analysed in 2 cm sections. Alkylphenols were found at levels above 1 µg g^{-1} at depths of up to 16 cm.

Alkylphenols are relatively hydrophobic, and will partition into the sediment in preference to the water column. Over time, transport and mixing mechanisms will dilute the alkylphenol concentration within the sediment. The depth of alkylphenols in this core sample would indicate that normal transport and mixing processes are being inhibited, and alkylphenols are building up in the sediment.

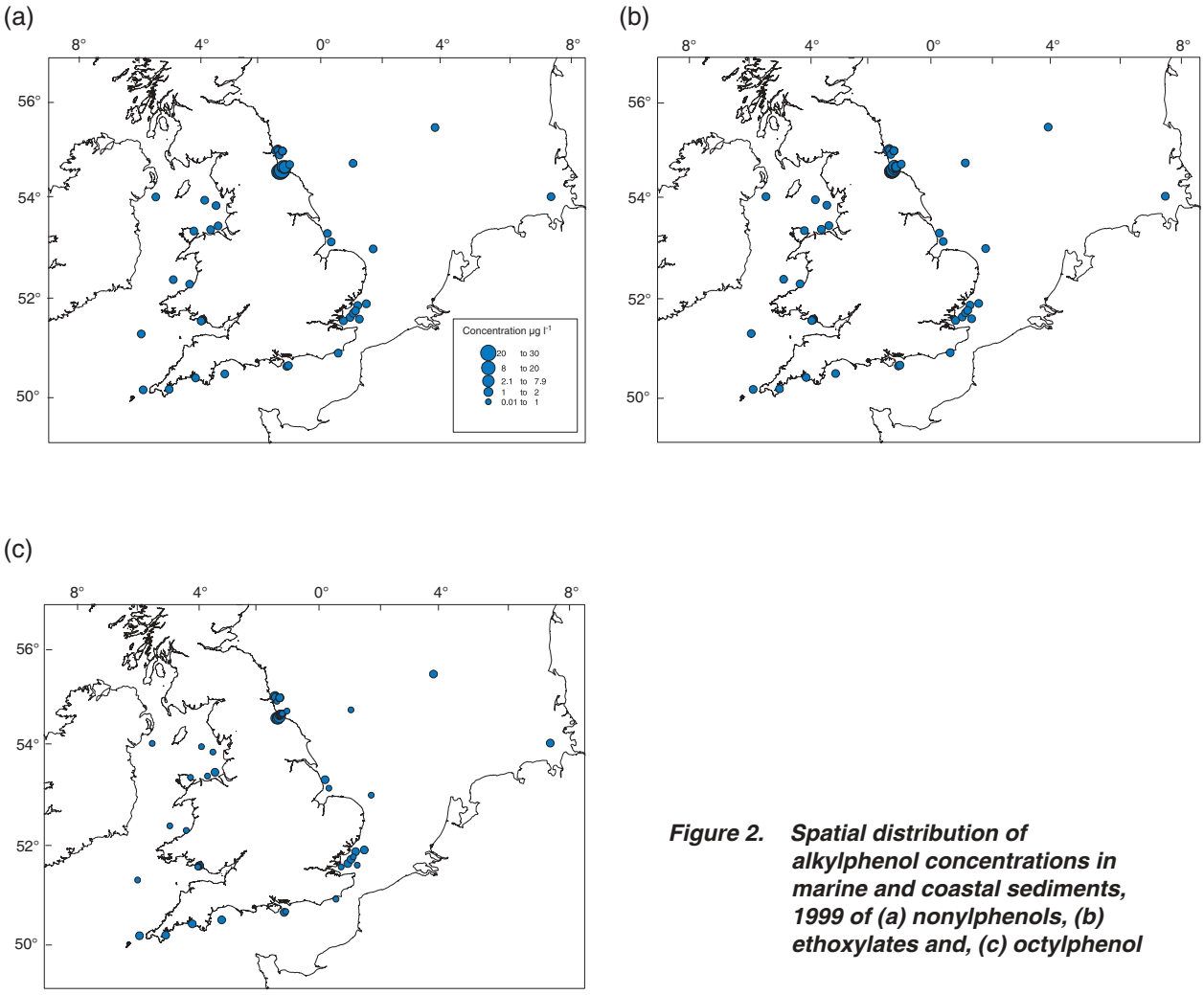

Figure 2. Spatial distribution of alkylphenol concentrations in marine and coastal sediments, 1999 of (a) nonylphenols, (b) ethoxylates and, (c) octylphenol

2. ANTIFOULING PAINT BOOSTER BIOCIDE CONTAMINATION IN HARBOURS, DOCKS AND MARINAS

2.1 Introduction

The term 'booster biocides' encompasses a group of compounds that are added to antifouling paints to enhance their performance in preventing the colonisation of boat hulls by algae and seaweed (See Thomas, 2001 for full review). Until September 2000, the Health and Safety Executive (HSE) approved eight biocides for use in antifouling paints on vessels in the UK. This has since been restricted to zinc pyrithione, zineb and dichlofluanid for boats <25 m in length with SeaNine 211, Irgarol 1051 and chlorothalonil also approved for use on boats >25 m (HSE, 2000). However, during the reporting period, paints containing Irgarol 1051 and diuron were still the most commonly used. The continued input of these biocides from the painted hulls of boats has led to reports of elevated concentrations of two of these biocides, diuron and Irgarol 1051, in areas of high yachting activity and low water exchange rates such as marinas (Ferrer

and Barceló, 1999; Thomas *et al*., 2000; Thomas *et al*., 2001; CEFAS, 2001). In a few marinas, these concentrations are sufficiently high to have the potential to pose a risk to aquatic life (Thomas *et al*., 2001). In the last AEMR (CEFAS, 2001) we reported data on the occurrence of these compounds in coastal and offshore waters. Here we report data on the occurrence of the booster biocides Irgarol 1051, diuron, SeaNine 211, TCMTB (Thiocyanomethylthio benzothiazole), chlorothalonil, and dichlofluanid and the booster biocide degradation products, 1-(3-chlorophenyl)-3,1-dimethylurea (CPDU), 1-(3,4-dichlorophenyl)-3-methylurea (DCPMU), 1-(3,4-dichlorophenyl)urea (DCPU), and (2-(tert-butylamino)-4-amino-6-(methylthio)-1,3,5-triazine (G526575) in harbours, docks and marinas.

2.2 Methods

Two analytical methods were used to determine booster biocide concentrations in sea water and sediments.

Irgarol 1051, diuron, SeaNine 211, TCMTB, GS26575, DCPMU, DCPU, CPDU

All three compounds were simultaneously determined by C18 Solid Phase Extraction (SPE) followed by high performance liquid chromatography coupled to mass spectrometry (HPLC-MS) (Thomas *et al*., 2002).

Dichlofluanid and chlorothalonil

Both compounds were simultaneously extracted by C18 SPE and quantified by gas chromatography coupled to mass spectrometry (Thomas *et al*., 2001).

2.3 Results and discussion

Surface waters

A summary of the booster biocide concentrations determined in seawater samples is presented in Table 3. Irgarol 1051 was detected in many of the samples collected (2 - 305 ng l^{-1}), however, the concentrations determined in marinas were higher than those found in estuaries and dock/port areas where boating activity is comparatively rare and water exchange much higher. GS26575 was determined at generally lower concentrations than Irgarol 1051 (1 - 59 ng l^{-1}) which suggests that the environmental transformation rate of Irgarol 1051 to GS26575 is relatively slow and that the rate of GS26575 degradation is greater than that of its formation. This is supported by laboratory data that show the rate of GS26575 degradation to be six times slower than that of Irgarol 1051 (Thomas *et al*., 2002). However, these data conflict with other studies which have shown Irgarol 1051 and GS26575 to have a similar seawater $t_{½}$ (Hall *et al*., 1999) and proposed that GS26575 has a greater environmental persistence than Irgarol 1051 (Okamura *et al*., 2000).

Diuron was determined in all the collected samples. The highest concentrations were measured in samples taken from the enclosed marina at Hythe. Concentrations above the limit of detection were also measured in samples collected from the Tees, Tyne and Clyde estuaries. It is suggested that the source of diuron in these estuaries is supplemented by non-antifouling paint inputs since the estuaries are subject to relatively low volumes of shipping and yachting when compared to Southampton Water. DCPMU, the primary product of aerobic diuron degradation, was only detected where diuron concentrations were >150 ng l^{-1}. DCPMU was determined to be present at between <1 ng l^{-1} and 78 ng l^{-1}. The secondary product of aerobic diuron degradation, DCPU, was only detected in samples collected from Hythe marina and at concentrations <6 ng l^{-1}. It would therefore appear that the main products of aerobic diuron degradation are found in areas where diuron concentrations are relatively high (e.g. an enclosed marina such as Hythe). The comparatively low persistence of these products when compared to diuron suggest that their mechanism of formation is much slower than that of their removal and it is therefore usual that only low ng l^{-1} concentrations of these compounds were measured. SeaNine 211, TCMTB, chlorothalonil, dichlofluanid, nor DCPMU were determined in any of the samples collected.

Marine sediments

Low concentrations of biocides were determined in marine sediments collected from Southampton Water (Table 4). Of the six biocides and four metabolites analysed, only Irgarol 1051, GS26575, and diuron were found at concentrations above the limits of detection of the methods used (0.1 µg kg^{-1}). Irgarol 1051 was detected at concentrations of between 0.3 and 3.5 µg kg^{-1}, diuron between 0.4 and 6.2 µg kg^{-1}, and GS26575 between 0.1 and 0.3 µg kg^{-1}. These concentrations are within the ranges reported previously (Thomas *et al*., 2000). When compared with the concentrations measured in surface waters this suggests that very little partition of all three compounds occur between water and sediment.

2.4 Conclusions

From the data presented it appears that Irgarol 1051 and diuron are the only antifouling paint biocides currently used in sufficient quantities to be measured in the marine environment. Unsurprisingly, samples collected from areas of high boating activity and low water exchange had the highest concentrations of biocides present. The data presented will provide a useful baseline for when antifouling paint biocide use patterns change with the proposed 2003 global ban on TBT and the UK HSE's September 2000 restrictions on booster biocide use.

Table 3. Concentrations of selected booster biocides in surface water samples collected from areas of boating and shipping activities in the UK

Station	Location	Position		Biocide Concentration (µg l^{-1})†					
		Latitude	Longitude	Irgarol 1051	GS 26575	Diuron	DCPMU	DCPU	CPMU
	Southampton Water								
1	Fawley Refinery	50° 50.245'N	01° 19.495'W	0.003	0.001	0.037	< 0.001	< 0.001	< 0.001
2	Cracknore Hard	50° 52.203'N	01° 23.119'W	0.004	0.002	0.041	< 0.001	< 0.001	< 0.001
3	Power Station	50° 53.629'N	01° 25.068'W	0.002	0.001	0.043	< 0.001	< 0.001	< 0.001
4	Docks (Upper)	50° 54.365'N	01° 25.922'W	0.002	0.001	0.035	< 0.001	< 0.001	< 0.001
5	Docks (Lower)	50° 53.922'N	01° 24.663'W	0.001	0.013	0.026	< 0.001	< 0.001	< 0.001
6	Ocean Village (Marina)	50° 53.699'N	01° 23.428'W	0.007	0.001	0.067	< 0.001	< 0.001	< 0.001
7	Hamble - Swanick Marina	50° 52.873'N	01° 17.988'W	0.028	0.004	0.260	< 0.001	< 0.001	< 0.001
8	Hamble - Mercury Yacht Harbour	50° 52.233'N	01° 18.550'W	0.030	0.005	0.258	< 0.001	< 0.001	< 0.001
9	Hamble - Port Hamble Marina	50° 51.608'N	01° 18.619'W	0.027	0.017	0.128	< 0.001	< 0.001	0.005
10	Hythe Marina 1	50° 52.448'N	01° 23.808'W	0.305	0.044	1.005	0.011	0.004	< 0.001
11	Hythe Marina 2	50° 52.518'N	01° 23.938'W	0.294	0.059	0.951	0.012	0.004	< 0.001
12	Hythe Marina 3	50° 52.443'N	01° 24.097'W	0.242	0.059	0.995	0.014	0.006	< 0.001
13	Hythe Marina 4	50° 52.334'N	01° 23.922'W	0.277	0.055	1.249	0.014	0.003	< 0.001
	River Tees								
14	D/S Dabholm Gut	54° 36.999'N	01° 09.072'W	< 0.001	0.008	0.297	0.028	< 0.001	< 0.001
15	Dabholm Gut	54° 36.270'N	01° 09.401'W	0.001	0.004	0.333	0.036	< 0.001	< 0.001
16	U/S Dabholm Gut	54° 36.069'N	01° 09.955'W	0.001	0.003	0.334	0.034	< 0.001	< 0.001
17	Port Authority	54° 35.550'N	01° 10.869'W	0.002	0.005	0.163	0.018	< 0.001	< 0.001
18	Mid Channel Buoy	54° 34.945'N	01° 12.571'W	0.003	0.004	0.430	0.074	< 0.001	< 0.001
19	Transporter Br.	54° 35.136'N	01° 13.864'W	0.003	0.004	0.462	0.055	< 0.001	< 0.001
	River Tyne								
20	Team Confluence	54° 57.481'N	01° 38.216'W	0.003	< 0.001	0.185	< 0.001	< 0.001	< 0.001
21	Tyne Bridge	54° 58.248'N	01° 35.632'W	0.009	< 0.001	0.186	< 0.001	< 0.001	< 0.001
22	Hebburn	54° 58.986'N	01° 31.935'W	0.005	< 0.001	0.254	0.026	< 0.001	< 0.001
23	Howdon STW	54° 59.263'N	01° 27.726'W	0.002	< 0.001	0.385	0.037	< 0.001	< 0.001
24	D/S Howdon STW	54° 59.782'N	01° 26.568'W	0.002	0.002	0.181	0.020	< 0.001	< 0.001
25	Lloyds Hayling	55° 00.577'N	01° 25.763'W	0.005	0.007	0.261	0.038	< 0.001	< 0.001
	River Clyde								
26	M8 Road Bridge	55° 51.344'N	04° 16.283'W	0.002	< 0.001	0.388	0.043	< 0.001	< 0.001
27	U/S Shieldhall STW	55°.51.610'N	04° 17.923'W	0.001	0.002	0.413	0.062	< 0.001	< 0.001
28	D/S Shieldhall STW	55° 52.172'N	04° 20.634'W	0.002	< 0.001	0.392	0.034	< 0.001	< 0.001
29	Cart Confluence	55° 52.762'N	04° 24.574'W	0.001	0.002	0.151	0.025	< 0.001	< 0.001
30	D/S Dalmuir STW	55° 53.785'N	04° 24.799'W	0.025	0.009	0.775	0.078	< 0.001	< 0.001
31	Erskine Bridge	55° 55.121'N	04° 27.688'W	0.004	0.003	0.262	0.021	< 0.001	< 0.001
	Firth of Forth								
32	Grangemouth	56° 02.179'N	03° 40.928'W	0.002	< 0.001	0.018	< 0.001	< 0.001	< 0.001
33	D/S Kibagie paper mill	56° 04.238'N	03° 43.912'W	< 0.001	< 0.001	0.016	< 0.001	< 0.001	< 0.001
34	Alloa STW	56° 06.240'N	03° 47.634'W	0.001	< 0.001	0.043	< 0.001	< 0.001	< 0.001
35	U/S Alloa STW	56° 06.592'N	03° 48.590'W	< 0.001	< 0.001	0.023	< 0.001	< 0.001	< 0.001
36	Forth Bridge	55° 59.874'N	03° 24.335'W	0.006	< 0.001	0.098	< 0.001	< 0.001	< 0.001

† *SeaNine 211, TCMTB, chlorothalonil, and dichlofluanid were not detected.*

Table 4. Concentrations of selected booster biocides in sediment samples collected from Southampton Water

Station	Location	Biocide concentration (µg kg^{-1})†		
		Irgarol 1051	GS26575	Diuron
1	Fawley Refinery	0.4	0.2	0.4
2	Cracknore Hard	0.6	0.1	1.4
3	Power Station	0.3	0.1	0.8
4	Docks (Upper)	0.3	0.1	0.7
5	Docks (Lower)	0.4	0.0	0.9
6	Ocean Village (Marina)	0.6	0.3	1.4
7	Hamble - Swanick Marina	1.1	0.2	2.3
8	Hamble - Mercury Yacht Harbour	1.2	0.2	1.7
9	Hamble - Port Hamble Marina	0.4	0.1	1.3
10	Hythe Marina	3.5	0.3	6.2

† *CPMU, DCPMU, DCPU, SeaNine 211, TCMTB, chlorothalonil and dichlofluanid were not detected.*

3. FURTHER INVESTIGATIONS INTO THE OCCURRENCE OF POLYBROMINATED DIPHENYL ETHER (PBDE) RESIDUES IN SEDIMENTS AND BIOTA

In AEMR No. 53 (CEFAS, 2001) we described the background to the work of the Burnham Laboratory in the area of brominated flame retardants and highlighted the occurrence of polybrominated diphenyl ether (PBDE) residues in pilot scale surveys. Here we review the ongoing work and the data collected so far. Much of this work has already been published, or is in the process of being published. Other work is presented for the first time.

3.1 Analytical methodology

PBDEs are amenable to similar techniques to those of other persistent organohalogens such as organochlorine pesticides and polychlorinated biphenyls. In collaboration with colleagues from other institutes we have been involved in extensive method development activities that have been validated through a series of small scale analytical comparison exercises and participation in the first world-wide inter-laboratory study on PBDEs. These interlaboratory trials have demonstrated that these methods are robust and valid for environmental studies. For further details see de Boer *et al.,* 2001 and de Boer, 2000.

3.2 Cormorant livers

Cormorants (*Phalocrocorax carbo*) are pisciverous birds common around the UK coast and increasingly common at inland sites where they exploit a range of fish species. They are, as top predators, a good indicator of environmental levels of contaminants/PBDEs. Livers from birds shot under licence were provided for analysis. Full details are given in Law *et al.* (2002).

Concentrations for the sum of 14 BDE congeners (Σ14BDE) ranged from 1.8-140 μg kg^{-1}. The now familiar BDE profile, often seen in a wide range of biota, is typically seen where the dominant congener is the 2',2'-Tetrabromodiphenyl ether (BDE47) followed by the pentabromo compounds 2,2',4,4',6-Pentabromodiphenyl ether (BDE100) and 2,2',4,4',5-Pentabromodiphenyl ether (BDE99) with lesser amounts of the hexabromo compounds 2,2',4,4',5,5'-Hexabromodiphenyl ether (BDE153) and 2,2',4,4',5,6'-Hexabromodiphenyl ether (BDE154). These compounds also are characteristic of the so call 'penta mix' formulations and probably reflect their use.

Summary BDE profiles for male and female cormorants in two sampling periods are presented graphically in Figure 3. The BDE profiles and concentrations are similar for both sets of samples. Figure 4 shows the concentrations of the dominant BDE47 congener. 6 of the 47 samples (13%) had BDE47 concentrations between 20 and 80 μg kg^{-1} wet weight. Whilst BDE47 was detected in the remainder of the samples, concentrations were below 20 μg kg^{-1} wet weight. No obvious pattern between age, sex or sampling location could be seen.

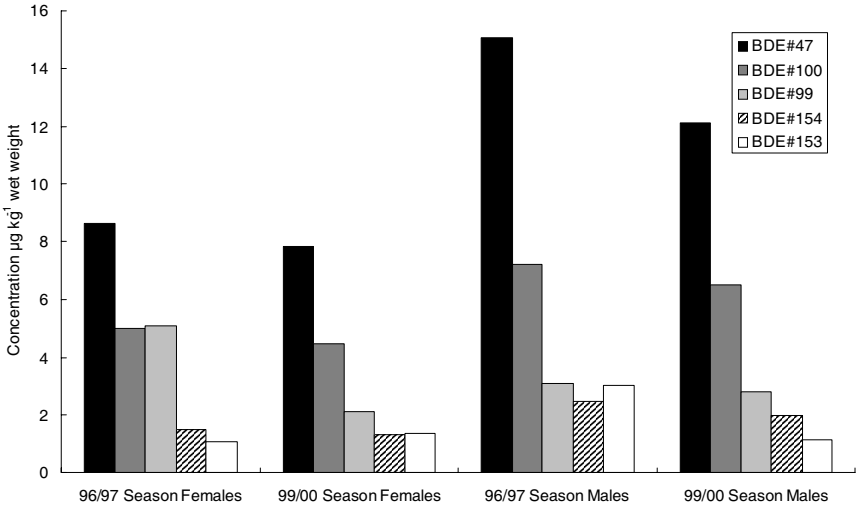

Figure 3. PBDEs in cormorant liver

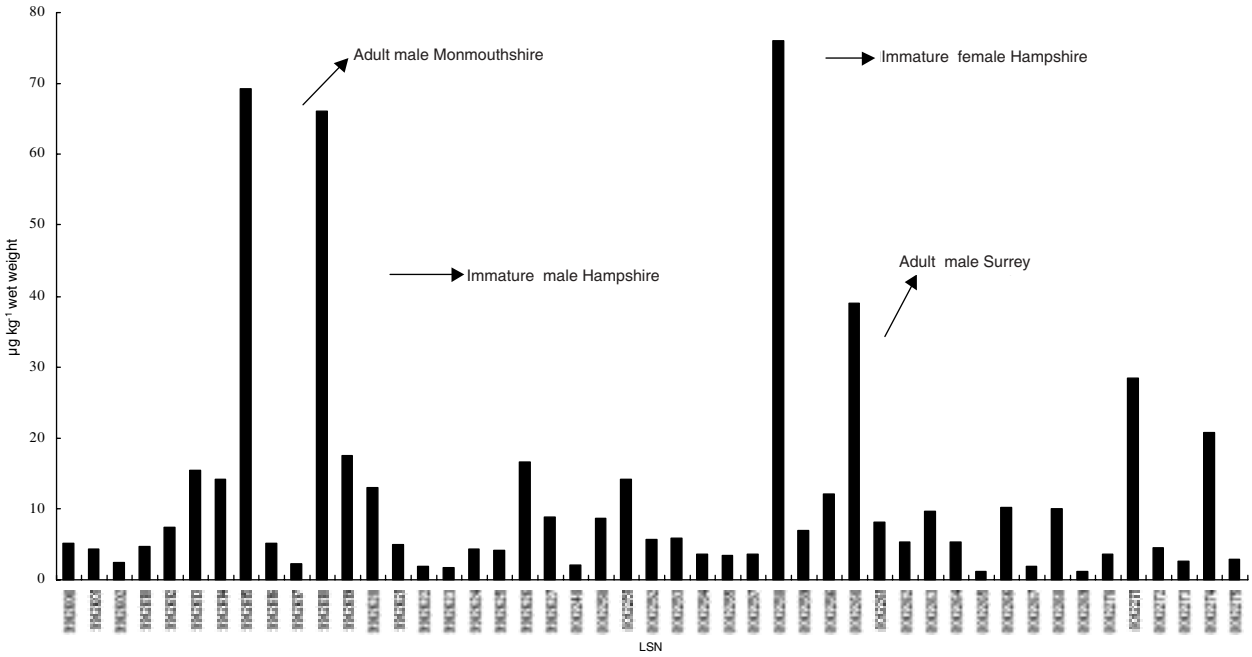

Figure 4. BDE47 in cormorant liver

3.3 Porpoise blubber

Common (harbour) porpoise (*Phocoena phocoena)* blubber samples were collected from bycaught or stranded animals from around the coast of England and Wales originally collected as part of the UK national marine mammals stranding programme (for details see Law *et al.,* 2002). Relative to the cormorant liver concentrations, Σ14BDE concentrations on a wet weight basis were up to two orders of magnitude higher with a range of 'not detected' to 6900 µg kg^{-1}, again with BDE47 dominating the profile contributing from 39 to 88% of the Σ14BDE concentrations.

Figure 5 shows the range of BDE47 concentrations seen. It is interesting to note that the animals with the three highest BDE47 values were all from the north east coast of England. This area is known to also contain elevated

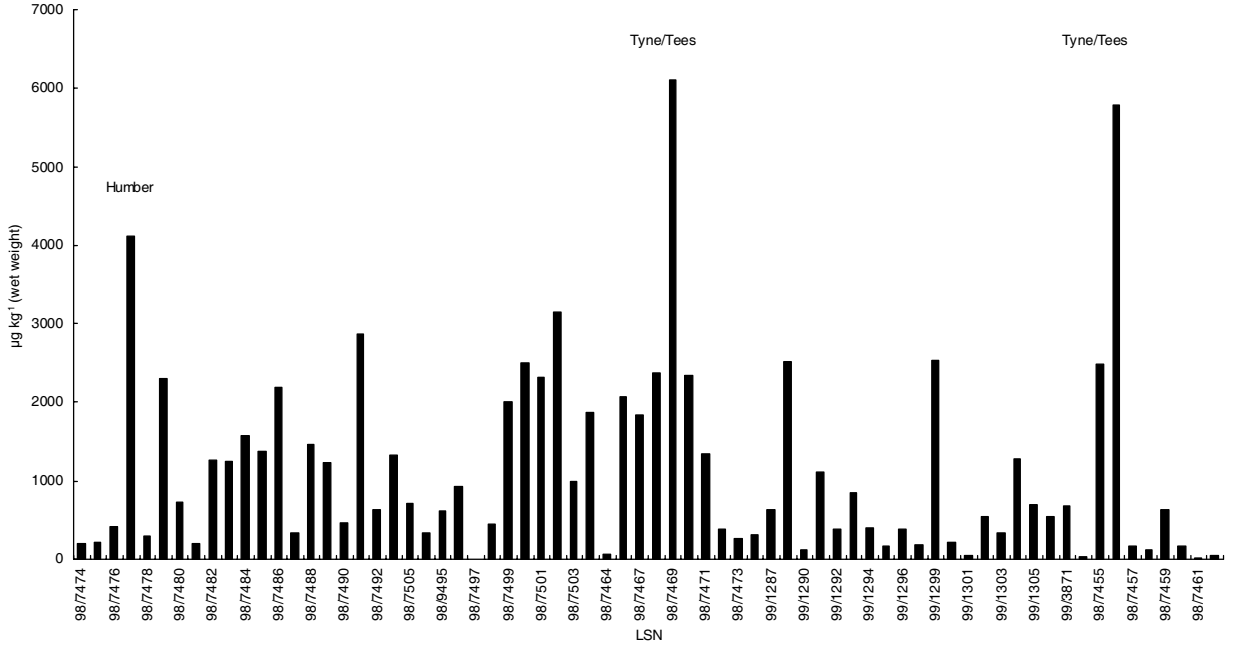

Figure 5. BDE47 in porpoise blubber

BDE concentrations in other biota and sediments which are likely to have resulted from discharges from the River Tees as a result of the historic manufacture and use of 'penta mix' compounds in this area.

3.4 Dab liver

Σ14BDE concentrations on a wet weight basis have also been determined in samples of dab liver (*Limanda limanda*) collected from a number of sites under the auspices of the UK NMMP. The data are presented in Table 5 and Figure 6.

BDEs (again with BDE47 being the most dominant congener) were detected in all samples with highest concentrations being seen in fish from the Sole Pit and off Anglesey.

3.5 Sediment samples from River Tees and Tees Estuary

As a follow on from the earlier reported pilot scale study investigating PBDE residues in Tees sediments (Allchin *et al.,* 1999), a comprehensive study for a broader range of 14 BDEs was conducted and partial results reported (DeBoer and Allchin, 2001). The full data set is presented here in Tables 6-10 and graphically in Figures 7-10. Data are presented in μg kg^{-1} dry weight. For a number of sites in the Lower Tees and Tees Estuary total organic carbon (TOC) and particle size analysis (PSA) were also performed. Although commonly used as data normalisers, no relationship could be found between BDE concentrations and TOC or PSA. These data are however available from the author if required.

Table 5. PBDEs in dab liver

Sample no.	Latitude/ Longitude	Location	% Lipid	μg kg wet^{-1} weight							
				BDE 28	BDE 75	BDE 71	BDE 47	BDE 66	BDE 77	BDE 100	BDE 119
00/675	54.070267° 1.799933°	Dogger	1.6	0.21	<0.5	0.4	13	0.65	<0.5	0.81	<0.5
00/682	54.070267° 1.799933°	Dogger	1.2	0.16	<0.5	0.88	11	0.72	<0.5	1.2	<0.5
00/689	54.070267° 1.799933°	Dogger	4	0.23	<0.5	0.47	11	0.71	<0.5	1	<0.5
00/696	54.070267° 1.179933°	Dogger	2.7	<0.5	<0.5	0.19	5.3	0.89	<0.5	0.82	<0.5
00/787	54.2431° 0.5004°	Sole pit	6.4	1.2	<0.5	2.5	108	2.3	1.1	7.9	<0.5
00/794	54.2431° 0.5004°	Sole pit	3.7	1.1	<0.5	2.7	65	1.9	0.91	5.2	0.39
00/801	54.2431° 0.5004°	Sole pit	3.1	0.48	<0.5	2.3	40	1.4	0.7	4.1	0.28
00/808	54.2431° 0.5004°	Sole pit	4.8	0.75	<0.5	2.8	82	2.1	<0.5	6	0.49
00/1444	53.35925° -4.140617°	Anglesey	7.7	0.62	0.17	4	89	1.6	<0.5	23	0.24
00/1451	53.35925° -4.140617°	Anglesey	4.9	3.4	0.23	17	241	3.5	0.57	42	0.57
00/1423	53.35925° -4.140617°	Anglesey	7.1	<0.5	<0.5	1.9	54	1.1	<0.5	17	<0.5
00/1430	53.35925° -4.140617°	Anglesey	5.6	<0.5	<0.5	3.2	100	1.9	<0.5	25	<0.5
00/1437	53.35925° -4.140617°	Anglesey	5.7	<0.5	<0.5	<0.5	4.8	<0.5	<0.5	2.1	<0.5
00/1209	54.076467° -8.137317°	German Bight	9.7	<0.5	<0.5	<0.5	4	<0.5	<0.5	0.84	<0.5
00/1216	54.076467° -8.137317°	German Bight	11	<0.5	<0.5	<0.5	2.5	<0.5	<0.5	0.76	<0.5
00/1223	50.86195° -0.799383°	Hastings	7.5	<0.5	<0.5	<0.5	13	<0.5	<0.5	2.4	<0.5
00/1231	50.86195° -0.799383°	Hastings	11	<0.5	<0.5	<0.5	9.6	<0.5	<0.5	1.9	<0.5
00/1238	50.86195° -0.799383°	Hastings	15	<0.5	<0.5	<0.5	6.6	<0.5	<0.5	1.7	<0.5
00/1245	50.86195° -0.799383°	Hastings	11	<0.5	<0.5	<0.5	8.2	<0.5	<0.5	2	<0.5
00/1252	50.86195° -0.799383°	Hastings	13	<0.5	<0.5	<0.5	6.8	<0.5	<0.5	<0.5	<0.5

Table 5. continued PBDEs in dab liver

LSN	Latitude/ Longitude	Location	% Lipid	µg kg wet^{-1} weight							
				BDE 99	BDE 85	BDE 154	BDE 153	BDE 138	BDE 190	BDE 209	Sum BDE
00/675	54.070267° 1.799933°	Dogger		0.61	<0.5	0.53	0.66	<0.5	<0.5	<0.5	16.9
00/682	54.070267° 1.799933°	Dogger		0.55	<0.5	0.65	0.61	<0.5	<0.5	<0.5	15.8
00/689	54.070267° 1.799933°	Dogger		0.53	<0.5	0.64	0.68	<0.5	<0.5	<0.5	15.3
00/696	54.070267° 1.179933°	Dogger		<0.5	<0.5	<0.5	1	<0.5	<0.5	<0.5	8.2
00/787	54.2431° 0.5004°	Sole pit		1.7	<0.5	3.6	2	<0.5	<0.5	<0.5	130.3
00/794	54.2431° 0.5004°	Sole pit		1.2	<0.5	3	1.7	<0.5	<0.5	<0.5	83.1
00/801	54.2431° 0.5004°	Sole pit		1.1	<0.5	2.3	1.3	<0.5	<0.5	<0.5	54.0
00/808	54.2431° 0.5004°	Sole pit		1.8	<0.5	3.8	2	<0.5	<0.5	<0.5	101.7
00/1444	53.35925° -4.140617°	Anglesey		1.3	<0.5	8.7	1.2	<0.5	<0.5	<0.5	129.8
00/1451	53.35925° -4.140617°	Anglesey		2.7	<0.5	19	2.9	<0.5	<0.5	<0.5	332.9
00/1423	53.35925° -4.140617°	Anglesey		0.9	<0.5	8.6	1	<0.5	<0.5	<0.5	84.5
00/1430	53.35925° -4.140617°	Anglesey		<0.5	<0.5	10	1.8	<0.5	<0.5	<0.5	141.9
00/1437	53.35925° -4.140617°	Anglesey		<0.5	<0.5	0.68	<0.5	<0.5	<0.5	<0.5	7.6
00/1209	54.076467° -8.137317°	German Bight		1.1	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	5.9
00/1216	54.076467° -8.137317°	German Bight		0.86	<0.5	0.71	<0.5	<0.5	<0.5	<0.5	4.8
00/1223	50.86195° -0.799383°	Hastings		<0.5	<0.5	1.5	<0.5	<0.5	<0.5	<0.5	16.9
00/1231	50.86195° -0.799383°	Hastings		<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	11.5
00/1238	50.86195° -0.799383°	Hastings		<0.5	<0.5	0.99	<0.5	<0.5	<0.5	<0.5	9.3
00/1245	50.86195° -0.799383°	Hastings		<0.5	<0.5	1.3	<0.5	<0.5	<0.5	<0.5	11.5
00/1252	50.86195° -0.799383°	Hastings		<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	6.8

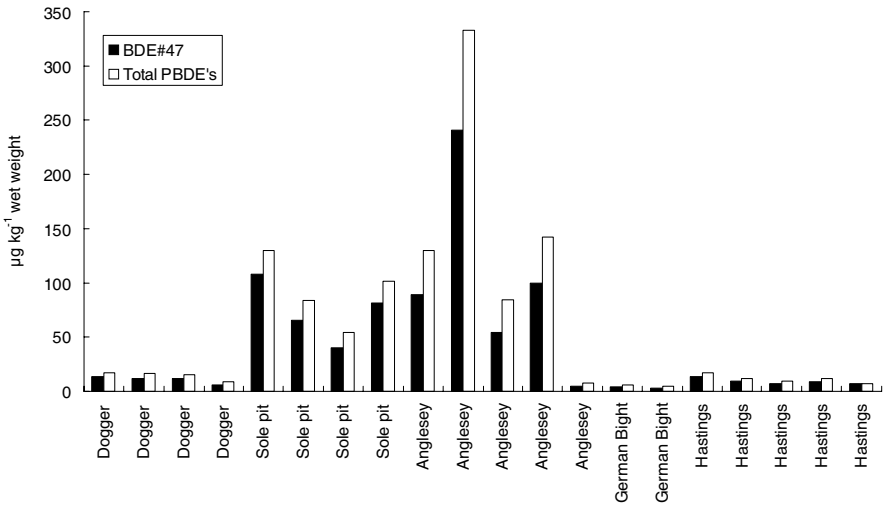

Figure 6. PBDEs in dab liver

Table 6. PBDEs in sediment - Upper Tees (Cow Green reservoir to Croft on Tees)

Sample no.	Latitude/ Longitude	Location	µg kg^{-1} dry weight (<2000 µm)							
			BDE28	BDE75	BDE71	BDE47	BDE66	BDE77	BDE100	BDE119
00/2590	54.67235° -2.231033°	Langdon Beck	<0.2	<0.2	<0.2	0.2	<0.2	<0.2	<0.2	<0.2
00/2591	54.62155° -2.081283°	R.Tees at Middleton in Teesdale	<0.2	<0.2	<0.2	0.2	<0.2	<0.2	<0.2	<0.2
00/2592	54.603683° -2.00655°	R.Tees at Eggleston Bridge	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
00/2593	54.528283° -1.892683°	R.Tees at Abbey Bridge - Barnard Castle	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
00/2594	54.541433° -1.778817°	R.Tees at Winston Gate Bridge	<0.2	<0.2	<0.2	0.36	<0.2	<0.2	<0.2	<0.2
00/2595	54.535° -1.674183°	R.Tees at Piercebridge	<0.2	<0.2	<0.2	0.27	<0.2	<0.2	<0.2	<0.2

			BDE99	BDE85	BDE154	BDE153	BDE138	BDE190	BDE209	Sum BDE (ex 209)
00/2590	54.67235° -2.231033°	Langdon Beck	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
00/2591	54.62155° -2.081283°	R.Tees at Middleton in Teesdale	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
00/2592	54.603683° -2.00655°	R.Tees at Eggleston Bridge	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
00/2593	54.528283° -1.892683°	R.Tees at Abbey Bridge - Barnard Castle	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	0.6	<0.2
00/2594	54.541433° -1.778817°	R.Tees at Winston Gate Bridge	0.3	<0.2	<0.2	<0.2	<0.2	<0.2	2	1
00/2595	54.535° -1.674183°	R.Tees at Piercebridge	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2

Table 7. PBDEs in sediment - Middle Tees (Croft on Tees to Tees Barrage)

Sample no.	Latitude/ Longitude	Location	µg kg^{-1} dry weight (<2000 µm)							
			BDE28	BDE75	BDE71	BDE47	BDE66	BDE77	BDE100	BDE119
00/2596	54.486033° -1.56215°	R.Tees upstream of confluence with R.Skerne at Croft-on-Tees	<0.2	<0.2	<0.2	0.21	<0.2	<0.2	<0.2	<0.2
00/2597	54.479533° -1.552417°	R.Tees downstream of confluence with R.Skerne at Croft-on-Tees	0.51	0.27	15	12	0.38	0.25	3.4	7.4
00/2605	54.48435° -1.5197°	R.Tees at Hurworth Bridge	<0.2	<0.2	<0.2	2	<0.2	<0.2	0.33	<0.2
00/2604	54.492233° -1.409167°	R.Tees at Newsham Grange	<0.2	<0.2	<0.2	0.94	0.44	<0.2	0.11	<0.2
00/2603	54.512117° -1.353983°	R.Tees at Yarm Bridge	<0.2	<0.2	<0.2	2.1	<0.2	0.12	0.38	<0.2
00/2601	54.537083° -1.32875°	R.Tees at Preston Hall	<0.20	<0.2	0.23	0.74	<0.2	<0.2	0.14	<0.2
00/2602	54.499717° -1.31495°	R.Leven at Leven Bridge	0.51	<0.2	<0.2	8.9	0.74	<0.2	1.3	5.4

			BDE99	BDE85	BDE154	BDE153	BDE138	BDE190	BDE209	Sum BDE (ex 209)
00/2596	54.486033° -1.56215°	R.Tees upstream of confluence with R.Skerne at Croft-on-Tees	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	1	<0.2
00/2597	54.479533° -1.552417°	R.Tees downstream of confluence with R.Skerne at Croft-on-Tees	25	1.2	2.1	4.2	0.47	0.71	107	73
00/2605	54.48435° -1.5197°	R.Tees at Hurworth Bridge	2.5	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	5
00/2604	54.492233° -1.409167°	R.Tees at Newsham Grange	0.42	<0.2	<0.2	<0.2	<0.2	<0.2	0.8	2
00/2603	54.512117° -1.353983°	R.Tees at Yarm Bridge	2.1	<0.2	0.25	0.31	<0.2	<0.2	4.8	5
00/2601	54.537083° -1.32875°	R.Tees at Preston Hall	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.05	1
00/2602	54.499717° -1.31495°	R.Leven at Leven Bridge	19	0.71	0.96	2.1	0.31	0.45	<0.05	40

Table 8. PBDEs in sediment - Lower Tees (Tees Barrage to Tees Mouth)

Sample no.	Latitude/ Longitude	Location	µg kg^{-1} dry weight (<2000 µm)							
			BDE28	BDE75	BDE71	BDE47	BDE66	BDE77	BDE100	BDE119
00/2600	54.565383° -1.285767°	R.Tees upstream of barrage (south bank)	0.3	<0.2	<0.2	4.2	0.32	<0.2	0.68	<0.2
00/2340	54.59° -1.143333°	Bamlett's Wharf	1.5	<0.2	<0.2	13.3	1.5	<0.2	1.4	<0.2
00/2341	54.590667° -1.243333°	Bamlett's Bight mid-channel	1.2	<0.2	<0.2	16.9	0.8	<0.2	4.3	<0.2
00/2342	54.5915° -1.242667°	Bamlett's Bight North Bank	1.9	<0.2	0.9	14.7	0.9	<0.2	3.8	<0.2
00/2343	54.5875° -1.236667°	Middlesbrough Wharf mid-channel	0.3	<0.2	0.7	2.9	<0.2	<0.2	<0.2	<0.2
00/2344	54.588° -1.236°	Middlesbrough Wharf north bank	0.42	<0.2	0.68	1.9	<0.2	<0.2	<0.2	0.33
00/2345	54.584667° -1.226°	Tees Transporter Bridge	1.1	0.33	6.6	8.8	1.3	<0.2	2	0.56
00/2346	54.585° -1.224167°	Tees Transporter Bridge	2.3	0.53	2.4	<0.2	<0.2	0.11	<0.2	3.5
00/2347	54.581333° -1.2105°	Tees Storage Company	1.9	<0.2	9.3	12	2	<0.2	<0.2	<0.2
00/2348	54.583833° -1.1845°	Cargo Fleet Wharf mid channel	0.4	<0.2	0.5	2.9	0.5	<0.2	0.3	0.34
00/2349	54.584667° -1.196167°	Cargo Fleet Wharf north bank	1.1	<0.2	5.1	8.4	<0.2	<0.2	1.1	0.25
00/2350	54.5875° -1.186667°	No. 25 Buoy South Bank	1.9	<0.2	6.7	0.46	2	<0.2	0.79	0.7
00/2351	54.588167° -1.187667°	No. 25 Buoy mid channel	1.3	<0.2	ND	6.2	<0.2	<0.2	ND	ND
00/2353	54.589167° -1.189667°	No. 25 Buoy north bank	0.99	<0.05	3.5	3.9	0.2	<0.05	<0.05	0.29
00/2352	54.597333° -1.10068°	ICI North Tees terminal mid-channel	1.8	0.26	6.5	11	2.3	0.11	1.9	0.36

			BDE99	BDE85	BDE154	BDE153	BDE138	BDE190	BDE209	Sum BDE (ex 209)
00/2600	54.565383° -1.285767°	R.Tees upstream of barrage (south bank)	7.2	0.17	0.62	0.89	0.13	<0.2	10.3	15
00/2340	54.59° -1.143333°	Bamlett's Wharf	17	0.9	1.1	1.7	0.3	<0.2	76	39
00/2341	54.590667° -1.243333°	Bamlett's Bight mid-channel	28	1.1	4	4.4	0.4	<0.2	378	61
00/2342	54.5915° -1.242667°	Bamlett's Bight North Bank	18	0.9	5.3	4.7	0.5	<0.2	74	52
00/2343	54.5875° -1.236667°	Middlesbrough Wharf mid-channel	16.0	0.6	4.9	3.9	0.4	<0.2	13	30
00/2344	54.588° -1.236°	Middlesbrough Wharf north bank	1.8	0.19	0.69	0.56	<0.2	<0.2	28	7
00/2345	54.584667° -1.226°	Tees Transporter Bridge	14.7	0.26	<0.2	2.4	0.33	<0.2	164	38
00/2346	54.585° -1.224167°	Tees Transporter Bridge	16.4	0.22	5.6	<0.2	<0.2	<0.2	177	31
00/2347	54.581333° -1.2105°	Tees Storage Company	16	0.23	3.2	3.4	<0.2	<0.2	306	48
00/2348	54.583833° -1.1845°	Cargo Fleet Wharf mid channel	4.9	0.29	0.42	0.61	0.21	<0.2	119	11
00/2349	54.584667° -1.196167°	Cargo Fleet Wharf north bank	<0.2	0.28	1.78	2.1	<0.2	<0.2	140	20
00/2350	54.5875° -1.186667°	No. 25 Buoy South Bank	<0.2	0.2	2.4	2.7	0.25	<0.2	165	18
00/2351	54.588167° -1.187667°	No. 25 Buoy mid channel	2.7	<0.2	0.8	1.3	<0.2	<0.2	55	12
00/2353	54.589167° -1.189667°	No. 25 Buoy north bank	<0.05	0.26	1.2	0.91	0.08	<0.05	55	11
00/2352	54.597333° -1.10068°	ICI North Tees terminal mid-channel	0.08	1.1	1.81	2.36	0.39	<0.05	327	30

Table 9. PBDEs in sediment - Tees Estuary (Tees Mouth and Tees Bay)

Sample no.	Latitude Longitude	Location	µg kg^{-1} dry weight (<2000 µm)							
			BDE28	BDE75	BDE71	BDE47	BDE66	BDE77	BDE100	BDE119
00/2356	54.604667° -1.163333°	ICI No.4 Buoy	1.6	0.15	0.94	12	2.5	<0.05	2.1	0.19
00/2357	54.61° -1.155°	Shell Oil jetty mid-channel	0.88	0.22	3.8	8.5	1.6	0.1	1.5	0.45
00/2358	54.6105° -1.159167°	Shell Oil jetty north bank	3	0.09	1.4	18	3.6	<0.05	3.2	2
00/2359	54.612667° -1.143333°	Entrance to Dabholm Gut	0.91	<0.05	0.05	11	0.08	<0.05	2.4	0.17
00/2360	54.6195° -1.153333°	East of No.15 buoy mid channel	1.6	0.15	4.9	0.06	2.7	0.16	1.8	0.64
00/2361	54.619833° -1.15°	East of No.15 buoy south bank	2.6	<0.05	10	18	4.2	<0.05	2.8	2
00/2332	54.626167° -1.180833°	Seal sands	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
00/2337	54.631167° -1.174167°	Seaton-onTees	<0.2	<0.2	<0.2	1.8	0.2	<0.2	0.36	<0.2
00/2338	54.631667° -1.155°	Off Bran sands mid-channel	3.4	<0.2	0.98	32.2	2.8	<0.2	4.5	<0.2
00/2702	54.686317° -1.150067°	Tees Bay G6	<0.05	<0.05	<0.05	0.15	<0.05	<0.05	<0.05	<0.05
00/2703	54.69405° -1.1203°	Tees Bay G4	0.04	<0.05	0.36	1.6	<0.05	<0.05	0.4	<0.05
00/2362	54.663333° -1.171667°	Tees Bay Off Seaton sands	<0.05	<0.05	<0.05	0.3	<0.05	<0.05	0.06	<0.05
00/2363	54.656333° -1.163333°	Tees Bay Off Seaton sands	<0.05	<0.05	<0.05	0.36	<0.05	<0.05	<0.05	<0.05
00/2704	54.6821° -1.0835°	Tees Bay G1	<0.05	<0.05	<0.05	0.23	<0.05	<0.05	0.06	0.03
00/2365	54.6485° -1.140833°	Tees entrance No.5 Buoy	<0.05	<0.05	0.19	0.85	0.14	<0.05	0.15	0.03
00/2701	54.665267° -1.143217°	Tees Bay G5	<0.05	<0.05	<0.05	0.12	<0.05	<0.05	0.05	<0.05
00/2700	54.6568° -1.114°	Tees Bay G3	<0.05	<0.05	<0.05	0.12	<0.05	<0.05	0.06	<0.05
00/2705	54.6616° -1.083483°	Tees Bay G2	<0.05	<0.05	0.05	0.37	<0.05	<0.05	0.09	0.04
00/2713	54.680367° -1.049783°	Tees Bay Inshore disposal site C4	0.42	0.01	0.16	3.6	0.67	<0.05	0.64	0.14
00/2367	54.636333° -1.110667°	Off Coatham sands	<0.05	<0.05	<0.05	0.14	<0.05	<0.05	<0.05	<0.05
00/2368	54.629167° -1.093°	Off Coatham sands	<0.05	<0.05	<0.05	0.19	0.06	<0.05	0.06	0.04
00/2706	54.673567° -1.027317°	Tees Inshore disposal site C6	<0.05	<0.05	<0.05	1.1	0.15	<0.05	0.17	0.05
00/2757	54.733183° -1.88305°	NMMP 295 Off Tees	<0.05	<0.05	<0.05	0.54	<0.05	<0.05	<0.05	<0.05

Table 9. continued. PBDEs in sediment - Tees Estuary (Tees Mouth and Tees Bay)

Sample no.	Latitude Longitude	Location	µg kg^{-1} dry weight (<2000 µm)							
			BDE99	BDE85	BDE154	BDE153	BDE138	BDE190	BDE209	Sum BDE (ex 209)
00/2356	54.604667° -1.163333°	ICI No.4 Buoy	21	1.3	1.7	0.06	0.37	<0.05	236	44
00/2357	54.61° -1.155°	Shell Oil jetty mid-channel	15	0.84	0.08	1.6	0.29	0.11	209	35
00/2358	54.6105° -1.159167°	Shell Oil jetty north bank	29	1.64	3.6	0.08	0.58	<0.05	246	66
00/2359	54.612667° -1.143333°	Entrance to Dabholm Gut	17	0.96	1.1	0.77	0.13	<0.05	1400	35
00/2360	54.6195° -1.153333°	East of No.15 buoy mid channel	21	1.2	1.4	2.3	<0.05	<0.05	281	38
00/2361	54.619833° -1.15°	East of No.15 buoy south bank	36	2.1	2.2	4.2	<0.05	<0.05	812	84
00/2332	54.626167° -1.180833°	Seal sands	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
00/2337	54.631167° -1.174167°	Seaton-onTees	3.6	<0.2	0.4	0.4	<0.2	<0.2	10.2	7
00/2338	54.631667° -1.155°	Off Bran sands mid-channel	38	1.6	3.7	4.6	0.5	<0.2	117	92
00/2702	54.686317° -1.150067°	Tees Bay G6	0.19	<0.05	0.05	0.07	<0.05	<0.05	2.8	<0.05
00/2703	54.69405° -1.1203°	Tees Bay G4	2.8	0.12	0.27	0.28	<0.05	<0.05	5	6
00/2362	54.663333° -1.171667°	Tees Bay Off Seaton sands	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
00/2363	54.656333° -1.163333°	Tees Bay Off Seaton sands	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
00/2704	54.6821° -1.0835°	Tees Bay G1	0.19	<0.05	0.06	0.07	<0.05	<0.05	<0.05	1
00/2365	54.6485° -1.140833°	R. Tees entrance No. 5 Buoy	1.2	<0.05	0.12	0.17	0.08	<0.05	9.6	3
00/2701	54.665267° -1.143217°	Tees Bay G5	0.11	<0.05	0.05	0.06	<0.05	<0.05	<0.05	<0.05
00/2700	54.6568° -1.114°	Tees Bay G3	0.13	<0.05	0.05	<0.05	<0.05	<0.05	<0.05	<0.05
00/2705	54.6616° -1.083483°	Tees Bay G2	0.46	<0.05	0.08	0.1	<0.05	<0.05	3.7	1
00/2713	54.680367° -1.049783°	Tees Bay Inshore disposal site C4	0.16	0.43	0.56	1	0.18	<0.05	17	8
00/2367	54.636333° -1.110667°	Off Coatham sands	0.13	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
00/2368	54.629167° -1.093°	Off Coatham sands	0.18	<0.05	0.05	0.06	<0.05	<0.05	<0.05	1
00/2706	54.673567° -1.027317°	Tees Inshore disposal site C6	1.2	0.08	0.14	0.17	<0.05	<0.05	17	3
00/2757	54.733183° -1.88305°	NMMP 295 Off Tees	0.63	<0.05	<0.05	<0.05	<0.05	<0.05	<0.5	1

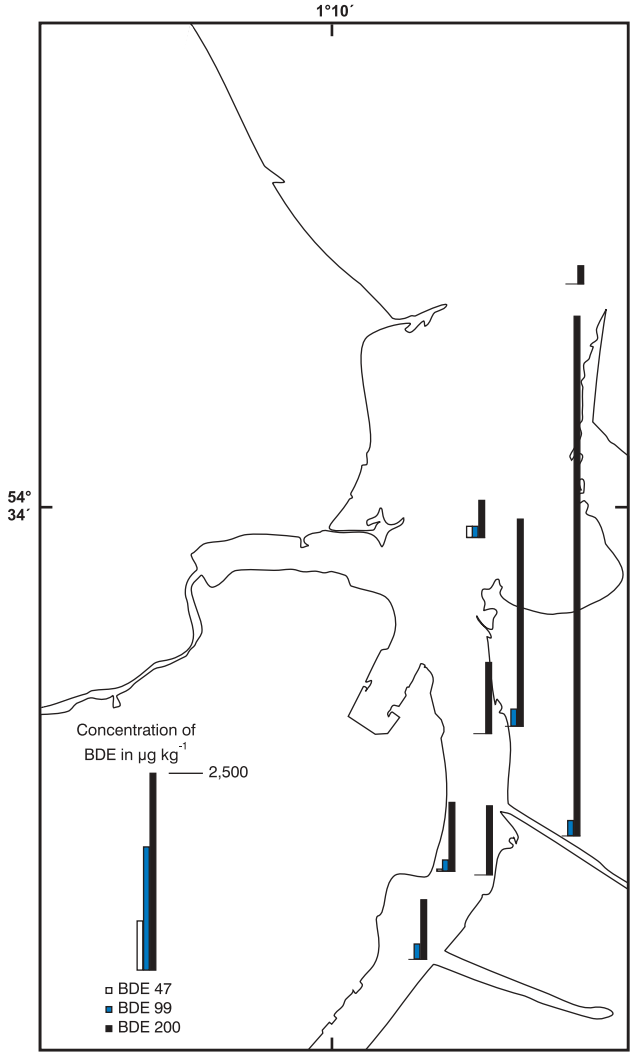

Figure 7. Tees Estuary BDE levels in biota

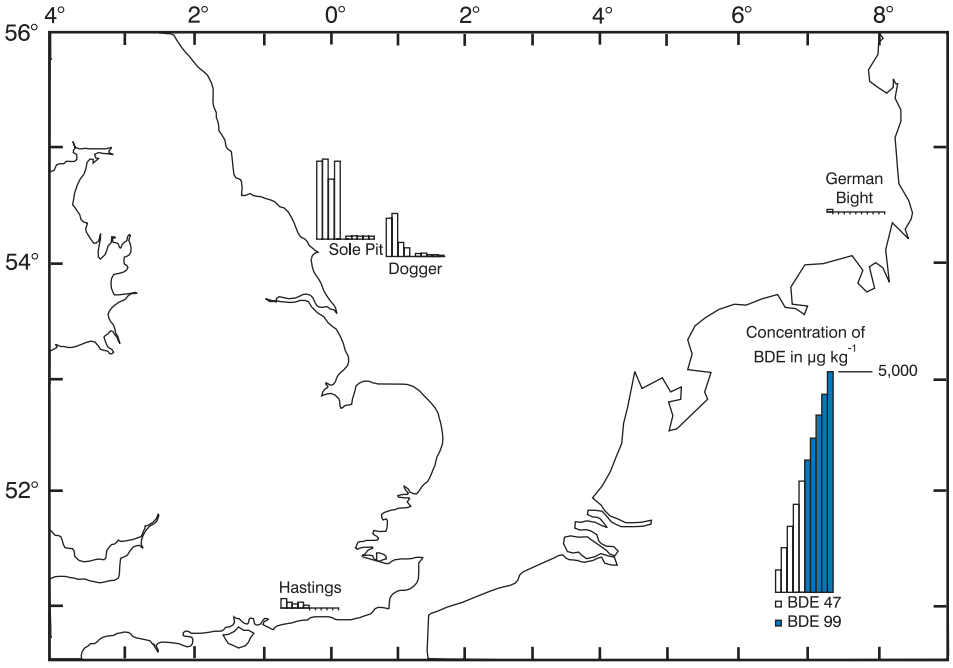

Figure 8. Concentrations of BDE in dab liver

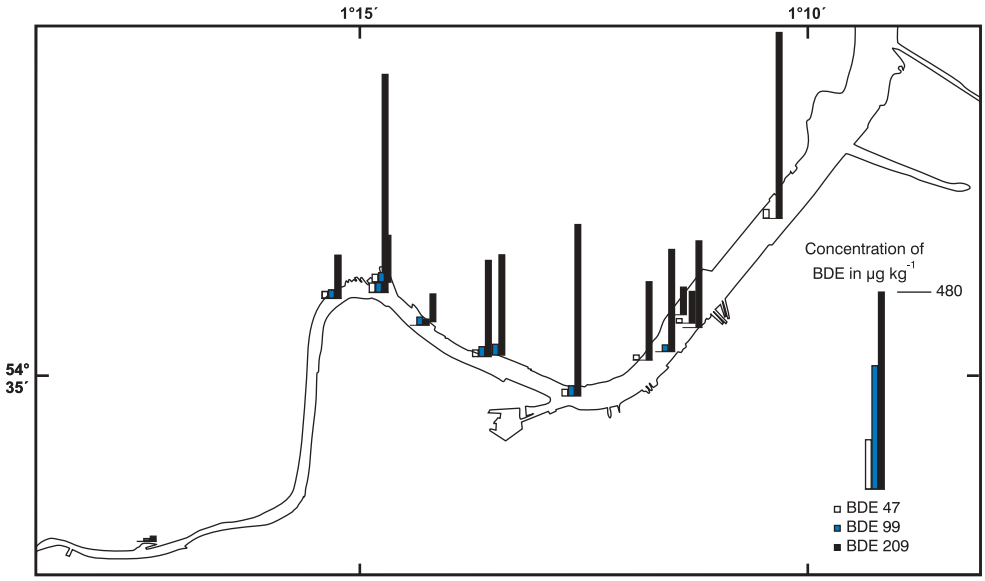

Figure 9. Lower Tees BDE levels in sediments

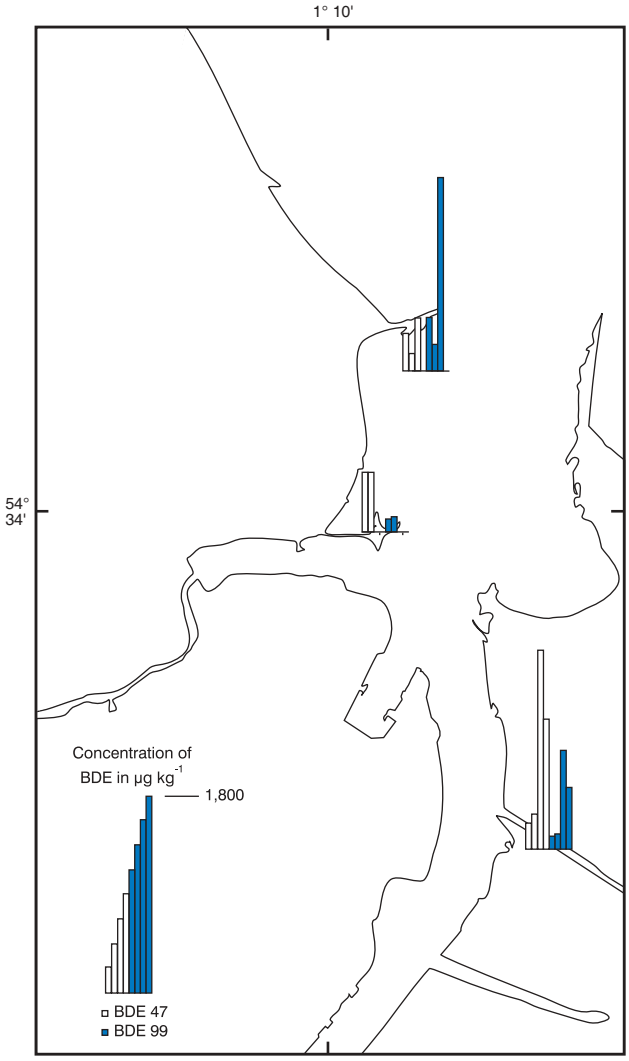

Figure 10. Tees Estuary (Tees Mouth and Tees Bay) BDE levels in sediments

For ease of data interpretation the survey area was divided into four sections. The Upper Tees (Cow Green reservoir to Croft on Tees), Middle Tees (Croft on Tees to Tees Barrage), Lower Tees (Tees Barrage to Tees mouth) and Tees Estuary (Tees Mouth and Tees Bay). The summary data for these four sections are presented graphically in Figure 11.

For the Upper Tees, BDE residues were generally absent or present at concentrations close to the detection limit, with the exception of BDE209 in a single sample from Winston Gate. This had a BDE209 concentration of 1 μg kg^{-1} dry weight.

Below the confluence of the River Skerne with the River Tees, BDE concentrations increased markedly.

BDE209 concentration in the Lower Tees sediments were often (but not exclusively) higher than the sum of the other BDE congeners studied. The BDE concentrations were highly variable in the estuary probably due to a combination of diffuse and point source inputs and the varied nature of the river beds which contains areas of gravel, sand, silt and cohesive muds (Table 10).

On leaving the estuary, BDE levels generally declined quite rapidly in the sandier sediments although there were localised pockets of elevated levels and there was some evidence of elevated levels of BDEs in the region of the main Tees dredged spoil disposal ground.

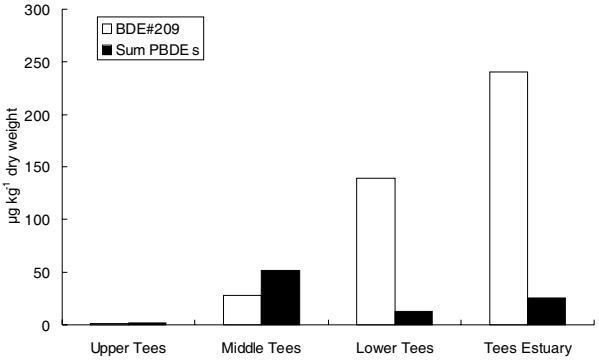

Figure 11. BDEs in the River Tees

3.6 Biota samples from the Tees Estuary

A number of samples of fish and invertebrate species were also collected from the Tees Estuary and BDE residues determined. The results are presented in Table 11. BDE residues were also commonly found in these samples.

Table 10. River Tees sediment summary data (BDE209)

	Dry weight	Normalised to <63 μm	Normalised to TOC
Upper Tees			
Mean	1.3		
Standard Deviation	0.99		
Range	<0.2 - 2		
Number	6		
Middle Tees			
Mean	28.4		
Standard Deviation	52.43		
Range	<0.05 - 288		
Number	6		
Lower Tees			
Mean	139.15	178.42	2331.97
Standard Deviation	116.67	143.67	2037.82
Range	10.3 - 378	12.17 - 474.22	181.66 - 7368.42
Number	15	15	15
Tees Estuary			
Mean	240.45	410.18	3637.85
Standard Deviation	398.39	701.98	4441.08
Range	<0.05 - 92	<0.05 - 2470	<0.05 - 13600
Number	22	16	16

Table 11. PBDEs in Tees biota

Sample no.	Species	Tissue	Latitude/ Longitude	Location	% Lipid	µg kg^{-1} weight wet							
						BDE 28	BDE 75	BDE 71	BDE 47	BDE 66	BDE 77	BDE 100	BDE 119
01/1231/1	Crangon	Whole	54.615° -1.151667°	North Gare	0.2	<0.2	0.01	0.41	0.7	0.05	<0.2	0.24	0.07
01/1231/2	Crangon	Whole	54.615° -1.151667°	North Gare	0.4	<0.2	<0.2	0.39	0.65	0.05	<0.2	0.23	0.07
01/1232	Nereis sp	Whole	54.615° -1.151667°	North Gare	0.4	0.03	0.03	0.35	1.9	0.18	<0.2	1.1	<0.2
01/1236/1	Asterias sp	Tentacle	54.653333° -1.163333°	Seaton	2.2	0.2	<0.2	0.87	12	0.35	<0.2	2.7	<0.2
01/1236/2	Asterias sp	Whole	54.653333° -1.163333°	Seaton	2.2	0.21	<0.2	0.82	12	0.35	0.1	2.5	<0.2
01/1244/1	Sprats	Whole	54.653333° -1.163333°	Dabholm	2	0.07	0.09	2.2	4.5	0.18	0.02	1.7	0.05
01/1244/2	Sprats	Whole	54.653333° -1.163333°	Dabholm	1.6	0.06	0.1	2.3	4.8	0.21	0.05	1.8	0.2
01/1252/1	Whiting	Whole	54.653333° -1.163333°	Dabholm	0.4	0.21	0.03	1.2	7.1	0.5	0.03	2	0.07
01/1252/2	Whiting	Muscle	54.653333° -1.163333°	Dabholm	0.6	0.18	0.02	1.1	6.9	0.48	0.02	1.9	0.07
01/1259	Flounder	Liver	54.615° -1.151667°	North Gare	7.5	0.01	<0.2	0.13	1.7	0.06	<0.2	0.39	0.04

					BDE 99	BDE 85	BDE 154	BDE 153	BDE 138	BDE 190	BDE 209	Sum BDE
01/1231/1	Crangon	Whole	54.615° -1.151667°	North Gare	0.95	0.03	0.17	0.2	<0.2	<0.2	<0.5	2.83
01/1231/2	Crangon	Whole	54.615° -1.151667°	North Gare	0.9	<0.2	0.17	0.19	<0.2	<0.2	<0.5	2.65
01/1232	Nereis sp	Whole	54.615° -1.151667°	North Gare	6.9	<0.2	1.43	1.1	<0.2	<0.2	<0.5	13.02
01/1236/1	Asterias sp	Tentacle	54.653333° -1.163333°	Seaton	2.5	<0.2	0.2	<0.2	<0.2	<0.2	<0.5	18.82
01/1236/2	Asterias sp	Whole	54.653333° -1.163333°	Seaton	2.4	<0.2	1	<0.2	<0.21	<0.2	<0.5	19.38
01/1244/1	Sprats	Whole	54.653333 -1.163333°	Dabholm	2.2	<0.2	0.65	0.26	0.08	<0.2	<0.5	12
01/1244/2	Sprats	Whole	54.653333° -1.163333°	Dabholm	2.2	<0.2	0.71	0.29	0.08	<0.2	<0.5	12.8
01/1252/1	Whiting	Whole	54.653333° -1.163333°	Dabholm	3.5	<0.2	0.86	0.28	0.03	<0.2	<0.5	15.81
01/1252/2	Whiting	Muscle	54.653333° -1.163333°	Dabholm	3.2	<0.2	0.79	0.25	0.03	<0.2	<0.5	14.94
01/1259	Flounder	Liver	54.615° -1.151667°	North Gare	0.44	<0.2	0.29	0.15	<0.2	<0.2	<0.5	3.21

3.7 Discussion

The interest in brominated flame retardants (especially BDEs) as environmental contaminants has grown in recent years, although they were first reported in the early 1980s (Andersson and Blomkvist, 1981). A review of the data available at the time (de Boer and Allchin, 2001) for Europe indicated that in some areas concentrations of BDE congeners associated with the 'penta mix' formulation may be declining whereas those of BDE209 may still be on the increase. Although high levels of BDE209 can be found in sediment levels, in most biota amounts remain very low, often below existing detection limits. This indicates that it has very limited bioaccumulative properties. Our investigations indicate that BDEs are common environmental contaminants in the UK, and can be found in sediments and in biota at all trophic levels. The elevated concentrations in the sediments of the River Tees and the associated dredging activities maybe a source to the wider North Sea (Boon *et al.,* 2002).

Restrictions in the manufacture and use of 'penta mix' formulations in the European Union (EU) should lead to a decline in concentrations of those congeners originating from these formulations. However for the time being the use of 'octa mix' and 'deca mix' formulations remain unrestricted. Questions remain over the bioaccumulative potential of these mixtures and indeed, the question of debromination of higher brominated congeners remains an issue where continued vigilance is required.

Further work is being conducted at the Burnham Laboratory and investigations are also underway on other brominated flame retardants of concern, most notably hexabromocyclododecane (HBCD) and tetrabromobisphenol A (TBBP-A).

4. WINTER 1999 AND 2000 NUTRIENT CONCENTRATIONS IN THE COASTAL WATERS OF ENGLAND AND WALES

4.1 Introduction

Under favourable conditions, nutrient enrichment of marine waters may give rise to eutrophication. The standards for judging the extent of eutrophication and the (eu)trophic status of marine waters are not fully developed, therefore, as an interim measure, and to allow historic comparisons, recourse is made to the monitoring of nutrient concentrations in coastal and offshore waters in winter (OSPARCOM, 1997). Nutrient concentrations alone are not direct evidence for eutrophication problems and there must also be comparable monitoring of biological and chemical indicators (chlorophyll, dissolved oxygen) in winter and summer to assess the trophic status of a given area. OSPAR (Oslo and Paris Commission) is developing common assessment methodologies for eutrophication status.

The monitoring undertaken under the auspices of the UK NMMP has been to determine winter (January to March) nutrient species (ammonium, nitrate, nitrite, phosphate and silicate) concentrations in the coastal waters of England and Wales. In addition, measurements have been taken of chlorophyll and dissolved oxygen concentrations to set the scene for the monitoring of possible eutrophication symptoms in the following spring and summer. The summer situation in the coastal waters of England and Wales is the subject of on-going work. One particular strategy that is being tested to better achieve our aim of monitoring the consequences of any nutrient enrichment is continuous measurement using automated measuring/sampling instrumentation.

4.2 Methods

All sampling and analysis was conducted using protocols which have been adopted by OSPAR as Eutrophication Monitoring Guidelines.

4.2.1 Sampling

A suite of water samples was taken at NMP sites in the coastal and offshore waters of England and Wales in January/February 1999 and England alone in 2000. Discrete surface and depth samples were taken at each station from the research vessel *CIROLANA*. Water samples were collected in Niskin bottles mounted on a CTD-rosette. Samples for nutrients and supporting parameters were handled, stored and pre-treated as discussed in the sampling and handling sections of the JAMP Eutrophication Monitoring Guidelines (OSPARCOM, 1997). At each site the spot samples reported here were collected within the 30 to 35 psu salinity range.

4.2.2 Sample analysis

Water samples were analysed immediately for ToXN (Total Oxidised Nitrogen), nitrite, phosphate, silicate and the supporting parameters; salinity, temperature, chlorophyll and suspended load. Nitrate concentration was determined by difference (ToxN minus nitrite concentration).

Nutrient determination was based on colourimetric methods developed by Bendschneider and Robinson (1952), Murphy and Riley (1962), Grasshoff *et al*. (1983) and Kirkwood (1996). Analytical quality assurance (precision and accuracy) was achieved by lab intercomparison procedures and by reference to the National Marine Analytical Quality Control (NMAQC) scheme.

Temperature and salinity were measured in-situ by CTD probes; calibration was achieved by reference to discrete samples measured using a Guildline 'Autosal' 8400 laboratory salinometer.

Chlorophyll was determined by filtering a known sample volume through Whatman glass-fibre filters and extracting into 90% acetone. A Turner Designs (Model 10) filter fluorometer was used to measure extracted pigment (Tett, 1987).

4.3 Results and discussion

4.3.1 Description of January/February 1998 NMP station nutrient distribution

Figures 12a to f display the spatial distribution of nutrient, dissolved oxygen and chlorophyll concentrations around England and Wales for the NMP sites sampled in January/February 1999 with Figures 13a to e showing the nutrient concentrations for England in 2000. A more restricted survey in 2000 was due to additional effort directed towards the deployment of automated data buoys (Smart Buoy) and the resulting pressures on research vessel survey time. As indicated in the legend, the diameter and shade of the circles is proportional to the parameter concentration. The actual sample concentration is listed next to the symbol.

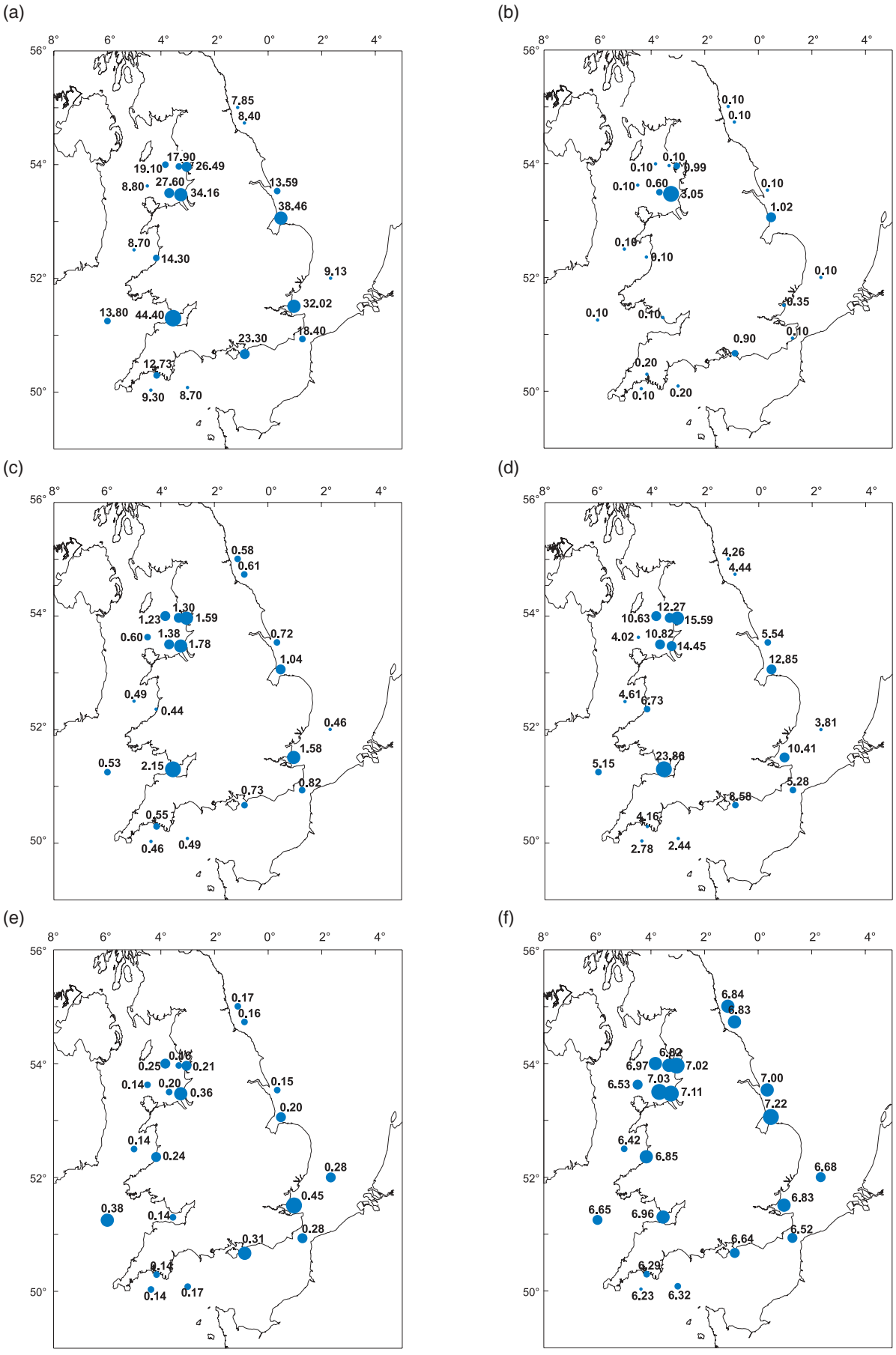

Figure 12. Surface concentrations at NMP stations in February 1999: (a) TOxN (µmol l^{-1}), (b) ammonia (µmol l^{-1}), (c) phosphate (µmol l^{-1}), (d) silicate (µmol l^{-1}), (e) chlorophyll (µg l^{-1}) and (f) oxygen (mg l^{-1})

TOxN, Nitrate and Nitrite
The TOxN concentration comprises of nitrate (NO_3) and nitrite (NO_2) species. Generally, the nitrite fraction is small (<1% of TOxN) compared to the nitrate contribution. TOxN concentrations are consistently lower in offshore waters than in the coastal samples which are influenced by nutrient inputs from rivers, direct discharges and diffuse run-off.

In 1999, TOxN concentration was highest in the Severn Estuary (44.4 µmol l^{-1}) and in the Thames, Wash and Liverpool bay areas, 32.02, 38.46 and 34.16 µmol l^{-1} respectively (Figure 12a). The Humber and the area off the Lune/Wyre also have high TOxN concentrations compared to other coastal sites. The winter nitrate concentrations in Atlantic water entering the North Sea are typically circa 12 µmol l^{-1} (North Sea Task Force, 1993) but lower concentrations (5.5 – 7.7 µmol l^{-1}) are found at some of the offshore NMP sites (Figure 12a). There is an emerging understanding that winter nutrient behaviour in the shelf seas is more dynamic than previously thought. It is important to understand the dynamics if winter values in the offshore waters are used as the baseline for seasonal changes in nitrogen and other nutrient concentrations.

In 2000, highest TOxN concentrations (Figure 13a) were found in the Thames and Humber regions (26.6 and 27.6 µmol l^{-1} respectively) with significantly lower concentrations elsewhere.

Ammonium
The concentrations of ammonium in 1999 are low (<1 µmol l^{-1}) in most estuarine, coastal and especially, offshore waters. However, there are several areas (Liverpool Bay, Thames, Solent, off Lune/Wyre and Humber/Wash) where surface water concentrations are obviously higher than at other sites (Figure 12b). This may be related either to the nature of the local estuaries, to the presence of large urban waste water discharges and also to the recycling of nitrogen. The concentration of ammonium is dependant on a range of factors and has varied at these sites year on year.

Offshore samples show consistently lower ammonium concentrations resulting from the reduction in the influence of estuarine inputs and progressive dilution of estuarine signals.

In 2000, ammonium concentrations are all <1.5 µmol l^{-1} (Figure 13b) with highest concentrations measured offshore in the North Sea. This reflects the inherent variability in the ammonium concentration showing both the transitory nature of ammonium and the difficulties in measuring low concentrations.

Phosphate
The spatial distribution of phosphate concentrations in 1999 largely follow those of TOxN. The Severn has the highest concentration of relatively elevated levels in the Thames, Liverpool Bay and Lune areas (Figure 12c). As with the other nutrients there is a decline in phosphate concentrations away from the input.

In 2000, phosphate concentrations (Figure 13c) are highest in the Thames and Humber regions with lowest concentrations elsewhere reflecting a similar pattern to TOxN.

Silicate
The spatial distribution of silicate concentration indicates that concentrations in 1999 are higher off the Severn, Thames, Wash, Liverpool Bay and Lune/Wyre (Figure 12d). All the other sample concentrations lie below 10 µmol l^{-1} and as with the other major nutrients, silicate concentrations decrease offshore.

An offshore gradient in silicate concentration is evident in 2000 (Figure 13d) with all concentrations <10.0 µmol l^{-1}. It is expected that the concentration of silicate, assuming no biological uptake in the estuaries will reflect the magnitude of freshwater discharge to the area.

Chlorophyll
Chlorophyll concentrations are chemical indicators of phytoplankton biomass. In January 1999 there are uniformly low levels (<0.5 µg l^{-1}) of chlorophyll in the coastal waters of England and Wales (Figure 12e). Chlorophyll levels in 2000 (Figure 13e) in English coastal waters were also low (<0.55 µg l^{-1}). Under ambient winter conditions the light levels are expected to be sufficiently low to prevent significant primary production and the development of phytoplankton biomass. Highest concentrations of chlorophyll in both years are present in the Thames.

Dissolved oxygen
Dissolved oxygen (DO) measurements were taken at depth in the water column. The range of dissolved oxygen concentrations measured during this survey ranged from 6 to 7 ml l^{-1}. This high level of dissolved oxygen concentration suggests that oxygen demand is low at these sites and that water mixing is good. These measurements will act as the basis for comparison with the situation in the spring and summer following the start of the growing season.

4.4 Conclusions

The main features of the spatial survey of winter 1999 and 2000 are:

- The offshore stations have lower nutrient concentrations as expected from the progressive dilution of terrestrial nutrient discharges (agricultural run-off, industrial and urban waste water discharges). The generally lower concentrations than those found in in-flowing Atlantic water indicate nutrient turnover in shelf waters even during winter.

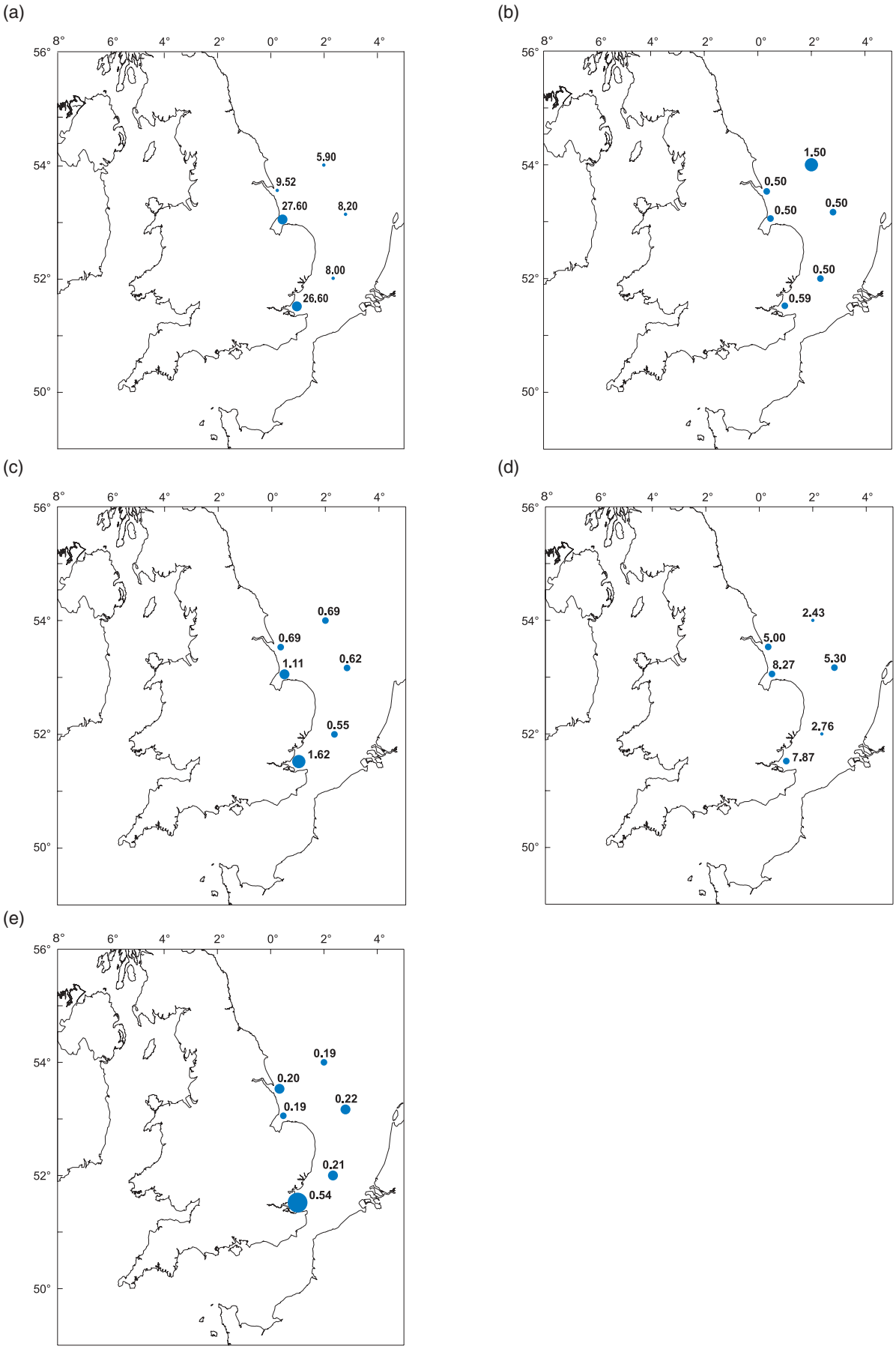

Figure 13. Surface concentrations at NMP stations in February/March 2000: (a) TOxN (µmol l^{-1}), (b) ammonia (µmol l^{-1}), (c) phosphate (µmol l^{-1}), (d) silicate (µmol l^{-1}) and (e) chlorophyll (µg l^{-1})

- Estuarine systems can be identified as the major sources of nutrients to the coastal waters of England and Wales. The Severn, Thames, Humber, Wash and estuaries discharging to Liverpool Bay are the main contributors of nutrients to the waters around England and Wales.

The variability of nutrient concentrations that result from discharge to coastal waters, as well as differences in the relative concentration of the different nutrient species (TOxN versus ammonium or phosphate) is a complex function of the processes taking place at a specific location. We seek to measure nutrient concentration in the winter when the, arguably, biological impact on the measured concentration is minimal. It is clear that in some circumstances this assumption may be incorrect and care should be taken in interpreting the presented dataset. Analysis of the temporal changes will form the subject of the next phase of NMMP sampling.

5. TRIAL MONITORING OPERATIONS USING AUTOMATED INSTRUMENTATION

We are seeking to improve measurement of nutrient status and ecosystem response through the use of new generation automated instrumentation and new observational platforms. In order to achieve this aim, CEFAS has developed Smart Buoy, an instrumented platform measuring NMMP parameters such as nutrient and chlorophyll concentration together with other environmental control variables.

5.1 Methods

Only a brief description of the methods will be given. The measurement of nutrient concentration reported here is restricted to nitrate and nitrite. Measurements are made using a NAS2E *in situ* nutrient analyser that employs a colourimetric method for determination of nitrate+nitrite concentration. The instrument is calibrated in the laboratory prior to field use by comparison of NAS2E estimates of nutrient concentration on discrete seawater samples with known concentrations. In the field, an on-board standard provides additional calibration data.

Chlorophyll concentration is determined using a fluorometer calibrated against extracted chlorophyll concentration from discrete samples collected in the field (Mills and Tett, 1990). Extracted chlorophyll concentration is determined according to the method in section 4.2.2.

5.2 Results and discussion

Data are shown in Figure 14 from an offshore NMP site number 475 (Outer Gabbard) in the southern North Sea. Nitrate (nitrate+nitrite) concentration was recorded hourly through April and into May 2000.

The preliminary data set presented illustrates the potential of automated *in situ* measurements to augment the current NMP programme and provide data suitable for analysis of temporal trends. The data shows the degree of variability that may be encountered in the parameters of interest and the potential shortfall of an annual survey in effectively measuring the overwinter nutrient concentration.

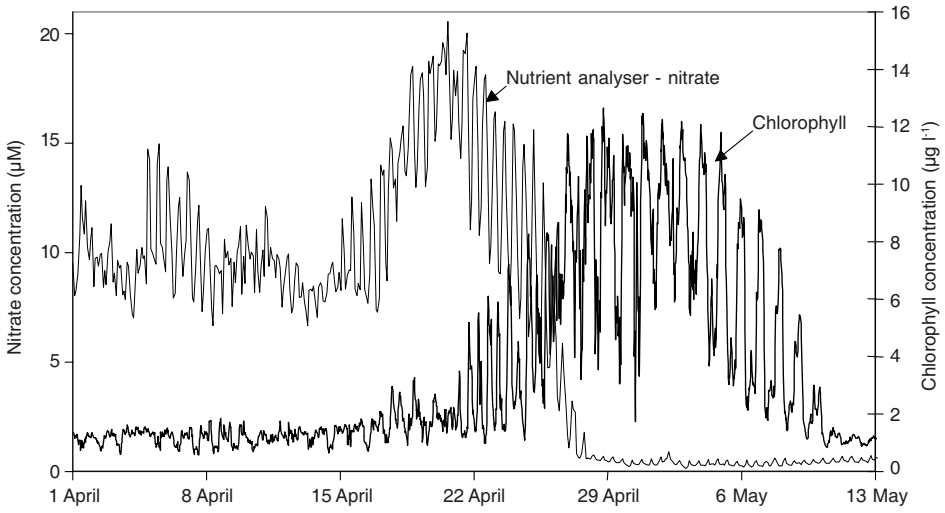

Figure 14. Time series of nitrate and chlorophyll concentration derived from measurements carried out on Smart Buoy in Spring 2000 at the Outer Gabbard in the southern North Sea

6. POLYCYCLIC AROMATIC HYDROCARBONS IN BIVALVE MOLLUSCS FROM DESIGNATED HARVESTING AREAS AROUND ENGLAND AND WALES

6.1 Introduction

The EU Shellfish Hygiene Directive 91/492/EEC (European Communities, 1991a) was implemented in Great Britain in 1993 by regulations made under the Food Safety Act 1990. Bivalve molluscs that are marketed must originate from production areas which have been classified according to the degree to which samples of shellfish from each area contain levels of *E.coli.* This determines whether the shellfish can go directly for human consumption or are required to be treated beforehand. The types of classification are as follows:

Class A - molluscs can be harvested for direct human consumption

Class B - molluscs can go for human consumption after purification in an approved plant or after relaying in an approved class A relaying area, or after an EC approved heat treatment process

Class C - molluscs can go for human consumption only after relaying for at least 2 months in an approved relaying area followed, where necessary, by treatment in a purification centre, or after an EC approved heat treatment process

Prohibited - shellfish harvesting is prohibited

When marketed for consumption, live molluscan shellfish must meet a number of 'end product' standards. These include a requirement that the shellfish should not contain toxic or objectionable compounds such as trace metals, organochlorine compounds, hydrocarbons and polycyclic aromatic hydrocarbons (PAHs), in such quantities that the calculated dietary intake exceeds the permissible daily intake. A study of commercial molluscs was carried out to provide information, which would help the United Kingdom (UK) to meet its commitments under the EU legislation.

Summaries of the first 2 years of this study were reported in CEFAS, 2000 and 2001. Full details can be found in Jones *et al*. (1998, 2000). A summary of the third and final year of the study is given below. Full details can be found in Jones *et al*. (2002).

6.2 Polycyclic aromatic hydrocarbons

PAHs arise mainly from either combustion sources (pyrolytic), such as forest fires, power generation and motor vehicle exhausts, where the unsubstituted parent compounds will predominate, or from crude and refined oils (petrogenic), where the alkylated PAH will predominate. Industrial sources of PAH include oil refinery operations, pyrolysis of wood to form charcoal, manufacture of electrolytic aluminium using graphite electrodes, coke production and the synthesis of chemicals from petroleum feedstocks. In addition, oil spills either accidental or deliberate, can cause high concentrations of PAHs and related compounds to enter the aquatic environment locally. Power generation using fossil fuels, industrial space heating and the combustion of fuels in internal combustion engines all produce PAH rich emissions (Neff, 1979; Gittins *et al*., 1997; Hankin *et al*., 1996; Taracredi and Cardenas, 1991 and Law and Biscaya, 1994).

PAHs are composed of three or more aromatic rings fused together, (naphthalene with only two fused rings is a bicyclic aromatic hydrocarbon, but is commonly studied with PAH). The low molecular weight PAHs of two to three rings (naphthalenes, phenanthrenes and anthracenes) exhibit significant acute toxicity to aquatic and terrestrial organisms. The high molecular weight PAHs of four to seven rings (chrysene, pyrenes and fluoranthenes) do not show this acute toxicity. However, all of the proven PAH carcinogens are in the high molecular weight group (Neff, 1979).

In this study, nineteen PAHs or groups were determined (Table 12) encompassing both petrogenic and pyrolytic sources; some of these are known mammalian carcinogens. Of these 19 PAHs, 4 have been identified as being of particular concern over their long term human health effects; benzo[*a*]pyrene (B*a*P), benz[*a*]anthracene, benzo[*b*]fluoranthene (B*b*F) and dibenz[*a,h*]anthracene (Department of Health, 1998); the structural isomer benzo[*k*]fluoranthene is not believed to be as biologically active as either B*b*F or benzo[*j*]fluoranthene. B*a*P however, is considered to be one of the most potent of the carcinogenic PAHs (Hardin *et al*., 1992). The PAHs themselves, due to their hydrophobic aromatic character, are not active but the metabolites produced during phase I oxidation may be carcinogenic (Smolowitz *et al*., 1992; Law and Hellou, 1999).

Table 12. PAHs determined in this study

Compound no.	IUPAC name	Abbreviation
1	naphthalene	N
2	methyl naphthalenes	C1-N
3	dimethyl naphthalenes	C2-N
4	trimethyl naphthalenes	C3-N
5	phenanthrene	P
6	anthracene	A
7	methyl phenanthrenes	C1-P
8	fluoranthene	Fl
9	pyrene	Py
10	methyl pyrenes	C1-Py
11	benz[*a*]anthracene	B*a*A
12	chrysene	Ch
13	2,3-benzanthracene	2,3-BA
14	benzo[*b*]+[*j*]+[*k*]fluoranthenes	BFs
15	benzo[*e*]pyrene	B*e*P
16	benzo[*a*]pyrene	B*a*P
17	perylene	Pe
18	indeno[1,2,3-*cd*]pyrene	I1,2,3-cdP
19	benzo[*ghi*]perylene	B*ghi*P

6.3 Sample collection

Between February 1995 and May 1996, approximately 200 samples of shellfish were collected from classified harvesting areas. These included samples of mussels (*Mytilus edulis*), cockles (*Cerastoderma edule*), Native oysters (*Ostrea edulis*) and Pacific oysters (*Crassostrea gigas*). Samples were collected and transported to the laboratory overnight in insulated containers. On arrival at the laboratory samples were frozen at –20°C until processing.

Samples consisted of 10 individuals for oysters and 50 individuals for mussels and cockles. For each sample, the shellfish were thawed and measurements of length, total weight and shell weight were recorded for each individual. The whole body tissue (as eaten) was then removed from the shell, bulked and homogenised and empty shell weight was recorded. Twenty-gram aliquots of homogenised tissue were stored in hexane-washed glass jars sealed with hexane-rinsed aluminium foil and screw caps, again at –20°C until submitted for chemical analysis. Hexane extractions were analysed for a suite of 19 PAHs using Fluorescence Spectrometry (UVF) and coupled gas chromatography-mass spectrometry (GC-MS).

6.4 Bivalve physiology

Bivalve molluscs can concentrate many chemical contaminants, including PAHs, by orders of magnitude above concentrations in the surrounding sea water. The degree to which contaminants are accumulated by bivalves depends on both abiotic physiochemical properties and biotic factors such as filtration rate, growth, reproductive condition and metabolism (Dame, 1996).

6.5 Results and discussion

6.5.1 Total PAH

Concentrations of ΣPAH (the sum of all PAH determined) (Figure 15) in cockles ranged from 26 to 492 µg kg^{-1} wet weight with a mean of 141 µg kg^{-1} wet weight. The highest concentrations (>400 µg kg^{-1},

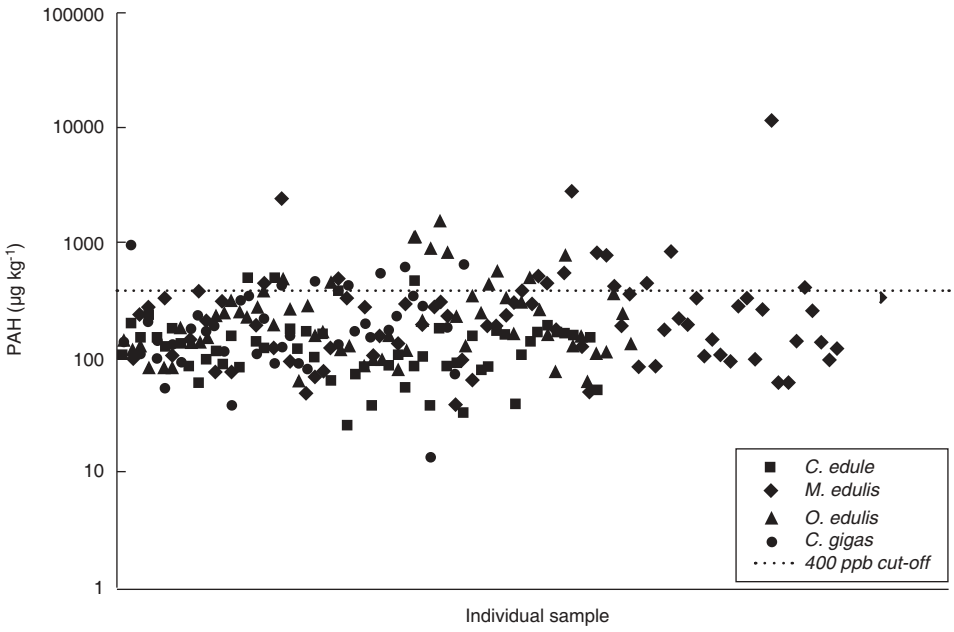

Figure 15. Total PAH levels in shellfish from designated bivalve production areas around England and Wales

selected as an arbitrary cut-off value) were recorded in samples from Swansea Bay and the Burry Inlet. Concentrations in mussels ranged from 40 to 2847 µg kg^{-1} wet weight (mean 423 µg kg^{-1}), with the highest concentrations (>400 µg kg^{-1}) recorded in samples from Exmouth, River Teign, Plymouth, Poole, Swansea Bay, Morecambe Bay, Wirral, Humber, Colwyn Bay and the Thames Estuary. The concentration in a sample from Tenby, to the East of Milford Haven in the Bristol Channel, contained 11,500 µg kg^{-1} wet weight, however this was collected in March 1996 shortly after the SEA EMPRESS oil spill and cannot therefore be regarded as typical for this location at other times. In Native and Pacific oysters, concentrations ranged from 63 to 1592 µg kg^{-1} wet weight and 14 to 942 µg kg^{-1} wet weight, respectively. Concentrations >400 µg kg^{-1} were recorded in samples from Portsmouth Harbour, Plymouth, Fal Estuary, Swansea, Medina, Thames Estuary, the Wash, Salcombe Estuary and Milford Haven.

The comparison of the data from this survey with that from other studies is difficult because of differences in methodology and in the range of PAHs analysed, and any such comparisons must be made with care. Nevertheless, the range of concentrations recorded in this survey, though large, does not appear to be unusual. Similar concentrations have been recorded in a number of other locations, including the United States coast and the Baltic Sea (Table 13). Concentrations at the lower end of the range only, have been recorded in areas off Denmark, Greece and in the Mediterranean Sea.

The overall mean concentration of ΣPAHs for all samples in this survey was 242 µg kg^{-1} (excluding the mussels from Milford Haven taken after the SEA EMPRESS incident). This is similar to the mean of 314 µg kg^{-1} recorded for samples of mussels and oysters collected during the 1995/96 Mussel Watch surveys, carried out by the National Oceanic and Atmospheric Administration (NOAA) (Mearns *et al*., 1999).

6.5.2 Carcinogenic PAH

Benzo[*a*]pyrene, benz[*a*]anthracene, benzo[*b*]fluoranthene and dibenz[*a,h*]anthracene, are of particular concern to human consumers because of their carcinogenic nature. Although no standards or guideline values have been set for PAHs in current UK legislation, the Food Standards Agency, advised by the Committee on Toxicology, have adopted a pragmatic guideline limit value of 15 µg kg^{-1} wet weight for any one of three named PAH (benz[*a*]anthracene, benzo[*a*]pyrene and dibenz[*a,h*]anthracene). These trigger values will be used to assess contamination of fish and shellfish following oil spills and other incidents and to justify fishery closures intended to protect human consumers.

Benzo[*a*]pyrene (B*a*P) concentrations (Figure 16) in cockles and Pacific oysters were in the ranges 0.1 to 17.0 µg kg^{-1} and 0.2 to 19.0 µg kg^{-1}, respectively. Higher concentrations were found in mussels and Native oysters, 0.2 to 64 µg kg^{-1} and 0.3 to 57 µg kg^{-1}, respectively. However, over 90% of all samples contained 15 µg kg^{-1} or less. Highest concentrations in

Table 13. Polycyclic aromatic hydrocarbon (PAH) concentrations (µg kg^{-1} wet weight) in bivalve molluscs from various locations

Area	PAH (µg kg^{-1})	Reference	Bivalve
US coastal sites	8 - 3,774	Mearns *et al*., 1999	Mussels & oysters
Hamilton Harbour (Ontario)	5 - 840	Marvin *et al*., 1994	*Dreissena polymorpha*
Yaquina Bay (Oregan)	14 - 1,320	Marvin *et al.,* 1994	*Mytilus edulis*
Black Sea (Crimean coast)	20 - 760	Shchekaturina *et al.,* 1995	*Mytilus galloprovincialis*
Denmark	0 - 110	Grandy and Spliid, 1995	*Mytilus edulis*
Thermaikos Gulf (Greece)	34 - 150	Kilikidis *et al.,* 1994	*Mytilus galloprovincialis*
Mediterranean Sea	5 - 78	Baumard *et al*., 1998	*Mytilus galloprovincialis*
Baltic Sea	18 - 780	Baumard *et al*., 1999	*Mytilus edulis*
England and Wales	14- 2,847	Jones *et al*., 2002 *(This survey)*	*Mixed*

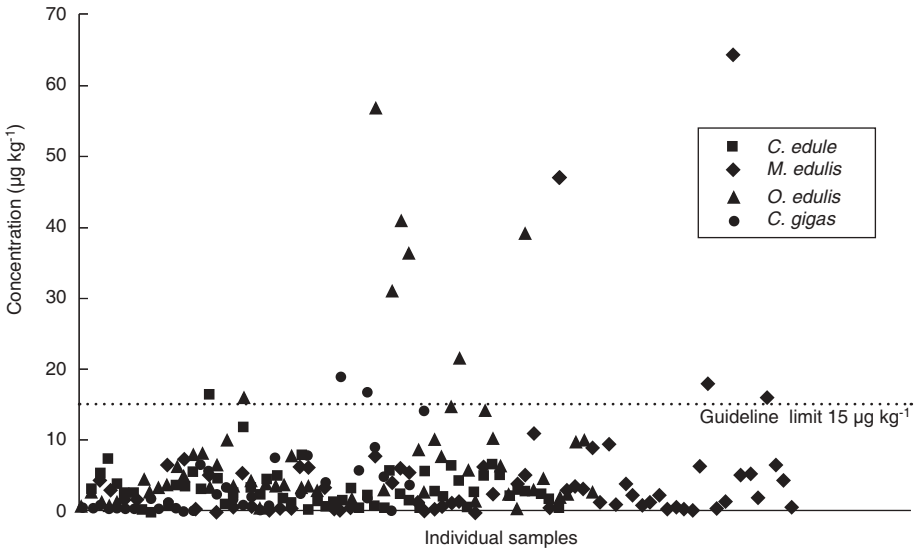

Figure 16. Concentration of benzo[a]pyrene in shellfish from designated bivalve production areas around England and Wales

cockles were recorded in samples from Swansea Bay and the Burry Inlet and in pacific oyster, from Milford Haven and the Fal. In Native oysters and mussels, concentrations above 15 µg kg^{-1} were recorded in samples from Thames, Plymouth, Swansea, Tamar and Portsmouth.

The concentrations of benz[*a*]anthracene (B*a*A) (Figure 17) ranged between 0.4 and 72.4 µg kg^{-1} and showed a similar pattern of distribution to benzo[*a*]pyrene. Approximately 90% of the samples contained concentrations of 15 µg kg^{-1} or below, 10% were between 15 and 25 µg kg^{-1} and only 2% were above 25 µg kg^{-1}. Highest concentrations were recorded in samples from the Burry Inlet, Swansea Bay, Dovey, Plymouth, Poole, Thames, Yealm and the Fal Estuary.

Concentrations of benzofluoranthenes (Figure 18) in this study represent the total concentration of a number of isomers, of which, benzo[*b*]fluoranthene and benzo[*j*]fluoranthene are the most biologically active. Concentrations ranged from 0.4 to 293 µg kg^{1}, with 50% falling at or below 15 µg kg^{1}. Although a considerably higher percentage of results fall above 15 µg kg^{1}, than for benzo[*a*]pyrene or benz[*a*]anthracene, this is to be expected since values represent several PAHs.

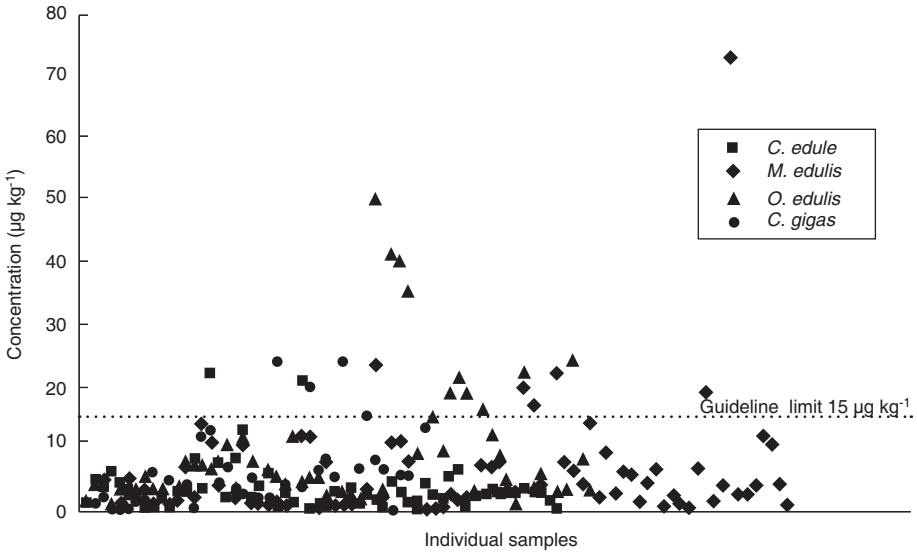

Figure 17. Concentration of benzo[a]anthracene in shellfish from designated bivalve production areas around England and Wales

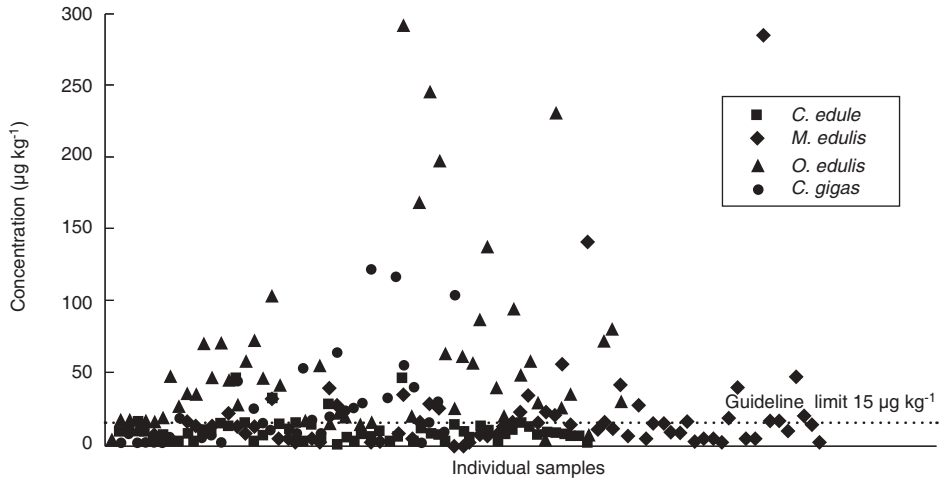

Figure 18. Concentration of benzofluoranthenes in shellfish from designated bivalve production areas around England and Wales

6.5.3 Pyrolytic vs petrogenic PAH

PAHs are generated by two main processes, combustion of organic matter at high temperatures and the release of petroleum. The profiles of pyrolitic and petrogenic PAHs differ and patterns of parent and alkyl-substituted PAH can be used to determine the likely source, particularly when used in conjunction with ttr-aromatic steroid biomarker compounds present in crude and some refined oils (see Bence *et al*., 1996; Boehm *et al*., 2001; and references therein). Pyrolitic PAH are characterised by the dominance of unsubstituted PAH with a wide range of molecular weights, whereas PAH from petrogenic sources are dominated by the low molecular weight PAH with a complete suite of alkylated PAHs.

In the majority of cockle samples, the PAH profiles exhibited both combustion and petroleum derived PAHs in roughly equal quantities. In the Thames Estuary area, PAH profiles showed a higher proportion of petrogenic PAHs towards the mouth of the estuary and off the east Essex coast. However, this appeared to be due to lower concentrations of combustion PAHs with distance from the source, rather than an increase in petrogenic PAHs. The possible sources of PAHs in this area include several oil and coal-fired power stations along the Thames, oil refineries/terminals at Thurrock and Canvey Island and a variety of industries including chemical and oil and gas related industries and metal works.

One sample of cockles, from Dovey on the West Coast of Wales, showed a significant predominance of pyrolitic PAH.

PAH profiles for Native and Pacific oysters showed a slight predominance of pyrolytic PAH in the majority of samples. However, samples of Pacific oysters from Boston in the Wash and Native oysters from the River Tamar, Plymouth, both showed a significant predominance of oil derived PAH. The chromatogram for the sample from the Wash indicated that the oil was weathered diesel oil, the source of which was likely to have been small boats or a localised spill. The chromatogram for the Plymouth sample on the other hand indicated contributions from both diesel and heavy fuel oil. Plymouth has a concentration of dock facilities, including the Naval Dock and also imports refined petroleum products from Milford Haven. Shipping is therefore the most likely source of the heavy fuel oil.

There was a predominance of petrogenic PAH in a high proportion of the mussel samples, particularly in samples from the Humber, Plymouth, Teign, Milford Haven, Colwyn Bay, Morecambe Bay and the Solway. The majority of these areas are highly industrialised estuaries; oil refineries and/or petrochemical industries and shipping are commonplace together with numerous other industries that produce PAHs. The data appear to suggest that mussels accumulate the lower weight, oil-derived PAH to a greater extent than other species of mollusc.

6.5.4 Seasonal variation

Several studies (Dunn and Stich, 1976; Fossato *et al*., 1979; Bender *et al*., 1986) have noted that PAH concentrations in marine organisms appear to show a seasonal variation, which may be caused by a number of factors that govern the uptake of contaminants in bivalves. Farrington *et al.* (1983), found large seasonal variations in the PAH concentrations in mussels from the east coast of the United States, where concentrations were elevated during the winter and spring and declined during the summer. Law *et al*. (1998), found a similar pattern whilst monitoring PAH in shellfish in the wake of the *SEA EMPRESS* oil spill, in Milford Haven in 1996. Concentrations of

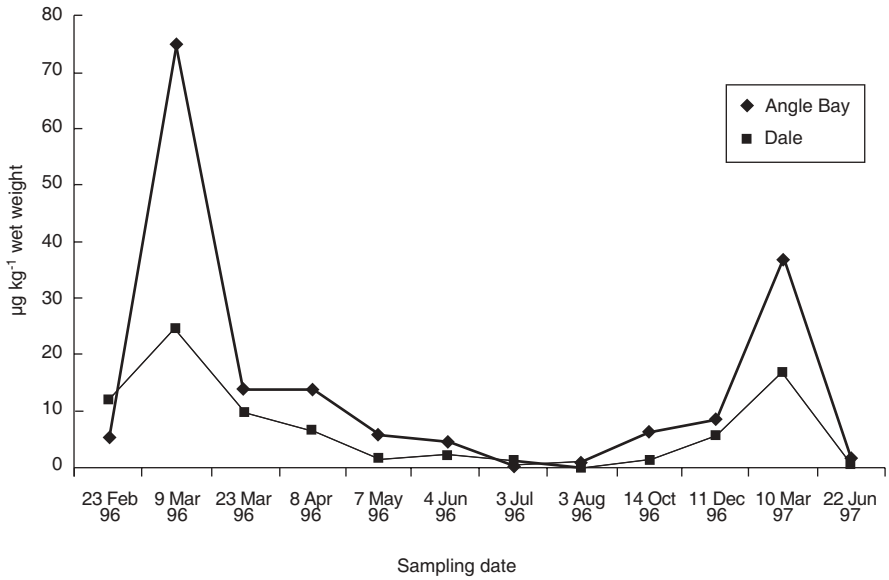

Figure 19. Benzo[a]pyrene in mussels (Sea Empress monitoring data)

benzo[*a*]pyrene in mussels from Angle Bay and Dale (Figure 19) exhibited peak concentrations in early March 1996, falling to close to zero in August 1996 and peaking again in March 1997.

This variability in PAH concentrations has been attributed to numerous factors including filtration rates, chemical species, temperature, environmental concentrations, spawning activity and other related factors. Mussels spawn during early spring and there is strong evidence, therefore, to suggest that spawning activity is an important factor in the variability of PAH tissue concentrations due to the lipophilicity of PAH and the mobilisation of lipid into the gametes.

Cockles also spawn during early spring whilst Native oysters spawn in late spring/summer. (Pacific oysters were thought not to spawn around the coast of England due to water temperatures not rising sufficiently, however, recent evidence of new spat suggests that water temperatures in some estuaries are occasionally sufficiently high for spawning to occur). A large proportion of the samples in this survey was collected during February/March 1996, i.e. prior to spawning.

In July 1997, approximately 40 sites were re-sampled. These mainly included sites where ΣPAH concentrations in 1996 were >400 µg kg^{-1}. The concentrations of ΣPAH in the July samples were lower than the 1996 spring samples, with concentrations ranging from 40-358 µg kg^{-1}. The most significant decreases were seen in mussels. This supports the work reported by Law *et al*. (2002) and indicates that spawning is a major factor in the elimination of PAHs from bivalves.

6.6 Conclusions

The final phase of this project has produced the first substantial dataset on the spatial distribution of PAHs in commercially exploited bivalve molluscs around England and Wales. There was some evidence of differences in the uptake of PAHs between species. In general, cockles tended to contain lower concentrations of ΣPAH than oysters and mussels. Relatively high concentrations (>400 µg kg^{-1}) were recorded in approximately 15% of the samples and included samples from each species. Some of the highest concentrations were recorded in areas associated with oil refineries/terminals, petrochemical industries, heavy shipping, power stations and other major sources of PAH inputs. Discharges from small boats, localised oil spills or high run-off, could be possible explanations for high concentrations observed in other areas.

Jones *et al*. (2002) calculated estimated dietary intakes of PAHs, from molluscs alone, for both typical and high level consumers of molluscs. However, it is not possible to assess the significance of these, as there are no Permitted Maximum Tolerable Daily Intakes (PMTDIs) for PAHs, due to the difficulties associated with establishing intake limits for carcinogenic substances.

There is considerable annual variation in the concentrations of PAHs in bivalves. Spawning activity appears to be an important factor, which can reduce PAH concentrations by as much as 80%. Clearly, when monitoring for PAHs, the time of sampling will have a significant influence on the results obtained and it is an important factor to consider when designing any sampling/monitoring plan and assessing/comparing data.

7. THE CONCENTRATIONS OF MERCURY IN FISH TAKEN FROM LIVERPOOL BAY IN 2000

Mercury levels in commercial fish species have been monitored by the Burnham Laboratory since the 1970s (e.g. Portmann, 1979). Early results indicated that the highest concentrations were found in four areas, Liverpool Bay, Morecambe Bay and Swansea Bay, all of which received discharges containing mercury from the chlor-alkali industry and in the outer Thames Estuary where a significant input was via sewage sludge disposal. Annual monitoring of these four areas commenced in the early 1980s, following the adoption in 1980 by the Paris Commission of the mercury Environmental Quality Standard (EQS), which required that in areas receiving significant mercury inputs, the concentration of mercury in a representative sample of fish flesh chosen as an indicator, should not exceed 0.30 mg kg^{-1} on a wet weight basis. Monitoring soon indicated that the concentrations of mercury in Swansea Bay and the Thames Estuary were no longer elevated to a level that could potentially exceed the EQS and in 1985 it was agreed that monitoring in these areas could cease. Mercury concentrations remained relatively high at this time in Liverpool and Morecambe Bays and results continued to be reported to OSPAR until 1994 (though at two yearly intervals from 1990), when it was agreed that the requirement for regular reporting could cease. Some re-assurance monitoring has been undertaken since that time and the results from the most recent survey of fish from Liverpool Bay, carried out in 2000, are summarised in Table 14. Flounder have become difficult to obtain in Liverpool Bay in recent years. In 2000, none were caught, despite efforts on three separate cruises, so the mean mercury concentration in 2000 is for five fish species, instead of the usual six. Relatively low mercury concentrations were recorded in some mid-summer samples.

The concentration of mercury in individual fish species is now very much less than the limit of 0.50 mg kg^{-1} set in European Community Decision 93/351/EEC on maximum limits for mercury in fishery products. The overall mean concentration was 0.16 mg kg^{-1}, so reassurance can continue to be given that the mercury EQS will not be breached.

Although not collected for time trend purposes, (for which a separate study has been taking place), the series of results obtained from Liverpool Bay over the 1982-2000 period (Table 15) gives some indication of the reduction in mercury concentrations undergone in fish taken from this area over the last 18 years.

Table 14. Liverpool Bay - Mercury concentrations found in fish muscle in 2000 (OSPARCOM 'upper' level category; >0.30 mg kg^{-1} wet weight). [EC maximum limit; 0.50 mg kg^{-1} wet weight]

Species	Number of fish analysed	Mean length (cm)	Mean mercury concentration in fish muscle (mg kg^{-1} wet weight)
Cod	45	28.8	0.08
Whiting	25	29.9	0.25
Dab	48	23.4	0.16
Flounder	0*	-	-
Plaice	50	27.9	0.15
Sole	47	25.5	0.16
		Mean, all fish 0.16	

** None caught in Liverpool Bay in 2000*

Table 15. Liverpool Bay - Time series of mean concentrations of mercury in fish flesh over the 1982-2000 period

Year	Concentration of mercury in mg kg^{-1} wet weight
1982/83	0.27
1984	0.31
1985	0.24
1986	0.24
1987	0.23
1988	0.22
1989	0.20
1990	0.19
1992	0.20
1994	0.17
1996	0.17
1998	0.16
2000	0.16

8. THE USE OF ETHOXYRESORUFIN-O-DEETHYLASE (EROD) IN FISH AS A BIOLOGICAL EFFECTS MONITORING TOOL

8.1 General introduction

The use of enzyme biomarkers as pollution monitoring tools is well established and their use by CEFAS has been explained in previous Aquatic Environment Monitoring Reports. This article reports the continued use of hepatic ethoxyresorufin-O-deethylase (EROD) as a marker of exposure to planar organics (especially PAH and PCB).

8.2 Introduction/methods

The Mixed Function Oxygenase (MFO) enzyme system is the primary detoxification pathway for a number of planar, organic contaminants, specifically PAHs and some PCBs and is induced in fish by exposure to such compounds. Cytochrome P4501A1 (CYP1A1) is the terminal component of the MFO system and EROD activity is CYP1A1 dependent, therefore, EROD represents a good marker of MFO induction.

The 1999 and 2000 data presented here represents the fourth and fifth consecutive years (see AEMR Nos. 51, 52 and 53 for previous results (CEFAS, 1998; 2000 and 2001)) that EROD data for dab (*Limanda limanda*) has been collected. Fish were collected during May and June for 1999 and 2000 respectively using *RV CIROLANA*'s Granton trawl. Once on deck, target species were separated into tanks containing flowing seawater. Dissections were performed within 1 hour of capture. The liver was excised and placed in a cryovial which was immediately placed in liquid nitrogen for storage. Notes were taken on fish condition, length, sex, gonad length and parasitism. Only fish over 10 cm were taken as samples.

Homogenate preparation

A 200 mg (± 10) slice of liver was homogenised with 1 ml of ice cold homogenising buffer (100 mM K_2HPO_4/ KH_2PO_4 pH 7.5, 1 mM EDTA, 1 mM dithiothrietol, 150 mM KCl) using six strokes of a Potter-Elvehjem automatic homogeniser set at 4000 rpm. The homogenates were then centrifuged at 10,000 g for 20 minutes in lidded eppendorf tubes using a refrigerated unit set at 4°C. Supernatents were removed and used as the raw enzyme solution.

EROD activity determination

EROD measurement was performed using the standard ICES method (Stagg and McIntosh, 1998). A Perkin Elmer LS50B fluorescence spectrometer set at 535 nm excitation and 580 nm emission with a cuvette stirring function was used. All assay reagents were kept at 20°C (±1) in a water bath so as to control the assay temperature. The reaction mixture, final volume 2 ml, contained 1.96 ml assay buffer (100 mM K_2HPO_4/ KH_2PO_4 pH 7.5, 100 mM KCl), 20 µl liver homogenate, 10 µl ethoxyresorufin substrate (0.4 mM in dimethyl sulphoxide, DMSO) and 10 µl of resorufin internal standard (25 µM, adjusted by absorbance to allow for variance in % purity in resorufin stock (see below) in DMSO). The standard equates to an addition of 250 pM of resorufin against which the assay was calibrated. The reaction was initiated by the addition of 10 µl NADPH (0.25 mM) and further emission readings were taken at the start and finish of a 60 second period from a linear section of the sample trace.

EROD activity was normalised to protein content and expressed as pM resorufin/min/mg protein. Protein analyses were carried out using a plate reader modification of the Bradford method (1976) with a bovine serum albumin standard.

8.3 Results/discussion

RV CIROLANA 3a/99

During this research cruise, dab (*Limanda limanda*) were collected from 16 sites in the North Sea and around the UK coast (Table 16). Data from the EROD analyses are shown in Table 17 and presented in Figure 20.

The most striking feature of the dataset is that male EROD is higher at all sites than that of the female. At some sites male EROD levels are 6-7 fold higher (e.g. Off Tees, Off Flamborough and NW Dogger). Furthermore, there is an apparent east/west coast divide in this pattern in that the highest male:female EROD ratios are east of the UK, in the North Sea, while those in the Irish Sea and Liverpool Bay are generally lower.

These inter-sex differences in EROD activity are inconsistent with the data for 2000 (see below) and that previously presented for years 1996-98 (CEFAS, 1998; 2000 and 2001). However, they are explained by the fact that the cruise in 1999 took place in early May, earlier than for all other years so far reported. The reproductive cycle of the dab in the North Sea has four distinct periods: prespawning (Sept-Dec), spawning (Jan-April), postspawning (May) and resting

Table 16. Sampling sites during RV CIROLANA 3a/99

Station no.	Location	NMP No.	Latitude	Longitude
1	The Wash	385	53º 09.772'N	00º 35.499'E
6	Lower Sole Pit	345	54º 03.327'N	01º 46.993'E
20	Amble	244	55º 15.144'N	01º 15.291'W
27	Off Tees	295	54º 45.344'N	00º 54.318'W
31	Off Flamborough		54º 15.107'N	00º 28.658'E
33	N.W.Dogger		54º 45.223'N	01º 18.735'E
35	Bremerhaven 9		55º 27.655'N	04º 05.802'E
38	Bremerhaven 1		54º 04.388'N	08º 07.182'E
59	Rye Bay		50º 52.345'N	00º 49.323'E
128	St Bees Head		54º 33.209'N	03º 50.345'W
143	Off Morecambe	795	53º 53.305'N	03º 25.362'W
145	Burbo Bight	705	53º 28.199'N	03º 22.514'W
156	Liverpool Bay 2		53º 23.626'N	03º 37.290'W
159	Red Wharf Bay	776	53º 21.555'N	04º 08.437'W
161	Outer Cardigan Bay	665	52º 23.344'N	04º 53.718'W
163	Inner Cardigan Bay		52º 17.507'N	04º 17.944'W

Table 17. Mean EROD (pM/min/mg protein) activity data for RV CIROLANA 3a/99

Location	All			Male			Female		
	No.	EROD	SD	No.	EROD	SD	No.	EROD	SD
The Wash	9	313.3	233.4	0	-	-	9	313.3	233.4
Lower Sole Pit	19	337.4	264.0	10	391.9	215.9	9	276.8	310.7
Off Amble	20	188.5	169.1	10	304.8	140.5	10	72.3	103.0
Off Tees	20	349.4	393.2	10	608.5	409.7	10	90.2	95.7
Off Flamborough	20	272.2	343.1	10	478.6	382.5	10	65.8	86.7
N W Dogger	20	455.3	396.9	10	785.8	291.7	10	124.7	68.6
Bremerhaven 9	20	136.0	120.1	10	196.1	112.5	10	75.9	98.9
Bremerhaven 1	20	560.0	465.1	10	923.4	366.1	10	196.6	170.7
Rye Bay	20	370.5	333.2	10	500.8	381.0	10	240.3	226.9
St Bees Head	20	628.8	291.0	10	808.7	229.0	10	449.0	233.4
Off Morecambe	20	923.7	354.7	10	927.2	411.1	10	920.2	310.8
Burbo Bight	20	491.3	317.9	10	533.0	211.4	10	449.5	405.9
Liverpool Bay 2	20	506.6	411.8	10	791.0	406.2	10	222.2	114.9
Red Wharf Bay	20	513.4	236.3	10	623.7	253.1	10	403.1	163.5
Outer Cardigan Bay	20	606.7	368.0	10	787.0	435.2	10	426.5	155.8
Inner Cardigan Bay	20	389.6	284.5	10	398.4	365.5	10	380.8	192.7

(June-August) (Kirby *et al.,* 1999a) though these can vary from year to year due to climatical factors. Lange *et al.* (1999) has shown that EROD activity levels in dab show a strong seasonal trend and that male levels are highest during the spawning period (April) and lowest during the postspawning/resting phase (August). Females do not appear to show as strong a trend. The male results reported here, from fish sampled in May 1999, were therefore higher because the fish were still at the tail-end of the spawning period.

While the interpretation of the data is made problematical by influences associated with the breeding cycle, there is still some evidence of inter-site differences that may be contaminant mediated. In male flounder, high EROD levels were to be found at sites associated with the Liverpool Bay/Irish Sea area: Off Morecombe (927 pM/min/mg pro.), St Bees Head (809 pM/min/mg pro.) and Outer Cardigan Bay (787 pM/min/mg pro.). In the North Sea a particularly high value (923 pM/min/mg pro.) was found in male flounder from the Bremerhaven Transect Station 1. Females also had generally high values at the same Liverpool Bay/Irish Sea stations as with the males but were low at sites in the North Sea including sites off of the North east coast. It is likely that the high levels associated with sites in Liverpool Bay and at Bremerhaven are due to contaminant exposure. However, high levels in relatively clean areas, such as outer Cardigan are more difficult to explain. It is likely that seasonal influences and migration have contributed to the pattern.

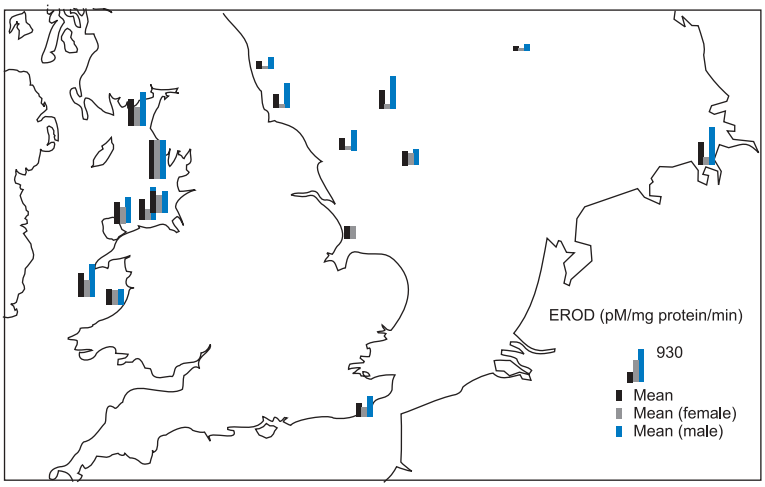

Figure 20(a). Mean EROD (pM/min/mg protein) activity data for RV CIROLANA 3a/99 for males and females

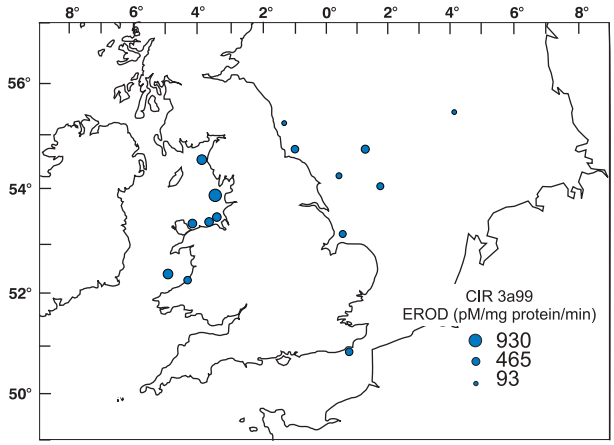

Figure 20(b). Mean EROD (pM/min/mg protein) activity data for RV CIROLANA 3a/99 for all dab caught

RV CIROLANA 4b/00

The survey of June 2000 saw dab sampled from 19 sites from around the UK coast (Table 18). Data from the EROD analyses are shown in Table 19 and presented in Figure 21. For this particular cruise, data was collected for the length and weight of each fish from which a condition factor (CF) was calculated. Furthermore, in order to ascertain maturity/reproductive state, the length of the gonads were measured and related to the body length (gonad length/body length x 100) to give a pseudo gonad somatic index (pGSI). These data are shown on Table 20.

In contrast to the 1999 survey, the data for each of the sexes reflect each other closely and therefore the amalgamated means can reasonably be used as definitive data. This reflects the fact that most of the samples were taken in late June during a time when both genders would be starting their reproductive 'resting' phase.

In general, the broad range of mean EROD activities reflect the range seen in 1999 but this time with the highest mean values being associated with sites on the east coast of the country. For example, Off Tees, Off Flamborough, West Dogger, Off Humber and the Firth of Forth gave mean EROD activity values of 785, 626, 574, 524 and 507 pM/min/mg pro. respectively (Table 19). On the west coast, the near shore sites in Liverpool Bay (Off Morecombe and Burbo Bight) once again provided the highest values. Particularly low mean ERODs were found at the South of Humber (23 pM/min/mg pro.) and Rye Bay sites (69 pM/min/mg pro.) and the putatively clean sites in the Irish and Celtic seas also provided low means of <200 pM/min/mg pro.

The 2000 data seems to reflect more closely the data previously gathered and generally agrees with known areas of organic contamination. However, correlations of size and reproductive parameters with EROD (Table

Table 18. Sampling sites during RV CIROLANA 4b/00

Station no.	Location	NMP No.	Latitude	Longitude
7	Celtic Deep		51º 10.19'N	05º 43.95'W
14	Off Cardigan Bay		52º 21.85'N	04º 43.95'W
28	Inner Cardigan Bay		52º 17.47'N	04º 54.23'W
32	Red Wharf Bay		53º 21.42'N	04º 17.88'W
34	Liverpool Bay		53º 28017'N	04º 07.58'W
38	Burbo Bight		53º 28.24'N	03º 42.39'W
55	Off Morecambe Bay	795	53º 55.31'N	03º 23.79'W
71	Morecambe Bay	805	54º 03.31'N	03º 23.64'W
74	Dundrum Bay		54º 05.20'N	03º 52.58'W
102	Firth of Forth		56º 33.69'N	05º 37.27'W
105	Off Amble		55º 19.23'N	01º 21.38'W
113	Tees		54º 42.91'N	01º 15.09'W
115	Hospital Ground		54º 30.91'N	00º 52.66'W
117	West Dogger	285	54º 47.24'N	02º 41.43'E
145	Flamborough		54º 15.59'N	01º 17.15'E
148	Off Humber	345	54º 04.96'N	00º 27.06'E
152	S. of Humber		53º 18.84'N	01º 48.60'E
158	Outer Gabbard	345	52º 02.54'N	00º 25.84'E
163	Rye Bay		50º 50.79'N	02º 06.62'E

Table 19. Mean EROD (pM/min/mg protein) activity data for RV CIROLANA 4b/00

Location	All			Male			Female		
	No.	EROD	SD	No.	EROD	SD	No.	EROD	SD
Celtic Deep	20	200.4	130.3	11	184.8	119.4	9	219.5	147.5
Off Cardigan Bay	20	124.5	108.2	10	75.9	50.3	10	173.2	130.1
Inner Cardigan Bay	20	174.9	207.5	-	-	-	20	174.9	207.5
Red Wharf Bay	20	152.4	164.4	10	155.0	205.5	10	149.7	121.8
Liverpool Bay	20	167.3	200.8	10	98.8	52.9	10	235.9	268.1
Burbo Bight	20	402.5	396.5	10	374.8	323.9	10	430.3	474.6
Off Morecambe Bay	20	369.7	253.1	10	272.5	211.1	10	466.9	263.9
Morecambe Bay	20	213.7	167.1	10	111.6	115.4	10	305.6	155.6
Dundrum Bay	15	405.3	171.9	10	397.3	173.5	5	421.2	187.5
Firth of Forth	20	506.9	286.1	10	618.1	273.9	10	395.8	265.2
Off Amble	20	470.4	351.9	10	476.1	326.2	10	464.7	393.7
Off Tees	20	785.1	504.7	10	699.3	509.2	10	871.0	511.8
Hospital Ground	20	181.0	176.6	10	90.1	81.1	10	271.9	202.2
West Dogger	20	573.5	369.7	11	656.9	411.2	9	471.6	303.2
Off Flamborough	20	626.3	338.2	10	767.0	354.5	10	485.7	268.1
Off Humber	19	523.5	253.8	9	580.7	318.8	10	472.1	179.7
S of Humber	20	23.0	16.7	10	16.8	12.9	10	29.2	18.3
Outer Gabbard	20	136.6	124.3	10	139.7	94.1	10	133.5	154.2
Rye Bay	20	66.8	39.0	10	57.4	33.4	10	76.2	43.6

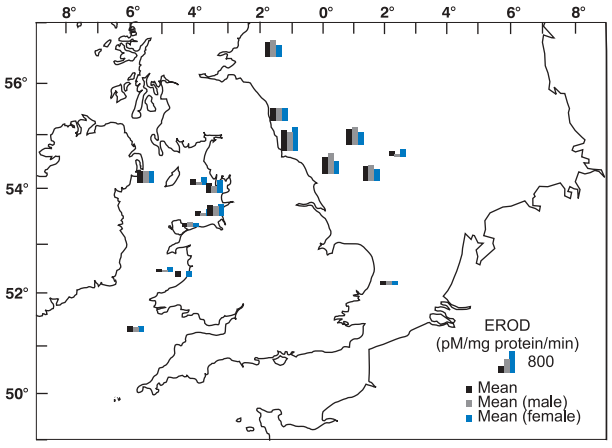

Figure 21(a). Mean EROD (pM/min/mg protein) activity data for RV CIROLANA 4b/99 for males and females

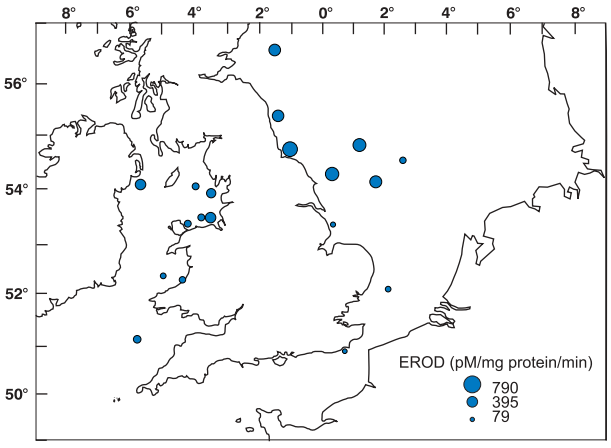

Figure 21(b). Mean EROD (pM/min/mg protein) activity data for RV CIROLANA 4b/99 for all dab caught

Table 20. Mean data for size and somatic indices of dab taken during RV CIROLANA 4b/2000

Location	Length (cm)			Weight (g)			Gonad Len.(cm)		pGSI *		Condition factor **		
	All	Male	Female	All	Male	Female	Male	Female	Male	Female	All	Male	Female
Celtic Deep	22.0	21.0	23.2	109.0	97.7	121.4	1.5	3.8	7.1	16.5	1.0	1.0	1.0
Off Cardigan Bay	20.5	18.9	22.1	91.3	65.6	117.0	1.5	3.0	7.9	13.7	1.0	0.9	1.0
Inner Cardigan Bay	18.5	-	18.5	63.2	-	63.2	-	2.3	-	12.2	1.0	-	1.0
Red Wharf Bay	21.8	21.1	22.5	127.8	114.4	141.1	1.7	3.1	8.0	13.5	1.2	1.2	1.2
Liverpool Bay	22.3	20.6	24.0	121.5	93.1	149.8	1.9	3.9	9.3	16.0	1.1	1.1	1.1
Burbo Bight	22.3	20.6	24.0	117.6	91.4	143.7	1.9	4.3	9.2	17.8	1.0	1.0	1.0
Off Morecambe Bay	20.8	19.5	22.1	108.3	86.6	130.0	1.7	3.6	8.8	16.2	1.2	1.1	1.2
Morecambe Bay	21.0	20.7	21.2	101.5	98.0	104.9	2.2	3.5	10.8	16.4	1.1	1.1	1.1
Dundrum Bay	17.1	17.7	15.8	54.5	60.9	41.6	1.6	2.3	8.8	14.6	1.1	1.1	1.0
Firth of Forth	22.7	19.1	26.2	75.1	61.8	88.4	1.6	4.9	8.5	21.5	0.9	0.9	0.8
Off Amble	21.4	21.2	21.5	96.6	95.6	97.5	2.2	4.2	10.2	19.1	1.0	1.0	1.0
Off Tees	21.7	20.2	23.1	103.0	81.4	124.6	2.0	5.2	9.6	22.6	1.0	1.0	1.0
Hospital Ground	22.7	22.5	22.9	126.4	124.7	128.0	2.1	4.1	9.3	17.9	1.1	1.1	1.1
West Dogger	21.7	21.0	22.6	99.8	89.5	112.3	2.1	4.9	10.0	21.4	1.0	0.9	1.0
Off Flamborough	20.1	18.8	21.3	79.3	63.8	94.8	1.8	4.9	9.4	23.2	1.0	1.0	1.0
Off Humber	21.4	20.9	21.9	103.8	96.4	111.1	2.0	3.9	9.4	17.9	1.0	1.0	1.1
S of Humber	26.2	25.8	26.6	189.3	177.5	201.1	1.8	4.2	6.0	15.7	1.0	1.0	1.1
Outer Gabbard	23.6	22.4	24.7	142.8	119.4	166.2	1.9	3.6	8.5	14.4	1.1	1.0	1.1
Rye Bay	21.3	19.7	22.8	108.4	84.1	132.6	1.6	2.6	8.1	11.4	1.1	1.1	1.1

** pGSI (pseudo gonad somatic index) = gonad length/body length x 100*
*** Condition Factor (CF) =(weight /(length)3) x 100*

21) suggest that the EROD activities seen may also be somewhat influenced by non-contaminant sources. The male data showed negative correlations with fish weight and condition factor of -0.52 and -0.51 respectively which goes someway to explaining the exceptionally low value at South of Humber as these were the largest fish (mean male length of 25.8 cm). Of perhaps more significance is the female EROD correlation to pGSI (r = 0.77) which suggests that even in June there is a residual effect on EROD of the breeding cycle.

Table 21. RV CIROLANA 4b/2000: Correlation (r values) analysis of EROD activity with other sample variables

	EROD		
	All	Male	Female
Length	-0.26	-0.41	-0.18
Weight	-0.47	-0.52	-0.36
Gonad Length	-	0.18	0.58
pGSI	-	0.46	0.77
Condition Factor	-0.4	-0.51	-0.27

8.3.1 5 year trends

The mean EROD activity results obtained during the 1999 and 2000 surveys were lower than those reported in previous years (CEFAS, 2000 and 2001) due to a change in methodology that was adopted following participation in an international ring test as part of the BEQUALM programme. The 1999/2000 assays were conducted using a standard ICES method (Stagg and McIntosh, 1998) to bring the laboratory in line with the more widely accepted technique. The change entailed minor alterations to buffers which caused negligible difference in assay response compared to that of the previous method (Stagg *et al*., 1995). However, the new procedures highlighted the % impurity of the resorufin used to prepare the standard for the assay and recommended the preparation of a 50 µM standard, which is adjusted by absorbance to ensure a true 25 µM concentration. Analyses showed our resorufin standard to be only 53% pure when compared against expected spectrophotometric parameters. Therefore previous assays in 1996-98, conducted using a supposed 25 µM standard, were in fact measured against only a 13.25 µM resorufin standard. For trend comparison over the 5 year study period, a factor of 0.53 has had to be added to the 1996-98 data to make them directly comparable.

The addition of the 1999/2000 datasets to the CEFAS EROD time series continues to indicate elevated levels of MFO activity in fish samples caught from the Liverpool Bay and north east coastal areas. Presumably these are associated with outputs of inducing compounds (PAH/PCB) from the Mersey and Tyne/Tees rivers respectively.

With only 5 years of surveys, trend analysis is still premature. It is made even more difficult by the fact that all sites cannot always be visited each year and by differing sampling times and changes in methodology. However, Figure 22 represents trend data for four of the most frequently sampled sites.

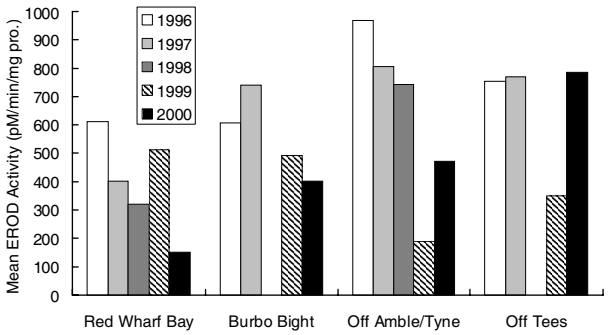

Figure 22. Five year trend data for mean EROD at selected sites

In referring to Figure 22 it is tempting to suggest that there is a downward trend in EROD activity at, for example, the Red Wharf Bay, Burbo Bight and Off Amble/Tyne sites. However, for the reasons outlined above it is far to early to draw any conclusions from the available data. A time series of at least 10 years will be required.

8.4 General conclusion

The data presented here continues to demonstrate the use of the EROD assay as a marker of organic exposure in marine fish species. The data continues to highlight the 'hotspots' off the north east and north west coasts of England and has now, in conjunction with data for previous years, started to form the basis of a time series for the analysis of exposure/effects trends. However, it is also clear that several more years of data from targeted sites will be required before any firm conclusions can be drawn. The surveys reported here also highlight the potential influence of the mating season and specimen size on EROD activity and, therefore, it will be important to standardise sampling time and size/sex class as much as possible in future years.

9. FISH PATHOLOGY AND DISEASE BIOMARKERS 1999-2000

9.1 Introduction

The use of fish diseases and pathological changes in the liver as indicators of environmental stress at the population level have been used for many years (Bucke *et al*., 1996; ICES 1997). The rationale for their inclusion in biological effects monitoring programmes has been provided in previous reports in this series. The main external conditions used for monitoring purposes include acute and healing ulcerations, lymphocystis, epidermal hyperplasia/papilloma and hyperpigmentation. Internally, the presence of hepatic lesions comprising of nodules and larger tumours has also become routine for health assessments of the target fish species for monitoring, namely the dab (*Limanda limanda*) and the flounder (*Platichthys flesus*). Although the aetiology of certain diseases is known, for example an iridovirus is known to cause lymphocystis (Bucke *et al*., 1983), the aetiology for others remains uncertain. However, the measurement of prevalence of these diverse conditions in individual fish provides the data required to produce an holistic assessment of the overall health status in the populations sampled. Recent attempts to assess long-term monitoring data have shown that it is possible to detect trends in disease prevalence that can, in conjunction with specific biomarker data, be used to provide greater confidence for the use of disease as an indicator of contaminant effect (Lang and Dethlefsen, 1996; Wosniok *et al*., 2000).

The histological assessment of liver pathology in dab and flounder has become a major component in the assessment of biological effects of contaminants and several categories of toxicopathic hepatocellular lesion have been identified in a number of fish species (Myers *et al*., 1987, 1992, 1998; Varanasi *et al*., 1987; Stein *et al*., 1990, 1992; Moore and Myers, 1994; Vethaak *et al*., 1996; Stentiford *et al*., 2002). Since toxicopathic lesions, in particular those considered to be neoplastic and pre-neoplastic, are increasingly regarded as end points of contaminant exposure (Myers *et al*., 1994, 1998; Bucke and Feist, 1993; Vethaak *et al*., 1996), their presence in wild fish populations is highly significant. Consequently, the current monitoring programme also incorporates measures of genotoxic damage at the molecular level, such as deoxyribose nucleic acid (DNA) adduct formation along with biomarkers of genotoxin exposure, such as EROD and bile metabolites.

In addition to the measurement of established disease and pathology biomarkers, the monitoring programme also seeks to identify conditions in the target species that may have potential for inclusion in the monitoring programme. As part of the current programme, the disease status of commercial fish species is also monitored to provide information on disease epidemics affecting commercial stocks (e.g. *Ichthyophonus* in North Sea herring) or which may cause aesthetic changes which render the fish unmarketable.

9.2 Materials and methods

A single dedicated cruise for monitoring fish diseases was conducted in each year (*RV CIROLANA* 3/99 and *RV CIROLANA* 4a/00. (For sites see Figure 23). A total of 18 sites were assessed for fish diseases during the two trips. One-hour tows using a Granton trawl fitted with a tickler chain and liner was used at all sites.

Sampling and disease reporting protocols followed those recommended by ICES (Bucke *et al*., 1996). Target fish species were the dab (*L. limanda*) and cod (*Gadus*

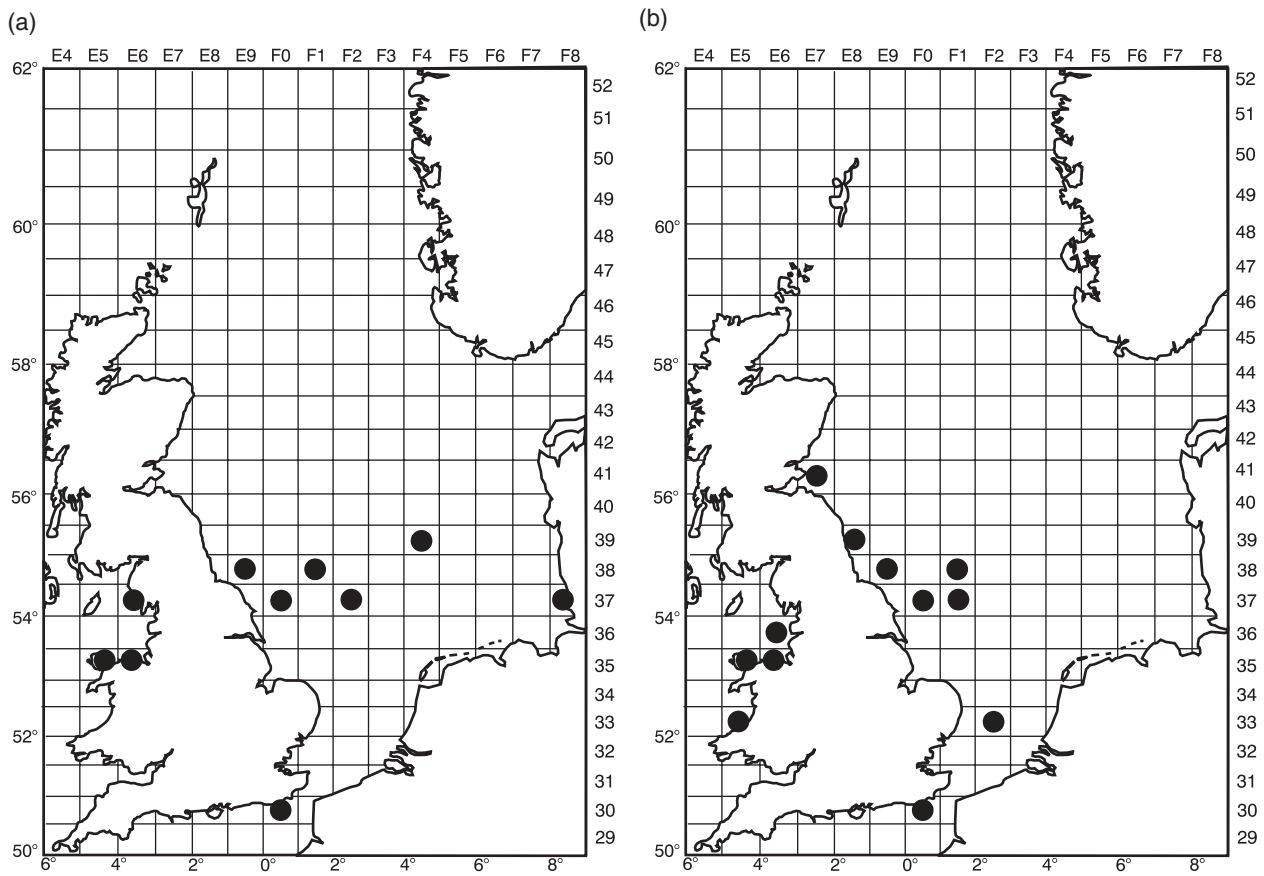

Figure 23. Areas sampled (●) in (a) 1999 and (b) 2000 for fish disease monitoring (by ICES rectangle)

morhua) for offshore locations and the European flounder (*P. flesus*) for inshore or estuarine stations. Where sufficient numbers of other fish species were caught, these were examined for significant diseases or parasites. In particular, herring (*Clupea harengus*) were examined macroscopically for the presence of the fungal pathogen *Ichthyophonus*. Routinely, all macroscopic liver nodules detected in dab, flounder and other flatfish species were preserved in 10% Neutral Buffered Formalin (NBF) for subsequent histological confirmation. Since it is known that flounder are susceptible to endocrine disruption (Allen *et al*., 1999), gonad samples were also taken from all flounder for histological assessment. Samples from dab were also taken to establish whether dab also exhibit the intersex condition.

In addition to the fish examined for externally visible diseases, standard sections of liver tissue from up to 50 dab of approximately 20 cm in length were sampled from each of the following areas in both years; Liverpool Bay, Red Wharf Bay, west Dogger, offshore from Flamborough and Rye Bay. In 1999, material from the two Bremerhaven stations, Burbo Bight, offshore from Tees and St. Bees was also obtained. In 2000, material from the Firth of Forth and offshore from Humber and Amble was also taken. Tissues were processed using internationally agreed standard histological techniques (CEFAS, 1999). Haematoxylin and eosin (H&E) stained histological sections were examined for the presence of the specific categories of hepatic pathology shown in Table 22. Although the range of possible lesions present in flatfish is large, the lesion categories used here have been restricted to those with greatest relevance as indicators of contaminant exposure. These include: foci of cellular alteration (FCA), benign and malignant tumours. All cases of the neoplastic lesions adenoma, cholangioma, hemangioma (putative) and carcinoma were incorporated into the final prevalence data. The prevalence of FCAs was recorded separately.

9.3 Results

9.3.1 Dab diseases

Disease prevalence and severity data, according to fish size group (see Bucke *et al*., 1996) are presented in Tables 23 and 24. The diseases recorded in dab were ulceration (acute and healing), epidermal papilloma, lymphocystis and macroscopic liver nodules (in fish greater than 20 cm in length). In addition, the prevalence of epidermal hyperpigmentation was also recorded. The summary data for the prevalence of these diseases are presented in Figures 24 (a to e) and 25 (a to e). The prevalence of histologically confirmed hepatic lesions found in dab from the larger size groups i.e. 20 to 24 cm and >25 cm in length, are given in Table 25. No evidence of intersex was detected in histological sections of the gonad from male dab.

Table 22. Criteria used in the assessment of hepatic pathology. Specific criteria agreed at BEQUALM workshop 11/99

1	NAD (no abnormalities detected)
	Early non-neoplastic toxicopathic lesions
2	Hydropic vacuolation
3	Phospholipidosis
4	Fibrillar inclusions
5	Hepatocellular and nuclear polymorphism
6	Spongiosis hepatis
	Foci of cellular alteration
7	Clear cell
8	Vacuolated
9	Eosinophilic
10	Basophilic
11	Mixed
	Benign neoplasms
12	Hepatocellular adenoma
13	Cholangioma
14	Hemangioma
15	Pancreatic acinar cell adenoma
	Malignant neoplasms
16	Hepatocellular carcinoma
17	Cholangiocarcinoma
18	Pancreatic acinar cell carcinoma
19	Mixed hepatobiliary carcinoma
20	Mixedangiosarcoma
21	Hemangiosarcoma
22	Hemangiopericytic sarcoma
23	Other
	Non Specific inflammatory lesions
24	Coagulative necrosis
25	Apoptosis
26	Lipoidosis
27	Hemosiderosis
28	Variable glycogen content
29	Melanomacrophage centres
30	Lymphocytic/monocytic infiltration
31	Granuloma
32	Fibrosis
33	Regeneration

9.3.2 Histological analysis of dab livers from selected areas in the North Sea and Irish Sea

Data from the histological screening of randomly selected livers from dab of approximately 20 cm in length from both cruises are presented in Figures 26 and 27. In 1999, fish from west Dogger and offshore from Flamborough showed the highest prevalence of macroscopic lesions and also had the highest prevalence of FCA. Fish captured from Burbo Bight, Bremerhaven 9 and the Liverpool Bay two sites also showed a higher prevalence of macroscopic lesions when compared to the reference station at Rye Bay where none were detected. In 2000, fish captured from west Dogger again had the highest prevalence of macroscopic and microscopic benign lesions. Prevalence of FCA was highest at the outer Humber, West Dogger and Flamborough sites. However the prevalence of these foci was also high at the reference station at Rye Bay. It should be noted that the prevalence of foci at the Rye Bay site was higher (18.4%) in 2000 than in 1999 (6%). In addition the prevalence of total hepatic pathologies showed a general increase over the period of 1997 to 2000, the majority of which can be attributed to an increased prevalence of inflammatory and non-specific lesions.

9.3.3 Cod diseases

Those fish captured were examined for the presence of external and internal diseases and parasites, including ulcerations, skeletal deformities (scoliosis and lordosis), pseudobranchial tumours, visceral granulomatosis and the parasites *Cryptocotyle* sp. and *Lernaeocera branchialis*, a pathogenic copepod gill parasite. In 1999, of the 23 cod that were examined for disease, one case of infection by *L. branchialis* and one case of skeletal deformity were noted. A total of 335 cod were caught in 2000. At Red Wharf, one case of skeletal deformity, 10 cases of *Cryptocotyle* sp. infection and 15 cases of *L. branchialis* infection were recorded from 63 fish examined. Of the 64 cod captured from Liverpool Bay, 3 were infected with *Cryptocotyle* sp. and 22 with *L. branchialis*. In the North Sea at the Amble site, two cases of *L. branchialis* infection were detected in the 89 fish captured. A total of 56 fish from the Tees, outer Gabbard and Farne Deep were free of infection with the parasite. Finally, of the 48 cod captured from the outer Humber station, one case of visceral granulomatosis and one case of skeletal deformity was recorded.

9.3.4 Examination of the total catch for significant diseases

Other species examined for externally visible pathology and parasites included haddock (*Melanogrammus aeglifinus*), whiting (*Merlangius merlangus*), herring (*Clupea harengus*), hake (*Merluccius merluccius*), mackerel (*Scomber scombrus*), brill (*Scophthalmus rhombus*) and flounder (*P. flesus*). A total of 450 haddock were examined. Low levels of disease were detected in those fish captured from Flamborough, 4 cases of *L. branchialis* infection and one of skeletal deformity were found in 12 fish examined in 1999 and 11 cases of *L. branchialis* infection from 50 fish examined in 2000. Offshore from the Tees, 6 of 30 haddock examined were found to be infected with the parasite in 1999, and in 2000, 11 fish were all infected. In contrast, this parasite was not detected in the 100 fish

Table 23. Summary catch data and disease prevalence in dab (Limanda limanda) by size category and disease severity on stations sampled during RV CIROLANA 3/99 disease survey

Area name (NMMP)	Size range (cm)	Numbers examined		No. and severity of disease cases Recorded according to ICES Guidelines (Bucke *et al*., 1996)												
		Male	Female	LY			U			E/P			HYP			LN
				1	2	3	1	2	3	1	2	3	1	2	3	
West Dogger	15-19	138	65	11	1	0	6	2	8	4	1	0	14	8	4	0
(286)	20-24	54	91	14	3	0	2	8	19	1	3	0	27	14	4	12
	>25	0	28	3	0	0	1	1	4	0	0	0	5	5	2	3
Rye Bay	15-19	95	105	1	0	0	13	1	0	0	1	0	3	1	0	0
(486)	20-24	40	89	1	0	0	13	5	1	2	0	0	4	1	1	0
	>25	0	17	1	1	0	1	1	1	3	0	0	2	0	3	0
Bremerhaven 9	15-19	130	70	9	2	0	10	6	6	2	0	0	5	1	0	0
	20-24	57	93	8	1	2	15	9	12	0	0	0	10	0	0	6
	>25	3	40	6	0	0	6	2	8	1	0	0	4	0	0	3
Flamborough	15-19	124	68	13	1	0	2	3	4	5	0	0	23	2	1	0
(344)	20-24	9	32	3	0	0	0	1	6	0	0	0	6	3	1	1
	>25	1	8	2	0	0	0	0	2	0	0	0	3	0	1	3
St Bees	15-19	165	38	4	0	0	15	1	2	5	0	1	0	0	0	0
(768)	20-24	18	27	1	0	0	0	2	0	1	0	0	0	0	0	1
	>25	0	5	0	0	0	0	0	0	0	0	0	0	0	0	1
Off Tees	15-19	60	64	5	1	0	2	0	4	6	0	0	6	1	0	0
(295)	20-24	17	45	2	3	0	0	0	1	1	0	0	3	1	0	0
	>25	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Bremerhaven 1	15-19	52	52	8	0	0	5	0	1	5	0	0	4	0	2	0
	20-24	18	124	8	0	0	2	3	13	9	1	1	16	3	0	4
	>25	0	104	7	1	1	7	1	6	2	0	0	21	4	2	4
Red Wharf	15-19	50	59	0	0	0	7	1	1	1	0	0	0	0	0	0
(776)	20-24	29	101	5	0	0	12	5	2	2	0	0	1	0	0	0
	>25	0	26	0	0	0	1	0	0	0	0	0	0	0	0	1
Liverpool Bay 2	15-19	77	24	0	0	0	15	3	5	0	0	0	0	0	0	0
(715)	20-24	55	87	2	0	0	18	4	2	1	0	0	0	0	0	7
	>25	0	47	1	0	0	8	1	7	2	0	0	1	0	0	3
Burbo Bight	15-19	140	63	3	0	0	23	1	7	7	1	1	0	0	0	0
(705)	20-24	46	95	2	0	0	6	3	12	1	0	0	0	1	0	6
	>25	0	11	1	0	0	2	0	0	0	0	0	0	0	0	1

Key: LY = Lymphocystis
U = Epidermal ulceration
E/P = Epidermal papilloma
HYP = Hyperpigmentation
LN = Macroscopic liver lesion

caught at the Firth of Forth site and the 100 fish caught at the Farne deep site visited in 2000. These results were consistent with previous findings. Other diseases detected in haddock were 8 cases of *Cryptocotyle sp.* infection in the 27 fish caught at inner Dundrum Bay and two cases of emaciation in the four fish caught offshore from the Humber. These were not attributable to *L. branchialis* infection. Five Angler fish (*Lophius piscatorius*) caught in outer Cardigan bay exhibited infections with the microsporean parasite *Spraguea lophii* which are commonly found in this species but did not show the pigment and ocular anomalies noted in previous years in angler fish from the Celtic Deep. A liver tumour detected in one Dover sole (*Solea solea*) caught off Flamborough was confirmed as an adenoma. *Ichthyophonus* was not detected in any of the 180 herring examined from the North Sea during the two cruises reported here. Normal tissues and examples of diseased tissue and parasites were taken from a range of fish and shellfish species for accession in the Registry of Aquatic Pathology (RAP) reference collection held at the CEFAS Weymouth Laboratory.

Table 24. Summary catch data and disease prevalence in dab (Limanda limanda) by size category and disease severity on stations sampled during RV CIROLANA 4a/00 disease survey

Area name (NMMP)	Size range (cm)	Numbers examined		No. and severity of disease cases Recorded according to ICES Guidelines (Bucke *et al*., 1996)												
		Male	Female	LY			U			E/P			HYP			LN
				1	2	3	1	2	3	1	2	3	1	2	3	
Inner Cardigan Bay	15-19	145	54	4	0	0	16	3	4	2	1	0	10	3	1	0
(656)	20-24	1	31	2	0	0	1	1	0	0	0	0	3	2	0	8
	>25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Red Wharf	15-19	37	63	0	0	0	8	1	0	1	0	0	1	0	0	0
(776)	20-24	16	107	0	0	0	10	3	3	1	0	0	1	0	0	1
	>25	0	124	6	1	0	6	5	6	6	1	0	1	0	0	9
Liverpool Bay	15-19	44	18	3	0	0	10	0	2	0	0	0	1	0	0	0
(706)	20-24	42	75	4	0	0	11	3	6	0	1	0	1	0	1	2
	>25	0	27	1	0	0	3	1	1	0	0	0	0	0	0	3
Liverpool Bay 2	15-19	60	40	2	0	0	19	8	2	1	1	0	0	0	0	0
(715)	20-24	26	74	2	0	0	25	6	4	2	0	0	1	0	0	1
	>25	0	91	0	0	0	16	8	5	1	1	0	2	1	0	7
Burbo Bight	15-19	54	46	2	0	0	13	3	3	0	0	0	1	0	0	0
(705)	20-24	14	102	2	0	0	17	7	5	2	0	0	0	0	0	0
	>25	0	18	1	0	0	1	1	2	1	1	0	0	0	0	2
Off Morecambe	15-19	37	63	0	0	0	9	0	2	0	0	0	0	0	0	0
(796)	20-24	25	87	1	0	0	14	2	1	1	0	0	0	0	0	0
	>25	0	51	2	0	0	9	3	0	1	0	0	1	0	0	0
Firth of Forth	15-19	66	34	9	2	0	1	1	2	7	1	0	8	3	0	0
	20-24	33	85	3	0	0	2	0	1	4	0	0	12	9	4	6
	>25	0	6	0	0	0	0	0	0	0	0	0	1	1	0	1
Amble	15-19	65	35	3	0	0	3	0	0	2	0	0	5	0	0	0
(244)	20-24	80	51	13	1	0	0	0	5	2	0	0	10	2	1	1
	>25	0	3	1	0	0	0	0	0	0	0	0	0	0	0	0
Off Tees	15-19	52	48	5	1	0	5	0	0	0	1	0	2	0	0	0
(295)	20-24	16	25	2	0	1	2	0	0	1	0	0	2	0	0	0
	>25	0	6	3	0	0	0	0	0	0	0	0	3	0	0	0
Northern Dogger	15-19	55	45	4	0	0	16	1	2	2	0	0	4	0	0	0
(Hospital Ground)	20-24	52	63	1	2	0	24	1	4	1	0	0	31	4	7	5
	>25	3	57	3	0	0	18	1	6	0	0	0	13	6	4	5
West Dogger	15-19	61	39	6	0	0	4	2	3	1	0	0	11	1	1	0
(286)	20-24	46	93	7	1	0	3	0	8	4	0	0	45	15	6	11
	>25	0	28	2	0	0	2	1	1	2	0	0	11	2	2	8
Flamborough	15-19	117	83	16	1	0	3	1	3	7	0	0	8	2	1	0
(344)	20-24	16	38	2	4	0	0	0	1	0	0	0	11	3	2	2
	>25	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0
Outer Humber	15-19	36	64	4	0	0	1	0	1	1	0	0	4	1	1	0
(345)	20-24	39	101	8	0	0	3	1	5	1	1	0	17	4	1	2
	>25	0	21	3	0	0	0	0	1	0	0	0	2	0	1	1
Outer Gabbard	15-19	22	42	1	0	0	1	0	0	2	0	0	0	0	1	0
(475)	20-24	31	59	0	0	0	4	1	2	1	0	0	7	2	0	0
	>25	1	19	0	0	0	2	0	0	1	0	0	1	1	1	1
Rye Bay	15-19	58	42	0	0	0	4	1	1	0	0	0	3	1	0	0
(486)	20-24	21	71	0	0	0	2	2	1	1	0	0	6	0	0	0
	>25	2	8	0	0	0	0	0	0	0	0	0	1	0	0	0

Key: LY = Lymphocystis
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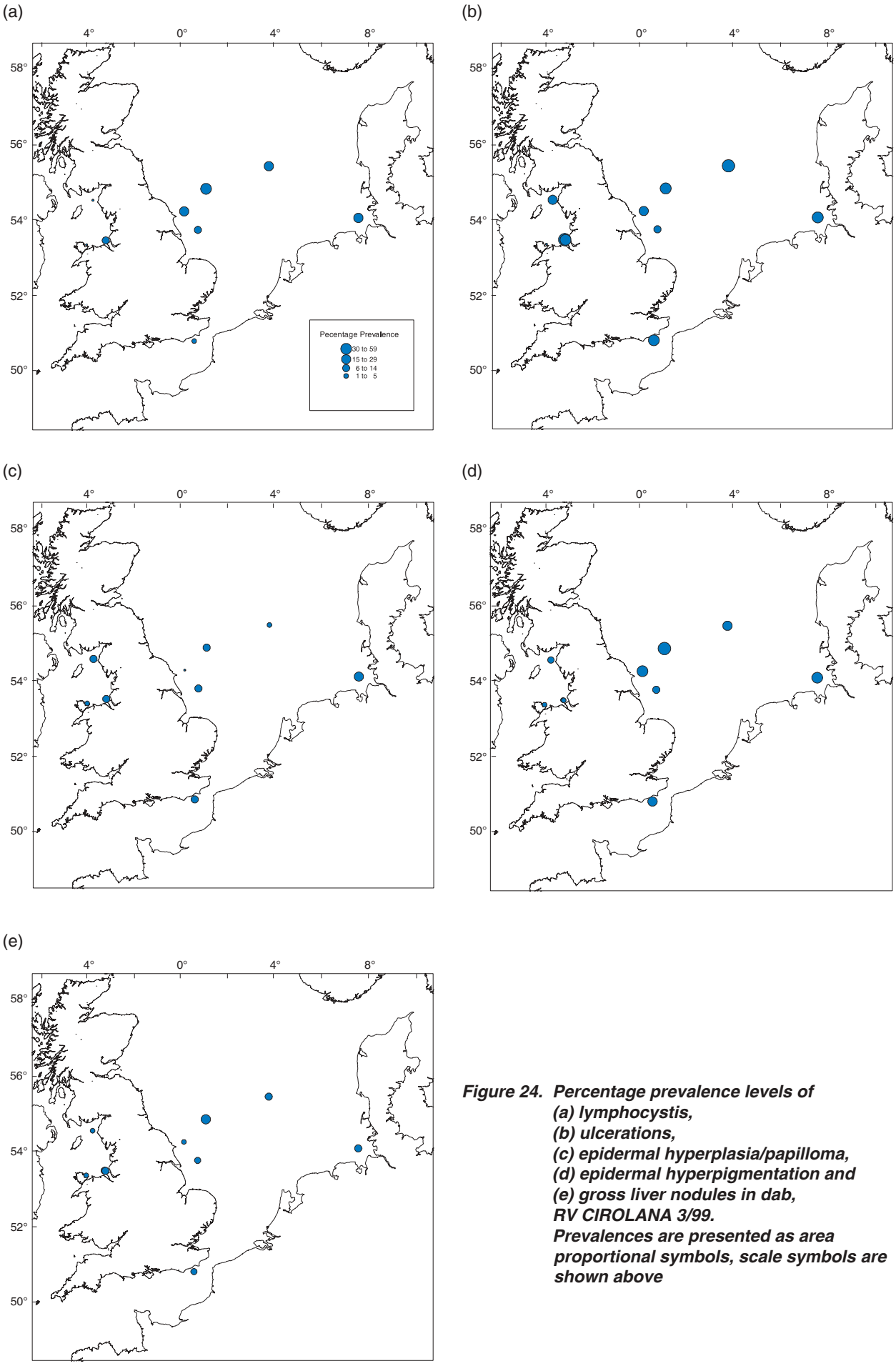

Figure 24. Percentage prevalence levels of (a) lymphocystis, (b) ulcerations, (c) epidermal hyperplasia/papilloma, (d) epidermal hyperpigmentation and (e) gross liver nodules in dab, RV CIROLANA 3/99. Prevalences are presented as area proportional symbols, scale symbols are shown above

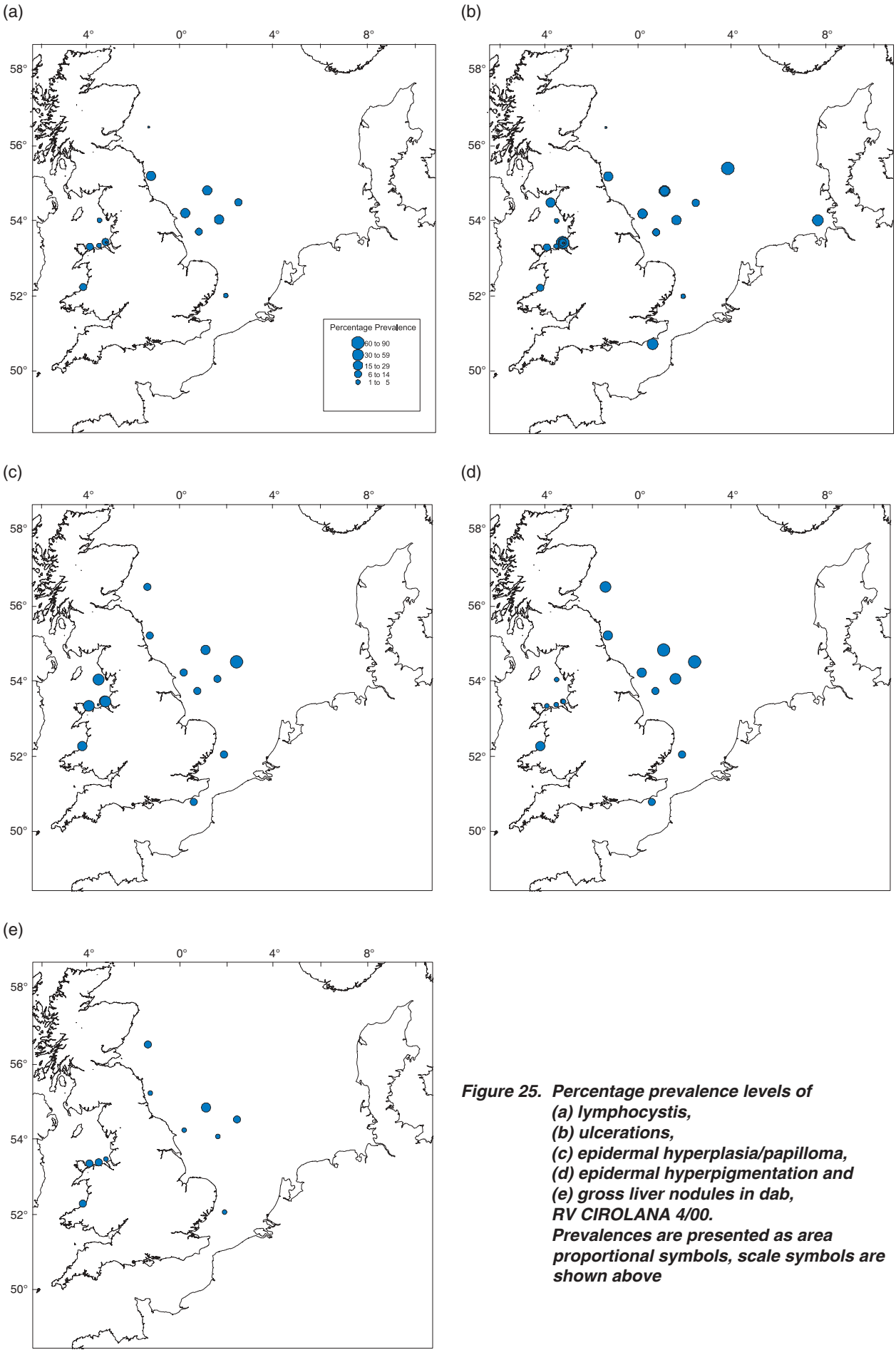

Figure 25. Percentage prevalence levels of (a) lymphocystis, (b) ulcerations, (c) epidermal hyperplasia/papilloma, (d) epidermal hyperpigmentation and (e) gross liver nodules in dab, RV CIROLANA 4/00. Prevalences are presented as area proportional symbols, scale symbols are shown above

Table 25. Histological confirmation and characterisation of specimens of macroscopic liver lesions observed in Dab >20 cm in length captured during RV CIROLANA 3/99 and 4a/00 disease surveys

Area name (NMMP)	Year	Latitude/ Longitude	Total no. dab examined	Macroscopic liver lesions		Histological lesion classification*										% confirmed (pre+) neoplastic lesions		
				No.	%	1	7	8	9	10	12	13	14	15	16	Tumour	FCA	NAD
West Dogger (286)	1999	54° 45.22'N 01° 18.76'E	173	15	8.7	1	0	0	1	0	13	0	0	0	0	7.5	0.6	0.6
	2000	54° 45.22'N 01° 18.76'E	167	19	11.4	3	0	1	0	0	15	0	0	0	0	9.0	0.6	1.8
Northern Dogger	2000	54° 30.91'N 02° 41.43'E	175	10	5.7	0	0	0	0	0	10	0	0	0	0	5.7	0.0	0.0
Rye Bay (486)	1999	50° 52.35'N 00° 42.39'E	146	0	0.0	0	0	0	0	0	0	0	0	0	0	0.0	0.0	0.0
	2000	50° 52.35'N 00° 42.39'E	102	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0
Outer Gabbard (475)	2000	52° 02.51'N 02° 06.67'E	110	1	0.9	0	0	0	0	0	1	0	0	0	0	0.9	0.0	0.0
Bremerhaven 9	1999	55° 27.66'N 04° 05.80'E	193	9	4.7	2	0	1	1	0	4	0	0	0	1	2.6	1.0	1.0
Flamborough (344)	1999	54° 15.1' N 00° 28.66'E	50	4	8.0	3	0	0	0	1	0	0	0	0	0	0.0	2.0	6.0
	2000	54° 15.11'N 00° 28.66' E	57	2	3.5	0	0	0	0	0	2	0	0	0	0	3.5	0.0	0.0
Amble	2000	55° 19.23'N	134	1	0.7	0	0	0	0	0	1	0	0	0	0	0.7	0.0	0.0
St Bees (768)	1999	54° 33.21'N 03° 50.35'W	50	2	4.0	2	0	0	0	0	0	0	0	0	0	0.0	0.0	4.0
Off Tees (295)	1999	54° 45.33'N	64	0	0.0	0	0	0	0	0	0	0	0	0	0	0.0	0.0	0.0
	2000	54° 45.33'N 00° 54.32'W	47	0	0.0	0	0	0	0	0	0	0	0	0	0	0.0	0.0	0.0
Outer Humber (345)	2000	54° 04.96'N 01° 8.60'E	161	3	1.9	0	0	0	0	0	2	0	0	0	1	1.9	0.0	0.0
Bremerhaven 1	1999	54° 04.39'N	246	8	3.3	0	0	0	0	2	3	0	0	0	3	2.4	0.8	0.0
Red Wharf (776)	1999	53° 21.55'N 04° 08.44'W	156	1	0.6	1	0	0	0	0	0	0	0	0	0	0.0	0.0	0.6
	2000	53° 1.42'N 04° 07.58'W	247	10	4.0	3	1	0	0	0	6	0	0	0	0	2.4	0.4	1.2
Liverpool Bay (706)	2000	53° 28.17'N 03° 42.39'W	144	5	3.5	1	0	0	0	1	3	0	0	0	0	2.1	0.7	0.7
Liverpool Bay 2 (715)	1999	53° 23.63'N 03° 37.29'W	189	10	5.3	1	0	0	0	1	8	0	0	0	0	4.2	0.5	0.5
	2000	53° 23.63'N 03° 37.29'W	191	8	4.2	0	0	0	0	1	7	0	0	0	0	3.7	0.5	0.0
Inner Cardigan Bay (656)	2000	52° 17.47'N 04° 17.88'W	32	8	25.0	2	1	0	1	0	3	0	0	0	1	12.5	6.3	6.3
Off Morecambe (796)	2000	55° 56.31'N 03° 23.64'W	163	0	0.0	0	0	0	0	0	0	0	0	0	0	0.0	0.0	0.0
Burbo Bight (705)	1999	53° 28.20'N 03° 22.51'W	152	7	4.6	3	0	0	0	1	3	0	0	0	0	2.0	0.7	2.0
	2000	53° 28.20'N 03°22.51'W	134	2	1.5	1	0	0	0	0	1	0	0	0	0	0.7	0.0	0.7
Firth of Forth	2000	56° 33.69'N 01° 21.38'W	124	7	5.6	2	0	0	0	3	1	0	0	0	1	1.6	2.4	1.6

** Lesion number as indicated in Table22*

Key: 1 = No abnormalities detected (NAD)

FOCI OF CELLULAR ALTERATION
7 = Clear cell foci (glycogen storage)
8 = Vacoulated focus (lipid storage)
9 = Eosinophilic focus
10 = Basophilic focus

BENIGN NEOPLASMS
12 = Hepatocellular Adenoma
13 = Cholangioma
14 = Hemangioma
15 = Pancreatic acinar cell adenoma

MALIGNANT NEOPLASMS
16 = Hepatocellular carcinoma

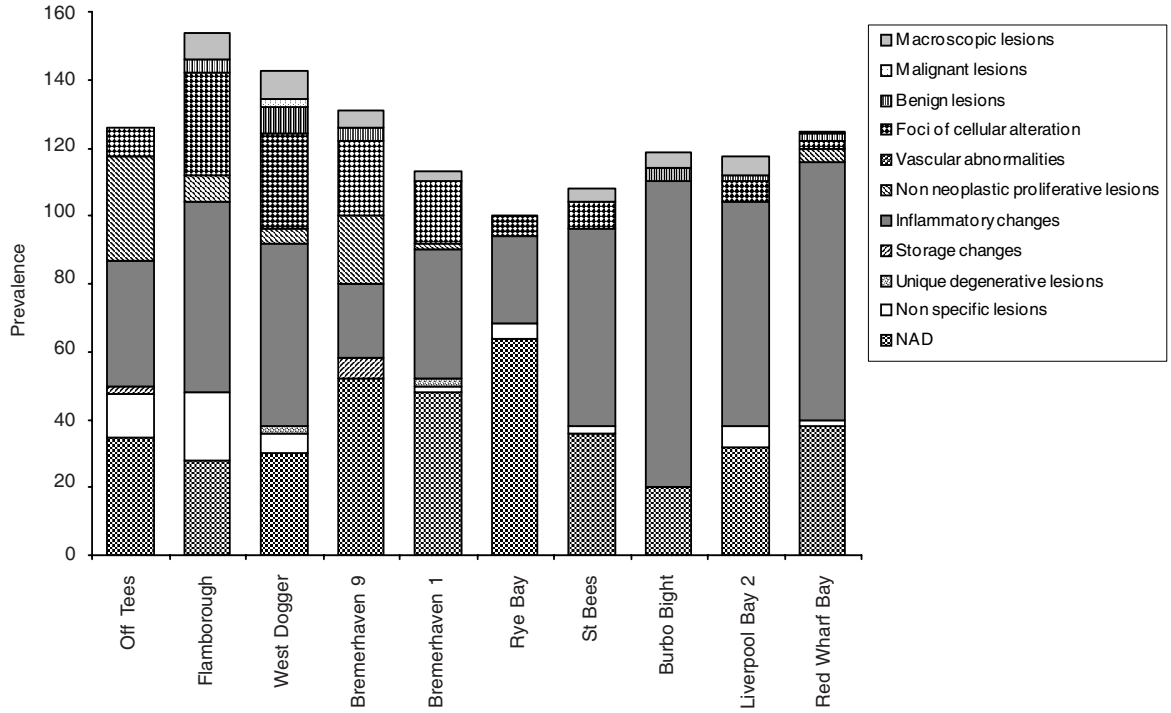

Figure 26. Histological and macroscopic screening for hepatic lesions in dab captured during RV CIROLANA 3/99 fish disease survey

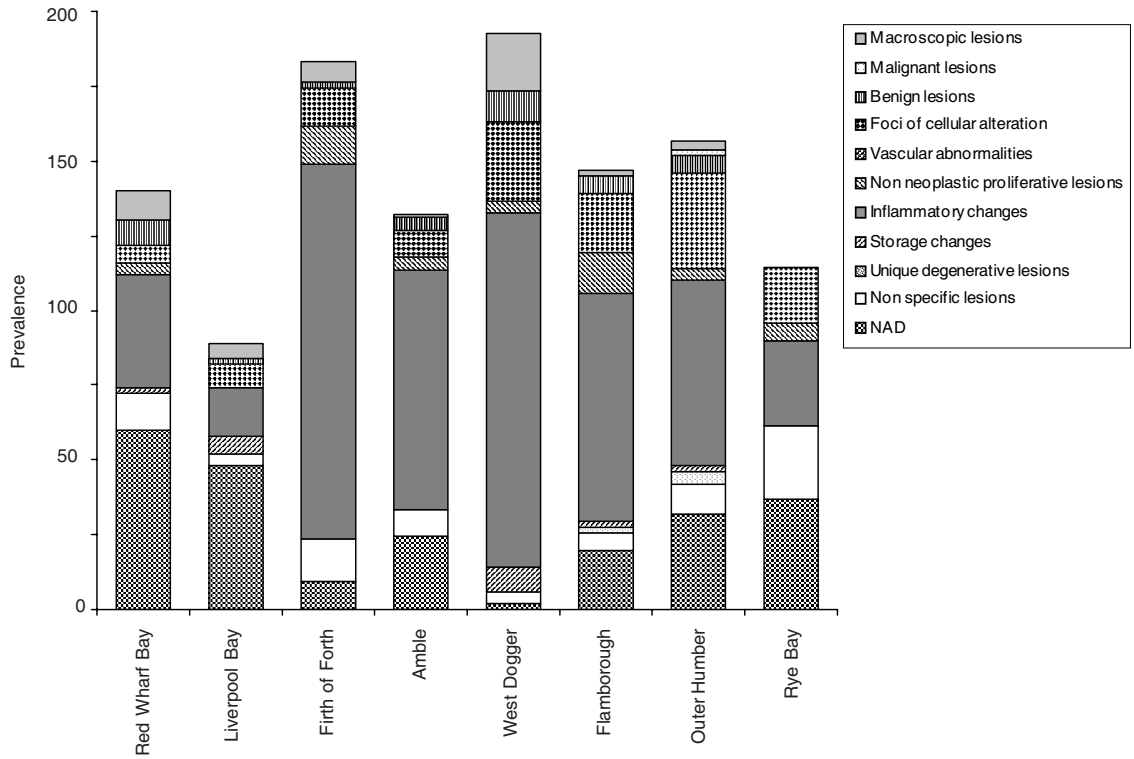

Figure 27. Histological and macroscopic screening for hepatic lesions in dab captured during RV CIROLANA 4a/00 fish disease survey

9.4 Conclusions

- Overall, the prevalence of external diseases of dab examined during the 1999 and 2000 monitoring periods reported here are similar to those previously described from the same sampling locations in previous years. The dab captured off Flamborough and at the western Dogger Bank continue to exhibit higher levels of disease than those fish captured at the Rye Bay reference site.
- Temporal trends in disease prevalence tend to be stable or slightly increasing.
- Hyperpigmentation was again a prominent condition noted in dab from several areas in the North Sea but generally only present at low prevalence in the Irish Sea and in the English Channel at Rye Bay. However a moderate prevalence was detected at Outer Gabbard and a high prevalence was detected at the Firth of Forth. The prevalence of hyperpigmentation has also apparently increased at the Cardigan Bay and West Dogger sites during the 1990s.
- The assessment of toxicopathic liver lesions is now routine in marine biological effects monitoring. The presence of macroscopic nodules or tumours was again noted in fish captured from numerous sites, with the highest prevalences recorded in fish from the Dogger Bank and Flamborough sites. This is consistent with previous findings. A high prevalence was also detected at the Cardigan Bay site, though it should be noted that due to low sample numbers from this site, this data should be treated with some caution. There was good correlation between the observation of gross liver lesions and their histological confirmation.
- Few liver lesions were detected in the other flatfish species examined. The presence of an hepatocellular adenoma in Dover Sole confirms our previous data that indicated that this species is susceptible to tumour formation.
- The disease status of other species appears to be at background levels, with no apparent emerging conditions of concern noted during the current survey.

10. MEASUREMENTS OF DNA ADDUCTS IN MARINE FLATFISH FROM OFFSHORE AND ESTUARINE LOCATIONS

10.1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a class of ubiquitous environmental contaminants found in marine sediments and waters associated with urbanised estuarine and coastal areas (CEFAS, 1998). The presence of PAHs in the marine environment is of concern because they are known to have mutagenic and carcinogenic properties. PAHs are hydrophobic in nature and accumulate in marine sediments, and benthic flatfish are therefore likely to be exposed to higher levels than pelagic fish species. Parent PAHs are extensively metabolised in fish and their measurement in tissue samples, by standard analytical methods, does not provide an adequate assessment of exposure. Furthermore, PAH metabolism may also result in the production of reactive intermediates that are able to bind to cellular macromolecules, such as DNA, and so exert genotoxicity. For example, while the majority of PAHs are rendered less harmful following cytochrome P450 mediated metabolism, others (particularly the 4-5 ringed PAHs) may form genotoxic metabolites with potentially mutagenic and/or carcinogenic properties (see Figure 28). The DNA adducts formed by the interaction of these reactive PAH metabolites with DNA are the precursors of DNA mutations, and as such are mechanistically linked to the initiation of cancer. Indeed, field and laboratory studies from both Europe and North America have identified PAHs as a causative agent in fish neoplasia (Myers *et al*., 1990, 1998; Stein *et al*., 1992; Baumann, 1998).

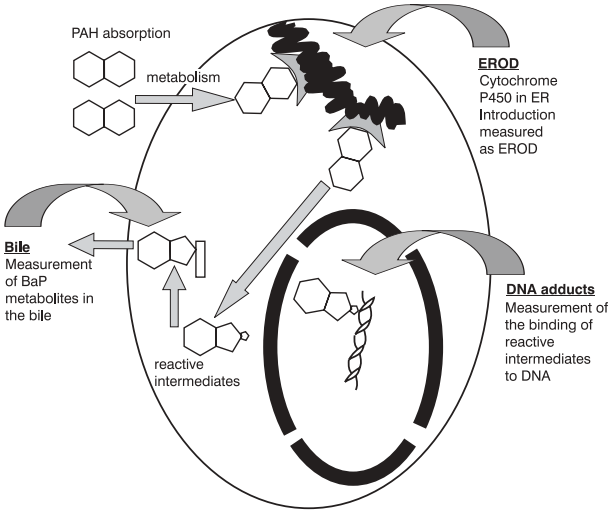

Figure 28. Schematic of the potential sequence of steps including PAH uptake, metabolism, detoxification and DNA adduct formation

Previous studies have supported the use of DNA adducts in selected aquatic species as a sensitive biomarker of environmental contamination (Harvey *et al*., 1997; CEFAS, 1998). The quantitative analysis of DNA adducts provides a measure of the biologically effective dose reaching a critical target site and thus integrates the multiple toxico-kinetic factors (i.e. contaminant bioavailability, metabolism and detoxification) involved in genotoxic exposure. In the assessment of exposure to genotoxic compounds and subsequent DNA

adduct formation there is a requirement for analytical methods that are extremely sensitive and applicable to a multitude of different genotoxic contaminants. Of the techniques currently available, these criteria have largely been satisfied by the ^{32}P-postlabelling assay for the detection of DNA adducts (Randerath *et al*., 1981; Harvey *et al*., 1997). The technique is applicable to any sample from which DNA can be isolated, and providing that the genotoxic adduct is amenable to the ^{32}P-labelling reaction and subsequent thin layer chromatography, its prior characterization (or genotoxic agent causing it) is not required. It is this last feature which makes the ^{32}P-postlabelling assay appropriate for aquatic biomonitoring, where the sources of exposure are likely to derive from complex mixtures of unknown chemicals. The technique is also extremely sensitive; it is capable of detecting one adducted nucleotide in 10^8 - 10^{10} undamaged nucleotides.

10.2 Methods

Flatfish species, dab (*Limanda limanda*) and European flounder (*Platichthys flesus*) more than 10 cm in length were collected by means of a Granton trawl or a 2 m beam trawl. Livers were immediately removed and stored in liquid nitrogen. DNA was isolated using a standard phenol/chloroform extraction procedure. All isolates were essentially free from RNA and protein contamination and were adjusted to a final concentration of 1 µg µl^{-1} and stored at -80ºC prior to ^{32}P-postlabelling. DNA adducts were determined using the butanol version of the ^{32}P-postlabelling assay (Gupta *et al*., 1985), as described previously (Harvey *et al*., 1997).

10.3 Results

10.3.1 Analysis of hepatic DNA adducts in dab collected from coastal and offshore NMMP sites

The total hepatic DNA adduct levels along with representative chromatograms of ^{32}P-postlabelled DNA from individual samples of dab hepatic tissue are displayed in Table 26 and Figure 29 a-c respectively. With the exception of outer Cardigan Bay (1998 and 1996) and off Tyne (1998) DNA adducts were detected in some fish from all NMMP sites analysed. In the

Table 26. Levels of hepatic DNA adducts (DNA adducts per 10^8 undamaged nucleotides) in dab and flounder from UK coastal waters

Date	Station	NMMP	DNA adducts per 10^8 undamaged nucleotides
Dab (offshore/coastal)			
6/2000 [c]	Firth of Forth	165	3.2 ± 0.8 [a] (7) [b]
6/2000	Amble	244	7.6 ± 2.0 (10)
6/1999	Amble	244	2.3 ± 1.0 (10)
6/2000	West Dogger	285	5.4 ± 1.0 (10)
6/1996	West Dogger	285	5.6 ± 2.3 (4)*
6/2000	Flamborough	344	2.0 ± 0.5 (10)
6/2000	Off Humber	345	4.3 ± 1.1 (10)
6/1996	Off Humber	345	7.6 ± 1.0 (4)*
6/1999	Rye Bay	486	2.1 ± 0.4 (10)
6/2000	Celtic Deep	605	10.6 ± 2.1 (10)
6/2000	Outer Cardigan Bay	665	6.0 ± 1.3 (10)
6/1999	Outer Cardigan Bay	665	10.0 ± 1.9 (9)
6/1998	Outer Cardigan Bay	665	0.0 ± 0.0 (5)*
6/1996	Outer Cardigan Bay	665	0.0 ± 0.0 (5)*
6/2000	Burbo Bight	705	2.3 ± 1.3 (10)
6/1999	Burbo Bight	705	6.1 ± 1.4 (10)
6/1998	Burbo Bight	705	5.6 ± 2.2 (10)
6/1996	Burbo Bight	705	16.2 ± 9.4 (4) *
6/2000	Liverpool Bay	715	7.5 ± 1.2 (10)
6/2000	Red Wharf Bay	776	8.2 ± 1.9 (10)
6/1999	Red Wharf Bay	776	12.7 ± 2.2 (10)
6/1998	Off Tyne		0.0 ± 0.0 (5)*
6/1996	Off Tyne		4.0 ± 2.6 (5)*
Flounder (Estuaries)			
5/2000	Tyne	/	7.4 ± 3.1 (6)
10/2000	Tyne	/	14.1 ± 2.7 (6)
10/2000	Tees	/	5.0 ± 1.5 (6)
10/2000	Mersey	/	7.4 ± 3.1 (5)
5/2000	Alde	/	1.7 ± 0.4 (6)
10/2000	Alde	/	3.0 ± 0.9 (6)

[a] *Mean adduct levels ± SE*
[b] *Numbers in parentheses represent number of individual liver samples analysed*
[c] *Sample date*
* *Number in parentheses represents number of pooled samples analysed (3 fish per pool)*

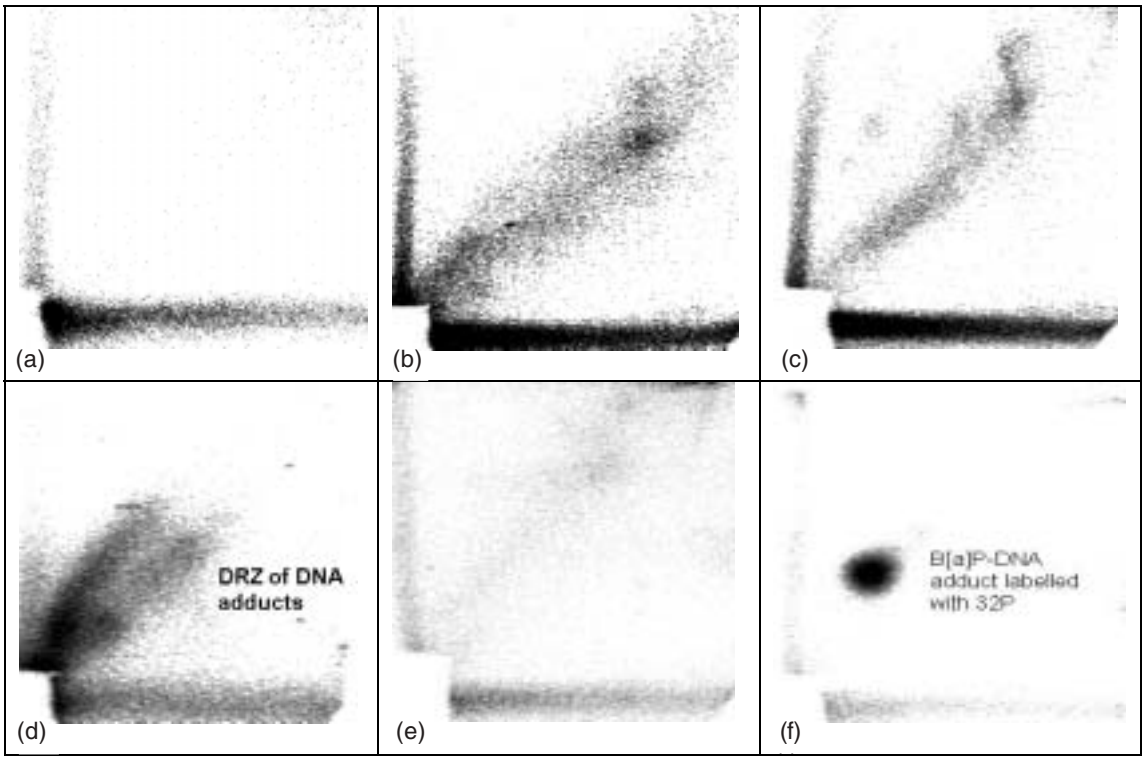

Figure 29. Typical DNA adduct profiles obtained from ^{32}P-postlabelling. (a) Dab Hepatic DNA from a sample collected from outer Cardigan Bay (1998), no DNA adducts are observed. (b) Diagonal radioactive zone (DRZ) of radiolabelled hepatic DNA adducts from a dab caught from Red Wharfe Bay (1999). (c) Representative hepatic DNA adducts profile from dab sampled from Burbo Bight (1998). (d) Flounder hepatic DNA sample collected from the Tyne (2000) displaying a clear DRZ of ^{32}P-labelled DNA adducts. (e) Flounder hepatic DNA sample from the Alde (2000) displaying a low level of DNA adducts. (f) Positive control consisting of dab hepatic DNA treated in vivo with 1.5 mM BaP diol-epoxide

majority of cases the DNA adduct profiles detected consisted of diagonal radioactive zones (DRZs) of DNA adducts indicative of exposure to complex mixtures of genotoxins (see Figure 29 b-c). DNA adduct levels varied both spatially and temporally within the sample sets analysed. For example, dab sampled from outer Cardigan Bay between 1996 and 1998 did not display any DNA adducts detectable under current assay conditions. This is in agreement with the view that outer Cardigan Bay is a suitable offshore reference site with minimal anthropogenic contamination (∑15PAH 26 µg kg^{-1}; Woodhead *et al*., 1999). In contrast, those dab caught at outer Cardigan Bay during 1999 and 2000 contained DNA adduct profiles characteristic of exposure to complex mixtures of carcinogenic metabolites.

10.3.2 Analysis of hepatic DNA adducts in flounder collected from the Alde, Tyne, Tees and Mersey

The total hepatic DNA adduct levels along with representative chromatograms of ^{32}P-postlabelled DNA from individual samples of flounder hepatic tissue are displayed in Table 26 and Figure 29 d-e respectively. A significantly higher level ($p<0.05$) of DNA adducts were detected in flounder caught from the contaminated Tyne estuary compared with fish from the reference Alde site during both the spring and autumn sampling periods. All DNA adduct profiles observed in flounder sampled from the Tyne consisted of a DRZ of ^{32}P-labelled adducts. In contrast, flounder DNA adduct profiles from the Alde lacked distinct DRZs and generally displayed lower levels of ^{32}P-labelled adducts. Previous studies have demonstrated DRZs to be characteristic of exposure to complex mixtures of aromatic and/or hydrophobic genotoxins, such as those formed by PAHs (Varanasi *et al*., 1987). PAH bile metabolite analysis of flounder sampled during May and October confirmed that fish sampled from the Tyne were indeed exposed to elevated levels of PAH compared with those residing in the Alde (data not shown). The hepatic DNA adduct levels detected in flounder from the Mersey and Tees during the autumn were elevated over those detected in the Alde. However, differences between sites were not statistically significant.

10.4 Discussion

10.4.1 Significance of hepatic DNA adducts detected in dab

Hepatic DNA adduct profiles consisting of DRZs were detected in dab at the majority of sites investigated. While such DNA adduct profiles are characteristic of exposure to complex mixtures of pollutants it is difficult to relate the levels of adducts to a specific group of environmental contaminants. However, the DRZs detected in this report share similarities in chromatographic characteristics with adduct profiles detected in wild fish collected from PAH contaminated environments (Myers *et al*., 1990; Lyons *et al*., 1999; Ericson *et al*., 1998). Significantly, similar DNA adduct profiles have been detected following laboratory exposure of marine flatfish to PAHs (CEFAS, 1998). It should be noted that previous national biological effects monitoring programmes in the North Sea have shown that in certain areas, dab exhibit relatively high prevalence of gross hepatic nodules (CEFAS, 1998). Consequently, it is likely that the detection of DNA adducts provides a sensitive measure of past and current carcinogenic exposure and is thus an extremely useful biomarker of species, population and ecosystem health.

10.4.2 Migrational influences affecting biomarker responses in dab

There are many problems arising from using a migratory species in biomonitoring program, not least the fact that determining previous exposure history is difficult. There is a paucity of data detailing the migrational movements of dab; the few studies conducted have suggested that populations of dab tend to be a temporary aggregation of fish originating from a larger area (Rijnsdorp *et al*., 1992). Therefore, the results of a biological effects monitoring program using dab or other migratory species can only be interpreted in general terms, especially when the endpoints measured are slowly induced or persistent once induced.

The results presented in this report demonstrate that the populations of dab around UK coastal waters are being exposed to genotoxins at levels sufficient to induce detectable levels of DNA adducts. However, due to the transient nature of the dab populations at the NMMP locations it is not possible to link site specific contamination with the induction of DNA adducts. This is highlighted by the fact that during 1999 and 2000, DNA adducts representative of PAH exposure were detected in dab at outer Cardigan Bay, a relatively uncontaminated site. Previous monitoring surveys (1996 and 1998) had not detected any DNA adducts in dab sampled from outer Cardigan Bay. This temporal variation in DNA adduct detection further highlights the problems associated with linking biomarker responses in a migratory species to point sources of contaminants and is thought to reflect the time prior to capture that populations have resided in contaminated areas. Once formed, DNA adducts, due to a low repair capacity, are relatively persistent and therefore reflect long-term PAH exposure. Previously, it has been demonstrated that DNA adducts may persist many months after the initial contaminant exposure. For instance, the persistence of DNA adducts was evident in English sole captured from a contaminated waterway and reared in clean conditions in the laboratory; greater than 50% of the DNA adducts present at the time of capture were still detectable following 60 days of depurination (Stein *et al*., 1995). The problem of migration by monitoring species has been previously highlighted. Cooreman *et al.* (1993) suggested that large variations in EROD activity between individuals at a single site could be attributed to local migrations and Kirby *et al.* (1999b) also suggested that EROD activity distribution patterns in dab after the *SEA EMPRESS* oil spill may have been blurred by migratory activity.

10.4.3 Significance of hepatic DNA adducts detected in flounder

Flounder usually confine themselves to their home estuaries for up to 8 months of the year and subsequently the DNA adduct data obtained is more likely to reflect local contamination levels than that obtained for dab. Fish sampled from the Tyne during both the spring and autumn periods exhibited patterns of aromatic/hydrophobic DNA adducts in the liver, typical of exposure to a complex mixture of PAHs (DRZs consisting of over-lapping adducts). Furthermore, the analysis of bile indicated that flounder collected from the Tyne had recently been exposed to PAH compounds, confirming that the high level of sediment associated PAHs found in the Tyne were bioavailable to the exposed fish. These findings support previous studies demonstrating that fish stocks inhabiting the Tyne are exposed to high levels of sediment associated PAH, and that a proportion of the bioavailable PAHs were being metabolized to carcinogenic metabolites (Lyons *et al*., 1999). Significantly, histopathological surveys have shown that flounder from the Tyne have elevated frequencies of known pre-cancerous lesions, specifically hepatocellular FCAs, when compared with fish collected from reference sites (DETR, 2001).

10.5 Conclusions

The results documented here provide the first detailed studies concerning the existing levels of DNA adducts in hepatic tissue of dab and flounder sampled from sites around the UK and North Sea coastal waters. The results point to several observations concerning the use of DNA adducts as dosimeters of environmental exposure to genotoxins.

Detection of DNA adducts
Dab and flounder both exhibited patterns of DNA adducts indicative of exposure to complex mixtures of genotoxins. The chromatographic characteristics of the DNA adduct profiles suggested that a proportion of the DNA adducts detected were a result of the bioavailability and biotransformation of sediment associated PAH. However, it should not be discounted that other (non-PAH) contaminants present in the environment also contributed to the over-all genotoxic response observed.

Migrational influences
The migration of dab is a complicating factor when trying to assess the results of any biomonitoring study. DNA adducts are relatively persistent once formed (may last several months) and as such the detection of DNA adducts in dab from one particular location may be a consequence of contaminant exposure unrelated to the site of capture. This data highlights the need for an integrated monitoring survey where biomarkers of recent exposure (days/weeks), such as EROD and bile metabolites, are used along-side DNA adducts which reflect cumulative contaminant exposure over a period of months.

Significance of DNA adducts
Previous studies have associated the presence of DNA adducts, indicative of exposure to complex PAH mixtures, with increases in the incidence of pre-cancerous and cancerous lesions in marine flatfish (Maccubin *et al*., 1990; Stein *et al*., 1990). The DNA adduct data presented here demonstrates that certain dab and flounder populations are being exposed to carcinogenic contaminants and these may, in part, be responsible for the detection of known pre-cancerous lesions observed in UK flatfish populations.

Explanation of Figure 29
Figure 29 is provided to allow a qualitative view of the types of DNA adduct profiles detected in the samples of dab and flounder investigated. Each figure (Figure 29a, 29b etc.) represents an autoradiograph of a chromatography plate onto which ^{32}P-labelled DNA from the particular sample under investigation has been spotted. DNA adducts (DNA modified by a specific genotoxic metabolite) will migrate to sections of the chromatography plate (this is dependent on both the chemical structure of the particular genotoxin and the chromatography conditions used). The adduct is then visualised by autoradiography and analytical imaging technologies due to the ^{32}P tags on the DNA. As observed in Figure 29f, B*a*P adducts migrate to a distinct spot approximately 45 degrees from the origin at the bottom left corner of the plate. As explained in the text, other PAH type adducts tend to migrate to similar positions along this 45 degree line. Therefore, when fish are exposed to complex mixtures of PAH, numerous PAH-DNA adducts are formed. This results in the broad band of radioactivity (termed diagonal radioactive zones, DRZ) detected in fish collected from polluted environments (Figure 29b-d). Figure 29a represents a sample where no DNA adducts were detected. The radioactivity detectable along the left-hand side and bottom edge of the plate represents unincorporated radioactive label and is not included in any analysis.

SEDIMENTS

11. ASSESSMENT OF SEDIMENT QUALITY: WHOLE SEDIMENT BIOASSAYS USING ARENICOLA MARINA AND COROPHIUM VOLUTATOR

11.1 Introduction

Since 1992, sediment toxicity has been evaluated using whole sediment bioassays, employing organisms that live in and feed directly on the sediment. These features make them better indicators of toxicity than techniques previously applied. Prior to 1992, sediment toxicity was assessed using the oyster embryo bioassay, exposing oyster embryos to sediment elutriates. Although this is a sensitive assay, it does not give a true reflection of sediment toxicity because it does not mimic the exposure of sediment dwelling animals.

Two whole sediment bioassays, using the polychaete *Arenicola marina* and the crustacean amphipod *Corophium volutator* have been developed which are now routinely used in the NMMP. *A. marina*, commonly known as the lugworm, is a surface deposit feeding polychaete, which inhabits intertidal and subtidal areas, whereas *C. volutator* is a marine amphipod which can be found on the foreshore of most unpolluted estuaries in the UK. These two assays were deployed aboard a CEFAS research vessel between 1992 and 1995. Since 1996, sediment samples have been taken and stored (either refrigerated or frozen) on board research vessels, before being transported back to the laboratory and bioassayed.

11.2 Methods and materials

11.2.1 Sediment collection

Intermediate and offshore sediments are collected using a Reineck Box corer. From each undisturbed core the surface 10 cm layer of sediment is removed and homogenised, from which a sample is taken for bioassay. A Day grab is sometimes used during periods of inclement weather or where the sediments are not suitable for coring. In estuaries, sediment samples are usually collected using a hand held van Veen grab. Reference sediment was collected from Shoeburyness, Essex and was used as the negative control.

11.2.2 Arenicola marina sediment bioassay

Animals were obtained from a local bait supplier and either used in the test the same day or kept in a 40 litre tank with a clean layer of reference sediment, running seawater and aeration, until ready for use. When the sediment samples had been thoroughly defrosted and homogenised they were placed into polythene sandwich boxes. The boxes were filled with a 4 cm depth of sediment (about 1 kg dry weight) and for each field and control sample, three replicates were set up. Filtered seawater (10 µm) was added 24 hours later to give a 3-4 cm layer on top of the sediment. Aeration was then added. The tanks were then left for another 24 hours. If the animals were held in a holding tank beforehand, they were first gently sieved from the tank and then added to the test. Five animals of approximately 1 g in weight were placed into each test container. After 10 days the contents of each test container were sieved and the number of surviving worms recorded. The number of casts produced on the surface of the sediment was counted daily during the exposure period to obtain a measure of the feeding rate of the worms; this was a sublethal as opposed to an acute (mortality) endpoint. The casts were smoothed over after each count. The survival results are expressed as percentage mortality after 10 days and the casting rate expressed as mean daily number of casts. The control mortality must not exceed 10 percent if the test is to be valid. ANOVA is used to assess whether there is a statistically significant difference between the control and test mortality, whereas the Dunnett's t-test is used to assess if the casting in the test sediment is significantly different from the casting in the control sediment.

11.2.3 Corophium volutator sediment bioassay

Animals were collected from a nearby muddy shore on the River Crouch estuary adjacent to the laboratory, sieved from their native sediment and maintained in a 40 litre aquarium with a layer of sediment, running seawater and aeration for a minimum period of five days before the start of the test. Sediment samples (thoroughly defrosted if frozen) were homogenised before being placed into 1 litre glass beakers. The beakers were filled with a 2 cm depth of sediment (about 300 g dry weight) and for each field and control sample, three replicates were set up. Filtered seawater (10 µm) was added 24 hours later to the 850 ml mark and aeration supplied. The beakers were then left for a further 24 hours, ten adult *Corophium* (4-6 mm in length) were added to each test beaker. After 10 days the contents of each test beaker was sieved, and the number of surviving amphipods recorded. The results are expressed as percentage mortality after 10 days. The control mortality must not exceed 20% if the test is to be valid. ANOVA is used to assess whether there is a statistically significant difference between the control and test mortality.

11.3 Results

RV CIROLANA, 30 April 1999 –20 May 1999

A total of 19 sediment samples were tested for toxicity using both the *C. volutator* and *A. marina* bioassays. Results are presented in Table 27. Only one sample produced a significant (p<0.01) acute response to *C. volutator* - Station 78, which is located off Plymouth. A total of 5 stations significantly (p<0.05) reduced feeding rate of *A. marina* compared to the control. Stations 37, 53, 57, 134 and 162 reduced casting rates to 52%, 53%, 60%, 57% and 48% of the control respectively.

RV CIROLANA, 10 June – 1 July 2000

A total of 21 sediment samples were tested for toxicity using both the *C. volutator* and *A. marina* bioassays. Results are presented in Table 28. None of the samples tested produced a significant acute response and only one sample (Station 27) significantly (p<0.05) reduced the feeding rate of *A. marina* (to 67% of the control).

11.4 Discussion

One sediment sample in 1999 produced an acute response with *C. volutator* (56.6% mortality). However, no significant mortality was seen in the *A. marina* bioassay. This highlights the importance of using a battery of tests when assessing sediment quality, as it is evident that the two test organisms differ in their susceptibility to the contaminants in the sediment.

The feeding rate of *A. marina* was also significantly reduced (from 40% - 52% of the control) at five stations (37, 53, 57, 134 and 162), indicating that at these stations the sediment is moderately contaminated. The use of a sublethal endpoint in the *A. marina* assay has proved to be very useful and indicates that the sediments are having a chronic effect rather than an acute effect. This highlights the need for development and application of chronic tests to assess the long-term effects of moderately and lightly contaminated estuarine, coastal and offshore sediments.

Table 27. RV CIROLANA, 30 April 1999 –20 May 1999: 10-day sediment bioassays using the lugworm Arenicola marina and the amphipod Corophium volutator

NMP station	Station	Actual Position		Location	*Corophium*	*Arenicola*	
		Latitude	Longitude		% mortality	% mortality	Mean daily number of casts
	Control			Shoebury Sands	3.3	13.3	3.46
NMP 385	2	53° 08.785'N	00° 33.518'E	Wash	3.3	6.7	3.79
NMP 375	3	53° 19.502'N	00° 25.319'E	Humber	6.7	6.7	5.125
NMP 244/246	21	55° 16.327'N	01° 15.355'W	Amble	0.0	0.0	3.88
NMP 295	29	54° 44.794'N	00° 54.016'W	Off Tees	6.7	6.7	5.13
	37	55° 29.771'N	04° 08.108'E	Bremerhaven 9	3.3	0.0	1.79*
	40	54° 04.774'N	08° 09.982'E	Bremerhaven 1	3.3	0.0	5.67
NMP 475	53	52° 04.987'N	02° 04.305'E	Outer Gabbard	0.0	13.3	1.83*
NMP 465	56	51° 31.232'N	00° 58.278'E	Thames Warp	3.3	13.3	4.88
	57	50° 56.361'N	01° 17.250'E	Varne	10.0	6.7	2.08*
	65	50° 40.749'N	00° 49.681'W	Selsey Bill	13.3	6.7	3.71
NMP 585	78	50° 02.158'N	04° 21.960'W	Off Plymouth	56.6*	6.7	4.33
	100	51° 15.119'N	06° 00.186'W	Celtic Deep	3.3	13.3	4.46
NMP 815	120	54° 04.028'N	05° 29.986'W	Dundrum Bay	10.0	0.0	3.46
NMP 805	134	54° 59.995'N	03° 50.012'W	SE Isle of Man	0.0	6.7	1.96*
NMP 795	144	53° 53.281'N	03° 25.545'W	Off Morcambe	3.3	0.0	3.71
	153	53° 28.288'N	03° 21.054'W	Burbo Bight	0.0	0.0	5.04
	160	53° 22.067'N	04° 11.350'W	Red Wharf	3.3	0.0	4.46
	162	52° 21.788'N	04° 54.092'W	Outer Cardigan	6.7	13.3	1.67*

** Denotes statistically significant from control (p<0.05), using ANOVA.*

Table 28. RV CIROLANA, 10 June-1 July 2000: 10-day sediment bioassays using the lugworm Arenicola marina and the amphipod Corophium volutator

NMP station	Station	Actual Position		Location	Corophium	Arenicola	
		Latitude	Longitude		% mortality	% mortality	Mean daily no. of casts
	Control			Shoebury Sands	5.0	6.7	3.4
	1	51° 32.74'N	03° 55.87'W	Swansea Bay	6.7	13.3	3.17
NMP665	15	52° 21.81'N	04° 54.06'W	Off Cardigan Bay	3.3	10	3.7
NMP656	27	52° 17.76'N	04° 17.25'W	Inner Cardigan Bay	6.7	6.7	*2.13
	33	53° 21.73'N	04° 09.20'W	Red Wharf Bay	3.3	0	3.2
NMP715	35	53° 29.79'N	03° 39.88'W	Liverpool Bay	3.3	13.3	3.2
	39	53° 28.31'N	03° 22.63'W	Burbo Bight	10.0	13.3	3.1
NMP705	40	53° 28.31'N	03° 15.37'W	Burbo Bight	10.0	0	2.6
NMP805	66	54° 01.01'N	03° 39.98'W	SE Isle of Man	6.7	0	4.1
NMP805	69	54° 01.04'N	03° 39.96'W	SE Isle of Man	0.0	0	4.8
NMP805	72	54° 03.35'N	03° 52.81'W	SE Isle of Man	13.3	0	4.6
NMP815	88	54° 06.23'N	05° 07.18'W	Dundrum Bay	6.7	0	3.8
NMP845	89	54° 42.28'N	05° 42.81'W	Belfast Lough	10.0	0	3.8
NMP825	98	54° 44.96'N	05° 36.81'W	Belfast Lough	3.3	0	3.3
NMP285	119	54° 46.46'N	01° 17.83'E	West Dogger	10.0	13.3	4.5
	129	55° 00.60'N	01° 16.57'W	Tyne Spoil Ground	6.7	0	3
	130	54° 59.34'N	01° 16.53'W	Tyne Spoil Ground	10.0	13.3	3.5
	141	54° 43.93'N	01° 08.38'W	Tees (Altenative tow)	3.3	13.3	3.6
NMP345	149	54° 04.08'N	01° 47.46'E	Off Humber	3.3	13.3	3
NMP385	155	53° 08.97'N	00° 34.30'E	Inner Wash	20.0	0	3.2
NMP465	160	51° 30.82'N	00° 58.24'E	Thames Warp	0.0	6.7	4.1
NMP485	166	50° 56.01'N	01° 16.82'E	Varne	16.7	0	2.8

** Denotes statistically significant from control (p<0.05), using ANOVA.*

Generally, little toxicity is observed at offshore stations, although a significant reduction in *A. marina* casting was observed at the offshore station Bremerhaven 9, located at Dogger Bank in the North Sea. This may be due to the fact that contaminants are able to re-concentrate at certain points, possibly due to current driven resuspension and deposition, and exert a certain degree of toxicity. Poor quality sediment has been identified at this location in previous studies (CEFAS, 1998).

All sediments sampled in 2000 were not acutely toxic. Although one sample (Station 27 - Inner Cardigan Bay) significantly reduced the feeding rate of *A. marina*, this was only by 37% and therefore the sediment is considered to be lightly contaminated.

The bioassay results from 1999 and 2000 show that sediment quality does seem to be improving at some sites, as evidenced by a decrease in mortality and an increase in casting rate since bioassays began in 1992 (CEFAS, 1998). Examples of such stations are Bremerhaven Station 1, Varne and West Dogger.

The toxicity data produced since 1992 clearly show that the main areas of concern are heavily industrialised estuarine sediments rather than offshore sediments. The NMMP programme will be concentrating on these areas in the coming years while still sampling some of the offshore stations.

12. PRELIMINARY RESULTS OF PAH ANALYSIS IN MARINE SEDIMENTS

To fulfil the requirements of the OSPAR monitoring programme, and to answer the question posed under JAMP issue 1.10 (Oslo and Paris Commissions, 1995), "What are the concentrations of PAH in the maritime area?" CEFAS have begun a 3 year sediment sampling programme. Starting from the year 2000, surface sediment samples were collected from the locations listed in Table 29.

In order to comply with the requirements for temporal trend monitoring, the sediment samples were collected during the month of June 2000 on *RV CIROLANA* 5/01. Unfortunately samples could not be collected from all of the NMMP sites as rough weather during the cruise disrupted the sampling programme. Five replicate samples were collected randomly within a 50 m radius at each station, observing the NMMP Green Book procedural guidelines for sediment sample collection. The PAHs were extracted from the sediment samples using alkaline saponification and analysed by coupled gas chromatography/mass spectrometry (Kelly *et al*., 2000).

A summary of the results obtained are given in Table 30 and presented in Figure 30.

Table 29. NMMP locations where surface sediment samples were collected

NMMP Code	Location	Site
245	Off Tyne NSTF14	NMMP Sediment Temporal Trend site
345	Off Humber/Wash NSTF53	NMMP Sediment Temporal Trend site
376	Off Wash	NMMP Sediment Temporal Trend site
466	Thames	
475	Thames Gabbard	NMMP Sediment Temporal Trend site
536	Lyme Bay	NMMP Sediment Temporal Trend site
605	Celtic Deep	NMMP Sediment Temporal Trend site
655	Cardigan Bay	NMMP Sediment Temporal Trend site
715	Liverpool Bay	NMMP Sediment Temporal Trend site
805	SE Isle of Man	NMMP Sediment Temporal Trend site

Table 30. ΣPAH results in ug kg^{-1} dry weight

Site and NMMP No	Naphthalene	Phenanthrene	Anthracene	Fluoranthene	Pyrene	Benz[*a*]anthracene	Chrysene	Benzo[*a*]pyrene	Benzo[*ghi*]perylene	Indeno[123-*cd*]pyrene	ΣPAH
Off Tyne 245	111	230	43	212	172	141	174	124	116	88	1411
Off Tyne 245	82	170	30	160	135	114	141	100	96	69	1098
Off Tyne 245	108	230	43	211	171	136	173	129	105	80	1387
Off Tyne 245	94	209	38	195	159	138	158	121	108	74	1295
Off Tyne 245	85	197	30	180	161	123	151	110	112	70	1219
Humber/Wash 345	10	23	2.4	25	19	13	19	11	28	29	180
Humber/Wash 345	11	27	2.4	28	21	16	19	15	31	31	202
Humber/Wash 345	5.9	13	1.0	13	10	8.4	8.2	6.4	16	15	97
Humber/Wash 345	11	23	2.1	26	19	14	19	14	30	31	188
Humber/Wash 345	11	25	2.4	27	19	13	19	13	29	29	189
Inner Dowsing 376	90	134	13	83	69	35	51	41	44	26	587
Inner Dowsing 376	87	299	25	75	63	33	48	43	42	25	740
Inner Dowsing 376	109	157	13	95	79	37	55	43	50	28	667
Inner Dowsing 376	83	113	11	68	56	33	42	35	36	21	498
Inner Dowsing 376	204	212	19	130	103	66	67	59	52	31	943
Thames 466	5.1	14	2.5	24	22	12	13	14	17	18	141
Thames 466	11	14	4.9	76	71	32	33	29	25	29	326
Thames 466	7.8	12	2.8	42	37	23	24	23	20	24	216
Thames 466	7.1	16	2.9	27	23	13	14	14	15	17	149
Gabbard 475	1.0	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	1.0
Gabbard 475	4.3	2.5	<0.1	<0.1	1.8	<0.1	0.9	<0.1	1.8	<0.1	11
Gabbard 475	1.4	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	1.4
Gabbard 475	1.5	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	1.5
Gabbard 475	2.0	1.7	<0.1	0.9	3.3	0.8	0.9	<0.1	2.3	<0.1	12
Lyme Bay 536	2.5	2.5	<0.1	6.5	4.9	6.3	4.6	5.3	3.4	4.0	40
Lyme Bay 536	1.9	11	2.3	26	20	17	16	19	7.1	8.7	129
Lyme Bay 536	1.8	2.1	<0.1	3.0	2.6	3.9	1.4	3.0	2.0	2.4	22
Lyme Bay 536	2.5	4.6	0.9	9.5	8.0	9	6.5	9.2	3.7	4.5	59
Lyme Bay 536	1.6	2.1	<0.1	4.0	2.9	3.1	2.3	3.0	1.9	2.5	23
Cardigan Bay 655	11	44	6.1	53	32	25	35	23	21	21	272
Cardigan Bay 655	15	53	8.0	72	46	28	47	29	23	23	343
Cardigan Bay 655	16	50	8.1	66	36	30	42	27	23	25	324
Cardigan Bay 655	15	55	7.7	68	39	29	45	29	22	22	333
Cardigan Bay 655	15	47	7.6	63	38	29	39	24	20	19	302
Liverpool Bay 715	1.0	3.1	0.6	2.6	2.7	1.0	1.9	1.9	1.9	2.0	19
Liverpool Bay 715	0.7	2.0	<0.1	1.8	2.0	0.9	1.3	1.3	1.1	1.0	12
Liverpool Bay 715	14	11	1.5	7.2	6.2	3.6	5.1	4.4	4.4	4.5	62
Liverpool Bay 715	<0.1	1.0	<0.1	0.7	0.9	0.6	<0.1	<0.1	<0.1	<0.1	3.2
Liverpool Bay 715	2.0	8.0	2.0	8.3	7.1	6.1	5.0	5.9	4.3	4.9	54
SE Isle of Man 805	11	32	4.2	41	37	20	24	29	30	33	260
SE Isle of Man 805	16	46	7.0	61	55	28	35	41	44	50	384
SE Isle of Man 805	12	33	4.4	48	41	18	25	29	30	34	273
SE Isle of Man 805	11	34	4.6	43	39	14	26	31	31	34	269
SE Isle of Man 805	7.7	26	3.3	34	30	14	20	22	25	27	209

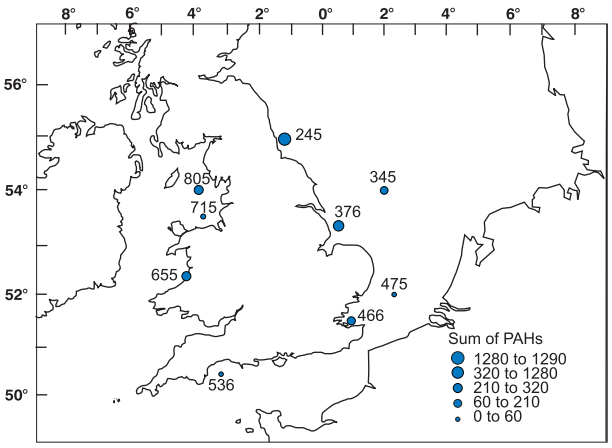

Figure 30. Sum of PAH (µg kg^{-1} dry weight) determined under the NMMP programme for each of the sites sampled

These initial data indicate that the Tyne is the area of highest contamination, as found during earlier studies undertaken within the spatial phase of the NMMP (MPMMG, 1998).

BENTHOS

13. THE UTILITY OF MEIOFAUNA IN THE NATIONAL MARINE MONITORING PROGRAMME (NMMP)

13.1 Summary

A study was conducted between 1997 and 1999 to investigate meiofauna assemblages (between 500 and 63 µm) from 12 inshore and offshore locations around the UK coast. The main objective was to relate the differences in meiofauna distribution patterns to a number of measured environmental variables and to establish more clearly the sensitivity of meiofauna communities to anthropogenic disturbance. Results from multivariate data analyses show that distinct spatial differences in species distribution patterns exist and that these correlate with the natural physical characteristics and concentrations of trace metals in the sediment. The inclusion of meiofauna in applied monitoring programmes offers the potential for improving the resolution of the spatial extent of anthropogenic impacts over that achievable from macrofauna investigations alone.

13.2 Introduction

Soft-bottom benthic infauna are most frequently used to monitor the biological effects of environmental change in the marine system. As a group they are largely sedentary and so must withstand the extremes of their local environment or perish. For reasons of convenience, most biological investigations have traditionally targeted the larger macroinfauna (i.e. animals living within sediments that are retained on 1000 or 500 µm meshes) that can readily be counted and identified, whereas the smaller meiofauna (between 500 and 63 µm) has been largely neglected in applied sampling programmes.

As a result of their high abundance, ubiquitous distribution, rapid generation times and fast metabolic rates, the meiofauna have an important role in ecosystem function. Thus the state of meiofauna assemblages may reflect the overall health of the marine benthos (Kennedy and Jacoby, 1999). By virtue of their dominance, universality and robust bodies, harpacticoid copepods and nematodes are the most promising components of the meiofauna for studies assessing effects of natural and anthropogenic disturbances on the marine environment (Sandulli and de Nicola-Giudici, 1990; Sandulli and de Nicola, 1991).
Studies of marine meiofaunal taxonomy and ecology have increased considerably in the last 20 years and meiobenthic assemblages have increasingly been used to assess the effects of perturbations in the marine environment. In the last 25 years more than 200 meiofauna papers have been published in a pollution context (Coull and Chandler, 1992).

In order to improve understanding of how meiobenthic communities respond to both anthropogenic impacts and natural environmental factors, 12 locations around the UK, some of them long-term NMMP stations, were sampled for meiofauna and a number of environmental

variables measured. The assessment of the relationship between meiofauna distribution patterns and trace metals in the sediment allows us to refine quality assessments of the marine environment and to establish more clearly the sensitivity of meiofauna communities to anthropogenic disturbance.

13.3 Material and methods

Sample collection

Sediment samples at all stations (Figure 31) were taken with the Bowers and Connelly Multicorer, which is designed to take four undisturbed sediment samples (23.76 cm^2 surface area each) simultaneously. The corer was deployed four times at each site. From each deployment, one whole sediment core was retained for meiofauna analysis and the top 5 cm of another core from the same deployment was retained for particle size and chemical analyses. Meiofauna samples were fixed in 7% formaldehyde in 63 µm filtered seawater. Samples for particle size and chemical analyses were frozen to a temperature of –20°C pending analysis.

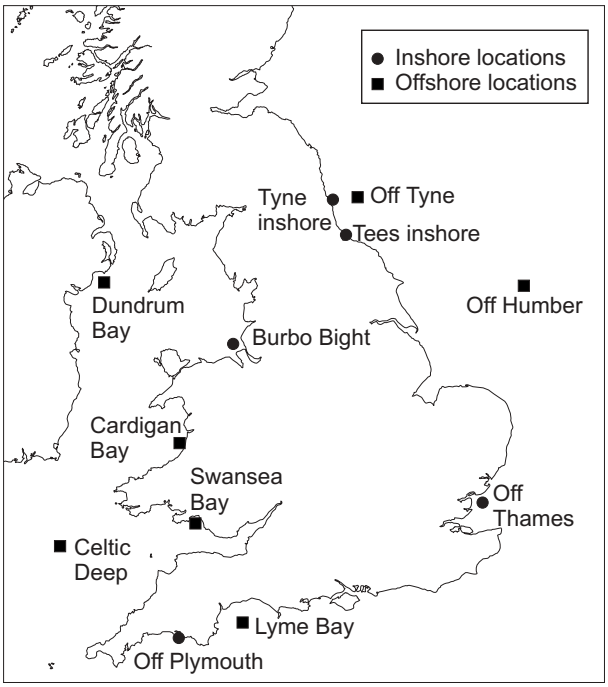

Figure 31. Inshore and offshore locations sampled between 1997 and 1999

Sample processing

Meiofauna samples were initially washed onto a 63 µm sieve to remove the fine silt fraction and the formalin. After decanting the samples five times onto a 63 µm sieve in order to separate the meiofauna and lighter sediment fractions from the coarser material, meiofauna was extracted with Ludox TM 40 with a specific gravity of 1.15 (Somerfield and Warwick, 1996). The extraction process was repeated three times.

The harpacticoid copepods were picked out of each sample under a binocular microscope and identified to species or genus level in hanging-drop mounts or by dissection. The remaining samples were evaporated slowly in anhydrous glycerol and evenly spread on microscope slides for identification and counting of nematodes. All nematodes were counted and the first 200 specimens on each slide identified to genus or species level.

Data processing

The depth of the sediment core obtained at each sampling site depends on the sediment compaction and the amount of weight loaded on the Multicorer. Due to the different depths of the sediment cores collected (3 to 13 cm inshore and 4 to 20 cm offshore), data analyses were performed based on the relative abundance of meiofauna species.

Non-metric Multi-Dimensional Scaling (MDS) ordination was applied using the Bray-Curtis similarity measure (Clarke and Warwick, 1994). Analysis of similarities (ANOSIM) (Clarke, 1993) was performed to test the significance of differences in meiofauna assemblage composition between samples. The nature of the community groupings identified in the MDS ordinations was explored further by applying the similarity percentages programme (SIMPER) to determine the contribution of individual species to the average dissimilarity between samples.

The RELATE programme was applied to test for significant relationships between similarity matrices based on relative meiofauna abundance and measured environmental variables. Additionally, the relationships between multivariate community structure and environmental variables were assessed using the BIOENV programme, defining suites of environmental variables which best explain the biotic structure (Clarke and Warwick, 1994).

13.4 Results

Sediment characteristics

The sediments collected at inshore locations were generally poorly sorted, with mean particle size diameters between 2.70 phi off the Thames and 4.85 phi in Swansea Bay (Table 31). The total organic carbon content was highest at the Tyne inshore location, probably largely natural in origin, due to higher burdens of coal particles in surface sediment (Buchanan and Longbottom, 1984) and in the Burbo Bight, probably largely anthropogenic in origin (Norton *et al*., 1984) and lowest off Plymouth. Concentrations of most trace metals were highest in the Burbo Bight, off Plymouth and at the Tyne inshore location. Lowest concentrations occurred off the Thames (Table 31).

The sediments collected at offshore locations all fell within the category of muddy sands or sandy muds and were generally poorly sorted, with sorting coefficients between 1.74 and 2.30, and poor in organic content, with the highest values being found off the Tyne (Table 32). Sediments collected in Lyme Bay were coarser than those collected at other offshore locations. The concentrations of trace metals were generally lower than at inshore locations. Concentrations of some metals were highest in the northern Irish Sea (Dundrum Bay) and lowest in the English Channel (Lyme Bay). Mercury concentrations off the Tyne were twice as high as at any other sampled offshore location but substantially lower than at most inshore locations (Table 32).

Table 31. Sediment characteristics at inshore locations

Particle size distribution

	Tyne inshore	Off Thames	Off Plymouth	Swansea Bay	Burbo Bight
Mean [phi]	3.41	2.70	4.22	4.85	3.12
Sorting	1.27	2.33	1.94	2.13	2.48
Skewness	2.72	0.31	0.94	0.65	0.98
Kurtosis	15.02	6.23	4.98	3.84	3.92
Gravel [%]	0.06	7.80	0.36	0.28	1.54
Sand [%]	82.91	73.45	53.23	33.85	67.19
Mud [%]	17.02	18.75	46.41	65.88	31.28
C [%]	2.7	1.9	1.3	1.4	2.5

Concentration of trace metals

	Tyne inshore	Off Thames	Off Plymouth	Swansea Bay	Burbo Bight
Cr [ppm]	50	43	27	30	67
Ni [ppm]	36	18	23	27	35
Cu [ppm]	49	15	61	20	37
Zn [ppm]	105	58	99	116	168
As [ppm]	19	7	23	12	14
Cd [ppm]	0.31	0.07	0.19	0.33	0.38
Pb [ppm]	49	29	81	42	91
Hg [ppm]	0.12	0.18	0.53	0.24	0.84

Table 32. Sediment characteristics at offshore locations

Particle size distribution

	Off Tyne	Off Humber	Lyme Bay	Celtic Deep	Cardigan Bay	Dundrum Bay
Mean [phi]	4.63	3.76	3.21	4.11	4.82	5.77
Sorting	1.74	1.89	2.05	1.79	2.30	2.00
Skewness	1.45	1.69	1.53	1.53	0.65	0.36
Kurtosis	5.43	5.73	5.53	5.84	3.25	3.71
Gravel [%]	0.03	0.10	0.30	0.04	0.15	0.08
Sand [%]	48.19	71.87	76.07	63.63	41.15	13.33
Mud [%]	51.78	28.03	23.63	36.33	58.70	86.59
C [%]	3.2	2.0	1.7	1.5	1.3	1.5

Concentration of trace metals

	Off Tyne	Off Humber	Lyme Bay	Celtic Deep	Cardigan Bay	Dundrum Bay
Cr [ppm]	52	51	27	36	44	56
Ni [ppm]	32	29	16	24	31	35
Cu [ppm]	18	16	8	21	19	21
Zn [ppm]	103	80	45	89	71	119
As [ppm]	10	12	8	12	13	7
Cd [ppm]	0.24	0.24	0.07	0.25	0.22	0.24
Pb [ppm]	48	31	18	25	41	47
Hg [ppm]	0.14	0.06	0.04	0.07	0.06	0.08

Though generally low, the concentrations of most trace metals, in particular copper and mercury, were higher at inshore than at offshore locations. This might indicate anthropogenic inputs in the inshore environment.

Meiofauna assemblage structure

The MDS ordinations for nematode and harpacticoid copepod assemblages collected at inshore and offshore locations are presented in Figures 32 and 33. Analysis of both the nematode and harpacticoid copepod components generally results in good discrimination between different locations. However, in contrast to the nematode assemblages (Figure 32a), harpacticoid copepod assemblages at the Tyne and Tees inshore locations and in the Burbo Bight do not form distinct clusters (Figure 33a). Despite these differences in multivariate patterns, results from the RELATE analyses indicate a statistically significant similarity in the biotic structure of nematode and harpacticoid copepod assemblages at inshore and offshore locations (inshore: $\rho = 0.20$, $p < 0.02$; offshore: $\rho = 0.35$, $p < 0.01$). ANOSIM and SIMPER results in Table 33 show that

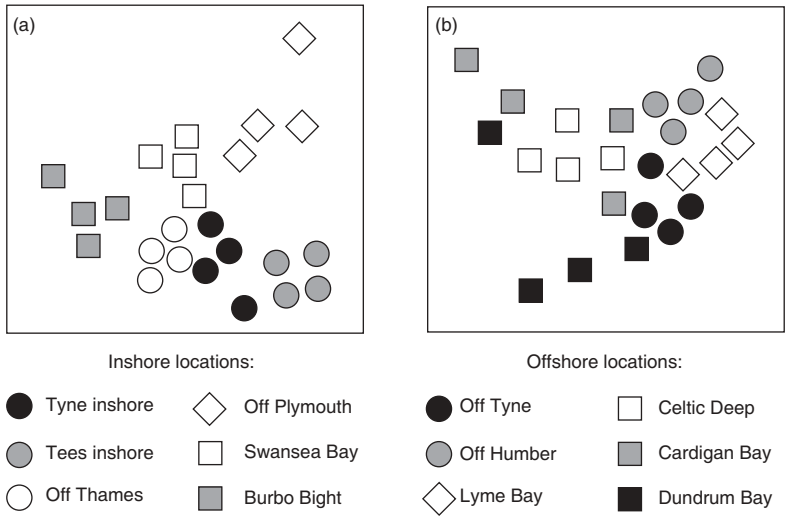

Figure 32. Non-metric multi-dimensional scaling (MDS) ordination based on relative abundance of nematode species. (a) inshore locations (stress = 0.13), (b) offshore locations (stress = 0.15)

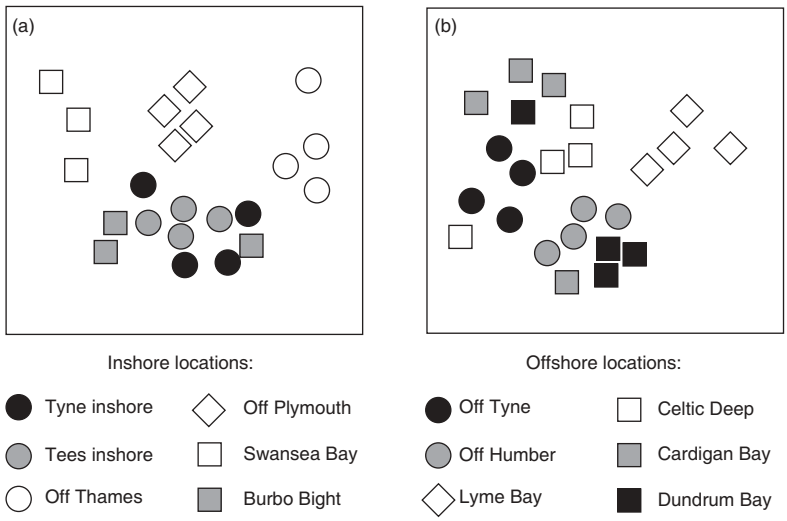

Figure 33. Non-metric multi-dimensional scaling (MDS) ordination based on relative abundance of harpacticoid copepod species. (a) inshore locations (stress = 0.12), (b) offshore locations (stress = 0.18)

Table 33. Dissimilarities [%] between nematode assemblages from inshore and offshore locations based on relative abundance. * denotes significant difference at p < 0.05

Inshore locations

	Tyne inshore	Tees inshore	Off Thames	Off Plymouth	Swansea Bay
Tees inshore	57*				
Off Thames	56*	65*			
Off Plymouth	72*	72*	72*		
Swansea Bay	59*	74*	60*	64*	
Burbo Bight	66*	80*	62*	78*	64*

Offshore locations

	Off Tyne	Off Humber	Lyme Bay	Celtic Deep	Cardigan Bay
Off Humber	56*				
Lyme Bay	53*	49*			
Celtic Deep	54*	55*	60*		
Cardigan Bay	55*	60*	62*	54*	
Dundrum Bay	59*	66*	64*	59*	56

Table 34. ***Dissimilarities [%] between harpacticoid copepod assemblages from inshore and offshore locations based on relative abundance. * denotes significant difference at p < 0.05***

Inshore locations

	Tyne inshore	Tees inshore	Off Thames	Off Plymouth	Swansea Bay
Tees inshore	60				
Off Thames	91*	89*			
Off Plymouth	84*	82*	93*		
Swansea Bay	94*	99*	100*	86*	
Burbo Bight	81	56	98*	95*	100

Offshore locations

	Off Tyne	Off Humber	Lyme Bay	Celtic Deep	Cardigan Bay
Off Humber	75*				
Lyme Bay	86*	80*			
Celtic Deep	71*	79*	82*		
Cardigan Bay	76	77*	87*	79*	
Dundrum Bay	78	58*	81*	79*	67

Table 35. Spearman rank correlations (ρ) between meiofauna assemblage structure and environmental variables at inshore and offshore locations

Inshore locations

	Particle size distribution		Concentration of trace metals	
	ρ	p	ρ	p
Nematodes	0.19	0.04	0.30	< 0.01
Harpacticoid copepods	0.35	< 0.01	0.43	< 0.01

Offshore locations

	Particle size distribution		Concentration of trace metals	
	ρ	p	ρ	p
Nematodes	0.45	< 0.01	0.25	< 0.01
Harpacticoid copepods	0.21	0.01	0.40	< 0.01

the species composition of nematode assemblages at all inshore locations were significantly different from each other at $p < 0.05$. The average dissimilarity between nematode assemblages was 67%. Nematode assemblages at all offshore locations, apart from the two northern-most locations in the Irish Sea (Cardigan Bay and Dundrum Bay), were significantly different from each other in terms of species composition with an average dissimilarity of 57%.

Despite a higher average dissimilarity of 87% inshore and 77% offshore, differences in harpacticoid copepod assemblage structure between samples collected in the Burbo Bight, the Tyne and Tees inshore locations and in Swansea Bay were not statistically significant at $p < 0.05$ (Table 34). This reflects the relatively higher variability of the harpacticoid copepod data. Additionally, differences between harpacticoid copepod assemblages collected off the Tyne and in the Irish Sea (Cardigan Bay and Dundrum Bay) were not statistically significant at $p < 0.05$.

Relationships between environmental variables and meiofauna assemblage structure

Results from the RELATE analyses in Table 35 reveal statistically significant relationships between meiofaunal distribution patterns and measured environmental variables at $p < 0.05$. Nematode and harpacticoid copepod species distribution patterns at inshore and offshore locations were significantly correlated with variables based on particle size distribution and concentrations of trace metals.

Inshore, a combination of the concentrations of chromium, arsenic and mercury best explained nematode assemblage structure ($\rho = 0.65$) and a combination of particle size and concentrations of chromium, zinc and cadmium produced the highest rank correlation coefficient for harpacticoid copepods ($\rho = 0.63$). A combination of particle size and arsenic concentration best explained nematode assemblage structure at offshore locations ($\rho = 0.41$). Observed patterns of harpacticoid copepod assemblages were mainly related to the sorting coefficient and the concentrations of chromium and copper in the sediment ($\rho = 0.46$).

There was no statistically significant co-variation of the concentrations of trace metals and the sand and silt/clay content of the sediments (p between 0.06 and 0.52) except for nickel ($p = 0.02$).

13.5 Discussion

Results from this study indicate that differences in species composition of the meiofauna assemblages between different inshore and offshore locations provide a sensitive and clear measure of environmental status. Meiofauna species distribution patterns were explained well by variables derived from particle size distributions and concentrations of trace metals. The concentrations of correlated trace metals were relatively low at most stations and an unlikely cause of biological effects. However, elevated concentrations of most trace metals at inshore locations compared to offshore might indicate anthropogenic impacts in the inshore environment and a correlation between the structure of meiofauna assemblages and unmeasured contaminants is possible.

A combination of particle size and concentrations of chromium, zinc, arsenic, mercury and cadmium best explained nematode and harpacticoid copepod assemblage structure at inshore locations. Relatively high concentrations of these trace metals in the Burbo Bight were clearly correlated with low diversity of meiofauna. This might indicate anthropogenic impact at this site. The disposal of sewage sludge into Liverpool Bay has been the subject of a long-term multidisciplinary scientific investigation since the early 1970s. This has shown that the addition of sludge is one of many factors – including the outflow from the polluted Mersey estuary - influencing the quality of the water and sediments in this area (Head, 1981; Norton *et al*., 1984; Rees *et al*., 1992).

Rank correlation coefficients between nematode and harpacticoid copepod assemblage structure and measured environmental variables were higher at inshore locations than offshore, indicating that other unmeasured but correlated factors, such as inter- and intraspecific competition could be more important in determining meiofauna assemblage structure in the offshore environment.

Monitoring of the effects of natural and man-made changes by examining meiofauna assemblage structure has proved useful. The clear separation of meiofauna assemblages collected at offshore and inshore locations suggests a high taxonomic resolution and a wide range of adaptations of nematodes and harpacticoid copepods to varying environmental conditions. Meiofaunal monitoring should be considered where there is difficulty in adequate sampling of the macrofauna, for example, due to impoverishment, and meiofauna might also be a useful monitoring tool to identify subtle changes in the ecosystem in response to contaminant inputs (Moore and Bett, 1989).

Management decisions aimed at protecting the marine environment against adverse effects of human activities and conserving biological diversity require a more holistic approach than hitherto. The use of both macrofauna and meiofauna techniques in routine monitoring therefore not only provides complementary information on environmental conditions and greater flexibility to meet site-specific study requirements but also widens the scope for evaluation of the status of the benthic ecosystem as a whole.

14. ADVICE ON FISHERY IMPLICATIONS OF PIPELINE DISCHARGES

This section gives a brief summary of activities carried out during 1999 and 2000 in connection with the provision of advice on fishery implications of pipeline discharges. The background to this work in relation to MAFF's responsibilities as a statutory consultee under the Water Resources Act 1991 (Great Britain Parliament, 1991) and the Environmental Protection Act 1990 (Great Britain Parliament, 1990) has been described in previous reports in this series (MAFF, 1991; 1992; 1993; 1994; 1995; CEFAS, 1997; 1998 and 2001).

During 1999, CEFAS assessed applications for a total of 541 individual discharges. As in previous years, the majority of the applications were for the discharge of domestic sewage, including storm and emergency sewage overflows. The applications included a sharp increase in storm sewage overflow applications; 255 in 1999 against 176 in 1998. Emergency sewage overflow applications were comparable with the previous years. A majority of the continuous sewage discharge applications made this year were for small domestic and workplace discharges, with only 16 of the 97 total being for upgrades at water treatment works. This accounts for the fall to 60% of those applications being for secondary treatment or higher, as opposed to 75% in 1998. There were a total of 17 applications including UV light treatment.

Numbers of trade effluent applications fell and most of those received involved updating and upgrading existing discharges. There were 63 applications in 1999 in comparison to 98 in 1998, which represents 13% of the total, against 25% in 1998. CEFAS asks that conditions are attached regarding the prevention of release of hazardous substances such as TBT, pesticides, oils and others. This is also the case with regard to surface water discharges, which can often drain commercial premises or areas of potential contamination such as roads and parking areas. There were few (12) applications for surface water discharges during 1999.

During 2000, CEFAS assessed applications for a total of 287 individual discharges. 231 of those were for the discharge of domestic sewage, including storm and emergency sewage overflows. There were 101 storm sewage and 66 emergency sewage applications made. A total of 64 continuous sewage discharge applications were received, with 34 of those from small domestic and workplace discharges and 30 from water treatment works or pumping stations. 61% of these were for secondary treatment or higher, consistent with 1999 figures. 6 discharges included UV light treatment.

There were only 34 trade effluent discharge applications in 2000, representing 12% of the total applications. 15 applications for surface water discharges were received.

The primary consideration with regard to most applications is the possible contamination of shellfish beds as a result of sewage discharges. The issues surrounding this have been described in previous reports (CEFAS, 1997; 1998 and 2001). Other issues that are considered during the consultation process are the possible effects on other fisheries and other users of the marine environment. Crustacean fisheries and fish nursery areas are often close to proposed discharge areas and can be impacted by various components of discharge effluents. Bathing quality is adequately covered by the EC Directive 76/160 (European Communities, 1976) and therefore the Environment Agency works to ensure that these standards are met. Consideration must be given to the possibility of components of the discharge settling, binding and building-up in sediments. The EC Directive 91/271 (European Communities, 1991b) concerns the standards of urban waste water treatment, which is considered by the Environment Agency during the application process. All applications, consents and authorisations continue to be entered onto a computerised database and Geographic Information System which contains details of all known discharges to saline water in England and Wales. This is proving to be a powerful management tool for integrating information on human activities, natural resources and environmental sensitivities in the marine and coastal zone.

15. LICENSING OF DEPOSITS IN THE SEA

15.1 Introduction

This section gives information about the licensing of deposits in the sea around the coasts of England and Wales during 1999 and 2000 under Part II of the Food and Environment Protection Act (1985) (FEPA) (Great Britain Parliament, 1985a). For convenience, licensing statistics for Scotland and Northern Ireland are included in this section to provide statistics for the UK as a whole.

15.2 Legislation and licensing authorities

The deposit of substances and articles in the sea, principally the dumping of dredgings (as opposed to discharge into the sea via pipelines) and the use of materials during construction and coast defence works, is controlled by a system of licences issued under Part II of FEPA. Certain operations (e.g. deposit of scientific instruments and navigation aids) are exempt from licensing under the Deposits in the Sea (Exemptions) Order 1985 (Great Britain – Parliament 1985b).

Following devolution in 1999, MAFF continued to license deposits in the sea around the Welsh coast on behalf of the Welsh Assembly. In Scotland, the licensing function became the responsibility of the Scottish Executive Rural Affairs Department (SERAD). In Northern Ireland, the issuing of licences remained the responsibility of the Environment and Heritage Service (EHS), an agency of the Department of the Environment for Northern Ireland.

15.3 Enforcement

Scientists from the CEFAS Burnham Laboratory have powers to enforce licence provisions. Visits are made to construction sites and disposal vessels. Samples are taken and records, including logbooks, are checked. Scientific staff carried out 19 inspections in 1999 and 44 in 2000.

Officers of the Department's Sea Fisheries Inspectorate (SFI) are charged with enforcing the provisions of FEPA (Part II) and undertake regular inspections from a network of port offices in England and Wales. During 1999 the SFI carried out 183 inspections in relation to marine construction works and the disposal of dredged material and fish waste at designated disposal sites. In 2000, the number of inspections increased to 266.

In England and Wales one warning letter was issued in 1999 in respect of the unlicensed redevelopment of a harbour.

Four warning letters were issued in 2000 for the following apparent infringements:

- deposit of dredged material by a harbour authority at an unlicensed disposal site;
- deposit of construction materials in connection with the redevelopment of a harbour without the authority of a licence;
- disposal of material by a private company in excess of the total quantity permitted by the FEPA licence;
- deposit of dredged material by a harbour authority without a licence.

In addition, following investigation by the SFI, three prosecutions were taken in 2000 for breaches of licensing controls. The master and owners of the dredger *LESSE* pleaded guilty at Workington Magistrates' Court on 15 February 2000, to two charges of depositing in the sea a total of 2324 cubic metres of spoil during the morning of 9 June 1999 without the authority of a licence. The master was fined £1,000 with £200 costs and the owners a fine of £4,000 with £505.50 costs.

The Council of the Borough of Gosport and May Gurney (Construction) Limited both pleaded guilty at Fareham Magistrates' Court on 6 November 2000, to one charge of depositing articles in the sea at Forton Lake between 1 September and 30 November 1999, without the authority of a licence. The defendants were each fined £6,000 and ordered to pay costs totalling £637.

Carmarthenshire County Council and Dean and Dyball Limited both pleaded guilty at Llanelli Magistrates' Court on 11 December 2000, to one charge of depositing articles in the sea at North Dock, Llanelli between 1 August and 3 December 1999, without the authority of a licence. The defendants were each fined £3,000 and ordered to pay costs totalling £578.30.

In Scotland, similar enforcement powers are held by certain authorised staff of the Fisheries Research Services (FRS) Marine Laboratory, Aberdeen and the Scottish Fisheries Protection Agency (SEPA). The FRS made 9 enforcement visits in both 1999 and 2000 and carried out 12 investigations visits in 1999 and 7 in 2000. The SEPA carried out 15 enforcement visits in 1999, and 12 in 2000. In Northern Ireland, EHS carried out 6 enforcement visits in both 1999 and 2000.

In 1999, EHS in Northern Ireland investigated a report that an oil rig, carrying out experimental trials just outside Belfast Lough, had jettisoned a number of 20 metre steel pipes 0.2 metres in diameter. One of these was reported to be protruding 0.3 metres from the sea surface, causing a hazard to navigation. EHS took statements under caution and a warning letter was sent to the drilling company. Most of the pipes were subsequently recovered, although some could not be found and were thought to have become buried in the sediment. Three investigations were carried out in 2000, two of which were reports of building materials being left on the beach. These were subsequently removed. The third related to reports of disposal of dredged material in an unlicensed area, but the logbooks appeared to be in order and it became clear that the reported activity had been washing the vessel's hold. No further action was taken.

15.4 Report on licensing activities

Tables 36-39b give details for the period 1996 to 2000 of the number of sea disposal licences issued,

the quantity of waste licensed and the quantity actually deposited, together with information on those contaminants in the wastes which the UK is required to report internationally to meet obligations under the OSPAR and London Conventions.

15.5 Licensing of minestone

In April 1999, a licence valid until the end of 2000, was issued to RJB Mining (UK) Ltd to authorise the use of minestone from the Ellington Colliery on the foreshore at Lynemouth Bay as an interim coast defence measure.

15.6 Licensing of dredged material disposal

Table 36 shows the number of licences issued for dredged material in 1999 and 2000, the quantity licensed and the quantity deposited together with figures for the quantity of a range of trace contaminants which enter the sea in the dredged material. A proportion of the trace metals in this dredged material are natural, but the mineral structure is such that it will not be available to marine organisms. Figures 34 a and b show the main disposal sites used in 1999 and 2000 and the quantities used at each site. Although applicants for licences are required to show evidence that they have considered alternative disposal options, including beneficial use, the problems of handling silty materials and matching timings of dredging with demand for sediments, have meant that most of the finer materials, in particular, are deposited at sea.

15.7 Other licensed activity

Under Part II of FEPA, licences are also required for certain other activities or deposits made below the mean high water spring tide mark for construction purposes. Each licence application is carefully considered, in particular, to assess the impact on tidal and intertidal habitat, hydrological effects, potential interference to other uses of the sea and any risk to human health. Further activities involve the use of tracers, the application of biocides, and burials at sea. Generally the anticipated environmental impact from deposit of these substances is minimal and little or no monitoring is required. Details of these licenses are shown in Table 37a and 37b. Licences have been authorised for the disposal of small amounts of fish waste, details of the quantities involved are given in Table 38. Table 39a and 39b shows the number of such licences issued in 1999 and 2000.

Table 36. Summary of dredged material licensed and disposed of at sea in 2000

Country	Year	Licences issued	Licensed quantity (Tonnes)	Wet tonnage deposited	Dry tonnage deposited	Quantities of metal contaminants in wastes deposited (tonnes)						
						Cd	Cr	Cu	Hg	Ni	Pb	Zn
England	1996	120	82,395,490	48,513,953	25,953,191	8.80	1,556	743	6.87	673	1,731	3,991
and Wales	1997	113	56,536,922	38,627,660	21,165,143	6.54	1,182	574	5.47	471	1,242	2,941
	1998	106	74,883,745	31,814,916	15,456,858	7.47	1,143	551	4.46	498	1,081	2,741
	1999	131	47,028,123	52,409,430	31,114,127	13.05	1,907	1,064	6.07	898	1,370	4,001
	2000	119	55,902,025	28,257,192	14,077,169	8.76	1,043	663	4.78	485	1,099	2,948
Scotland	1996	30	3,971,045	2,601,864	1,174,999	0.40	56	89	0.73	26	81	155
	1997	29	3,910,900	2,436,745	1,045,762	0.22	46	50	0.66	25	69	153
	1998	22	5,917,150	3,106,253	1,284,550	0.45	118	131	0.97	38	128	311
	1999	30	4,044,300	2,352,954	945,563	0.25	57	55	0.78	36	66	130
	2000	30	6,135,400	4,155,018	2,034,213	0.51	87	80	1.79	73	139	298
Northern	1996	6	166,000	135,550	106,768	0.05	2	2	0.01	3	2	4
Ireland	1997	7	206,000	176,919	122,289	0.17	1	1	0.03	1	1	5
	1998	11	1,121,300	803,181	617,503	0.32	16	7	0.20	17	9	33
	1999	5	1,923,000	2,058,506	768,609	0.54	32	21	0.56	18	23	92
	2000	3	3,950,000	640,815	455,222	0.13	45	7	0.05	13	14	42
UK Total	1996	156	86,532,535	51,251,367	27,234,957	9.25	1,613	835	7.61	701	1,814	4,149
	1997	149	60,653,822	41,241,324	22,333,194	6.93	1,230	624	6.16	497	1,312	3,100
	1998	139	81,922,195	35,724,350	17,358,911	8.24	1,278	689	5.63	553	1,218	3,084
	1999	166	52,995,423	56,820,890	32,828,299	13.85	1,997	1,141	7.41	953	1,459	4,223
	2000	152	65,987,425	33,053,025	16,566,605	9.39	1,176	750	6.63	571	1,252	3,289

Notes: Tonnages deposited relate to quantities in the calendar year 2000, which may be covered by 2 or more licences, including one or more issued in 1999.

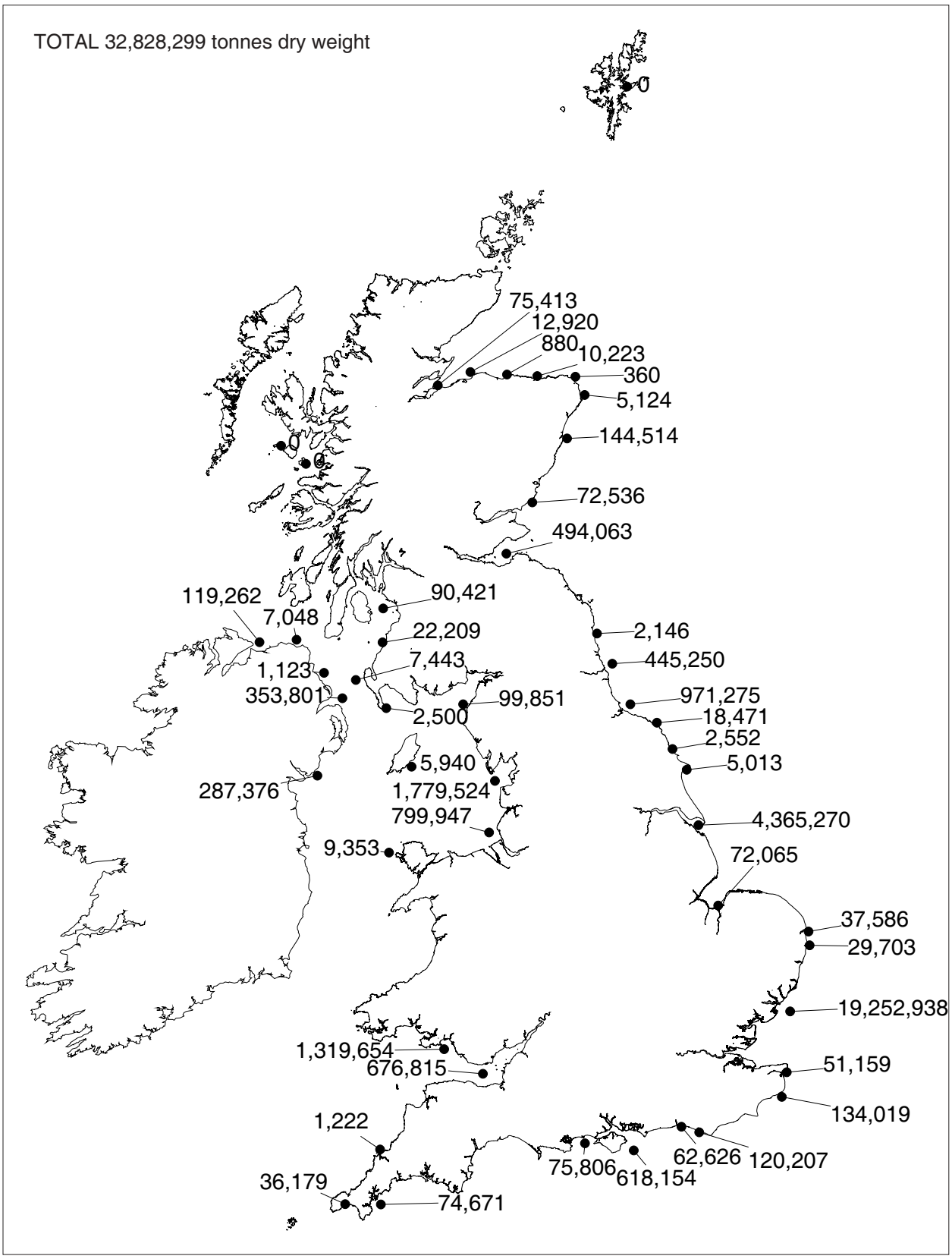

Figure 34(a). UK Dredged material disposal sites and amounts deposited in tonnes dry weight for 1999

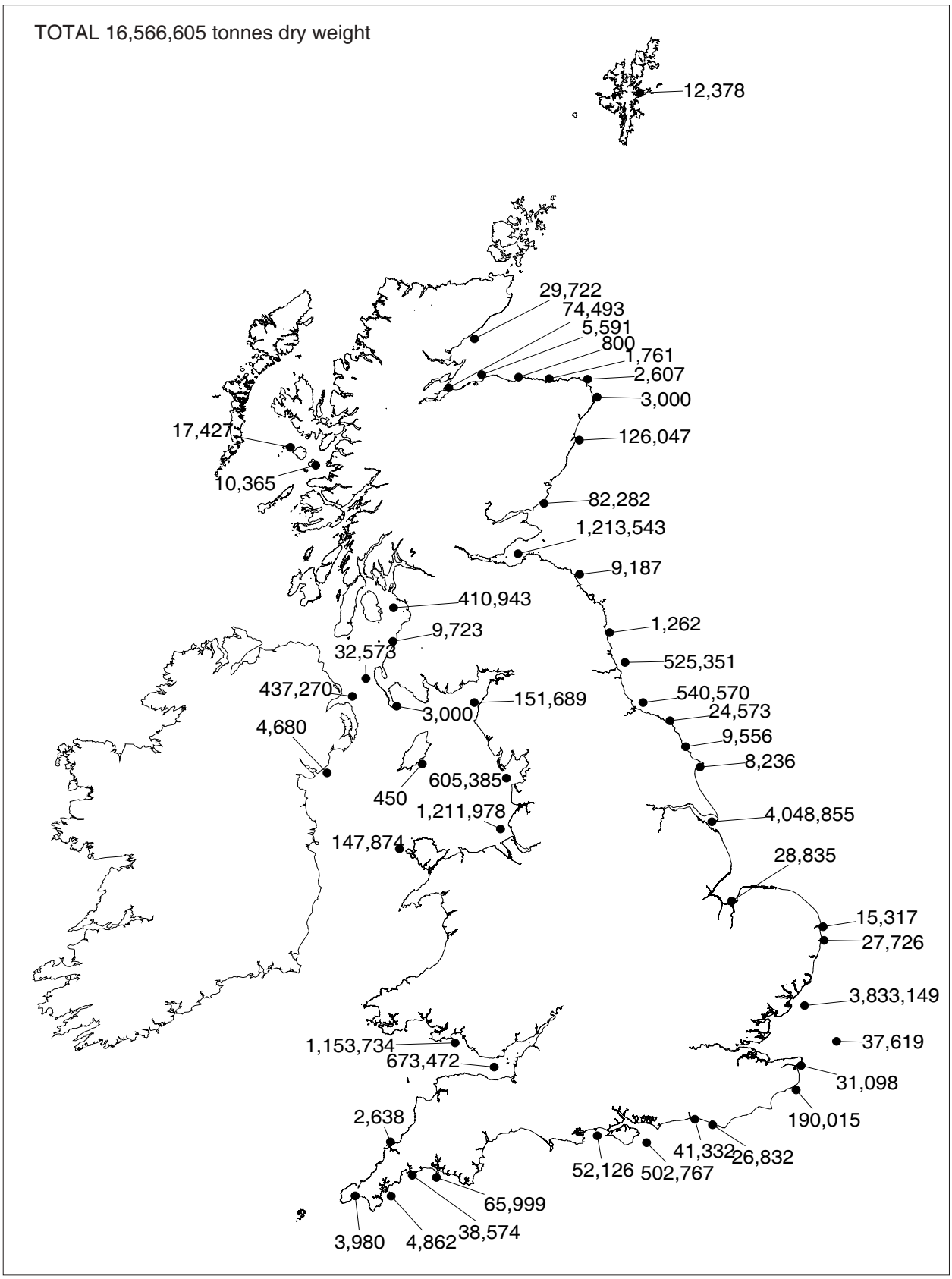

Figure 34(b). UK Dredged material disposal sites and amounts deposited in tonnes dry weight for 2000

Table 37(a). Other categories of licences issued in 1999 (number of licences)

Licence category	Year	England and Wales	Scotland	Northern Ireland	Total
Construction - new and renewal	1999	278	125	14	417
Tracers, biocides etc.	1999	14	2	0	16
Burial at Sea	1999	16	3	0	19

Table 37(b). Other categories of licences issued in 2000 (number of licences)

Licence category	Year	England and Wales	Scotland	Northern Ireland	Total
Construction - new and renewal	2000	271	126	15	412
Tracers, biocides etc.	2000	11	0	0	11
Burial at Sea	2000	18	0	0	18

Table 38. Summary of fish waste licensed and disposed of at sea in 2000

Country	Year	Licences issued	Licensed quantity (Tonnes)	Wet tonnage deposited	Dry tonnage deposited
England and Wales	1996	1	750	16	16
	1997	1	750	747	747
	1998	0	0	52	52
	1999	1	1,000	956	956
	2000	1	1,000	1,559	1,559
Scotland	1996	0	0	0	0
	1997	2	262	51	41
	1998	2	515	142	114
	1999	1	200	137	110
	2000	1	200	45	36
Northern Ireland	1996	0	0	0	0
	1997	0	0	0	0
	1998	0	0	0	0
	1999	0	0	0	0
	2000	0	0	0	0
UK Total	1996	1	750	16	16
	1997	3	1,012	798	788
	1998	2	515	194	166
	1999	2	1,200	1,093	1,066
	2000	2	1,200	1,604	1,595

Note: For information on licensed quantities and tonnages deposited see footnote to Table 36.

Table 39(a). Fish waste licensed for disposal at sea in 1999[1]

Country	Licensed Quantity (Tonnes)[1]	Company and source of waste	Disposal sites	Quantity Deposited (Tonnes)[1]
England	1,000	Quay Fresh & Frozen Foods Ltd, New Quay	New Quay	956
Scotland	0	Orkney Fishermen's Association, Stromness	Stromness C	80
			Stromness B	0
	200	Orkney Fishermen's Association, Stromness	Stromness C	57
			Stromness B	0

Notes: No fish wastes were licensed or disposed of in Northern Ireland during the period covered by this report.
[1] All figures are for tonnage in wet weight.
For information on licensed quantities and tonnages deposited, see footnote to Table 36.

Table 39(b). Fish waste licensed for disposal at sea in 2000[1]

Country	Licensed Quantity (Tonnes)	Company and source of waste	Disposal sites	Quantity Deposited (Wet tonnes)	Quantity Deposited (Dry tonnes)
England	1,000	Quay Fresh & Frozen Foods Ltd, New Quay	New Quay	1,559	1,559
Scotland	200	Orkney Fishermen's Association, Stromness	Stromness C	45	36
			Stromness B	0	0

Notes : No Fish Wastes were licensed or disposed of in Northern Ireland during the period covered by this report.
For information on licensed quantities and tonnages deposited see footnote to Table 36.

16. CHARACTERISATION OF DREDGED MATERIAL AT SOURCE: ORGANIC MATTER AND PARTICLE SIZE

16.1 Introduction

The deposit of material at sea is controlled by MAFF under Part II of the Food and Environmental Protection Act (Great Britain Parliament, 1985a). Every application for a licence to dredge and dispose at sea must satisfy certain criteria. Licence applicants are required to submit samples for analysis to CEFAS prior to dredging and sea disposal, following OSPAR Commission guidelines for the management of dredged sediment (OSPAR,1993).

Under FEPA, there is also a requirement to have regard to the practical availability of any alternative methods of dealing with dredged material. As an alternative to the conventional sea disposal route, beneficial use schemes such as the construction of sea defences or restoration of mud flats are under study.

A CEFAS project is presently examining the implications of the nature and quality of dredged material for its beneficial placement in the coastal environment on behalf of DEFRA. A more detailed evaluation of the characteristics of licensed dredged materials will help in the decision-making process regarding the use of conventional sea disposal routes, and on the assessment of alternative beneficial uses of dredged material.

This report provides preliminary results on the characterisation of licensed dredged material at source with respect to particle size, organic carbon and nitrogen content.

16.2 Materials and methods

Licence applicants were required to take evenly-spaced samples, after the recommendations of the OSPAR Commission guidelines, to give a good representation of the areas to be dredged. Samples were taken from the upper layers of *in-situ* sediments using a stainless steel scoop. Samples enclosed in labelled polythene bags provided by CEFAS were kept as close to freezing as possible prior to transportation.

Preparation of collected samples for analysis on particle size, organic carbon and nitrogen content was carried out at the Burnham laboratory. Collected material was

subsampled for particle size analysis. Each subsample was wet-sieved to obtain the silt/clay fraction (<63 µm) and dry-sieved to obtain the gravel fraction (>2 mm), the coarse sand fraction (<2 mm - >500 µm) and the medium and fine sand fraction (<500 µm - >63 µm). Organic carbon and nitrogen concentrations were determined using a Leeman CE4440 elemental CHN analyser, after removal of carbonates with 8% HSO_3 (Shaw, 1959). The organic carbon and nitrogen concentrations were determined on the whole sample with the exception of a few samples (* in Table 40) where determinations were carried out on the silt/clay (<63 µm) fraction.

16.3 Results

The dredged material analysed as part of the FEPA licensing process in England and Wales over 3 years (1999-2001) was mainly sandy mud or mud. There were only two locations where the sediment was predominantly sandy: Kings Lynn and Staithes. The silt/clay content for the majority of the locations was higher than 60% (Table 40, Figure 33).

In order to accurately characterise the dredged material at source, samples were taken from different points at each site. There was significant variability within the silt/clay fraction, organic carbon and nitrogen values at certain locations (Figures 34 and 35).

The values were shown to be highly variable between the different locations sampled throughout England and Wales. Silt-clay content ranged from 1 to 99%, organic carbon content from 1 to 18% and nitrogen from 0.01 to 0.5%.

This report presents the mean values (Table 40) in order to get a preliminary overview of between-site variability.

The mean values of organic carbon content found in the dredged material at source ranged between 0.26 and 7.38% (Table 40). This range is higher than the levels usually found at UK sea disposal sites; for example, the maximum values of organic content on sediments found off the Mersey estuary were from 3 to 4% (Somerfield *et al*., 1995; Boyd *et al*. 2000). Maximum mean values recorded in the Celtic Sea were 3.2% (Rowlatt and Lovell, 1994).

Mean values of nitrogen content ranged between 0.01 and 0.50% (Table 40). This range is comparable with values typically recorded at dredged material disposal sites (Somerfield *et al*., 1995; Boyd *et al*., 2000). Maximum values of nitrogen content (0.34%) found in fine sediments at several sites off the UK coastal environment (CEFAS, unpublished data) are below the maximum range of dredged material (0.50%).

Organic carbon and nitrogen content showed minimum mean values at Staithes and Kings Lynn harbour, which also have the lowest percentage of silt/clay (<8%). Samples taken from the Tyne estuary Quay show the highest percentage of silt/clay, organic carbon and nitrogen (Table 40). The organic carbon content of sediments from Cardiff dredged material at Barry Dock showed the highest mean value (7.38%); however the mean silt/clay fraction (62%) is not exceptionally high.

An analysis of variance showed that the relationship between the mean values of silt/clay and organic carbon is not statistically significant ($p>0.10$; $n=42$; $df=40$).

Thus there are several locations (Figures 34 and 35) such as Felixstowe, Humber, Maryport, Poole, Portsmouth, Plymouth, and Swansea, where the silt/clay content is higher than 80%, but the organic carbon content is not exceptionally high (<4%) (Table 40).

General trends and relationships between silt/clay, organic carbon and nitrogen content are not applicable to all the England and Wales locations considered in this study.

16.4 Discussion

The results indicate that fine-grained dredged material, which typically arises from the need to maintain access to harbours and docks, may contain significant amounts of organic carbon, which may have implications for the responses of the benthic biota following sea disposal or 'beneficial use'.

It is recognised that there are limitations involved in the use of samples from the superficial layers of sediments, as these may not be wholly representative of the total amounts that are eventually dredged.

The very diverse character of the UK geology may contribute, to some extent, to the variability of the silt/clay, organic carbon and nitrogen content found in sediments at the different locations throughout England and Wales.

Follow-up analyses of the relationships between physical characteristics, organic content and contaminant concentrations in dredged material at source, and of any links with the environmental status of receiving areas following disposal, will be the subject of future reporting.

Table 40. Total Organic Carbon, Nitrogen content and particle size characterisation in dredged material collected at source between 1999-2001 (mean values shown)

Location	Site	Number of samples	% Silt/clay	%TOC	%ON	% Gravel	% Coarse Sand	% Medium & Fine Sand
Cardiff	Cardiff, Barry Dock	5	61.91	7.38	0.34	0.76	3.65	12.65
	Cardiff Docks	10	62.77	2.88	0.26	0.22	3.18	23.06
Chichester and Hayling Island	Chichester, Northney Marina	3	72.18	1.96	0.22	4.10	10.08	13.64
	Hayling Island, Sparks Marina	6	60.21	1.37	0.19	0.00	0.53	29.20
Felixstowe	Felixstowe Harbour	6	88.62	1.53	0.12	1.80	1.35	4.74
Harrington	Harrington Harbour	2	66.96	2.61	0.17	0.46	1.12	28.08
Holyhead	Holyhead Harbour	5	64.59	1.41	0.15	6.97	4.02	11.69
Humber	Humber, N.Killinghome*	15	-	3.71	0.16	-	-	-
	Humber, Immingham	8	89.79	2.83	0.16	0.01	0.22	9.98
	Humber, King George Dock	5	87.38	2.80	0.17	0.49	0.79	17.03
Kings Lynn	Kings Lynn Harbour	2	7.65	0.38	0.02	0.32	0.53	70.98
Maldon	River Blackwater	2	73.63	2.22	0.25	1.79	10.94	13.64
Maryport	Maryport Harbour	5	91.26	3.02	0.28	0.16	2.03	6.58
Mersey	River Mersey, Garston	5	74.59	2.47	0.23	0.31	0.64	24.46
	Mersey Docks	2	45.96	2.16	0.12	-	-	-
	Mersey Docks	7	45.95	1.15	0.17	0.08	0.67	39.80
	River Mersey	5	53.94	1.78	0.15	0.43	2.77	51.00
Morecambe	Morecambe, Heysham	3	68.69	1.16	0.11	0.29	0.28	30.74
Newport	Newport, Eastern Wharf *	2	56.02	2.78	-	0.00	2.08	42.95
Pembroke	Pembroke Dock	3	73.06	2.90	0.26	7.96	2.79	16.19
Poole	Poole, Lake Yard Marina	3	68.10	3.07	0.33	0.42	1.15	16.71
	Poole, Rockley Park	2	88.90	3.10	0.29	0.27	1.09	9.73
Portsmouth	Portsmouth Harbour	2	98.94	1.01	0.09	0.41	1.05	0.00
Plymouth	Plymouth, Rame Head	5	76.33	3.24	0.33	1.94	1.36	10.84
	Plymouth, Queen Anne's Battery*	4	83.99	2.85	-	0.00	2.46	13.55
Scarborough	Scarborough Harbour	4	36.42	4.49	0.19	0.13	0.63	11.72
Southampon Water	Southampton, River Itchen*	6	75.92	1.97	0.20	0.37	8.69	15.02
	Southampton, Marchwood	4	69.56	2.12	0.21	2.61	2.59	11.49
	Southampton, Dibden Bay	2	40.40	1.45	0.15	45.61	4.37	10.43
	Southampton Ocean Village	5	68.42	2.51	0.23	0.72	10.10	20.76
	Southampton, Itchen, Vosper Thornycroft	3	74.80	2.31	0.28	0.41	1.05	23.75
	Southampton, Fareham	2	71.81	5.15	0.33	4.32	4.02	10.37
	Southampton, Hythe shore	4	-	1.82	0.17	-	-	-
	Southampton, Hythe Harbour*	5	52.70	2.10	-	24.86	10.05	12.40
	Southampton, Hythe Marina	3	75.30	1.95	0.17	1.48	2.77	20.46
Staithes	Staithes Harbour	2	2.57	0.26	<0.01	0.58	7.15	97.71
Swansea	Swansea Docks	2	90.68	3.83	0.31	0.00	3.13	6.20
	Swansea, Port Talbot*	6	60.66	5.24	-	0.55	3.86	34.96
Tees and Hartlepool	Tees and Hartlepool Harbours	3	37.74	3.45	0.25	0.27	0.93	34.23
Tyne	Tyne, Engine Works Quay	3	84.76	5.85	0.32	1.11	1.68	6.53
	Tyne, West Quay	2	80.69	7.21	0.50	0.00	0.59	10.22
	Tyne, Shields Harbour Reach	5	61.26	6.08	0.20	0.79	2.22	25.62
Silloth	Silloth Dock	4	73.78	3.16	0.27	5.10	2.41	18.72
Watchet	Watchet Harbour	3	76.45	2.47	0.18	3.16	3.41	10.14

** Organic carbon and nitrogen content were analysed from the fine fraction (<63 µm) of sediment*

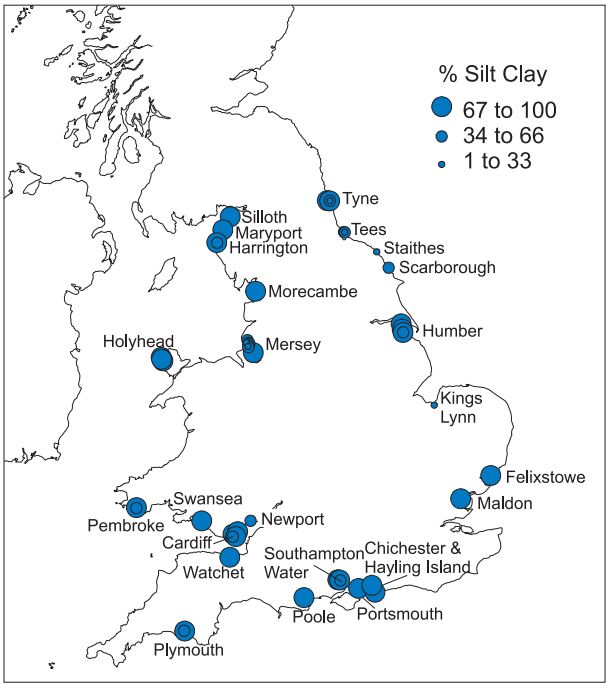

Figure 34. Content (%) of silt clay in UK dredged material collected at source between 1999-2001

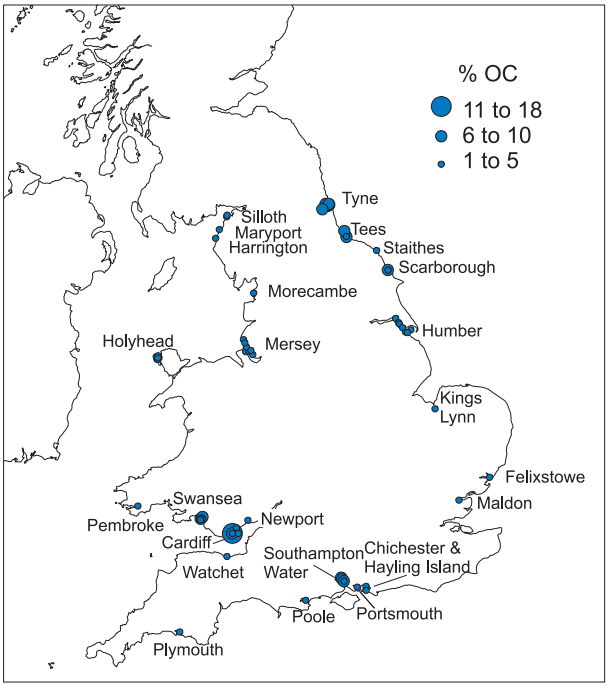

Figure 35. Content (%) of organic carbon in dredged material 1999-2001

17. AN ACOUSTIC ASSESSMENT OF THE PLACEMENT OF CAPITAL DREDGED MATERIAL AT THE ROUGHS TOWER DISPOSAL SITE

17.1 Introduction

In 1998, Harwich Haven Authority applied for a licence to dispose of approximately 29 million tonnes of capital material resulting from major port development work. The capital material was predominantly composed of sand, gravel and rock and consolidated clay. After consultation it was decided that this material should go to the Roughs Tower disposal site. The preliminary stage of the disposal exercise involved the placement of a clay bund around the western and northern perimeter of the licensed area. As further capital material was produced during 1999, it was placed initially within the north-western area of the site behind the clay bund. The material placed in the north-western sector of the site was then capped with a 'sprinkling' of gravel on completion of this phase of the operation. The remainder of the capital material, consisting of a mixture of unconsolidated sand, gravel, rock and consolidated clay, was placed over much of the remainder of the site, with the intention of ensuring that water depths were not reduced to less than 8 m at the Lowest Astronomical Tide (LAT). The material disposed of during this phase of the operation was not 'capped' with gravel. To conclude the exercise, more consolidated material was placed along the eastern and southern perimeters of the site. The site was closed for capital disposal in March 2000 and finally closed for all dredged material disposal activities, including small amounts of maintenance dredgings, in September 2001.

Table 41. Quantities of capital material disposed of at Roughs Tower between November 1998 and March 2000

Date	Quantity disposed (Wet weight tonnes)
Nov – Dec 1998	1,991,356
Jan – Dec 1999	23,468,545
Jan – March 2000	4,219,998

The Roughs Tower Disposal site is located off the East coast of England, approximately 13 km south-east of Harwich (Figure 36). Water depths at the site range from 13 metres to 6.5 metres at the LAT. The site was licensed in 1971 for the disposal of both capital and maintenance dredged material from a number of ports on the East Coast, including Harwich and Felixstowe. CEFAS have undertaken regular monitoring work at the site and the results of this work have been reported

Figure 36. Location of the Roughs Tower disposal site

periodically (MAFF, 1990; MAFF, 1991; Rowlatt and Limpenny, 1987). The results of monitoring work carried out at the site in 1995 and 1997 are reported elsewhere in this document.

17.2 Methods

In May 2000, a comprehensive sidescan sonar survey was carried out over the site to assess the distribution of the capital material immediately following the disposal operation. The survey was carried out using a Datasonics™ SIS 1500 digital chirps sidescan system and the raw data was georeferenced and mosaiced using Delphwin™ post-processing software (Figure 37). Line bathymetry data was collected simultaneously with the sidescan survey and an example of a profile from this survey is shown in Figure 38. Groundtruthing of the acoustic survey was carried out in 2000 using both Hamon and Shipek grabs. Seabed video images were also collected from within the site during a further survey conducted in 2001, using a camera mounted on the Hamon grab. This imagery was used to assess the status and stability of the surface of the capital material at the seabed.

17.3 Results

The presence of the capital dredged material is clearly discernible from the output of the sidescan sonar survey (Figure 39). The difference in appearance between the capital material (mounds of sand and gravel, with consolidated clay) and the surrounding substrata (predominantly stable, sandy gravel and mobile sand) is immediately apparent from the acoustic record. Where present as a continuous layer over the seabed, the dredged material appears as a coarse mottling on the sidescan image and where less densely distributed, individual disposal events can be discerned. Substrata surrounding the disposal site appear to comprise of largely featureless sandy gravels and gravelly sands to the east and south, and more mobile sandy substrata to the north of the site. The limit of the distribution of the dredged material over the seabed is shown in Figure 37. All of the material appears to have been deposited within the site and, excepting the northern extremes of the disposal site, the licensed area was almost completely covered by the capital material.

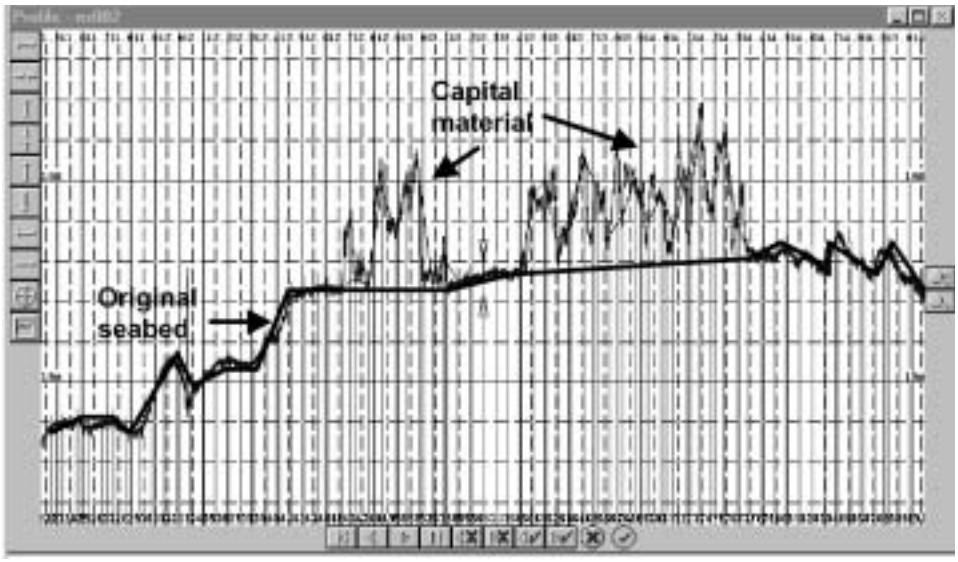

Figure 37. Bathymetric profile across the eastern part of the disposal site produced from the survey carried out in 2000. The heavy black line depicts the approximate original seabed prior to capital disposal. Horizontal distance shown is approximately 2.5 km Depth graduations are in 1 m intervals. Data has not been corrected for tidal variation

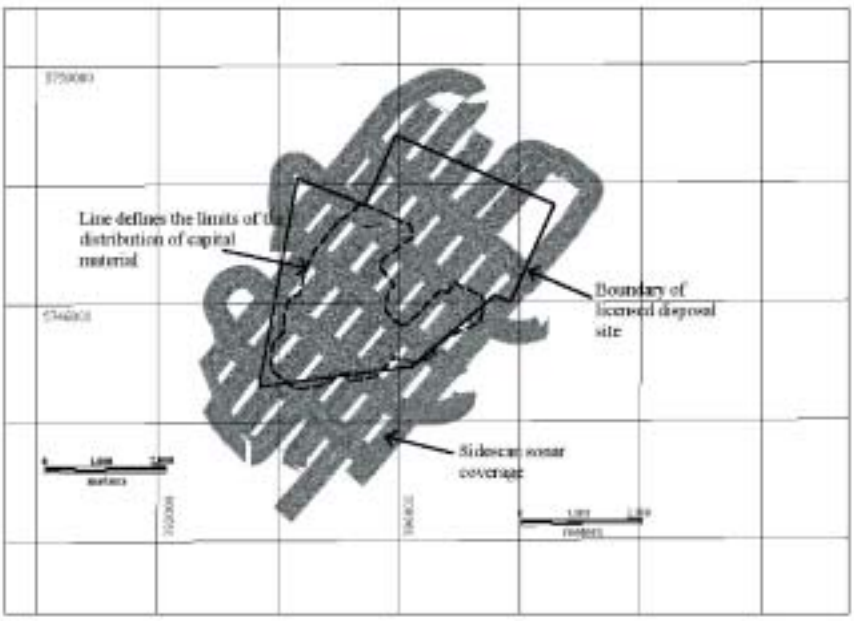

Figure 38. Sidescan sonar mosaic of the survey carried out in 2000. The dashed line defines the limits of the capital material placed within the licensed area

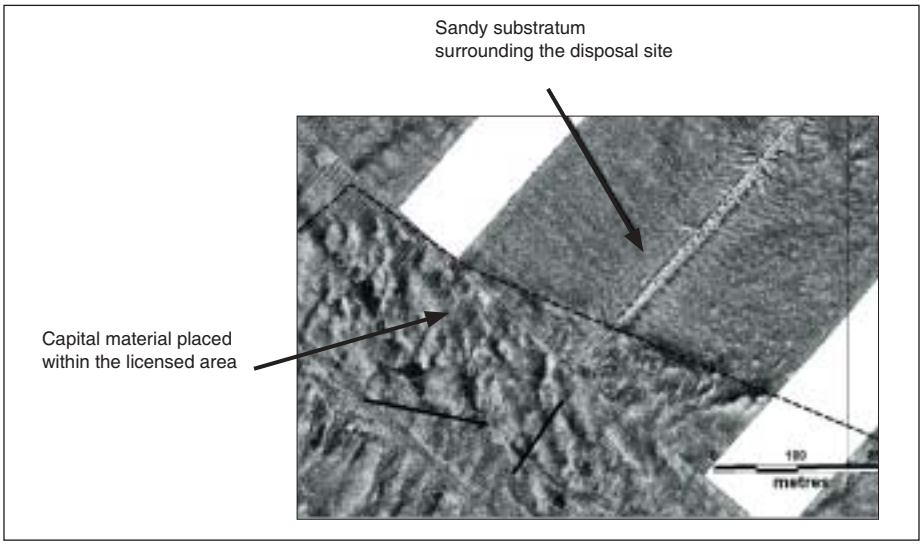

Figure 39. Sidescan sonar image demonstrating the contrast between the capital material within the confines of the licensed area and the surrounding substrata

Individual disposal events can be identified in the central eastern part of the site. A bathymetric profile across the north-eastern part of the site provides an illustration of the extent to which the water depths have changed as a result of the disposal operation (Figure 38). In this instance the dredged material mound rises to a maximum height of 3 m above what is considered to be the original seabed The bathymetric profile also shows the uneven and 'hummocky' nature of the dredged material *in-situ* on the seabed, compared to the substrata which surround the site. The extremely turbid conditions at the site prevented the collection of good quality video images. However, the video images that were collected of the dredged material on the seabed suggest that clay is exposed at the seabed over some parts of the site and that some erosion of this material is occurring.

17.4 Discussion

The acoustic survey work completed in 2000 confirms that all of the capital material generated as part of the port re-development project, was placed within the confines of the licensed area. The material forms large mounds, at least 3 m high, on the seabed causing an uneven terrain. The uneven nature of the seabed may make the area more attractive to some commercial species of shellfish. Work is in progress to evaluate the effectiveness of this site as a lobster habitat.

It is not clear how effective the gravel sprinkling exercise has been. Grab and video evidence from 2001 suggests that clay is exposed to some degree at the seabed within the site. If this is the case, it is likely that a combination of strong tidal streams and wave action in this shallow water environment will cause some erosion and transport of this material away from the site. Further studies will continue to monitor the physical and biological status of this disposal site.

RADIOACTIVITY

18. RADIOACTIVITY IN UK COASTAL WATERS

18.1 Introduction

Seawater surveys support international studies concerned with the quality status of coastal seas (e.g. OSPAR, 2000) and provide information which can be used to distinguish different sources of man-made radioactivity (e.g. Kershaw and Baxter, 1995). In addition, the distribution of radioactivity in seawater around the British Isles is a significant factor in determining the variation in individual exposures at coastal sites, as seafood is a major contribution to food chain doses. Therefore a programme of surveillance into the distribution of key radionuclides is maintained using research vessels and other means of sampling. Detailed historical data on ^{134}Cs and ^{137}Cs in seawater have been published in a series of reports to aid model development (Camplin and Steele, 1991; Baxter *et al*., 1992; Baxter and Camplin, 1993a-c) and have been used to derive dispersion factors for nuclear sites (Baxter and Camplin, 1994). Data have also been used to examine the long distance transport of activity to the Arctic (Kershaw *et al*., 1999b).

In recent years (since 1994), discharges of ^{99}Tc from the British Nuclear Fuels plc (BNF) facilities at Sellafield have increased significantly against the overall trend of most other radionuclides. Results for the ^{99}Tc migration behaviour have afforded the opportunity to substantiate and extend the information obtained from the earlier ^{137}Cs data. The distribution of ^{99}Tc in waters around the British Isles prior to, and immediately after, the increased ^{99}Tc discharges indicated a rapid advection of ^{99}Tc within and from the Irish Sea to the north of Scotland as compared with previous estimates (Leonard *et al*., 1997a,b).

18.2.1 Sampling

The research vessel programme on radionuclide distribution currently comprises cruises in the Irish Sea, Scottish waters and the North Sea every two or three years. Large volume surface seawater samples (50 litres) were collected, using the ships pumped supply, during cruises of the CEFAS research vessels, *RV CIROLANA* and *RV CORYSTES*. Surveys of the Bristol Channel and Irish Sea were carried out in September 1999 and of the Bristol Channel and North Sea in September 2000.

18.2.2 Sample analysis

Samples were filtered (0.45 µm) to separate dissolved and particulate phases. Dissolved ^{137}Cs analyses involved pumping filtered seawater, acidified with nitric acid, through cartridges filled with ASG resin (ammonium duodeca-molybdophosphate on silica gel) to extract caesium. ^{3}H analyses involved double distillation of water samples under alkaline conditions and in the presence of holdback carriers to ensure chemical separation from all gravimetric and radiometric interference. Subsamples of distillate were assayed for ^{3}H using a Packard Tri-Carb 2550 TR/LL liquid scintillation counter.

18.3 Results and discussion

The results of the seawater surveys are given in Figures. 40a-f.

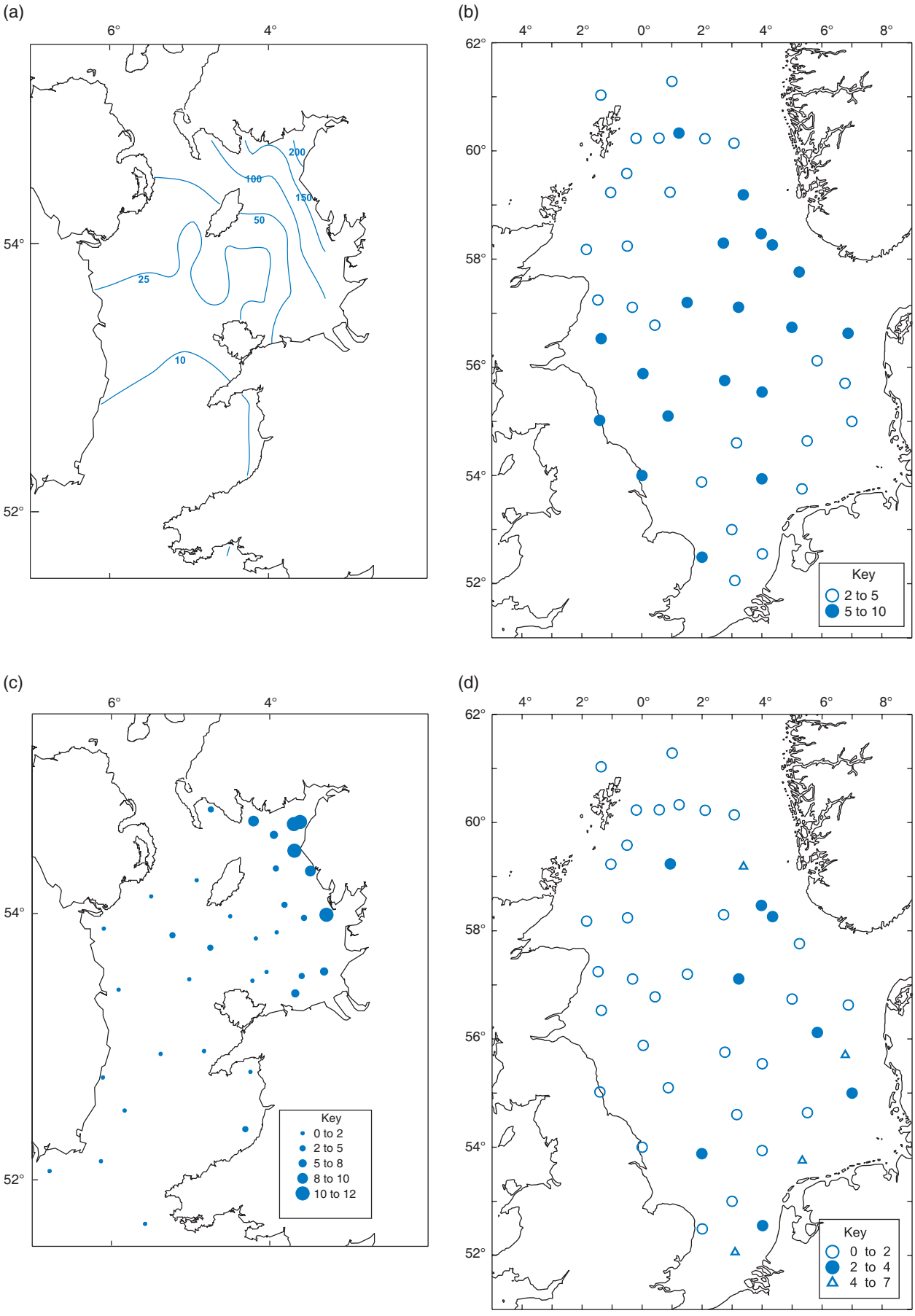

Figure 40. ***^{137}Cs (mBq kg^{-1}) and ^{3}H (Bq kg^{-1}) concentrations in surface seawater from the UK and European continental shelf. (a) Dissolved ^{137}Cs in the Irish Sea (September 1999), (b) Dissolved ^{137}Cs in the North Sea (August -September 2000), (c) ^{3}H in the Irish Sea (September 1999), (d) ^{3}H in the North Sea (August -September 2000)***

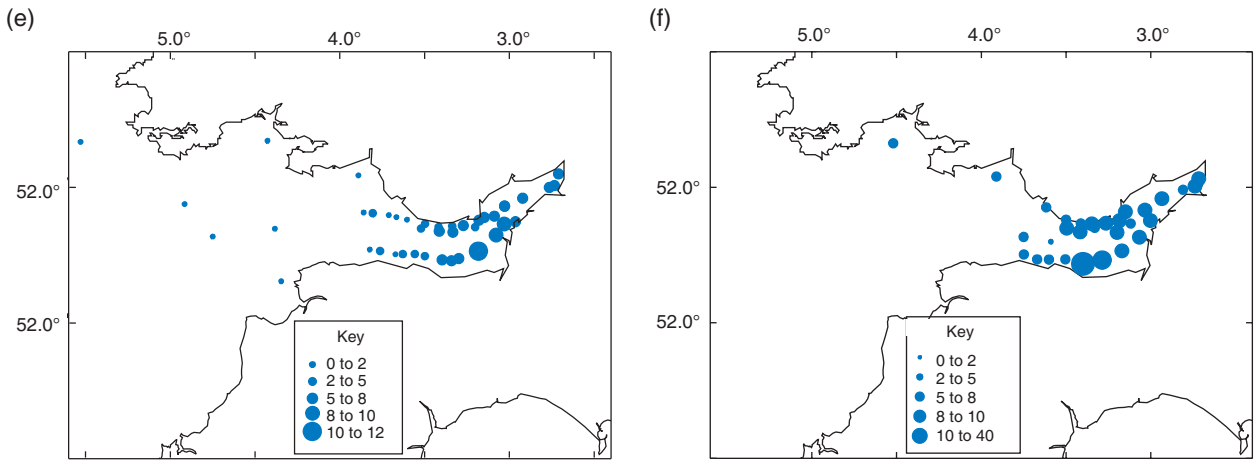

Figure 40. continued: ^{3}H (Bq kg^{-1}) concentrations in surface seawater from the UK and European continental shelf. (e) ^{3}H in the Bristol Channel (September 1999), (f) ^{3}H in the Bristol Channel (September 2000)

18.3.1 ^{137}Cs distribution

The Irish Sea ^{137}Cs data (Figure 40a) indicate that levels along a large section of the British coastline, extending from Liverpool Bay in the south to the Mull of Galloway in the north (typically 100-200 mBq kg^{-1}), were significantly greater than those observed along the Irish coastline (typically 10-50 mBq kg^{-1}). The ^{137}Cs contours extend parallel to the Cumbrian coastline with some anticlockwise displacement towards the Mull of Galloway in the north and Liverpool Bay in the south. Comparison of ^{137}Cs concentrations in the North Channel and at the southern entrance to the northern Irish Sea shows the predominant northwards migration. The ^{137}Cs overall distribution is in line with that expected from our knowledge of mean surface water circulation in the Irish Sea (Dickson, 1987). The predominant flow of water is northward via input of Atlantic water from St. George's Channel to the west of the Isle of Man. A minor component of the flow enters the eastern Irish Sea to the north of Anglesey and moves anti-clockwise round the Isle of Man before rejoining the main flow to exit through the North Channel.

The ^{137}Cs concentrations observed here are only a small percentage of those prevailing in the late 1970s. Levels as high as 30,000 mBq kg^{-1} have been observed in the vicinity of the Sellafield outfall (Baxter *et al.*, 1992) when discharges from Sellafield were substantially greater. Indeed, differences between the $^{137}Cs/^{90}Sr$ ratio in Sellafield discharges and seawater indicate that ^{137}Cs remobilisation, from sediments contaminated by large discharges in the 1970s, is presently the predominant (~90%) source term to the water column (Leonard *et al.*, 1998).

The North Sea ^{137}Cs data (Figure 40b) indicate that levels ranged from ~2 mBq kg^{-1} up to ~10 mBq kg^{-1} (i.e. 5 fold variation). Concentrations were, therefore, significantly less than those observed in the Irish Sea. Indeed, at some sampling sites, concentrations were only marginally elevated above the 'background' level (~2 mBq kg^{-1}) due to global fallout from atmospheric testing of nuclear weapons in the 1950s and early 1960s. These data also provide some indication that water flowing out of the Baltic Sea, as well as the Irish Sea, represents a continuing source of ^{137}Cs to the North Sea. This is because the Baltic Sea was more heavily contaminated than the North Sea by ^{137}Cs arising from the Chernobyl accident in 1986, together with the fact that the outflow from the Baltic Sea is restricted (Kershaw and Baxter, 1995).

18.3.2 ^{3}H distributions

Levels of ^{3}H in the Irish Sea (Figure 40c) were below the limit of detection (<2 Bq kg^{-1}) for a large proportion of the survey area. However, the impact of discharges from Sellafield and the Heysham nuclear power plant was apparent along the Cumbrian and southern Scottish coastline, extending from Morecambe Bay in the south to Luce Bay in the north. Along this section, ^{3}H concentrations were in the range 10-15 Bq kg^{-1}. Surface seawater concentrations in the vicinity of Sellafield were, therefore, similar to those in the Severn estuary near the points of release from the Amersham Biosciences radiopharmaceutical plant at Cardiff and the Hinkley Point nuclear power station (Figures 40e-f). ^{3}H concentrations in the North Sea (Figure 40d) were also below the limit of detection

(<2 Bq kg^{-1}) for a large proportion of the survey area. The highest concentration (6.1 Bq kg^{-1}) was in waters bordering the continental coastline, possibly due to releases from the La Hague (France) nuclear fuel reprocessing plant.

In the Bristol Channel (Figures 40e-f), the greatest ^{3}H concentrations (~ 10 Bq kg^{-1}) were observed on the English side of the Bristol Channel, in the vicinity of the Hinkley nuclear power plant, compared with ~7 Bq kg^{-1} in the vicinity of Amersham Biosciences radiopharmaceutical plant at Cardiff. The impact of the ^{3}H inputs into the Severn estuary was most apparent upstream of both these points of discharge. Concentrations were noticeably elevated (>5 Bq kg^{-1}) to the eastern limit of the survey area. This is to be expected given the tidal nature of the Severn estuary. Tidal current speeds generally exceed 1.5 m s^{-1} at springs and 0.75 m s^{-1} at neaps, meaning water parcels can move up to 25 km during a flood or ebb tide (Uncles, 1984). Outside of the typical tidal excursion, ^{3}H concentrations decreased rapidly with distance downstream of the points of discharge (i.e. in a westerly direction). Levels in the mouth of the Bristol Channel were below the limit of detection (2 Bq kg^{-1}).

18.3.3 Other radionuclides

^{99}Tc concentrations in seawater are now decreasing following the substantial increases observed since 1994. This pattern reflects the discharges of ^{99}Tc from Sellafield. The results of research cruises to study this radionuclide have been published by Leonard *et al*. (1997a and b; 2001). Trends in plutonium and americium concentrations in seawater of the Irish Sea have been considered by Leonard *et al*. (1999). A full review of the quality status of the north Atlantic has been published by OSPAR (2000).

19. RADIONUCLIDE CONCENTRATIONS IN DREDGED SEDIMENT

19.1 Introduction

In England and Wales, DEFRA issues licences to operators for the disposal of dredged material under the Food and Environment Protection Act, 1985 (Great Britain Parliament, 1985a). The protection of the marine environment is considered before a licence is issued. Since dredge material may contain radioactivity, assessments are undertaken where appropriate for assurance that there is no significant foodchain or other risk from the disposal. In 1999 and 2000, specific assessments were carried out for the disposal of spoil from Whitehaven Harbour in Cumbria and the Tamar Estuary near Devonport. Whitehaven Harbour is known to contain significantly enhanced quantities of natural and artificial radionuclides as a legacy of spillage of phosphate ore whilst unloading ships and large discharges in the 1970s from Sellafield. The Tamar estuary contains low levels of artificial radionuclides due to discharges from the submarine related operations at Devonport and from other widespread sources such as weapon test fallout.

19.2 Materials and methods

Samples of surface sediments and core samples were collected from a variety of locations to ensure the data provided representative information. Particular consideration was given to areas of fine sediment, which tend to contain higher radionuclide concentrations. Radionuclide assay was achieved using gamma-ray spectrometry by which it is possible to simultaneously measure a wide range of radionuclides commonly found in radioactive wastes. Additional analyses for the Whitehaven samples were by radiochemical separation and beta counting using gas proportional detectors for ^{210}Pb and alpha counting using alpha spectrometry for ^{210}Po.

19.3 Results and discussion

Results from the sediment analyses are provided in Tables 42 and 43.

Assessment of these data indicated that the impacts of radioactivity associated with the disposals of material from Whitehaven Harbour and the Tamar estuary should not give cause for concern since they are small compared to other sources of radioactivity in the marine environment. Guidance on exemption criteria for radioactivity in relation to sea disposal is available from the International Atomic Energy Agency (IAEA, 1999).

Table 42. Radioactivity in sediment dredged from Whitehaven Harbour, Cumbria, 1999

Location and depth	Radioactivity concentration (dry)[a], Bq kg^{-1}														
	^{60}Co	^{106}Ru	^{125}Sb	^{137}Cs	^{154}Eu	^{155}Eu	^{210}Po	^{210}Pb	^{212}Pb	^{214}Bi	^{226}Ra	^{228}Ac	^{233}Pa	^{234}Th	^{241}Am
A Top	30	56	<5.7	1100	17	6.0	170	170	38	63	65	36	18	900	1100
A Middle	6.5	15	11	990	18	<4.8	130	150	38	43	47	37	87	870	1300
A Bottom	3.2	<12	<4.9	910	12	<4.9	410	340	36	160	200	30	27	2600	740
B Top	23	67	<5.0	890	12	<4.5	150	150	40	54	63	36	*	1000	1000
B Middle	4.0	<14	<5.7	1100	16	<5.9	520	560	43	220	260	40	38	3700	1200
B Bottom	5.9	<15	<6.3	1900	14	<5.9	450	460	42	190	220	33	*	3100	1100
C Top	<1.0	<11	<4.3	270	<3.4	<5.0	380	710	50	340	400	43	*	3200	850
C Middle	1.8	<18	<8.0	2800	20	<6.8	300	440	45	150	190	38	*	1600	2200
C Bottom	<0.53	<6.1	<2.0	130	<1.6	<2.7	22	35	23	49	56	18	*	500	120
D Top	19	42	<4.7	810	12	<4.3	180	200	38	93	110	26	*	520	890
D Middle	6.4	<14	<5.9	1100	18	9.6	200	240	36	70	88	37	45	610	1500
D Bottom	4.0	<14	<5.6	1100	19	<5.7	750	730	45	270	320	48	34	1500	1300

** Not detected by the method used*
[a] All short-lived nuclides assumed to be unsupported

Table 43. Radioactivity in sediment to be dredged from the Tamar Estuary near Devonport, 2000

Location and depth (m)		Radioactivity concentration (dry)[a], Bq kg^{-1}						
		^{40}K	^{137}Cs	^{212}Pb	^{214}Bi	^{226}Ra	^{228}Ac	^{234}Th
D1	surface	710	6.9	39	26	31	32	100
D2	"	690	6.1	38	34	28	30	56
D3	"	680	7.2	39	34	31	29	85
D4	"	580	5.5	33	26	24	29	57
D5	"	710	7.7	39	25	30	29	37
D6	"	570	1.4	32	22	23	27	35
D7	"	710	6.7	38	23	30	32	59
D8	"	710	4.9	34	22	26	29	56
D9	"	690	6.1	38	25	30	31	61
D10	"	660	1.1	29	20	21	26	39
D11	"	680	1.1	39	22	27	36	45
D12	"	440	3.0	24	22	20	22	33
D13	0.8	680	<0.59	35	22	26	34	58
D15	2.1	630	<0.59	35	20	27	31	54
Vc8	1-1.25	650	<0.59	42	23	30	37	54
Vc67	1-1.25	540	<0.59	30	20	24	29	41
Vc88	1-1.25	700	<0.59	42	31	30	40	69
Vc95	2-2.25	540	<0.59	26	18	20	26	38

[a] All other gamma emitting radionuclides were present at less than the limits of detection, generally ~1 Bq kg^{-1}

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ANNEX 1. Areas of work and staff responsible for the projects

SEA WATER

Alkylphenols in seawater and marine sediments	J. Balaam
Antifouling-paint booster biocide contamination	K. Thomas
PBDE residues in selected matrices	C. Allchin
Winter nutrients in coastal waters	S. Malcolm
Smartbuoy developments and data	S. Malcolm

BIOTA

PAHs in bivalve molluscs	J. Jones
Mercury concentrations in fish	A. Franklin

BIOLOGICAL EFFECTS

EROD data	M. Kirby
Fish disease monitoring	G.Jones/S. Feist
DNA adducts in marine flatfish	B. Lyons

SEDIMENTS

Sediment bioassays	S.Kirby/Y. Allen
PAHs in sediments	C. Kelly

BENTHOS

Meiofauna research	M. Schratzberger

DISPOSAL AT SEA

Advice on fishery implications of pipeline discharges	M. Pendle
Licensing of deposits in the sea	C. Vivian
Characterisation of dredged material	E. Garnacho
Rough's Tower studies	D. Limpenny

RADIOACTIVITY

Radioactivity data	B.Smith/D.McCubbin