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AQUACULTURE SCIENCE

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Number 53

Monitoring and surveillance of non-radioactive contaminants in the aquatic environment and activities regulating the disposal of wastes at sea, 1998

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Staff responsible for the projects described in this report are listed in Annex 1.

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FOREWORD

Aquatic Environment Monitoring Report No. 53 collects together work carried out in 1998 by CEFAS scientists in support of our monitoring and surveillance duties (see overleaf). The information covers both general quality monitoring at marine and coastal sites and site-specific work carried out in support of risk assessment 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) – the Green Book which is available in downloadable format from the Fisheries Research Services, Aberdeen web site. 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 considered likely to pose specific risks. Measurement of alkylphenols and polybrominated diphenylethers due to OSPAR concerns are two examples of these special surveys and results are reported in Chapters 1 and 3.

The growing list of contaminants of concern dictate that it is impossible to measure all contaminants in marine waters and, even if we did so, 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 then to understand causal factors. To this end we have developed a cascade of methods to describe early warning signs (Chapter 10), effects at an individual level (Chapters 9 and 14) and small and large scale studies at the community level (Chapters 12 and 13).

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

α-HCH	Alpha isomer of hexachlorocyclohexane
γ-HCH	Gamma isomer of hexachlorocyclohexane (lindane)
ABS	Acrylonitrile butadiene
ACh	Acetylcholine
AChE	Acetylcholinesterase
ACTC	Acetylthiocholine
ADI	Acceptable daily intake
AGDS	Acoustic Ground Discrimination System
AP	Alcohols
APEC	Carboxylic acids
APEOs	Alkylphenol polyethoxylates
As	Arsenic
BEQUALM	Biological Effects Quality Assurance in Monitoring programmes
BPEO	Best Practicable Environmental Option
CB	Chlorinated biphenyl/chlorobiphenyl
CEFAS	Centre for Environment, Fisheries and Aquaculture Science
ChE	Cholinesterase
CPT	Copper pyrithione
DANI	Department of Agriculture for Northern Ireland
DBDE	Decabromodiphenyl ether
DBT	Dibutyltin
DDE	Dichlorodiethylene
DDT	Dichlorodiphenyltrichloroethane
DEFRA	Department for Environment, Food and Rural Affairs
DETR	Department of Environment, Transport and the Regions
DMSO	Dimethyl sulphoxide
DNA	Deoxyribose nucleic acid
DTI	Department of Trade and Industry
DTLR	Department for Transport, Local Government and the Regions
DTNB	Dithiobisnitrobenzoate
EC	European Community
EQS	Environmental quality standard
EROD	Ethoxyresorufin-O-deethylase
EU	European Union
FCA	Foci of cellular alteration
FEPA	Food and Environment Protection Act
GC-FPD	Gas chromatography coupled to flame photometric detection
GC-MS	Gas chromatography – mass spectrometry
GC-MS (SIM)	Gas chromatography – mass spectrometry single ion monitoring
HCB	Hexachlorobenzene
HIPS	High impact polystyrene
HPLC-MS	High performance liquid chromatography coupled to mass spectrometry
HYP	Hyperpigmentation
ICES	International Council for the Exploration of the Sea
JAMP	Joint Assessment and Monitoring Programme
LOD	Limits of detection
MA	*Myxobolus aeglifini*
MAC	Maximum allowable concentration
MAFF	Ministry of Agriculture, Fisheries and Food
MBT	Monobutyltin
MDS	Multi-dimensional scaling
MFO	Mixed function oxygenase
NBF	Neutral buffered formalin
NICI	Negative ion chemical ionisation
NMAQC	National Marine Analytical Quality Control Scheme
NMMP	National Marine Monitoring Programme
NP	Nonylphenol
NP2EO	Diethoxylate

NPEO	Nonylphenol monoethoxylate
OD	Optical density
OP	Octylphenol
PAH	Polyaromatic hydrocarbon
PBDE	Polybrominated diphenyl ether
PCB	Polychlorinated biphenyl
PTDI	Provisional tolerable daily intake
QSAR	Quantitative structure-activity relationship
OSPAR	Olso and Paris Commission
RAP	Registry of Aquatic Pathology
RV	Research vessel
SERAD	Scottish Executive Rural Affairs Department
SFI	Sea Fisheries Inspectorate
SFPA	Scottish Fisheries Protection Agency
SIMPER	Similarities percentages programme
SOAEFD	Scottish Office Agriculture, Environment and Fisheries Department
SPE	Solid phase extraction
TBT	Tributyl tin
TCMS	Tetrachloromethylsulfonul
TCMTB	Thiocyanomethylthio benzothiazole
TDE	Dichlorodiethane
TOxN	Total oxidised nitrogen
UK	United Kingdom
ZPT	Zinc pyrithione

1. ALKYLPHENOLS IN SEA WATER AND MARINE SEDIMENTS SAMPLED IN COASTAL AND OFFSHORE WATERS AROUND ENGLAND AND WALES

1.1 Alkylphenols in seawater

1.1.1 Introduction

Alkylphenol polyethoxylates (APEOs) are non-ionic surfactants used extensively in 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). APEOs have previously been used in the oil and gas industries as rig washers and cuttings cleaners, where they are discharged directly into the sea without treatment (Blackburn *et al*., 1999). Their use in this area is being phased out, and ten major manufacturers of polyacrylamide emulsion compounds have agreed a Europe-wide exclusion of APEOs by the year 2001 (Environmental Data Services Report, 1999). However, this only covers an estimated 1-2% of total APEO consumption within the European Community (EC). Chemical companies in the United States and Japan have formed an alliance to protect APEOs from environmental legislation. Due to the growing concern over their environmental effects, nonylphenol ethoxylates have been banned from cleaning products in Germany and Switzerland (Environmental Data Services Report, 1999) and a voluntary ban is in force within Europe on the household use of these products. In the UK, it is estimated that the total consumption of APEOs is 15-19,000 tonnes per annum (Department of the Environment, 1993) and that approximately 37% of these are discharged into rivers and estuaries, mainly via sewage effluents.

Alkylphenol surfactants have been suggested as remediators for contaminated soils and aquifers, without a full investigation into the effect that the surfactant itself will have on the surrounding environment. Their extensive use has led 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. Surfactants are usually used in aqueous solutions and so metabolites are often formed during the breakdown of products during waste water treatment. APEOs break down to give short chain ethoxylates, carboxylic acids (APEC) and alcohols (AP), namely nonylphenol (NP) and octylphenol (OP) derivatives. These breakdown products have been identified as having the ability to mimic natural hormones by interacting with the oestrogen receptor and they have the potential to bioaccumulate (Ahel *et al*., 1996). Exposure to NP has been found to increase the production of oestrogen-responsive proteins such as vitellogen and zona radiata protein in fish (Arukwe *et al*., 1997). This oestrogenicity has 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*., 1993).

1.1.2 Methods

Water sampling, using UK National Marine Monitoring Programme (NMMP) guidelines, was conducted at a total of 52 sites during two research vessel (RV) cruises, *RV CIROLANA* 3a/98 and 3b/98. Of these, 19 were from NMMP stations. Samples were taken at offshore, intermediate and estuarine sites, in order to elucidate trends in these areas. Filtered (dissolved) and unfiltered (total) samples were analysed for NP, nonylphenol mono (NPEO)- and diethoxylate (NP2EO), and OP.

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

1.1.3 Results

Results are presented in Table 1 and Figure 1.

The limits of detection (LOD) for the extraction plus analytical methods were evaluated by spiking replicates of reagent water with all target analytes, and then

serially diluting the resulting sample extract. The concentration at which the signal-to-noise ratio approximated to 3 was taken to be the LOD. The LOD for both total and dissolved samples were found to be 0.01 µg l^{-1} for NP, 0.06 µg l^{-1} for NPEO + NP2EO, and 0.01 µg l^{-1} for OP.

In the unfiltered samples, concentrations of total NP ranged from below the LOD (0.01 µg l^{-1}) to 5.2 µg l^{-1}. Dissolved NP was found to range from below the LOD to 4.2 µg l^{-1}. Concentrations greater than 1 µg l^{-1} were found in the vicinity of the Isle of Man, Amble (on the north east coast) and the Tyne, the Tees and the Humber river estuaries.

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

NMP	Location	Position		Nonylphenol		Ethoxylates*		Octylphenol	
Site				Total	Dissolved	Total	Dissolved	Total	Dissolved
	North Farne	55° 38.250'N	01° 30.410'W	0.89	1.2	3.0	2.1	0.04	0.01
	Amble	55° 16.888'N	01° 15.116'W	5.0	3.3	8.1	5.1	0.52	0.27
	North Reference Site - Tyne	55° 11.135'N	01° 22.442'W	0.40	<0.01	<0.06	<0.06	<0.01	<0.01
	Disposal Site	55° 03.202'N	01° 16.988'W	0.91	0.24	<0.06	0.62	4.1	0.05
	Mouth of Tyne	55° 01.388'N	01° 23.263'W	2.0	0.44	1.2	0.66	0.15	0.09
	Mouth of Tyne	55° 01.388'N	01° 23.263'W	1.7	1.7	2.2	2.4	0.12	0.15
245	Tyne	55° 00.400'N	01° 08.150'W	0.45	0.50	<0.06	<0.06	3.5	1.6
	Southern Reference Site	54° 59.625'N	01° 14.117'W	0.72	0.24	<0.06	<0.06	3.05	0.08
	TBT Tees Transect - TE2	54° 39.906'N	00° 59.643'W	0.28	<0.01	<0.06	<0.06	<0.01	<0.01
	TBT Tees Transect - TE8	54° 40.679'N	01° 02.177'W	0.28	<0.01	<0.06	<0.06	<0.01	<0.01
	TBT Tees Transect - TE9	54° 42.286'N	01° 04.272'W	0.30	<0.01	<0.06	<0.06	<0.01	<0.01
295	Tees Bay	54° 42.891'N	00° 52.819'W	0.62	0.34	0.65	0.50	0.05	0.02
295	Tees Bay	54° 42.891'N	00° 52.819'W	0.78	0.41	<0.06	<0.06	0.04	0.03
	Tees Mouth	54° 41.044'N	01° 07.966'W	2.0	1.0	<0.06	<0.06	0.09	0.05
	Tees Mouth	54° 41.044'N	01° 07.966'W	1.65	1.35	<0.06	<0.06	0.08	0.15
	Tees - Bamletts Wharf	54° 35.440'N	01° 14.600'W	0.22	0.17	2.6	1.6	0.03	0.02
	Tees - Transporter Bridge	54° 35.100'N	01° 13.450'W	0.66	0.50	1.9	0.76	0.06	0.05
	Tees - Telecom Wharf	54° 35.030'N	01° 11.700'W	0.48	0.38	3.2	1.4	0.05	0.03
	Tees - No. 23 Buoy	54° 35.840'N	01° 10.400'W	0.78	0.50	4.0	3.9	0.08	0.06
	Tees - Ramp Outfall	54° 36.280'N	01° 09.800'W	0.67	0.42	3.2	1.9	0.06	0.04
	Tees - Outfalls	54° 37.190'N	01° 09.000'W	2.6	1.6	5.0	4.1	0.15	0.11
285	West Dogger	54° 49.875'N	01° 19.816'E	0.63	0.80	<0.06	<0.06	0.05	0.03
	Bremerhaven 6	54° 43.863'N	05° 33.120'E	1.5	0.49	<0.06	<0.06	0.05	0.03
	Off Flamborough	54° 02.078'N	01° 45.677'E	2.2	1.7	1.3	1.84	0.12	0.10
345E	Humber/Wash	53° 59.989'N	02° 00.017'E	0.99	0.42	<0.06	<0.06	3.2	1.4
	Bremerhaven 1	54° 04.169'N	08° 07.075'E	0.54	0.91	<0.06	<0.06	0.05	0.03
	North Tail Dogger	53° 30.790'N	04° 10.464'E	1.4	1.1	<0.06	<0.06	0.07	0.07
	Arsenic 6	53° 25.950'N	03° 48.110'E	2.2	1.1	0.63	0.79	0.20	0.08
375	Humber	53° 32.040'N	00° 35.085'E	2.0	1.1	<0.06	1.4	0.13	0.07
387	Inner Wash	53° 09.134'N	00° 34.473'E	1.1	2.9	1.5	0.46	0.07	0.05
	Rotterdam Transect 2	51° 58.080'N	03° 51.040'E	1.1	0.46	1.6	1.6	0.05	0.06
	Barrow Deep	51° 43.627'N	01° 23.202'E	0.17	0.24	0.81	1.0	0.01	0.01
	Barrow Deep	51° 39.942'N	01° 17.379'E	0.24	0.20	1.4	0.99	0.01	0.01
	Barrow Deep	51° 35.539'N	01° 12.062'E	0.25	1.4	0.74	0.83	0.02	<0.01
485	South Varne	50° 56.050'N	01° 16.760'E	1.7	1.1	<0.06	0.56	0.16	0.07
	Rye Bay	50° 39.520'N	01° 41.380'E	0.90	0.51	1.4	0.51	0.08	0.03
	Isle Of Wight	50° 36.116'N	00° 56.798'W	<0.01	<0.01	0.08	0.48	<0.01	<0.01
495	NMP 495	50° 40.780'N	01° 50.450'W	0.28	0.16	0.35	0.55	<0.01	<0.01
537	Lyme Bay	50° 31.456'N	03° 13.028'W	0.29	0.24	0.46	0.12	0.06	0.10
536	Lyme Bay	50° 25.845'N	03° 07.016'W	0.13	<0.01	0.14	0.46	<0.01	<0.01
585	Off Plymouth	50° 02.050'N	04° 21.850'W	0.19	0.74	0.36	0.43	0.02	0.03
595	Western Approaches	48° 29.373'N	07° 59.844'W	0.27	0.26	0.40	1.3	0.04	0.06
605	Celtic Deep	51° 15.118'N	05° 59.774'W	<0.01	<0.01	0.13	0.29	<0.01	<0.01
605	Celtic Deep	51° 15.092'N	05° 59.743'W	0.37	0.33	0.48	1.0	0.06	0.10
665	Outer Cardigan Bay	52° 22.510'N	04° 54.098'W	0.31	0.34	1.7	3.7	<0.01	<0.01
655	Inner Cardigan Bay	52° 21.610'N	04° 10.685'W	1.5	0.21	0.97	0.55	<0.01	<0.01
776	Red Wharfe Bay	53° 21.810'N	04° 08.295'W	0.91	0.25	0.45	0.78	0.10	0.22
715	Liverpool Bay	53° 29.891'N	03° 41.430'W	0.92	0.23	2.8	0.47	0.12	0.48
705	Liverpool Bay	53° 28.251'N	03° 21.745'W	0.49	0.28	1.1	0.80	0.11	0.04
795	Off Morcambe Bay	53° 55.717'N	03° 22.841'W	2.2	4.2	0.41	2.9	<0.01	<0.01
805	SE Isle of Man	54° 00.102'N	03° 49.899'W	5.2	2.9	1.7	0.22	<0.01	<0.01
815	Dundrum Bay	54° 04.090'N	05° 29.762'W	2.0	1.7	0.34	0.19	0.14	<0.01

** Sum of NP mono- and diethoxylates*

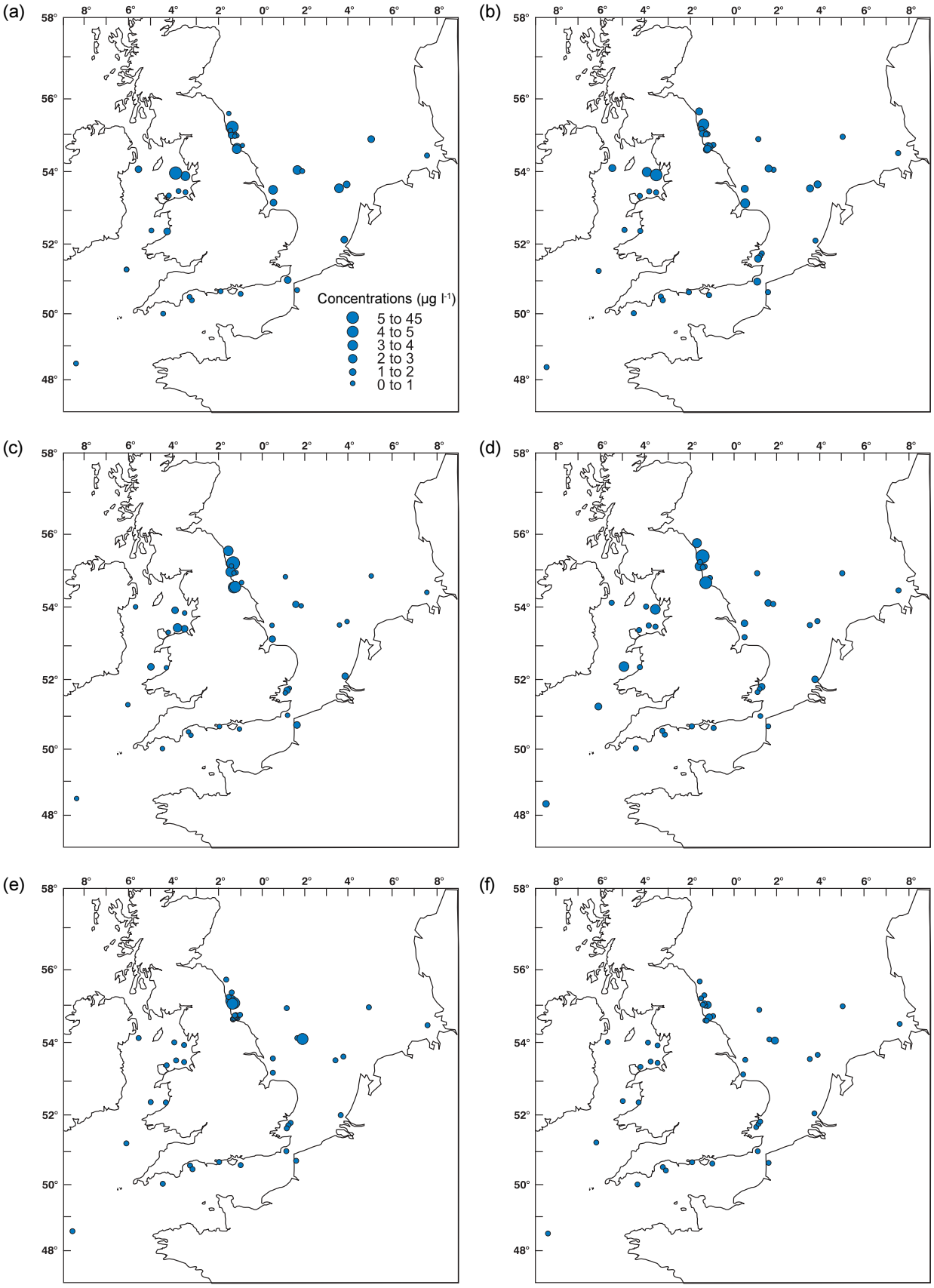

Figure 1. ***Spatial distribution of alkylphenol and alkylphenol ethoxylate concentrations in marine and coastal waters around England and Wales, 1998 of (a) total nonylphenols, (b) dissolved nonylphenols, (c) total mono- and diethoxylates, (d) dissolved mono- and diethoxylates, (e) total octylphenol and (f) dissolved octylphenol***

Total concentrations of NPEO and NP2EO ranged from below the LOD (0.06 µg l^{-1}) to 8.1 µg l^{-1}. The maximum was detected at Amble (not an NMMP site). The range of concentrations for dissolved mono- and diethoxylates was from below the LOD to 5.1 µg l^{-1}. The maximum was also found at Amble. No ethoxylates were detected at Bremerhaven (north of Germany), Dogger Bank, the Wash, the Tyne, Tees Bay and Tees Mouth. Conversely, inside the Tees Estuary, relatively high concentrations (up to 5 µg l^{-1}) of ethoxylates were detected, indicating that the ethoxylates break down or are diluted in saline water very quickly once away from the source of contamination. There are many industries operating from the shores of the Tees, which may contribute towards the relatively high concentrations found inside the estuary.

Total OP ranged from below the LOD (0.01 µg l^{-1}) to 4.1 µg l^{-1}. Most sites were found to have relatively low concentrations of OP (below 0.2 µg l^{-1}), with the exception of samples taken at NMMP 345 Humber/ Wash, NMMP 245 Tyne, Tyne Southern Reference Site and Tyne Disposal Site. Dissolved OP ranged from below the LOD at North Farne on the north-east coast, to 1.6 mg l^{-1} at NMMP 245 off the Tyne. The only other site with a concentration in excess of 0.5 µg l^{-1} was NMMP 345 Humber/Wash.

Concentrations in the dissolved phase were invariably lower than in the corresponding unfiltered samples, with differences being more marked in areas of high turbidity (e.g. Tees Ramp Outfall, just off ICI Terminal No. 4).

1.1.4 Discussion

Discharge limits for APEOs have been set by the Environmental Quality Standard (EQS) steering group (Matthiessen, pers. comm.) on the basis of a quantitative structure-activity relationship (QSAR). Maximum allowable concentrations (MACs) for NP and OP are 2.5 µg l^{-1}. In this study, NP exceeded this limit at three sites; NMMP 805 (SE Isle of Man), Amble, off Flamborough and Tees Outfalls. OP exceeded the MAC value at four different sites; NMMP 245 (Tyne), NMMP 345 (Humber/ Wash), Tyne Southern Reference site and Tyne Disposal Site.

MACs for NP mono- and diethoxylates are 3.3 and 4.3 µg l^{-1} respectively, which tentatively leads to an allowable concentration of 7.6 µg l^{-1} for the sum of the mono- and diethoxylates. This limit was exceeded at only one site in this study, Amble.

The majority of the highly contaminated samples in this study were found in coastal or estuarine areas, of high industrial activity, with contamination likely to originate from industrial or domestic discharges through sewage treatment works.

Further monitoring is required to confirm some of the results obtained e.g. the high concentrations found at Amble for NP mono-and diethoxylates, so that their significance in the aquatic environment can then be assessed.

1.2 Alkylphenols in sediment

1.2.1 Methods

Sampling was carried out using UK NMMP guidelines at a total of 44 sites during two research cruises, *RV CIROLANA* 3a/98 and 3b/98. Of the 44 sediment samples, 16 were from NMMP stations. Samples were taken from offshore, coastal and estuarine sites, using a Day Grab. Analysis was carried out on the <2 mm fraction.

Samples were extracted with 3 x 25 ml ethyl acetate after addition of butylphenol internal standard. Extracts were reduced to approximately 1 ml, cleaned up using 10% deactivated alumina and eluted with dichloromethane. Analysis was carried out by GC-MS in full scan mode.

1.2.2 Results

Results are presented in Table 2 and Figure 2.

The LOD for the extraction plus analytical methods was evaluated by spiking replicates of uncontaminated sediment with all target analytes, and then serially diluting the extract that resulted. The concentration at which the signal-to-noise ratio approximated to 3 was taken to be the LOD. The LOD was found to be 0.2 µg g^{-1} for NP, 1 µg g^{-1} for NPEO + NP2EO, and 0.01 µg g^{-1} for OP.

In almost all samples, concentrations of NP were found to be below the LOD (0.2 µg g^{-1}), except for those taken in or around the Tees. All of the sites within the Tees had a concentration of greater than 2 µg g^{-1}, with a maximum of 42 µg g^{-1} at Tees Outfalls. NP mono- and diethoxylates were below the LOD (1 µg g^{-1}) at all stations. OP was found to be above the LOD (0.01 µg g^{-1}) at very few sites, and the concentrations found at those sites were below 0.06 µg g^{-1}.

1.2.3 Discussion

Concentrations were low or undetectable at intermediate or offshore sites. At present, 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. This and other UK estuaries such as the Aire, which has been found in previous studies to contain high levels of alkylphenols (Blackburn *et al*., 1999), which should be further monitored. The Tees is an ideal candidate for

Table 2. Concentrations of alkylphenol and alkylphenol ethoxylates (μg g^{-1}) in sediment

NMP Site	Location	Position		Nonylphenol	Ethoxylates*	Octylphenol
	Amble	55° 16.888'N	001° 15.116'W	<0.19	<1.00	<0.01
	Northern Reference Site - Tyne	55° 11.213'N	001° 22.456'W	<0.19	<1.00	<0.01
	Disposal Site	55° 03.235'N	001° 16.967'W	<0.19	<1.00	<0.01
245	Tyne	55° 00.475'N	001° 08.261'W	<0.19	<1.00	<0.01
	Southern Reference Site	54° 59.724'N	001° 14.035'W	<0.19	<1.00	<0.01
	TBT Tees Transect - TE2	54° 39.906'N	000° 59.643'W	0.34	<1.00	<0.01
	TBT Tees Transect - TE8	54° 40.679'N	001° 02.177'W	7.1	<1.00	0.03
	TBT Tees Transect - TE9	54° 42.286'N	001° 04.272'W	0.20	<1.00	0.01
	Tees - Bamletts Wharf	54° 35.440'N	001° 14.600'W	2.6	<1.00	<0.01
	Tees - Transporter Bridge	54° 35.100'N	001° 13.450'W	2.0	<1.00	<0.01
	Tees - Telecom Wharf	54° 35.030'N	001° 11.700'W	2.8	<1.00	<0.01
	Tees - No. 23 Buoy	54° 35.840'N	001° 10.400'W	8.6	<1.00	<0.01
	Tees - Ramp Outfall	54° 36.280'N	001° 09.800'W	13	<1.00	<0.01
	Tees - Outfalls	54° 37.190'N	001° 09.000'W	42	<1.00	<0.01
	Off Flamborough	54° 02.078'N	001° 45.677'E	<0.19	<1.00	<0.01
345	Humber/Wash	53° 59.950'N	001° 59.975'E	<0.19	<1.00	<0.01
387	Inner Wash	53° 09.134'N	000° 34.473'E	<0.19	<1.00	<0.01
	Rotterdam Transect 2	52° 28.898'N	004° 23.239'E	<0.19	<1.00	<0.01
	Barrow Deep	51° 43.627'N	001° 23.202'E	<0.19	<1.00	<0.01
	Barrow Deep	51° 39.942'N	001° 17.379'E	<0.19	<1.00	0.03
	Barrow Deep	51° 35.539'N	001° 12.062'E	<0.19	<1.00	0.06
485	South Varne	50° 56.050'N	001° 16.760'E	<0.19	<1.00	0.01
	Rye Bay	50° 39.520'N	001° 41.380'E	<0.19	<1.00	<0.01
	Isle Of Wight	50° 36.116'N	000° 56.798'W	<0.19	<1.00	<0.01
536	H7 Lyme Bay	50° 25.762'N	003° 07.114'W	<0.19	<1.00	<0.01
536	H9 Lyme Bay	50° 25.665'N	003° 07.106'W	<0.19	<1.00	<0.01
536	H4 Lyme Bay	50° 25.961'N	003° 07.104'W	<0.19	<1.00	<0.01
536	H2 Lyme Bay	50° 25.983'N	003° 07.519'W	<0.19	<1.00	<0.01
536	H1 Lyme Bay	50° 25.833'N	003° 07.276'W	<0.19	<1.00	<0.01
495	Selsey Bill	50° 31.456'N	003° 13.028'W	<0.19	<1.00	0.01
585	Off Plymouth	50° 02.050'N	004° 21.850'W	<0.19	<1.00	0.02
605	Celtic Deep	51° 15.092'N	005° 59.743'W	<0.19	<1.00	<0.01
605	Celtic Deep	51° 15.118'N	005° 59.774'W	<0.19	<1.00	0.03
	Swansea Bay	51° 31.514'N	003° 55.403'W	<0.19	<1.00	0.01
	Swansea Bay	51° 32.613'N	003° 55.318'W	<0.19	<1.00	<0.01
	Swansea Bay	51° 32.979'N	003° 52.495'W	<0.19	<1.00	<0.01
665	Outer Cardigan Bay	52° 22.510'N	004° 54.098'W	<0.19	<1.00	0.01
655	Inner Cardigan Bay	52° 21.528'N	004° 10.481'W	<0.19	<1.00	<0.01
776	Red Wharfe	53° 21.810'N	004° 08.295'W	<0.19	<1.00	0.01
715	Liverpool Bay	53° 29.891'N	003° 41.430'W	<0.19	<1.00	<0.01
705	Liverpool Bay	53° 28.251'N	003° 21.745'W	<0.19	<1.00	<0.01
705	Off Morcambe Bay	53° 55.717'N	003° 22.841'W	<0.19	<1.00	0.02
805	SE Isle of Man	54° 00.008'N	003° 49.986'W	<0.19	<1.00	0.01
815	Dundrum Bay	54° 04.031'N	005° 29.913'W	<0.19	<1.00	0.05

* *Sum of NP mono- and diethoxylates*

examination as it has a concentration of industrial activity. In 1998, the Tees Bay area (outside the industrial estuary) had levels of NP below the LOD, even though levels within the estuary were very high. This may be because the sediment in the Tees Bay area is very sandy, and so does not hold much organic matter. Either the NP is not detected due to this low adsorption of organics, or the transportation out of the estuary is very poor, and so all the contamination is contained within the estuary. More sites within the estuary (high and low flow rate sites) should be sampled to get a clearer picture of the transportation of contaminants.

Continuation of this study will provide information on the retention of these contaminants within the sediment matrix. As APEO surfactants are phased out in industry, the proportion of contamination contributed by industrial and domestic effluent will also become clearer. If it is shown that a large proportion of the contamination found is from domestic sewage, more emphasis may be put on finding alternatives to APEOs in domestic applications.

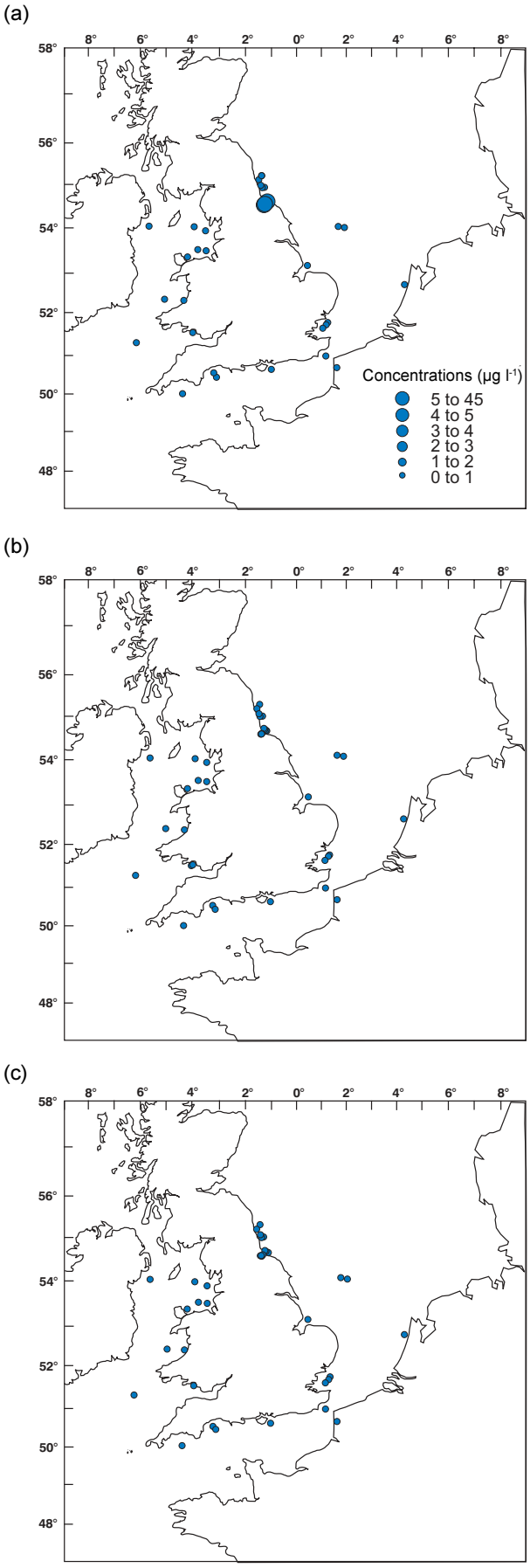

Figure 2. ***Spatial distribution of alkylphenol concentrations in marine and coastal sediments, 1998 of (a) nonylphenol, (b) ethoxylates and (c) octylphenol***

2. ANTIFOULING PAINT BOOSTER BIOCIDE CONTAMINATION IN COASTAL AND OFF-SHORE WATERS AROUND ENGLAND AND WALES

2.1 Introduction

Antifouling paints contain toxic biocides to prevent marine life from colonising the bottoms of boats. These biocides are constantly released from the painted surface into the surrounding waters. Prior to a ban on vessels <25 m in 1989, tributyl tin (TBT) was widely used as a biocide on all vessels. Since this ban, organic booster biocides have been developed to improve the efficacy of both copper and TBT based formulations. Along with TBT, eight organic booster biocides are currently approved for use in the UK; Irgarol 1051, diuron, zinc pyrithione (ZPT), dichlofluanid, thiocyanomethylthio benzothiazole (TCMTB), tetrachloromethylsulfonul (TCMS) pyridine, Kathon 5287 and zineb (Chadwick, pers. comm.). The data reported here represent the findings of a temporal survey of Southampton Water, the Crouch Estuary and Plymouth Sound during the 1998 yachting season, and a spatial survey of off-shore stations during an *RV CIROLANA* cruise in 1998.

2.2 Methods

Four analytical methods were used to determine TBT, Irgarol 1051, diuron, ZPT, dichlofluanid, TCMTB, TCMS pyridine and Kathon 5287 in sea water:

TBT

Analysis was carried out using simultaneous reduction of the alkyl tin to the hydride and dichloromethane extraction with the addition of a tripropyltin chloride internal standard. The extract was reduced in volume and analysed by gas chromatography coupled to flame photometric detection (GC-FPD) (Waldock *et al.*, 1989).

Irgarol 1051, Chlorothalonil, and Dichlofluanid

All three compounds were simultaneously determined by C18 SPE followed by GC-MS single ion monitoring (GC-MS(SIM)) (Fileman *et al.*, in prep.).

Diuron, TCMTB, TCMS pyridine, and Kathon 5287

All four compounds were simultaneously extracted by C18 SPE and quantified by high performance liquid chromatography coupled to mass spectrometry (HPLC-MS) (Thomas, 1998).

Zinc pyrithione

Samples collected for the analysis of ZPT were determined by transchelation to the Cu^{II} complex. The copper pyrithione (CPT) was then extracted, as described above, and analysed by HPLC-MS (Thomas, 1999).

2.3 Results and discussion

2.3.1 Coastal survey

Seawater samples were collected from stations on Southampton Water, the Crouch Estuary and Plymouth Sound in January (off-season) and from April, monthly through until October. The results of the off-season survey are presented in Table 3. One feature of the data is that only TBT, Irgarol 1051 and diuron are present at detectable concentrations in the samples obtained from the Crouch Estuary, Southampton Water, Sutton Harbour and Hythe Marina. A recent UK Environment Agency R&D Report (Environment Agency, 1998) suggested that only diuron, Irgarol 1051, ZPT and dichlofluanid were in use in the UK. The absence of TCMS pyridine, TCMTB, Kathon 5287 and chlorothalonil in this survey appears to confirm this, however, ZPT and dichlofluanid only occupy <5% of the market share and it is therefore likely that the amounts of these compounds released from antifouling coatings is not sufficient to increase the environmental concentrations above the LOD of 1-20 ng/l. Another factor that may affect the determined concentrations of ZPT is that it is reported to have a half-life ($t_{1/2}$) of less than 24 h (Turley *et al*., 1999).

A summary of the results of the yachting season surveys are presented in Table 4. As with the background survey, the major feature of the data is that only TBT, Irgarol 1051 and diuron were present at detectable concentrations in the samples.

Table 3. Summary of 1998 yachting season monitoring data, April to October

Sample station	Location	Min.-Max. biocide† concentration (median)		
		TBT (ng l^{-1})	Irgarol 1051 (ng l^{-1})	Diuron(ng l^{-1})
Southampton Water				
1	Beaulieu @ Penerley Fm	<1 (<1)	<1-3 (<1)	<1-5 (<1)
2	Beaulieu @ Bucklers Hard	<1-2 (2)	1-47 (22)	<1-141 (106)
3	Beaulieu @ Exbury River	<1-23 (4)	3-20 (9)	9-82 (32)
4	Calshot	<1-10 (5)	<1-7 (4)	4-58 (9)
5	Hythe Marina	<1-8 (<2)	118-403 (208)	112-6742 (632)
6	Cracknore Hard	<2-29 (4)	3-7 (4)	6-47 (25)
7	Fawley Refinery	<1-61 (<3)	<1-19 (4)	4-70 (24)
8	Docks (Upper)	<1-39 (19)	3-4 (3)	3-110 (21)
9	Docks (Lower)	<4-45 (17)	3-6 (5)	3-97 (21)
10	Ocean Village (Marina)	13-33 (27)	8-24 (13)	4-405 (82)
11	Itchen Mouth	<1-11 (6)	4-16 (8)	14-104 (32)
12	Netley Abbey Castle	<1-11 (10)	4-9 (6)	4-465 (30)
13	Hamble - Port Hamble Marina	<1-16 (3)	7-94 (27)	44-613 (150)
14	Hamble - Mercury Yacht Harbour	<1-17 (6)	22-141 (33)	113-214 (162)
15	Hamble - Swanwick Marina	<1-12 (4)	19-102 (39)	19-487 (117)
16	Hamble Mouth	<4-59 (5)	7-25 (13)	27-438 (52)
17	Outer Water	<1-2 (2)	2-33 (2)	1-23 (14)
18	Hill Head	<1-7 (2)	<1-8 (3)	1-27 (13)
19	Upper Hamble	<1-7 (4)	22-38 (31)	39-116 (101)
Sutton Harbour				
1	Melampus	<1-2 (<1)	<1-4 (1)	1-45 (8)
2	Mallard Beacon	<1-7 (<2)	<1-4 (1)	3-16 (8)
3	Sutton Harbour Berth B24/26	<1-40 (20)	4-69 (16)	40-331 (137)
4	Sutton Harbour Berth E2	<1-17 (<1)	9-65 (14)	12-189 (115)
5	Queen Ann's Battery	<1-39 (4)	<1-35 (3)	4-72 (26)
6	Sutton Harbour by Lock	<1-49 (30)	10-84 (16)	23-334 (93)
River Crouch				
1	Bridgemarsh Island	<1-3 (<1)	4-11 (7)	5-226 (32)
2	Fambridge YC	<1 (<1)	1-19 (11)	12-172 (44)
3	Crouch Outer	<1-4 (<2)	<1-4 (3)	6-46 (25)
4	Burnham Marina	<1-6 (<2)	12-49 (24)	36-305 (88)
5	Ringwood Bar	<1-1 (<1)	<1-18 (5)	6-60 (11)
6	Burnham Lab	<1-3 (<1)	2-19 (5)	8-64 (15)

† *All other biocides below LOD*

Table 4(a). Off-season (January) concentrations of TBT and booster biocides† measured in water samples from the Crouch Estuary during 1998

Station No.	Location	Latitude	Longitude	Tide (± HW)	TBT‡ (ng l^{-1})	Irgarol 1051 (ng l^{-1})	Diuron (ng l^{-1})	Salinity (‰)	pH	Temperature (°C)	Number of vessels	Proximity to agricultural land
1	Bridgemarsh Island	51° 37.89'N	00° 44.38'E	0	<2.7	9.1	2.1	29.0	8.2	5.1	0	Arable
2	Fambridge YC	51° 38.20'N	00° 40.52'E	+0:45	<2.4	9.4	4.5	27.7	8.6	5.0	25	Arable
3	Crouch Outer	51° 37.74'N	00° 48.19'E	+1:40	<4.0	<1.0	0.6	30.3	8.4	5.2	0	Arable
4	Burnham Marina	51° 37.33'N	00° 54.15'E	+2:30	2.0	15	117	32.8	8.3	5.5	≤250	Parkland
5	Ringwood Bar	51° 37.34'N	00° 49.61'E	+3:00	1.8	9.0	2.9	31.1	8.3	5.6	2	Arable
6	Burnham Lab	51° 37.40'N	00° 49.55'E	+3:30	1.2	8.1	3.9	30.1	8.2	5.2	0	Arable

Table 4(b). Off-season (January) concentrations of TBT and booster biocides measured in water samples from Sutton Harbour during 1998

Station No.	Location	Latitude	Longitude	Tide (± HW)	TBT‡ (ng l^{-1})	Irgarol 1051 (ng l^{-1})	Diuron (ng l^{-1})	Salinity (‰)	pH	Temperature (°C)	Number of vessels	Proximity to agricultural land
1	Sutton Harbour Berth 24/26	50° 22.17'N	04° 08.80'W	-2:30	78	71	<1	30.7	8.01	7.6	-	none
2	Sutton Harbour Berth E2	50° 22.09'N	04° 07.99'W	-2:05	56	55	25	31.3	8.06	8.1	-	none
3	Queen Ann's Battery	50° 21.85'N	04° 07.92'W	-1:38	45	80	8.5	30.7	8.06	7.2	-	none
4	Sutton Harbour Fish Market	50° 22.04'N	04° 07.89'W	-1:18	2.4	11	8.7	32.3	8.11	8.9	-	none

Table 4(c). Off-season (January) concentrations of TBT and booster biocides† measured in water samples from Southampton Water during 1998

Station No.	Location	Latitude	Longitude	Tide	TBT‡ (ng l^{-1})	Irgarol 1051 (ng l^{-1})	Diuron (ng l^{-1})	Salinity (‰)	pH	Temperature (°C)	Number of vessels	Proximity to agricultural land
1	Beaulieu @ Penerley Fm	50° 49.90'N	01° 27.62'W		<4.0	<1.0	<1.0	8.2	34.7	4.8	0	arable
2	Beaulieu @ Bucklers Hard	50° 48.13'N	01° 25.38'W		<3.0	6.7	1.3	8.6	33.0	5.9	-	arable
3	Beaulieu @ Quay	50° 47.18'N	01° 23.40'W		21	2.0	<1.0	8.4	29.9	5.0	-	upstream
4	Calshot	50° 49.23'N	01° 18.53'W		<10	6.0	1.2	8.3	32.4	5.5	-	none
5	Hythe Marina	50° 52.48'N	01° 23.91'W	Locked	14	1421	101	8.3	32.9	6.0	>500	none
6	Cracknore Hard	50° 53.82'N	01° 25.59'W		19	4.7	1.7	8.3	30.5	6.3	-	none
7	Fawley Refinery	50° 50.31'N	01° 19.70'W		No	Sample	collected	-	-	-	-	none
8	Docks (Upper)	50° 54.37'N	01° 25.92'W		10	<1.0	1.5	-	-	-	-	none
9	Docks (Lower)	50° 53.92'N	01° 24.66'W		33	5.5	2.8	-	-	-	-	none
10	Ocean Village (Marina)	50° 53.70'N	01° 23.43'W		22	61	4.7	-	-	-	-	none
11	Itchen Mouth	50° 53.33'N	01° 23.08'W		12	-	2.2	-	-	-	-	none
12	Netley Abbey Castle	50° 52.99'N	01° 22.08'W		6.1	4.7	1.6	-		-	-	none
13	Hamble - Port Hamble Marina	50° 51.65'N	01° 18.75'W		4.3	1.6	3.8	8.3	29.9	4.7	-	none
14	Hamble - Mercury Yacht Harbour	50° 52.22'N	01° 18.71'W		6.1	22	4.4	8.1	34.0	5.9	-	none
15	Hamble - Swanwick Marina	50° 52.92'N	01° 18.07'W		5.5	32	3.7	8.1	35.2	5.2	-	none
16	Hamble Mouth	50° 50.91'N	01° 18.41'W		3.4	6.1	2.6	7.4	0.0	2.5	-	none
17	Hill Head	50° 49.27'N	01° 15.25'W		<4.0	1.2	<1.0	8.1	29.0	4.0	-	none

† Concentrations of TCMTB, Kathon 5287, TCMS pyridine, dichlofluanid, chlorothalonil and zinc pyrithione all below the LOD.
‡ < indicates where the LOD for the method is higher than the stated value of 1 ng l^{-1}. Often due to the low recovery of internal standard due to the presence of compounds such as hydrocarbons which interfere with the FPD detection.

TBT

The concentration of TBT in samples obtained from the Crouch Estuary at the start of the yachting season was generally around the EQS of 2 ng l^{-1}, with the highest concentration determined in Burnham Marina (6 ng l^{-1}; Table 3). There was a higher concentration of TBT in the samples collected at the start of the yachting season when compared to those collected in January. It appears that there is some additional input at this time; possibly through the application of fresh coatings. This is also the case for a number of other marinas. The concentration of TBT in samples collected from the Crouch then declined to below the LOD for the remaining months. The concentrations determined within Sutton Harbour were generally higher (1-39 ng l^{-1}; Table 3). TBT concentrations outside the locked area were below the LOD for the method and suggest that the presence of commercial fishing vessels (>25 m in length) and limited water exchange are contributory factors resulting in the higher concentrations determined within the lock. For Southampton Water, most samples contained TBT at detectable concentrations at the very beginning of the season (Table 3). At the mouth of the estuary, concentrations decreased to below the LOD for most of the year. However, at the docks, Ocean Village marina, and particular marinas on the Hamble, TBT concentrations remained higher than the EQS of 2 ng l^{-1}. There appeared to be no consistent pattern of a seasonal decrease of TBT, with concentrations suddenly rising during mid summer. Once again, the concentrations determined are significantly below those measured pre-ban, however, elevated concentrations within certain marinas, in particular Hythe, suggest some illegal application of TBT-containing coatings to vessels <25 m. In open water well away from the influence of dockyard activity, TBT concentrations were still detectable in most samples, and in the area of Fawley refinery where large ships load and unload oil products, the highest recorded concentration was 61 ng l^{-1}. It must, however, be emphasised, that the highest concentrations determined in this survey are, in general, orders of magnitude lower than those determined in areas of high boating activity before the ban (Waite *et al*., 1991).

Irgarol 1051

Irgarol 1051 was detected in most samples with the highest concentrations (up to 403 ng l^{-1}) recorded in Hythe marina during the yachting season, with levels peaking in early summer. Concentrations of Irgarol 1051 in samples collected from the Crouch estuary and most of the Southampton Water sampling stations are similar to those previously reported for UK estuaries (<1-40 ng l^{-1}) by Gough *et al.* (1994) and Zhou *et al.* (1996).

Higher concentrations were predictably determined in marinas (e.g. Hythe marina and Sutton Harbour) due to a higher density of boats. The result from the sample collected from Hythe Marina in January 1998 (1421 ng l^{-1}; Table 4c) is higher than any previously reported concentration of Irgarol 1051 in the UK (Gough *et al*., 1994; Zhou *et al*., 1996; Scarlett *et al*., 1997) and may be due to the fact that boats are present within the marina throughout the year. Other contributing factors may be the use of high pressure hosing and repainting of boats and the limited flushing of the harbour outside the yachting season, when lock gates are frequently closed. Concentrations within this range have also been reported in samples obtained from the Côte d'Azur, France (110-1700 ng l^{-1}; Readman *et al*., 1993; Tolosa *et al*., 1996), Swedish marinas (10-100 ng l^{-1}; Dahl and Blanck, 1996), and the Spanish Mediterranean coast (10-180 ng l^{-1}; Ferrer *et al*., 1997; Ferrer and Barceló, 1999).

Diuron

Off-season concentrations of diuron were generally low with only two samples exceeding 100 ng l^{-1}. Both these samples were collected from marinas, Hythe Marina, Southampton Water and Burnham Marina, Crouch Estuary. This is similar to the concentrations observed in Swedish Marinas (10-100 ng l^{-1}; Dahl and Blanck, 1996) and in marinas and estuaries on the Spanish Mediterranean Coast (10-180 ng l^{-1}; Ferrer *et al*., 1997).

At the majority of sample locations used for this survey, diuron was determined at much higher concentrations following the start of the yachting season when compared to the data obtained during the off-season survey. Significant increases were observed in the Crouch Estuary, particularly at Burnham Marina (100-300 ng l^{-1}). Generally, diuron was determined at higher concentrations than TBT and Irgarol 1051 and, more importantly, at higher concentrations than have been previously reported for marinas (10-180 ng l^{-1}, Dahl and Blanck, 1996; Ferrer *et al*., 1997). The ubiquitous presence of diuron is unsurprising since it appears to be the most commonly used antifouling paint booster biocide in the UK, whilst also having widespread non-antifouling uses.

2.3.2 Off-shore survey

An off-shore survey was carried out during June and July with samples collected from around the UK coast from the *RV CIROLANA*. Sample locations were selected on their proximity to areas of high in-shore boating activity and off-shore shipping activities. The results of the survey are presented in Table 5 and it is clear that TBT and biocide concentrations, in the majority of samples collected, were below the LOD for each method. TBT and Irgarol 1051 concentrations are below the LOD for all stations except those in the Solent. This is understandable since the density of boats painted with both biocides in off-shore areas is low, becoming greater in near-shore areas such as the Solent. The concentrations of diuron observed were slightly higher,

Table 5. Off-shore concentrations of TBT and booster biocides

Station No.	Location	Position		TBT (ng l^{-1})	Irgarol 1051 (ng l^{-1})	Diuron (ng l^{-1})
1	Swansea Bay	51° 32.50'N	03° 55.30'W	<2	<1	<1
2	Milford Haven	51° 39.85'N	05° 09.03'W	<3	<1	<1
3	Outer Cardigan Bay (NMP 665)	52° 23.60'N	04° 53.52'W	<4	<1	<1
4	Inner Cardigan Bay (NMP 655)	52° 21.61'N	04° 10.68'W	<3	<1	<1
5	Dundrum Bay (NMP 815)	54° 04.09'N	05° 29.76'W	<5	<1	<1
6	SE Isle of Man (NMP 805)	54° 00.12'N	03° 49.95'W	<2	<1	1
7	Off Morcambe Bay (NMP 705)	54° 00.08'N	03° 48.85'W	<1	<1	2
8	Burbo Bight	53° 28.31'N	03° 15.63'W	<1	<1	8
9	Liverpool Bay (NMP 715)	53° 29.87'N	03° 41.45'W	<1	<1	3
10	Red Wharfe Bay (NMP 776)	53° 21.70'N	04° 09.09'W	<1	<1	2
11	Celtic Deep	48° 29.93'N	08° 00.07'W	<6	<1	<1
12	Western Approaches	48° 29.89'N	08° 00.00'W	<2	<1	<1
13	Off Plymouth (NMP 585)	50° 02.05'N	04° 21.85'W	<2	<1	1
14	Lyme Bay	50° 31.46'N	03° 13.03'W	<3	<1	<1
15	Solent 1	50° 39.52'N	01° 41.38'W	<5	<1	3
16	Solent 2	50° 43.51'N	01° 29.42'W	2	1	7
17	Solent 3	50° 45.93'N	01° 20.79'W	<2	1	9
18	Solent 4 (Calshot spit)	50° 49.26'N	01° 18.56'W		2	11
19	Solent 5 (Hamble Mouth)	50° 50.94'N	01° 18.43'W		6	29
20	Solent 6	50° 45.50'N	01° 06.96'W	5	1	8
21	Solent 7	50° 40.78'N	01° 50.45'W	<2	1	4
22	Rye Bay	50° 39.52'N	01° 41.38'E	<1	<1	1
23	South Varne	50° 56.05'N	01° 16.76'E	<1	<1	<1
24	Rotterdam transect 1	51° 29.47'N	02° 55.06'E	<2	<1	7
25	Rotterdam transect 2	51° 58.08'N	03° 51.04'E	<2	<1	8
26	Rotterdam transect 3	52° 29.20'N	04° 23.24'E	<1	<1	10
27	Waddeneilanden	53° 25.95'N	03° 48.11'E	<1	1	<1
28	Rotterdam transect 4	52° 29.20'N	04° 23.24'E	<1	<1	<1
29	Rotterdam transect 5	53° 47.13'N	06° 11.99'E	<1	<1	6
30	Bremerhaven transect 1	54° 04.20'N	08° 07.06'E	<1	<1	<1
31	Bremerhaven transect 6	54° 43.86'N	05° 33.12'E	<1	<1	<1
32	North Tail Dogger	53° 30.79'N	04° 10.46'E	<1	<1	<1
33	West Dogger (NMP 285)	54° 49.86'N	01° 20.14'E	<1	<1	<1
34	North Farne	55° 38.25'N	01° 30.42'E	<1	<1	<1
35	Amble	55° 16.59'N	01° 15.12'E	<3	<1	<1
36	Mouth of Tyne	55° 01.44'N	01° 18.36'E	<1	<1	6
37	NMP295 Tees Bay	54° 42.87'N	00° 52.85'E	<2	<1	<1
38	Tees Mouth	54° 41.04'N	01° 07.97'E	<1	<1	<1
39	Off Humber	54° 02.08'N	01° 45.68'E	<1	<1	<1
40	Inner Wash NMP	53° 07.52'N	00° 31.67'E	<1	<1	<1
41	Humber NMP	53° 32.04'N	00° 35.09'E	<1	<1	<1
42	Off Lowestoft	52° 27.09'N	01° 45.03'E	<1	<1	<1

with detectable concentrations consistently present in areas such as the North Irish Sea, Solent and Rotterdam highway. Increased concentrations of diuron in the Solent are expected, since, as with Irgarol 1051, a large contribution of diuron is supplied from the high density of small boats in the area. The samples obtained from the stations termed 'Rotterdam Transect' were obtained from a transect taken along the busy shipping lanes off the Netherlands coast. These shipping lanes are some of the busiest in the world, with over 60% of North Sea shipping movements and around 100 ship movements a day taking place (ICONA, 1992; Davies *et al.*, 1998). These sampling stations represent locations where biocide inputs from the normal use of the compounds are likely to be at their highest. Even here, TBT and Irgarol concentrations were at or below the LOD for each method.

2.4 Conclusions

TBT, Irgarol 1051 and diuron are the only antifouling paint biocides currently used in sufficient quantities to be measured in the marine environment. As would be expected, samples collected from areas of high boating activity and low water exchange had the highest concentrations of biocides present. Biocide contamination in off-shore areas was low, with only diuron being detected at concentrations above the LOD of the analytical methods used. The survey demonstrates that after a 10 year ban on the use of TBT on vessels <25 m, TBT concentrations have been reduced in the UK aquatic environment. Additionally, the data provided on the level of organic booster biocide contamination will allow an assessment to be made on whether these compounds pose any environmental risk.

3. INVESTIGATIONS INTO THE OCCURRENCE OF POLYBROMINATED DIPHENYL ETHER (PBDE) RESIDUES IN SELECTED MATRICES

3.1 Introduction

Polybrominated diphenyl ethers (PBDEs) are extensively used as additive flame retardants in polymeric materials such as acrylonitrile butadiene (ABS) and high impact polystyrene (HIPS) and as such find many applications in both domestic and commercial situations (Hedemalm *et al*., 1995).

The use of flame retardants is increasing on a global scale. In 1992, 600,000 tonnes were consumed world-wide (OECD, 1994) with 150,000 tonnes of these being brominated compounds (WHO/IPCS, 1994) and of these, 40,000 tonnes were PBDEs, including 30,000 tonnes of the decabromodiphenyl ether (DBDE).

PBDE's have the general molecular formula $C_{12}H_{10-n}Br_nO$ where n = 1-10 giving 209 possible individual congeners. The systematic number system of Ballschmiter and Zell, used for describing the structure of PCBs, which also have 209 congeners, has been generally adopted for PBDEs. Commercial formulations are largely classified by the PBDE congener content, thus 'penta mixes' contain mainly tetra to penta congeners, 'octa mixes' contain mainly hexa to nona congeners and 'deca mixes' predominantly DBDE. Draft European Union (EU) risk assessments will recommend restrictions on the use of the penta and octa mixes, but it is thought that the production and use of the deca mix is likely to increase. Table 6 shows percentage composition of the technical mixes.

PBDEs can be considered to be environmentally stable and persistent compounds, being resistant to acids, bases, reducing and oxidising compounds. They are hydrophobic, lipophilic and have become ubiquitous environmental contaminants being reported in diverse environmental compartments and locations (Sellstrom *et al*., 1993; Jansson *et al*., 1987; Watanabe *et al*., 1987).

PBDEs have been manufactured in England and continue to be widely used. A previous pilot scale survey (Allchin *et al*., 1999) reported PBDEs quantified on a largely commercial formulation basis, but with some congener data, in samples of fish and shellfish as well as sediment. As a result of the widespread occurrence of PBDEs demonstrated by this survey, the Burnham Laboratory has expanded its research interests in these compounds and now has numerous studies underway. As well as investigating improvements in analytical methodology, CEFAS is conducting surveys designed to determine the distribution and fate of PBDEs in marine mammals, cormorants, sediments and fish and shellfish. The latter falls under the auspices of the NMMP for which CEFAS is conducting a special spatial survey. The full results of these programmes will be reported elsewhere in due course.

Earlier work reported PBDEs on a largely commercial formulation basis, however, more recently, a number of individual PBDE congeners have become commercially available and Marsh *et al*. (1999) reports the synthesis and characterisation of 32 PBDEs. The gas chromatographic identification and quantification of PBDEs in a commercial penta mix has also recently been described (Sjödin *et al*., 1997). CEFAS are currently using a mix of 14 individual PBDEs, which includes the dominant congeners found in the commercial penta mixes, plus the DBDE (as the commercial formulation Great Lakes DE-83R) to quantify residues. Table 7 identifies those PBDE congeners currently being studied at the Burnham Laboratory. Two congeners BDE#47 and 99 account for

Table 7. PBDE Congeners currently being studied at the CEFAS, Burnham Laboratory

PBDE number *	Structure
28#	2,4,4'
47#	2,2',4,4'
66#	2,3',4,4'
71	2,3',4',6
75	2,4,4',6
77	3,3',4,4'
85#	2,2',3,4,4'
99#	2,2',4,4',5
100#	2,2',4,4',6
119	2,3',4,4',6
138#	2,2',3,4,4',5'
153#	2,2',4,4',5,5'
154#	2,2',4,4',5,6'
190	2,3,3',4,4',5,6
209	2,2',3,3',4,4',5,5',6,6'

** Numbered according to Ballschmiter and Zell*
 # Present in commercial penta mixes

Table 6. Percentage BDE congeners present

Technical product	Tri-BDE's	Tetra-BDE's	Penta-BDE's	Hexa-BDE's	Hepta-BDE's	Octa-BDE's	Nona-BDE's	Deca-BDE
PentaBDE	0-1	24-38	50-62	4-8				
OctaBDE				10-12	43-44	31-55	9-11	
DecaBDE							0.3-3	97-98

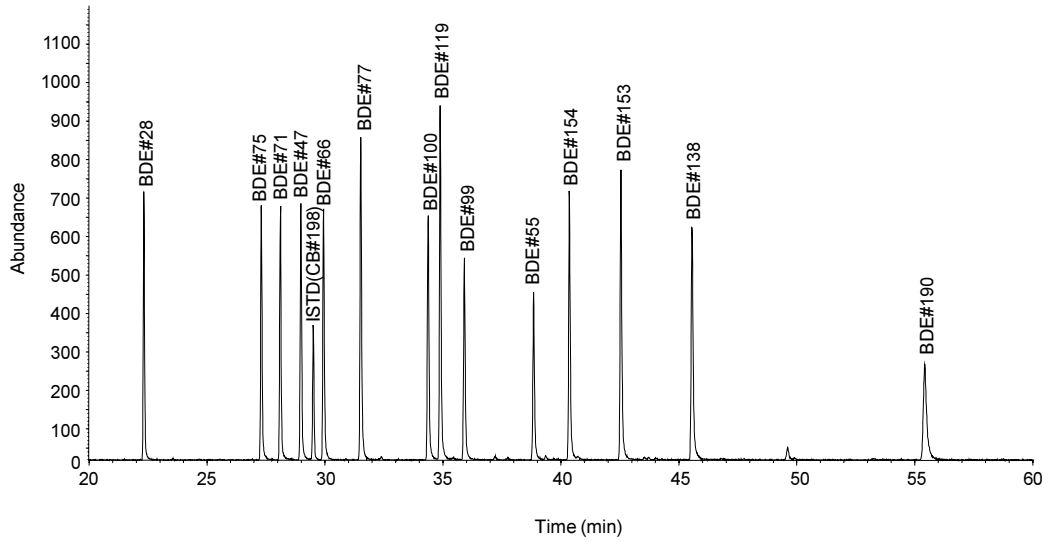

Figure 3. An example chromatogram of a PBDE standard mix

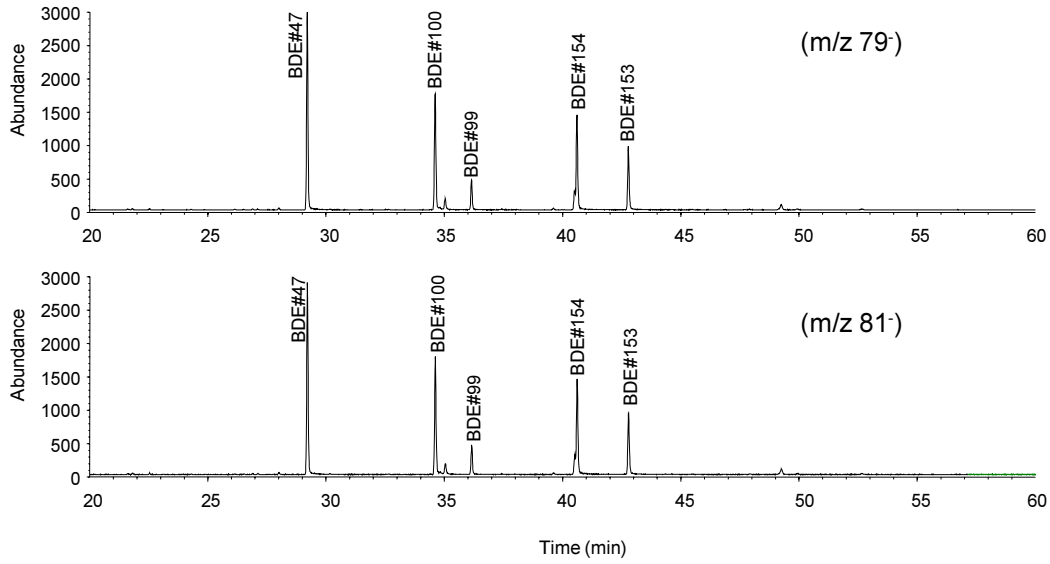

Figure 4. Cormorant liver BDE profile

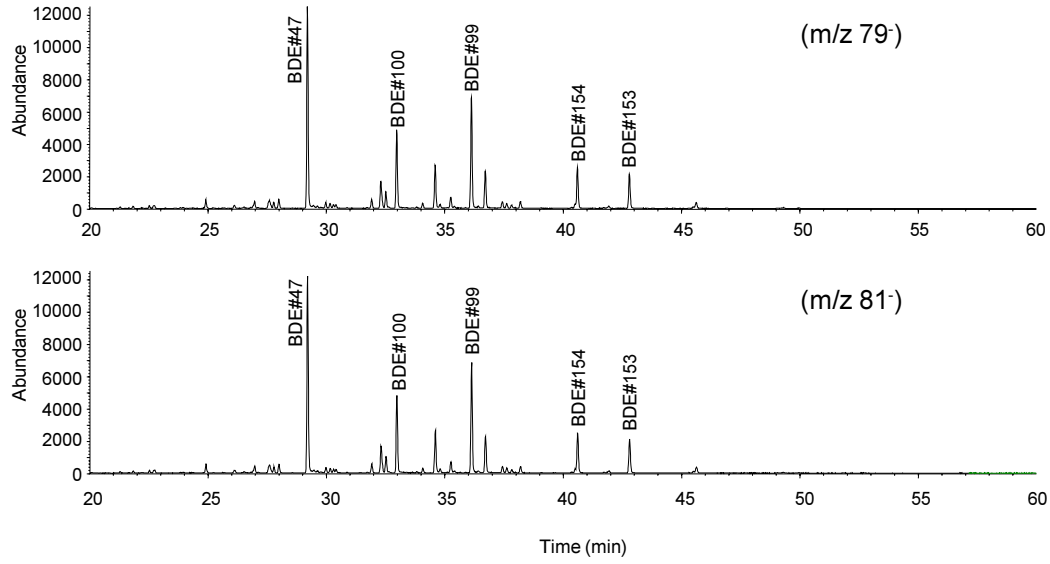

Figure 5. Harbour porpoise blubber BDE profile

35% and 37% respectively of the PBDE content of the penta mix Bromkal 70-5DE (Sjödin *et al.*, 1997).

3.2 Analytical methodology

PBDEs are amenable to similar analytical methodology to that employed for other organohalogens such as PCBs or DDT, although care should be exercised to optimise each stage of the process. As PBDEs are complex mixtures, particular care should be exercised in choosing a suitable analytical column to facilitate adequate resolution between congeners. Column dimensions of 50-60 m length and an internal diameter of 0.25 mm with a mid to low polarity stationary phase of around 0.25 µm have been found to be particularly useful.

Previous work has utilised electron capture detection. More recently, bench top mass spectrometers with either quadrupole or ion trap configurations have found widespread use, being used in both electron impact and chemical ionisation modes. CEFAS have found the use of negative ion chemical ionisation (NICI) with methane as a reagent gas monitoring the bromide ions at m/z 79^- and 81^-, to be a particularly robust, specific and sensitive method allowing quantitation in the low picogramme range of most PBDEs.

The high molecular weight and thermal instability of DBDE makes it's determination difficult and the use of shorter (15 m) analytical columns are preferred to avoid on-column degradation. Even so, the correct choice of stationary phase is important because even similar phases from different manufactures can lead to degradation. Also, because of its photo instability, the use of amber laboratory glassware is to be recommended.

Figure 3 shows an example chromatogram of a PBDE standard mix. Figures 4-5 show typical profiles from a cormorant liver and marine mammal (porpoise blubber).

4. WINTER 1998 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 a variety of symptoms which are defined in European Law and international agreements as 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.

The monitoring undertaken under the auspices of the UK NMMP has been to determine winter (January to March) nutrient (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.

4.2 Methods

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

4.2.1 Sampling

A suite of water samples was taken at NMMP sites in the coastal and offshore waters of England and Wales in January/February 1998. Discrete surface and depth samples were taken at each station from the *RV 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 detailed in the sampling and handling sections of the JAMP (Joint Assessment and Monitoring Programme) Eutrophication Monitoring Guidelines (OSPARCOM, 1997). At each site the spot samples reported here were collected within the 30 to 35 salinity range. The measured concentrations have not been normalised to salinity.

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 laboratory 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 NMMP station nutrient distribution

Figures 6a to 6d display the spatial distribution of nutrient concentrations around England and Wales for the NMMP sites sampled in January/February 1998. 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.

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.

The TOxN concentration is highest in the Severn Estuary (95.4 µmol l^{-1}) and in the Thames, Wash and Liverpool bay areas, 89.9, 23.7 and 26.9 µmol l^{-1} respectively (Figure 6a). The Humber and the area off

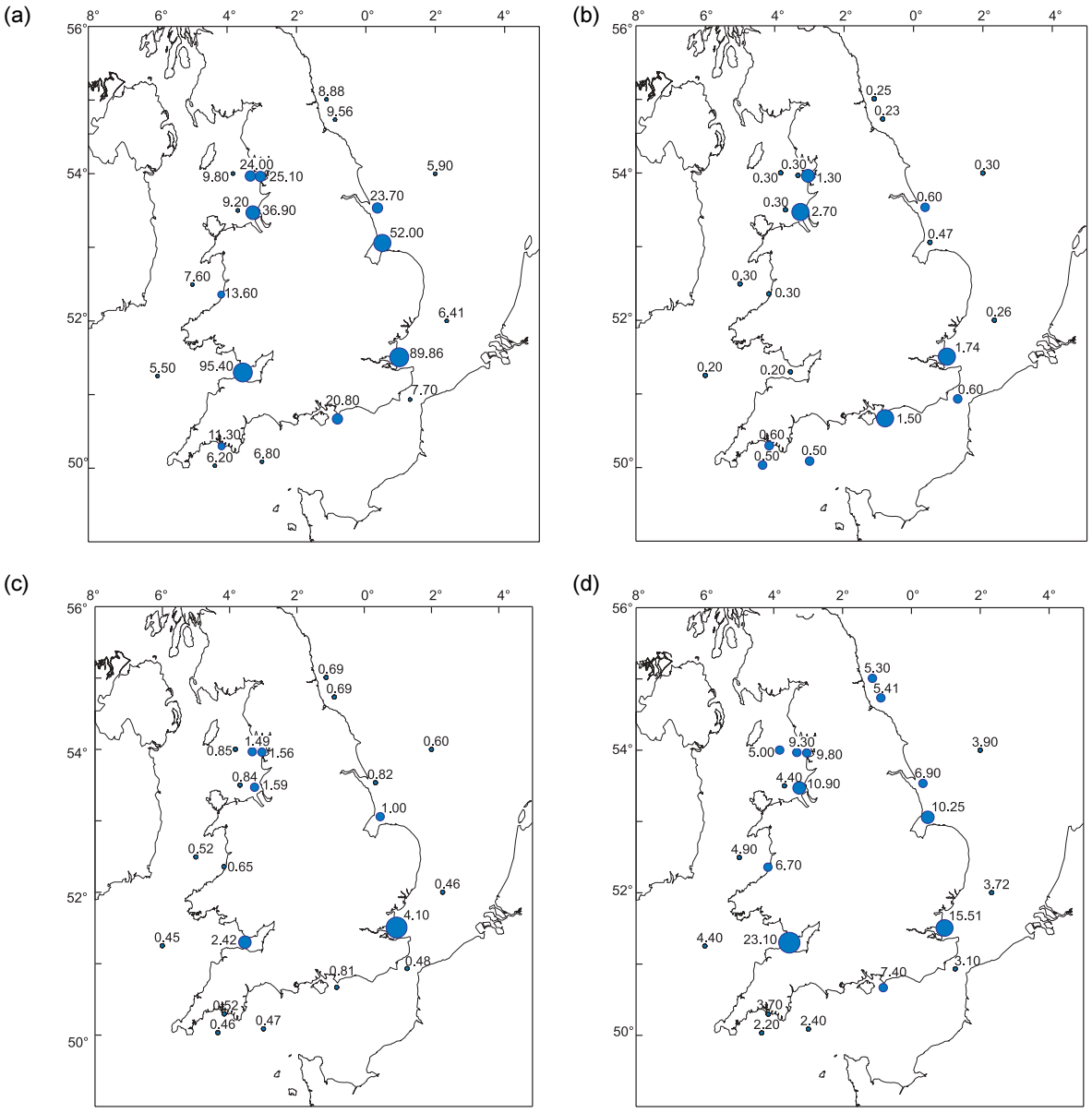

Figure 6. Surface concentrations (µmol l^{-1}) at National Monitoring Plan stations in January/February 1998: (a) TOxN, (b) ammonia, (c) phosphate, (d) silicate

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 the offshore NMMP sites (Figure 6a). There is an emerging understanding that winter nutrient behaviour in the shelf seas is more dynamic than previously thought. It is important to understand these dynamics if winter values in the offshore waters are used as the baseline for seasonal changes in nitrogen and other nutrient concentrations.

Relatively high nitrite concentrations (2 to 5% of TOxN) are found in Liverpool Bay and at the Selsey Bill site. The use of nitrite concentration as an indicator of disturbance to the nitrogen cycle is being investigated.

Ammonium

The concentrations of ammonium are low (<1.0 µmol l^{-1}) in most estuarine, coastal and especially, offshore waters. However, there are several areas (Liverpool Bay, Thames, Solent and off Lune/Wyre) where surface water concentrations are obviously higher than at other sites (Figure 6b). 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 dependent on a range of factors and has varied at these sites year on year.

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

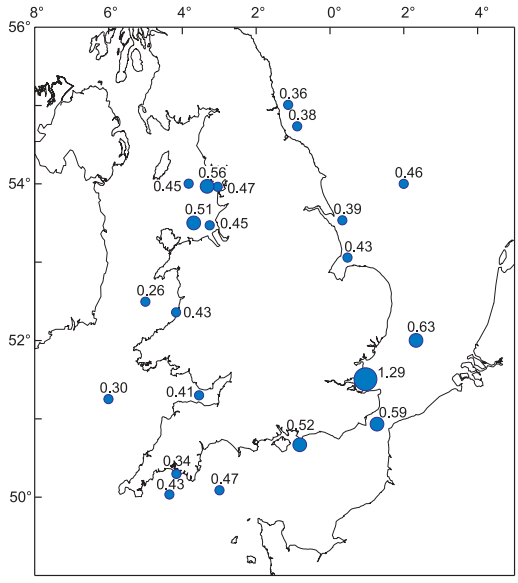

Figure 7. Surface concentrations of chlorophyll (µg l^{-1}) at National Monitoring Plan stations in January/February 1998

Phosphate

The spatial distribution of phosphate concentrations largely follow those of TOxN. The Thames and Severn areas have the highest concentrations (Figure 6c). The exception is the Humber site which shows much lower phosphate concentrations in comparison to nitrate. As with the other nutrients there is a decline in phosphate concentrations away from the input.

Silicate

The spatial distribution of silicate concentration indicates that concentrations are higher off the Severn, Thames, Wash and Liverpool Bay (Figure 6d). All the other sample concentrations lie below 10 µmol l^{-1} and as with the other major nutrients, silicate concentrations decrease offshore. 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 can be used as an indication of phytoplankton biomass. In January there are uniformly low levels (<1.0 µg l^{-1}) of chlorophyll in the coastal waters of England and Wales. Under ambient winter conditions, the temperature and light levels are expected to be sufficiently low to prevent significant primary production (Figure 7) and the development of phytoplankton biomass. Higher concentrations of chlorophyll are present in the Thames. These higher concentrations of chlorophyll occur in the areas of higher ammonium concentration, which has been identified as a preferential nutrient for phytoplankton.

Dissolved oxygen

Dissolved oxygen (DO) measurements were taken at depth in the water column. The range of DO concentrations measured during this survey ranged from 6 to 7 ml l^{-1}. This high level of DO 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 January/ February 1998 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.

- 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.

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 the specific location. Nutrient concentrations are therefore measured 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.

At most of the sites the level of phytoplankton biomass appears to be very low, which is the expected winter situation. This information will be used as a yardstick for comparison with the spring and summer situation which will be presented in a separate report.

BIOTA

5. PESTICIDE RESIDUES AND PCBS IN BIVALVE MOLLUSCS FROM DESIGNATED HARVESTING AREAS AROUND ENGLAND AND WALES AND THEIR SIGNIFICANCE TO HUMAN HEALTH

5.1 Introduction

In 1992, the EU Shellfish Hygiene Directive 91/492/EEC (European Communities, 1991a) came into force in England and Wales and was implemented under the Food Safety (Live Bivalve Mollusc and Other Shellfish) Regulations, 1992. This was replaced by The Food Safety (Fishery Products and Live Shellfish) (Hygiene) Regulations, 1998. The Directive lays down health conditions for the production and placing on the market of live bivalve molluscs for human consumption. Bivalve molluscs sold commercially must originate only from production areas designated under the Directive and these areas are classified according to the extent of *E.coli* contamination in the shellfish, which determines their conditions of sale.

In addition, shellfish must comply with other parameters defined in the Directive end product standard which include not containing toxic or objectionable compounds such as trace metals, organochlorine compounds, hydrocarbons and polyaromatic 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 that would help the UK to meet its commitments under the EU legislation. A summary of the first year of this study was reported in CEFAS, 2000. A summary of the second year of the study is given below. Full details can be found in Jones *et al*. (1999a).

5.2 Materials and methods

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 individual oysters, and 50 individual 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 the empty shell weight recorded. Twenty-gramme 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 pesticide residues and a suite of 25 individual CBs, using gas liquid chromatography. Full details of the method of analysis can be found in Allchin *et al*. (1989).

5.3 Results and discussion

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

5.3.1 Pesticide residues

Pesticides are widely used in agriculture and have, in the past, also been used in a variety of industries. Dichlorodiphenyltrichloroethane (DDT) for instance was widely used as an insecticide from the 1940s, until the 1970s. It was also used extensively, along with dieldrin in woollen mills and by dry cleaners for mothproofing. Lindane has been used as a broad-spectrum insecticide since the early 1950s for purposes that include treatment of seeds and soil, application on trees, timber and stored materials, treatment of animals against ectoparasites and in public health (WHO, 1991). Toxic doses of these compounds can cause chloracne, blindness and oedema. DDT can also effect the nervous system.

As party to the International Conference on the Protection of the North Sea, the UK is committed to reducing by the year 2000, discharges and emissions of substances such as pesticide residues, which are toxic and persistent and liable to bioaccumulate, to levels close to zero, with the aim of their eventual elimination. Since the 1960s, the use of pesticides in the UK has been subject to either voluntary control or national legislation and since 1986, pesticide use has been tightly controlled under the Control of Pesticides Regulations. Approvals for many pesticides have been withdrawn, or limited to a few specific cases for which effective alternatives are not available. The sale and use of DDT was banned in the 1970s.

Pesticide residues mainly enter the marine environment via agricultural run-off and industrial waste discharges. Although the use of many of these chemicals is no longer permitted, their persistence in the environment means that they can still accumulate in fish and shellfish tissue to significant concentrations. Pesticide residues and their metabolites are stored in lipid rich tissues. Many organisms have an annual cycle in lipid storage and utilisation and this may produce a related annual cyclic pattern in the concentrations of these compounds within organisms.

HCB *(Hexachlorobenzene)*
Concentrations of HCB in all of the samples were at or below the LOD, 0.001 mg kg^{-1} wet weight.

α-HCH *(Alpha isomer of hexachlorocyclohexane)*
Concentrations in cockles, Native oysters and Pacific oysters were all at or below the detection limit of 0.001 mg kg^{-1} wet weight. Concentrations in mussels ranged between <0.001 and 0.003 mg kg^{-1} wet weight, although the majority of the results were below the detection limit.

γ-HCH *(Gamma isomer of hexachlorocyclohexane (Lindane))*
γ-HCH is the primary constituent, at not less than 99%, of the insecticide Lindane (WHO, 1991). Again, concentrations in the majority of samples were at or below the detection limit of 0.001 mg kg^{-1} wet weight. Positive values were recorded in only a handful of samples with maximum concentrations reaching only 0.003 mg kg^{-1} wet weight.

Dieldrin
Concentrations of dieldrin in all but one sample of cockles were at or below the LOD, 0.001 mg kg^{-1} wet weight. Most of the sites where positive values were recorded were located in the Thames Estuary. The Humber yacht club and the Fal were the only other sites outside the Thames Estuary that had values above the detection limit, at 0.001 and 0.004 mg kg^{-1} wet weight, respectively. Concentrations of dieldrin in mussels and oysters ranged between <0.001 and 0.007 mg kg^{-1} wet weight. Highest concentrations were found in samples from the Thames Estuary and Southampton Water.

DDT
The term DDT is generally understood throughout the world and refers to *p,p'*-DDT. The term is also applied to commercial products consisting predominantly of *p,p'*-DDT with smaller amounts of other compounds (WHO, 1989). Different organisms metabolise DDT via different pathways. The two major metabolites are dichlorodiethylene (DDE) and dichlorodiethane (TDE). DDE is the more persistent, but not all organisms produce DDE from DDT. The alternative route of metabolism via TDE leads to more rapid elimination (WHO, 1979).

p,p'-DDT
Concentrations in all but one sample of cockles were at or below the LOD, 0.001 mg kg^{-1} wet weight, the exception being the sample from the Fal with a concentration of 0.002 mg kg^{-1} wet weight. Concentrations in mussels and oysters were between <0.001 and 0.003 mg kg^{-1} wet weight.

p,p'-DDE
Concentrations in cockles were at or below the LOD, 0.001 mg kg^{-1} wet weight, with the exception, again, of the sample from the Fal with 0.002 mg kg^{-1} wet weight. Concentrations in mussels and oysters ranged between <0.001 and 0.006 mg kg^{-1} wet weight. Highest concentrations were found in mussels from Blackledge (River Roach).

p,p'-TDE

Concentrations in cockles were at or below the LOD, 0.001 mg kg^{-1} wet weight, except for the Fal, at 0.002 mg kg^{-1} wet weight. Concentrations in mussels were between <0.001 and 0.033 mg kg^{-1}, the maximum was found in the sample from Wonderland (Humber Estuary). However, the mean concentration in mussels (for samples with concentrations above detection limits) was 0.003 mg kg^{-1} wet weight, an order of magnitude less than the maximum concentration found. Concentrations in Native and Pacific oysters ranged between <0.001 and 0.005 mg kg^{-1} wet weight.

ΣDDT

The highest concentration of ΣDDT recorded was 0.038 mg kg^{-1} wet weight (< values were taken as being equal to the LOD so these values may be slightly high).

5.3.2 Polychlorinated biphenyls (PCBs)

PCBs were industrially manufactured and widely used in the electrical industry. With four or more substituted chorines per molecule, biphenyls become non-flammable with high electrical resistance, withstanding temperatures of up to 700°C. Formulations such as Aroclor, Phenclor and Kaneclor were produced for use in capacitors and transformers, gas turbines, vacuum pumps, adhesives and textiles. Although PCBs are of low acute toxicity, effects are cumulative with prolonged exposure and can result in effects on the skin and liver. In addition, there is evidence to suggest that PCBs may be carcinogenic (WHO, 1992), though this is inconclusive.

In 1976, the estimated cumulative world production of PCBs, since manufacture began in 1930, was in the order of 1 million tonnes (WHO, 1976). More than one-half had entered dumps and landfills, whilst much of the remainder had entered the environment by the disposal of industrial wastes into rivers and coastal waters, by leakage from non-enclosed systems, or by volatilisation into the atmosphere from incineration. The ultimate reservoirs of PCBs in the environment are mainly sediments of rivers and coastal waters.

Manufacture of PCBs ceased in the UK in 1977 and their manufacture and general use is no longer permitted under the Control of Pollution (Supply and Use of Injurious Substances) Regulations 1986 (S.I. 1986 No. 902), as amended. Continued concern regarding the effects of PCBs on sea mammals led to agreement by the Third North Sea Conference, in 1990, to phase out and destroy any remaining identifiable PCBs by the end of 1999. Council Directive 96/59/EEC on the controlled disposal of PCBs and equipment containing PCBs was adopted on the 16 September 1996 and the UK action plan for the phasing out and destruction of PCBs to meet this commitment was published on the 21 March 1997 (DOE, 1997). Under the Environmental Protection (Disposal of PCBs and other dangerous Substances) (England and Wales) Regulations 2000 (S.I. 2000 No. 1043), which came into force on the 4 May 2000, the holding of PCBs and equipment contaminated by PCBs will no longer be legal after the 31 December 2000, except for purposes concerned with the decontamination or disposal of PCBs or for the purposes of analysis or research into the effects of PCBs.

PCBs are resistant to degradation by normal processes and therefore continue to be widely present in the environment and food albeit at very low levels.

As with pesticides residues, PCBs accumulate in lipid-rich tissues and concentrations in some organisms are therefore also likely to be subject to an annual cyclic pattern.

Chlorinated biphenyls

Concentrations of individual CBs in cockles were, generally, at or below the LOD of 0.001 mg kg^{-1} wet weight. The highest value of 0.006 mg kg^{-1} wet weight, for CB#153, was found in the sample from the Fal. Concentrations of individual CBs in mussels and Native oysters were mainly below the detection limit, with maximum concentrations of 0.008 mg kg^{-1} wet weight (Thames estuary and Plymouth) and 0.011 mg kg^{-1} wet weight (Plymouth), respectively, for CB#110. In Pacific oysters, individual CB concentrations ranged from <0.001 to 0.004 mg kg^{-1} wet weight.

Concentrations of Σ25CBs (Figure 8) (< values taken as equal to the LOD, these figures will therefore be an over estimate) were generally low in cockles with maximum concentrations of 0.045 mg kg^{-1} wet weight. In mussels, concentrations ranged from 0.025 to 0.064 mg kg^{-1} wet weight, with relatively high concentrations in areas such as Plymouth, Taw/Torridge, Liverpool and Morecambe Bays and the Thames Estuary. Concentrations recorded in Pacific and Native oysters were up to 0.035 and 0.07 mg kg^{-1} wet weight respectively, with relatively high concentrations found off the North Kent coast, Chichester, Langstone and Portsmouth Harbours, Plymouth, the Fal and the Thames Estuaries and the Rivers Exe and Yealm.

There are no internationally agreed safety limits for the consumption of CBs. The strictest guideline for PCBs in molluscs is in Norway/Sweden at 2 mg kg^{-1} wet weight (CB congeners not specified) (OSPAR, 1990). The maximum concentration for Σ25CBs in this survey was 0.07 mg kg^{-1} wet weight, well below this guideline value.

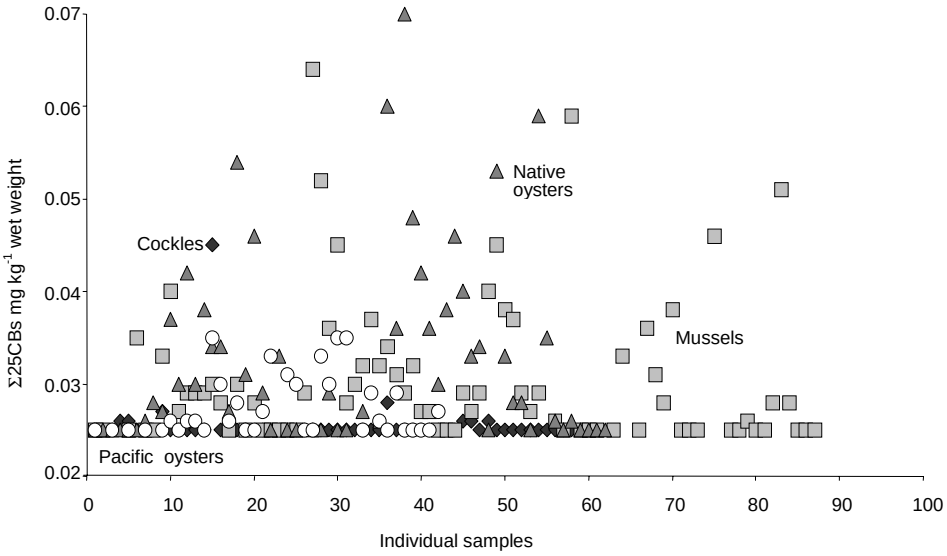

Figure 8. Total chlorinated biphenyls (Σ25 CBs) in shellfish from designated bivalve production areas around England and Wales

5.4 Conclusions

The concentrations of pesticide residues found in this survey were low in all the samples, with maximum concentrations of 0.007 mg kg^{-1} (excluding the value for TDE in mussels from the Humber at 0.033 mg kg^{-1}) and were similar to those reported in MAFF (1993). Jones *et al*. (1999a) calculated estimated dietary intakes of pesticide residues, from molluscs alone, for high level consumers of molluscs, all of which represented less than 2% of established Acceptable Daily Intakes (ADIs)/ Provisional Tolerable Daily Intakes (PTDIs).

Concentrations of CBs were also generally low and again similar to those previously reported in MAFF (1993). The highest concentration of Σ25CBs recorded in this survey was well below the strictest guideline value in existence for PCBs in molluscs.

Several studies have noted that concentrations of some chemical contaminants in bivalve molluscs tend to show a seasonal variation, which may be influenced by a number of factors that govern the uptake of contaminants in marine organisms (Jones *et al.,* 1998; 1999b). Pesticide residues and PCBs are lipophilic and as with many other organisms, bivalves have an annual cycle in lipid storage and utilisation. Spawning activity, for instance, appears to be a major factor in the variation of polyaromatic hydrocarbon (PAH) concentrations which are also lipophilic (Jones *et al.,* 1999b). PAH concentrations can decrease by up to 80% as lipid stores are mobilised into the gametes and then removed on spawning. It is probable therefore, that concentrations of pesticide residues and PCBs may fluctuate in a manner similar to PAHs. However, this is likely to be of little consequence to consumers, since the levels of pesticide residues and PCBs in this survey were low and a significant number of the samples were collected when lipophilic contaminants were likely to be at their highest, particularly in mussels and cockles.

In summary, the results from this survey indicate that the concentrations of pesticide residues and PCBs in bivalve molluscs from designated harvesting areas around the UK are low and even extreme consumption of molluscs is unlikely to cause calculated dietary intakes to exceed ADIs.

6. THE CONCENTRATIONS OF MERCURY IN FISH TAKEN FROM LIVERPOOL BAY AND MORECAMBE BAY IN 1998

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 EQS. This 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

Table 8. Mercury concentrations found in fish muscle in 1998 (OSPARCOM 'upper' level category; >0.30 mg kg^{-1} wet weight) [EC maximum limit; 0.50 mg kg^{-1} wet weight]

Area	Species	Number of fish analysed	Mean length (cm)	Mean mercury concentration in fish muscle (mg kg^{-1} wet weight)
Liverpool Bay	Cod	10	30.4	0.10
	Whiting	40	28.6	0.30
	Dab	45	22.9	0.17
	Flounder	2	28.0	0.10
	Plaice	25	27.1	0.14
	Sole	50	24.3	0.16
			Mean, all fish	0.16
Morecambe Bay	Cod	2	29.3	0.04
	Whiting	50	32.6	0.29
	Dab	47	25.6	0.17
	Flounder	55	31.4	0.20
	Plaice	25	27.1	0.09
	Sole	40	25.9	0.15
			Mean, all fish	0.16

elevated to a level potentially exceeding 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, carried out in 1998, are summarised in Table 8. 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 in fish from both areas was 0.16 mg kg^{-1}, so reassurance can continue to be given that the mercury EQS will not be breached.

Table 9. Time series of mean concentrations of mercury in fish flesh

Year	Concentration of mercury in mg kg^{-1} wet weight	
	Liverpool Bay	Morecambe Bay
1982/83	0.27	0.29
1984	0.31	0.27
1985	0.24	0.20
1986	0.24	0.24
1987	0.23	0.23
1988	0.22	0.23
1989	0.20	0.19
1990	0.19	0.20
1992	0.20	0.14
1994	0.17	0.18
1996	0.17	0.17
1998	0.16	0.16

Although not collected for time trend purposes (for which a separate study has been taking place), the series of results obtained from Liverpool and Morecambe Bays over the 1982-1998 period (Table 9) gives some indication of the reduction in mercury concentrations in fish taken from these areas over the last 16 years.

7. BUTYLTIN COMPOUNDS IN PELAGIC CETACEANS

In the previous report in this series, data were reported for butyltin compounds in liver tissues from seals and porpoises inhabiting the coastal waters of England and Wales (Law *et al.,* 1998). Concentrations of tributyltin (TBT), dibutyltin (DBT) and monobutyltin (MBT) were determined in the livers of stranded or bycaught grey seals *Halichoerus grypus* and harbour porpoises *Phocoena phocoena.* Summed concentrations of these three compounds ranged up to 640 µg kg^{-1} wet weight, with lower concentrations being found in seals than porpoises. These data indicated that butyltin contamination is widespread in marine mammals around England and Wales, and taken with other data worldwide, suggest that these compounds may now be ubiquitous in coastal areas frequented by shipping. In order to assess the extent of butyltin contamination in other marine mammal species, the earlier study has been extended to pelagic marine mammal species feeding over the outer areas of the continental shelf and the continental slope, and in deep ocean waters. These data have been reported in the literature (Law *et al*., 1999) and are summarised here.

Concentrations of butyltins were determined in the liver tissues of 16 marine mammals, including two mysticete and ten odontocete species. Liver is the preferred tissue for analysis, as it exhibits the highest concentrations of butyltins in marine mammals. The animals studied were stranded on the coasts of England and Wales, and tissue samples were collected within the marine mammal strandings programme funded by the Department of Environment, Transport and the Regions (DETR), and operated by the Institute of Zoology and the Natural History Museum. The aim was to analyse samples from as wide a range of pelagic species as possible, with priority being given to adults, and to animals which were classified as freshly dead or only slightly decomposed when examined after stranding. The animals sampled included 2 Risso's dolphins (*Grampus griseus*), 2 white-beaked dolphins (*Lagenorhyncus albirostris*), 2 common dolphins (*Delphinus delphis*), 2 striped dolphins (*Stenella coeruleoalba*), and 1 specimen each of the long-finned pilot whale (*Globicephala melas*), white-sided dolphin (*Lagenorhyncus acutus*), pygmy sperm whale (*Kogia breviceps*), Sowerby's beaked whale (*Mesoplodon bidens*), Blainville's beaked whale (*Mesoplodon densirostris*), Northern bottlenose whale (*Hyperoodon ampullatus*), the fin whale (*Balaenoptera physalus*), and the minke whale (*Balaenoptera acutorostrata*). Both of the mysticete species (fin and minke whales) are essentially open ocean animals which feed upon euphausiids (mainly *Meganyctiphanes norvegica* in the North Atlantic) and some fish. All of the odontocete species feed primarily in deep waters over continental shelves and slopes, or in oceanic waters, mostly on cephalopods and a variety of fish species (Evans, 1987; Martin, 1990). The animals were stranded between February 1992 and November 1998.

The results of analyses are given in Table 10. DBT was detected in all samples, TBT was detected in 9 and MBT in 3 of the 16 samples analysed. The summed concentrations of butyltins in liver ranged from 19 to 312 μg kg^{-1} wet weight. This can be compared with the concentrations in porpoise and grey seal livers from coastal waters around England and Wales, which ranged from 22-640 and <12 to 22 μg kg^{-1} wet weight, respectively (Law *et al*., 1998). In those samples about 20% of the total butyltin burden in liver was TBT, with DBT the major component. In the pelagic mammals analysed in the current study, the proportions were similar, with DBT averaging 77% (range 39-100%) of the total butyltin concentrations.

Data on organotin concentrations in marine mammals are relatively scarce, and those relating to pelagic animals even more so. No data have previously been published for animals from the Atlantic Ocean, for direct comparison with the present study. Worldwide, data have been reported for toothed whales of nine species and a single baleen whale, a minke whale from the Antarctic. Butyltin compounds could not be detected in the minke whale, but that animal aside, the overall concentration range reported was similar to that found in the present study. Thus, summed butyltin concentrations (ΣBT) in liver of the various species ranged from 2.0 to 400 μg kg^{-1} wet weight, and once again DBT predominated. The fact that butyltin residues could be detected in a minke whale from the NE Atlantic but not from the Antarctic, presumably indicates that significant butyltin contamination has not spread as yet to the most remote waters of the world's oceans.

Table 10. Concentrations of butyltin compounds in the livers of pelagic cetaceans (μg kg^{-1} wet weight). Data for ΣBTs calculated using zero for undetected values

Reference no.	Species*	Sex	Length (cm)	Age (yrs)	Date	Location	TBT	DBT	MBT	ΣBTs
SW1994/5	WSD	M	228	9	19/01/94	Rhossili Bay, West Glamorgan	< 3	29	< 3	29
SW1995/145	WBD	F	257	> 15	31/12/95	Sizewell, Suffolk	38	101	< 3	139
SW1998/154	WBD	F	215	5	06/08/98	Blyth, Northumberland	36	134	< 4	170
SW1996/40	SD	M	219	20	13/02/96	Greatstone-on-Sea, Kent	77	84	< 3	161
SW1996/121	SD	M	219	17	16/07/96	Ramsey Island, Pembrokeshire	82	230	< 4	312
SW1998/104	CD	F	208	12	10/06/98	Aberaeron, Ceredigion	68	195	< 4	263
SW1998/148	CD	F	209	24	05/08/98	Saundersfoot, Pembrokeshire	53	132	< 4	185
SW1992/213	RD	F	262	3	26/11/92	Borth, Ceredigion	19	62	< 3	81
SW1994/39	RD	M	207	nk	18/03/94	Fishing Cove, Gunwalloe, Cornwall	33	26	7	66
SW1997/162	PW	M	502	nk	25/10/97	Beadnell, Northumberland	< 3	19	3	22
SW1992/13	FW	F	1660	nk	04/02/92	Dane's Dyke, East Yorkshire	< 3	19	< 3	19
SW1996/162	MW	F	467	nk	29/10/96	Purfleet, Essex	< 4	56	< 4	56
SW1997/159	PSW	F	276	nk	17/10/97	Manorbier, Pembrokeshire	< 4	50	35	85
SW1998/81	SBW	M	444	nk †	30/04/98	Mablethorpe, Lincolnshire	24	34	< 4	58
SW1993/78	BBW	F	411	> 21	18/07/93	Aberaeron, Ceredigion	< 6	33	< 5	33
SW1998/189	NBW	F	610	nk	06/11/98	West Kirby, Wirral	< 3	28	< 3	28

† *the teeth of this animal were very worn and could not be used for age determination*

* *WSD, white-sided dolphin; WBD, white-beaked dolphin; SD, striped dolphin; CD, common dolphin; RD, Risso's dolphin; PW, long-finned pilot whale; FW, fin whale; MW, minke whale; PSW, pygmy sperm whale; SBW, Sowerby's beaked whale; BBW, Blainville's beaked whale; NBW, Northern bottlenose whale*

nk age not known

These data indicate the widespread distribution of butyltin residues in deep offshore waters and the oceanic food chains of both mysticetes and odontocetes. The impact of this contamination by tributyltin compounds is, however, harder to assess. Further studies are needed in order to elucidate the potential impact of butyltin body burdens on individual marine mammals and populations, if TBT inputs continue. In particular, the possible impairment of immune function and consequent vulnerability to disease should be investigated, as these compounds may act additively with other contaminants such as organochlorine pesticides and chlorobiphenyls to which immuno-suppressive properties have been ascribed (Ross *et al.*, 1996).

8. POTENTIAL LINKS BETWEEN CONTAMINANT BURDENS AND INFECTIOUS DISEASE MORTALITY IN HARBOUR PORPOISES STRANDED AROUND ENGLAND AND WALES

The question of whether environmental pollution is affecting marine mammal populations is unresolved, although there is evidence that exposure to toxic contaminants can cause immunosuppression and disease. Two recent studies (Bennett *et al*., in press; Jepson *et al*., in press) tested the hypothesis that increased contaminant levels in the tissues of harbour porpoises *Phocoena phocoena* may predispose them to infection. The porpoises were derived from the marine mammal strandings programme funded by the Department of Environment, Transport and the Regions (DETR), and operated by the Institute of Zoology and the Natural History Museum in England and Wales. Contaminant analyses in the tissues are conducted by CEFAS. Animals selected for analysis were stranded between 1990-96, and were either fresh or only slightly decomposed. Detailed pathological investigations were conducted according to standard *post-mortem* protocols (Law, 1994). For statistical treatment the data available were divided into two groups based on the assigned cause of death. Animals diagnosed to have died due to physical trauma, by-catch or dystocia were included in the physical trauma category, whilst those diagnosed to have died due to disease processes caused by one or more infectious agents were included in the infectious disease category.

Metals

Mean liver concentrations of mercury, selenium, zinc, and the mercury:selenium molar ratio, were significantly higher in the porpoises that died of infectious disease (n = 49) than in those that died from physical trauma (n = 37). Elevated zinc levels are known to occur in diseased animals and probably represent a response to disease rather than a cause, as zinc is an essential trace element, and necessary to maintain the integrity of the immune system. Liver concentrations of chromium, cadmium, copper and lead did not differ between the two groups. The mercury:selenium ratio reflects the detoxification process within marine mammals whereby toxic methylmercury ingested from the diet is converted to mercuric selenide, an inert compound which is deposited in the liver (Law, 1996). Methylmercury could play a significant role, as it is both more toxic and more readily bioaccumulated than inorganic mercury, and is also known to impair both the primary and secondary immune response. Further work is required however to evaluate whether chronic exposure to mercury in this form may have presented a toxic challenge to the porpoises that succumbed to infectious diseases.

Chlorinated biphenyls

Blubber concentrations of 25 individual CB congeners were significantly higher in the infectious disease group (n = 33) than in the physical trauma group (n = 34). The mean summed CB concentrations were 31.1 mg kg^{-1} in the former group, and 13.6 mg kg^{-1} in the latter group (both expressed on a lipid basis). The statistical association between high blubber CB concentrations and mortality due to infectious disease was not confounded by any other measured variables which could affect CB concentrations, including age, sex and nutritional status. The immunosuppressive nature of CBs has been well documented, suggesting that the association observed in this study is indicative of a causal relationship.

Further studies

Additional studies are underway and will seek to utilise larger datasets in order to test more robustly for potential relationships between organochlorines and organobromine compounds (such as the flame retardant compounds), metals, and the health status of harbour porpoises. The possibility of additive or synergistic effects between these various contaminant groups must also be considered in future.

9. FISH PATHOLOGY AND DISEASE BIOMARKERS 1998

9.1 Introduction

The use of fish as target organisms in environmental monitoring programmes is well established and a number of International Council for the Exploration of the Sea (ICES) member countries incorporate measurements of externally visible fish diseases as indicators of environmental stress at the population level (Bucke *et al*., 1996; ICES, 1997). The main conditions used for monitoring purposes are acute and healing ulcerations, lymphocystis, epidermal hyperplasia/papilloma and hyperpigmentation. Internally, the presence of hepatic

lesions comprising of nodules and larger tumours have also become an important component of disease assessments in the target fish species for monitoring, namely the dab (*Limanda limanda* L.) and flounder (*Platichthys flesus* L.). The aetiology of several diseases regularly recorded is known. Ulcerations are likely to result from a variety of causes, including physical trauma and subsequent infection with bacteria and other opportunistic organisms. Lymphocystis disease is caused by an iridovirus. Infected cells become greatly hypertrophied, forming clusters of nodules on the surface of the fish (Bucke *et al*., 1983). Occasionally, lymphocystis nodules occur on the gills and in internal organs. Epidermal hyperplasia and papilloma has been recorded from many fish species from contaminated and relatively clean environments (Cross, 1986; Baumann *et al*., 1987; Hayes *et al*., 1990; Bowser *et al*., 1991; Poulet *et al*., 1984). It is not yet clear whether infectious agents are involved in the development of epidermal papillomas, but viral particles have been recorded from these lesions in some fish species (see Baumann, 1992 and Grizzle and Goodwin, 1998).

There are clearly difficulties in attempting to understand the myriad of factors, including both biological and environmental variables that influence disease prevalence. However, long-term monitoring can 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).

In addition to the monitoring of externally visible disease conditions, an increasingly significant component of this work involves the histological assessment of liver pathology in dab and flounder. The liver is the main organ for the detoxification of xenobiotics and several categories of toxicopathic hepatocellular lesion have been identified in a variety of fish species (Myers *et al.*, 1987, 1991, 1992, 1998; Varanasi *et al*., 1987; Stein *et al*., 1990, 1992; Moore and Myers, 1994; Vethaak *et al*., 1996). Guidelines for the methodology for sampling, diagnostic criteria and reporting results for liver pathology assessments have been developed through ICES activities. Since toxicopathic lesions, in particular neoplastic and pre-neoplastic lesions are increasingly regarded as end points of contaminant exposure (Myers *et al*., 1991, 1994, 1998; Bucke and Feist, 1993, Vethaak *et al*., 1996), their presence in wild fish populations is significant. Current monitoring programmes also incorporate 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 with samples also taken for complimentary chemical analysis.

The disease monitoring programme also seeks to detect the presence of emerging disease conditions in the target species that may have significance for monitoring purposes. In addition, the disease status of commercial fish species is also monitored to provide warning of disease epizootics which may be a significant threat to specific fish populations (e.g. *Ichthyophonus* in North Sea herring) or render fish unsightly and likely to be rejected for human consumption.

9.2 Materials and methods

A single dedicated cruise for monitoring fish diseases was conducted (*RV CORYSTES* cruise 1/98, 6-22 January, 1998; for sites see Figure 9). Additional disease data and samples for histopathological studies were collected during a second, integrated cruise (*RV CIROLANA* 3b/98, 19 June to 8 July; for sites see Figure 9). A total of fourteen areas were investigated including stations in the North Sea off the north-east coast (Amble), Humber and Flamborough Off Grounds, the western Dogger Bank and Rye Bay in the eastern English Channel. Irish Sea stations included Liverpool Bay, Red Wharf Bay and Cardigan Bay. Stations along a transect from Bremerhaven (not included in Figure 9) to the north-eastern Dogger Bank were also visited during the integrated survey to improve the interpretation of UK monitoring data and where necessary, sampling sites were adjusted to provide greater correlation with NMMP

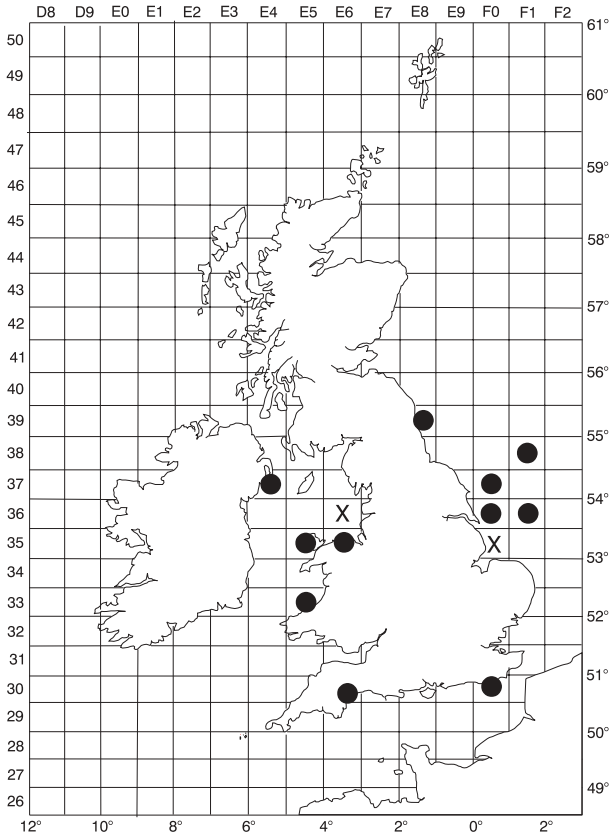

Figure 9. Areas sampled for fish disease monitoring (by ICES rectangle)
● Area sampled
X Insufficient data

stations. Stations at the Wash and Morecambe Bay did not provide a viable catch of fish for disease monitoring purposes. Standard one-hour tows were made with a Granton trawl fitted with a tickler chain and liner.

Sampling and disease reporting protocols followed those recommended in the ICES guidelines (ICES, 1996). Target fish species were the dab and cod (*Gadus morhua*) for offshore locations and the European flounder for inshore or estuarine stations. Where sufficient numbers of other fish species were caught, these were examined for significant diseases or parasites. Herring (*Clupea harengus*) and sprat (*Sprattus sprattus*) in particular, 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 Dietrich's fixative or neutral buffered formalin (NBF) for subsequent histological confirmation. Because it has been established that flounder are susceptible to endocrine disruption, gonad samples were also taken from both of these species for histological assessment.

In addition to the fish examined for externally visible diseases, standard sections of liver tissue from up to 50 dab approximately 20 cm in length were sampled from each of the following areas; Humber, Sole Pit, Flamborough, West Dogger, Dundrum, Liverpool Bay, Rye Bay, Cardigan Bay, Morecambe Bay and three stations along the Bremerhaven transect (ICES rectangles, 36F0, 36F1, 37F0, 38F1, 37E4, 35E6, 30F0, 33E5, 36E6, 37F8, 38F5 and 40F4 respectively). Tissues were processed using standard histological techniques (Bucke, 1994). Detailed recommendations on the diagnosis and reporting of histological liver lesions are included in the report of an ICES Special Meeting, held at CEFAS Weymouth, 22-25 October, 1996 (ICES, 1997). The guidelines included in that report were followed for the investigations reported here and haematoxylin and eosin stained histological sections were examined for the presence of the specific categories of hepatic pathology shown in Table 11. Although the range of possible lesions present in flatfish is large, the lesion categories used have been restricted to those with greatest relevance as indicators of contaminant exposure. These comprise foci of cellular alteration (FCA), benign neoplasms (e.g. hepatocellular adenoma) and malignant tumours, which are rarely reported in dab.

Table 11. Categories of histopathological lesions used for the assessment of hepatic pathology in dab (adapted from the list of lesions given by ICES, 1997)

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 Results

9.3.1 Dab diseases

Disease prevalence data according to fish size group and disease severity (Bucke *et al*., 1996) are presented in Tables 12 and 13. Dab diseases recorded 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 hyperpigmentation was also recorded. The overall summary data for these diseases are presented in Figures 10 (a) to (e) and 11 (a) to (e). The prevalence of histologically confirmed hepatic lesions found in dab from the larger size groups i.e. 20 to 24 and >25 cm in length are given in Tables 14 and 15. All cases of the neoplastic lesions, adenoma, cholangioma, putative hemangioma and malignant tumours were incorporated in the final prevalence data. FCA precursors to visible nodules are also included separately.

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 approximately 20 cm in length from both cruises are presented in Figures 12 and 13. Of particular importance are the FCA, benign and malignant neoplasms (see Table 11). Fish from the Bremerhaven transect stations and Dogger Bank exhibited the highest prevalences of these lesions with fish from Humber and Sole Pit also showing an increased prevalence when compared to the reference station at Rye Bay. Prevalence compared to that recorded from the 1997 sampling programme was generally slightly reduced. Unique degenerative lesions were only found in samples from Cardigan Bay and the Bremerhaven 1 station. Non-specific lesions were generally present at higher levels in samples obtained from the summer cruise, as were inflammatory changes.

9.3.3 Cod diseases

Sufficient cod for disease investigations were obtained from six areas during the first cruise; Red Wharf Bay, Liverpool Bay, Rye Bay, Flamborough, Humber Rough and Amble (ICES rectangles 35E5, 35E6, 30F0, 37F0, 36F0 and 39E8 respectively). A total of 1104 fish were caught, with numbers of males and females being approximately equal. Results are summarised in Table 16. Fish were examined for the presence of external and internal diseases and parasites which included ulcerations, skeletal deformities (scoliosis and lordosis), pseudobranchial tumours, visceral granulomatosis and the parasites *Cryptocotyle* sp. and *Lernaeocera branchialis*, a pathogenic copepod gill parasite. The general disease prevalence remains very low. At Rye Bay only 2.1% of fish were ulcerated with 9.3% harbouring *L. branchialis* parasites. None exhibited visceral granulomatosis, skeletal deformities or the presence of pseudobranchial tumours. Parasitism with *Cryptocotyle* was detected in 7.4% of fish from Red Wharf Bay and in 5.6% of fish from Liverpool Bay. (Total catch prevalence of fish harbouring *L. branchialis* was 5.3%). All other diseases were present at levels between 0.5 and 1.6%. During the second survey, sufficient numbers of cod were obtained only from Off Amble (39E8) where 100 fish were caught (38 males, 62 females). Two cases of *L. branchialis*, and one of X-cell were noted. One hundred fish were also caught at the Humber station (36F0) where two ulcers, one case of skeletal deformity, a possible case of epidermal papilloma and two cases of visceral granulomatosis were recorded.

9.3.4 Examination of the total catch for significant diseases

Examination of other species including haddock (*Melanogrammus aeglifinus*), whiting (*Merlangius merlangus*), herring, gurnard (*Eutrigla gurnardus*), plaice (*Pleuronectes platessa*), brill (*Scophthalmus rhombus*) and flounder (*P. flesus*) revealed generally low levels of disease. *Ichthyophonus* was not detected in North Sea herring examined during the two cruises reported here in 1998. Normal tissues as well as examples of disease were taken from a range of fish and shellfish species for the Registry of Aquatic Pathology (RAP) reference collection held at CEFAS Weymouth Laboratory. Samples of mullet (*Mugil cephalus*) collected from Rye Bay revealed a single case of hepatocellular FCA, the first record of this lesion in this species. Of thirty-five plaice livers from Carmarthen Bay, two basophilic FCA's and three cases of non-specific pathology were seen. An additional specimen of plaice and one of brill from Lyme Bay were found to harbour macroscopic liver lesions which were confirmed as hepatocellular adenomas. Eighty-seven flounder from the German Bight (Bremerhaven 1) were examined and two cases of lymphocystis and one of hyperpigmentation were detected. Twenty flounder livers from this location were examined histologically. Six cases of non-specific lesions, in particular storage conditions and inflammatory changes were detected. From the station at the eastern Dogger Bank, twenty-one Long Rough dab were examined, two acute ulcers and one mild case of hyperpigmentation noted. Ninety flounder from The Wash (ICES rectangle 35F0) were examined and one liver nodule (histologically confirmed as adenoma) and one case of lymphocystis were noted. Thirty-five male gonad samples were taken for histological screening, one case of intersex was found in a flounder from The Wash and an additional case from inner Liverpool Bay, this condition not having been noted in the open sea previously.

9.4 Conclusions

External disease prevalence for dab examined during the two monitoring periods reported here are similar to the prevalence in previous years at the same sampling locations. Dab populations off Flamborough and at the western Dogger continue to exhibit higher levels of disease than at the Rye Bay reference area. However, differences in the prevalence of certain disease conditions were apparent between the winter and summer sampling periods. This was most obvious for acute and healing ulcerations, where higher prevalences at several sampling stations in the summer period were recorded. This phenomenon was particularly prominent in dab examined in Rye Bay, Liverpool Bay and the western Dogger Bank and has also been noticed during other disease surveys (Wosniok *et al*., 1999). Other externally visible diseases also showed seasonal differences in prevalence. Epidermal hyperplasia/ papilloma showed the opposite trend to acute ulceration with generally decreased prevalence in the summer. However, at several locations (e.g. Rye Bay) this difference was only slight. Hyperpigmentation prevalence varied between sampling locations and seasonally. It is difficult to explain these differences

Table 12. Summary catch data and disease prevalence in dab (Limanda limanda) by size categories and disease severity at stations sampled in the North Sea and Irish Sea for fish disease monitoring, RV CORYSTES Cruise 1/98

Area name (NMP)	ICES Rect.	Size Range (cm)	Numbers examined		No. and severity of disease cases Recorded according to ICES Guidelines (*Bucke et al.*, 1996)												
					LY			E/P			U			HYP			LN
			Male	Female	1	2	3	1	2	3	1	2	3	1	2	3	
Carmarthen Bay	30E6	15-19	109	138	0	0	0	0	0	0	0	0	0	0	0	0	0
		20-24	34	33	0	1	0	0	0	0	0	0	0	3	0	0	0
		>25	12	25	0	0	0	0	0	0	1	0	0	0	0	0	0
Rye Bay	30F0	15-19	200	100	7	0	0	2	0	0	1	0	0	5	0	0	0
(486)		20-24	99	232	5	0	0	5	4	1	3	0	0	26	2	1	6
		>25	4	79	3	0	0	1	0	3	4	0	0	7	1	1	5
Cardigan Bay	33E5	15-19	178	107	3	0	0	1	1	3	5	1	0	6	1	1	0
(655)		20-24	28	169	2	0	0	3	1	4	6	2	0	0	1	1	10
		>25	0	38	0	0	0	0	0	0	1	0	0	0	0	0	3
Red Wharf	35E5	15-19	207	193	3	0	0	1	2	0	0	0	0	0	0	0	0
(776)		20-24	47	171	3	0	0	1	3	3	2	1	0	0	0	0	1
		>25	0	12	0	0	0	0	1	2	0	0	1	0	0	0	1
Liverpool Bay	35E6	15-19	177	123	5	0	0	2	5	7	1	0	0	0	0	0	0
(715)		20-24	56	177	1	0	0	7	10	5	7	2	0	0	0	0	5
		>25	0	26	0	0	0	0	2	3	1	0	0	0	0	0	4
Humber	36F0	15-19	125	75	12	1	0	0	1	1	2	0	0	15	3	0	0
(377)		20-24	34	35	2	0	0	1	0	2	2	0	0	10	4	1	1
		>25	1	20	0	0	0	0	1	1	1	0	1	3	0	0	1
Humber Rough	36F0A	15-19	13	11	1	0	0	0	0	0	0	0	0	0	0	0	0
		20-24	4	36	3	0	0	0	0	2	0	0	0	4	0	0	0
		>25	1	54	3	0	0	0	1	3	2	0	0	7	6	2	7
Sole Pit	36F1	15-19	86	158	4	0	2	1	2	1	6	0	7	12	3	0	0
		20-24	45	239	6	1	2	6	6	9	4	1	12	23	20	8	6
		>25	0	51	0	0	0	0	2	3	3	0	2	14	6	3	10
Dundrum	37E4	15-19	148	62	14	0	0	0	1	0	3	0	0	0	0	0	0
(815)		20-24	8	70	4	0	0	0	1	1	2	0	0	1	0	0	1
		>25	0	12	1	0	0	0	1	0	0	0	0	0	0	0	1
Flamborough	37F0	15-19	115	260	13	0	0	0	2	0	3	0	0	20	1	0	0
(344)		20-24	78	110	7	0	0	0	1	5	5	1	0	33	12	5	5
		>25	3	28	3	0	0	0	0	1	1	0	0	6	1	3	1
W. Dogger	38F1	15-19	321	79	24	0	0	0	6	17	6	2	0	29	3	1	0
(286)		20-24	287	144	20	1	0	4	12	27	4	1	0	70	7	5	27
		>25	11	49	0	1	0	0	4	5	1	0	0	9	4	0	5

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

since the aetiologies of these conditions in dab have not been conclusively established. However, it is generally accepted that microrganisms play an important role in their development and it is known that temperature is a major influence affecting both the immune system of the fish and the pathogenicity of the fish pathogens. Hyperpigmentation was again a prominent condition noted in dab from several areas in the North Sea but only present at low prevalence in the Irish Sea and in the English Channel at Rye Bay. The aetiology of the condition is still not known but it is now recognised that several other flatfish species exhibit the condition. However, in species such as Long Rough dab examined during the 1998 cruises, the extent of the hyperpigmentation is generally much lower than that seen in dab (*Limanda limanda)*.

Table 13. Summary catch data and disease prevalence in dab (Limanda limanda) by size categories and disease severity at stations sampled in the North Sea and Irish Sea for fish disease monitoring, RV CIROLANA Cruise 3b/98

Area name (NMP)	ICES Rect.	Size Range (cm)	Numbers examined		No. and severity of disease cases Recorded according to ICES Guidelines (*Bucke et al.*, 1996)												
					LY			E/P			U			HYP			LN
			Male	Female	1	2	3	1	2	3	1	2	3	1	2	3	
Rye Bay (486)	30F0	15-19	153	67	0	0	0	3	0	0	5	4	0	5	0	0	0
		20-24	87	112	4	0	0	5	0	0	6	5	6	8	1	0	0
		>25	9	23	0	0	0	0	1	0	1	3	1	1	0	0	0
Cardigan Bay (655)	33E5	15-19	129	83	3	0	0	2	0	0	4	3	3	9	1	1	0
		20-24	6	157	2	0	0	4	0	0	1	8	0	3	2	1	11
		>25	0	3	0	0	0	0	0	0	0	0	1	0	0	0	1
Red Wharf (776)	35E5	15-19	63	37	0	0	0	2	0	0	5	2	0	0	0	0	0
		20-24	12	88	0	0	0	0	0	0	4	2	1	0	0	0	3
		>25	0	50	0	0	0	0	0	0	3	1	0	0	0	0	1
Liverpool Bay (715)	35E6	15-19	124	78	3	0	0	0	0	0	12	14	2	0	0	0	0
		20-24	61	166	1	0	0	2	0	0	12	14	9	1	0	0	8
		>25	0	51	1	0	0	3	0	0	3	5	8	0	0	0	6
Flamborough (344)	37F0	15-19	133	74	17	2	0	0	1	0	3	1	0	20	0	2	0
		20-24	9	10	0	0	0	0	0	0	0	0	0	4	2	1	1
		>25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
W. Dogger (286)	38F1	15-19	161	40	19	0	0	2	1	0	27	3	9	22	8	4	0
		20-24	105	128	6	1	0	2	1	1	17	5	17	42	18	6	20
		>25	1	15	1	0	0	0	0	0	0	0	2	1	5	1	7
Off Amble (244)	39E8	15-19	55	49	3	1	0	1	0	0	3	0	2	2	0	1	0
		20-24	61	63	10	1	1	3	1	0	5	2	3	6	3	1	2
		>25	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Bremerhaven 1	37F8	15-19	70	132	0	1	0	2	0	0	6	4	1	5	0	0	0
		20-24	17	227	5	0	0	12	1	0	10	7	4	11	4	1	0
		>25	6	95	4	0	0	3	1	0	1	1	4	5	3	2	1
Bremerhaven 7	38F5	15-19	150	150	7	1	0	2	0	0	11	1	6	1	1	0	0
		20-24	66	163	7	1	0	2	0	0	10	1	6	7	1	0	2
		>25	0	105	0	0	0	0	0	0	0	0	0	0	1	0	0
Bremerhaven 7	40F4	15-19	121	79	10	1	0	1	0	0	34	2	4	1	0	0	0
		20-24	102	116	18	0	1	1	1	0	64	7	8	15	1	1	1
		>25	12	69	7	2	0	1	0	0	18	7	4	3	1	1	7

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

The application of toxicopathic liver lesions is now routine in marine biological effects monitoring. The presence of macroscopic nodules or tumours was again identified in a number of areas with the highest prevalences recorded in fish from the Dogger Bank. This is consistent with previous findings. However, in all areas sampled apart from Rye Bay, the prevalence of liver nodules was higher in the fish sampled during the summer. Similarly, the prevalence of histological lesions in the specimens taken from the same populations for liver pathology screening was also higher during the summer cruise. This is difficult to explain since there is strong evidence that these lesions can be induced following exposure to contaminants and that no infectious aetiology has yet been demonstrated. It is possible that physiological parameters influence the development of these lesions in the relatively short term, but this is counter to other evidence that macroscopic lesions have a long latency period (Vethaak *et al*., 1996). There was good correlation between the observation of gross liver lesions and the confirmation of these histologically.

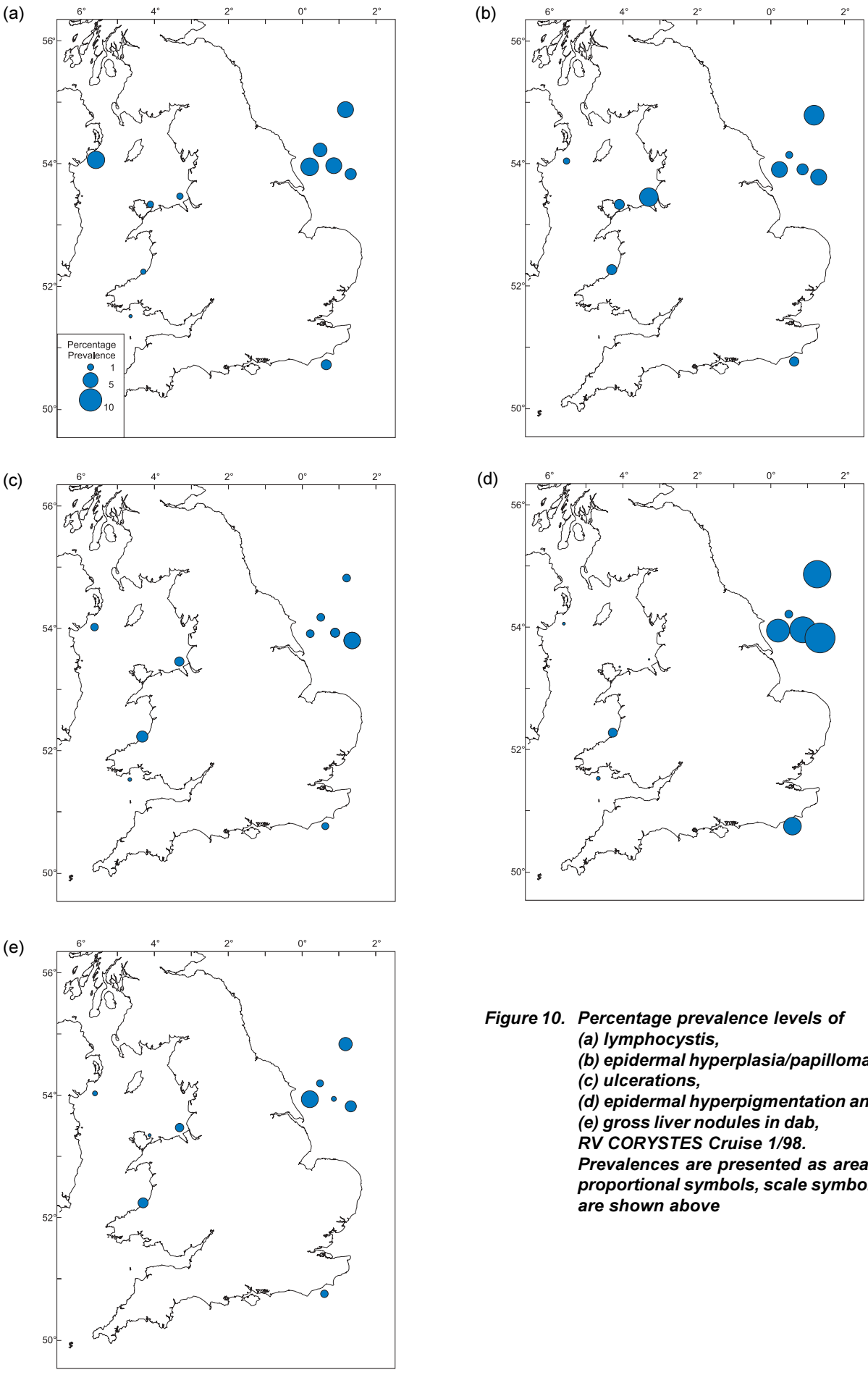

Figure 10. ***Percentage prevalence levels of (a) lymphocystis, (b) epidermal hyperplasia/papilloma, (c) ulcerations, (d) epidermal hyperpigmentation and (e) gross liver nodules in dab, RV CORYSTES Cruise 1/98. Prevalences are presented as area proportional symbols, scale symbols are shown above***

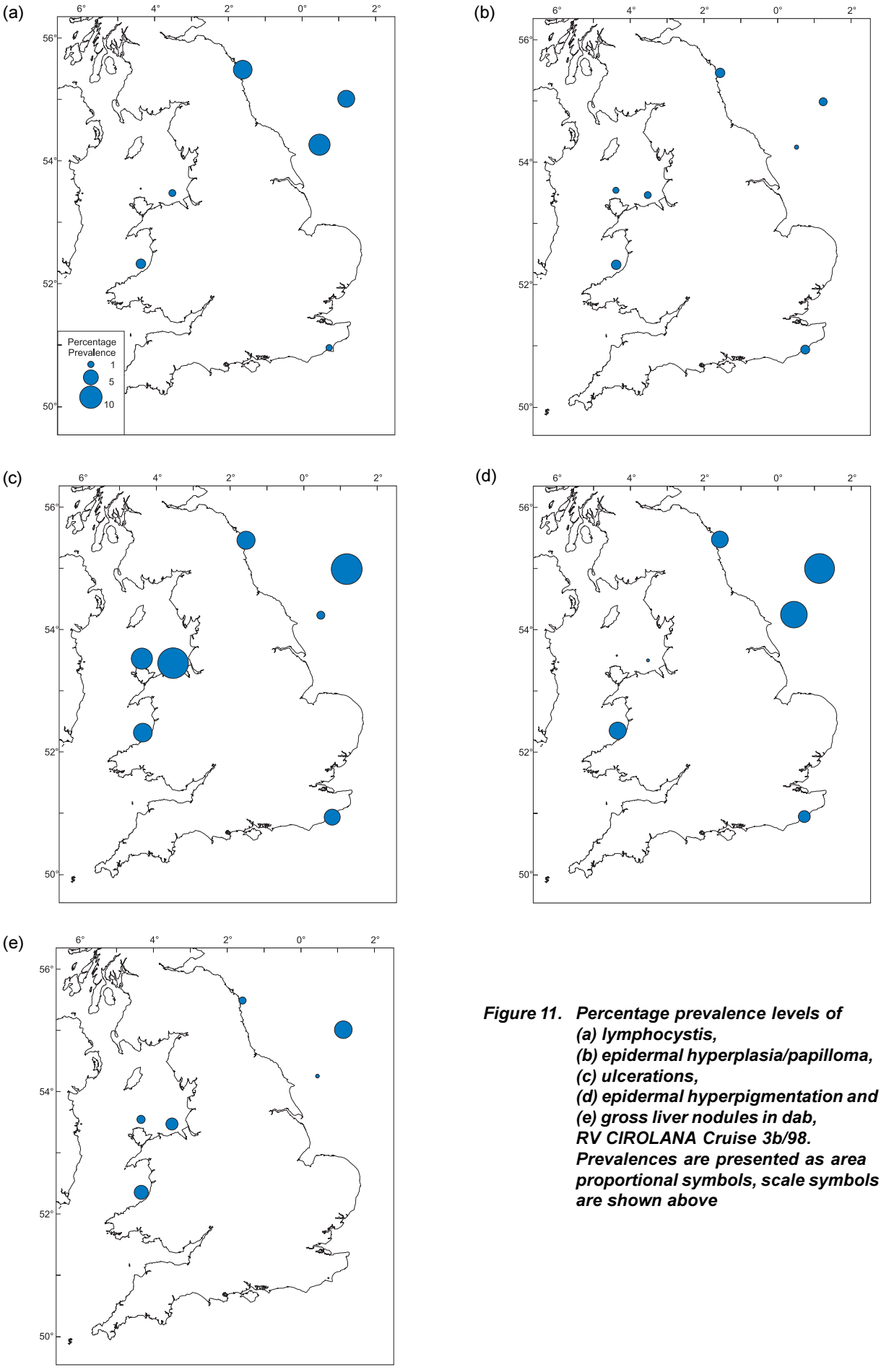

Figure 11. ***Percentage prevalence levels of (a) lymphocystis, (b) epidermal hyperplasia/papilloma, (c) ulcerations, (d) epidermal hyperpigmentation and (e) gross liver nodules in dab, RV CIROLANA Cruise 3b/98. Prevalences are presented as area proportional symbols, scale symbols are shown above***

Table 14. Histological confirmation and characterisation of specimens of macroscopic liver lesions observed in the two larger sizes of dab sampled in the North Sea and Irish Sea. RV CORYSTES Cruise 1/98

Area name (NMP)	ICES Rect.	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
Carmarthen Bay	30E6	51° 33.42'N 04° 40.18'W	104	0	0.0	0	0	0	0	0	0	0	0	0	0	0.0	0.0	0.0
Rye Bay (486)	30F0	50° 45.71'N 00° 44.42'E	414	11	2.7	2	0	2	0	1	7	0	0	0	2	2.2	0.7	0.5
Cardigan Bay (655)	33E5	52° 16.24'N 04° 18.57'W	235	13	5.5	0	0	0	0	0	14	0	0	0	0	6.0	0.0	0.0
Red Wharf (776)	35E5	53° 21.20'N 04° 07.35'W	230	2	0.9	0	0	0	0	0	2	0	0	0	0	0.9	0.0	0.0
Liverpool Bay (715)	35E6	53° 28.69'N 03° 17.71'W	259	9	3.5	0	0	0	0	1	8	0	0	0	0	3.1	0.4	0.0
Humber (377)	36F0	53° 57.51'N 00° 57.34'E	90	2	2.2	0	0	0	0	0	2	0	0	0	0	2.2	0.0	0.0
Humber Rough	36F0	53° 57.06'N 00° 16.90'E	95	7	7.4	0	0	0	1	0	9	0	0	0	1	10.5	1.1	0.0
Sole Pit	36F1	53° 49.68'N 01° 25.54'E	335	16	4.8	2	0	0	4	1	13	0	0	0	0	3.9	1.5	0.6
Dundrum (815)	37E4	54° 04.04'N 05° 37.36'W	90	2	2.2	0	0	0	0	0	2	0	0	0	0	2.2	0.0	0.0
Flamborough (344)	37F0	54° 13.17'N 00° 34.47'E	219	6	2.7	0	0	1	1	2	3	0	0	0	3	2.7	1.8	0.0
W. Dogger (286)	38F1	54° 51.74'N 01° 16.59'E	491	32	6.5	1	1	3	4	8	23	0	0	0	6	5.9	3.3	0.2

** Lesion number as indicated in Table 11*

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

Occasionally, multiple lesions were detected in the histological sections resulting in a higher prevalence figure for confirmed tumours. This was regularly observed in the batches of livers sampled as part of the histological screening programme. This aspect of the monitoring has confirmed that liver histopathology can be used as a sensitive tool to detect toxicopathic lesions in dab and other European flatfish species. Several categories of lesion were detected, including unique degenerative lesions not observed in those livers taken for confirmatory diagnosis of macroscopic lesions. Since the microscopic appearance of tissue lesions can be variable, it is vitally important that quality assurance procedures for the methodology and especially diagnostic criteria to be used in reporting histopathological data are in place. This requirement for fish disease monitoring as well as other biological effects monitoring techniques are the subject of a current European Commission funded programme 'Biological Effects Quality Assurance in Monitoring programmes' (BEQUALM).

Relatively few liver lesions were detected in other flatfish species collected during the two cruises. The presence of an hepatocellular adenoma in plaice confirms previous data that indicate this species to be susceptible to tumour formation (CEFAS, 2000). Although thirty-seven plaice livers were examined, most of these fish were small and probably less than three years old. The prevalence of hepatic pathology could be expected to increase in older fish. Unfortunately, few large plaice were caught during the two cruises. Plaice and other commercial fish species examined showed few external signs of disease.

Disease data generated from the UK fish disease monitoring programme will be included in the UK NMMP database and as with previous data will be available to OSPAR via the ICES Environmental Data Centre.

Table 15. Histological confirmation and characterisation of specimens of macroscopic liver lesions observed in the two larger sizes of dab sampled in the North Sea and Irish Sea. RV CIROLANA Cruise 3b/98

Area name (NMP)	ICES Rect.	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
Rye Bay (486)	30F0	50° 51.404 N 00° 47.283'E	231	0	0.0	0	0	0	0	0	0	0	0	0	0	0.0	0.0	0.0
Cardigan Bay (655)	33E5	52° 17.256'N 04° 17.297'W	166	12	7.2	0	0	1	2	2	9	0	1	0	0	6.0	3.0	0.0
Red Wharf (776)	35E5	53° 21.800'N 04° 09.842'W	150	4	2.7	0	0	0	0	2	4	0	0	0	0	2.7	1.3	0.0
Liverpool Bay (706)	5E6	53° 28.105'N 03° 22.568'W	278	14	5.0	0	0	0	0	4	10	0	2	0	0	4.3	1.4	0.0
Flamborough (344)	37F0	54° 15.092'N 00° 28.337'E	19	1	5.3	0	0	0	0	0	1	0	0	0	0	5.3	0.0	0.0
W. Dogger (286)	38F1	54° 47.851'N 01° 14.150'E	249	27	10.8	1	1	4	4	7	26	0	0	0	1	10.8	6.4	0.4
Off Amble (244)	39E8	55° 17.759'N 01° 14.986'W	125	3	2.4	1	0	0	0	1	1	0	0	0	0	0.8	0.8	0.8
Bremerhaven 1	37F8	54° 04.601'N 08° 09.657'E	345	1	0.3	0	0	0	0	0	1	0	0	0	0	0.3	0.0	0.0
Bremerhaven 7	38F5	54° 43.896'N 05° 33.067'E	334	2	0.6	0	0	0	0	1	1	0	0	0	0	0.3	0.3	0.0
Bremerhaven 9	40F4	55° 28.193'N 04° 07.133'E	299	8	2.7	0	0	1	0	5	8	0	0	0	0	2.7	2.0	0.0

** Lesion number as indicated in Table 11*

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

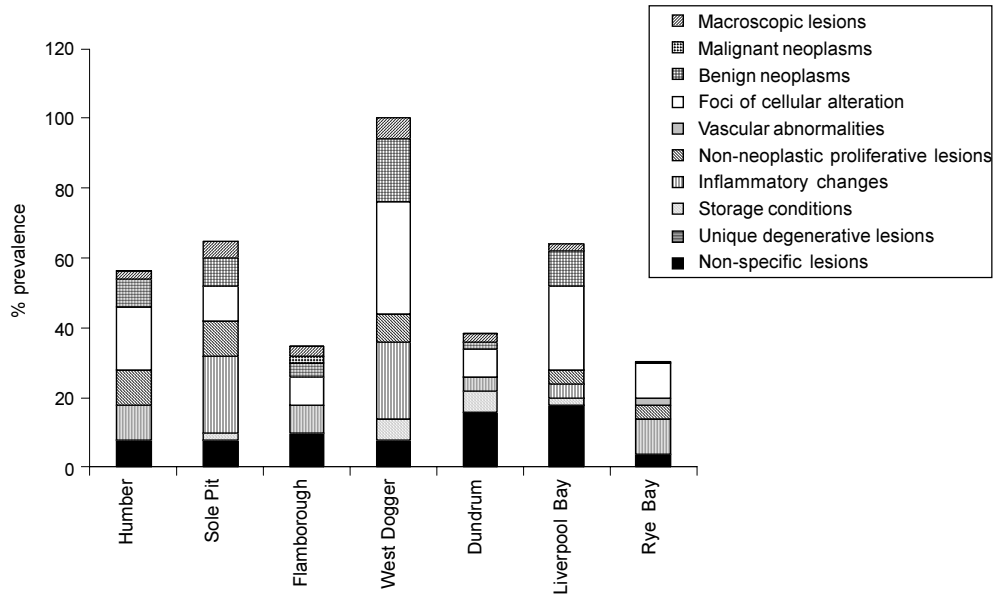

Figure 12. Histological characterisation of hepatic lesions in the two larger size categories of dab sampled on stations in the North Sea and Irish Sea, RV CORYSTES Cruise 1/98

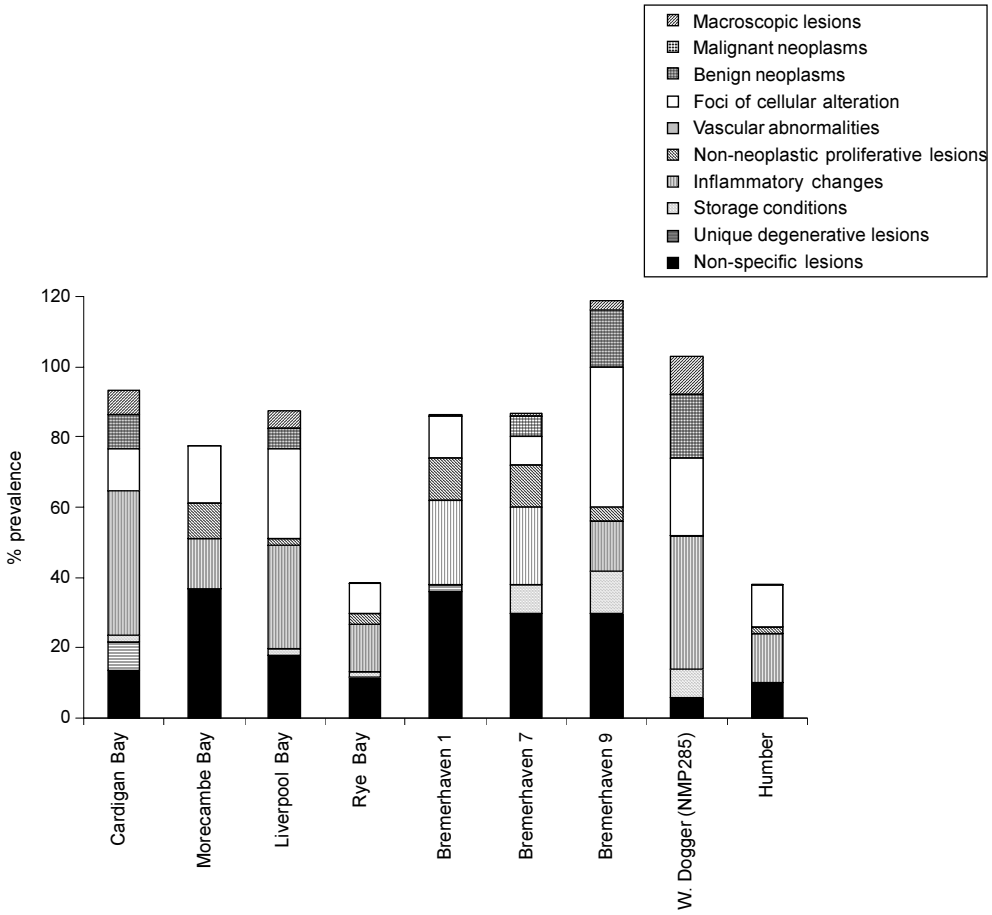

Figure 13. Histological characterisation of hepatic lesions in the two larger size categories of dab sampled on stations in the North Sea and Irish Sea, RV CIROLANA Cruise 3b/98

Table 16. Disease prevalence in cod (Gadus morhua) sampled in the North Sea and Irish Sea (data from both cruises combined)

Area	ICES Rectangle	Total No. examined	Percentage of disease cases recorded according to ICES (1996)					
			ULC	SKD	PBT	CR	LB	MA
Flamborough	37F0	144	0.0	0.7	0.0	0.0	0.7	1.4
Amble	39E8	84	1.2	1.2	0	0.0	3.6	0
Humber	36F0	311	0.3	0.0	0	0.0	3.9	0
Liverpool	35E6	177	0	0.6	0	5.6	11.9	0
Red Wharf	35E5	244	0	0	0	7.4	3.3	1.6
Rye	30F0	140	2.1	0	0	0.0	9.3	0

Key: ULC = Ulcers
SKD = Skeletal deformity
PBT = Pseudobranchial 'tumour'
CR = Cryptocotyle
LB = Lernaeocera branchialis
MA = Myxobolus aeglifini

10. THE USE OF ENZYME BIOMARKERS IN FISH AS BIOLOGICAL EFFECTS MONITORING TOOLS

10.1 General introduction

The use of enzyme biomarkers as pollution monitoring tools is well established and their use by CEFAS has been described in previous Aquatic Environment Monitoring Reports. This section reports the continued use of two important enzyme biomarkers in fish; i) hepatic ethoxyresorufin-O-deethylase (EROD) as a marker of exposure to planar organics (especially PAH and PCB), and ii) muscular ChE as a marker of exposure to neurotoxins (especially organophosphate and carbamate pesticides).

10.2 EROD

10.2.1 Introduction and 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 1998 data presented here represents the third consecutive year (see CEFAS (2000) for previous results) that EROD data for dab (*Limanda limanda*) has been collected. Fish were obtained by Granton trawl during an *RV CIROLANA* cruise - June/July 1998. 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. 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 (50 mM TRIS pH 7.5, 1 mM EDTA, 1 mM dithiothrietol, 150 mM NaCl) 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 a lidded eppendorf tube 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 a modification of the method described in Stagg *et al.* (1995). 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 pH 7.5 TRIS, 100 mM NaCl), 20 ml liver homogenate, 10 ml ethoxyresorufin substrate (0.4 mM in dimethyl sulphoxide (DMSO)) and 10 ml of resorufin internal standard (25 mM 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 ml NADPH (0.25mM) and emission readings were recorded at 0, 15, 30, 45 and 60 seconds post addition.

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.

10.2.2 Results and discussion

RV CIROLANA 3b/98
EROD activity results for the last three years monitoring are summarised in Table 17. Figures 13-15 graphically represents the data collected in those years. The highest EROD values in 1998 (>1000 pM min^{-1} mg^{-1} protein) are associated with the North East coast and Liverpool Bay. The results also include two samples taken along the Bremerhaven transect in the German Bight which also showed high EROD activities. One slightly anomalous result was the relatively high levels apparent on the Dogger bank.

Low activity levels (<525 pM min^{-1} mg^{-1} protein) were evident in samples taken from well flushed coasts/bays with lower contaminant inputs (e.g. Off Wash, Rye Bay, Lyme Bay and Cardigan Bay).

Cruise data continues to prove difficult to explain in some instances (e.g. the high EROD activities in two from the last three years on the Dogger Bank) and suggests that much more needs to be known about the

Table 17. Hepatic EROD Activity in dab

Site	Mean hepatic EROD (pM/min/mg protein)								
	RV CIROLANA 6b/96 (July)			RV CIROLANA 5b/97 (June)			RV CIROLANA 3b/98 (June)		
	n	Mean	SD	n	Mean	SD	n	Mean	SD
Morecambe Bay	20	1040	606	20	2083	1139	19	1176	841
Liverpool Bay	20	983	707	20	1782	858	20	1456	955
Burbo Bight	20	1146	960	20	1397	789	-	-	-
SE Isle of Man	20	601	368	20	869	837	-	-	-
Red Wharf Bay	20	1156	907	20	757	599	20	602	389
Dundrum Bay	-	-	-	16	1286	603	20	880	578
Inner Cardigan Bay	-	-	-	20	792	470	21	520	467
Outer Cardigan Bay	12	864	479	20	808	587	-	-	-
30 miles SW of Milford	20	2549	1400	-	-	-	-	-	-
St. Brides Bay	20	1035	619	-	-	-	-	-	-
Carmarthen Bay - East	22	1655	1156	20	691	663	-	-	-
Carmarthen Bay - Mid	6	1429	948	20	1081	945	-	-	-
Inner Carmarthen Bay	24	1604	1046	20	1323	813	-	-	-
Carmarthen Bay - West	20	1592	739	20	993	543	-	-	-
Celtic Deep	-	-	-	20	562	287	-	-	-
Lyme Bay	-	-	-	-	-	-	20	245	165
Rye Bay	-	-	-	-	-	-	20	385	251
Off Tyne/Amble	20	1828	1167	20	1520	1350	20	1400	789
Off Tees	20	1424	820	20	1454	710	-	-	-
Off Flamborough	-	-	-	-	-	-	20	1569	752
Off Humber	20	449	234	20	580	335	20	998	685
The Wash	10	830	653	-	-	-	20	218	259
Dogger Bank	20	1343	902	20	668	479	20	1261	1125
West Dogger Bank	-	-	-	-	-	-	20	873	569
Bremerhaven 1	-	-	-	-	-	-	20	1010	870
Bremerhaven 7	-	-	-	-	-	-	20	1135	1007

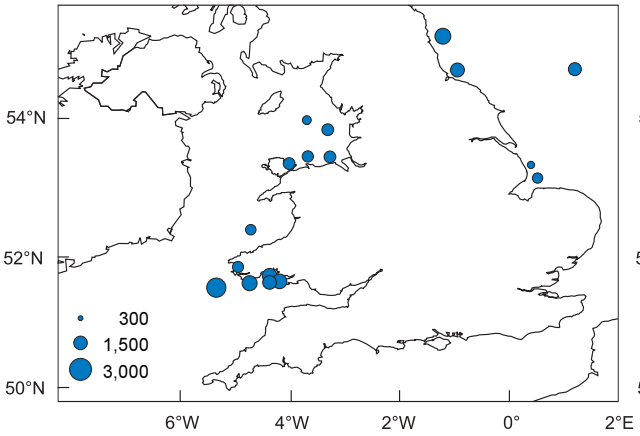

Figure 13. Hepatic EROD (pM/min/mg protein) activity in dab from RV CIROLANA 6b/96 (July)

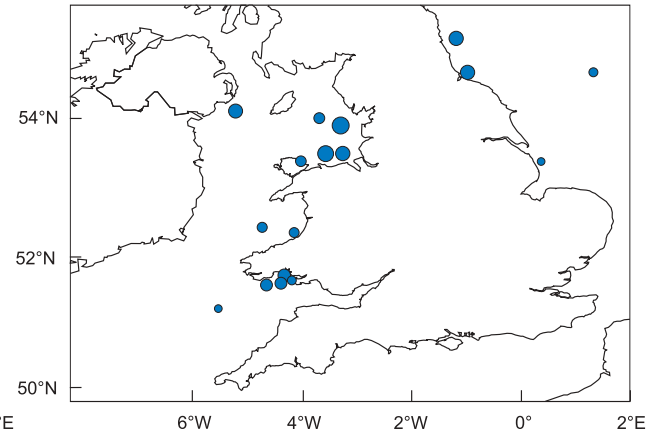

Figure 14. Hepatic EROD (pM/min/mg protein) activity in dab from RV CIROLANA 5b/97 (June)

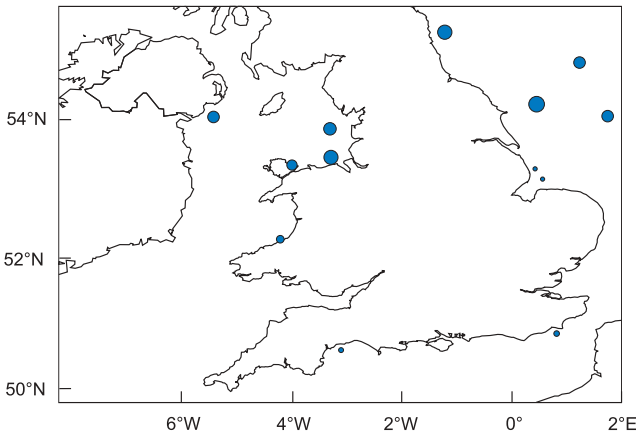

Figure 15. Hepatic EROD (pM/min/mg protein) activity in dab from RV CIROLANA 3b/98 (June)

environmental influences on MFO activity before the monitoring data can be more confidently used to interpret biological effects of contaminants. However, general but clear patterns of induction have emerged over the three annual datasets. Sites in Liverpool Bay and off the north east coast of England routinely have significantly elevated EROD activity whilst those in areas such as Cardigan Bay have been consistently low. These general trends now demonstrated over a 3 year survey period provide conclusive evidence that fish populations in certain areas are undergoing long-term exposure to MFO inducing chemicals. It has been shown that flounder (*Platichthys flesus*) populations in the estuaries of the Mersey, Tyne and Tees exhibit significantly elevated levels of EROD activity (Kirby *et al.* 1999) and the monitoring data presented here strongly supports the idea that the coastal populations of other fish species, such as dab, adjacent to these estuarine outflows are also being affected. Research work to investigate the possible effects of long-term exposure to MFO inducers (specifically PAH) is ongoing at the CEFAS Burnham Laboratory.

10.3 Cholinesterase (ChE)

10.3.1 Introduction and methods

Acetylcholine (ACh) is the primary neurotransmitter in the sensory and neuromuscular systems in fish. As such, the activity of this system is vital to normal behaviour in this group (Payne *et al.,* 1996) and it represents a prime target on which toxicants can realise a detrimental effect. The levels of ACh at a neuro-junction must be carefully regulated and this is done by the activity of the enzyme acetylcholinesterase (AChE) which degrades the ACh into the inactive products choline and acetic acid which are reabsorbed and used as raw materials for the continued production of ACh. Inhibition of the AChE enzyme will result in a build up of ACh causing a continuous and excessive stimulation of the nerve/muscle fibres which will result in tetany and eventual paralysis and death. Some of the most potent AChE inhibitors are the organophosphate and carbamate pesticides and it is primarily with the environmental monitoring of these chemicals in mind that we have applied the ChE technique.

The data presented here represents that obtained during the annual monitoring survey carried out in 1997. These routine determinations looked at ChE activity levels in the muscle tissue of dab (*Limanda limanda)* and plaice (*Pleuronectes platessa*). Samples were taken from aboard the *RV CIROLANA* in June/July 1997 using a Granton trawl. Once on deck, dab were separated into tanks containing flowing seawater. Dissections were performed within 1 hour of capture. A strip of muscle (approx.1-2 cm^3) was removed from the dorsal surface near to the spine 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. Fish over 10 cm were retained as samples.

Homogenate preparation

Samples were kept at -80°C for no longer than 4 months before the assays were performed. Approximately 1g of muscle tissue was placed in 10 mls of homogenising buffer (0.1M pH7 TRIS/HCl containing 0.1% Triton X100) in a suitably sized, clean glass beaker. The muscle was processed using an Ultra-Turrax homogeniser for 15-20 seconds whilst keeping the beaker on ice to minimise the temperature increase. The crude homogenate was then decanted into an eppendorf tube and centrifuged at 10,000 g for 20 minutes. The resultant supernatants were used as the ChE source.

AChE activity determination

AChE activity determinations were performed using a modification of the technique described by Bocquene and Galgani (1996). Briefly, using a 96-well microplate, assays were performed in quadruplicate with each test well initially containing 10 µl of supernatant, 340 µl of assay buffer (0.1M pH 7 Tris/HCl – no Triton X100) and 20 µl of 0.01M dithiobisnitrobenzoate (DTNB). Blanks contained no supernatent and 350 µl of buffer. Assay reactions were initiated by quick addition, via stepping pipette, of 10 µl of 0.1M acetylthiocholine (ACTC) which acted as the substrate. The plate was then placed in a microplate reader set to read absorbance at 412 nm and optical density (OD) readings were taken every 15 seconds for 1 minute. The assay was temperature controlled at 25°C and all reagents were brought to this temperature prior to use.

AChE activity was normalised to protein content and expressed as mU min^{-1} mg^{-1} protein (1U = 1mOD unit). Protein analyses were carried out on the same muscle homogenate as the AChE activity measurements using a plate reader modification of the Bradford method (1976) with a bovine serum albumin standard.

10.3.2 Results and discussion

RV CIROLANA 6b/97

The muscle ChE activities in dab and plaice samples are shown in Table 18 and represented in Figure 16. The data show clearly that ChE levels vary significantly between sites around the UK.

The plaice data show that the sites associated with the Liverpool Bay area (Liverpool Bay, Burbo Bight and Morecambe Bay) yield samples which have substantially lower muscular ChE levels than at other areas. High activities are generally associated with offshore and bay areas.

Table 18. Muscle ChE (mU/min/mg protein) activities in muscle tissue from dab and plaice in 1997

Sampling Location	Dab			Plaice		
	n.	ChE	SD	n.	ChE	SD
Swansea Bay	-	-	-	20	11368	2557
Mid Carmarthen Bay	20	3686	848	20	10371	2266
Rhossili Bay	20	3988	796	-	-	-
Inner Camarthen Bay	20	4408	902	20	11839	2911
West Camarthen Bay	20	4474	1447	20	10093	2614
Celtic Deep	20	3565	969	20	11458	2851
Inner Cardigan Bay	20	5263	1119	20	11119	3063
Outer Cardigan Bay	20	3024	635	-	-	-
Red Wharf Bay	20	2491	695	20	11520	3250
Liverpool Bay	20	3881	867	20	8216	3371
Burbo Bight	20	2892	957	20	8355	2237
Morecambe Bay	20	2756	522	20	8751	1740
SE Isle of Man	20	3303	1051	20	10627	3431
Dundrum Bay	16	4216	1202	16	14081	8009
Off Tyne	20	4174	1453	-	-	-
Dogger Bank	20	2834	985	-	-	-
Off Tees	20	4036	1106	20	13026	2817
Off Humber	20	4341	1822	-	-	-

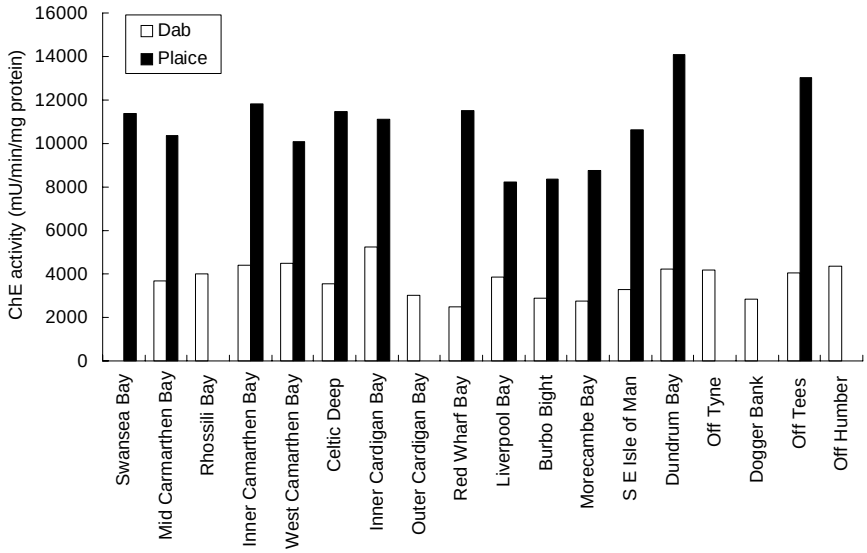

Figure 16. ChE activities in muscle of dab and plaice from RV CIROLANA 5b/97

The dab data reflect this effect to an extent, with sites associated with the Liverpool Bay area also yielding the lowest ChE activities (Burbo Bight, Morecambe Bay and Red Wharf Bay) along with those dab taken from the Dogger Bank. Moreover, the samples exhibiting high ChE activity were once again generally from offshore or bay areas (Carmarthen Bay, Cardigan Bay etc.).

The low activity associated with sites in and around the Liverpool Bay area was reflected to a slightly lesser extent in the 1996 ChE data (see CEFAS, 2000) and is consistent with the fact that Kirby *et al.* (2000) demonstrated that flounder (*Platichthys flesus*) from the Mersey estuary consistently had the lowest muscular ChE activity in comparison to a range of other UK estuaries. Furthermore, this is the second consecutive set of monitoring data for dab that has demonstrated low ChE activity in samples caught from the Dogger Bank and similar results were reported by Galgani *et al.* (1992). Whilst the cause of this is unclear, the evidence strongly supports the fact that the results gained on the Dogger are 'real'.

10.4 General conclusion

The data presented here shows that the use of both EROD and ChE enzyme assays are useful tools for the monitoring of environmental quality in UK waters. The three years of EROD and two years of ChE data reported so far, establishes convincing evidence of 'hotspots' for pollutant effects. For EROD, the results repeatedly 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. The ChE data, although only from two years, already shows consistently low activity in Liverpool Bay and the Dogger Bank. Whilst the Liverpool Bay results could be associated with output from the Mersey, a plausible theory for the Dogger Bank phenomenon remains to be established.

Samples are currently being processed for EROD and ChE activity from 1999 and 1998/1999 respectively. These results will enable the initiation of a time series data set which it is hoped will contribute to our understanding of longer term trends in sub-lethal effects of pollutants. Furthermore, now it has been established that certain coastal fish populations are subject to chronic stress due to exposure to important contaminant groups, research is now required to investigate the potential for long-term impacts on factors such as reproductive competency.

SEDIMENTS

11. AVAILABILITY OF ARSENIC IN SEDIMENTS TO BENTHOS IN THE SOUTHERN NORTH SEA

Previous work has shown the existence of relatively high concentrations of arsenic in sediments off the north east coast of Norfolk (Whalley *et al.,* 1999). The current work used opportunistic sampling of benthos to assess the availability of arsenic to biota on the seabed. Concentrations of arsenic in *Asterias rubens* and *Pagurus* spp. from various locations in the southern North Sea are presented. These include comparative data previously collected for a joint MAFF- and DoE-funded project into metal concentrations in sediments and benthos in the Dogger Bank region (Whalley *et al.*, 1997).

Arsenic concentrations were measured in the disc- and leg-meat of *A. rubens* and in the body meat of *Pagurus*. For *A. rubens*, lengths were measured along the maximum leg-to-leg distance, and for *Pagurus*, across the carapace. Concentrations generally declined with increasing size in *A. rubens*. Data for *Pagurus* showed analytical reproducibility of the arsenic concentrations as being better than 8% (n = 7).

Figure 17 shows arsenic (As) concentrations (wet weight) in *A. rubens* and *Pagurus* spp. Highest concentrations were found in animals collected off the south western side of the Dogger Bank (18 mg kg^{-1} As in *Pagurus* and up to 21 mg kg^{-1} in *A. rubens*) (Whalley *et al.*, 1997), and in *A. rubens* from the outer German Bight (up to 16 mg kg^{-1}).

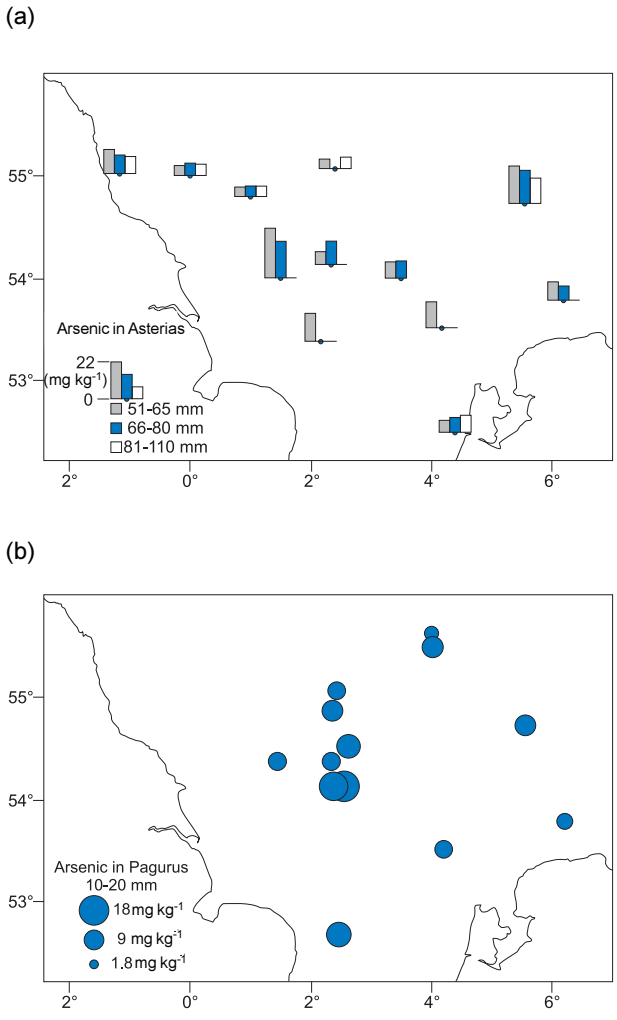

Figure 17. Arsenic concentrations (wet weight) in (a) Asterias in size ranges between 51 and 110 mm and (b) Pagurus

Arsenic concentrations in *A. rubens* collected off Norfolk averaged 12 mg As kg^{-1}, where the mean for all data shown was 9 mg kg^{-1} (95% confidence interval = 3.0). In *Pagurus*, the arsenic concentration averaged 13 mg kg^{-1} compared with an overall survey mean of 10 mg kg^{-1} (95% confidence interval = 1.7). After accounting for analytical variability, that for *A. rubens* was within the expected range of results, but that for *Pagurus* was slightly higher. Further data are required to confirm these findings; although the concentrations may be slightly elevated over the mean concentration, they are within the range found elsewhere in the southern North Sea.

Given the apparent geochemical relationship between arsenic and iron in sediments (Whalley *et al*., 1999), it was interesting that a linear relationship was found between iron and arsenic in *A. rubens* ($r^2 = 0.521$, n = 14, $P < 0.01$) and this may provide some indication as to the route of uptake of arsenic. This relationship was not observed in *Pagurus*.

BENTHOS

12. DEVELOPMENTS IN MAPPING SEABED BIOTOPES USING BIOLOGICAL AND REMOTE SENSING (ACOUSTIC) TECHNIQUES

12.1 Introduction

Recent advances in acoustic technologies are offering new insights and opportunities to explore and map seabed habitats. Benthic studies have traditionally used grabs and/or dredges to quantify the invertebrate fauna of the sea floor. The data generated from such techniques provides single, geographically separated points of data across the area of seabed under investigation. In order to produce biotope maps from such sources of data it is necessary to interpolate between these data points. However, interpolation has the potential to overlook discrete seabed features and/or biological assemblages, which may lie between sample stations. For this reason the use of acoustic techniques to assist in mapping the geographical distribution of biotopes (physical habitats and their associated biological assemblages) can be seen to have many potential advantages, including the prospect of 100% coverage of the seabed as resources allow or priorities dictate.

The production of high-resolution biotope maps of the seabed may assist in future site-specific environmental assessments of potential aggregate dredging areas, and would be of value during any subsequent environmental monitoring activities. For this reason, a programme of research funded by MAFF was initiated by CEFAS in April 1998 to investigate the utility of several acoustic remote sensing techniques, used in conjunction with biological sampling and underwater video surveys, for mapping biotopes on coarse substrates. Many of the techniques under development would also be of direct relevance to a number of other environmental management applications (e.g. environmental monitoring of disposal sites/SACs etc.). The account below reports on the progress to date in this project.

12.2 Evaluation of survey methodology

Selection of acoustic systems

The rapid pace of developments in acoustic technology in recent years has led to the availability of a wide range of affordable acoustic systems. As an essential precursor to meeting the objectives of the present project, a range of swathe bathymetric, side scan sonar and acoustic ground discrimination systems (AGDS) were evaluated for their potential as effective mapping tools. Many of these systems were deployed over the same area of seabed, which allowed a direct comparison to be made of each system's ability to efficiently characterise the physical nature of the sediments and associated features. Factors such as speed of survey, ease of deployment, cost of system and, most importantly, quality of out-put, were assessed and compared.

A digital side scan system and two AGDS systems were judged to be the most appropriate technologies to suit the requirements of the research programme relative to the resources available. Side scan sonar produces high-resolution images across a "swathe" of seabed, and can record textural and topographic features of the seabed at a distance of up to 200 m either side of the ships track. This has the potential, if needs require, to produce a detailed image of the seabed covering 100% of the survey area. In contrast, AGDS uses a single beam echo sounder to discriminate between different sediment properties and provide bathymetric data. Both AGDS systems only provide information on the area of seabed

directly below the ship. By using a combination of side scan sonar and AGDS over an area of seabed, along with strategically placed grab samples and underwater video deployments for ground-truthing, information about the nature and distribution of sediment types can be produced which is considerably more detailed than would be possible using conventional (e.g. non-acoustic) methods.

Pilot survey 1998 - Eastern Isle of Wight

Using a combination of acoustic techniques, an area of seabed 3 km by 13 km off the eastern coast of the Isle of Wight was intensively surveyed (Figure 18). Acoustically distinct areas of differing substrata and seabed features were identified (Figure 19) and this information was used to direct subsequent sampling. A variety of sampling devices (grabs, dredges and cameras) were used to collect biological and sedimentological data, in order to assess the most appropriate techniques for determining the varied habitats prevalent in the survey area. Successful collection of biological and sediment samples from areas of coarse substrata can be difficult due to the hard nature of the seabed. Previous studies of unconsolidated, coarse sediments have employed a limited number of dredges and grabs specifically designed to sample these substrata (Kenny and Rees, 1994; Cabioch, 1984), or have used non-destructive techniques such as video and photography to record sediment forms and epibenthos distributions (Service and Magorrian, 1997). A 0.1 m^2 Hamon grab was the primary tool selected for the collection of biological and sediment data from the preliminary survey area, due to its ability to collect samples from unconsolidated, coarse sediments. Samples were also collected using an anchor dredge, and video footage of the seabed was recorded using a drop camera frame fitted with an underwater camera.

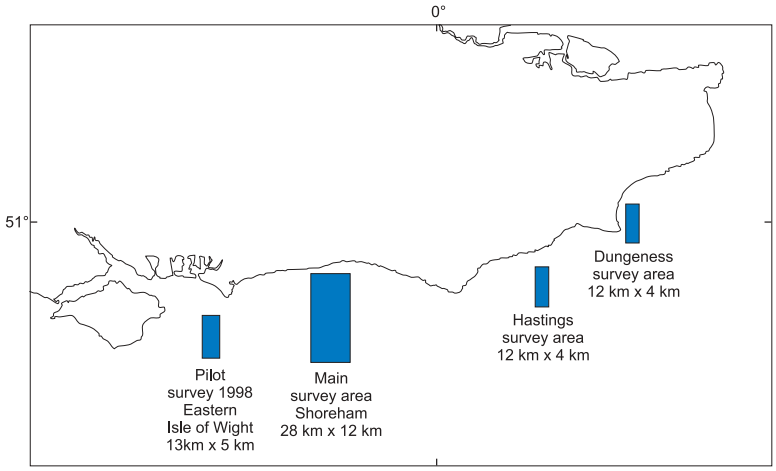

Figure 18. Location of Habitat Mapping surveys

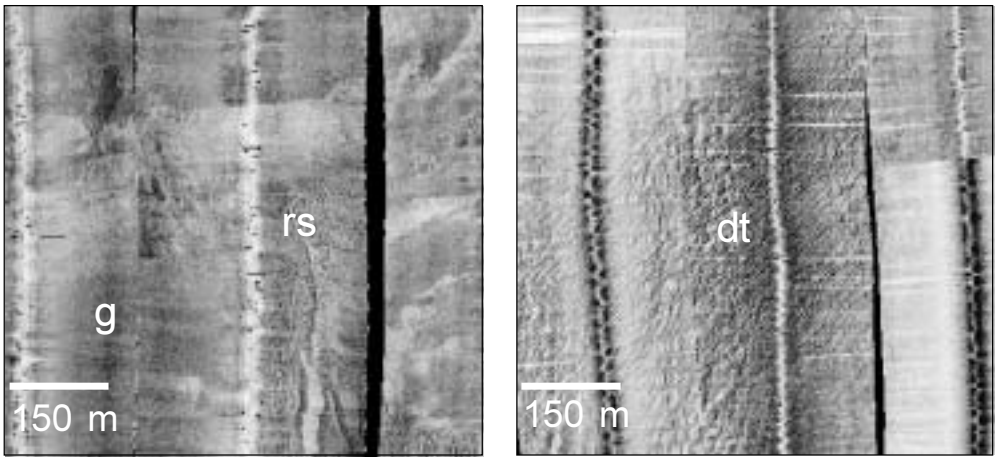

Figure 19. Side scan sonar images (EG&G DF100) illustrating examples of acoustically distinct substrate types: g - gravel; rs - rippled sand; dt - dredge tracks

Results from this preliminary survey revealed complex relationships between the fauna and habitat type derived from acoustic information, reflecting the small-scale variability of the sediments within the survey area, which is characteristic of the seabed in this region (Jenkinson, 1972). In order to widen the scope of the study, and to develop the biotope mapping methods further, follow up surveys were conducted at a number of other locations exhibiting a wider range of physical and biological gradients. The seabed at these locations included, in some cases, the apparent presence of distinctive boundaries between adjacent habitats of discrete, homogeneous sediment types. Such an approach is aimed to maximise the chances of a successful outcome in the evaluation of the use of acoustic and ground-truth methods for mapping the distribution and extent of biotopes. The outcome was also intended to provide a wider context for the occurrence of small-scale sediment heterogeneity, in areas such as the Eastern Isle of Wight, and the most appropriate means to account for this.

Main survey 1999 - Shoreham

An area of seabed in the English Channel off Shoreham (12 x 28 km), with strong biological and physical gradients, displaying a high level of sediment homogeneity within habitat boundaries, was selected as the main site for study. A further two smaller areas were also selected for study, one offshore from Hastings, and the other to the East of Dungeness (Figure 18). Both of these sites contain similar sediment types to those encountered off Shoreham, but have greater small-scale spatial complexity in the arrangement of their sediment types. The Shoreham and Dungeness areas were intensively surveyed in July 1999 using a combination of acoustic techniques. Treating each site separately, the acoustic data were used to divide each area into acoustically distinct regions, following a similar procedure to that used in the pilot survey. These distinct regions were then sampled during a follow up 'ground-truth' and biological survey in August 1999, using a suite of sampling techniques. An identical procedure was carried out at the Hastings site during surveys in October and November 1999.

Although processing of biological samples and accompanying data sets is not yet complete, a number of important considerations concerning suitable survey methodology for the mapping of seabed biotopes have become apparent. On several occasions during the pilot survey, there were discrepancies between the predicted sediment type determined from the acoustic records, and the actual sediment type collected during the physical sampling programme. In an effort to elucidate the reasons for these discrepancies, a Hamon grab was fitted with a video camera, positioned to obtain an image of the surface sediments of the seabed directly adjacent to the sampling bucket of the grab. This grab was used for the collection of biological samples during the 1999 surveys, and proved to be a useful tool. It revealed that thin surface deposits of one type of sediment can often 'mask' the dominant sediment type below. For example, sidescan and AGDS data may suggest that an area of seabed consists of sand, whereas a grab sample may reveal the substrate to be primarily gravel. This information can only be obtained using a combination of techniques (e.g. physical sampling combined with video footage), and such an approach becomes an essential procedure when attempting to map biotopes, due to a need to describe both the biological and physical attributes of the habitat.

Samples were also collected using a 'Rallier du Baty' dredge (Cabioch, 1984), a heavy-duty sampling tool that is suited for use on coarse substrates. Samples collected from the same area using a dredge differ from those collected using a grab due to the nature of the sampling gear. A dredge removes material across an area of seabed which can have the result of 'averaging' the associated biological and physical attributes, particularly in areas of spatially complex, heterogeneous substrates. This is in contrast to samples collected by grabs, which remove material from a single point on the seabed, and therefore have the benefit of sampling from one discrete biotope. By collecting samples using both the Rallier du Baty dredge and the Hamon grab, an assessment can be made of which technique is most suited for use in biotope mapping surveys.

Using a range of sampling techniques will also provide a comparison of how biotope interpretations vary depending on which sampling techniques are used to collect biological data. For example, the final biotope classification is strongly dependant on the type of gear used, with different techniques placing emphasis on different fractions of the biological assemblages (e.g. grabs collecting small infaunal organisms, dredges and trawls collecting larger mobile species). These issues and others will be addressed following the processing of biological samples and further analysis of acoustic and photographic data sets.

13. LABORATORY EXPERIMENTS ASSESSING THE EFFECTS OF THE DEPOSITION OF DREDGED MATERIAL ON THE STRUCTURE OF MEIOFAUNA ASSEMBLAGES

13.1 Introduction

The disposal of dredged material at sea in UK waters is licensed under the Food and Environment Protection Act 1985 Part 2 (Great Britain Parliament, 1985a). Criteria, which must be satisfied before a licence is issued, relate

to the chemical quality of the material, the quantity to be disposed of, its nature and origin and its predicted impacts at the disposal site.

Although chemical analyses of dredged material provide an indication of the relative degree of contamination, they do not provide a measure of any biological effects nor an estimate of the potential for such effects (Reynoldson and Zarull, 1989; Long, 1992; Somerfield *et al*., 1994; Langston *et al*., 1999). The assessment of the potential biological impacts of the deposition of dredged material often requires direct tests involving bioassays on individuals or populations of selected macrofauna species. However, results from single species bioassays cannot provide a full picture of the impact of contaminants on assemblages of organisms. Because of their close association with the sedimentary environment, high abundance and diversity, short generation times and benthic larvae, the meiobenthos are ideal organisms with which to study the effects of contaminants (Coull and Chandler, 1992). To date, there is no published information on studies assessing both the effects of the degree of sediment contamination, and the role of burial associated with the deposition of dredged material, which may have significant ecological effects independent of the effects of associated contaminants (Somerfield *et al*., 1995). Therefore, two microcosm experiments were conducted to evaluate the relative contribution of these factors to observed changes in nematode assemblages in response to the simulated disposal of uncontaminated and contaminated sediment under controlled laboratory conditions.

13.2 Collection of sediments

a. Uncontaminated estuarine deposits

Estuarine sand was collected from Shoebury (Outer Thames Estuary) at low water. A spade was used to a depth of 5 cm in order to transfer sand into a bucket. The poorly sorted sediment (s = 1.3 Φ) had a median particle diameter of 108 µm and consisted of 91% sand (63 µm - 2 mm) and 9% mud (<63 µm). The sediment contained 2.1% of total organic carbon.

Estuarine mud was collected locally from the Crouch Estuary at Creeksea. Mud from mean low water level was scraped into a bucket from the top 2 cm using a spade. The very poorly sorted sediment (s = 2.8 Φ) had a median particle diameter of 63 µm and consisted of 6% gravel (>2 mm), 36% sand and 58% mud. The sediment contained 1.2% of total organic carbon.

b. Contaminated sublittoral deposits

Sublittoral mud from areas proposed for dredging was collected from the River Mersey and the River Tees. A stainless steel van Veen grab (0.1 m^2) was deployed from a boat, sediment was then scooped into a bucket and transported to the laboratory. In contrast to the brown estuarine sediments, the Tees and Mersey sediments were black, indicating anoxia.

Sediment collected from the River Tees was poorly sorted (s = 1.8 Φ), had a median particle diameter of 63 µm and consisted of 66% sand and 34% mud. The sediment contained 6.1% of total organic carbon. The poorly sorted Mersey sediment (s = 2.0 Φ) had a median particle diameter of 63 µm and consisted of 73% sand and 27% mud. The sediment contained 3.1% of total organic carbon.

Concentrations of trace metals were considerably higher in the sediments from the Tees and Mersey compared to estuarine sediments. Values were generally higher in the Tees than in the Mersey sediment.

c. Estuarine mud and meiofauna

Intertidal estuarine mud along with the indigenous meiofaunal community was collected from Creeksea (see above). On return to the laboratory, sediment was homogenised by gentle hand-stirring with a large spatula. Sediment was allowed to acclimatise at a temperature of 15ºC for two hours before subsamples for the microcosms were taken.

13.3 Experimental set-up

Defaunation of deposits

Two weeks prior to setting up the experiments, deposits were defaunated. The sediments were thawed at room temperature for 48 hours and frozen to a temperature of -20°C for 12 hours. This process was repeated three times. Weighed amounts of defaunated sediment were then frozen to a temperature of -20°C until required for the experimental treatments.

Experimental microcosms

Experimental microcosms consisted of glass cylinders (internal diameter 4.5 cm, height 45 cm) closed with a rubber bung at the bottom. A 3 mm thick glass disc was placed on top of the bung to prevent direct contact between the sediment and the rubber material (Figure 20). 70 g (= 3 cm) of homogenised estuarine mud collected the same day was washed into the cylinders with filtered seawater of natural salinity (33 S).

All microcosms were run as closed systems with aeration for two months at a temperature of 15°C. To prevent microalgal growth, the experiments were conducted in the dark.

Experimental treatments

After an acclimatisation period of one week, defaunated deposit was added to the treatments in different frequencies up to an eventual depth of 6 cm. There were four treatments in Experiment 1 and six treatments in Experiment 2 with four replicates each (Table 19).

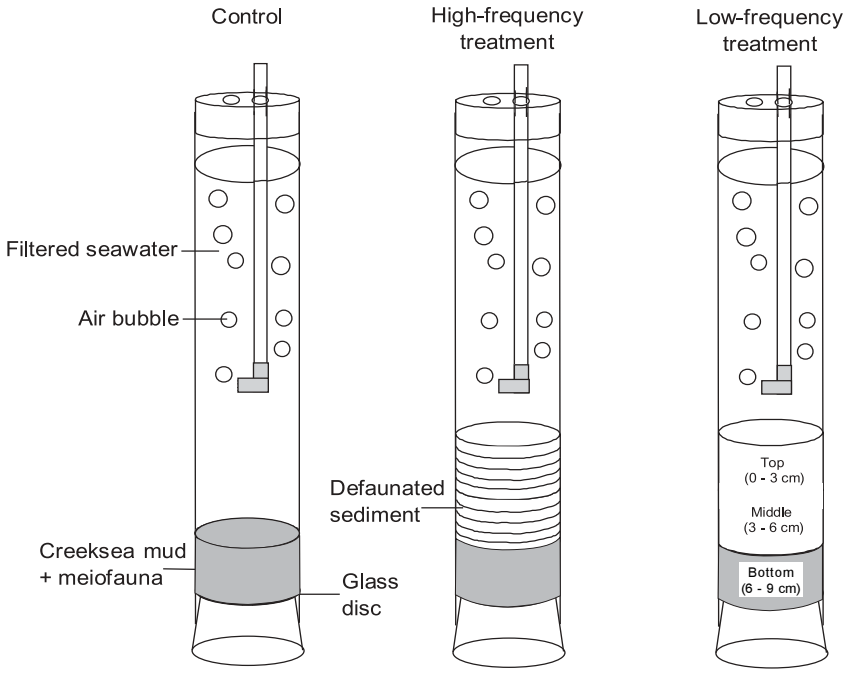

Figure 20. Schematic diagram of control microcosms and experimental treatments

Table 19. Experimental treatments

Code	Experimental treatment
Experiment 1:	
Control	None
S10	Estuarine Shoebury sand added in 10 small doses of 0.6 cm
S1	Estuarine Shoebury sand added in one single dose of 6 cm
C10	Estuarine Creeksea mud added in 10 small doses of 0.6 cm
C1	Estuarine Creeksea mud added in one single dose of 6 cm
Experiment 2:	
Control	None
C10	Estuarine Creeksea mud added in 10 small doses of 0.6 cm
C1	Estuarine Creeksea mud added in one single dose of 6 cm
T10	Tees sediment added in 10 small doses of 0.6 cm
T1	Tees sediment added in one single dose of 6 cm
M10	Mersey sediment added in 10 small doses of 0.6 cm
M1	Mersey sediment added in one single dose of 6 cm

Sediment colour, conductivity, pH and water temperature were recorded on 10 occasions during the experiments. Two thirds of the water was then siphoned out of each microcosm and defaunated sediment, which had been defrosted the previous day, was washed into the treatments with filtered seawater. All microcosms were then filled up with fresh filtered seawater. The treatment of microcosms ceased one week before the end of the experiments in order to allow the nematode assemblages to respond to the final addition of defaunated sediment.

At the end of the experiments after 58 days, the supernatant water in the microcosms was siphoned out carefully and the sediment cores from the treatments were sectioned into slices of 3 cm depth (top, middle, bottom layer, Figure 20). All samples were fixed in 4% formalin.

13.4 Sample processing

After washing the samples onto a 63 µm sieve, meiofauna was extracted with Ludox (McIntyre and Warwick, 1984; Somerfield and Warwick, 1996). The extraction was repeated three times. Subsamples of the extracted material (10%) were evaporated slowly in anhydrous glycerol and mounted evenly spread on slides for identification and counting. Nematodes were identified to genus or species level.

13.5 Data processing

Total number of individuals and species richness (Margalef's d) were calculated to describe nematode assemblage structure. Bartlett's test was used to test for homogeneity of variance. The significance of differences between treatments was tested applying one-way ANOVA. The Tukey HSD multiple comparisons test was used in pairwise comparisons of controls and treatments.

Non-parametric multi-dimensional scaling (MDS) ordination using the Bray-Curtis similarity measure was applied to species abundance data and analysis of similarities (ANOSIM) (Clarke, 1993) was performed to test the significance of differences in nematode assemblage composition between treatments. The similarity percentages program (SIMPER) was applied to determine the contribution of individual species to the average Bray-Curtis dissimilarity between treatments (Clarke and Warwick, 1994).

13.6 Results

Univariate analyses

The graphical summary of univariate indices for nematode assemblages from the controls and experimental treatments in Figure 21 shows a clear effect of the frequency of deposition in both experiments. Total nematode abundance was significantly higher in the controls than in the top sediment layer of all treatments except the treatments S10 and C10. Species richness in this layer was significantly lower in the low-frequency treatments than in the controls.

The deposition of uncontaminated estuarine sediment in multiple doses (treatments S10 and C10) did not significantly affect univariate indices in the top 3 cm of the sediment. All univariate indices were significantly higher in the top layer of the high- than in the low-frequency treatments, whereas significant differences between Tees and Mersey treatments of similar deposition frequency were not detected.

In the middle and bottom sediment layer, significant reductions in univariate measures occurred for most treatments compared with control microcosms. Univariate measures were lower in the treatments than in the controls.

Multivariate analyses

The MDS ordination for nematode assemblages in the controls and the top sediment layer (0 – 3 cm) of the treatments based on untransformed species abundance data is presented in Figure 22. Type of deposited sediment and frequency of deposition strongly affected migration and survival rates of nematodes. High- and low-frequency treatments cluster separately from each other.

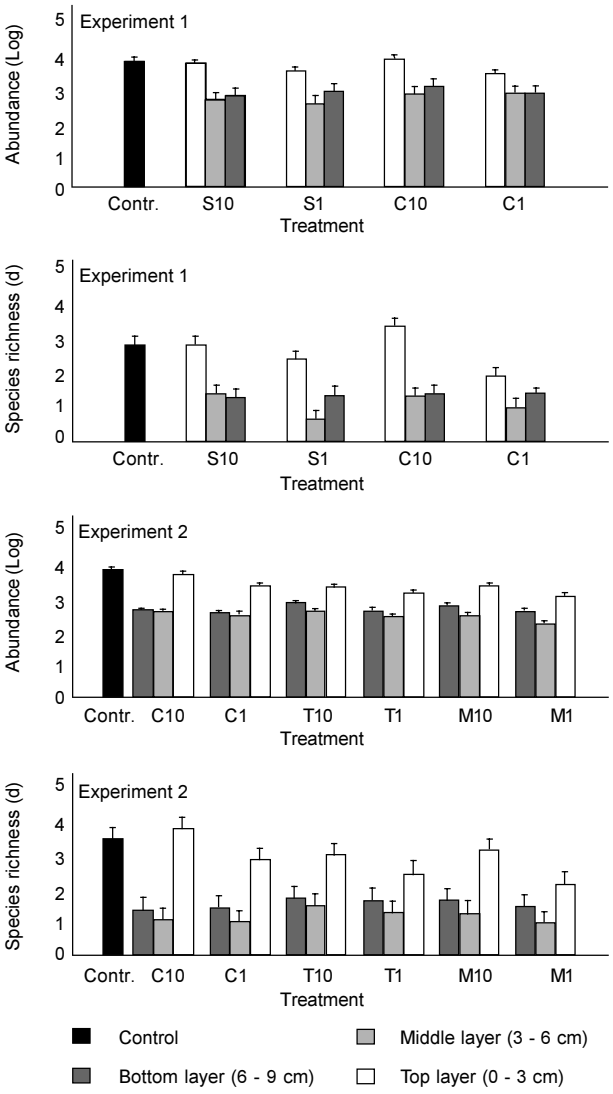

Figure 21. Graphical summary of means and 95% pooled confidence intervals of univariate indices for nematode assemblages from controls and experimental treatments. Codes for microcosms as in Table 19

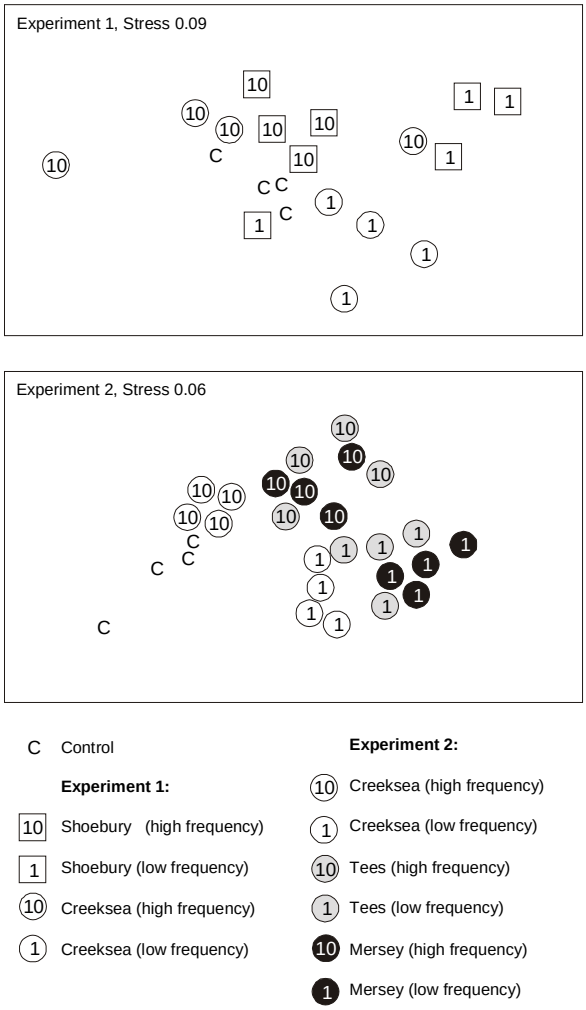

Figure 22. Multi-dimensional scaling ordination for experimental microcosms based on untransformed species abundance data. Codes for microcosms as in Table 19

In Experiment 1, nematode assemblages from the top sediment layer (0 – 3 cm) of the high-frequency treatments S10 and C10 were more similar to those extracted from the controls than their respective low-frequency treatments S1 and C1. Most of the changes in assemblage structure, however, were not statistically significant at $p < 0.05$ (Table 20).

Results from Experiment 2 reveal a significant effect of the experimental treatments on the structure of nematode assemblages (Table 20). All treatments were significantly different from the controls and all treatments except the high-frequency treatments T10 and M10 and the low-frequency treatments T1 and M1 were significantly different from each other.

Table 20. Dissimilarities [%] between nematode assemblages from the top sediment layer (0-3 cm) based on untransformed species abundance data. * denotes significant difference at p < 0.05

Experiment 1:

	Control	S10	S1	C10
S10	32*			
S1	47	46		
C10	39	42	52	
C1	40*	42*	44	53

Experiment 2:

	Control	C10	C1	T10	T1	M10
C10	40*					
C1	60*	53*				
T10	62*	48*	49*			
T1	70*	60*	36*	47*		
M10	61*	45*	47*	37	44*	
M1	76*	69*	45*	54*	32	51*

Results from the SIMPER analyses reveal that significant differences between nematode assemblages from the controls and those from the top sediment layer of the treatments mainly resulted from changes in the abundances of dominant species, including *Sabatieria punctata*, *Aponema torosa* and *Terschellingia communis* in Experiment 1 and *Viscosia viscosa*, *S. punctata* and *Molgolaimus demani* in Experiment 2. These species responded characteristically when exposed to the deposition of uncontaminated estuarine sand and mud and contaminated sediment from the Tees and the Mersey.

Survival and migration rates of *Terschellingia communis* were significantly higher in native estuarine mud than in estuarine sand. Numbers of most species were higher in the uncontaminated than the contaminated treatments. In contrast to most species, the numbers of *Sabatieria punctata* were higher in the low-frequency treatments than in their respective high-frequency treatments.

Abundances of all species except *Molgolaimus demani* were significantly higher in the top sediment layer of the experimental treatments than in the middle and bottom layer.

13.7 Discussion

Results from univariate and multivariate data analyses showed that most nematode species were capable of migrating into uncontaminated estuarine sand and mud and sublittoral Tees and Mersey sediment which was contaminated with heavy metals. As evident from the MDS ordinations, the type of deposited sediment and the frequency of deposition strongly affected nematode assemblage structure. Migration and survival rates were highest in native estuarine mud and higher in high- than in low-frequency treatments.

In contrast to the oxic, organic-poor estuarine sediments, the sediments collected in the Tees and the Mersey were anoxic, organic-rich muds which have often been reported to be sites of accumulation of trace metals (Hirst and Aston, 1983). Assemblages from the Tees and Mersey treatments were less similar to the controls than those from the Shoebury and Creeksea treatments. This could have been due to relatively high metal and/or low oxygen concentrations in the contaminated sediments.

Differences in assemblage structure were more pronounced when the same type of sediment was deposited in different frequencies than when different sediment types were deposited in similar frequencies. This indicates that the nematodes were more affected by the frequency of deposition than by the type of deposit. To a certain extent, the high-frequency treatments simulated natural deposition in hydrodynamically active environments with small volumes of sediment deposited at regular intervals. Derived from an estuarine intertidal environment, Creeksea nematodes are adapted to tolerate regular burial caused by shifting sediments. In the low-frequency treatments, however, the depth of the deposit exceeded the ability of some species to migrate to the sediment surface.

The concentration and bioavailability of trace metals is strongly influenced by a suite of physical, chemical and biological factors in the sediments, including the adsorption to organic matter and occlusion in iron and/or manganese oxyhydroxides (Reynoldson, 1987; Bryan and Langston, 1992; Long, 1992; Comber *et al*., 1995; Jones and Turki, 1997; Chapman *et al*., 1998; Langston *et al*., 1999).

Although not measured in the microcosm experiments, iron and manganese concentrations are usually high in Mersey and Tees sediments (Hirst and Aston, 1983; Langston, 1986; Bryan and Langston, 1992) which will, together with high organic content, have reduced the

bioavailibility of metals. Reduced bioavailability of metals and the tolerance of estuarine nematodes to metal contamination (Howell, 1982; Millward and Grant, 1995; Austen and Somerfield, 1997) might explain the relatively high migration and survival rates of most nematode species in the Tees and Mersey treatments.

The microcosm experiments provided a means of assessing the response of nematodes to the simulated deposition of uncontaminated and contaminated sediment under replicated, controlled and repeatable conditions. Nematodes showed a clear species-specific response depending on the frequency of deposition and the chemical quality of the deposited material (e.g. metal and oxygen concentrations). The observed trends demonstrate the potential of microcosm experiments in simulating 'worst case' scenarios at disposal sites and in testing the quality of contaminated dredged material at the licensing stage i.e. prior to disposal.

14. THE USE OF SEDIMENT BIOASSAYS TO ASSESS THE QUALITY OF MARINE SEDIMENTS

14.1 Introduction

Since 1992, the toxicity of sediments has been assessed using whole sediment bioassays, using organisms that live in and feed directly on the sediment. These features make them a better indicator of toxicity than techniques previously applied; prior to 1992 the oyster embryo bioassay was used which involved 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 experienced by sediment dwelling animals.

Two whole sediment bioassays were developed using the polychaete lugworm *Arenicola marina* and the crustacean amphipod *Corophium volutator,* and were deployed for the first time aboard a MAFF research vessel in 1992. After the success of the initial trials reported in MAFF (1993 and 1994) and Thain *et al.* (1996), the bioassays were used between 1992 and 1995 at most of the intermediate and offshore NMMP sites and some additional locations in estuaries. Since 1996 the samples have been taken on board research vessels and stored, either refrigerated or frozen, then brought back to the laboratory and bioassayed within a week.

14.2 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 a negative control.

14.3 Materials and methods

14.3.1 *Arenicola marina*

Arenicola marina, commonly known as the lugworm, is a surface deposit-feeding polychaete which inhabits intertidal and subtidal areas. 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. Every day during the exposure period the number of casts produced on the surface of the sediment was counted to obtain a measure of the feeding rate of the worms; a sublethal endpoint as opposed to an acute endpoint. The casts were smoothed over after each count.

14.3.2 *Corophium volutator*

Corophium volutator is a marine amphipod, which can be found on the foreshore of most unpolluted estuaries in the UK (Lincoln, 1979). Animals were collected from a nearby muddy shore on the River Crouch estuary, adjacent to the laboratory. They were collected and sieved from their native sediment and maintained in a 40 litre aquaria with a layer of sediment, running seawater and aeration for a minimum period of five days before the start of the test. When the sediment samples had been thoroughly defrosted and homogenised they were 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 aerated. The beakers were then left for a further 24 hours. For each test beaker, ten adults (4-6 mm in length) were added. After 10 days the contents of each test beaker were sieved and the number of surviving amphipods recorded.

14.4 Results

RV CIROLANA, 19 June – 7 July 1998

A total of 25 sediment samples were tested for toxicity using both the *C. volutator* and *A. marina* bioassay. Results are shown in Table 21. Only one sample (Stn 72) showed significant acute toxicity to *C. volutator* (17% mortality); this was a sample taken at Rotterdam Transect 1. Two sediments, Rotterdam Transect 1 and Inner Wash, produced a significantly lethal response compared to the control in *A. marina* (100% mortality in both cases), but sediments from a total of 13 stations significantly reduced feeding rate compared to the control.

14.5 Discussion

Station 72 (Rotterdam Transect 1) significantly reduced survival of both *A. marina* and *C. volutator*. In addition, station 113 (Inner Wash) also significantly reduced survival of *A. marina*. However, these results are most likely due to the nature of the substrate, rather than any toxicity, as both sediment samples consisted of gravel sized particles of shell and stone.

At both stations the majority of *A. marina* buried into the sediment at the start of the test, but were unable to feed (no casts were formed on the surface) or build burrows, therefore, they subsequently resurfaced and died. Although there was a statistically significant effect on survival of *C. volutator* at station 72, the mean mortality was only 17% and cannot be considered to be biologically significant. The losses were probably due to a less than optimum substrate particle size distribution. As expected, feeding behaviour in *A. marina* was the most sensitive of the endpoints used. Overall, the sediments sampled were, generally, of low toxicity and exerted only sublethal effects, indicating low to moderate contamination.

Table 21. RV CIROLANA, 19 June-7 July 1998: 10-day sediment bioassays using the lugworm Arenicola marina and the amphipod Corophium volutator

NMP station	Station	Actual position		Location	*Corophium*	*Arenicola*	
					% mortality	% mortality	Mean daily no. of casts
				Shoebury Sands (Control)	0	7	3.6
	1	51° 32.974'N	3° 52.459'W	Swansea Bay (Inner dredge spoil ground)	3	13	*2.73
	2	51° 32.188'N	3° 55.147'W	Swansea Bay (Mid dredge spoil ground)	3	0	*1.7
705	52	53° 28.310'N	3° 15.634'W	Burbo Bight	3	53	*1.3
776	56	53° 21.746'N	4° 09.234'W	Red Wharfe Bay	0	0	3.1
	72	51° 29.738'N	2° 55.416'E	Rotterdam Transect 1	*17	*100	*0.03
	81	53° 25.827'N	3° 47.950'E	Arsenic 6	3	20	3.2
	82	53° 26.032'N	4° 41.370'E	Rotterdam Transect 2	7	13	*2.6
	84	54° 04.150'N	8° 07.100'E	Bremerhaven 1	0	7	*2.7
	85	54° 43.811'N	5° 33.140'E	Bremerhaven 7	0	7	3.3
285	87	54° 47.414'N	1° 14.111'E	West Dogger	10	20	3.2
	90	55° 15.700'N	1° 15.600'W	Amble	3	13	3.5
295	99	54° 42.884'N	0°52.842'W	Tees Bay	3	0	3.5
				Shoebury Sands (Control)	0	7	3.1
	57	51° 15.070'N	5° 59.850'W	Celtic Deep	0	7	3.5
	71	50° 56.070'N	1° 16.900'E	South Varne	10	0	2.6
	73	51° 58.305'N	3° 50.902'E	Rotterdam Transect 3	3	0	3.6
	84	54° 04.150'N	8° 07.100'E	Bremerhaven 1	7	0	*2.1
	96G	54° 59.520'N	1° 15.983'W	Tyne spoil ground	3	13	3.1
	96H	55° 01.535'N	1°17.005'W	Tyne spoil ground	0	7	*1.57
	113	53° 09.100'N	0° 34.500'E	Inner Wash	3	*100	*0
				Shoebury Sands (Control)	7	13	3.5
665	6	52° 23.633'N	4° 53.517'W	Outer Cardigan Bay	13	13	*0.96
805	45	54° 00.008'N	3° 49.986'W	SE Isle of Man	20	7	4.1
715	55	53° 29.883'N	3° 41.435'W	Liverpool Bay	20	7	*2
	70	50° 52.050'N	0° 48.353'E	Rye Bay	3	13	*1.29
	86	55° 31.393'N	4° 10.975'E	Bremerhaven 9 (North Tail Dogger)	13	0	3.9
	111	54° 02.232'N	1° 45.443'E	Off Humber NMP	0	20	*1.17

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

15. CHLOROBIPHENYL CONTAMINANTS IN DREDGED SEDIMENT IN THE 1990s

15.1 Introduction

The removal of sediment by dredging operations and the disposal of material to sea requires a licence under the Food and Environment Protection Act, part II (1985) (CEFAS, 1997). Before a licence is granted to deposit material to sea, certain chemical contaminants in dredged sediments are analysed and a risk assessment is undertaken to determine the potential hazard of the deposit to the marine environment. The assessment of each licence requires careful consideration of a variety of physical parameters (e.g. geological origin, hydrological cycling and sedimentation patterns), biological impacts (e.g. disturbance of ecosystems including species diversity and abundance) and chemical contamination (levels of both organic and inorganic contaminants). As a consequence, a range of contaminants have been measured in dredged sediments at a number of sites around England and Wales. There is now, as a result of this large amount of information, an extensive dataset pertaining to PCB concentrations in dredged sediments. These compounds were chosen for review because they are regarded as high risk chemicals due to their persistence and ability to bioaccumulate (Shifrin and Toole, 1998). These chemicals are classified as hazardous substances (OSPAR, 1998).

The purpose of this review is to establish the spatial distribution of PCBs in dredged sediments in England and Wales between 1990 and 1999, and to calculate an average concentration of PCBs in dredged sediments in estuaries and at other sites in the coastal zone. Using this information, it may be possible to indicate sites where PCB levels have changed over time and potential 'hotspots' of historic contamination.

A summary of licensing activity, in particular, chlorobiphenyl (CB) contaminants in dredged sediments between 1990 and 1994 was presented in CEFAS (1997). This review identified not only areas where high concentrations of CBs were found but also industrial areas exposed to local or prolonged discharges of PCBs. The review also provided an initial record of changing CB concentrations over time. However, at some sites, these changes were difficult to determine over such a short timescale because of the nature of sampling under FEPA (1985) (CEFAS, 1997). A review of biphenyl concentrations over the last decade will provide a longer timescale to identify changes of CB concentrations in dredged sediments around England and Wales.

15.2 Methods

The guidelines for sampling dredged sediments are provided by the Oslo Paris Commission (OSPAR, 1993). The sampling of dredged sediments is required when an applicant applies for a new licence or a renewal of a licence. The guidelines recommend sampling every 3 to 5 years depending on the perceived environmental sensitivity (CEFAS, 1997) although in some instances sampling is carried out annually. Due to the nature of this sampling, not every site is sampled annually or in the same location (CEFAS, 1997). The sample locations are selected by CEFAS to reflect areas where sediments are fine enough to retain contaminants and are close to known or potential point sources. The sampling is also directed towards encompassing a range of sites within the proposed dredged area.

The concentration of chemicals at selected sampling sites are therefore not typical of background UK levels of contaminants. These sediments represent the more highly contaminated sites being located close to navigation activities, agriculture, industry or areas that have a historic legacy of contamination from past industrial processes and activities. The dredged sediments therefore represent material that may contain elevated levels of some contaminants.

The analytical methodology used to determine CBs in dredged sediments has broadly remained the same since 1990, except for minor modifications, and a comparison of data over this timescale is therefore possible (Allchin *et al*., 1989). The methodology is widely published and CEFAS has been involved with improving the quality of the analytical chemistry it provides by being actively involved with quality control workshops such as QUASIMEME. The detection limit for individual congeners is 0.2 $\mu g\ kg^{-1}$ but is lower for some individual congeners. A total of 25 separate CB congeners ($\sum$25CBs) are routinely determined in dredged sediments prior to licensing a disposal. These are CB#18, CB#28, CB#31, CB#44, CB#47, CB#49, CB#52, CB#66, CB#101, CB#105, CB#110, CB#118, CB#128, CB#138, CB#141, CB#149, CB#151, CB#153, CB#156, CB#158, CB#170, CB#180, CB#183, CB#187 and CB#194.

15.3 Results

Since 1990, there have been a total of 922 measured concentrations of ∑25CBs in dredged sediments at various sites around England and Wales (Reed *et al*., 2000a). The concentrations of biphenyls measured in dredged sediments at these sites provides information of the spatial distribution of ∑25CBs in inner estuaries, marinas, docks, harbours, ports and other industrialised areas in the coastal zone around England and Wales.

The location of samples taken under FEPA, Part II (1985) have been subdivided into regions around England and Wales. These regions were defined as follows: Northeast England, East England, South England, South Wales and Northwest England, and these are presented in Figure 23. Within each region the data were separated into two areas: the outer estuary (light circles) and inner estuary sites (dark circles). The inner estuary sites included docks, harbours, marinas and ports, and outer estuary sites were generally located along the coastline. The separation of the data is intended to identify sites that are located close to industrialised areas (e.g. docks, marinas and harbours) and, as such, were potential sources of contamination in the coastal zone.

The annual median concentrations of ∑25CBs were calculated to investigate temporal changes of PCBs in dredged sediments in estuaries and at sites in the coastal environment at locations around England and Wales. The median concentration of ∑25CBs ($\mu g\ kg^{-1}$) and the standard deviations (half of the difference between the 25 and 75 percentiles) in dredged sediments within each region are presented in Table 22. Where sampling occurred more than once at a site, the change (%) of ∑25CBs was calculated for these sites.

There were several sites around England and Wales, both in the inner and outer estuaries, where concentrations of ∑25CBs in dredged sediments had reduced over time. These sites included: River Tyne, Seaham, River Orwell, Plymouth, Wareham, Pembrokeshire coast, outer River Taff, outer River Usk (Newport), Swansea Docks and River Mersey. The largest reductions of ∑25CBs were in the River Orwell, Pembrokeshire coast and River Mersey. At some sites, however, concentrations of ∑25CBs in dredged sediments were highly variable making it difficult to establish obvious trends over time.

In contrast, several sites showed an increase in median concentrations of ∑25CBs over the last decade. These sites were located in the River Blyth, River Tees, River Humber, Langstone Harbour, Dover Harbour, Poole, Solent, Southampton Water, Avonmouth, Barry Docks, Cardiff Docks, Milford Haven, Newport Docks, Barrow-in-Furness and Heysham. There was a wide range of median concentrations at all these sites (0–188.4 ± 106.4 $\mu g\ kg^{-1}$ dw) and even where there were increases in ∑25CBs concentrations, some sites contained quite low levels (Reed *et al*., 2000b).

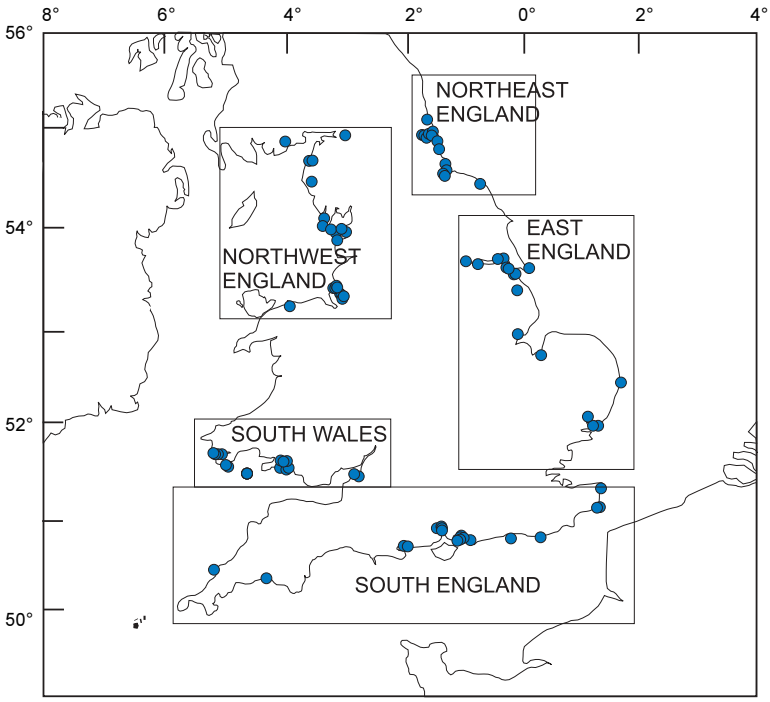

Figure 23. Locations of regions and sites where dredged sediments were sampled in England and Wales between 1990-1999 under FEPA, Part II (1985)

The geographical and temporal patterns of ∑25CBs in dredged sediments between 1990 and 1999 are presented in Figure 24. The guidelines proposed by Wells *et al*. (1989) were employed to assess the contamination levels in sediments (CEFAS, 1997). The highest concentrations of ∑25CBs in dredged sediments were measured in the Rivers Blyth, Tees, Tyne, Mersey, Orwell and Humber and in dockyards in Swansea, Barry, Cardiff, Newport and Avonmouth. The highest concentration of ∑25CBs was 3.5 mg kg^{-1} measured in dredged sediments in the River Blyth (Reed *et al*., 2000b). The proportion of sites where dredged sediments contained ∑25CBs >100 µg kg^{-1} was approximately one third during the last decade. Between 1990 to 1999, the number of individual sites with ∑25CBs >100 µg kg^{-1} had increased, especially over the last 5 years.

The average (mean) concentration of ∑25CBs for sites located in the industrialised areas, including docks, harbours, ports and marinas was 86.50 ± 250.7 µg kg^{-1} (n = 800). In contrast, the average (mean) concentration of ∑25CBs at sites located in the coastal zone was 77.7 ± 117.1 µg kg^{-1} (n = 122).

15.4 Discussion

There was a wide range of ∑25CBs measured in dredged sediments and concentrations were highly variable between regions around England and Wales. The highest concentrations of ∑25CBs were consistently in the Northeast, South Wales and Northwest England. These areas were highly industrialised in the past and have a legacy of contamination spanning many years and in some cases many decades. Today, these areas are still considered to be hotspots of contamination due to historic processes, application, use and disposal (Proudfoot, *pers. comm*., 1998).

The widespread and continuously high concentrations of ∑25CBs in South Wales was investigated by CEFAS and the Environment Agency and a review of dockyard procedures was undertaken by Associated British Ports. Further investigations at sites locally have been undertaken (Reed and Waldock, 1998; Reed *et al*. 2000c). Sea disposal is only acceptable for low level concentrations and therefore dredging sites that contain high and consistently high PCB levels have limited options for disposal of the dredged material. In these situations, it is important to investigate whether contamination is historic or is a result of continuing practices locally.

In 1994, the elevated concentrations in the River Blyth, in the Northeast region, was a specific case where current shipbreaking practices resulted in sediments being heavily contaminated with PCBs in a particular section of the river. This area was excluded from the sea disposal licence and the contaminated sediments were removed to landfill (CEFAS, 1997). Concentrations of PCBs were reduced in a similar way in the River Orwell following a specific contamination incident.

The increasing percentage of sites with ∑25CBs >100 µg kg^{-1} around England and Wales does not reflect the general trend of declining PCB concentrations in other countries (Pavlou *et al*., 1982; Laane *et al*., 1999; Venkatesan *et al*., 1999). The number of sites containing high concentrations of ∑25CBs is not representative of sediment quality generally in England and Wales (Reed *et al*., 2000b). This is a consequence of the sampling design which is directed towards sediments containing the highest possible concentrations of contaminants. However, the consistently high concentrations of PCBs in sediments over long timescales is a concern but is not surprising due to the persistence of these compounds (Jones and de Voogt, 1999). The concentrations of ∑25CBs in dredged sediments at some sites provides further evidence that PCBs are not declining over this timescale. The lack of degradation of PCB concentrations was also recorded for PCB concentrations measured in sewage sludge in the Firth of Clyde, UK (Kelly and Ball, 1995; Kelly and Campbell, 1995).

15.5 Conclusions

PCB concentrations in dredged sediments between 1990 and 1999 have shown that there are a number of sites that have decreasing concentrations of PCBs over the last decade and this is encouraging. However, there are sites that have been identified where there are consistently high or increasing concentrations of PCBs. These sites are often located in or close to industrialised areas. The wide range of PCB concentrations in dredged sediments illustrates how spatially variable the levels are in sediments both locally and in regions around England and Wales.

In areas where PCB concentrations are high, additional sampling was undertaken to investigate the extent of contamination locally. In some cases, extensive surveys have established areas of contamination and PCB contaminated sediments have been removed from these areas prior to dredging. Further monitoring of heavily contaminated sites is required to determine the spatial distribution of PCBs, the congener profiles and changes of PCB concentrations over time (Reed *et al*., 2000c). These compounds will remain a problem for future monitoring of dredged sediments and DEFRA will need to continue to measure PCBs before a licence is approved for sea disposal.

Table 22. Median PCB Concentrations (µg kg^{-1}) and standard deviations (half of the difference between the 25 and 75 percentiles) in surficial dredged sediments sampled in outer and inner estuaries under FEPA (1985) between 1990-1999

Region	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	% Reduction
NORTHEAST ENGLAND											
Outer Estuary											
Hartlepool				11.7±2.5 (4)							-
Off Blyth			80.9±21 (5)			52.7±37.3 (10)	159.2±106.4 (15)				I
Off Seaham				73.4±40.1 (3)					8.7±5.8 (4)		88
Inner Estuary											
River Blyth			71.7±12 (32)						83.7±56.8 (10)		I
River Esk, Whitby				10.3±2.4 (2)							-
River Tees						0±0 (6)	121.7±57.3 (8)	15.2±5.4 (9)	71.2±31.5 (17)		I
River Tyne		36.4±1.7 (2)	31.2±0 (1)	81.7±109.7 (11)		34.9±6.3 (8)		58.3±7.5 (3)	25.9±15.6 (29)		29
River Wear				18.3±2.8 (6)							-
EAST ENGLAND											
Outer Estuary											
Off Lowestoft (R. Waveney)				3.1±6.6 (3)							-
Off River Humber	6±0 (2)										-
Inner Estuary											
King's Lynn, Great Ouse (The Wash)				10.1±8.8 (3)							-
River Humber			28.3±7.9 (13)	26.5±15.4 (13)	31.2±9.1 (21)	133.6±0 (1)			28.4±3.4 (9)		I
River Orwell				28.4±9.3 (20)	1.7±1.3 (23)						94
The Haven, Boston (The Wash)					3.8±1.3 (6)						-
SOUTH ENGLAND											
Outer Estuary											
Off Eastbourne						2.7±1.5 (6)					-
Off Langstone Harbour			11.8±5.1 (6)		25.3±5.2 (3)						I
Off Newhaven				0.4±0.2 (2)							-
Inner Estuary											
Chichester Harbour					7.23±0 (1)						-
Dover (Harbour)					0.3±0.9 (10)	1.85±1.3 (7)					I
Emsworth Yacht Harbour, Chichester									34.5±1.6 (5)		-
Exmouth					34.5±6.9 (2)						-
Gosport									25.03±19.7 (16)		-
Holesbay									46.30±10.4 (3)		-
Langstone Harbour				12.6±0 (1)	8.5±7.4 (30)	16.3±19.8 (8)	14.8±1.2 (6)	32.0±29.8 (12)			I
Newquay									48.3±26.2 (6)		-
Plymouth			39.2±8.6 (2)	26.7±5.3 (23)	21.2±8.4 (41)	13.3±2.9 (4)	26.2±4.1 (4)	26.8±2.6 (15)		11.9±12.2 (4)	70
Poole								10.9±8.4 (10)	345.4±229.4 (5)		I
Port Solent Marina										8.1±0.7 (4)	
R. Stour				11.7±0 (1)							-
River Stour, Kent (Nr. Sandwich)			3.6±0 (1)								-
Solent									5.2±1.6 (4)	27.2±4.8 (5)	I
Southampton Water			12.8±0 (1)	30±0.7 (3)	31.7±10.2 (13)			13.2±1.9 (2)			I
The Manacles					188.4±32.3 (4)						-
Tyneham						63.9±34 (7)					-
Wareham							26.6±0 (1)	6.4±1.8 (3)			76

Table 22. continued

Region	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	% Reduction
SOUTH WALES											
Outer Estuary											
Off Carmarthen Bay			105.5±34 (6)								-
Off Mumbles								103.9±2.2 (4)			-
Off Pembrokeshire Coast						76.4±81.4 (5)		0±0.3 (9)			100
Off River Taff, Outer Cardiff Bay					111.4±0.1 (2)			97.5±0 (1)	109.4±0 (1)		2
Off River Usk, Newport					123.6±23.7(4)			96.3±3.1 (2)	101.6±3.5 (3)		18
Port Talbot Approach Channel					53.7±3 (2)						-
Swansea Approach Channel					50.8±3.2 (3)	53.2±8.7 (8)	123± 4.2 (3)	51.9±9.2 (4)	48.9±0 (1)		4
Inner Estuary											
Avonmouth			54.6±0 (1)			77.2±4.6 (4)					I
Barry Docks				70.1±1 (2)		187.8±21.8 (4)					I
Cardiff Docks					126.1±12.5 (30)			122.1±23 (17)	133.4±29.7 (13)		I
Milford Haven				3.5±1.8 (2)		0±8.8 (8)	12.7±0 (1)				I
Neath								1.3±0 (1)			-
Newport Docks					131.2±8.9 (6)			131.5±26.4 (10)	141.4±40 (15)		I
Port Talbot					57.7±4.8 (3)						-
Swansea Docks					125.4±27 (8)			156.8±101.7 (7)	100.6±24.6 (16)		20
NORTHWEST ENGLAND											
Inner Estuary											
Barrow-in-Furness					0.6±3.6 (6)				5.5±0.4 (2)		I
Conwy								10.8±2.7 (6)			-
Heysham				1.3±1.5 (4)					8.1 ±0(1)		I
Maryport					4.0±2.1 (7)						-
Off Heysham									5.6±0 (1)		-
River Lune				2.3± 0 (1)							-
River Mersey	69.0±13.5 (13)	50.1±1.8 (3)	56.1±16.6 (13)		93.1±53.3 (26)	139.8±41.8 (5)		164.0±710.2 (3)	54.8±22.4 (32)	5.3±0.8 (2)	92
River Wyre				0±2.7 (3)							-
Solway									9.5±1.2 (2)		-
St. Bees Head							12.8±5 (5)				-

- = Only one observation
I = % Increase
In brackets, number of samples analysed.

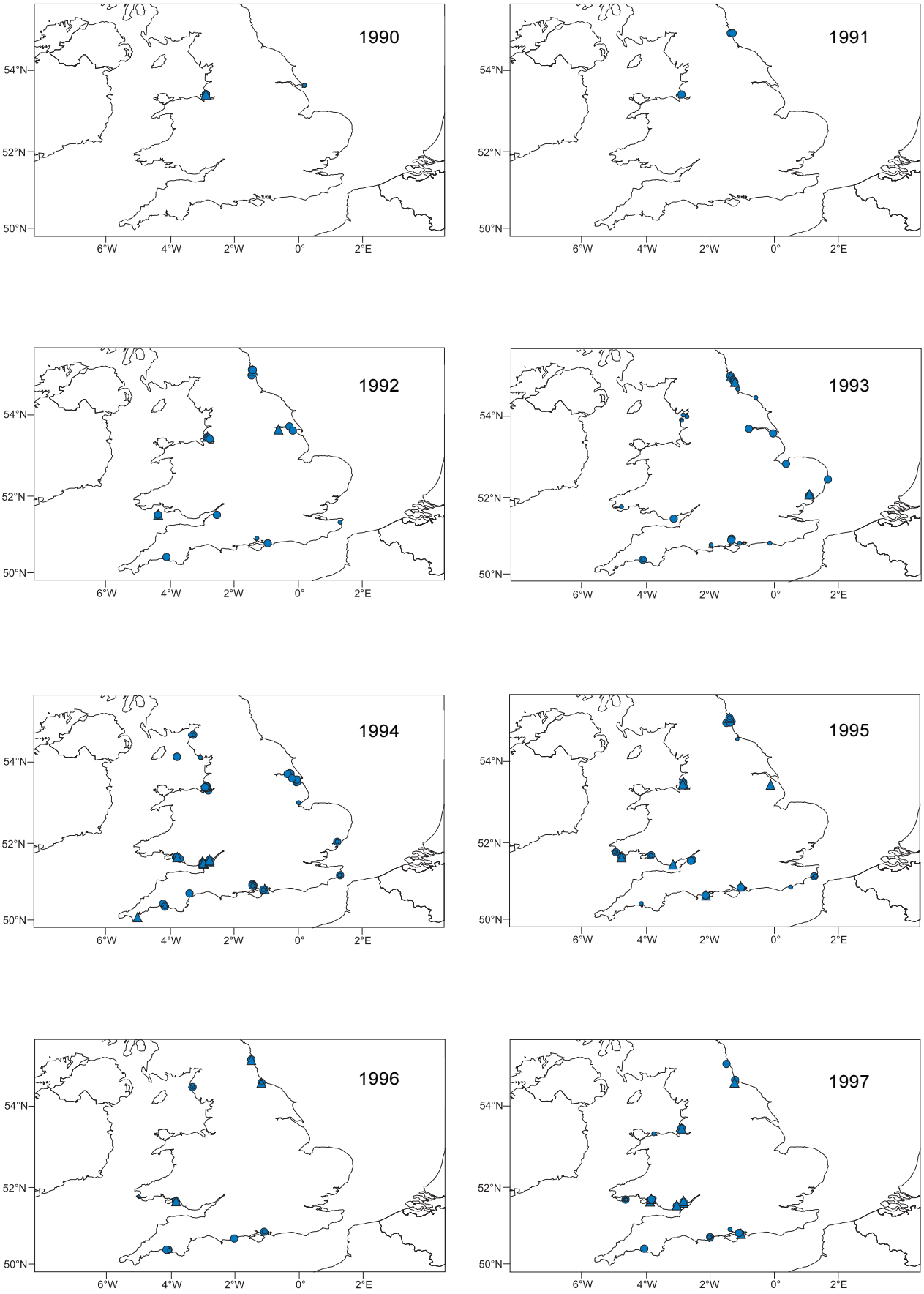

Figure 24. Distribution of Σ25CBs in dredged sediments in England and Wales between 1990 and 1999

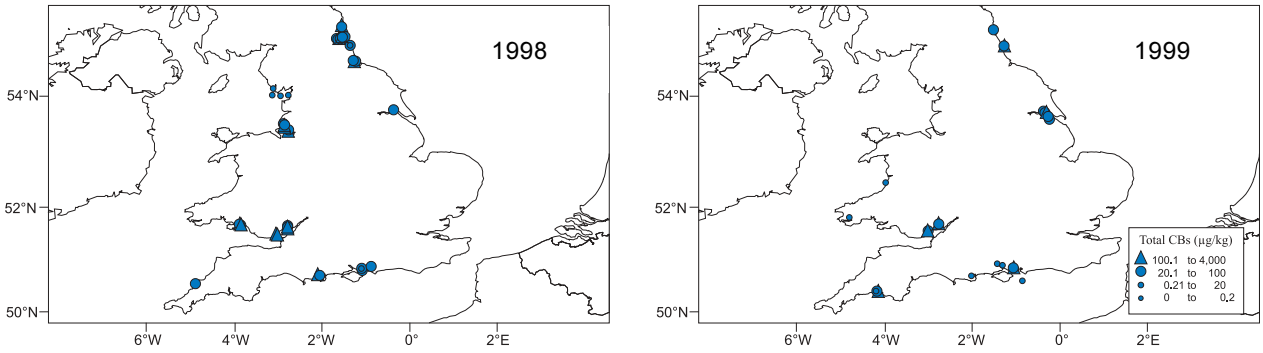

Figure 24. continued: Distribution of Σ25CBs in dredged sediments in England and Wales between 1990 and 1999

16. LICENSING OF DEPOSITS IN THE SEA

16.1 Introduction

This section gives information about the licensing of deposits in the sea around the coasts of England and Wales during 1998 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.

16.2 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). During the period covered by this report, the licensing function in England and Wales rested with MAFF (acting on behalf of the National Assembly for Wales for Welsh licences), and in Scotland by the Scottish Office Agriculture, Environment and Fisheries Department). In Northern Ireland, the issuing of licences was the responsibility of the Environment and Heritage Service, an agency of the Department of the Environment for Northern Ireland.

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). There was no change in the arrangements for licensing in Northern Ireland.

16.3 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 29 inspections in 1998. The Sea Fisheries Inspectorate (SFI) with staff based on the coast, reports unlicensed activities and enforces licence conditions relating to construction and the disposal of wastes at designated disposal areas. 344 inspections were made in 1998.

In Scotland, similar enforcement powers are held by staff of the SOAEFD Marine Laboratory, Aberdeen and the Scottish Fisheries Protection Agency (SFPA). SOAEFD Marine Laboratory staff made 8 enforcement visits and carried out 6 investigation visits in 1998 and the SFPA carried out 11 enforcement visits and 2 investigation visits. In Northern Ireland, 6 enforcement visits were carried out and 1 investigation visit in 1998.

During the year, one investigation was carried out regarding an allegation of dumping other than at a licensed site. However it became clear that the material was being used to build a bund with which to lessen the tidal effects of siltation within a marina. It was brought to the attention of the company manager that this operation was licensable and an application was applied for and granted, and no further action was deemed necessary.

A further case was investigated involving the alleged unlicensed deposit of silt. In this case the contractor denied that an unlicensed operation had taken place but admitted that he had undertaken agitation dredging (which falls outside the scope of the Act). In the absence of firm evidence to refute the masters' claim, it was decided to send the contractor a warning letter drawing attention to the provisions of FEPA and to the penalties it provides for breach of the Act.

The majority of the 19 enforcement visits undertaken in Scotland were to ensure that due regard was being paid to the conditions attached to licences e.g. where consultees had highlighted environmental sensitivities immediately adjacent to licensed operations. In a few cases, enforcement visits were undertaken during the licence assessment period in order to establish the precise nature and location of proposed works. Of the 8 investigations undertaken in Scotland by SOAEFD Marine Laboratory and SFPA, 5 resulted in no action being taken as the works were found to be exempt from licensing, while in the other 3 cases, retrospective licences were applied for and subsequently granted. In all these cases, those undertaking the works were told to stop activity pending investigation.

In Northern Ireland, enforcement action was taken against WAM (GB) LTD, Civil Engineering Contractors. WAM (GB) LTD received a sea disposal licence in October 1997 to dispose of dredged material from Carrickfergus harbour, Co Antrim. During February 1998, the Fisheries Division of the Department of Agriculture for Northern Ireland (DANI) reported suspected incidents of disposal taking place short of the dumpsite. On investigation, this was confirmed and in March 1998 a warning letter was issued stating that should a similar incident occur in the future, the licence would be revoked. DANI again reported disposal outside the licensed area in April and the licence was revoked later that month.

16.4 Report on licensing activities

Tables 24-28 give details for the period 1994 to 1998 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.

16.5 Licensing of minestone

In April 1998, RJB's licence was renewed to use minestone from the Ellington Colliery on the foreshore at Lynemouth Bay as an interim coast defence measure.

16.6 Licensing of sewage sludge disposal

1998 was the final year for which sea disposal of sewage sludge was permitted and Table 24a gives details of the licences issued. Total quantities of key metallic contaminants in sewage sludge actually disposed of at sea are shown in Table 24b. Figure 25 shows the location of the disposal sites for sewage sludge and quantities deposited at each site in 1998.

Table 24(a). Sewage sludge licensed for disposal at sea in 1998

Country	Licensed Quantity (Tonnes) [1]	Company and source of waste	Disposal sites	Deposited Quantity (Tonnes) [1]
England and Wales	159,090	Anglian Water (Tilbury STW)	Barrow Deep	268,667
	249,500	Northumbrian Water (Howdon, Chester-le-Street, Cramlington, Washington STWs)	Tyne	396,630
	52,500	Northumbrian Water (Portrack, Billingham, Guisborough, Ayton STWs)	Tyne	70,594
	1,250,000	North West Water (Davyhulme, Liverpool, Warrington STWs)	Liverpool Bay	1,218,289
	380,000	Southern Water (Woolston, Portswood, Millbrook, Slowhill Copse STWs)	Nab Tower	237,758
	37,500	South West Water (Countess Wear STW)	Lyme Bay	49,561
	36,600	South West Water (Plympton, Radford, Camel's Head, Ernesettle, Ivybridge, Saltash, Newton Ferrers STWs)	Plymouth	34,173
	1,800,000	Thames Water (Crossness STW)	Barrow Deep	768,836
	3,875,000	Thames Water (Beckton, Riverside and Deephams STWs)	Barrow Deep	1,598,935
Scotland	400,000	Lothian Regional Council	St Abb's Head/ Bell Rock	375,079
	2,500,000	Strathclyde Regional Council	Garroch Head	1,631,600
Northern Ireland	0	Dept. Environment (Northern Ireland)	Belfast Sludge	283,380 [2]

Notes: (1) All figures are for tonnage in wet weight unless indicated otherwise
(2) 283,380 t disposed of by DOE (NI) Water Services under an administrative authorisation
STW = Sewage Treatment Works
Tonnages deposited relate to quantities in the calendar year 1998, which may be covered by 2 or more licences, including one or more issued in 1997

Table 24(b). Summary of sewage sludge licensed and disposed of at sea in 1998

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 and Wales	1994	12	7,911,970	7,474,849	216,208	0.94	45	123	0.69	13	81	217
	1995	12	7,941,000	7,525,746	211,268	0.89	44	121	0.56	13	83	218
	1996	11	8,405,500	7,477,458	182,099	0.66	38	97	0.45	11	70	178
	1997	9	7,681,001	7,334,999	171,091	0.56	30	88	0.38	8	57	157
	1998	9	7,949,767	4,535,084	108,815	0.36	19	56	0.24	5	35	101
Scotland	1994	2	3,000,000	1,930,510	60,242	0.14	27	29	0.10	3	16	42
	1995	2	3,000,000	1,919,950	69,747	0.18	25	25	0.08	4	18	52
	1996	2	3,000,000	2,057,265	80,593	0.17	25	28	0.11	4	17	84
	1997	2	3,000,000	2,078,579	77,079	0.11	24	29	0.11	4	17	101
	1998	2	2,900,000	2,006,679	72,616	0.10	14	18	0.10	6	12	60
Northern Ireland	1994	0	0	251,860 %	12,719	0.03	4	4	0.03	1	2	12
	1995	0	0	285,229 *	15,231	0.03	6	6	0.02	1	2	23
	1996	1	60,000	375,136 #	13,918	0.02	7	5	0.03	1	3	22
	1997	0	241,920	241,920 $	10,136	0.02	3	4	0.02	1	2	12
	1998	0	283,380	283,380 $	11,874	0.02	3	5	0.03	1	2	11
UK Total	1994	14	10,911,970	9,657,219 %	289,169	1.10	77	156	0.83	17	99	270
	1995	14	10,941,000	9,730,925 *	296,246	1.10	75	152	0.66	18	103	293
	1996	14	11,465,500	9,909,859 #	276,610	0.85	71	130	0.59	15	91	285
	1997	11	10,922,921	9,655,498 $	258,307	0.70	57	121	0.51	13	76	270
	1998	11	11,133,147	6,825,143 @	193,304	0.47	35	79	0.37	12	49	172

Notes: % Includes 251,860 t disposed of by DOE(NI) Water Services under an administrative authorisation
** Includes 285,229 t disposed of by DOE(NI) Water Services under an administrative authorisation*
 # Includes 329,015 t disposed of by DOE(NI) Water Services under an administrative authorisation
$ Includes 241,920 t disposed of by DOE(NI) Water Services under an administrative authorisation
@ Includes 283,380 t disposed of by DOE(NI) Water Services under an administrative authorisation
For information on licensed quantities and tonnages deposited see footnote to Table 24(a)

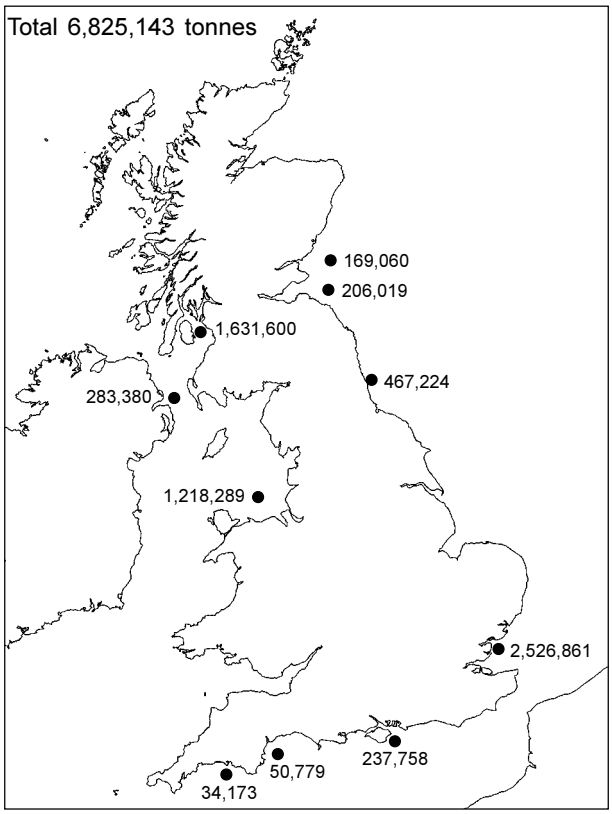

Figure 25. UK sewage-sludge disposal sites and amounts deposited in tonnes for 1998

The quantities of contaminants deposited in the sea in sewage sludge have continued to decline. Table 25 sets out how the aggregate totals authorised for disposal between 1989 and 1998 compared with the licensed levels in 1987. As indicated in previous reports these are now close to background levels, with most of these contaminants coming from general domestic sources.

16.7 Licensing of dredged material disposal

Table 26 shows the number of licences issued for dredged material in 1998, 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 is natural, but the mineral structure is such that it will not be available to marine organisms. Figure 26 shows the main disposal sites used in 1998 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.

Table 25. Contaminants in sewage sludge authorised for disposal in the North Sea from 1989 to 1998 compared against estimated quantities (tonnes) in 1987

Year	Cd	Cr	Cu	Hg	Ni	Pb	Zn
1987	3.7	56.2	133.6	1.2	19.4	146.4	468.2
1989	3.7	55.4	133.0	1.2	19.2	143.9	460.0
1990	3.4	49.7	132.5	1.2	18.6	136.0	435.6
1991	2.4	43.5	128.4	1.1	15.9	126.2	340.0
1992	2.3	43.8	127.1	1.1	15.8	125.3	339.4
1993	2.0	39.3	123.0	1.0	14.9	114.8	295.5
1994	1.2	28.7	120.3	0.8	14.0	91.8	212.8
1995	1.1	26.7	116.4	0.7	13.2	90.0	205.1
1996	0.7	21.0	100.5	0.6	10.9	80.2	174.1
1997	0.6	20.5	95.9	0.5	10.5	73.4	162.8
1998	0.5	12.7	78.4	0.4	11.1	55.7	133.4

Table 26. Summary of dredged material licensed and disposed of at sea in 1998

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 and Wales	1994	106	53,187,009	37,219,028	21,395,174	9.55	1,385	823	6.21	683	1,502	3,655
	1995	109	54,300,948	35,215,611	17,941,131	5.83	1,298	625	5.19	548	1,380	3,161
	1996	120	82,395,490	48,513,953	25,953,191	8.80	1,556	743	6.87	673	1,731	3,991
	1997	113	56,536,922	38,627,660	21,165,143	6.54	1,182	574	5.47	471	1,242	2,941
	1998	110	75,168,342	31,814,916	15,456,858	7.47	1,143	551	4.46	498	1,081	2,741
Scotland	1994	23	3,643,250	1,822,053	820,368	0.87	42	36	0.49	20	56	122
	1995	32	6,186,600	4,782,421	2,204,223	1.12	155	120	3.45	66	153	349
	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
Northern Ireland	1994	5	113,200	91,314	59,067	0.01	0	0	0.00	0	0	1
	1995	9	335,280	249,593	170,297	0.24	2	1	0.05	2	2	8
	1996	6	166,000	135,550	106,768	0.05	2	2	0.01	3	2	4
	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
UK Total	1994	134	56,943,459	39,132,395	22,274,610	10.44	1,427	860	6.70	703	1,558	3,777
	1995	150	60,822,828	40,247,625	20,315,652	7.19	1,455	746	8.69	616	1,535	3,517
	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	143	82,206,792	35,724,350	17,358,911	8.24	1,278	689	5.63	553	1,218	3,084

Notes: For information on licensed quantities and tonnages deposited see footnote to Table 24(a)

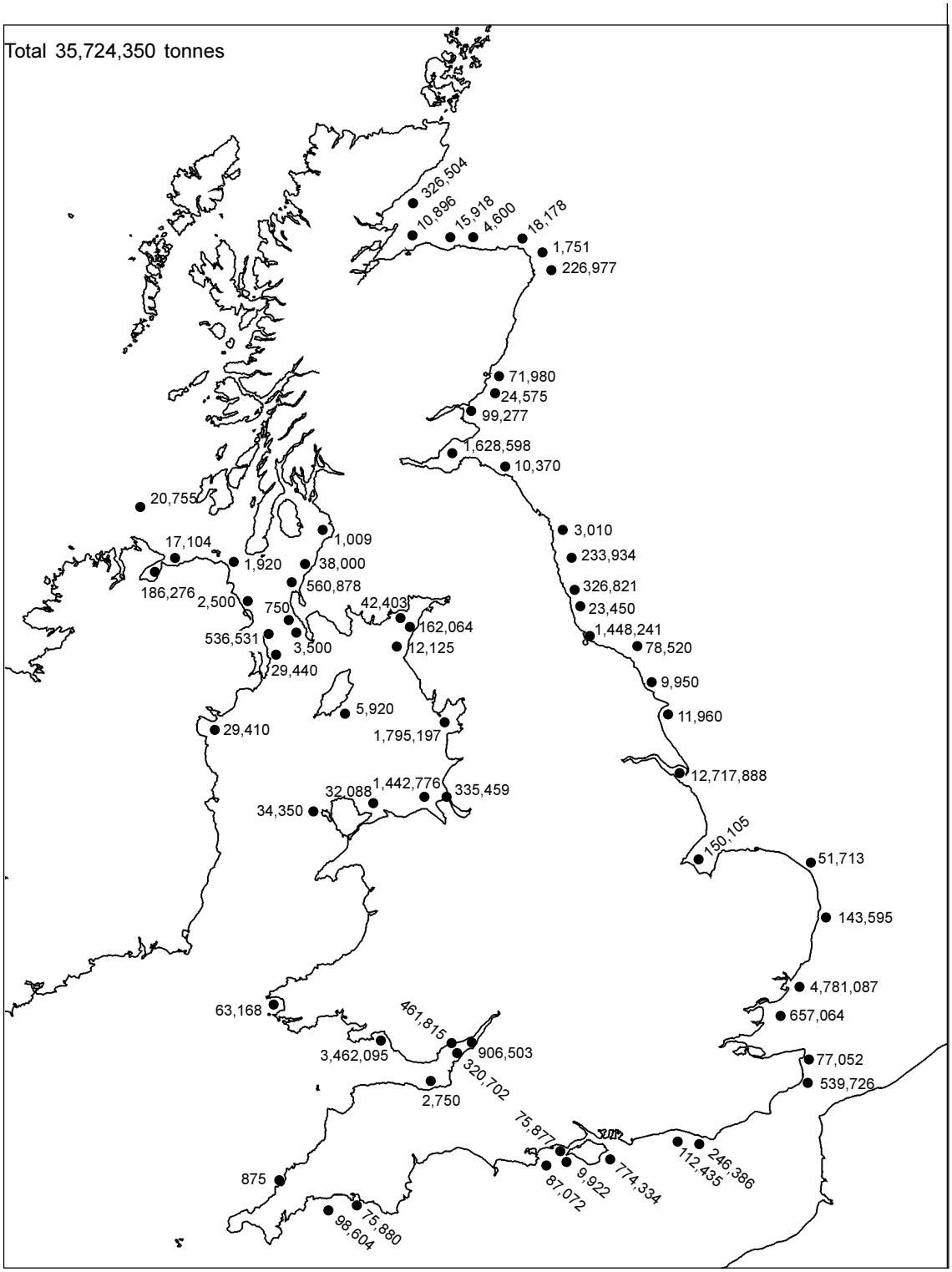

Figure 26. UK dredged material disposal sites and amounts deposited in tonnes for 1998

16.8 Other licensed activity

Under Part II of FEPA, licences are also required for certain other activities or substances in the sea below high water spring tide mark. 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.

Such licences have authorised the disposal in 1998 of small amounts of fish waste, details are given in Tables 27a and 27b. 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. Table 28 shows the number of such licences issued in 1998.

Table 27(a). Fish waste licensed for disposal at sea in 1998 [1]

Country	Licensed Quantity (Tonnes)	Company and source of waste	Disposal sites	Quantity Deposited (Tonnes)
England	0	Quay Fresh & Frozen Foods Ltd, New Quay	New Quay	52
Scotland	0	Orkney Fishermen's Association, Stromness	Stromness C	0
			Stromness B	0
	200	Orkney Fishermen's Association, Stromness	Stromness C	45
			Stromness B	0
	315	Orkney Fishermen's Association, Stromness	Stromness C	97
			Stromness B	0

Notes:[1] 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 24(a)

Table 27(b). Summary of fish waste licensed and disposed of at sea in 1998

Country	Year	Licences issued	Licensed quantity (tonnes)	Wet tonnage deposited	Dry tonnage deposited
England and Wales	1994	0	0	0	0
	1995	0	0	0	0
	1996	1	750	16	16
	1997	1	750	747	747
	1998	0	0	52	52
Scotland	1994	0	0	0	0
	1995	0	0	0	0
	1996	0	0	0	0
	1997	2	262	51	41
	1998	2	515	142	114
Northern Ireland	1994	0	0	0	0
	1995	0	0	0	0
	1996	0	0	0	0
	1997	0	0	0	0
	1998	0	0	0	0
UK. Total	1994	0	0	0	0
	1995	0	0	0	0
	1996	1	750	16	16
	1997	3	1012	798	788
	1998	2	515	194	166

Notes: For information on licensed quantities and tonnages deposited see footnote to Table 24(a)

Table 28. Other categories of licences issued in 1998

Licence category	Year	England and Wales	Scotland	Northern Ireland	Total
Construction - new and renewal	1998	240	95	12	347
Tracers, biocides etc.	1998	9	2	0	11
Burial at Sea	1998	11	0	0	11

17. ADVICE ON FISHERY IMPLICATIONS OF PIPELINE DISCHARGES

This section gives a brief summary of activities carried out during 1998 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 and 1998).

During 1998, CEFAS assessed applications for a total of 482 individual discharges. As in previous years, the majority of the applications were for discharge of domestic sewage, including storm and emergency sewage overflows.

Most of the applications for continuous sewage discharges were for upgrading of long-standing discharges in order to meet the requirements of EC Directive 76/160 concerning the quality of bathing water (European Communities, 1976) and EC Directive 91/271 concerning urban waste water treatment (European Communities, 1991b). Nearly 75% of these applications were for secondary treatment or higher, compared with only 10% in 1992 and 24% in 1993. An increasing number of discharges are also being treated by sterilisation using UV light which, provided it is carried out all year round and not just during the bathing season, will help to significantly reduce levels of microbiological contamination of shellfish.

A number of individual improvement schemes introduced to meet the requirements of Directives 76/160 and 91/271 have resulted in significant reduction of contamination of nearby shellfisheries such that the classification under EC Directive 91/492 (European Communities, 1991c) has improved. Such improvements in classification benefit the shellfish industry by reducing the processing requirements prior to marketing for human consumption. There has not, however, been any overall reduction in the proportion of English and Welsh harvesting areas included in the two most contaminated classes (C and Prohibited). This may reflect the fact that improvement schemes to date have not been specifically directed at improving shellfisheries and the influence of contaminating factors other than continuous sewage discharges.

The most frequent issue to be addressed over the year was in relation to storm sewage overflows to shellfish areas. For overflows which impact on Designated bathing waters, there is a statutory requirement to restrict spill frequency to three per bathing season. There is currently no such requirement for shellfish waters and many of the applications received during the year were for high frequency overflows to shellfish areas. In many cases, it was not possible to carry out any assessment of risk, since no information on spill frequency was available. During the year, CEFAS has continued to stress to the Environment Agency the importance of these issues in relation to shellfish hygiene and has given a number of presentations in an attempt to raise the profile of fish and shellfish issues in relation to discharges.

Emergency overflows only come into operation when there is a major failure at the sewage treatment works or pumping station. If this should occur, it may cause severe contamination of shellfish in the area. In advising on such applications, CEFAS therefore asks for inclusion of a consent condition which requires that the local food authority be notified as soon as possible after an emergency spill so that they can take whatever action they consider necessary to protect public health.

About 25% of the applications received during 1998 were for discharge of trade effluent. A small number of these were for new processes. As the legislation requires new processes to be designed according to the Best Practicable Environmental Option (BPEO), these are not normally likely to have any adverse fishery implications. The consultation process nevertheless provides CEFAS with an opportunity to ensure that fishery and related matters are not overlooked in the setting of consent conditions. However, the majority of trade effluent discharge applications received during the year were part of an ongoing review of existing processes. Many of these have given cause for concern in the past, or else were not previously assessed by CEFAS. This review process provides a valuable opportunity to ensure that factors of concern to MAFF (e.g. discharge of potential endocrine disrupters) are gradually brought under tighter control.

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 an increasingly useful management tool for integrating information on human activities in the marine and coastal zone.

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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

BIOTA

Pesticides and PCBs in bivalve molluscs	J. Jones
Mercury concentrations in fish	A. Franklin
Butyltin compounds in cetaceans	R. Law
Potential links between contaminant burdens	R. Law
Marine fish diseases	S. Feist

BIOLOGICAL EFFECTS

The use of enzyme biomarkers	M. Kirby

SEDIMENTS

Arsenic in North Sea sediments	C. Whalley

BENTHOS

Mapping using biological and remote sensing	C. Brown
Meiofauna research	M. Schratzberger
Bioassays for marine sediment quality	Y. Allen

DISPOSAL AT SEA

CBs in dredged sediment	J. Reed
Licensing of deposits in the sea	C. Vivian
Advice on fishery implications of pipeline discharges	F. Franklin