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**Monitoring and surveillance of  
non-radioactive contaminants  
in the aquatic environment and activities  
regulating the disposal of wastes at sea,  
1997**

LOWESTOFT  
2000

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# CONTENTS

Page

FOREWORD

BACKGROUND TO THE WORK

## SEA WATER

<b>1. Winter nutrients in the coastal waters of England and Wales</b> .....	7
1.1 Introduction .....	7
1.2 Methods .....	7
1.2.1 Sampling .....	7
1.2.2 Sample analysis .....	7
1.3 Results and discussion .....	7
1.3.1 Description of January 1997 NMP station nutrient distribution .....	7
1.4 Conclusions .....	10

## BIOTA

<b>2. Heavy metals in bivalve molluscs from designated harvesting areas around England and Wales</b> .....	11
2.1 Introduction .....	11
2.2 Materials and methods .....	11
2.3 Results and discussion .....	11
2.4 Conclusions .....	16
<b>3. Arsenic speciation in fish muscle around the coasts of England and Wales</b> .....	16
3.1 Introduction .....	16
3.2 Method .....	17
3.3 Results .....	17
3.4 Conclusion .....	18
<b>4. PAH levels in fish and shellfish, post Sea Empress oil spill</b> .....	18

## SEDIMENTS

<b>5. Total arsenic in sediments from the western North Sea and the Humber Estuary</b> .....	21
5.1 Introduction .....	21
5.2 Method .....	21
5.3 Results and discussion .....	21

## BIOLOGICAL EFFECTS

<b>6. The use of enzyme biomarkers in biological effects monitoring</b> .....	23
6.1 General introduction .....	23
6.2 Ethoxyresorufin-O-deethylase (EROD) .....	23
6.2.1 Introduction .....	23
6.2.2 Methods .....	24
6.2.3 Results .....	24
6.2.4 Discussion .....	26
6.2.5 Conclusions .....	29
6.3 Acetylcholinesterase (AChE) .....	30
6.3.1 Introduction .....	30
6.3.2 Method .....	30
6.3.3 Results .....	33
6.3.4 Discussion .....	34
6.3.5 Conclusion .....	37
6.4 General conclusion .....	37
<b>7. Oestrogenic endocrine disruption in flounder (<i>Platichthys flesus</i> L.) from United Kingdom estuarine and marine waters</b> .....	37
7.1 Introduction .....	37
7.2 Executive summary .....	37

/continued:

<b>8.</b>	<b>Monitoring and surveillance for disease in marine fish 1997: Monitoring programme for externally visible disease biomarkers and hepatic pathology in marine fish</b>	<b>39</b>
8.1	Introduction	39
8.2	Methods	39
8.3	Results	41
8.4	Discussion	44

## **BENTHOS**

<b>9.</b>	<b>Epifaunal studies around the United Kingdom coastline</b>	<b>45</b>
9.1	Introduction	45
9.2	Methods	45
9.3	Results	46
9.4	Discussion	54
<b>10.</b>	<b>A comparison of benthic changes at a dredged material disposal site</b>	<b>55</b>
10.1	Introduction	55
10.2	Methods	56
10.3	Results	57
10.4	Discussion	59

## **DISPOSAL AT SEA**

<b>11.</b>	<b>Metal concentrations in dredged sediment</b>	<b>60</b>
11.1	Introduction	60
11.2	Sampling dredged material	60
11.3	Methods	60
11.4	Results	60
11.5	Discussion	60
11.6	Conclusion	69
<b>12.</b>	<b>TBT concentrations in dredged sediment</b>	<b>69</b>
12.1	Introduction	69
12.2	Sampling of dredged material for TBT	69
12.3	Methods	69
12.4	Results	69
12.5	Discussion	69
12.6	Conclusion	73
<b>13.</b>	<b>Advice on fishery implications of pipeline discharges</b>	<b>74</b>
<b>14.</b>	<b>Licensing of deposits in the sea</b>	<b>75</b>
14.1	Introduction	75
14.2	Legislation and licensing authorities	75
14.3	Enforcement	75
14.4	Report on licensing activities	76
14.5	Licensing of minestone disposal	76
14.6	Licensing of sewage sludge disposal	76
14.7	Licensing of dredged material disposal	78
14.8	Other licensed activity	78
<b>15.</b>	<b>References</b>	<b>81</b>
	<b>Annex 1. Areas of work and staff responsible for the projects</b>	<b>89</b>
	<b>Annex 2. Standards/guidelines for contaminants in fish and shellfish</b>	<b>90</b>



## FOREWORD

This is the ninth report in a series of CEFAS publications on monitoring and surveillance activity. This report describes work carried out in 1997 by scientists working in the Environment Group at CEFAS. The information covers both general quality monitoring at marine and coastal sites and site-specific work carried out in support of risk assessments in support of regulatory work.

Comprehensive spatial surveys of contaminants in coastal waters were reported in 1995/96 in this series and form the basis for long-term environmental quality monitoring. The strategy for the future programme (the National Marine Monitoring Programme -NMMP) 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 polycyclic aromatic hydrocarbons in the context of oil spills is one example of these special surveys and some results are reported here in Chapter 4.

The use of biological effects techniques to provide information on impacts of particular groups of compounds is also a requirement of the NMMP. Results are provided here for the use of ethoxyresorufin-O-deethylase and acetylcholine esterase as indicators of impacts of PAH/PCB and organophosphorus compounds respectively. Such techniques form part of a cascade of methods used to describe occurrence of contaminants through to biological effects at an individual level (fish disease) and population level (community change). We are at an early stage in evaluating the utility of such techniques and are participating in quality assurance schemes both nationally via the NMMP and internationally via QUASIMEME and BEQUALM (see later).

Monitoring in support of risk assessments allows us to ensure that our decision making process is valid. The results provided here for sampling of dredged material for TBT has allowed us to delineate the legacy of contamination resulting from uncontrolled discharges of TBT from dry docking practices and to exclude heavily contaminated material from that allowed for sea disposal. The information has been invaluable in underlining the need for better control of discharges under Integrated Pollution Control, supporting the complete ban on the use of TBT as an antifouling biocide and protection of coastal waters.

This report is available in downloadable format from the CEFAS web site: [www.cefas.co.uk](http://www.cefas.co.uk)



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## BACKGROUND TO THE WORK

As an Executive Agency of the Ministry of Agriculture, Fisheries and Food, CEFAS carries out work in support of Government objectives to:

- Protect public health in relation to food and to animal diseases transmissible to humans
- Sustain and enhance the rural and marine environments and public enjoyment of the amenities they provide and to promote forestry
- Conserve fish stocks for future generations and secure a sustainable future for the sea fishing industry
- Ensure that farmed animals and fish are protected by high welfare standards and do not suffer unnecessary pain or distress.

Environment work 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. CEFAS is directly involved in advising on UK and international legislation and in developing policy relating to management of the aquatic environment. CEFAS provides 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 the work is to provide advice to MAFF 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) MAFF has the responsibility to license and control the deposit of material to sea. Following the cessation of disposal of sewage sludge to sea, licensed materials are predominantly sediments, 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 make regular visits to licence holders to ensure any stipulated licence conditions are being met. Conducting monitoring programmes in support of risk assessments enables MAFF to ensure the effectiveness of the assessment process and provides a basis for decisions on future policy for management of marine resources. The Environment Group monitors environmental conditions at marine disposal sites and compares 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), MAFF 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.

CEFAS also provides advice to MAFF, DTI and DETR 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 MAFF'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 across 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 MAFF 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.

The Environment Group has over 50 years experience in aquatic studies. During this period the Group has made a number of significant contributions to environmental protection and as a consequence of its work has 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.cefasc.co.uk](http://www.cefasc.co.uk)).

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# SEA WATER

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## 1. WINTER NUTRIENTS IN THE COASTAL WATERS OF ENGLAND AND WALES

### 1.1 Introduction

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

The monitoring undertaken under the auspices of the UK National Monitoring Programme (NMP) has been to determine winter (January to March) nutrient species (ammonium, nitrate, nitrite, phosphate and silicate) concentrations in the coastal waters of England and Wales. In addition, measurements have been taken of chlorophyll and dissolved oxygen concentrations to set the scene for the monitoring of possible eutrophication symptoms in the following spring and summer.

### 1.2 Methods

All sampling and analysis was conducted using protocols which have subsequently been adopted by the Oslo and Paris Commissions (OSPAR) as Eutrophication Monitoring Guidelines (OSPARCOM, 1997).

#### 1.2.1 Sampling

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

within the 30 to 35 psu salinity range. Further samples were collected to define the nutrient versus salinity relationship and will be used in a later report.

#### 1.2.2 Sample analysis

Water samples were analysed directly 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).

### 1.3 Results and discussion

#### 1.3.1 Description of January 1997 NMP station nutrient distribution

Figures 1(a-f) display the spatial distribution of nutrient concentrations around England and Wales for the NMP sites sampled in January 1997. The diameter and shade of the circles is proportional to the parameter concentration. The actual sample concentration is listed next to the symbol.

#### **Total oxidised nitrogen (TOxN), nitrate and nitrite**

The total oxidised nitrogen concentration comprises of nitrate (NO<sub>3</sub>) and nitrite (NO<sub>2</sub>) 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 run-off.

Total oxidised nitrogen concentration is highest in the Severn Estuary ( $63.6 \mu\text{mol l}^{-1}$ ) and in the Thames, Humber and Liverpool Bay areas, 31.6, 36.7 and  $33.8 \mu\text{mol l}^{-1}$  respectively (Figure 1(a)). The Wash and the area off the Lune/Wyre also have high TOxN concentrations compared to other coastal sites. The winter nitrate concentrations in Atlantic water entering

the North Sea are typically circa  $12 \mu\text{mol l}^{-1}$  (North Sea Task Force, 1993) but lower concentrations are found at the offshore NMP sites (Figure 1(b)). Winter values in the offshore waters of the North Sea are the baseline for seasonal changes in nitrogen and other nutrient concentrations ( $0.8 \mu\text{mol l}^{-1}$  and  $6 \mu\text{mol l}^{-1}$  for phosphate and silicate respectively).

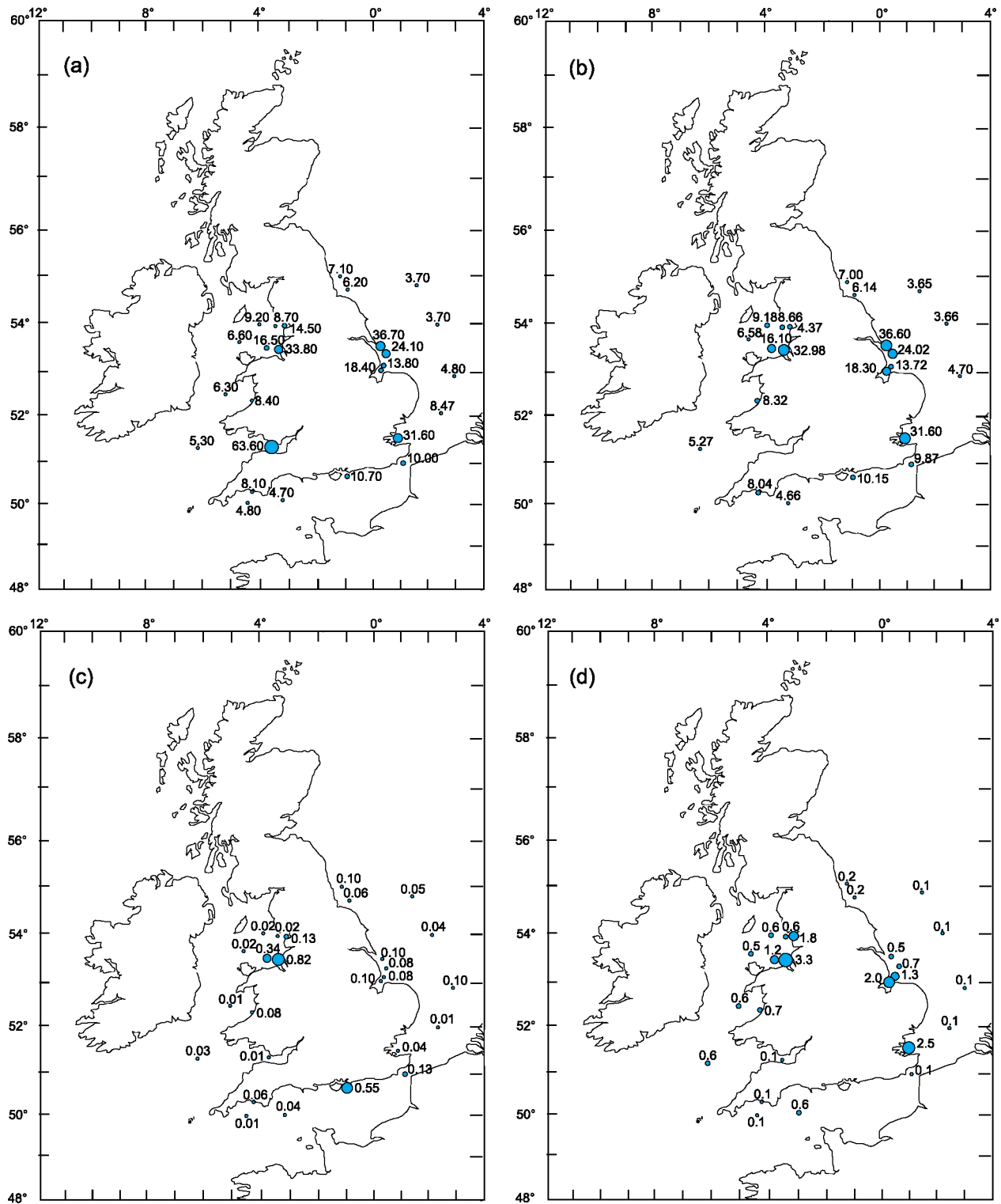


Figure 1. Coastal distributions ( $\mu\text{mol l}^{-1}$ ) at NMP stations (January 1997) of (a) ToxN (Total Dissolved Nitrogen), (b) nitrate, (c) nitrite and (d) ammonium

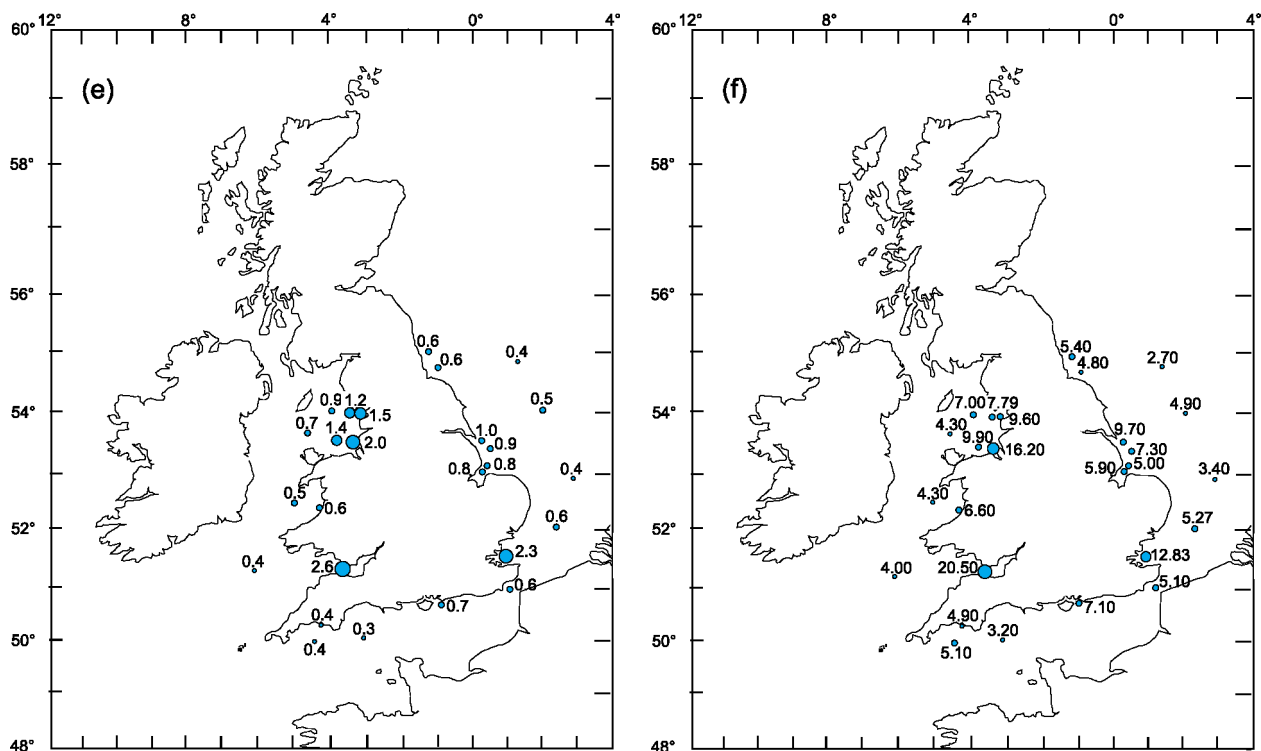


Figure 1. Coastal distributions ( $\mu\text{mol l}^{-1}$ ) at NMP stations (January 1997) of (e) Phosphate and (f) Silicate

Relatively high nitrite concentrations (2 to 5% of  $\text{TOxN}$ ) are found in Liverpool Bay and at the Selsey Bill site (Figure 1(c)). The use of nitrite concentration as an indicator of disturbance to the nitrogen cycle is being investigated.

#### **Ammonium**

The concentrations of ammonium are low ( $<1 \mu\text{mol l}^{-1}$ ) in most estuarine, coastal and especially, offshore waters. However, there are several areas (Thames, Wash, Liverpool Bay and off Lune/Wyre) where surface water concentrations are obviously higher than at other sites (Figure 1(d)). This may be related either to the nature of the local estuaries or to the presence of large urban waste water discharges.

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.

#### **Phosphate**

The spatial distribution of phosphate concentrations largely follows those of  $\text{TOxN}$ . The Severn, Thames, Liverpool Bay and Lune/Wyre areas have the highest concentrations (Figure 1(e)). 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**

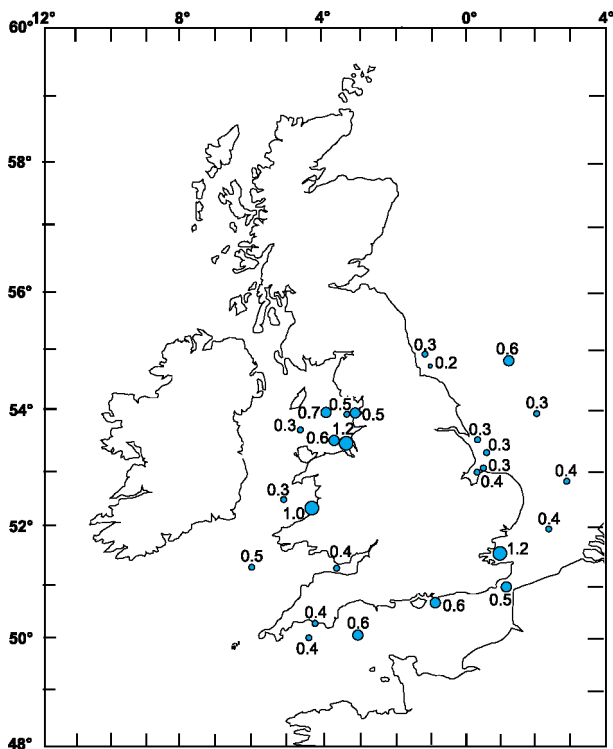
The spatial distribution of silicate concentrations indicates that concentrations are higher off the Severn, Thames and Liverpool Bay (Figure 1(f)). All the other sample concentrations lie below  $10 \mu\text{mol l}^{-1}$  and as with the other major nutrients, silicate concentrations decrease offshore. It is expected that the concentration of silicate, assuming no biological uptake in the estuaries will reflect the freshwater discharge to the area.

#### **Chlorophyll**

Chlorophyll concentrations can be used as an indication of phytoplankton biomass. In January there are uniformly low levels ( $<1.5 \mu\text{g l}^{-1}$ ) 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 2) and the development of phytoplankton biomass. Higher concentrations of chlorophyll are present in the Thames and Liverpool Bay. 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 dissolved oxygen concentrations measured during this survey ranged from 6 to  $7 \text{ ml l}^{-1}$ . This high level of dissolved oxygen concentration suggests that oxygen demand is



**Figure 2. Chlorophyll coastal distribution ( $\mu\text{g l}^{-1}$ ) at NMP stations (January 1997)**

low at these sites and that water mixing is good. These measurements act as the basis for comparison with the situation in the spring and summer following the start of the growing season.

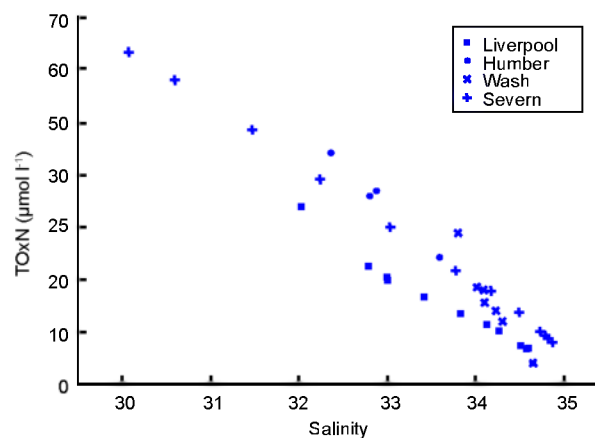
### 1.4 Conclusions

The main features of the spatial survey of January 1997 are:

- The more 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) and generally lower concentrations than those found in in-flowing Atlantic water.

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

The variability of nutrient concentrations that result from discharge to coastal waters, as well as differences in the relative concentration of the different nutrient species (TOxN versus ammonium or phosphate), is a complex function of the processes taking place in a specific location. We seek to measure nutrient concentration in the winter when the, arguably dominant, 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. However, for most of the sites the level of phytoplankton biomass appears to be very low. Further insight into input and process variability can be gained from the interpretation of nutrient versus salinity plots. Examples from this dataset are shown in Figure 3 and will form the basis for a separate report. This information will be used as the yardstick for comparison with the spring and summer situation.



**Figure 3. Surface water TOxN (Total Oxidised Nitrogen) concentrations for Liverpool Bay, the Humber, Wash and Severn NMP transects in January 1997**



## 2. HEAVY METALS IN BIVALVE MOLLUSCS FROM DESIGNATED HARVESTING AREAS AROUND ENGLAND AND WALES

### 2.1 Introduction

Data for contaminants in shellfish from around the coast of England and Wales have, until now, mainly been gathered for general food assurance purposes, or as an indicator of the quality of coastal/estuarine waters. In 1992, the European Union, Shellfish Hygiene Directive 91/492/EEC (European Communities, 1991(a)) came into force in England and Wales and was implemented under the Food Safety (Live Bivalve Mollusc and Other Shellfish) Regulations, 1992 (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 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 end product standard, which include not containing toxic or objectionable compounds such as trace metals, organochlorine compounds and hydrocarbons, in such quantities that the calculated dietary intake exceeds the permissible daily intake. A study of commercial molluscs was carried out to provide information which would help the UK to meet its commitments under the EU legislation. A summary of the first year of this study is given below, full details can be found in Jones *et al.*, 1998.

In the first year of the programme samples of cockles, mussels and oysters were collected and analysed for a suite of eleven metals.

### 2.2 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  $-20^{\circ}\text{C}$  until processing.

Samples consisted of 10 individual oysters, and 50 individuals for mussels and cockles. For each sample, the shellfish were thawed and measurements of length, total weight and shell weight were recorded for each individual. The whole body tissue (as eaten) was then removed from the shell, bulked and homogenised and empty shell weight was recorded. Ten-gramme aliquots of homogenised tissue were stored in glass jars, again at  $-20^{\circ}\text{C}$  until submitted for chemical analysis.

Microwave digests were analysed for trace metals using a combination of atomic absorption spectrophotometry (for Cu, Fe, and Zn), atomic fluorescence spectrometry (for Hg) and inductively coupled plasma-mass spectrometry (for Ag, As, Cd, Cr, Ni, Pb and Se) (Jones and Laslett, 1994).

### 2.3 Results and discussion

Bivalves can concentrate many chemical contaminants, including metals, by orders of magnitude above concentrations in the surrounding sea water. The degree to which any contaminant is accumulated will depend on both abiotic physiochemical properties and biotic factors such as filtration rate, growth, reproductive condition and metabolism (Dame, 1996).

#### *Silver*

Silver is naturally present in sea water. Increases above background concentrations may result from discharges from electroplating, photochemical and food processing industries or from its use in the manufacture of coinage, tableware, mirrors, and jewellery.

Concentrations (all concentrations are wet weight) ranged from  $<0.001$  to  $2.4\text{ mg kg}^{-1}$ . Concentrations in cockles and mussels were very variable and spread across the whole range, with mean concentrations of  $0.31\text{ mg kg}^{-1}$  and  $0.11\text{ mg kg}^{-1}$  respectively. The concentrations in both species of oysters fell within a much narrower range,  $0.12$ - $1.0\text{ mg kg}^{-1}$ , with means of  $0.31\text{ mg kg}^{-1}$  for Native oysters and  $0.29\text{ mg kg}^{-1}$  for Pacific oysters. Highest concentrations ( $>0.7\text{ mg kg}^{-1}$ ) were found in several samples of cockles from the Thames Estuary, mussels from the Blackwater and the North Kent coast, and Native oysters from Langstone Harbour and the Solent.

### Arsenic

High arsenic levels in soil and water can arise naturally from the presence of arsenical ores. Additional industrial contamination mainly results from the exploitation of these deposits for their metal content; firstly from tipping of mine waste containing arsenic and secondly, pollution from the metal smelting process.

Concentrations of total arsenic ranged from 0.62 to 4.3 mg kg<sup>-1</sup>, with the exception of one sample of Native oysters from the Fal which contained 13 mg kg<sup>-1</sup>.

The distribution of concentrations was similar in all four species with mean concentrations of 1.4 mg kg<sup>-1</sup> for cockles, 2.2 mg kg<sup>-1</sup> for mussels and 1.5 mg kg<sup>-1</sup> for both species of oysters. These levels were similar to those reported in MAFF (1998). Highest concentrations (>3 mg kg<sup>-1</sup>) were recorded in mussels from the Colne, Humber, Scarborough, Tenby, Colwyn Bay and Blyth.

The Fal region is well known for its high mineralogical content. This area has been historically exploited leaving a network of mine shafts, many of which discharge metal-rich water in to the estuary. The high concentration of arsenic in the sample of oysters from this area is therefore not unexpected. The Humber has in the past, been subject to significant arsenic discharges from a non-ferrous smelter situated at North Ferriby, which has now closed. Steel works, smelters and gas or coal power stations are possible sources of the relatively high concentrations in other areas.

The majority of total arsenic detected, is present in the form of organic compounds such as arsenobetaine, whilst it is the inorganic forms which are most toxic. Only around 1 to 3 percent of the total arsenic present in fish and shellfish is in the inorganic form (Edmonds and Francesconi, 1993; Buchet *et al.*, 1994; CEFAS, unpublished data).

### Cadmium

Major sources of cadmium to the environment include iron and steel production, refuse incineration, fossil fuel combustion and sewage sludge deposition. Until recently, cadmium was primarily used in the UK for pigment manufacture, nickel-cadmium batteries, plating and as a stabiliser for PVC plastics. Its use in these processes was effectively banned by Directive 89/677/EEC, (European Communities, 1991(b)) which came into force on 31 December 1992 and was implemented in the UK on 31 July 1993 (Great Britain-Parliament, 1993).

The distribution pattern for cadmium was similar in mussels and Pacific oysters (mean concentrations 0.26 mg kg<sup>-1</sup> and 0.21 mg kg<sup>-1</sup>, respectively) with slightly higher concentrations found in Native oysters (mean 0.46 mg kg<sup>-1</sup>) (Figure 4). Concentrations in cockles (mean 0.06 mg kg<sup>-1</sup>) were lower than mussels and oysters by approximately a factor of 10. Similar levels were reported in MAFF, 1992, 1993 and 1998. The highest concentrations found in this survey were in two samples of Native oysters from Porthcawl and horse mussels (*Modiolus modiolus*) from Silloth (2.4, 2.1 and 1.9 mg kg<sup>-1</sup>, respectively).

The European Commission has put forward a Draft Regulation setting maximum limits for lead and cadmium in food (European Communities, 1997). The proposed limit for cadmium in molluscs is 1.5 mg kg<sup>-1</sup> wet weight. Only samples from two areas contained concentrations above this level. Although the Maryport bed at Silloth is in a classified area, the horse mussels are not apparently commercially harvested due to their large size. The Bristol Channel is a known area of relatively high metals contamination, due to inputs from domestic and industrial outfalls and run-off from mineralised zones of north Devon (Abdullah *et al.*, 1972; Abdullah and Ireland, 1986; Vivian, 1980). The elevated cadmium concentrations in oysters from

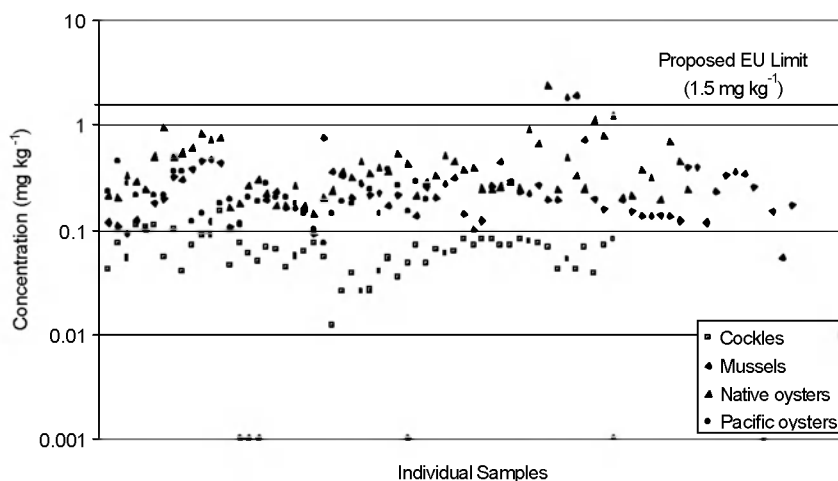


Figure 4. Concentration of cadmium in shellfish from designated bivalve production areas in England and Wales



Porthcawl are probably due to inputs from industries such as the smelting operations in Avonmouth, steel works at Newport and the coal fired power station at Aberthaw. Concentrations below, but close to, the proposed limit at 1.1 and 1.2 mg kg<sup>-1</sup>, respectively, were found in samples of Native oysters from the Swale and the North Kent coast.

### **Chromium**

Chromium is used in the production of alloy steels, chrome plating and tanning leather and in the manufacture of refractory bricks, pigments and wood preservatives. The main sources of inputs to the aquatic environment are sewage, and industrial waste discharges particularly from metallurgical industries.

For the majority of samples, concentrations of chromium fell in the range 0.1 to 3.0 mg kg<sup>-1</sup>, with no discernible differences in the distribution patterns between species. Mean concentrations were 0.99 mg kg<sup>-1</sup> for cockles, 1 mg kg<sup>-1</sup> for mussels and 1.3 and 0.89 mg kg<sup>-1</sup>, respectively, for Native oyster and Pacific oysters. These levels are above those reported in a recent survey of market samples (MAFF, 1998) but slightly lower than mean concentrations reported previously for these species, by the CEFAS Laboratory (MAFF, 1985). Concentrations >5 mg kg<sup>-1</sup> were found in cockles from the Blackwater (5.7 mg kg<sup>-1</sup>); mussels from the North Kent coast (6.6 mg kg<sup>-1</sup>), Portland (5.3 mg kg<sup>-1</sup>) and Silloth (5.4 mg kg<sup>-1</sup>); Pacific oysters from Blakeney (6.7 mg kg<sup>-1</sup>) and Native oysters from Chichester Harbour (9.4 mg kg<sup>-1</sup>) and North Kent coast (6.1 and 8.1 mg kg<sup>-1</sup>).

### **Copper**

Copper is naturally present in sea water but additional inputs come from its use in the electrical industry for electroplating and also the production of pigments and dyes and the manufacture of bronzes, brass and other copper alloys. Copper compounds are used in agriculture, especially as fungicides and insecticides and copper-containing antifouling paints are a further source of copper inputs to the marine environment, particularly in areas of high boating/shipping activity such as harbours and marinas. Their use has increased significantly since the phasing out of TBT based antifouling paints in the late 1980s and they are now a significant source of local inputs in areas away from industrial activity.

Concentrations of copper in cockles and mussels were relatively low ranging from 0.19 to 9.1 mg kg<sup>-1</sup> with a mean of 1.2 and 1.8 mg kg<sup>-1</sup>, respectively. The highest concentrations were in cockles from the Thames Estuary and in mussels from the Fal (9.1 mg kg<sup>-1</sup>). Concentrations in oysters were between 10 and 100 times higher, ranging between 5.3 to 106 mg kg<sup>-1</sup> (mean 41.6 mg kg<sup>-1</sup>) in Pacific oysters (with the exception of one sample from Taw/Torridge which contained 494 mg kg<sup>-1</sup>) and 12 to 211 mg kg<sup>-1</sup> (mean

86.3 mg kg<sup>-1</sup>) in Native oysters. These values are in agreement with earlier data published by CEFAS (MAFF, 1992, 1993 and 1994) and consistent with the recent data for market samples of cockles and mussels (MAFF, 1998).

The highest concentrations of copper were found in samples from areas such as the Thames Estuary, North Kent coast, Portsmouth Harbour, Plymouth Taw/Torridge and the Fal. These areas receive discharges and emissions from metallurgical industries and copper is one of the main metals that have been mined in the Fal area for centuries.

The recommended limit for copper in food (Ministry of Food, Food Standards Committee, 1956) is 20 mg kg<sup>-1</sup> but higher levels are permitted in shellfish if copper is of natural occurrence. Oysters are known to accumulate some metals such as copper and zinc more readily and to higher concentrations than other shellfish and the concentrations recorded in this survey are not unusual.

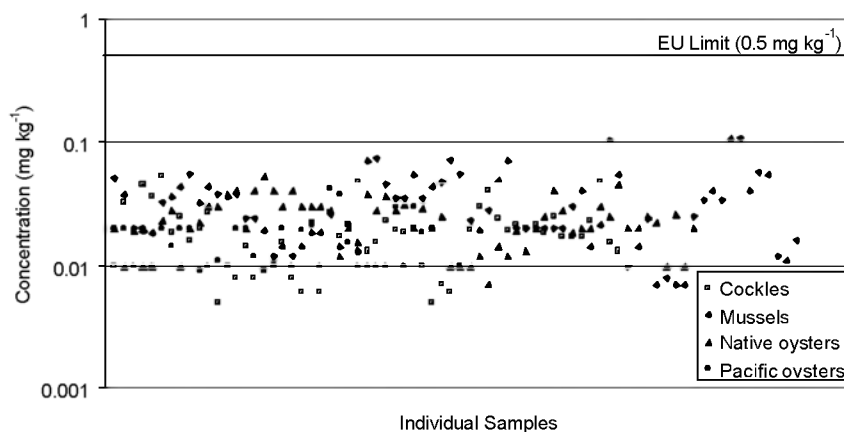
### **Iron**

Iron is the most abundant metal in the earth's crust. It is mainly used in the iron and steel works industry, but is also used in petroleum refining and chemical and fertiliser production. The main sources of iron to the marine environment are geological weathering and mine effluents, industrial effluents and emissions and the corrosion of iron and steel. As with most of the other metals, highest concentrations of iron were found in samples from areas with associated industrial inputs or inputs from mineralised zones.

Concentrations of iron were generally higher in cockles and mussels although 20% of Native oysters had concentrations between 100 and 400 mg kg<sup>-1</sup> (means 98 and 67 mg kg<sup>-1</sup>, for Native and Pacific oysters, respectively). The highest concentrations were found in cockles from Blakeney (700 mg kg<sup>-1</sup>), the Blackwater (1010 mg kg<sup>-1</sup>) and Anglesey (728 mg kg<sup>-1</sup>), mussels from the Thames Estuary (457 mg kg<sup>-1</sup>) and the North Kent coast (423 mg kg<sup>-1</sup>), Native oysters from the Fal (383-412 mg kg<sup>-1</sup>) and Newtown (379 mg kg<sup>-1</sup>) and Pacific oysters from the River Exe (535 mg kg<sup>-1</sup>).

### **Mercury**

The major source of mercury in the environment is the natural degassing of the earth's crust which is estimated to produce up to 125,000 tonnes year<sup>-1</sup> (WHO, 1989). Anthropogenic sources arise from the mining and smelting of mercury ores and the use of mercury in industries such as the chloralkali, electrical equipment and paint industries. Releases into the environment also occur from the burning of fossil fuels and the production of cement, steel and phosphate. Mercury discharges by the chloralkali industry and other sectors are now subject to the provisions of EC Council Directives 82/176/EEC (European Communities, 1982) and 84/156/EEC (European Communities, 1984).



**Figure 5. Concentration of mercury in shellfish from designated bivalve production areas in England and Wales**

Concentrations of mercury (Figure 5) for all species were in the range 0.005 - 0.11 mg kg<sup>-1</sup>. Highest concentrations were recorded in mussels from Menai Strait-west (0.071 and 0.074 mg kg<sup>-1</sup>), Morecambe Bay (0.072 mg kg<sup>-1</sup>), Liverpool Bay (0.071 mg kg<sup>-1</sup>), Colwyn Bay (0.11 mg kg<sup>-1</sup>) and Lynmouth (0.11 mg kg<sup>-1</sup>).

Although the use of mercury has reduced due to recent legislation, high concentrations are still present in sediments in areas associated with industrial applications of mercury. Morecambe Bay and Liverpool Bay are subject to discharges from the chlor-alkali industry and as such are known 'hot spot' areas for mercury. The relatively high levels recorded in samples from these areas are not therefore unexpected. The source of contamination in the Lynmouth sample is likely to be a combination of both the natural and industrial inputs to the Bristol Channel.

European Commission Decision 93/351/EEC (European Communities, 1993) set a maximum limit for the mean total mercury content of the edible parts of fishery products, of 0.5 mg kg<sup>-1</sup> wet weight. Concentrations in all the samples in this survey were well below the limit.

### **Nickel**

Nickel is a ubiquitous trace metal. It is introduced in to the marine environment by the natural erosion of soils and rocks and by removal from the atmosphere, surface run-off and the discharge of industrial wastes. Nickel is extracted from mined ore and used in the production of stainless steel and other nickel alloys. Nickel compounds are also used as catalysts, pigments and in nickel-cadmium batteries. The primary sources of nickel emissions to the atmosphere are the combustion of fossil fuels, waste incineration, mining and production, steel and cement manufacture and electroplating.

Lowest concentrations of nickel were found in oysters, 0.02-4.7 mg kg<sup>-1</sup> in Native oysters and 0.05-3.4 mg kg<sup>-1</sup> in Pacific oysters. Concentrations in mussels were

generally similar to, or slightly higher than oysters (0.13-4.8 mg kg<sup>-1</sup>). Concentrations in cockles were up to ten times higher, ranging between 2.3 and 17 mg kg<sup>-1</sup>, (excluding the one sample of 0.23 mg kg<sup>-1</sup>). This is considerably higher than the mean concentration for shellfish (0.61 mg kg<sup>-1</sup>) reported in MAFF, 1998.

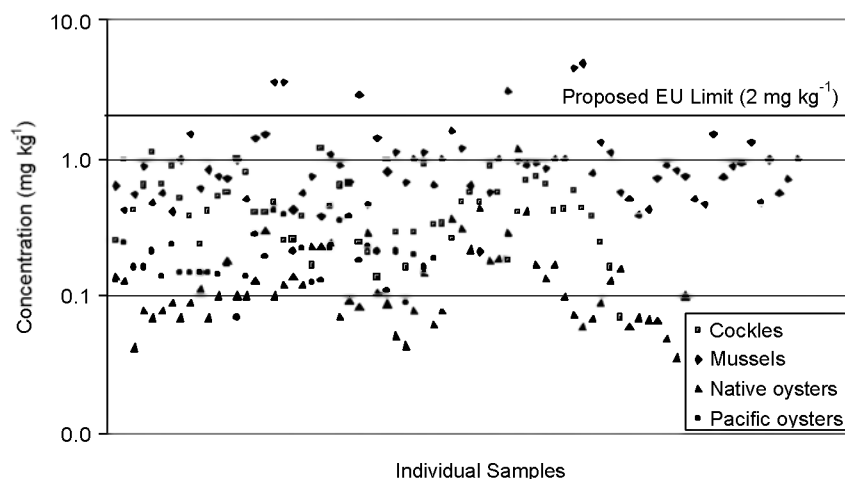
The highest concentrations of nickel were found in areas such as the Thames estuary, North Kent coast, Humber and Silloth, all of which house industries associated with nickel emissions, e.g. oil and gas fired power stations, cement works, metal refining and steel works.

### **Lead**

Lead occurs naturally in sea water from the weathering of rocks and volcanic activity. Additional inputs arise from lead mining, smelting and processing and the burning of fossil fuels. Lead is used in the construction and automotive industries for the production of roofing materials, guttering and flashing and for the manufacture of petrol additives and batteries. Lead is also used for radiation shields, paints, plastics, ammunition and cable sheathing.

Mean concentrations of lead (Figure 6) were similar in both species of oysters at 0.15 mg kg<sup>-1</sup> for Native oysters and 0.20 mg kg<sup>-1</sup> for Pacific oysters. Concentrations in cockles and mussels were generally higher with means of 0.49 mg kg<sup>-1</sup> and 1.1 mg kg<sup>-1</sup>, respectively). These values were consistent with previous data (MAFF, 1992, 1993 and 1998).

The UK limit for lead in food is currently 1 mg kg<sup>-1</sup>, set under the Lead in Food Regulations 1979 (Great Britain - Parliament, 1979). A more specific limit of 10 mg kg<sup>-1</sup> was set for lead in shellfish. Concentrations in all samples were below this UK limit. In the European Commission Draft Regulation setting maximum limits for lead and cadmium in food (European Communities, 1997), the proposed limit for lead in molluscs is 2.0 mg kg<sup>-1</sup> wet weight.



**Figure 6. Concentration of lead in shellfish from designated bivalve production areas in England and Wales**

Concentrations in cockles, oysters and the majority of mussels were all below the proposed limit. Six samples of mussels contained concentrations of lead above the limit: Plymouth (3.6 mg kg<sup>-1</sup>), Dovey (2.9 mg kg<sup>-1</sup>), Anglesey (2.4 mg kg<sup>-1</sup>), Liverpool Bay (3.1 mg kg<sup>-1</sup>) and Silloth (4.5 and 4.9 mg kg<sup>-1</sup>), most likely due to industrial inputs.

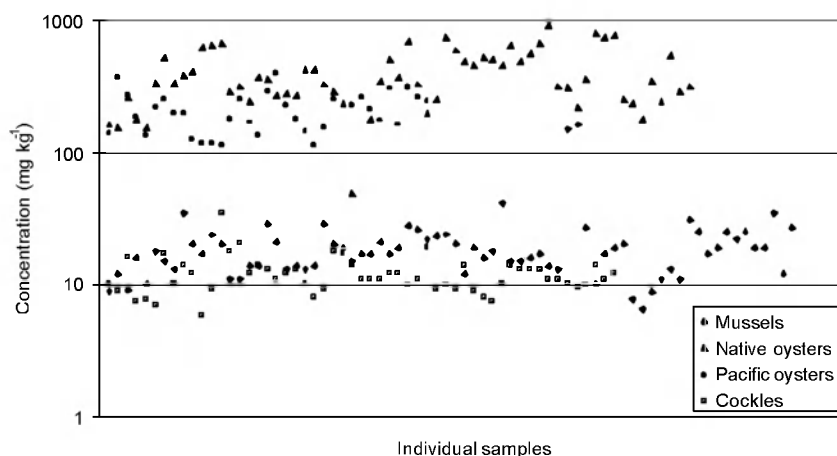
### Selenium

Selenium is usually obtained commercially as a by-product of electrolytic refining processes of copper, nickel and zinc ores (MAFF, 1998). It is used in the manufacture of glass, plastics, ceramics, pigments, lubricants, electronic components and shampoos and is also a constituent of some dietary supplements. Several of these industries are located in areas where the highest concentrations of selenium were found in bivalves, i.e. paint and plastics manufacture and electronic and mechanical engineering.

Concentrations in all species were generally between 0.3 and 1.0 mg kg<sup>-1</sup> and similar to levels reported for cockles and mussels in MAFF, 1998. Slightly higher concentrations (up to 4.0 mg kg<sup>-1</sup>) were present in a number of samples of mussels and Native oysters, with relatively high concentrations (>2.0 mg kg<sup>-1</sup>) found in oysters from the Blackwater (3.6 mg kg<sup>-1</sup>) and the North Kent coast (3.1 mg kg<sup>-1</sup>), and mussels from Anglesey (2.4 mg kg<sup>-1</sup>).

### Zinc

Concentrations in cockles and mussels were relatively low (5.7-40 mg kg<sup>-1</sup>, mean 12.1 mg kg<sup>-1</sup> and 22 mg kg<sup>-1</sup>, respectively (Figure 7)). Concentrations in oysters were generally between 100 and 1000 mg kg<sup>-1</sup>, i.e. 10 to 100 fold higher than cockles and mussels (mean 200 mg kg<sup>-1</sup> for Pacific oysters and 400 mg kg<sup>-1</sup> for Native oysters). These values are consistent with previous CEFAS data (MAFF, 1992 and 1993).



**Figure 7. Concentration of zinc in shellfish from designated bivalve production areas in England and Wales**

The highest concentrations were found in areas such as the Fal, Bristol Channel, Thames estuary, Liverpool Bay, North Kent coast and Plymouth. The overall mean for all mollusc samples was 154 mg kg<sup>-1</sup>.

Zinc occurs widely in a number of minerals and is easily extracted from its ores. It is used in plating for the protection of steel against corrosion, in the manufacture of a number of alloys including brass and in storage batteries. It is also used as a dietary supplement. Sources of zinc in the environment include natural weathering of rocks, mining and, metal refining and smelting. There is also recent evidence that the erosion of zinc-based sacrificial anodes in ships and boats is a significant contributor to estuarine zinc budgets (Matthiessen *et al.*, 1999). As might be expected, the highest concentrations of zinc were found in areas receiving industrial inputs of metals or high natural inputs.

The guideline set for zinc in food by the Food Standards Committee (Ministry of Food, 1953) is 50 mg kg<sup>-1</sup>, with higher levels permitted in food which naturally contains more, such as shellfish. Although the concentrations in all samples of oysters were well above this general guideline they are not considered to be of concern.

## 2.4 Conclusions

The initial phase of this project has produced the first substantial dataset on the spatial distribution of trace metal contaminants in commercially exploited bivalve molluscs.

There was evidence of considerable differences in the levels of uptake of various metals, between species. Cockles contained the highest concentrations of Ni and Fe and the lowest concentrations of Cd, Cu and Zn. Oysters contained the highest concentrations of Cd, Cu and Zn and the lowest concentrations of Pb and Ni. Whilst mussel contained the highest concentrations of Pb and As.

The highest concentrations were found in areas associated with high levels of industrial activity, particularly metallurgical industries or in some cases high shipping/boating activity, or highly mineralised areas.

The results indicate that concentrations of metals in most bivalve molluscs from commercial harvesting areas are below existing standards and guidelines in England and Wales and relevant EU proposed limits. Jones *et al.* (1998) calculated estimated total dietary intakes of metals for high-level consumers of molluscs, all of which fell below established Permitted Tolerable Weekly Intakes and Permitted Maximum Tolerable Daily Intakes (PMTDIs) recommended by the Joint

Evaluation Committee on Food Additives. In all cases where PMTDIs were available, the estimated total dietary intake of metal represented less than 50% of the recommended limit.

It is known from various studies, including monitoring work carried out by CEFAS during the Wheal Jane mine incident (Fowler and Oregoni, 1976; Forstner and Wittmann, 1979; MAFF, 1993 and 1994; Law *et al.*, in press), that there is a seasonal variation in the concentrations of metals such as cadmium, copper and zinc and other contaminants, in some species of molluscs. In Native oysters, metal concentrations tend to peak between April and July, whilst in mussels there is evidence to suggest concentrations peak in February/March. The majority of samples in this survey were collected during February/March and therefore the levels of metals present in the samples may not necessarily represent annual peak concentrations, particularly for Native oysters. Further work is required to determine in more detail the influence of seasonal variation on the levels of contaminants, in all commercial species of bivalve molluscs, in order to aid the interpretation of results and develop the most effective sampling and monitoring programme.

## 3. ARSENIC SPECIATION IN FISH MUSCLE AROUND THE COASTS OF ENGLAND AND WALES

### 3.1 Introduction

Arsenic is classified under list II of the EC regulation 76/464/EEC and has a maximum permissible limit of 1.0 mg kg<sup>-1</sup> under the Arsenic in Food Regulation, 1959 (SI 1959, No: 831). This limit is often exceeded by marine biota by up to 20 to 30 times. However, the main arsenic species found in marine biota is the metabolite arsenobetaine which is not thought to have mammalian toxicity.

Following concern over weapons disposal in the area of Beaufort's Dyke in the Irish Sea, samples of fish muscle were tested from around the coast of England and Wales and, in particular, the north Irish Sea. Previous work at the CEFAS Burnham Laboratory had shown that the inorganic fraction of arsenic in fish from the Humber Estuary was very low (Table 1). The aims of this study were firstly, to confirm that the most toxic forms of arsenic, inorganic arsenic III and V were present in very low concentrations and secondly, to confirm which species were present in the fish muscle. Most of these samples had previously been tested for total arsenic and the majority were found to have relatively high concentrations of arsenic.

**Table 1. Comparison of total arsenic concentration with inorganic arsenic concentration**

Species	Date	Total Arsenic mg kg <sup>-1</sup> wet weight	Inorganic Arsenic mg kg <sup>-1</sup> wet weight
Plaice	Jul-85	9.2	0.033
Plaice	Jun-86	15	0.084
Flounder	Jul-85	14	0.041
Flounder	Jun-86	11	0.04
Sole	Jul-85	15	0.021
Sole	Jun-86	10	0.039
Sole	Aug-86	22	0.06
Cod	Jul-85	9	0.009
Cod	Jun-86	9.3	0.023
Whiting	Jul-85	12	0.013

Arsenic can exist in many forms in the environment. In marine biota the main forms thought to be present are: - Inorganic As III and As V and organic forms of methylarsenic, MMA and dimethyl arsenic, DMA and the metabolites arsenocholine, ASC and arsenobetaine, ASB, although other forms of reducing sugars are known to be present. The known toxicity order is:-

As III > As V > MMA > DMA > ASC > ASB

### 3.2 Method

The method used for this investigation for arsenic speciation was developed by Thomas and Sniatecki (1995). The method uses ion-pair reversed phase chromatography coupled to inductively coupled plasma-mass spectrometry.

Samples were extracted using a methanol water mixture 75:25 V/V on up to 2 g of wet tissue in a centrifuge tube. This was sonicated for five minutes and centrifuged, the extract was then transferred to a round bottomed flask. This extraction was repeated five times. The methanol was then reduced in a rotary

evaporator to ~10 mls and quantitatively transferred to a 50 ml flask.

An isocratic Waters pump running at 0.9 mls min<sup>-1</sup> was connected to a Hamilton PRP-1 resin based reverse phase column. Injection was manual with a Rheodyne 7125 valve with a 50 µl loop. The mobile phase was 0.5 mmol<sup>-1</sup> tetrabutyl ammonium phosphate buffered with sodium hydrogen phosphate to pH 9. Connection to the VG2+ Plasma mass spectrometer, ICP-MS, was made using a 0.5 m peek tubing of 0.17 mm internal diameter to a V-grooved nebuliser and double pass spray chamber cooled to 8°C. The ICP-MS was set up as normal and tuned on mass 75 with a 10 ppb As III solution before connection to the HPLC system.

Thomas and Sniatecki (1995) showed the elution order for the arsenic species to be ASC, ASB, AS III, DMA, MMA, AS V. For this work only the standards ASB, DMA, AS III and AS V were present.

### 3.3 Results

Table 2 gives the results for the fish samples analysed in this study. Where no standard was present but a signal appeared on the chromatogram a '+' is shown, and where no signal was found a '-' is shown. The inorganic fraction showed no arsenic V in any of the samples, although a trace of arsenic III was found in some samples up to 0.16 mg kg<sup>-1</sup>, which is consistent with previous data. Figure 8 shows a standard of 4 species of arsenic at 5 µg kg<sup>-1</sup> ASB, AS III, DMA and AS V with clear separation. Figure 9 shows a typical chromatogram of a sample of fish muscle, with all the arsenic in the form of arsenobetaine. As expected, all of the results show that the predominant arsenic species present in all samples is in the form of arsenobetaine. This is further illustrated in Figure 10 where the concentrations of arsenic species are compared to the concentrations of total arsenic found.

**Table 2. Arsenic speciation**

Sample	Area	ASC mg kg <sup>-1</sup> wet weight	ASB mg kg <sup>-1</sup> wet weight	AS III	DMA	MMA	AS V	Total
Dorm-2	Refmat	+	13.6	<0.01	0.5	-	<0.01	18
Cod	Irish Sea	+	13.8	<0.01	0.51	-	<0.01	14
Pollack	Irish Sea	+	28 <sup>#</sup>	<0.01	0.7	-	<0.01	26
Whiting	Irish Sea	+	11.2	<0.01	0.4	-	<0.01	12
Cod	Irish Sea	+	8.2	<0.01	0.12	-	<0.01	7.9
Cod	Western Isles	-	14.6	<0.01	0.02	-	<0.01	15
Haddock	Western Isles	-	12.9	0.16	<0.01	-	<0.01	12
Dab	Humber	-	6.4	0.07	<0.01	-	<0.01	*
Cod	Channel	-	3.3	0.05	<0.01	-	<0.01	*

Note: - Not present on chromatogram

+ Present but not calibrated

\* Not available

<sup>#</sup> The analytical error for arsenic species analysis is higher than that for total arsenic analysis, this occasionally results in arsenic species values being higher than the total arsenic value

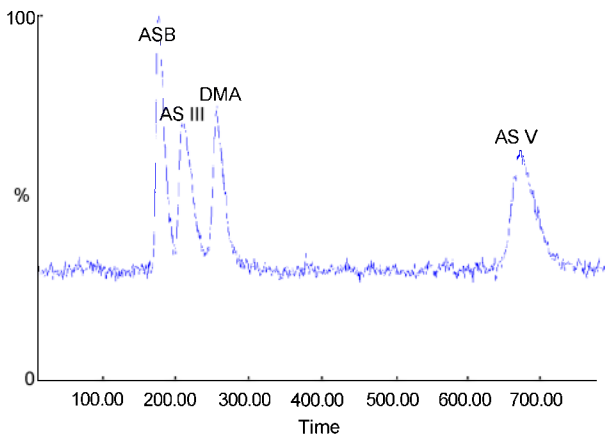


Figure 8. Combined arsenic standard at 5 µg kg<sup>-1</sup>

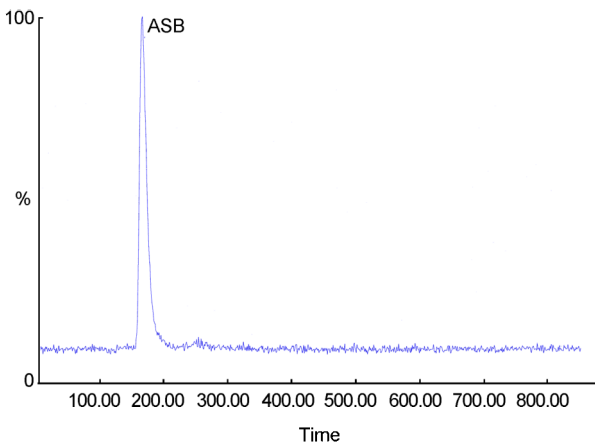


Figure 9. Chromatogram of arsenic species in flounder muscle

### 3.4 Conclusion

The data suggest that the high concentrations of arsenic found in fish muscle do not pose a health risk since the main arsenic species present is arsenobetaine and the inorganic fraction is well below the 1.0 mg kg<sup>-1</sup> limit. Further work could be carried out to include a greater number of samples and other fish species. Shellfish from around the coasts should be analysed to confirm that the dominant arsenic species present is in the safe form of arsenobetaine.

## 4. PAH LEVELS IN FISH AND SHELLFISH, POST SEA EMPRESS OIL SPILL

Following the grounding of the oil tanker *SEA EMPRESS* at the mouth of Milford Haven in 1996, a major monitoring programme was undertaken. Fisheries in the area were closed under the Food and Environment Protection Act (1985) as a precaution, in order to protect consumers. The closure area included about 2,000 km<sup>2</sup> of the coastal waters off south-west Wales, and all the rivers discharging into this area (Figure 11).

Monitoring work conducted by CEFAS had two main aims; firstly, to support the closure order, and secondly, to contribute to the overall impact assessment being conducted by the Sea Empress Environmental Evaluation Committee. Although this effort was primarily directed towards the analysis of biota samples (particularly commercial fish and shellfish species), other biological samples, seawater and sediments, were also analysed in order to yield the fullest possible

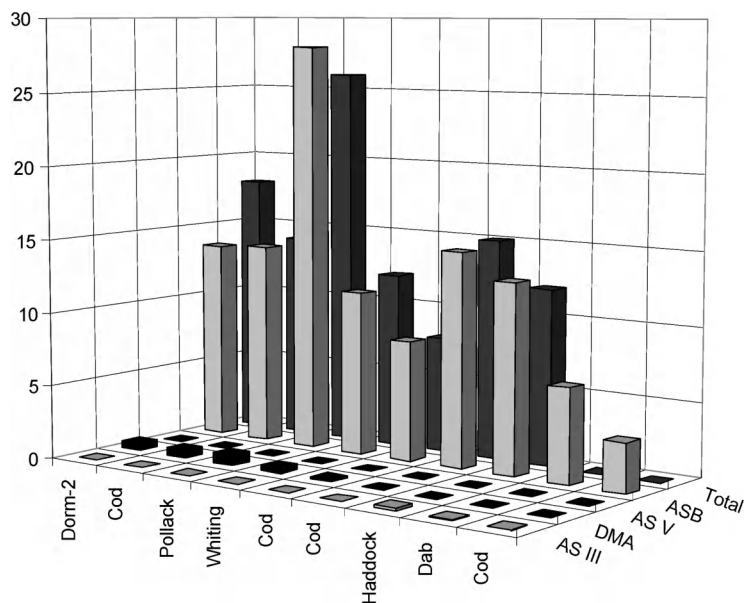
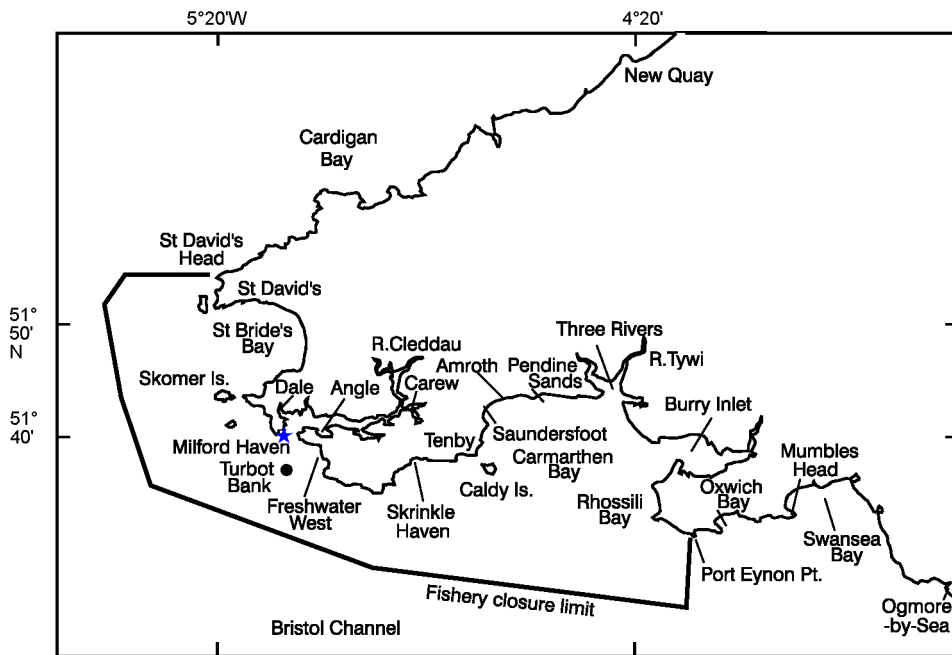


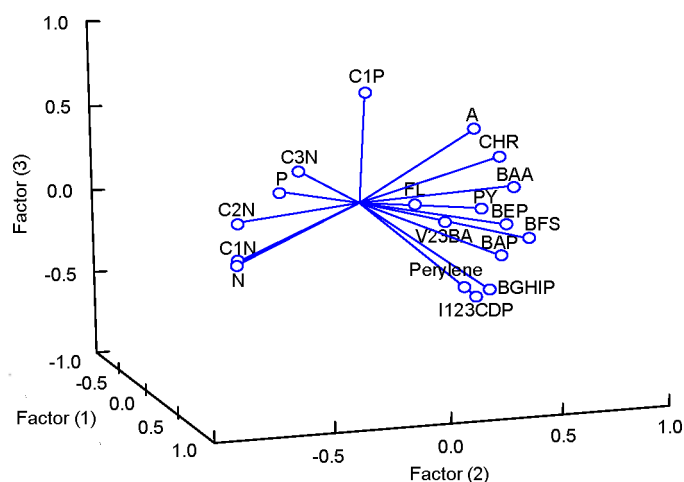
Figure 10. Comparison of arsenic species



**Figure 11.** The coast of south-west Wales affected by oil spilled from the SEA EMPRESS. The line indicates the outer boundary of the fisheries closure area designated under the Food and Environment Protection Act, 1985, and the star symbol marks the SEA EMPRESS grounding site at St. Ann's Head

dataset for impact assessment. The data from the monitoring study were used to follow the uptake and elimination of oil and polycyclic aromatic hydrocarbons (PAH) by animals, and so to manage the fishery controls. In the event, contamination declined relatively rapidly, and finfisheries in the area were opened within 3 months of the spill, and all other fisheries within 19 months.

In contrast to fish and crustacea, which generally took up only low concentrations of 2- and 3-ring PAH (presumably across their gill surfaces directly from the dissolved phase), bivalve molluscs in the spill area rapidly accumulated a wider range of PAH (2- to 6-ring compounds). Application of principal components analysis to the PAH dataset for cockles, mussels and oysters together yielded the plot given in Figure 12.



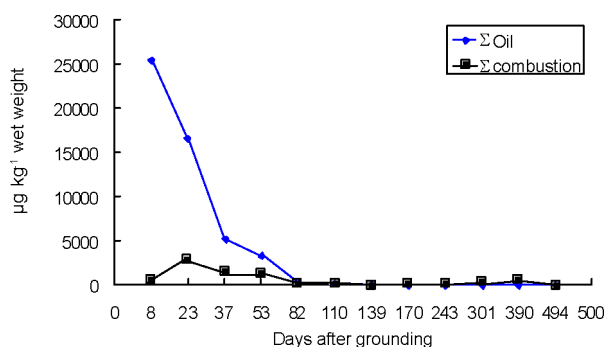
**Figure 12.** Plot of PAH data for bivalve molluscs (cockles, mussels and oysters) derived from principal component analysis. The first two principal components accounted for 68% and 12% of the total variance respectively. This plot shows the vectors for primarily oil-derived PAH (naphthalene and C<sub>1</sub> to C<sub>3</sub>-naphthalenes, and phenanthrene and C<sub>1</sub>-phenanthrenes) clustering away from the combustion PAH



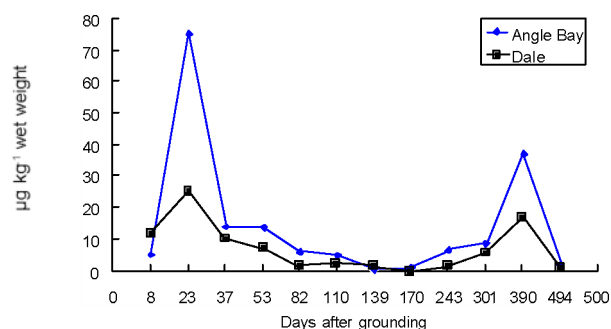
This shows the coefficients of the first three components plotted against each other, and suggests a differing of behaviour between two groups of PAH compounds. In the plot the PAH are separated into two groups, one containing naphthalene, the C<sub>1</sub>- to C<sub>3</sub>-naphthalenes, phenanthrene and the C<sub>1</sub>-phenanthrenes; and the other containing anthracene, fluoranthene, pyrene, benzanthracenes and chrysene, benzofluoranthenes and benzopyrenes, indeno[1,2,3-*cd*]pyrene and benzo[*ghi*]perylene.

In essence this corresponds to one group primarily derived from oil, and a second primarily derived from combustion processes. Figure 13 illustrates the time trend observed at Angle Bay over approximately 500 days following the grounding for PAH grouped by their distribution in Figure 12 as  $\Sigma$ Oil and  $\Sigma$ Combustion. Eight days after the grounding the  $\Sigma$ Oil concentrations have peaked, as oil is no longer being released at the entrance to Milford Haven and bulk oil has moved to the east, whilst concentrations of the  $\Sigma$ Combustion are highest in samples 23 days after the spill began. In both cases concentrations decline until mid-summer, but the  $\Sigma$ Combustion concentrations rise again approximately 1 year post-grounding whilst  $\Sigma$ Oil does not. Figure 14 shows the concentrations of benzo[*a*]pyrene at Angle Bay and Dale over the same period, both exhibiting peak concentrations 1 year apart (in March) and falling close to zero in August 1996. Although there may be some contribution to the first of these peaks from the *SEA EMPRESS* incident, the pattern appears to be primarily seasonal. A similar inusoidal variation in the concentration of benzo[*a*]pyrene by season has been reported in mussels from List-Königshafen/Sylt in Germany (Jacob *et al.*, 1996; 1997). It is likely that three factors contribute to this seasonal variation:

1. higher concentrations of high-MW PAH in the water column during the winter deriving from increased power generation, space heating, atmospheric deposition, enhanced run-off and freshwater inputs, sediment resuspension, etc. Increased photodegradation during summer could also act to reduce PAH concentrations.
2. although the ability of mussels to metabolise PAH is limited in comparison with fish, they do possess a mixed-function oxygenase system and so have some metabolic capacity for these compounds. As the body temperature of mussels will rise and fall with the water temperature then it is likely that this capacity will be reduced in the winter when water temperatures are low, as enzymatic reactions within the mussels' tissues will proceed more slowly.
3. progressive lipid storage through the autumn and winter occurs as gonad development proceeds in preparation for spawning in the spring. The increase in the level of triglycerides and phospholipids in mussel tissue, which peaks just prior to spawning, will lead to



**Figure 13. The time trend observed at Angle Bay over approximately 500 days following the grounding, for PAH grouped as  $\Sigma$ Oil and  $\Sigma$ Combustion in accordance with the groups identified in Figure 12**



**Figure 14. The concentrations of benzo[*a*]pyrene in mussels from Angle Bay and Dale over the 500 days following the grounding of the *SEA EMPRESS***

higher body burdens of lipophilic contaminants (such as PAH); these contaminants will be released with the gametes at spawning, thereby leading to a lesser body burden in the adult mussels. Spawning is followed by a period of rapid growth as temperatures rise and productivity in coastal waters increases, thereby reinforcing the decline in PAH concentrations.

It is not known at present which of these processes dominates in this situation, and it will be necessary for other populations to be studied in order to elucidate this. It would, for instance, be useful to conduct time-trend studies on molluscs which do not normally undergo spawning (such as farmed Pacific oysters, *Crassostrea gigas*, in the UK), and on mussel populations which spawn either at different times of year or on more than one occasion.

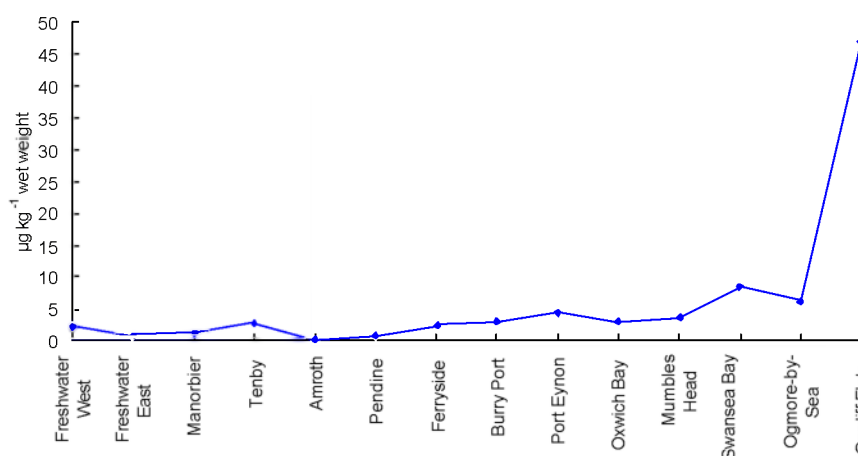
In March and October 1996, additional samples of mussels were collected along a transect from New Quay in Cardigan Bay to Ogmores-by-Sea in the Bristol Channel, through the fishery closure area and well



beyond the limits both to the north of St. David's and the east of Port Eynon. Figure 15 shows the benzo[a]pyrene concentrations found in the eastern part of the transect in October (supplemented by a few winter samples taken in February), from Freshwater West by the mouth of Milford Haven, to Cardiff. This indicates a steady increase in benzo[a]pyrene concentration from Amroth (on the north shore of Carmarthen Bay) along the Bristol Channel to the east, with the highest value in the sample from Cardiff Flats. This is probably related to the trend in urban and industrial development, with the majority of the population of South Wales being concentrated in the cities of Swansea, Cardiff and Newport to the east of the study area. The River Severn (which feeds into the upper reaches of the Bristol Channel) also delivers water from a large catchment area, so overall PAH

inputs are likely to be much greater further up the Bristol Channel.

This study demonstrated once more that PAH are readily accumulated by filter-feeding bivalves following oil spills. Not all PAH absorbed were predominantly related to the oil spilled, and both spatial and temporal variations have been seen which are related to other (both pre-existing and continuing) sources in the area. In addition, seasonal variations were observed in PAH and benzo[a]pyrene levels which are related directly to the physiology of molluscs. All of these factors will need to be taken into account when planning monitoring studies, whether following future spill incidents or in investigations of chronic inputs of PAH from specific or diffuse sources (e.g. Mussel Watch programmes) and food quality assurance programmes.



**Figure 15. Benzo[a]pyrene concentrations in mussels from the eastern part of the transect sampled in winter (October to February). This section runs from Freshwater West by the mouth of Milford Haven to Cardiff**

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## SEDIMENTS

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### 5. TOTAL ARSENIC IN SEDIMENTS FROM THE WESTERN NORTH SEA AND THE HUMBER ESTUARY

#### 5.1 Introduction

In previous surveys of North Sea sediments, arsenic has often been omitted from the range of metals analysed. In this study archived samples from these earlier surveys were analysed and the data combined with recent results for the Dogger Bank, to examine the distribution of total arsenic in sediments from the

western North Sea and Humber Estuary. Historically, the Humber has been subjected to a large point discharge of arsenic from an industrial source, and so there is interest in investigating the extent of contamination in the area.

#### 5.2 Method

Samples were collected during various surveys over the years 1991-1995. The <2 mm fraction of the sediments was digested using a mixture of hydrofluoric acid and either nitric acid or *aqua regia* with microwave heating (Jones and Laslett, 1994). Analyses of certified reference materials showed that the data collected were accurate and comparable.

**Table 3. Regression equations for arsenic versus aluminium, rubidium or iron in the western North Sea and Humber Estuary**

Metals	Region	Regression equation	r <sup>2</sup>	n	P
As v Al	Humber	[As] = 5.32 . [Al] + 9.00	0.750	16	***
	W North Sea	[As] = -1.19 . [Al] + 16.78	0.003	195	ns
	Dogger Bank	[As] = 6.09 . [Al] - 3.23	0.203	258	***
As v Rb	Humber	[As] = 0.50 . [Rb] + 3.84	0.746	16	***
	W North Sea	[As] = -0.29 . [Rb] + 22.06	0.020	195	ns
	Dogger Bank	[As] = 0.18 . [Rb] + 0.83	0.046	257	*
As v Fe	Humber	[As] = 8.09 . [Fe] + 6.81	0.747	16	***
	W North Sea	[As] = 15.40 . [Fe] - 4.12	0.541	195	***
	Dogger Bank	[As] = 7.31 . [Fe] - 0.06	0.406	258	***

Notes: [As] = As  $\mu\text{g g}^{-1}$   
[Rb] = Rb  $\mu\text{g g}^{-1}$   
[Al] = Al %  
[Fe] = Fe %

\*\*\* = significant at  $P < 0.001$  level  
\* = significant at  $P < 0.1$  level  
ns = not significant

### 5.3 Results and discussion

The  $<2 \mu\text{m}$  fraction of the sediment is one of the major sinks for contaminants introduced to natural waters. This is largely due to the presence of mineral clays, and to organic coatings and iron and manganese (oxy-) hydroxide coatings on clays. Such substances scavenge dissolved trace metals from the water column and can act to transport metals into the sediment. This fine fraction can be taken into account by normalisation of the metal concentration to a measure of the dominant metal-binding component, such as the aluminium concentration for clays (ICES, 1993; Loring, 1991; Rowlett and Lovell, 1994), or iron for the iron (oxy-) hydroxide coatings (Whalley *et al.*, 1997).

The normaliser showing the strongest relationships with arsenic across the survey area is iron (Table 3). Arsenic is known to have a high affinity with iron (oxy-) hydroxide coatings on sediment particles (Belzile and Tessier, 1990; Fuller *et al.*, 1993; Howard *et al.*, 1984; Langston, 1983; Raven *et al.*, 1998; Waychunas *et al.*, 1993). It is important to use an appropriate normaliser where the residuals are to be used in assessing the relative degree of contamination. Iron is acceptable here not just because it is statistically significant, but also because there is strong geochemical validity for the approach. Figure 16 shows a map of the arsenic data plotted as positive or negative residuals from the arsenic *versus* iron regression model.

The raw arsenic concentration data show several areas of relatively high arsenic concentrations, notably in areas off north Yorkshire, the Humber, off Norfolk, the outer Thames Estuary and off the south-west Dogger Bank. After normalisation against iron (Figure 16), all areas except those off north Norfolk and the Thames appear much reduced in significance. The Thames Estuary has a history of arsenical waste disposal, but possible reasons for the unexpectedly high values off Norfolk are less clear.

The low residual values observed in the Humber and its plume area are of particular interest, since this estuary has been assumed to be a significant source of arsenic to the North Sea, although some evidence suggests that the metal is retained within the estuary (Kitts *et al.*, 1994). The arsenic data presented here, which show only slightly elevated absolute concentrations in bottom sediments, might be explained either by dilution into the Humber's high suspended load, or by particulate transport away from the region. These particulates may be phytoplankton (Millward *et al.*, 1997) or fine sediments with iron oxyhydroxide coatings. The Humber Estuary receives a large amount of iron waste (Millward and Glegg, 1997), providing a considerable pool of material to which arsenic might sorb. Another unresolved problem, which may or may not be related, is the high concentration of arsenic seen in some sediments off north east Norfolk. The circulation pattern of the North Sea might lead to the suggestion that arsenic from the Humber is being transported to this area; interestingly, the North Sea project showed high particulate iron concentrations in suspended sediments off the coast of Norfolk (Tappin *et al.*, 1995). As yet, there is no hydrographic evidence for settling out of particles off north east Norfolk, which would be required if there was an association with the Humber plume. An alternative explanation may be that drilling has brought arsenic-rich marine shales to the surface, since the affected area coincides with the main group of English North Sea gas fields. Further work is clearly required before the source of the arsenic observed off north east Norfolk can be identified.

#### Acknowledgements

This article summarises a paper in Marine Pollution Bulletin - Whalley *et al.* (1999), and is based on a report for the DETR (Rowlett, S., Whalley, C., Bennett, M. and Jones, B. (1998) *Arsenic in sediments and benthos in the western North Sea, Humber Estuary and Dogger Bank*. DETR report CW0709, CEFAS, Lowestoft, pp26 (plus figures)).

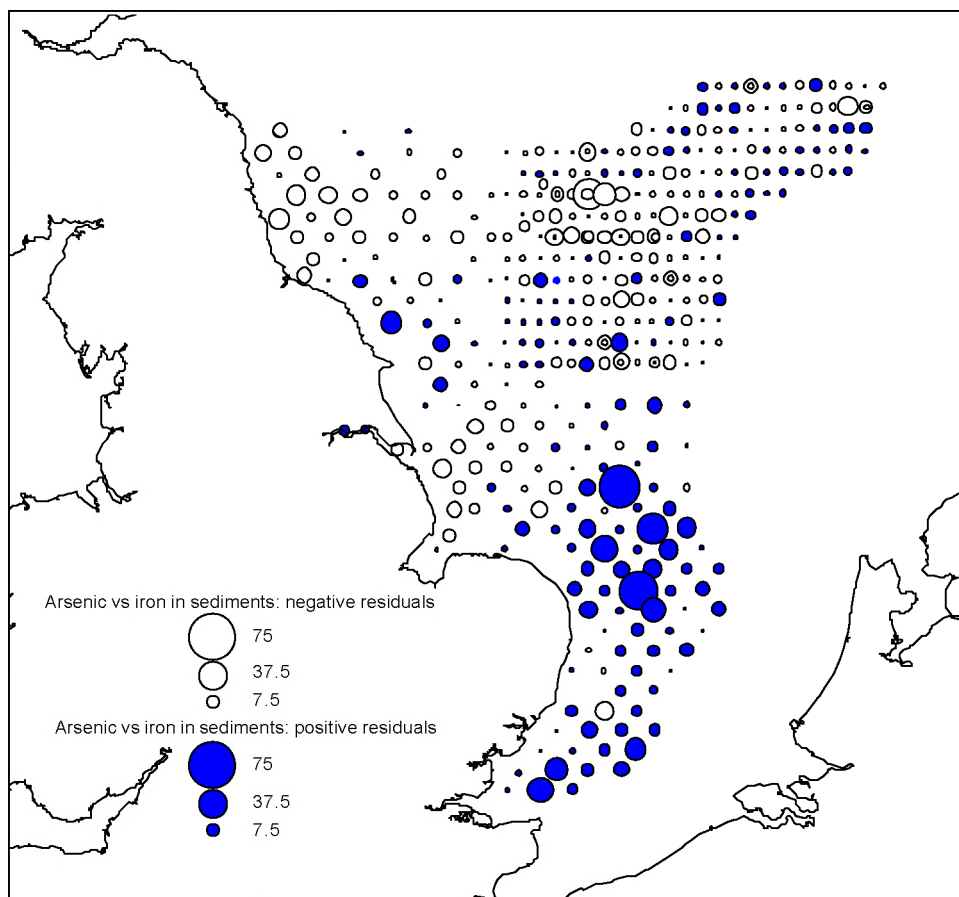


Figure 16. Residual arsenic normalised to iron in the western North Sea

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## BIOLOGICAL EFFECTS

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### 6. THE USE OF ENZYME BIOMARKERS IN BIOLOGICAL EFFECTS MONITORING

#### 6.1 General introduction

A wide variety of potentially harmful contaminants enter the estuarine and coastal waters of the UK. Conventional bioassay methods and chemical analysis techniques have allowed scientists to assess the concentrations of specific pollutants in water and sediment and the potential biological effects of these levels on parameters such as survival, growth and reproduction. However, the need to detect and assess the effects of contamination at ever lower concentrations and in ever more complex mixtures have led to the development of a wide range of sub-lethal indicators of exposure to, and effects of, contaminants

and other environmental stressors (Livingstone *et al.*, 1997). These indicators are collectively referred to as 'biomarkers' and can be either specific (monitoring the presence/effects of specific chemical classes) or general. This section describes the use of two specific enzyme biomarkers (EROD and AChE) by CEFAS to monitor the exposure and effects of specific contaminants in estuarine and coastal fish populations.

#### 6.2 Ethoxyresorufin-O-deethylase (EROD)

##### 6.2.1 Introduction

The mixed function oxygenase (MFO) enzyme system is the primary detoxification pathway for a number of planar, organic contaminants, specifically polycyclic aromatic hydrocarbons (PAH) and some polychlorinated biphenyls (PCB) 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.

Induction of the MFO system, whilst detoxifying certain xenobiotics, may also have deleterious effects. First, many carcinogenic and genotoxic compounds only become harmful after transformation to active forms by the MFO system (e.g. formation of benzo[*a*]pyrene diol-epoxide from the parent compound). Secondly, since essential endogenous substances such as steroids are regulated by the system, abnormally elevated MFO activity could have a detrimental effect upon an organism's normal functions such as growth or reproduction. With this in mind the measurement of EROD levels in fish has become an important element of CEFAS' monitoring programme and is used as a monitor for contaminant exposure and as an indicator of potential future health problems for the fish population. Recent work has been published in Kirby *et al.* 1999(a) and Kirby *et al.* 1999(b).

## 6.2.2 Methods

### **Fish species**

Three fish species have been routinely targeted as sentinel organisms: dab (*Limanda limanda*), plaice (*Pleuronectes platessa*) and flounder (*Platichthys flesus*).

### **Vessel/Trawl gear**

Coastal and offshore samples were taken from aboard the CEFAS Research Vessels, *CORYSTES* and *CIROLANA*, but for nearshore and estuarine sampling smaller chartered trawlers or Environment Agency survey vessels were used. A 2 or 3 metre beam trawl towed at 3-4 knots for 30 minutes to minimise the stress to the catch was used.

### **Processing**

Once on deck, target species were separated into tanks containing flowing sea water. 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 ( $\pm$  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 dithiothreitol, 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. Supernatants 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 ( $\pm$ 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  $\mu$ l liver homogenate, 10  $\mu$ l ethoxyresorufin substrate (0.4 mM in dimethyl sulphoxide, DMSO) and 10  $\mu$ l of resorufin internal standard (25  $\mu$ M 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  $\mu$ l NADPH (0.25 mM) 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.

### **Statistical analysis**

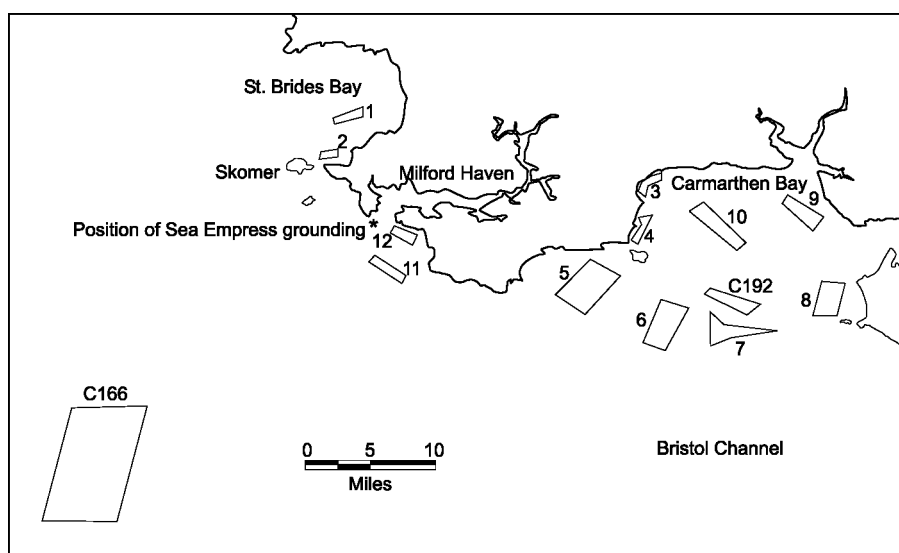
Sampling data were analysed for significant inter-site differences for EROD activity using single factor ANOVA. Certain data subsets were subject to a more in-depth statistical analyses including determination of the significance of inter-site differences between EROD activity, mean length and sex. Correlations were also determined between EROD activity (male and female), length, gonado-somatic index (GSI) (female only) and water depth.

## 6.2.3 Results

### 6.2.3.1 SEA EMPRESS

The EROD assay was deployed in response to the *SEA EMPRESS* oil spill (15 February 1996) in order to determine the extent of exposure, to oil-derived PAH in the local dab and plaice populations. Samples were collected on two separate occasions, once from *CORYSTES* within two weeks of the spillage and a more comprehensive survey was conducted in May 1996 using a chartered local fishing vessel, the *PROVIDER*. The trawl areas are shown in Figure 17.

During the *CORYSTES* cruise the target species were not caught in abundance. In fact dab and plaice were only caught in sufficient numbers from 5 stations: Stn 1 - St. Brides Bay, Stn 4 - Tenby, Stn 7 - Off Carmarthen, Stn 8 - Rhossili Bay and Stn 10 - Central Carmarthen. However, in May 1996 a much wider range of sites was sampled. The differential EROD levels between sites on both sampling occasions are shown in Table 4.



**Figure 17. Areas trawled for dab and plaice samples during research cruises, CORYSTES 3b/96 and PROVIDER, May 1996**

**Table 4. Hepatic EROD activity levels (pM/min/mg protein) in dab and plaice sampled after the SEA EMPRESS oil spill**

Station	Location	February 1996 (CORYSTES 3b/96)		May 1996 (PROVIDER)	
		Dab	Plaice	Dab	Plaice
1	St Brides Bay	2041	388	1496	404
2	St Brides Bay - Off Skomer	-	-	1624	418
3	Saundersfoot/Amroth	-	-	-	714
4	Tenby	1740	556	2122	812
5	Off Manorbier	-	-	2860	768
6	Offshore Caldey	-	-	2634	738
7	Offshore Carmarthen	325	334	3619	1032
8	Rhossili Bay	247	217	3062	672
9	Off Pembrey	-	-	3551	632
10	Central Carmarthen	1624	464	2915	982
11	Turbot Bank	-	-	4909	785
12	Freshwater West	-	-	3918	840
C166	Offshore Milford (Reference)	-	-	3888	-
C192	Carmarthen Bay	-	-	3799	-

The samples at each site were not homogeneous with respect to sex, size and gonadal development due to the fact that enough fish of one sex and size range were often not available. Therefore, the apparent trends in the pooled EROD data must be treated with caution and reviewed with reference to the size/sex composition of the samples. The possible influences of size, sex and gonadal maturation on EROD are discussed further below.

### 6.2.3.2 CIROLANA 6b/96 and CIROLANA 5b/97

The assay was deployed during the summer research cruises of 1996 and 1997. During these cruises hepatic EROD activity in dab was determined from a wide range of coastal and offshore sites. The results from both cruises are shown in Table 5.

**Table 5. Mean hepatic EROD activities (pM/min/mg pro.) in dab from CIROLANA 6b/96 and 5b/97**

Location	EROD (pM/min/mg protein)	
	CIROLANA 6b/96	CIROLANA 5b/97
Inner Cardigan Bay	-	792
Station 189 (Outer Cardigan Bay)	864	808
Dundrum Bay	-	1286
Station 191 (Red Wharf Bay)	1156	757
Station 199 (Liverpool Bay)	983	1782
Station 200 (Burbo Bight)	1146	1397
Station 217 (Morecambe Bay)	1040	2083
Station 218 (S.E. Isle of Man)	601	869
Station 152 (30m SW of Milford)	2549	-
Celtic Deep	-	562
Station 247 (Off Tyne)	1828	1520
Station 252 (Dogger Bank)	1343	668
Station 258 (Off Tees)	1424	1454
Station 259 (Off Humber)	449	580
Station 260 & 261 (The Wash)	830	-

### 6.2.3.3 Estuaries 1997

The assay had been successfully deployed in coastal and offshore situations using both dab and plaice as the target species. However, the relatively low levels of contamination at these sites made contaminant-induced EROD difficult to discern from other influential factors on the biomarker (e.g. temperature, sexual maturity etc). To assess its use in more contaminated environments the assay was deployed in a number of English estuaries using flounder as the target species. The sampling areas are shown in Figure 18. Mean EROD activities at each estuarine site are displayed in Table 6. Significantly elevated levels, compared to the Alde reference site, were evident in ten out of the 16 stations sampled. The sites from the Mersey and Tees showed the greatest induction, reaching a maximum of 161.6 pM/min/mg protein, a four fold increase in induction over the reference level, at the Bromborough site in the Mersey. EROD activities from all the sites sampled are represented in Figure 19.

### 6.2.4 Discussion

#### 6.2.4.1 SEA EMPRESS

The first observation is that the EROD levels tended to be higher in May than in February for both species. Dab from Rhossili Bay and Off Carmarthen showed an eleven-fold increase in activity in May when compared to the earlier sampling date. However, those from off Tenby had similar levels and those from St Brides Bay were in fact lower. Plaice also exhibited higher levels in May by a factor of 2-3 fold except in St Brides Bay where, as with dab, the levels were similar at both sampling times.

It is not clear what could account for the differences in EROD activity ranking for the two periods and why the distribution was so dissimilar for the two occasions. However, it is feasible that the induction pattern in February was at least partially influenced by the, still recent, hydrocarbon spillage, because the nearest site to

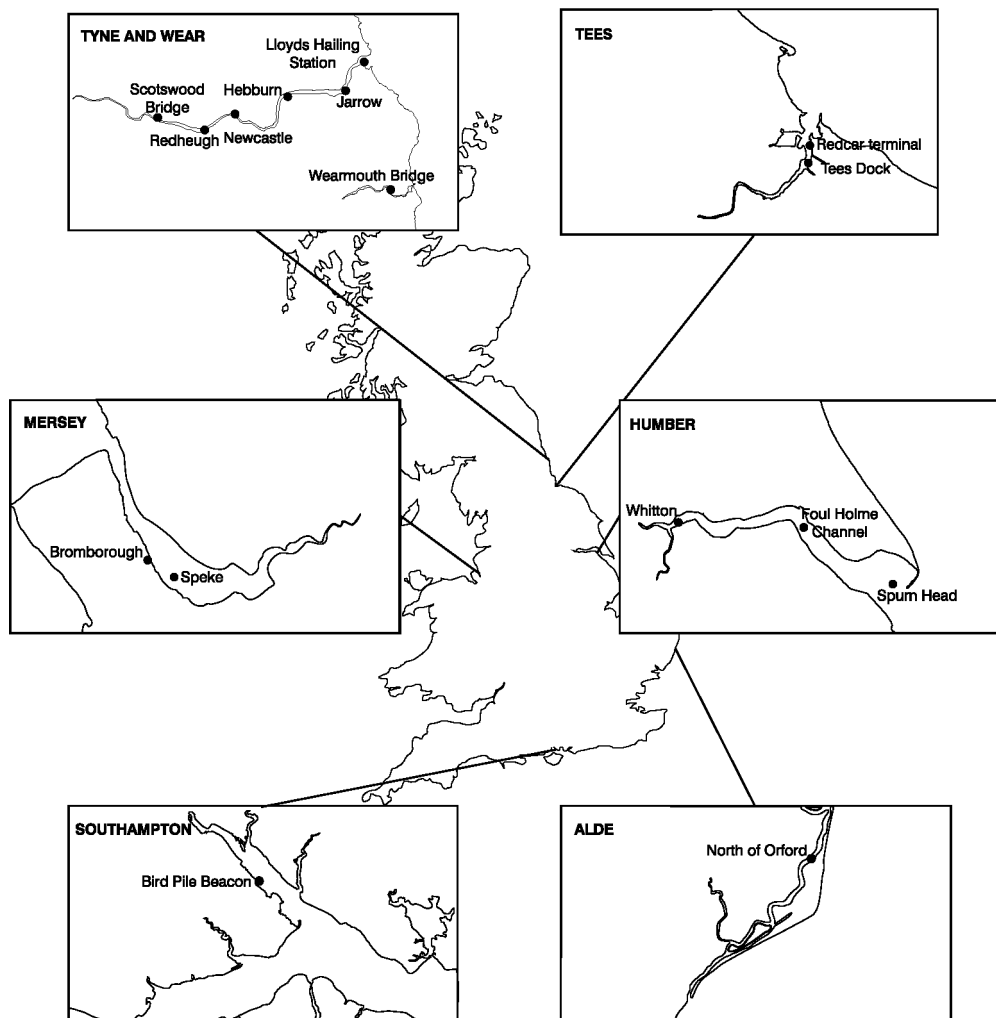


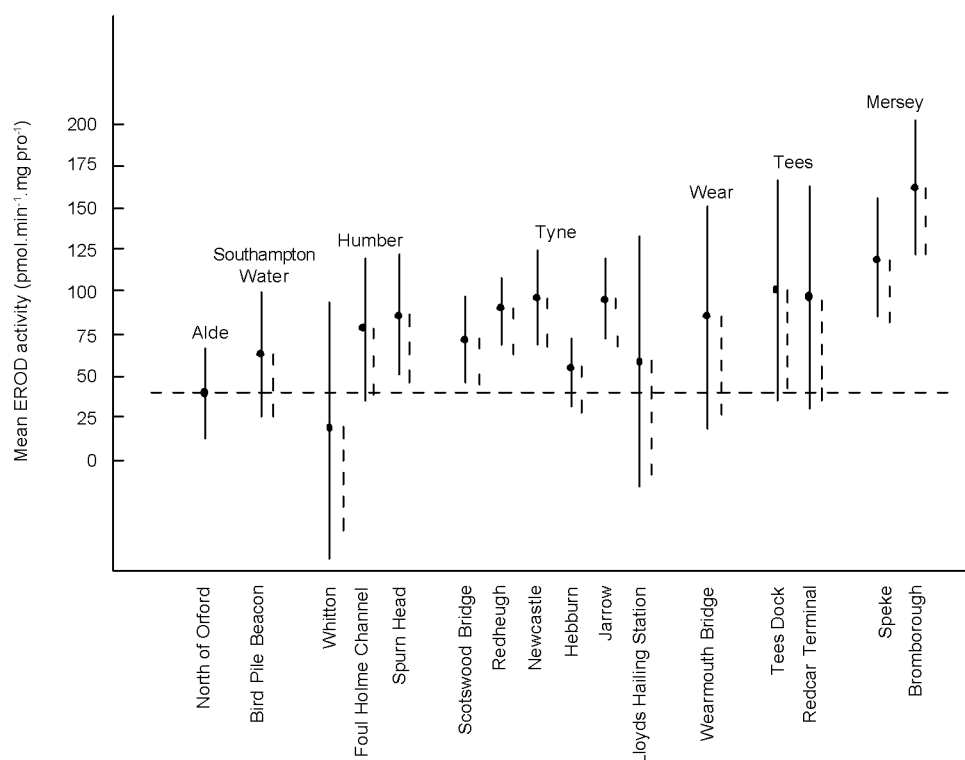
Figure 18. Estuarine sampling locations



**Table 6. Mean EROD and other variables in Flounder from English estuaries (1997)**

Location	Samples(n)	EROD (pmol/min/mg protein)	Length (cm)	Weight (g)	GSI Female	GSI Male	HSI	CF
<b>Alde (Reference)</b>								
North of Orford	24	38.7	25.4	156.8	0.72	0.76	1.03	0.95
<b>Mersey</b>								
Speke	16	117.9 *	25.0	185.3	0.82	-	1.35	0.96
Bromborough	11	161.6 *	28.1	238.2 *	0.98	-	1.58 *	0.98
<b>Tees</b>								
Tees Dock	4	99.8 *	23.9	171.4	1.47	0.17	1.77 *	1.26 *
Redcar Terminal	4	95.6 *	22.0	125.3	1.04	0.08	1.66 *	1.17 *
<b>Humber</b>								
Whitton	3	17.4	22.4	106.0	1.40	-	1.27	0.94
Foul Holme Channel	9	77.0	21.9	117.8	1.11	-	1.79 *	0.98
Spurn Head	13	84.6 *	25.4	181.6	1.27	0.86	0.92	1.07 *
<b>Southampton Water</b>								
Bird Pile Beacon	12	62.1	27.2	218.5	2.52 *	1.36	1.42 *	0.95
<b>Wear</b>								
Wearmouth Bridge	4	84.6 *	20.4	99.6	0.69	0.18	1.44	1.08 *
<b>Tyne</b>								
Scotswood Bridge	26	70.4 *	29.7 *	288.1 *	2.36 *	1.35	1.70 *	1.05 *
Redheugh	41	90.0 *	27.2	237.2 *	1.72 *	1.37	1.78 *	1.05 *
Newcastle	22	95.8 *	25.4	183.7	1.53	0.56	1.68 *	1.02
Hebburn	28	54.3	24.2	176.3	1.94 *	0.80	1.59 *	1.08 *
Jarrow	30	94.8 *	20.9 *	105.5	1.51	0.16	1.74 *	1.04 *
Lloyds Hailing Station	3	57.6	28.2	271.6	2.54	0.27	2.03 *	1.19 *

\*Significantly different ( $p < 0.05$ ) from the Alde reference site



**Figure 19. EROD activities in flounder collected from English estuaries in 1997**

the spillage (St Brides Bay) exhibited comparatively high EROD activity and those sites exhibiting low EROD activity (i.e. Off Carmarthen and Rhossili Bay) were known to be less heavily contaminated than the others (Law *et al.* 1997). In the samples from May, no

obvious correlation between known oil contamination and EROD activity was evident but it seems likely that the high EROD activities associated with the two sites nearest the spillage, Freshwater West and Turbot Bank, were still a residual effect of the spillage. EROD

fluctuations at the other sites were more likely influenced by other variables known to affect EROD activity e.g. temperature, sex, maturity etc.

In this particular set of data the most influential factor on the EROD activity values in dab was a seasonal phenomenon. Dab in this area spawn from December – March and are in a ‘post-spawning’ phase from April – May. Therefore the fish sampled during this study were in a spawning or post-spawning condition. Lange *et al.* (1995) have shown that EROD activity rises steadily during spawning and reaches a maximal level in the post-spawning phase. The gonado-somatic index (GSI) is an indicator of a fish’s reproductive state and is normally defined as the percent weight of gonads compared to total body weight. However, due to the difficulties associated with weighing specimens at sea a ‘pseudo’ GSI (pGSI) was calculated in female specimens only as the average length of the gonads expressed as a percentage of the total body length.

In dab, a fairly strong negative correlation was observed between female pGSI and EROD ( $r = -0.53$ ). The female dab pGSI value at the offshore reference station C166 was the lowest observed at 9.3. This low figure shows that the female dab at this station had relatively underdeveloped or regressed gonads compared to the fish at inner stations. This low pGSI at the reference station helps explain the high level of female EROD activity, a mean figure of 4105 pM/min/mg protein which was the second highest, only exceeded by fish taken from Turbot Bank. It is known that EROD activity in gonadally mature females is significantly lower than immature or spent females (Elskus *et al.* 1992) in Winter Flounder and that this phenomenon is linked to the elevated levels of the sex hormone oestradiol and its ability to act as a P450/EROD suppressant. Therefore, it may be assumed that the offshore dab in this study were not mature and therefore had lower levels of oestradiol resulting in the higher levels of EROD activity observed. Other factors such as gender, size, migration, temperature and depth were also investigated but effects due to these were probably masked by the seasonal fluctuations in EROD associated with the spawning season.

No corresponding correlation between pGSI and EROD was found in plaice, most of the fish in this study were probably immature. It could, therefore, be argued that plaice represented a better species to use to monitor the effects of the spill. However, the high EROD activity levels associated with the dab at the sites near to the spill were not repeated in the plaice. No easy explanation exists for this but it may be that plaice were migrating at a greater rate at this time and therefore spatial trends with this species may be difficult to interpret in this instance.

#### 6.2.4.2 *CIROLANA 6b/96 and CIROLANA 5b/97*

It is known that EROD activity in flatfish varies temporally on a seasonal basis connected to the spawning season. The results from the two summer cruises also suggest that EROD activity levels vary spatially around the UK.

The results from *CIROLANA 6b/96* revealed an unusually high result (2549 pM/min/mg protein) in those dab taken 30 miles off Milford Haven. It was tempting to suggest that this was a residual effect of the *SEA EMPRESS* oil spill but this was not borne out by other results from nearer the spill (not reported here). In fact, the results appear to be explained by the fact that these fish had by far the smallest GSI and therefore it seems logical to assume that this high value was a result of the presence of regressed or immature gonads which could mean a relatively low level of circulating oestrogen which acts as an MFO inhibitor. However, whilst the high levels obtained in those fish sampled off the Tyne and Tees could be associated with contamination from the adjacent estuaries these values coincided with the largest GSI figures. These data are very difficult to interpret. The fact that there was a strong correlation of female GSI with latitude (0.83), would suggest that spatially disparate populations can be at different stages in their reproductive cycle which can have a large influence on the MFO system, and therefore cannot be easily compared.

In contrast, the results from the *CIROLANA 5b/97* cruise appeared to offer a relatively clear pattern of EROD induction. Of the 13 stations sampled there was a clear split between those showing relatively high levels of induction (>1280 pM/min/mg protein) and those with relatively low levels (< 870 pM/min/mg protein.). Again the interpretation is slightly confused by the fact that female GSI and EROD induction are quite well correlated in fish on the east of the country whereas those on the west show no such relationship. In general, correlations are not apparent across all sites but those showing high levels were all taken in the vicinities of contaminated estuaries and those showing low levels were from offshore sites or relatively uncontaminated bays.

The cruise data has been very difficult to interpret and suggests that much more needs to be known about the environmental influences on MFO activity before the monitoring data can be more confidently used.

#### 6.2.4.3 *Estuaries 1997*

It is clear from the estuary survey that the mixed function oxygenase (MFO) enzyme system in flounder



is significantly induced in a number of English estuaries (Table 6; Figure 19) compared to the clean control estuary of the River Alde. These results presumably reflect the anthropogenic contamination of estuaries such as the Mersey, Tees and Tyne, and suggest that the flounder (and probably other fish) populations in these areas are subject to sub-lethal contaminant stress. However, it should also be borne in mind that the endocrine system in the most EROD-induced fish was highly disturbed (Allen *et al.*, 1997, 1999; Matthiessen *et al.*, 1998), with high levels of plasma vitellogenin occurring in male fish from the Mersey, Tees and Tyne. It is also possible that the MFO system was responding to oestrogenic contaminants such as ethynyl estradiol or nonylphenol (which have been shown to inhibit EROD activity in the Atlantic salmon, *Salmo salar* (Arukwe *et al.*, 1997)) in addition to planar PCBs or PAH (some of which are themselves oestrogenic or anti-oestrogenic). The survey nevertheless allowed a tentative ranking of contamination in the estuaries in terms of their EROD inducing capabilities in flounder: Mersey, Tees > Tyne, Humber, Wear, Southampton Water > Alde.

If the EROD results obtained from the River Tyne are observed in isolation it is evident that in this estuary there are inter-site differences in EROD activity with the mean activity at Hebburn (54.3 pM/min/mg protein) being significantly less than mean EROD activity at three other Tyne stations: Redheugh, Newcastle and Jarrow.

Several parameters including gender, length, weight, GSI, hepato-somatic index (HSI) and condition factor (CF) were also measured and correlated against EROD activity. The resultant correlation coefficients for these analyses across all estuaries and more specifically in the Tyne estuary are shown in Table 7. In this study no significant inter-gender difference was demonstrated in mean EROD activities from any of the sites where sufficient numbers of each sex were collected. Further scrutiny of Table 6 reveals that whilst there is some indication of a relationship between size (length and weight) and EROD activity in male flounder, by far the most consistently correlated parameter is GSI. EROD activity in flounder has already been shown to exhibit high levels of seasonal fluctuation but these influences

appear to be at a minimum during the period June to October (Eggens *et al.*, 1996). The influence of reproduction may significantly obscure the desired biomarker response (Goksøyr *et al.*, 1996) and therefore the samples taken in this study were timed to coincide with a period when reproductive influences were thought to be minimal. In general this appears to be the case, and so we are confident that the inter-estuary differences shown are contaminant induced, with oestrogens and their mimics possibly contributing somewhat to the overall effect. However, correlation analysis (Table 7) of mean GSI with mean EROD does suggest that even in the optimal sampling period there may be a residual effect of the reproductive cycle on the MFO system in some estuaries. For example, the female samples from the Tyne sites showed a high negative correlation ( $r = -0.84$ ) of EROD with GSI which reflects a trend that is more evident during the reproductive season of several flatfish species (Eggens *et al.*, 1996; Kirby *et al.*, 1999(a); Elskus *et al.*, 1992).

## 6.2.5 Conclusions

The EROD assay has been deployed in three separate ways; to monitor effects of a specific incident (*SEA EMPRESS*), for estuarine water quality assessments and for nearshore/offshore marine monitoring. In each case it has successfully differentiated significant inter-site differences.

There are several strong influences on the MFO system other than the effects of contaminants including gender, spawning stage, size and temperature. In the three applications outlined above it is clear that the spawning stage of the fish, especially, can have a very significant influence on the level of EROD activity to such an extent as to mask contaminant influences. This influence is particularly strong during the spawning and post-spawning period as was evident in the studies of the *SEA EMPRESS* spillage.

Another factor that appears to complicate interpretation, is when a comparison is attempted between sites and areas of a dissimilar nature. This appears to have been the case when assessing the cruise data that is collected from sites from all around the UK. Although some

**Table 7. Linear correlation coefficients (*r*) for mean EROD with other variables in estuarine samples 1997**

	Number of Stations	Length	Weight	GSI	HSI	CF
<b>All Estuaries</b>						
All	16	-0.09	0.05	-	0.21	0.22
Female	13	0.09	0.14	-0.33	0.31	0.24
Male	7	-0.54	-0.37	-0.44	0.11	0.52
<b>Tyne Only</b>						
All	6	-0.55	-0.60	-	-0.17	-0.67
Female	5	0.01	-0.24	-0.84	-0.07	-0.80
Male	5	-0.51	-0.51	-0.43	0.09	-0.63

trends are apparent and in general the levels were much higher near contaminated estuaries than, for example at offshore sites, anomalous and not easily explicable results often occur especially when comparing spatially disparate sites. In surveys such as this, more information is required on the genetic and climatic influences on the MFO system before interpretation can be improved.

For the estuarine study the technique does appear to have been very successful at differentiating between the areas, and a tentative ranking of contamination, in terms of EROD activity, of the estuaries sampled was enabled (highest activity first): Mersey, Tees > Tyne, Humber, Wear, Southampton Water > Alde. The fact that no inter-gender differences were apparent during this study and no consistent correlation was found between EROD activity and other measured parameters suggests that anthropogenic contamination was almost certainly the main cause of inter-estuary variation in EROD activity.

Livingstone *et al.* (1997) mention several instances where increased EROD activity in fish have been linked to higher order effects such as disease and reproduction. These studies have shown that the MFO system of flatfish is significantly elevated above baseline levels in certain areas of UK waters. Whilst the MFO system is essential for detoxification of certain xenobiotics and the metabolism of some endogenous compounds (for example steroid hormones and vitamins), its induction may also produce deleterious side effects. First, whilst many xenobiotics are rendered less harmful, others form carcinogenic or genotoxic compounds after transformation by the MFO system (e.g. formation of benzo[*a*]pyrene diol-epoxide from the parent PAH compound). Secondly, since essential endogenous substances such as steroid hormones are metabolised by the MFO system, its elevated activity could have serious repercussions for an organism's normal reproductive development. However, it is also possible that heavy exposure to exogenous oestrogenic hormones and their mimics is actually causing some of the EROD induction observed. On balance, it seems possible that dab and plaice but certainly flounder populations (and probably other species) in industrialised English estuaries are facing a significant threat to their long-term health and viability from anthropogenic contamination. More research is required to establish the significance of these findings in terms of ecosystems and fisheries.

## 6.3 Acetylcholinesterase (AChE)

### 6.3.1 Introduction

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 CEFAS has applied the AChE technique. More recently CEFAS has been experimenting with the reactivation of inhibited AChE, ideally up to normal levels, using an *in-vitro* technique. This would allow the determination of 'real' levels of environmental inhibition and the differentiation between those areas that are truly exhibiting neurotoxicity and those which have low AChE activities for other reasons.

### 6.3.2 Method

#### *Fish species*

Two fish species were targeted as sentinel organisms: dab (*Limanda limanda*) for all near- and offshore sites (*CIROLANA* 6b/96) and flounder (*Platichthys flesus*) for estuarine work. Dab have been used to monitor the presence of marine neurotoxicity previously (Glagani *et al.*, 1992; Bocquene *et al.*, 1993), but although AChE has been measured in the European flounder before (Stieger *et al.*, 1989), this appears to be the first time that the species has been used for environmental monitoring of AChE inhibition.

#### *Vessel/Trawl gear*

As for EROD, coastal and offshore samples were taken from aboard the *CIROLANA* and for nearshore and estuarine sampling smaller chartered trawlers or Environment Agency survey vessels were used. A 2 or 3 metre beam trawl towed at 3-4 knots for 30 minutes to minimise the stress to the catch was used. For most areas several tows were required to recover a satisfactory sample.

#### *Processing*

Once on deck, target species were separated into tanks containing flowing sea water. Dissections were performed within 1 hour of capture. A strip of muscle (approx. 1-2 cm<sup>3</sup>) 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 taken 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 ml 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 cholinesterase 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 0.01 M dithiobisnitrobenzoate (DTNB). Blanks contained no supernatant and 350 µl of buffer. Assay reactions were initiated by quick addition, via stepping pipette, of 10 µl of 0.1 M 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/mg protein (1 U = 1 mOD 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.

### ***Reactivation assays***

At the time of writing, the reactivation technique had only been briefly investigated. The technique involves the addition of 10 µl 0.5 mM pyridine 2-aldoxime methiodide (2-PAM) to the reaction well with the supernatant in (with an equivalent reduction in buffer volume) and reading the AChE activity after 1 hours incubation at 20°C. Theoretically all the activity restored, as compared to the original assay, after this period was that amount inhibited by neurotoxins.

### ***Water sample acquisition and insecticide residue enrichment***

Samples of estuarine, coastal and marine waters were acquired between May and June 1997 from the *RV CIROLANA* (*CIROLANA* 6a/97 + 6b/97; Table 8). Six estuaries were also sampled (at low water) between May and October 1997 using chartered or Environment

Agency coastal vessels. A total of seventy-three, 2.7 l samples were taken at 1 m below the surface and separate samples were obtained for organophosphorus (OP) and carbamate (C) analyses. Duplicates were also taken from one-in-every-ten samples. These were fortified with either a solution of OPs or Cs in the range of 91 to 234 ng l<sup>-1</sup> to provide information on recovery efficiencies of the extraction methodologies.

For OPs, the pH of the sample was reduced to approximately 6 and deuterated Parathion-methyl was added as a surrogate standard. Enrichment of the OP insecticides was achieved *in situ* by solid phase extraction using pre-washed and conditioned octadecylsilane (C18) bonded cartridges. Samples for C analysis were extracted at the native pH of the sample using a styrene divinylbenzene (ENV+) sorbent. All cartridges were stored at -10°C prior to elution, pre-concentration and analysis.

### ***Mass spectrometric analysis***

Thirty-five OP and twenty C compounds including several transformation products (TP) were selected for screening. These were chosen on the basis of their use in the UK and their listings in List I of the EC Directive on Dangerous Substances in Water (76/464/EEC), the UK 'Red List' of substances for priority action and the 'North Sea Conference' List. All OP analyses were performed using a high resolution GC system connected to an Ion Trap Detector. The Mass Spectrometer was operated in positive, electron impact ionisation and full scan mode. Individual OPs were analysed to determine their retention times and to provide spectral information of mass fragmental ions. For positive confirmation of an OP analyte in a sample extract, three designated ions were required to be evident with the correct relative abundances.

For the analysis of the C compounds, LC-atmospheric pressure chemical ionisation-MS was deployed. The MS was operated in positive ionisation mode and mass spectra of individual compounds were obtained to choose quantitation ions. Time scheduled selected ion monitoring (SIM) was performed during the screening of sample extracts.

Limits of quantitation (LOQ) of each insecticide were determined from analysing serially-diluted blank extracts fortified with the range of OPs and Cs. LOQs were defined as the mass of the analyte (translated to mass per volume of sample) injected that resulted in a peak height-to-baseline noise >10 at a precision level of 10 %. Values of <50 ng l<sup>-1</sup> for all compounds were achieved. For OPs, LOQs ranged from 10 to 50 ng l<sup>-1</sup> and lower LOQs were determined for Cs, ranging from 2 to 30 ng l<sup>-1</sup>. Where a positive detection of an insecticide was apparent, extracts were re-analysed along with matrix-matched calibration standards.

**Table 8. Location of water sample sites, 1997**

Sampling Date	Station	Locality	Latitude	Longitude
<b>CIROLANA 5a/97</b>				
25/05/97	6	North Norfolk	53° 14.69'N	01° 08.71'E
26/05/97	20	NMP 345 - Off Humber/Wash	54° 00.00'N	02° 00.00'W
27/05/97	41	Off Tees	54° 41.90'N	01° 03.58'W
27/05/97	42	Bamletts Wharf - Tees	54° 35.40'N	01° 15.20'W
27/05/97	43	Tees - Transporter Bridge	54° 35.10'N	01° 13.55'W
27/05/97	44	Tees - ICI No. 4 Buoy 'g'	54° 36.28'N	01° 09.80'W
27/05/97	45	NMP 325 - Gare Breakwater	54° 38.91'N	01° 08.45'W
28/05/97	95	NMP 295 - Tees	54° 42.26'N	00° 55.88'W
29/05/97	113	NMP 245 - Off Tyne	55° 00.49'N	01° 07.94'W
29/05/97	117	Off Scarborough	54° 17.50'N	00° 12.50'E
30/05/97	119	NMP 375 - Humber	53° 32.00'N	00° 20.00'E
31/05/97	127	Harwich	51° 52.34'N	01° 32.35'E
01/06/97	146	East of North Foreland	51° 25.19'N	01° 43.20'E
01/06/97	147	South Varne	50° 58.32'N	01° 13.74'E
01/06/97	148	CS-1 Buoy	50° 32.72'N	00° 02.74'E
02/06/97	150	NMP 495 - Selsey Bill	50° 40.70'N	00° 49.60'W
03/06/97	201	NMP 515 - East Brambles Bell	50° 47.15'N	01° 13.55'W
03/06/97	202	NMP 505 - Dock Head	50° 52.90'N	01° 23.50'W
03/06/97	203	NMP 525 - Hook Horn	50° 49.47'N	01° 18.30'W
04/06/97	217	NMP 585 - Off Plymouth Sound	50° 02.00'N	04° 22.00'W
05/06/97	219	Off Lands End	50° 00.03'N	05° 49.80'W
05/06/97	221	NMP 605 - Celtic Deep	50° 15.06'N	06° 00.04'W
05/06/97	234	Mumbles/Swansea Bay	51° 31.20'N	03° 56.29'W
<b>CIROLANA 5b/97</b>				
09/06/97	1	NMP 615 Nash Point	51° 18.10'N	03° 32.80'W
09/06/97	4	Swansea Bay - Disposal Point	51° 34.89'N	03° 55.35'W
09/06/97	6	Mid Carmarthen	51° 33.34'N	04° 32.51'W
09/06/97	7	West Carmarthen St. Govans	51° 33.11'N	04° 51.46'W
12/06/97	19	Celtic Deep - NMP	51° 14.44'N	05° 58.83'W
12/06/97	21		52° 21.49'N	04° 10.51'W
13/06/97	36	NMP 665 - Outer Cardigan Bay	52° 30.13'N	04° 59.92'W
13/06/97	38		53° 21.69'N	04° 08.89'W
13/06/97	41		53° 28.62'N	03° 41.56'W
14/06/97	44	NMP 705	53° 28.27'N	03° 28.36'W
14/06/97	45	Anchor Station - Mersey	53° 29.25'N	03° 13.82'W
14/06/97	46	Tranmere - Mersey	53° 23.70'N	02° 59.70'W
14/06/97	47	Seacombe Ferry - Mersey	53° 24.56'N	03° 00.48'W
14/06/97	48	C20 Buoy - Mersey	53° 28.00'N	03° 03.00'W
14/06/97	49	Crosby Channel - Mersey	53° 31.80'N	03° 08.00'W
15/06/97	52	NMP 795	53° 58.01'N	03° 20.00'W
15/06/97	55		53° 56.00'N	03° 49.95'W
16/06/97	68		54° 04.00'N	05° 30.12'W
16/06/97	80	15 Buoy - Northern Ireland	54° 44.00'N	05° 36.04'W
16/06/97	81	6 Buoy - Northern Ireland	54° 42.33'N	05° 42.52'W
16/06/97	82	Northern Ireland	54° 40.10'N	05° 48.40'W
16/06/97	83	Northern Ireland	54° 38.68'N	05° 52.10'W
19/06/97	84	Anchor St. Tyne	55° 01.31'N	01° 23.60'W
19/06/97	85	Team Mouth	54° 57.51'N	01° 38.01'W
19/06/97	86	International Paints	54° 57.52'N	01° 33.17'W
19/06/97	87	Downstream Howden	54° 59.30'N	01° 28.36'W
19/06/97	89	Fishing Station	55° 17.78'N	01° 14.66'W
19/06/97	92	Blyth - Anchor	55° 07.26'N	01° 27.95'W
19/06/97	93	Blyth - Inner	55° 08.52'N	01° 31.24'W
19/06/97	94	Blyth - Harbour	55° 07.47'N	01° 29.75'W
20/06/97	96	Dogger Bank	54° 50.12'N	01° 19.99'E
21/06/97	110	Fishing Station	54° 43.28'N	01° 08.25'W
22/06/97	120	Fishing Station	53° 21.30'N	00° 24.57'E
22/06/97	123	Fishing Station	53° 03.56'N	00° 28.40'E
<b>Estuaries</b>				
05/13/97	1	Severn - Beacon Lower Shoots	51° 32.53'N	02° 44.03'W
05/13/97	2	Severn - Inshore North Avonmouth	51° 31.01'N	02° 42.76'W
05/14/97	3	Severn - Peterstone-NMP63	51° 29.62'N	03° 00.40'W
05/14/97	4	Severn - NMP 60	51° 28.50'N	03° 01.87'W
05/14/97	5	Severn - Swansea Bay	51° 27.42'N	03° 40.66'W
09/02/97	1	Mersey - Bromborough		
09/03/97	2	Mersey - Speke		
09/09/97	1	Tamar	50° 25.64'N	04° 11.89'W
10/97	1	Humber	53° 36.87'N	00° 04.26'W
10/97	3	Humber - Boreham Shoal	53° 36.43'N	00° 05.95'W
10/97	4	Humber	53° 39.77'N	00° 10.50'W
10/97	8	Humber - Whitton		
10/97	9	Humber Bridge	53° 42.42'N	00° 27.21'W
10/97	3	Tyne - Newcastle	54° 58.14'N	01° 36.14'W
10/97	4	Tyne - Scotswood	54° 58.21'N	01° 41.84'W
10/97	5	Tyne - Redheugh	54° 57.83'N	01° 39.98'W

### 6.3.3 Results

#### 6.3.3.1 CIROLANA 6b/96

The assay was deployed during the summer research cruise of 1996. During this cruise AChE activity in dab muscle was determined from a wide range of coastal and offshore sites. The results are shown in Table 9. Clear and significant ( $p < 0.05$ ) inter-site differences were demonstrated between certain sites.

**Table 9. Mean AChE activity (mU/min/mg protein) in dab muscle tissue sampled on CIROLANA 6b/96**

Station No.	Location	n	Mean AChE
152	30m SW Milford	20	7162.8
153	St Brides Bay	20	8330.1
158	Carmarthen Bay - East	22	6351.5
159	Carmarthen Bay - Mid	24	6899.9
163	Carmarthen Bay	24	6463.4
164	Carmarthen Bay - West	20	5973.5
189	Outer Cardigan Bay	12	7102.0
191	Red Wharf Bay	20	5922.4
199	Liverpool Bay	20	7010.0
200	Burbo Bight	20	6148.4
217	Morecambe Bay	20	6276.8
218	S.E. Isle Of Man	20	5799.6
247	Off Tyne	20	6054.8
252	Dogger Bank	20	4643.3
258	Off Tees	20	6683.0
259	Off Humber	20	6336.6
260	The Wash	13	9107.4

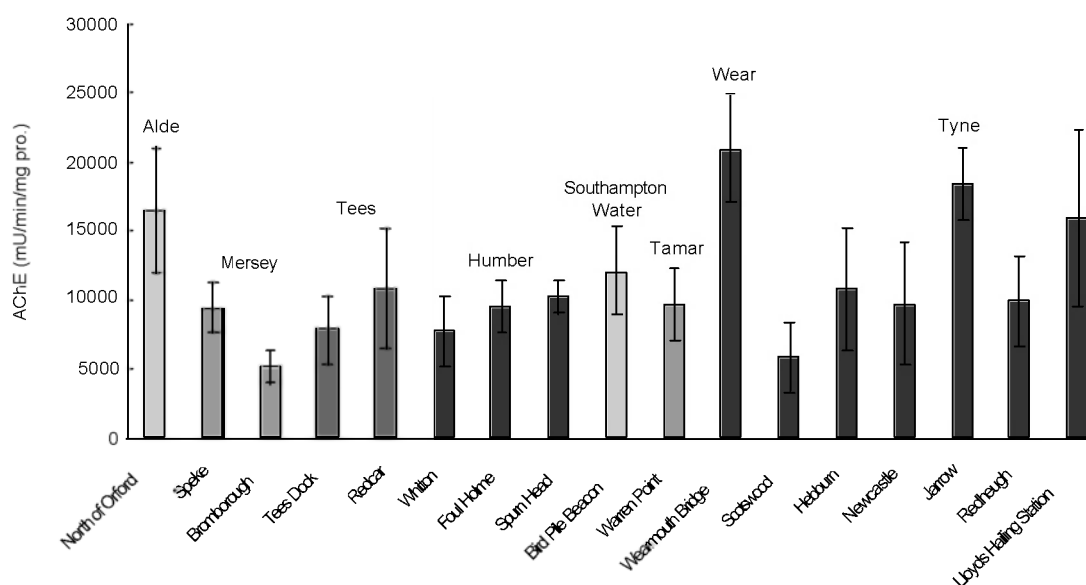
Of particular interest, was the very low mean value gained in samples from the Dogger Bank (4646 mU/min/mg protein). This was significantly ( $p < 0.05$ ) lower than that gained from all other sites and represents a 35-50% inhibition of activity when compared to the sites with the highest AChE levels. The highest mean figures were from The Wash (9107), St Brides Bay (8330) and SW of Milford Haven (7163), however, the figure from The Wash included only females and due to high variability was not proven to be significantly higher than at other sites.

Inter-gender differences were only significant ( $p < 0.05$ ) at 3 out of 15 sites. In the samples from these areas (S.E. Isle of Man, Off Tyne and Dogger Bank) the female fish showed higher levels of AChE activity. No link was apparent between EROD and fish length, or GSI in pooled gender data or for either sex.

#### 6.3.3.2 Estuaries 1997

In late 1997, samples were obtained opportunistically from flounder caught from 17 sites in 8 English estuaries. Mean AChE activities are shown in Figure 20. Mean activities at each site were statistically compared using single factor ANOVA to the mean gained from a site in a clean estuary, the River Alde, Suffolk.

In total, 13 out of the 16 sites compared to the Alde showed significantly ( $p < 0.05$ ) depressed AChE activities. The only sites that showed 'near normal' levels were from the Wear (but this was a mean of only 4 samples) and the two outer sites of the Tyne, Jarrow and Lloyds



**Figure 20. AChE activity in flounder muscle tissue from estuarine sites showing 95% C.L.**

hailing station. Whilst the Alde showed a mean activity of 16573 mU/min/mg protein, Bromborough Port on the Mersey showed a mean of only 5206 mU/min/mg protein and Scotswood Bridge on the Tyne only showed 5894 mU/min/mg protein which are the equivalent of 68% and 64% inhibition of AChE activity respectively. Other low spots included sites on the Tees and Humber which showed >50% inhibition.

#### ***Spatial distribution of insecticides water samples - 1997***

Thirty one of the 73 sample stations including marine, coastal and selected estuaries, showed evidence of OP contamination and 14 different analytes were identified, with Chlorfenvinphos and Diazinon being the most abundant (Table 10). In general, only one OP compound was found at the majority (23 out of 31) of the stations. The maximum number of OPs found at any one station was three. These were detected at the Gare's breakwater in the lower Tees Estuary (St 45; NMP 325) and at Whitton (St H8), in the Humber Estuary and Chlorfenvinphos was common to both locations. Concentrations ranged from 12 to 72 ng l<sup>-1</sup>. Azinphos-methyl exhibited the maximum concentration (St 20; *CIROLANA 5a/97*; NMP 345 off Humber/Wash). The lower value, also found in the Humber (H8) was measured for Chlorfenvinphos.

There was no evidence of OP contamination in offshore water samples with the exception of St 117 (*CIROLANA 5a/97*; off Scarborough, North Sea) where Chlorfenvinphos was detected at a concentration of 32 ng l<sup>-1</sup>. The presence of OP compounds was confined to estuaries and coastal waters where residue levels of up to 60 ng l<sup>-1</sup> were found. Diazinon was common to waters taken from the Blyth Estuary and at an average concentration of 30 ng l<sup>-1</sup>. Azinphos-methyl was present in the outer Tyne Estuary (*CIROLANA 5a/97*; St 113) whereas Diazinon was detected upstream and five months later. Diazinon and Chlorfenvinphos were evident in the Tees Estuary and at an average levels of 35 and 40 ng l<sup>-1</sup>. Samples from the Humber Estuary also exhibited Diazinon and at an average concentration of 43 ng l<sup>-1</sup>. The only occurrence of Dichlorvos was in Southampton Water (*CIROLANA 5a/97*; NMP 525, Hook Horn). Pirimiphos-methyl and the sulphoxide TP of Phorate were also found here and at levels of 39 and 25 ng l<sup>-1</sup>, respectively. The Severn Estuary showed evidence of Chlorpyrifos, Phorate sulphoxide and Dimethoate with the latter compound approximating to the LOQ of 20 ng l<sup>-1</sup>. Both the *RV CIROLANA* and estuarine study samples from the Mersey resulted in three OP compounds being identified and Fenthion and Chlorpyrifos were common at levels approximating to 20 ng l<sup>-1</sup>.

Thirteen sample stations showed evidence of C insecticides and a total of nine compounds were positively identified. Of these, the most abundant were Carbofuran and Primicarb. Mean concentrations were 18 ± 9 (n=4) and 25 ± 12 (n=3) ng l<sup>-1</sup>. With the exception of waters from the Severn Estuary

(*CIROLANA 5b/97*; St 1, NMP 615; Nash Point), Sts 85 and 86 (*CIROLANA 5b/97*, Team Mouth and 'International Paints', Tees), only one C compound was found at most (>75 %) of the stations. Concentrations ranged from 8 to 37 ng l<sup>-1</sup>. The minimum and maximum values were detected from waters from the Mersey (M2) Estuary and from Nash Point, South Wales (*CIROLANA 5b/97*; St 1), respectively. Ethiofencarb was common at both of these locations.

As it was shown by the distribution of OP insecticides, carbamates were only evident in estuarine and coastal waters. They were also present at similar locations to those contaminated with OPs. However, there was no evidence of these insecticides in the Tyne and Mersey Estuaries or Southampton Water. The sulphone TP of Methiocarb was found in the sample taken from the estuary mouth of the Blyth and at a level of 28 ng l<sup>-1</sup>. Four Cs in the range of 18 to 32 ng l<sup>-1</sup> were identified in the Tees Estuary including Carbofuran-3-OH - the TP of Carbofuran. From samples taken from the Humber Estuary, Ethiofencarb was quantified at levels <10 ng l<sup>-1</sup>. Four compounds were also detected in the Severn Estuary and South West Wales coastal stations of the Bristol Channel. Of these, Primicarb was found in two of the samples and at an average concentration of 32 ng l<sup>-1</sup>.

#### **6.3.3.3 Reactivation assays**

At the time of reporting no validated reactivation results are available. However, preliminary studies have shown the technique to work in trout tissue although more work is required before optimal reactivation of inhibited AChE activity is achieved in flounder/dab muscle.

#### **6.3.4 Discussion**

##### **6.3.4.1 *CIROLANA 6b/96***

The assay has demonstrated that even at near- and offshore locations there is a substantial variance in the activity of AChE in dab muscle. It is highly unlikely that in these areas these differences are due to biologically significant concentrations of anthropogenic neurotoxins. However, this incidence of relatively low AChE activity on the Dogger Bank is not isolated as Galgani *et al.* (1992) also discovered a suppression of AChE activity in dab muscle on the Dogger Bank as compared to another offshore site on a transect from the Rivers Elbe/Weser.

The cause for this phenomenon is unclear but theories include an accumulation of neurotoxic pollutants on the Dogger Bank, possible migration of flounder to this area from contaminated sites or a high occurrence of algal toxins from cyanobacterial blooms which have also been shown to be strong AChE inhibitors. However, none of these proposals have conclusive evidence to back them up.



**Table 10. Concentrations of organophosphorus and carbamate insecticides (>LOQs; ng l<sup>-1</sup>) in estuarine, coastal and marine water samples from England and Wales, 1997**

Compound (in elution order)	LOQ (ng l <sup>-1</sup> )	Sample Station Number																																									
		CIROLANA 5a/97										Estuaries																															
		20	42	44	45	113	117	147	201	202	203	234	1	4	7	46	48	49	55	85	86	87	92	93	94	94	SI	S3	S4	S5	M1	M2	TAI	HI	H4	H8	H9	T5					
<b>Organophosphorus</b>																																											
Dichlorovos	30								52																																		
Heptenophos	30			39																																							
Dimethoate	20								24																																		
Diazinon	20						47										36				43		26	34														57	39	28			
Disulfoton	30																																									32	
Etrimfos	10																																									26	
Pirimiphos-methyl	20								39																																		
Phorate sulphoxide	30						36																																				
Fenthion	10															13																										29	
Chlorpyrifos	10				18									23																											34		
Parathion-ethyl	10													17																													
Chlorfenvinphos	10				63		32								31																										18		
Triazophos	20			37																																							
Azinphos-methyl	30	72	46	37	54																																				48		
<b>Carbamates</b>																																											
Carbendazim	15																																										29
Carbofuran-3-OH	10				26										17																												
Carbofuran	2											29							9	13	21																						
Bendiocarb	2																		18																								
Carbaryl	2																																										
Ethiofencarb	5																																									8	
Pirimicarb	2																																										
Methiocarb	5																																									28	
Methiocarb sulphone	20																																									28	

S = Severn

M = Mersey

TA = Tamar

H = Humber

T = Tyne

### 6.3.4.2 Estuaries 1997

It is apparent from these studies that inter- and intra-estuarine populations of flounder are exhibiting significantly different levels of muscle AChE activity. If one made the assumption that the fish taken from the control estuary, the Alde, were showing 'normal' levels of activity then many of those from other estuarine sites were showing very high levels (>50%) of muscle AChE inhibition. Stansley (1993) measured brain AChE activity in dead and moribund fish from a number of cases and found that at these advanced stages of poisoning the AChE activity was inhibited by 50-87%. Zinkl *et al.* (1991), however, stated that the level of resistance to brain AChE inhibition does appear to vary dependent on species. This present study has shown muscle AChE inhibition of 50-68% in certain estuarine sites compared to the Alde. However, these fish did not appear to be showing obvious external signs of dis-orientation or other symptoms of neurotoxin poisoning.

It appears to be the case that high levels of AChE inhibition can be less tolerated in nervous tissue than in muscular tissue. Numerous studies have shown that different tissues (brain, muscle, liver, gill etc.) show differential levels of sensitivity to AChE inhibition therefore conclusions made on the basis of activities in one tissue type only must be treated with caution. It could be that the trends in muscle AChE shown in this study were not mirrored in other tissues.

The occurrence of apparently low AChE activity in estuaries such as Mersey, Tees, Tyne etc. do, however, contribute to the evidence suggesting that the fish populations in these areas are under significant contaminant assault. Other studies conducted at the same time in these estuaries have shown other evidence of biological effects on the MFO system (Kirby *et al.*, 1999(b)) and the reproductive system (Matthiessen *et al.*, 1998).

Concomitant studies undertaken in 1997 of AChE determination in demersal fish and the analysis of OPs and Cs have revealed very little evidence of these chemicals in open, marine waters surrounding England and Wales. Where insecticides have been detected, these have been confined to estuarine and coastal locations and at concentrations in the <100 ng l<sup>-1</sup> range. There was evidence of AChE suppression in Flounder sampled from the Mersey, Tees and Tyne. On examination of the chemical data from these respective estuaries, it was also apparent that several OPs and Cs were present. Within the Mersey Estuary, Chlorfenvinphos, Fenthion, Diazinon and Ethiofencarb were detected at levels ranging from 8 to 37 ng l<sup>-1</sup>. Studies in the Tees showed a suite of OPs including Trizophos, Azinphos-methyl, Heptenophos, Chlorpyrifos and Chlorfenvinphos. A range of 18 to 63 ng l<sup>-1</sup> was measured. Additionally, Carbaryl and the TP of Carbofuran (Carbofuran-3-OH) were also found here and at <30 ng l<sup>-1</sup>. Diazinon and Azinphos-methyl

were quantified in the Tyne at concentrations of 54 and 28 ng l<sup>-1</sup>, respectively.

With the exception of Heptenophos and Ethiofencarb, both exhibiting aqueous solubilities of >1800 mg l<sup>-1</sup>, the detected insecticides have solubilities in the range of <1 to approximately 120 mg l<sup>-1</sup>. The former insecticides are very water-soluble and can be easily mobilised in the aquatic environment. It can also be expected that their bio-concentration factors are to be low in comparison to the less polar insecticides. The presence of the more non-polar chemicals and their mode of transportation from source was likely to have been associated with suspended particulate matter as opposed to the soluble phase. Since insecticide concentrations were operationally-defined as 'total' (i.e. particulate plus soluble phases), it is possible that evidence of the more soluble insecticides was a product of 'fresh' inputs to the Mersey at Speke and in the lower Tees estuary.

A major route for these insecticides to enter the marine environment is via the transportation of surface water (Strum *et al.*, 1999). Sources of these compounds include unintentional drift from aerial spraying of agricultural crops (Ferando *et al.*, 1992), watershed drainage and irrigation channels (Croll, 1991), industrial and or domestic effluent discharge and accidental spillage (Ibrahim *et al.*, 1998; Raven and George, 1989). Compared with organochlorine (OC) pesticides, these chemicals are relatively non-persistent and exhibit low bioaccumulation (Guzzella *et al.*, 1997). They also undergo several breakdown processes including photolysis, hydrolysis and microbial degradation (Chambers and Levi, 1992; Ferrando *et al.*, 1992; Sanchez *et al.*, 1997). As a result, they have half lives ranging from hours, days and up to several weeks and degradation is faster than OCs.

In the present study and where insecticides have been found, only the more persistent compounds have been detected and at levels (<100 ng l<sup>-1</sup>) up to three orders of magnitude lower than sub-lethal concentrations. In addition, it should be noted that the recovery of AChE inhibition by fish following an exposure is, in general, prolonged in relation to the persistence of these pesticides in the aqueous environment (Carr *et al.*, 1997). Although residues both in fish tissue and in water can degrade within several days, AChE activity in the brain normally remains inhibited for several weeks (Finlayson and Rudnicki, 1985).

### 6.3.4.3 Reactivation assays

Tissue samples are still available from the estuarine sites sampled in 1997. The successful application of a reactivation technique to these samples may allow reason for low AChE in certain areas to be explained. For example, evidence of reactivation would suggest that the low level is possibly due to pesticide inhibition whereas little evidence of reactivation would suggest that the fish



have low levels due to other, possibly natural, reasons e.g. environmental temperature. Furthermore, Martin *et al.* (1981) have suggested a method by which reactivation techniques can be used to differentiate between organophosphate and carbamate poisoning.

### 6.3.5 Conclusion

AChE inhibition in fish muscle has been shown to be a powerful tool by which differences between near/offshore and estuarine populations can be revealed. Whilst it can be clearly demonstrated in controlled conditions that these effects could be caused by OP and C pesticides, the environmental data is still not easily interpretable. However, there is no question that low levels of AChE could compromise fish populations in their ability to behave, migrate or reproduce normally. The chemical analysis of waters for OP and C residues and the use of reactivation techniques should allow better interpretation of results in the future.

## 6.4 General conclusion

The use of biomarkers in biological monitoring is being extensively utilised by CEFAS. The above report focuses on two such methods that have proven to be useful in the monitoring of biological effects. Other methods exist which are currently being used by CEFAS (e.g. vitellogenin production to detect effects of oestrogens and their mimics, DNA adducts to detect effects of genotoxins) and still more that are currently being assessed (e.g. metallothionein for metal exposure, and lysosomal membrane fragility as a measure of general health). Many of the effects detected with these biomarkers are intimately entwined and dependent upon each other and therefore the way forward in monitoring is to deploy a suite of biomarkers in order to more fully understand the integrated effects of a mixture of contaminants as is present in the environment.

CEFAS is currently undertaking research in order to link these sub-lethal biomarkers with the occurrence of higher order effects such as reproductive capability and disease susceptibility and their potential to act as 'early warning systems' for such effects.

## 7. OESTROGENIC ENDOCRINE DISRUPTION IN FLOUNDER (*PLATICHTHYS FLESUS* L.) FROM UNITED KINGDOM ESTUARINE AND MARINE WATERS

### 7.1 Introduction

The following account is a summary of a report recently published by CEFAS (Matthiessen *et al.*, 1998). The

report describes one aspect of a phenomenon known as endocrine disruption (ED) which concerns the interference of environmental chemicals with the normal functioning of the endocrine (hormone) system. The aspect considered is that of substances in the UK marine environment which are able to mimic female vertebrate hormones (oestrogens) such as 17 $\beta$ -oestradiol, and thereby cause inappropriate feminisation of male fish (and other organisms).

### 7.2 Executive summary

It is already known that natural and synthetic oestrogenic hormones, and at least one industrial chemical, in sewage treatment works (STW) discharges are causing widespread feminisation of male river fish in the UK. This feminisation involves the unnatural synthesis of the yolk-precursor protein vitellogenin (VTG), and the induction of a condition known as ovotestis, in which egg cells develop in otherwise normal testes. Fish with this condition are described as 'intersex' although they remain genetically male. It is thought that this process may be damaging the ability of some freshwater fish to breed, but this has not yet been unequivocally established.

The present investigation set out to look for similar effects in the sea, and has focused on the common estuarine flatfish, the flounder (*Platichthys flesus* L.). This species, although not the basis of a significant fishery in the UK, was considered suitable for this survey because it is heavily exposed to contaminants associated with the muddy sediments in which it lives. Furthermore, it spends most of its life in its home river and estuary, only venturing offshore to breed. This means that studying feminisation in flounder will reveal something about the geographical location of the oestrogenic discharges which are contributing to any effects.

The primary techniques used in this survey included the measurement of VTG in male and female flounder, a search for feminised testes and other intersex conditions, and measurement of gonadal weights and sex ratios. Supplementary information was provided by chemical analysis for a range of contaminants in flounder liver tissue, measurement of certain steroid hormones in flounder, and measurement of the degree of induction of the cytochrome P450 mixed-function oxidase enzymes, a major system by means of which fish are able to degrade and excrete unwanted substances.

Vitellogenin levels in male blood plasma in the period autumn 1996 to spring 1998 were found to be significantly elevated (in comparison with a clean reference site on the Alde estuary) in at least one sample from most of the 11 estuaries investigated. The exceptions were the Tamar and the Dee where fish appeared entirely normal. In broad terms, the degree of

oestrogenic contamination as measured by male vitellogenesis in the various estuaries can be ranked in the following descending order: Tees > Mersey > Tyne > Wear = Humber = Clyde = Southampton Water = Thames > Crouch > Dee = Tamar. VTG concentrations in Tees, Mersey and Tyne fish were extremely high (>100,000 ng ml<sup>-1</sup>), and often exceeded those normally found in sexually mature females. There were no major differences between VTG levels measured in 1996/97 and 1997/98.

Flounder were also sampled from 5 coastal sites, and from several sites in the central southern North Sea. In all cases, VTG was significantly induced, and in the case of fish from Liverpool Bay and Red Wharf Bay (Anglesey), the degree of induction was large (although not as large as in the Mersey). Laboratory experiments indicated that VTG in male fish disappears rather slowly, with a half-life of about 2 weeks, and it is therefore suggested that the high VTG in fish at these two sites could have been induced in an estuary (probably the Mersey) before they migrated to sea to breed. However, local oestrogenic contamination at coastal and offshore sites cannot be entirely ruled out.

At most locations, ovotestis conditions in male flounder were entirely absent. However, in 1996, 17% of Mersey fish showed this abnormality, with large numbers of primary and secondary egg cells (oocytes) in the testes. In 1997, 9% of male Mersey fish and 7% of male Tyne fish contained ovotestis. In a few cases, eggs were fully developed with yolk granules. Most testes did not show gross morphological abnormalities related to oestrogenic exposure, although one testis from the Mersey appeared to be almost entirely composed of eggs. Unexpectedly, in view of the high levels of VTG induction, no intersex fish were seen in the Tees, but this is probably due to the fact that the sample size from this estuary was very small. Broadly speaking, ovotestis only seems to occur in flounder populations when mean plasma VTG levels in adults exceed 100,000 ng ml<sup>-1</sup>.

Abnormal sex ratios were not seen in any estuary, although this is based on histological and not genetic data; i.e. it is possible that some of the overt females may have been genetic males. There was no consistent pattern to the distribution of testis:body weight ratios (i.e. gonadosomatic ratio), but hepatosomatic ratios (i.e. liver:body weight) were increased in males which had elevated VTG. This shows that oestrogenic exposure had caused abnormal liver growth (hypertrophy) in order to synthesise VTG, thus placing strain on the metabolism of these fish.

Due to resource limitations, only female Dee estuary flounder were sampled for natural steroid hormones (in September 1997). Male hormones (the androgens 11-ketotestosterone and testosterone) were not detectable, while the oestrogen 17 $\beta$ -oestradiol was only present at a

low level in one fish. This was probably due to the season of sampling, and it would be desirable to conduct more analyses of this type in order to establish whether hormone metabolism is altered in the more oestrogen-contaminated fish.

The pattern of induction of the P450 mixed function oxidase system in flounder liver (as measured by the activity of the enzyme ethoxyresorufin-*o*-deethylase, or EROD) showed considerable similarities with the pattern of VTG induction, with highest EROD activity in the Mersey, Tees and Tyne, intermediate activity in the Humber, Wear and Southampton Water, and low activity in the Aide reference site. It is possible that the EROD was being induced by the same substances responsible for oestrogenicity, but even if not, there may still be reproductive implications because the P450 system is responsible for metabolising steroids. The main EROD inducers are the planar polychlorinated biphenyls (PCBs), polychlorinated dibenzodioxins and furans (PCDDs and PCDFs), and polycyclic aromatic hydrocarbons (PAHs), several of which are known to have endocrine disrupting properties.

In broad terms, the flounder from the Mersey and Tees were the most contaminated with organochlorines and PAHs, although appreciable amounts of PCBs were also present in Thames and Tamar fish. However, the levels of these contaminants in liver were rather low (max. median  $\Sigma$ PCB = 1.3 mg kg<sup>-1</sup> wet wt.; max. median  $\Sigma$ DDT = 0.5 mg kg<sup>-1</sup> wet wt.; max.  $\Sigma$ PAH = 0.4 mg kg<sup>-1</sup> wet wt.). It is doubtful whether these residues made a major positive or negative contribution to the observed oestrogenic effects, but they at least indicate that the fish which were most contaminated with common manmade chemicals were also those most impacted by oestrogenic hormones and/or their mimics. It is clear that more investigative chemistry is required to identify the causative compounds.

It seems unlikely that the major causative substances will prove to be oestrogenic hormones derived from domestic sewage, although these materials are almost certainly contributing to the observed effects. There is little correlation between VTG induction and the volumes of domestic sewage discharged to each estuary, but there is a much clearer relationship with the volume of industrial effluent. Again, this does not prove a causal link with industrial chemicals, but it suggests that non-hormonal substances are major players.

The full biological implications of these results are not yet understood, but it is a possibility that flounder in the most contaminated estuaries (Tees, Mersey, Tyne) have impaired reproductive output and reduced overall fitness. Whether this is likely to have long-term implications for the survival of these flounder populations is unknown, but it seems unlikely that flounder in the UK as a whole are seriously endangered from oestrogenic exposure. However, it should be noted that the most oestrogen-

sensitive part of the flounder's life cycle (the larva undergoing gonad formation) occurs at sea where contaminant levels are relatively low. It is therefore likely that species which breed in estuaries will be experiencing more serious effects.

It is recommended that further research is conducted in order to clarify the following:-

- What are the major oestrogenic substances in UK estuaries and what are the main sources?
- What are the implications for the reproductive output (and hence for populations) of fish species which breed in the more contaminated situations?
- What impacts are oestrogens having on the reproductive output of other estuarine organisms (e.g. crustaceans and molluscs)?
- Are UK estuaries significantly contaminated with other endocrine disruptors such as androgen and thyroid hormone mimics, and their antagonists?

Many of these questions are being addressed by a new 4-year research programme (**Endocrine Disruption in the Marine Environment, or EDMAR**) funded mainly by the British Government (Ministry of Agriculture, Fisheries and Food - MAFF; Department of Environment, Transport and the Regions - DETR; Environment Agency - EA; Scotland and Northern Ireland Forum for Environmental Research SNIFFER), but also by the European Chemical Industry Association (CEFIC). The programme is being managed by the Centre for Environment, Fisheries and Aquaculture Science (CEFAS), but other collaborating organisations include Fisheries Research Services (FRS) Marine Laboratory, Aberdeen, Zeneca Brixham Environmental Laboratory, and the Universities of Liverpool and Plymouth.

## **8. MONITORING AND SURVEILLANCE FOR DISEASE IN MARINE FISH 1997: MONITORING PROGRAMME FOR EXTERNALLY VISIBLE DISEASE BIOMARKERS AND HEPATIC PATHOLOGY IN MARINE FISH**

### **8.1 Introduction**

The use of disease biomarkers in marine environmental assessment is well established and is incorporated in the suite of biological effects techniques in the Joint Assessment and Monitoring Programme (JAMP) developed by the Oslo and Paris Commissions (OSPAR).

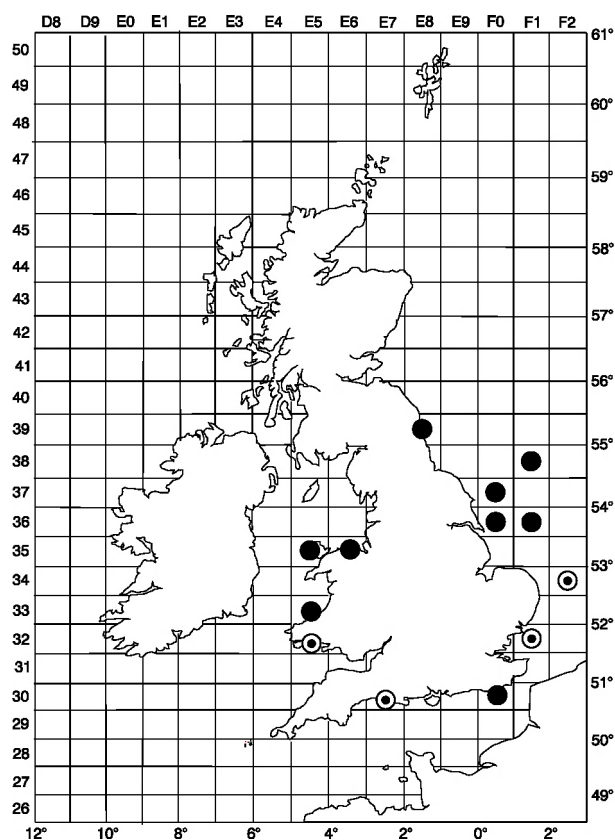
The JAMP will form the basis of the five Quality Status Reports (QSRs), and together, the data from these will be combined in the QSR 2000 for the maritime area of the Oslo and Paris Conventions. Data on the prevalence of externally visible diseases and macroscopic liver nodules in marine fish populations have been used as a monitoring tool for the assessment of environmental impacts of anthropogenic contaminants for several years. It is well recognised that several externally visible diseases of marine fish have infectious aetiologies and are therefore subject to naturally occurring variables including biological and environmental factors which may affect disease susceptibility. In addition, spawning migrations of the target species have made it difficult to spatially correlate disease prevalence with contaminant concentrations in specific sampling areas. Nevertheless, long term monitoring is able to detect trends in disease prevalence at the population level and, when used in conjunction with specific biomarkers for contaminant exposure greater confidence can be placed in disease data as an indicator of contaminant effect (Lang and Dethlefsen, 1996; Stagg, 1998). For this reason MAFF (CEFAS) routinely undertake an integrated biological effects monitoring programme, incorporating the collection of samples for assessment using other established biomarkers included in JAMP as well as samples for complementary chemical analysis.

The information is used to investigate temporal and spatial changes in disease prevalence in the main target species dab (*Limanda limanda*) and flounder (*Platichthys flesus*). In certain other commercial species the programme aims to detect the presence of emerging disease conditions and assess their significance in biological effects monitoring. Methodologies for all aspects of the disease monitoring have been developed through the activities of the International Council for the Exploration of the Sea (ICES) and include sample collection, disease diagnosis, recording and data submission (ICES, 1996). Consequently, basic quality assurance procedures for disease monitoring are in place.

Increasing importance is given to the analysis of liver pathology in flatfish since there is some evidence that neoplastic and non-neoplastic liver lesions in some flatfish species can be induced by exposure to certain organic contaminants (Myers *et al.*, 1991, 1994; Bucke and Feist, 1993, Vethaak *et al.*, 1996). For this reason, the current MAFF (CEFAS) monitoring programme targets the liver for a variety of biomarker investigations including histopathology as well as assays for EROD activity (Section 6 of this report) and evidence of genetic damage.

### **8.2 Methods**

A single dedicated cruise for monitoring fish disease was conducted (*RV CIROLANA* Cruise 2/97, 11-25 February, 1997). Additional samples for histopathological studies



**Figure 21. Areas sampled for fish disease monitoring (by ICES rectangle)**  
 ● Sufficient dab caught for external disease data  
 ⊙ Insufficient dab caught

were collected during a second cruise (*RV CIROLANA 5/97*, 9-22 June, 1997). A total of nine 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 (Figure 21). A station at Dundrum Bay (ICES rectangle 37E4) was not sampled because of adverse weather conditions and stations at Carmarthen Bay, Lyme Bay, Smiths Knoll and the outer Thames estuary did not provide a viable catch of fish for external 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 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 and flounder were preserved in Dietrich's fixative for histological confirmation.

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

Lesion classification	Relative importance (1-3) as biomarker of contaminant exposure/toxicity
1. NAD (No abnormalities detected)	
<i>Non-specific lesions</i>	
2. Coagulative necrosis	2
3. Apoptosis	2
4. Hyaline inclusion bodies	2
<i>Unique degenerative lesions*</i>	
5. Cellular & nuclear polymorphism	1
6. Megalocytic hepatitis	1
7. Spongiosis hepatis	3
<i>Storage conditions</i>	
8. Lipoidosis	3
9. Hemosiderosis	3
10. Variable glycogen content	3
11. Fibrillar inclusions	3
<i>Inflammatory changes</i>	
12. Melanomacrophage centres	3
13. Lymphocytic/monocytic infiltration	3
14. Granuloma	3
15. Fibrosis	3
<i>Non-neoplastic proliferative lesions</i>	
16. Regeneration	2
17. Bile ductular epithelial cell proliferation	2
18. Bile duct hyperplasia	2
19. Cholangiofibrosis/adenofibrosis	2
<i>Vascular abnormalities</i>	
20. Peliosis hepatis	3
<i>Foci of cellular alteration</i>	
21. Clear cell foci (glycogen storage)	1
22. Vacuolated focus (lipid storage)	1
23. Basophilic focus	1
24. Eosinophilic focus	1
<i>Benign neoplasms</i>	
25. Adenoma (basophilic)	1
26. Adenoma (eosinophilic)	1
27. Adenoma (clear cell)	1
28. Cholangioma	1
29. Hemangioma	1
30. Pancreatic acinar cell adenoma	1
<i>Malignant neoplasms</i>	
31. Hepatocellular carcinoma	1
32. Cholangiocellular carcinoma	1
33. Mixed hepatobiliary carcinoma	1
34. Hemangiosarcoma	1
35. Hemangiopericytic sarcoma	1

\* *Hydropic vacuolisation of biliary epithelial cells and/or hepatocytes is an important category of lesion, which has been recorded in flounder and plaice. It appears to be rare or absent in dab*

In addition to the fish examined for externally visible diseases, standard sections of liver tissue from 50 dab, greater than 20 cm in length, were sampled from each of the following areas: Sole Pit, Flamborough, Liverpool Bay, western Dogger, Cardigan Bay, and Rye Bay (ICES rectangles 36F1, 37F0, 35E6, 38F1, 33E5, and 30F0 respectively). In addition, 16 dab and 32 flounder livers were sampled from Carmarthen Bay and Lyme Bay respectively. Tissues were processed using standard histological techniques (Bucke, 1994). Detailed recommendations on the diagnosis and

reporting of histological liver lesions have been the subject of an ICES Special Meeting, held at CEFAS Weymouth, 22-25 October, 1996 (ICES, 1997). The guidelines included in the report were followed for the investigations reported here. Haematoxylin and eosin stained histological sections were examined for the presence of the specific categories of hepatic pathology. Although the range of possible lesions present in flatfish is large, they are conveniently grouped in different lesion categories which in turn are ranked (1 to 3) as an indication of their relative importance as indicators of contaminant exposure Table 11.

### 8.3 Results

#### *Dab diseases*

Disease prevalence data according to fish size group and disease severity (ICES, 1996) are presented in Table 12. Dab diseases recorded were ulceration (acute and healing), epidermal papilloma, lymphocystis and macroscopic liver nodules in fish >20 cm in length. In addition, the prevalence of hyperpigmentation was also recorded. The mean prevalence, combining data for all size groups per station, for these diseases are presented in

Figure 22. 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 Table 13. Only cases of the benign and malignant neoplastic lesions, adenoma, cholangioma, hemangioma and carcinomas are incorporated in the final prevalence data.

#### *Histological analysis of flatfish livers from selected areas in the North Sea, English Channel and Irish Sea*

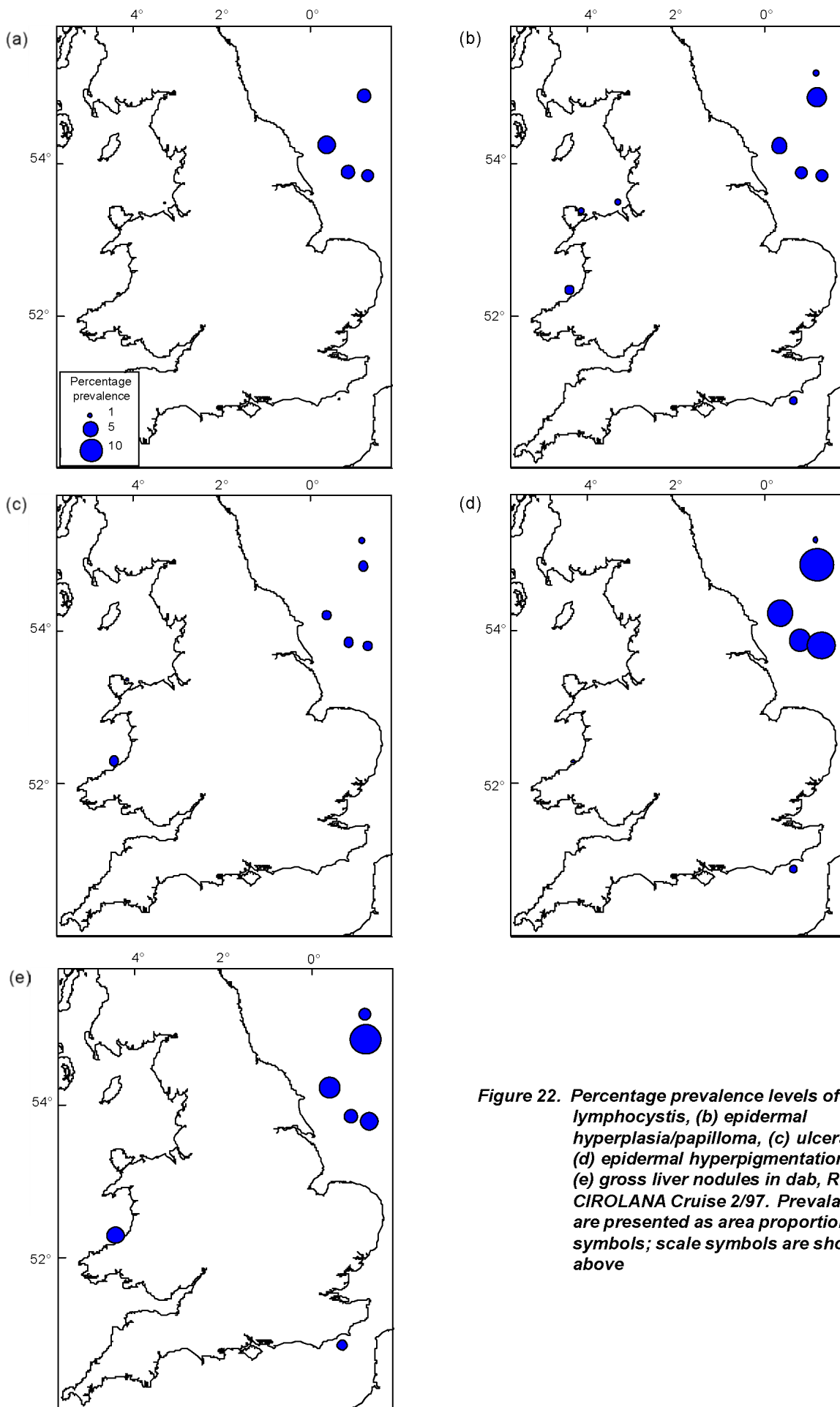
Data from the histological characterisation of randomly selected livers from dab >20 cm in length sampled from six areas in the North Sea and Irish Sea are presented in Figure 23. Of particular importance are the foci of cellular alteration (FCA), benign and malignant neoplasms (lesion numbers greater than 20 in Table 11). Fish from the Dogger Bank exhibit the highest prevalence of these lesions with fish from Flamborough and Sole Pit also showing an increased prevalence when compared to the reference station at Rye Bay. Although liver pathology was detected in fish from Liverpool Bay and the nearby location at Red Wharf Bay during the general disease survey, prevalence was low. The histological analysis confirmed the gross disease data. Samples from Cardigan Bay and to a lesser extent from

**Table 12. Summary catch data and disease prevalence in dab (*Limanda limanda*) by size categories and disease severity on stations sampled in the North Sea and Irish Sea for fish disease monitoring**

Area name (NMP)	Station	ICES Rectangle	Size Range (cm)	Numbers examined		Number and severity of disease cases Recorded according to ICES (1985)														
				Male	Female	LY			E/P			U			HYP			LN		
						1	2	3	1	2	3	1	2	3	1	2	3			
Sole Pit	3-8	36F1	15-19	187	0	11	1	1	3	4	5	7	0	0	15	4	4	0		
			20-24	33	0	9	1	0	3	4	3	5	2	0	32	6	15	8		
			>25	0	0	1	0	0	0	0	2	0	1	0	13	5	4	9		
Humber (377)	10-12	36F0	15-19	180	0	11	1	0	1	1	6	2	0	0	8	2	1	0		
			20-24	60	0	15	1	0	1	5	6	7	3	0	27	8	11	9		
			>25	2	0	7	0	0	3	2	6	2	1	1	23	13	3	13		
Flamborough (344)	13-15+17	37F0	15-19	188	0	25	3	0	2	2	2	1	0	0	18	1	4	0		
			20-24	52	0	12	2	1	3	6	10	6	0	0	33	11	4	11		
			>25	1	0	2	0	1	2	0	15	7	0	0	17	7	8	17		
Amble (244)	21,22	39E8	15-19	52	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
			20-24	10	0	0	0	0	0	1	0	0	0	0	1	0	0	1		
			>25	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0		
W. Dogger (286)	18-20	38F1	15-19	274	0	8	2	1	3	5	5	3	0	0	25	11	3	0		
			20-24	220	0	13	1	0	6	6	7	7	1	1	35	13	11	29		
			>25	3	0	2	0	0	4	2	13	1	0	0	13	8	7	28		
Red Wharf (776)	27-29	35E5	15-19	29	0	0	0	0	1	0	0	2	0	0	0	0	0	0		
			20-24	1	0	0	0	0	1	1	1	2	0	0	0	0	0	1		
			>25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Liverpool Bay (706)	23-26	35E6	15-19	82	0	0	0	0	1	1	0	0	1	0	0	0	0	0		
			20-24	12	0	1	0	0	1	5	4	2	0	0	0	0	0	0	1	
			>25	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0		
Cardigan Bay (656)	30-32	33E5	15-19	83	0	0	0	0	1	1	3	6	2	0	0	2	0	0		
			20-24	1	0	1	0	0	0	0	1	3	0	0	0	0	0	6		
			>25	0	0	0	0	0	1	0	2	0	0	0	0	0	0	3		
Rye Bay (486)	41-45	30F0	15-19	116	0	1	0	0	1	0	0	0	0	0	3	0	1	0		
			20-24	10	0	0	0	0	3	1	2	0	1	0	2	1	0	1		
			>25	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1		

Key: LY = Lymphocystis  
 E/P = Epidermal Papilloma  
 U = Epidermal Ulceration  
 HYP = Hyperpigmentation  
 LN = Macroscopic Liver Lesion



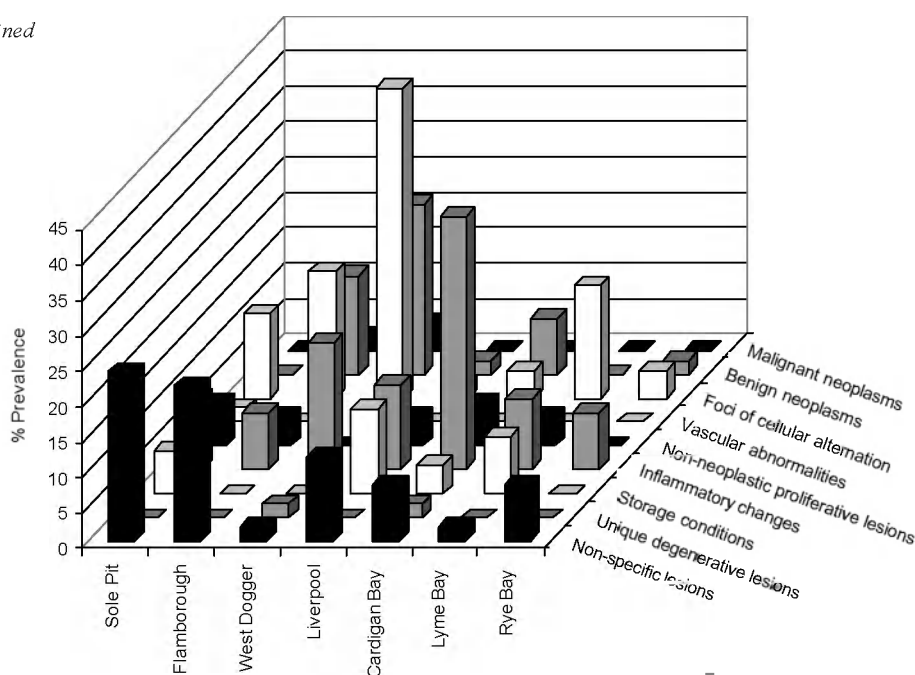


**Figure 22. Percentage prevalence levels of (a) lymphocystis, (b) epidermal hyperplasia/papilloma, (c) ulcerations, (d) epidermal hyperpigmentation and (e) gross liver nodules in dab, RV CIROLANA Cruise 2/97. Prevalances are presented as area proportional symbols; scale symbols are shown above**

**Table 13. Histological confirmation and characterisation of specimens of macroscopic liver lesions observed in two larger sizes of dab sampled in the North Sea and Irish Sea**

Area name (NMP)	ICES Rect.	Latitude/ Longitude	Total no. dab examined	Macroscopic Liver lesions		Lesion classification						Confirmed neoplastic lesions		
				No.	%	AD	CH	HE	PF	NEC	OT	NAD	No.	%
Sole Pit	36F1	53° 48.63'N 01° 23.36'E	294	17	5.8	8			1	2	5	1	8	2.7
Humber (377)	36F0	53° 51.87'N 00° 57.23'E	550	22	4.0	16			5		1		16	2.9
Flamborough (344)	37F0	54° 14.82'N 00° 28.19'E	390	28	7.2	15		1	7	1	3	1	16	4.1
Amble (244)	39E8	55° 14.57'N 01° 15.33'W	35	1	2.9	1							1	2.9
W. Dogger (286)	38F1	54° 54.22'N 01° 18.28'E	419	57	13.6	40		1	11		4	1	41	9.8
Red Wharf (776)	35E5	53° 21.56'N 04° 07.29'W	210	1	0.5	1							1	0.5
Liverpool Bay (706)	35E6	53° 28.86'N 03° 16.11'W	1090	1	0.1								0	0.0
Cardigan Bay (656)	33E5	52° 15.08'N 04° 21.29'W	143	9	6.3	7					1	1	7	4.9
Rye Bay (486)	30F0	51° 32.60'N 04° 44.77'W	133	2	1.5	1			1				1	0.8

Key: AD = Adenoma  
 CH = Cholangioma  
 HE = Hemangioma  
 PF = Preneoplastic Foci (Basophilic, Foci of Cellular Alteration, Clear Cell Focus)  
 NEC = Necrosis  
 OT = Other (Granuloma, Cyst, Storage Change)  
 NAD = No Abnormalities Detected  
 NEO = Neoplasia  
 NE = Not Examined



**Figure 23. Histological characterisation of hepatic lesions in the two larger size categories of dab and flounder sampled from stations in the North Sea and Irish Sea, 1997 (1 hour tows using a Granton trawl)**

the Dogger Bank showed a high prevalence of inflammatory changes. These were predominantly associated with increased numbers of melanomacrophage centres and varying degrees of cellular infiltration.

Although relatively few flounder were caught, thirty fish for histological assessment were obtained from Lyme Bay. Of these, livers from six exhibited FCAs, but none with more advanced benign or malignant lesions were found. Of five flounder caught at Carmarthen Bay, one



exhibited a hepatic adenoma and another a basophilic FCA. From samples obtained later in the year (*RV CIROLANA 5/97*) 10% of dab sampled from Carmarthen Bay exhibited vacuolated or basophilic hepatic FCAs. Thirty plaice (*Pleuronectes platessa*) livers were also examined. Only one case of adenoma was found, no other pre-neoplastic lesions were detected. A single case of liver nodule was found in a four-beard rockling (*Enchelyopus cimbrius*) caught in Cardigan Bay, the lesion was confirmed as a basophilic FCA.

### **Cod diseases**

Sufficient cod for disease investigations were obtained at only three areas, Red Wharf Bay, Rye Bay and Amble (ICES rectangles 35E5, 30F0 and 39E8 respectively) with a total of 358 fish being caught, including a small number of samples obtained at west Dogger. 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 the pathogenic copepod gill parasite *Lernaeocera branchialis*. As has been reported previously (MAFF, 1995, 1998), the general disease prevalence remains very low. At Rye Bay only one ulcerated fish was found and none exhibited skeletal deformities, visceral granulomatosis or the presence of pseudobranchial tumours. Parasitism with *Cryptocotyle* was detected in nine fish from Red Wharf Bay and in a single fish from Rye Bay. Seventeen fish were infected with *L. branchialis* and several of these fish were anaemic and emaciated. The number of parasites affecting individual fish varied from one to four and did not seem to be related to the degree of emaciation. In a few additional fish, chronic inflammatory lesions were observed on, or close to the gill arches, associated with localised necrosis of the primary gill filaments. These lesions were considered to have resulted from old infections with *L. branchialis* since, in several cases, remnants of the holdfast were still present.

### **Examination of the total catch for significant diseases**

Examination of other species including haddock (*Melanogrammus aeglefinus*), whiting (*Merlangius merlangus*), herring, gurnard (*Eutrigla gurnardus*), plaice and flounder revealed generally low levels of disease. Of sixty-one haddock sampled from Amble, twenty-three were infected with *L. branchialis*, two exhibited skeletal deformities and an unidentified gill tumour was detected in one fish. At Red Wharf Bay, several whiting and a pollack (*Pollachius pollachius*) were found to be heavily infected with *Cryptocotyle* and a single whiting was affected by epidermal hyperplasia. No evidence of *Ichthyophonus* was found in samples of herring and sprat.

## **8.4 Discussion**

The overall prevalence rates of the externally visible diseases, lymphocystis, epidermal hyperplasia/papilloma, and acute and healing ulcers in dab are broadly consistent

with the levels detected in previous surveys (CEFAS, 1998) with the highest levels being recorded off Flamborough and at West Dogger (ICES rectangles 37F0 and 38F1 respectively). The reference area at Rye Bay exhibited low levels of disease as was expected, based on previous findings. However, the extremely low prevalence of disease in the Irish Sea stations Liverpool Bay and Red Wharf Bay was unexpected and inconsistent with previous data (MAFF, 1995(a); CEFAS, 1998). The reasons for the decrease in prevalence are not known, but were not due to insufficient numbers of fish caught for examination. The prevalence of hyperpigmentation appears to be increasing in several areas, particularly in North Sea areas Sole Pit and West Dogger (ICES rectangles 36F1 and 38F1 respectively). The aetiology of this condition is still unknown although histologically it is clear that the discolouration of affected fish results from a marked increase in the numbers of pigment containing cells in the dermal layers (CEFAS, 1998). The lack of an inflammatory response in most cases may indicate that an infectious agent is not involved in the formation of these lesions. Further investigations are required in order to determine the cause and pathogenesis of this condition.

In most areas the prevalence of macroscopic liver nodules was lower than in previous years. This appears to be a continuing trend and is supported by data submitted to ICES from other countries bordering the North Sea (ICES, 1998). However, the prevalence of histologically confirmed liver nodules (benign and malignant neoplasia) was significantly higher in fish sampled from West Dogger than at other areas sampled. This finding was supported by the histological data obtained from the samples of randomly selected livers from dab from the same area. These also exhibited higher levels of microscopic lesions than in fish sampled from other areas.

The use of histological biomarkers for detecting biological effects of contaminants is now well established as a recommended monitoring technique for biological effects of contaminants within the OSPAR Convention area (Stagg, 1998). The results from this study demonstrate the utility of histological screening in detecting a wide variety of neoplastic and non-neoplastic lesions in the dab and flounder, including those fish that do not exhibit macroscopic lesions. In addition, there is evidence that certain categories of non-neoplastic liver lesions are capable of being used to detect early biological damage in sub-adult flatfish (Myers *et al.*, 1998) and these may become more important as biomarkers in dab and flounder. However, for current monitoring purposes using dab, pre-neoplastic FCA and hepatocellular adenomas in particular, are the most relevant lesion types to target since malignant neoplasms are relatively rare and may be expected to become more so if the current declining trend in the prevalence of macroscopic liver lesions continues. It remains to be seen whether this decline will also occur in the prevalence of toxicopathic hepatic lesions.

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# BENTHOS

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## 9. EPIFAUNAL STUDIES AROUND THE UNITED KINGDOM COASTLINE

### 9.1 Introduction

The benthic macrofauna sampled by Day grab at 'intermediate' and 'offshore' National Monitoring Plan stations is described in CEFAS (1998). The following account summarises the outcome of a parallel study of animals inhabiting the surface of the seabed (the epifauna) sampled by beam trawl at NMP and additional stations around the England and Wales coastline and offshore. An earlier progress report was given in MAFF, 1995(b); a full account of both infaunal and epifaunal surveys is given in Rees *et al.* (1999).

Typical epifaunal taxa include starfish, crabs and shrimps, and hence this component of the benthic fauna is likely to be the most familiar to non-specialists and the public at large. Many epifaunal species have an important role in the marine food chain (e.g. Collie *et al.*, 1997). However, this group has received much less scientific attention than the infauna, largely due to sampling difficulties. The present survey aimed to redress the balance by providing an insight into the distribution and relative abundance of epifaunal taxa over a much wider geographical range than has previously been attempted in surveys of benthic populations around the UK coastline.

### 9.2 Methods

#### *Field sampling*

Samples were obtained by MAFF research vessels from 69 stations between 1992 and 1996 (Figure 24).

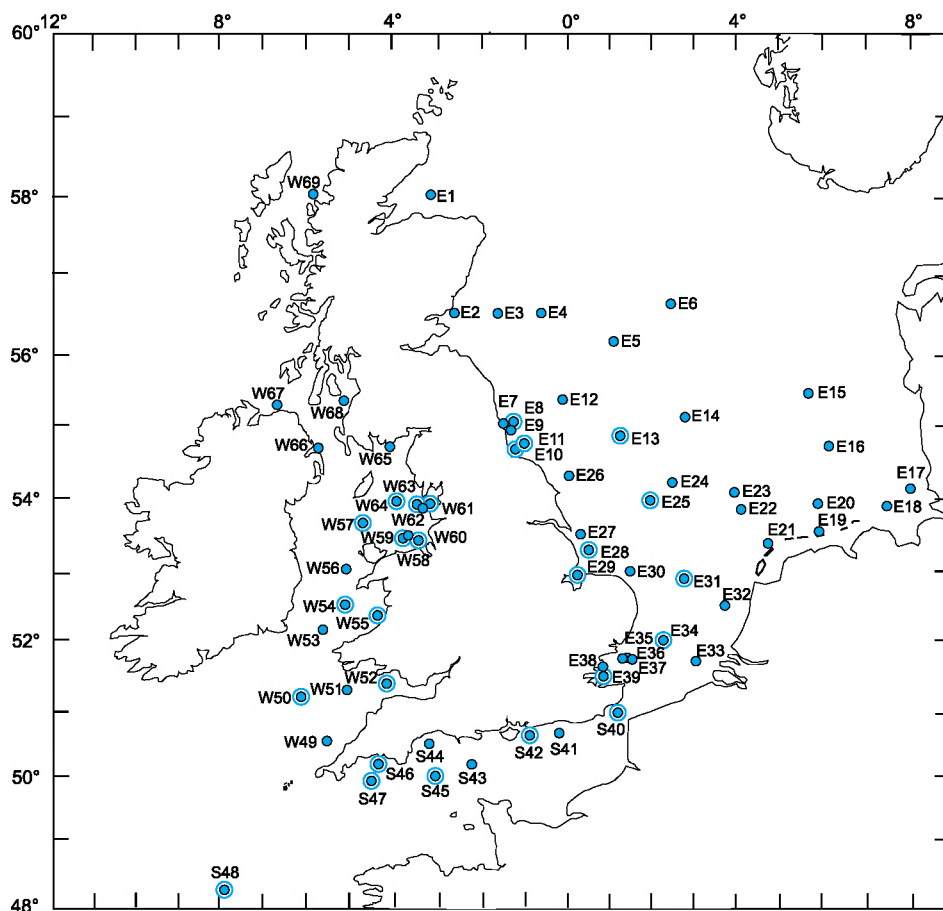


Figure 24. Locations of 2-metre beam trawl stations. (Encircled stations were also sampled by Day grab as part of the UK National Monitoring Programme)

A standard two-metre Lowestoft beam trawl (see Riley *et al.*, 1986) with a 3 mm mesh cod-end liner was deployed for 5-10 minutes across each station at a speed of approximately 0.5 m.s<sup>-1</sup>. The 'start' (locking of winch following seabed contact) and 'end' (commencement of hauling) positions were recorded. Tow length averaged 400 m, but varied substantially (sd = 290) depending on tidal current velocity and wind strength at the time of sampling. On retrieval of the trawl, an estimate of sample volume was made, along with a summary of the contents, noting especially the presence of stones, rock, etc., and any anthropogenic debris. The sample was then sorted on deck over a 5 mm mesh sieve, with specimens being identified and enumerated either immediately or in the ship's laboratory. Any problematic specimens were preserved in formalin for further identification back at the Laboratory.

The presence of infaunal organisms arising from the fouling of soft sediments was noted, as were occurrences of pelagic species such as jellyfish. However, these records were excluded from the final compilation of the data. The former included most polychaete worms (with the exception of encrusting forms such as *Sabellaria* and *Filograna*, and surface-dwelling specimens within the family Aphroditidae), bivalve molluscs (excepting taxa with a surface-dwelling habit such as *Chlamys* and *Modiolus*) and burrowing echinoderms such as *Echinocardium* and *Amphiura*. In the following account, the faunal data are reported as numbers per tow, i.e. unadjusted for tow length.

#### Laboratory analysis

Motile taxa not dealt with at sea were identified to species level, as far as possible, with the use of a range of standard taxonomic keys. In the preparation of fauna lists, nomenclature followed that of Howson (1987).

A ranked descriptor of the predominant substratum type along trawl tows was derived from a combination of records of trawl contents, particle size analyses of sediments from grabs where available, and information from Admiralty Charts:

Code	Description	Rank
M	Mud	1
M(S)	Sandy mud	2
S(M)	Muddy sand	3
S	Sand	4
S(Sh)	Shelly sand	5
G(S)	Sandy gravel	6
G(Sh)	Shelly gravel	7
G	Gravel	8

Whilst useful as a crude indicator of sediment 'coarseness', these summaries were inadequate to categorise some of the admixtures encountered, for example, gravel and mud (see Section 9.4 Discussion).

#### Data treatment

Inter-relationships between the following variables (log-transformed, where necessary, to reduce skewness in the data) were examined using Pearson product moment correlation coefficients:

maximum spring tidal current strength (m.s<sup>-1</sup>), depth (m), average winter surface-water temperature and salinity, latitude, longitude, sediment type (1-8, see below), tow length (m), numbers of taxa and numbers of individuals per trawl tow.

Average surface-water temperature and salinity in winter (February) were selected because only limited data at the seabed were available, especially for the western UK coast (Lee and Ramster, eds, 1981). However, these authors note that differences in temperature between surface and bottom over most of the North and Irish Seas are minimal at this time of year. They also note the more conservative nature of salinity relative to temperature, with only very small differences in values between winter and summer both at the surface and at the seabed, and the similarity in distributional trends between surface and bottom, for the area covered by the present study.

Multivariate classification analysis was conducted on presence/absence data using the Sorensen coefficient (see Clarke and Warwick, 1994) and group-average sorting (Lance and Williams, 1967). Relationships between the ranked dissimilarity matrices and different combinations of environmental variables were examined using the method described by Clarke and Ainsworth (1993) : sub-sets which best explained the biological variability were identified by the highest correlation coefficients ( $p_w$ ).

## 9.3 Results

#### Sediments

Sampling stations in the southern North Sea were characteristically sandy in nature (Figure 25(a)). Sand and mud in varying proportions were associated with stations in inner Liverpool and Morecambe Bays (north west England) and off the Tyne (north east England). Deeper-water samples in the northwestern North Sea had an appreciable gravel component (although the prevailing sediment type is, in parts, finer : see Section 9.4 Discussion), as did those in coastal waters off eastern England, where coarse deposits are widespread. Samples from the English Channel, Celtic and Irish Seas were also predominantly gravelly in nature, again reflecting the generally coarser sediments in these areas.

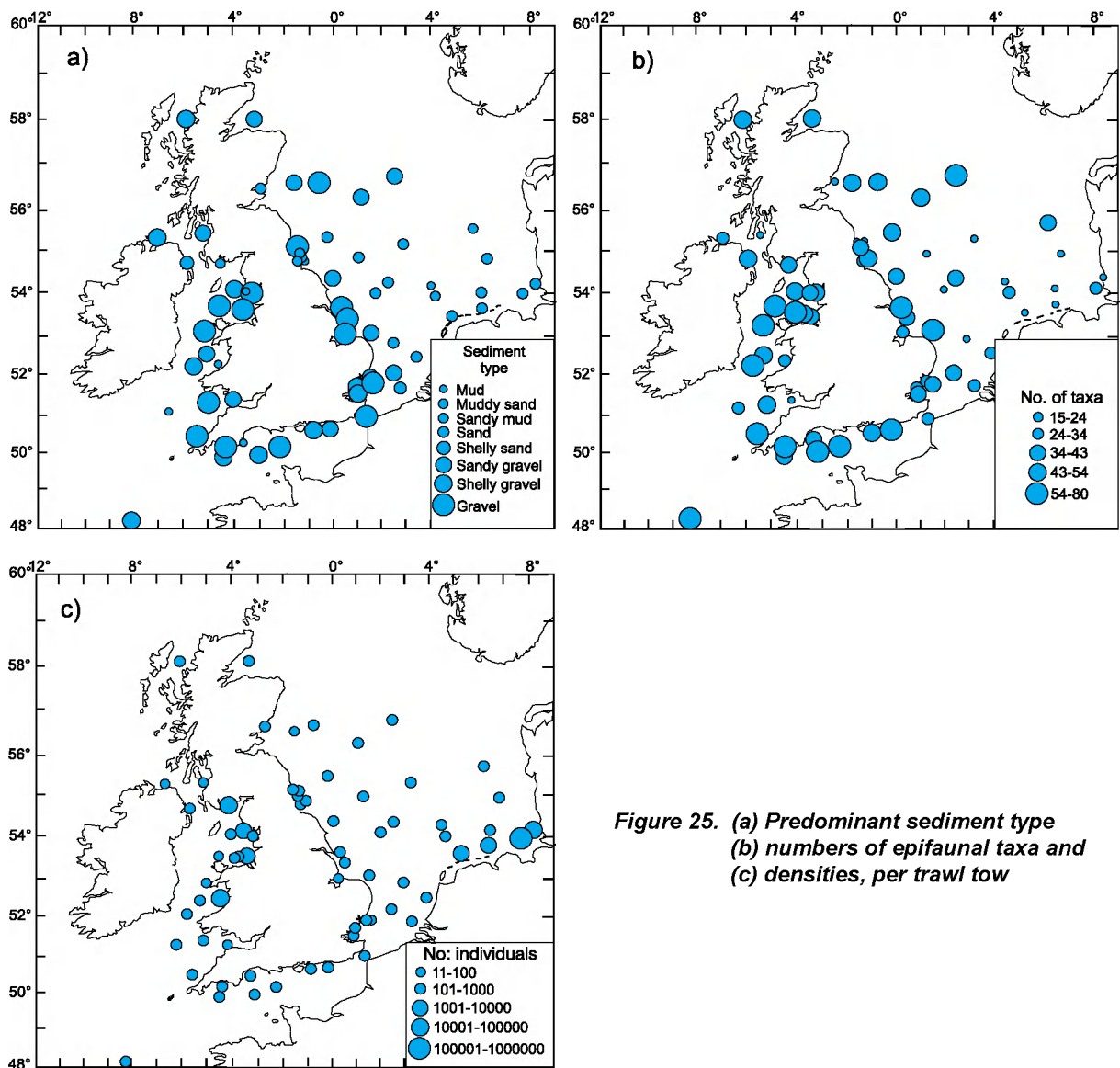


Figure 25. (a) Predominant sediment type (b) numbers of epifaunal taxa and (c) densities, per trawl tow

### Litter content

Litter of recent human origin was identified at 60% of stations. However, the quantity was minimal except in some coastal areas, notably the Thames Estuary and Liverpool Bay, where items arising from pipeline discharges of domestic sewage or disposal of sewage sludge from ships were encountered.

### Fauna

The apportioning of taxa across major groups (Table 14(a)) showed that bryozoans, molluscs and crustaceans were dominant, with hydroids and fish also being well represented. The total across all animal groups amounted to 414 taxa. The six most frequently encountered taxa (Table 14(b)) were Paguridae (hermit crabs), *Asterias rubens* (common starfish), *Ophiura* spp (brittle-stars), *Liocarcinus holsatus* (swimming crabs), *Crangon allmanni* (brown shrimp) and Gobiidae (gobies). These are all motile, in contrast to the remaining four (the hydroid *Hydrallmania falcata*, the

barnacle *Balanus crenatus* and the bryozoans *Electra pilosa* and *Flustra foliacea*) which are sessile in habit. Overall, relatively few taxa were widespread throughout the sampling area and 132 (about 30%) were single occurrences.

A comparison of Figure 25(a) and (b) shows that, in the North Sea, higher numbers of taxa tend to be associated with coarser substrata to the north of the survey area, and along parts of the eastern English coastline. Elsewhere, gravelly substrata in the English Channel and areas adjacent to the western UK coast also supported a generally high epifaunal diversity. The distribution of animal densities (Figure 25(c)) is more even across the survey area, although very high numbers were occasionally encountered at inshore locations. For example, in the German Bight, Liverpool Bay and Solway Firth, dense populations of the brittle star *Ophiura* were sampled.

**Table 14. Numbers of epifauna taxa allocated to each major faunal group (a); top ten ranked taxa in terms of frequency of occurrence in trawls (b)**

(a)	
Group	No. of taxa
Bryozoa	80
Mollusca	66
Crustacea	62
Hydrozoa	43
Pisces	40
Porifera	36
Echinodermata	26
Anthozoa	24
Ascidiacea	23
Other groups	14
<i>Total</i>	414

(b)	
Taxon	Frequency (max = 69)
Paguridae	64
<i>Asterias rubens</i>	57
<i>Ophiura</i> spp	48
<i>Liocarcinus holsatus</i>	47
<i>Crangon allmanni</i>	45
Gobiidae	45
<i>Hydrallmania falcata</i>	43
<i>Balanus crenatus</i>	43
<i>Electra pilosa</i>	42
<i>Flustra foliacea</i>	40

The numbers of taxa encountered at each station for each of the major faunal groups (Figure 26(a)-(i)) further illustrate the capacity of coarser substrata to support a higher epifaunal diversity, especially of sessile groups (Figure 26(a)-(c), (f) and (h)). Molluscan diversity (Figure 26(e)) is also noticeably higher at coarse ground locations. The distributions of crustacean and echinoderm taxa (Figure 26(d) and (g)) are more equitable, while a greater variety of fish were found in the southern North Sea and SE Irish Sea (Figure 26(i)).

Statistical relationships between numbers of taxa and individuals per trawl and a range of environmental variables were explored through correlation analysis (Table 15). There was a significant positive correlation between sediment 'coarseness' and log. numbers of taxa, and a significant negative relationship with log. densities. The former may be explained by the capacity of the mixed gravelly areas to provide a much greater array of attachment points and refuges, compared with uniform muddy areas (see Figure 25(b)). The latter reflects a capacity for muddy sand or muddy deposits, especially close inshore near to major estuaries, to support very high densities of common species such as brittle-stars (see Figure 25(c)) which, in turn, accounts for the significant negative relationship between counts, water depths and salinity (Table 15).

A significant negative correlation between numbers of taxa and longitude (i.e. numbers tend to increase in a

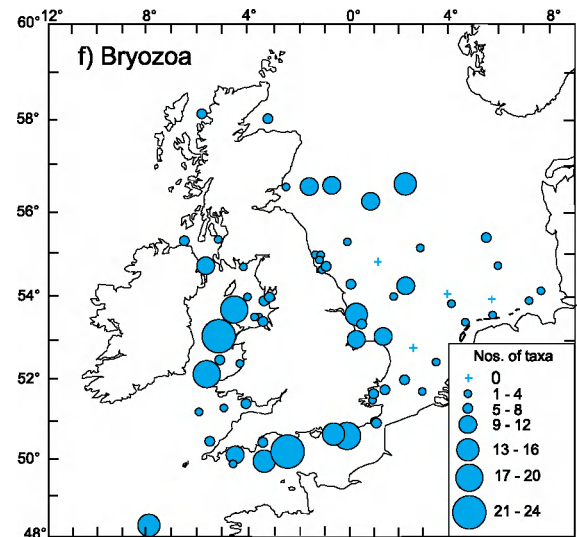
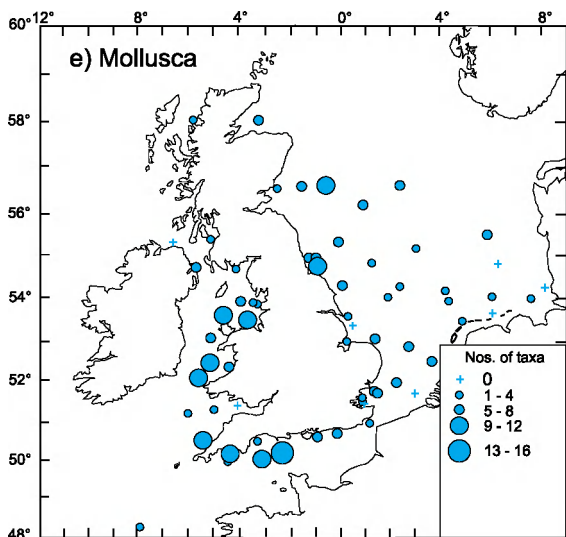
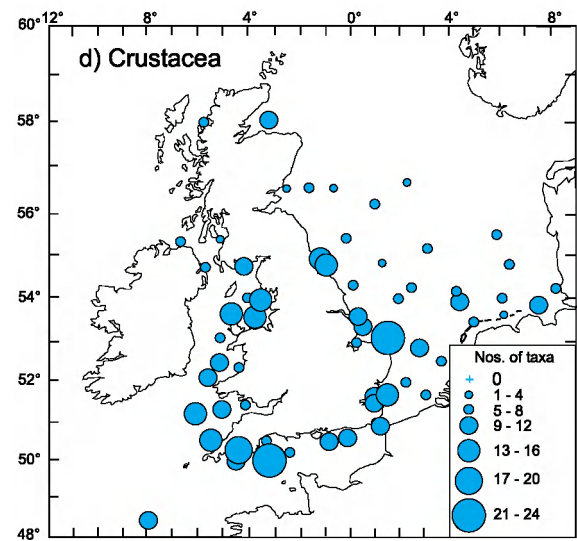
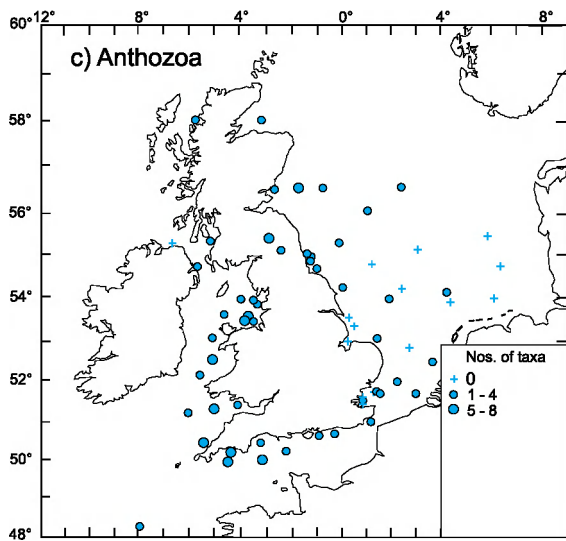
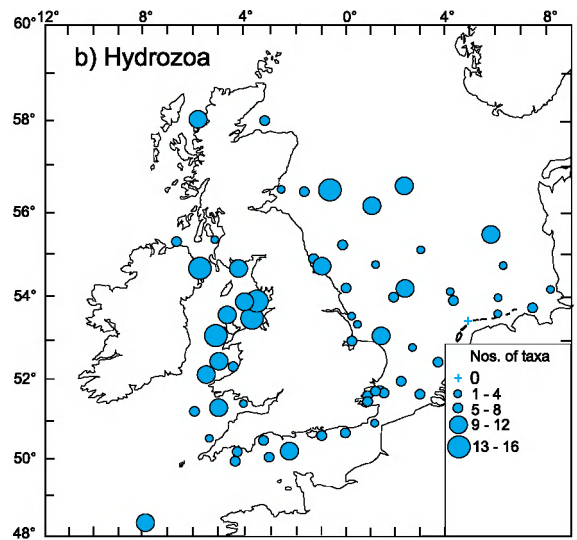
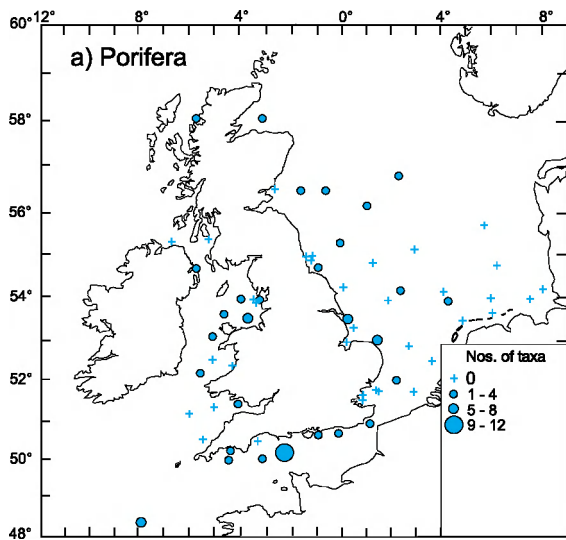
westerly direction) appears to be largely a function of coincident trends in temperature, depth and, to a degree, substratum type. There was also a discernible underlying trend towards increased winter surface temperature in a north-south direction which, along with substratum type, might help to explain a comparable, but less pronounced, trend in numbers of taxa. There was no significant correlation between faunal measures and maximum spring tidal current strength, despite a significant positive correlation between this variable and sediment 'coarseness', and a significant negative correlation with latitude. Although not significant, a weak positive relationship between numbers of taxa and current velocity ( $p = .054$ ) contrasts with a significant negative relationship between the two for infaunal data (see Rees *et al.*, 1998 and 1999).

Relationships between epifaunal and environmental data were further examined using the procedure of Clarke and Ainsworth (1993). Maximum correlation ( $p_w = 0.47$ ) arose from a combination of five variables: latitude (0.30), sediment type (0.29), log. depth (0.27), maximum spring tidal current strength (0.14) and winter temperature (0.26). These are listed in rank order of their contributions to ascending best-variable combinations, and the figures in parentheses are correlation coefficients for each variable when tested singly against the biological data. As was the case with numbers and densities of taxa (Table 15), there was no significant influence of tow length ( $p_w = -0.01$ ).

Multivariate classification analysis was conducted on presence/absence data and eight faunal assemblages were identified (Figure 27). These broadly correspond with differences associated with substratum type, and are therefore most easily described in these terms (Figure 28), although it is recognised that sub-clusters, especially within groups 7 and 8, will repay further investigation in relation to species distributions. A biogeographical influence is suggested by, for example, the distinction between a 'NW North Sea' station group and a 'W Channel/W Coast' station group, both of which are characterised by a gravelly component to samples. However, other groupings (especially in coastal areas) comprised stations from widely separated geographical locations. Six stations remained 'unclassified' in this scheme. They were generally located in inshore areas, typically contained relatively low numbers of taxa and, in four cases, were intermediate in character to other major cluster groups.

Average numbers of taxa for each cluster group (Table 16) show that there is a trend of increasing numbers with increasing sediment 'coarseness', the major distinction arising from an appreciable gravelly component to samples in the four right-hand groups (Groups 6, 3, 1 and 8). Lowest numbers were found in predominantly sandy areas of the southern North Sea (Group 4), and highest numbers in a 'stony ground' assemblage mainly off the western UK coast (Group 8).





**Figure 26 (a-f).** Numbers of taxa per station for the major epifaunal groups

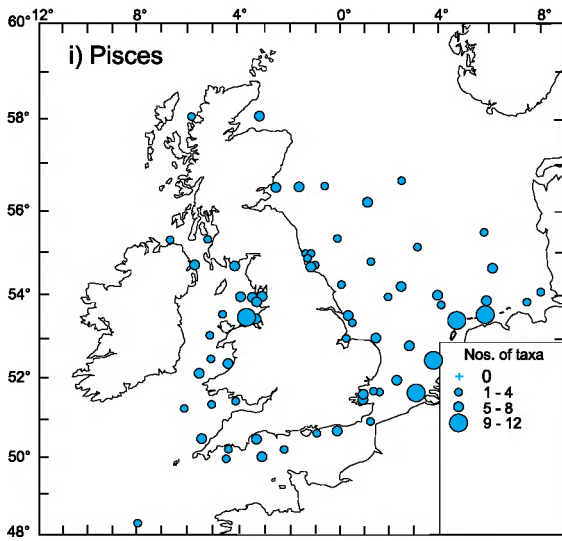
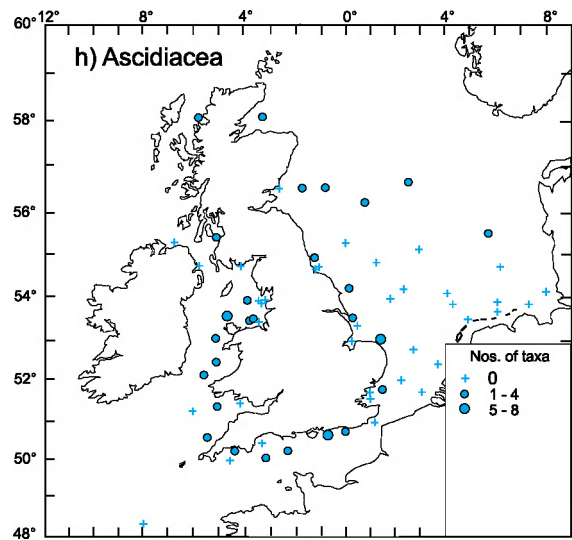
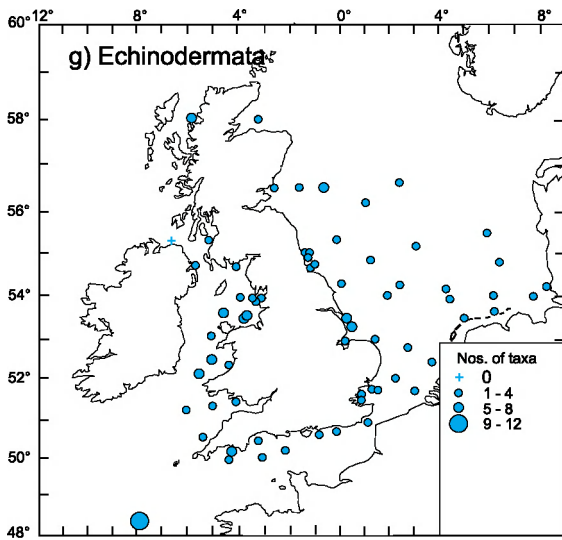
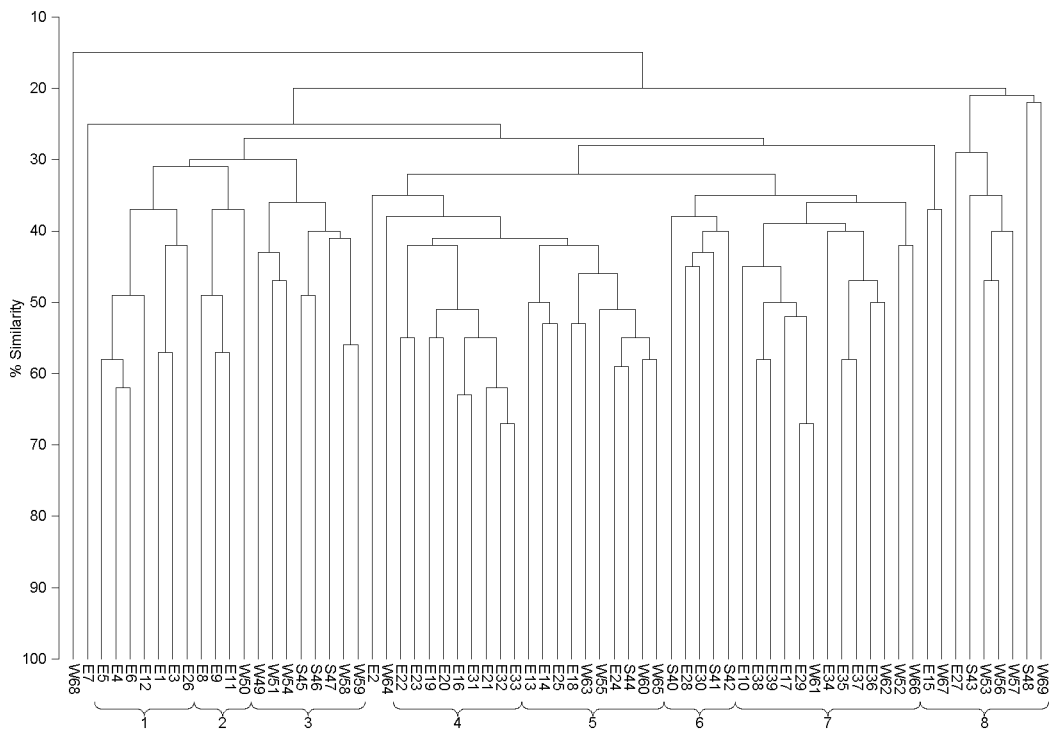


Figure 26(g-i) Numbers of taxa per station for the major epifaunal groups

Table 15. Correlation matrix for biological and environmental variables at 2-metre beam trawl stations (n = 69). Significance levels : \* .05 - .01; \*\* .01 - .001; \*\*\* <.001

	Current speed	Log depth	Temperature	Salinity	Latitude	Longitude	Sediment type	Log tow length	Log taxa	Log counts
Current speed										
Log depth	-0.26									
*										
Temperature	ns	0.61								
***										
Salinity	ns	0.48	0.61							
***		***								
Latitude	-0.44	ns	-0.47	ns						
***			***							
Longitude	-0.25	-0.41	-0.75	ns	ns					
*	***	***								
Sediment type	0.37	ns	0.36	ns	-0.24	-0.29				
**			**		*					
Log two length	ns	ns	ns	ns	ns	-0.25	ns			
						*				
Log taxa	ns	0.34	0.51	ns	-0.25	-0.47	0.51	ns		
		**	***		*	***	***			
Log counts	ns	-0.31	ns	-0.46	ns	ns	-0.44	ns	ns	
		*		***			***			





**Figure 27. Outcome of cluster analysis of presence/absence data for the epifauna from 2-metre beam trawls**

Cluster groups can be further characterised in terms of the most frequently occurring taxa (Table 16). The contributions of sessile and motile species to these lists again serves to highlight the importance of substratum type in determining the character of epifaunal assemblages.

Offshore stations in the North Sea are grouped into three regions. The southern part supports a relatively sparse epifauna characteristic of sandy substrata, with limited scope for the establishment of sessile species. Indeed, of the 12 most frequently occurring taxa (Table 16, Group 4), the only sessile species is the hydroid *Hydractinia echinata*, which was recorded as an epigrowth on the shells of hermit crabs which are, of course, themselves motile in habit. The central part encompasses the relatively shallow Dogger Bank area. Sediments here are slightly muddier in nature and, while still lacking an appreciable gravel component, have the capacity to support marginally greater numbers of sessile taxa than sandy stations to the south. The epifauna shares similarities with comparable near-shore muddy sand substrata in the German Bight and eastern Irish Sea (Figure 28 and Table 16, Group 5). Finally, samples from the deeper northern part were more gravelly in nature, allowing colonisation by a wider array of attached species. As a result, higher numbers of taxa are found here, compared with offshore stations to the south (Figure 25(b)), and there is a more equitable balance between sessile and motile species among the most frequently occurring taxa (Table 16, Group 1).

Towards the NE English coast, muddier sediments prevail, and these are characterised by the presence of *Nephrops norvegicus*, and a reduced frequency of occurrence of sessile species (Group 2 in Table 16). These conditions are mirrored in a station in the Celtic Deep off the western UK coast which was linked with the NE group.

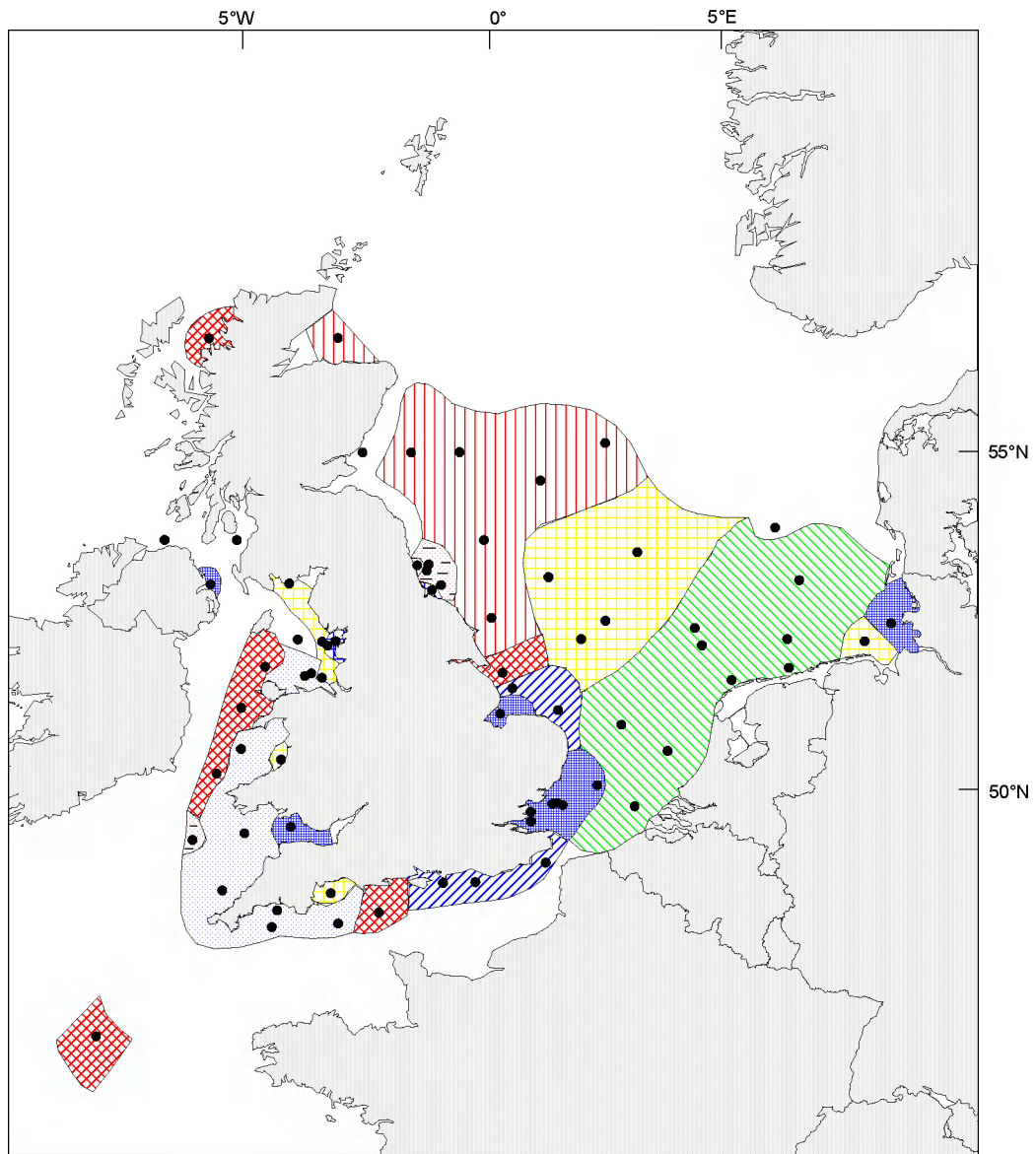
Influences associated with estuarine efflux are evident at near-shore sites off the estuaries of the Elbe/Weser, Thames and Tees, within the Wash, Bristol Channel and Morecambe Bay, and off Belfast Lough where a comparable 'estuarine' fauna is encountered (Figure 28). The assemblage is notable for the frequent occurrence of the brown shrimp *Crangon crangon* and the pink shrimp *Pandalus montagui* (Table 16, Group 7), both of which are commonly encountered within estuaries as well as coastal waters. Numbers of taxa at these stations are similar to those in the Dogger Bank area.










Elsewhere, gravelly samples collected off the south and west coasts supported a similar fauna characterised by high numbers of taxa (Figure 25(b)). The frequent occurrence of the echinoderm *Psammechinus miliaris*, and the rarity of the large gastropods *Neptunea antiqua* and *Colus gracilis* in the western assemblage helped to distinguish it from the 'NW North Sea' assemblage (Table 16, Groups 3 and 1, respectively). The higher frequencies (and densities) of the shrimps *Pandalus montagui* and *Crangon allmanni* in an 'E Channel/E Coast' gravelly cluster (Table 16, Group 6) suggested

**Table 16. Most frequently occurring taxa in cluster Groups 1-8**

**Groups are ranked from left to right according to the contribution of sessile taxa, which are highlighted. In the column adjacent to each group, numbers of occurrences (n) appear above the total sample number.**

Group 4: 'S North Sea sandy'	n	Group 2: 'Muddy'	n	Group 5: 'Near-shore muddy sand/Dogger Bank'	n	Group 7: 'Estuarine'	n	Group 6: 'E Channel/ E Coast gravelly'	n	Group 3: 'W Channel/ W Coast gravelly'	n	Group 1: 'NW North Sea gravelly'	n	Group 8: 'Stony'	n
Paguridae	9	<i>Hydrallmania falcata</i>	4	Paguridae	10	Paguridae	13	<i>Hydrallmania falcata</i>	5	Serpulidae	8	<i>Hydractinia echinata</i>	7	<i>Sertularia cupressina</i>	7
<i>Liocarcinus holstius</i>	9	<i>Laonidea flexuosa</i>	4	<i>Asterias rubens</i>	10	<i>Electra pilosa</i>	13	Paguridae	5	Paguridae	8	<i>Alcyonium digitatum</i>	7	<i>Hydrallmania falcata</i>	6
Gobiidae	9	<i>Aphrodita aculeata</i>	4	<i>Ophiura</i> spp	10	<i>Asterias rubens</i>	13	<i>Macropodia</i> spp	5	<i>Hyas coarctatus</i>	8	Serpulidae	7	Serpulidae	6
<i>Hydractinia echinata</i>	8	<i>Crangon allmanni</i>	4	<i>Liocarcinus holstius</i>	9	<i>Pandatus montagui</i>	12	<i>Alcyonidium diaphanum</i>	5	<i>Ophiura</i> spp	8	Paguridae	7	<i>Flustra foliacea</i>	6
<i>Arneglossus laterna</i>	8	<i>Nephrops norvegicus</i>	4	<i>Hydractinia echinata</i>	8	<i>Sertularia cupressina</i>	11	<i>Vesicularia spinosa</i>	5	<i>Alcyonium digitatum</i>	7	<i>Neptunea antitica</i>	7	<i>Calyceella syringa</i>	5
<i>Limanda limanda</i>	8	Paguridae	4	<i>Hydrallmania falcata</i>	8	<i>Balanus crenatus</i>	10	<i>Flustra foliacea</i>	5	<i>Adamisia carcinopados</i>	7	<i>Colus gracilis</i>	7	<i>Abietmaria abietina</i>	5
<i>Biglossidium luteum</i>	8	<i>Astropecten irregularis</i>	4	<i>Aphrodita aculeata</i>	8	<i>Crangon crangon</i>	10	Gobiidae	5	<i>Balanus crenatus</i>	7	<i>Asterias rubens</i>	7	Paguridae	5
<i>Crangon allmanni</i>	7			<i>Electra pilosa</i>	8					<i>Macropodia</i> spp	7	<i>Suberites domuncula</i>	6	<i>Calliostoma zizyphinum</i>	5
<i>Crangon crangon</i>	7			<i>Astropecten irregularis</i>	8					Anonimidae	7	<i>Clytia hemisphaerica</i>	6	Anonimidae	5
<i>Asterias rubens</i>	7			<i>Callionymus lyra</i>	8					<i>Psammechinus militaris</i>	7	<i>Epizoanthus incrustatus</i>	6	<i>Crista eburnea</i>	5
<i>Ophiura</i> spp	7			<i>Biglossidium luteum</i>	8					<i>Hyas coarctatus</i>	6	<i>Alyonidium mytili</i>	6	<i>Alyonidium mytili</i>	5
<i>Callionymus lyra</i>	7													<i>Escharella immersa</i>	5
														<i>Serripocellaria scriptosa</i>	5
Number of samples	9		4	10	13	5	8	7	5	8	8	7	7		
Average number of taxa (95% CL)	22 (19-25)	35 (20-51)	31 (25-37)	33 (27-39)	50 (29-71)	58 (46-70)	49 (43-55)	64 (53-75)							



- |    |   |  |    |  |  |
|----|---|--|----|--|--|
| 1. |  | NW North Sea gravelly<br>( <i>Neptunea antiqua</i> / <i>Colus gracilis</i> )                     | 6. |  | E Channel/E Coast gravelly<br>( <i>Vesicularia spinosa</i> /<br><i>Alcyonidium diaphanum</i> ) |
| 2. |  | Muddy<br>( <i>Nephrops norvegicus</i> )  | 7. |  | Estuarine<br>( <i>Pandulus montagui</i> /<br><i>Crangon crangon</i> )                          |
| 3. |  | W Channel/W Coast gravelly<br>( <i>Adamsia carciniopados</i> /<br><i>Psammechinus miliaris</i> ) | 8. |  | Stony<br>( <i>Abietinaria abietina</i> /<br><i>Alcyonidium mytili</i> )                        |
| 4. |  | S North Sea sandy<br>( <i>Crangon spp.</i> / <i>Limanda limanda</i> )                            |    |  | Unclassified   |
| 5. |  | Nearshore Muddy sand/<br>Dogger Bank   |    |  |  |

**Figure 28.** The distribution of epifaunal assemblages derived from cluster analysis of presence/absence data. Where possible, distinctive taxa (or combinations of taxa) among those most frequently occurring are also listed against cluster type

that these are more subject to coastal influences than the predominantly offshore stations on the western side. This was also supported by the close affinity of this group with the 'Estuarine' assemblage, in the dendrogram output from cluster analysis (Figure 27).

Another coarse ground cluster (defined as 'stony' in Figure 28) comprises stations which are widely separated both geographically and in terms of depth. For example, it includes a station off the Humber at 20 m, a station off the Pembrokeshire coast of Wales at 100 m and one in the South West Approaches at 170 m, albeit linked at a low level of similarity. Highest numbers of taxa generally occurred within this group, the most frequently occurring of which were largely sessile forms, especially hydroids and bryozoans (Table 16, Group 8).

## 9.4 Discussion

Multivariate analysis of the epifaunal data revealed a number of similar assemblages inhabiting comparable environments across the large geographical range of the survey area. In other cases, biogeographical factors, such as increasing winter temperatures to the south and west, probably contributed to differences in the complement of species between assemblages, although intercorrelation among many of the environmental variables under consideration complicates the identification of causal influences. Nevertheless, substratum type appeared to be the main structuring force, with significantly enhanced diversity on coarser ground, as would be expected given the scope for colonisation of this more complex habitat by a wider array of sessile species. Thus sandy areas of the southern North Sea were relatively impoverished, while samples with an enhanced gravelly or stony component collected in areas to the north of the Dogger Bank, parts of the English east coast, the English Channel and Celtic Seas, generally supported the richest assemblages.

Inshore, the outflow from major estuaries was also an influential factor, with a distinctive assemblage type developing, irrespective of geographical location. Nearby muddy sand locations in the inner German Bight and NE Irish Sea were characterised by high densities of the brittle-star *Ophiura*, which matched the earlier findings of Riese and Bartsch (1990) and Rees *et al.* (1992), respectively, and indicates an enhanced food supply at these locations.

Within the English Channel, the distinction between a western and eastern gravelly fauna broadly corresponds with the boundary for 'W Channel' species identified by Holme (1966), and may be linked to the west-east transition from deeper, stratified to shallower, mixed waters. From the results of photographic and acoustic surveys of the sea bed, Holme and Wilson (1985) highlighted the importance of tidally-induced sand

transport in determining the structure of benthic assemblages at a predominantly coarse-ground location SW of the Isle of Wight. Different successional states in the fauna encountered over short distances could be explained by the degree of abrasion or overburden. Species largely characteristic of 'sub-climax' assemblage types were frequently encountered in Channel trawl samples during the present study, but the relatively large tow distances would be expected to integrate across small-scale patchiness and hence preclude finer-scale resolution.

Mackie *et al.* (1995) have recently reported on surveys of the macrobenthic infauna and epifauna of the southern Irish Sea. Multivariate analysis of the data revealed a similar species-rich offshore gravelly assemblage adjacent to the western Wales coastline (equivalent to the 'stony' group of Figure 28), trending to a muddy fauna in the Celtic Deep area. The sessile epifauna was relatively uniform in character throughout the survey area, with local topography and sediment type being more influential than geographical location.

For the North Sea area, comparable but more intensive surveys of the epifauna encompassing the region sampled in the present study have been reported by, for example, Dyer *et al.* (1983), Frauenheim *et al.* (1989) and Duineveld and van Noort (1990). Despite differences in sampling methodology, all three identified a broad division between a 'northern' and 'southern' fauna lying along the 50 m contour, i.e. the northern edge of the Dogger Bank, a finding which is paralleled by the outcome of the present survey. This division of the North Sea was similar to that proposed by Glemarec (1973), based upon thermal stability of the water-column, and is supported by the recent work of Jennings *et al.* (in press) who identified a combination of depth and the difference between winter and summer bottom temperatures as factors which best explained trends in the North Sea epifauna sampled by 2 m beam trawl. In a more limited epifaunal survey of the south-eastern and central North Sea, Kunitzer (1990) similarly distinguished between a northern and southern fauna, citing earlier observations on the boundary between stratified and mixed waters, and on the influence of different water masses (N Atlantic and Channel inflow to the northern and southern North Sea, respectively) in explanation. She also identified a transitional assemblage, comparable with that of Group 5 in Figure 28, occupying stations to the north-east of the Dogger Bank.

Duineveld and van Noort (1990) found no significant relationship between the distribution of epifaunal assemblages and that of sediment type, at least as measured by the percentage silt/clay content of grab samples. The authors speculated that the paucity of attached epifaunal species (such as anemones, ascidians and sponges) in the southern North Sea might be due to the high intensity of commercial beam trawling. Collie

*et al.* (1997) observed a reduction in bryozoans and hydroids at gravelly locations on Georges Bank (NW Atlantic) subject to disturbance by commercial dredging. While the present survey also found a low frequency of occurrence of sessile taxa in the southern North Sea, evidence of a causal relationship with fishing activity still remains inconclusive, since the prevailing sandy sediments would be expected to naturally restrict their occurrence. On a wider scale, Jennings *et al.* (in press) found no relationship between epifauna distributions and fishing effort in the North Sea within ICES rectangles, but cautioned that the available information was too imprecise to establish whether or not commercial trawling had actually occurred across individual sampling stations.

Basford *et al.* (1989) sampled the epifauna of the northern North Sea using a 2 m Agassiz trawl with a 2 cm end-mesh, and identified depth and sediment characteristics as the principal determinands of assemblage type. Only the southernmost part of their grid overlapped with the present survey but, despite differences in sampling methodology, a number of larger taxa co-occurred, for example, the anthozoans *Alcyonium digitatum*, *Hormathia digitata* and *Bolocera tueidae*, the bryozoan *Flustra foliacea* and ascidians, all indicative of a coarser sediment type in this area. In a comparison between the outcome of infaunal and epifaunal surveys in the northern North Sea, Basford *et al.* (1990) noted that, in contrast to the former, the role of sediment type in determining the distribution of epifauna assemblages was apparently secondary to that of depth. However, they recognised the possibility that, at many stations, sediment samples from grabs may be inadequate to account for variation in the habitat along trawl tows.

International surveys of demersal fish, conducted over a number of years under ICES auspices, and Rogers *et al.* (1998) report similar trends in species numbers to those of the epifauna in the present study. Namely lower species richness especially in the uniform sandy area of the SE North Sea, compared with the English Channel and the UK west coast, where a higher diversity is supported by the generally coarser substrata.

Trawl sampling of the epifauna has the advantage of providing an integrated sample over a much wider area than is feasible with grabs, and is an essential procedure for assessing the larger, rarer 'megafaunal' taxa (McIntyre, 1978). Another advantage of trawling for larger organisms is that much of the material may be processed on deck immediately after collection. However, there are inherent uncertainties over the performance of the trawl at the seabed, especially across coarser substrata. Therefore, it is preferable to treat the data on a presence/absence or, at most, 'semi-quantitative' basis in the absence of replication.

It is most important to recognise that, as with demersal fish population surveys, the results from trawl sampling of the epifauna are 'operationally determined', i.e. are subject to systematic error or bias associated with the design specification (width of beam, mesh sizes of 'belly' and 'cod end' and so on). Consistency in equipment design and in deployment practices is therefore essential, especially for the assessment of temporal trends.

One important operational problem is the tendency of the trawl to select for larger particles, where present. This may result in a bias towards the sampling of attached species, especially colonial hydroids and bryozoans. Thus the description of a 'gravelly' fauna from a trawl tow may, in some circumstances, provide a misleading impression of the predominant sediment type (and faunal assemblage) along its entirety, especially when the data are treated on a presence/absence basis. Caution must therefore be exercised in drawing inferences about habitat type from the content of trawl tows, and an agreed procedure for describing faunal assemblages arrived at.

Useful working descriptions of the environment along trawl tows are difficult to establish, especially where a variety of sediment types are encountered. There is a need to assess the feasibility of developing an integrated and cost-effective measure of such variability, for example, with the use of combined acoustic and photographic techniques such as those employed by Holme and Wilson (1985), Sotheran *et al.* (1997) and Service and Magorrian (1997), as occasional 'spot' samples of sediments using grabs may inadequately convey the complexity of the environment in many areas.

#### *Acknowledgements*

We are grateful to Gerd Konnecker (consultant) for identification on contract of sessile colonial taxa from trawl samples.

## **10. A COMPARISON OF BENTHIC CHANGES AT A DREDGED MATERIAL DISPOSAL SITE**

### **10.1 Introduction**

Published studies of the biological effects of the disposal of maintenance dredgings have historically relied on an examination of macrofaunal organisms (Harrison, 1967; Flint, 1979; van Dolah *et al.*, 1984; Wildish and Thomas 1985; Rees *et al.*, 1992; Harvey *et al.*, 1998). These studies show wide differences in the responses of the fauna to disposal. Such studies



demonstrate the need for site-specific surveys for evaluating the consequences of disposal. Recently it has emerged that meiofaunal taxa are sensitive to the disposal of dredgings (Somerfield et al., 1995) and therefore may provide an alternative to more conventional approaches for assessment.

Prior to adopting such a method in routine monitoring programmes it was necessary to establish the utility of meiofaunal taxa for assessing disturbance from dredgings disposal at a range of sites. The present report forms part of a series of investigations designed to compare the responses of nematode and macrofaunal assemblages to the disposal of dredged material varying both in sedimentological composition and contaminant burden with a view to producing a generic model suitable for application at other locations.

## 10.2 Methods

### *Field sampling*

The Lune Deep disposal site is located at about 35 m depth within the greater Morecambe Bay area in the north-eastern Irish Sea and receives about 0.9 million tonnes of maintenance dredgings annually. At the south-western end of the Lune Deep, peak tidal velocities run parallel to the Deep with flood directed currents of 0.8 knots being slightly stronger than ebb directed currents.

Three replicate Day grab and Multicore samples (cores of 55 mm internal diameter) were collected at 7 stations along a transect through the Lune Deep dredged material disposal site in line with the prevailing tidal currents. Two of the stations were located within the dredged material disposal site (Stations 1 and 2).

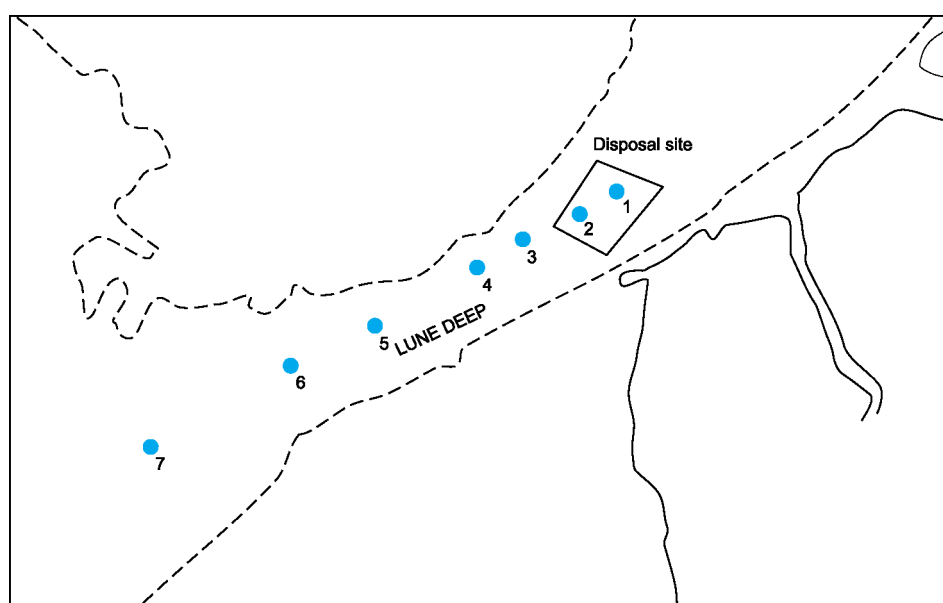
Station 3 and 4 were located peripheral to the site (~0.5 km and 1.5 km respectively) and Stations 5, 6 and 7 were more distant (3.5, 5.6, 8.3 km) (see Figure 29). Both macrofaunal and nematode samples were preserved separately in 4% formalin in sea water with added Rose Bengal (a vital stain). The top 5 cm of sediment from additional cores from 3 separate deployments of the Bowers and Connelly Multiple Mini-Corer, at each station were also retained for analyses of particle size, trace metals and organic content. Due to difficulties sampling the coarse sediments found within the disposal area, only one core was taken from Station 1 and two from Station 2 for trace metals analysis. Sediment samples were stored at -20°C pending analysis.

### *Laboratory analysis*

Core samples for nematode analysis were processed according to the procedures described by Somerfield and Warwick (1996) in their laboratory meiofauna manual. Macrofaunal specimens were identified to species level as far as possible using a range of standard taxonomic keys.

After thawing, sediment samples were wet-weighted on a 63 µm mesh to provide an estimate of the silt and clay fraction. The coarser fraction (sand and gravel) was then oven dried for 12 hrs at 100°C and fractionated over a series of sieves at 0.5 phi intervals.

Organic carbon and nitrogen concentrations were determined in the <63 µm fraction using a Perkin Elmer 240 CHN analyser, after removal of carbonates with 8% HSO<sub>3</sub> (Shaw, 1959). The concentration of a range of trace metals was determined following the procedures outlined in Jones and Laslett (1994).



**Figure 29.** The location of the sampling stations in relation to the Lune Deep dredgings disposal site

### Data analysis

The PRIMER software package (see Clarke and Warwick, 1994) was employed for analysis of species abundance data. Differences in the diversity indices between stations were evaluated through calculation of Least Significant Intervals (LSIs). This method assumes that where means and LSIs do not overlap there is a statistically significant difference at the 95% probability level (Andrews *et al.*, 1980). Square-root transformations were used to reduce the influence of numerically dominant species in the multivariate analyses.

## 10.3 Results

### Benthic fauna

Adverse effects of dredgings disposal on the macrofaunal assemblages were not detectable from estimates of the numbers of species ( $\log 1+S$ ) or species richness ( $d$ ). Indeed, a station within the disposal site (Station 1) was found to be the richest in terms of its species complement (Figure 30). However, both measures of diversity ( $H'$ ) and evenness ( $J$ ) were reduced at Station 1 compared to adjacent stations (Stations 2, 3, and 4).

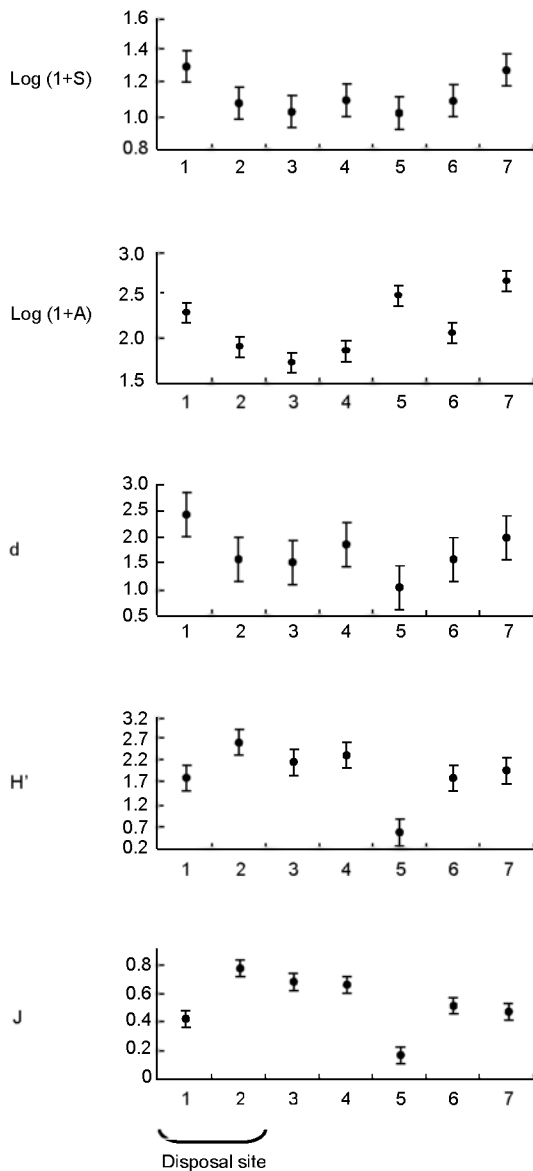


Figure 30. Means and 95% LSD intervals for univariate measures of MACROFAUNA community structure along the LUNE DEEP transect.

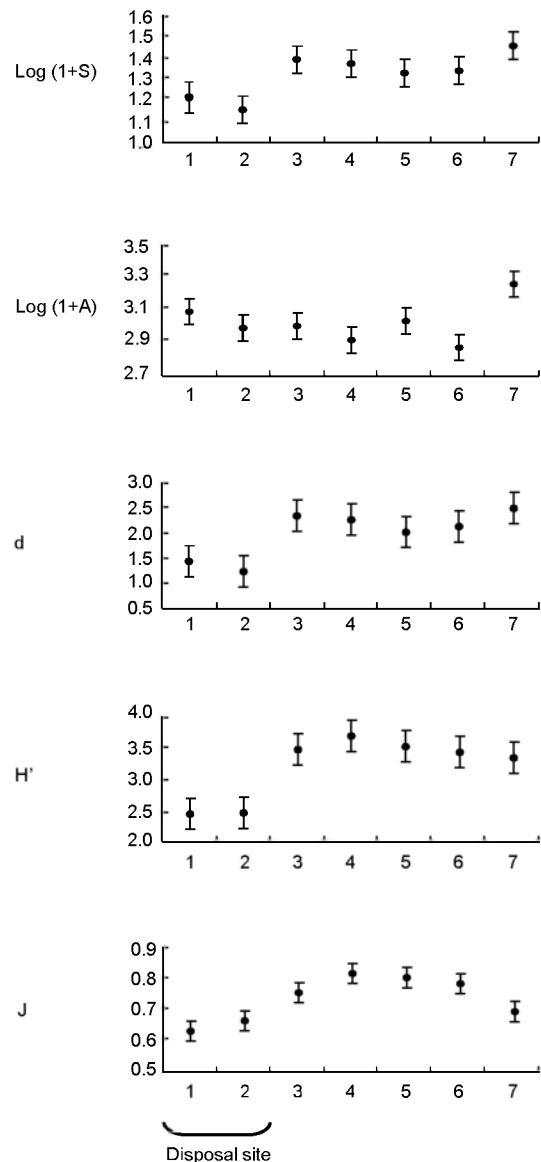


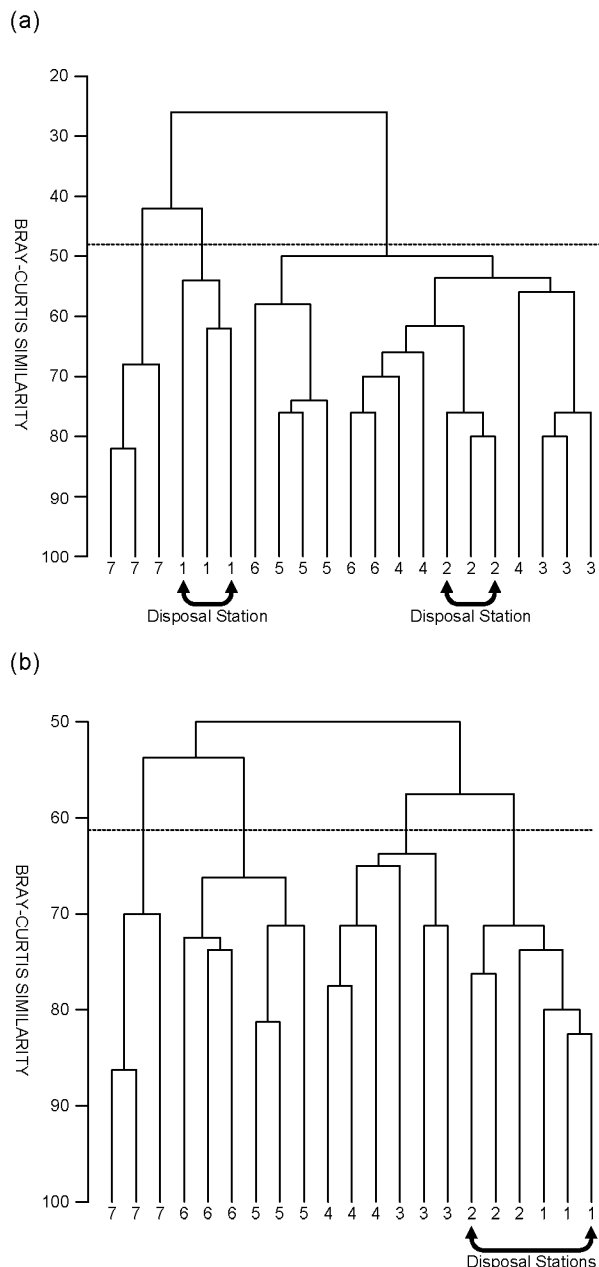
Figure 31. Means and 95% LSD intervals for univariate measures of NEMATODE community structure along the LUNE DEEP transect.



The pattern of disturbance from dredgings disposal was more clearly discernible with the nematode data. Significantly reduced values ( $p < 0.05$ ) of all diversity measures, excepting abundance (Log 1+A), were recorded within the disposal site at Stations 1 and 2 (Figure 31). Effects of disposal also appear to be detectable at Stations (3 and 4) peripheral to the disposal site. This is manifested by slightly higher numbers of species (Log 1+S) and species richness (d) at stations 3 and 4 compared with other stations to the south (Figure 31). Furthermore, both Shannon-Wiener diversity ( $H'$ ) and evenness (J) values show a trend of increasing diversity away from the disposal site, which peaks at station 4. This may indicate an additional “enrichment” effect associated with the disposal of dredgings but the effect is marginal, since there is no evidence for the proliferation of nematode species characteristic of organically enriched situations. Differences in the counts of nematodes (Log 1+A) are less distinct and can be attributed to elevations in abundance of the *Sabatieria pulchra* group and *Daptonema tenuispiculum* within the disposal site, which mask reductions in the abundance of many other taxa.

Cluster analysis of the macrofaunal assemblages show several clusters at the 50% Bray Curtis similarity level (Figure 32). On the left hand side of the dendrogram there is a loose affinity between a station within the disposal site (1) and the most southwestern station (7) along the transect. The overwhelming feature of these two stations is the numerical dominance of the opportunistic polychaete *Lagis koreni* and the bivalve mollusc *Mysella bidentata*. The second major cluster is more complex, and consists of a group of stations (2, 3, 4 and 6) which were typified as having a predominately sandy sediment containing a relatively sparse fauna. The low density of macrofauna was probably due to frequent disturbance of the sandy sediment by tidal currents. Station 5 is distinct from this main group of stations; its separation probably results from the large numbers of *Nucula nitidosa* and corresponds with slightly muddier sediment.

The dendrogram derived from square-root transformed nematode data revealed the presence of several major clusters (Figure 32). The disposal site Stations (1 and 2) formed a distinct grouping that was linked with Stations (3 and 4) adjacent to the site. A further cluster consisted of Stations (5, 6, and 7) located at the southern end of the transect. Thus the arrangement of stations is suggestive of a gradual change in nematode species composition as a function of distance from the disposal operation. Patterns in the distribution of nematode assemblages were therefore consistent with the effects of disposal activity, both at the site itself, and peripherally in line with the tidal axis.



**Figure 32. Cluster analysis of square-root transformed macrofauna (a) and nematode (b) data for stations 1-7 along LUNE DEEP transect.**

#### **Environmental variables**

Most of the sediments sampled consisted of slightly muddy sands, except a station in the disposal site (Station 1) and in the southwest of the survey area (Station 7) where there was an increase in the proportion of fines (Table 17). Core sediments from the disposal site were found to have an underlying anoxic muddy layer, but there was no evidence for the transport of material (in the form of clay lumps or black mud) to stations adjacent to the site. A single core retrieved from Station 1 within the disposal site had slightly enhanced levels of Zn and Hg compared with sediments retrieved from other stations (Table 17).

**Table 17. Mean concentrations (mg kg<sup>-1</sup> dry weight) of trace metals and other environmental variables at Stations 1 to 7 from core samples**

Station	%C	%OrgC	% N	% s/c	% S	% G	Cd	Cr	Cu	Hg	Ni	Pb	Zn	As
1	2.31	1.51	0.12	32.99	59.07	7.95	0.27	58	29	0.64	39	75	170	13
2	1.82	0.96	0.09	12.30	87.69	0.01	0.46	48	18	0.26	21	38	84	12
3	2.03	1.08	0.11	12.46	87.5	0.04	0.26	47	20	0.37	29	46	111	7
4	1.99	0.88	0.09	8.44	91.55	0.01	0.25	39	23	0.27	24	45	75	9
5	2.34	1.38	0.13	14.38	85.11	0.51	0.23	52	27	0.47	30	63	105	12
6	2.25	1.38	0.14	8.8	91.14	0.06	0.28	56	29	0.38	31	61	83	11
7	2.39	1.38	0.13	38.51	61.22	0.27	0.38	56	27	0.47	33	66	115	12

## 10.4 Discussion

In general, the Lune Deep possessed an impoverished macrofaunal community, the composition of which appeared indicative of an area subject to physical disturbance. However, one station within the disposal site had the greatest number of macrofaunal taxa and several possible explanations exist for this. The disposal of dredgings may have increased the range of sediment types within the disposal area leading to the diversification of microhabitats and thereby providing opportunities for colonisation by a wider range of species (MPMMG, 1996). In addition, the disposal of cohesive muddy or coarse sediments in high-energy environments such as the Lune Deep may also confer a degree of temporary stability in the receiving area, and hence increase the potential for colonisation by opportunistic taxa such as *Lagis koreni* and *Mysella bidentata*. It has also been suggested that newly deposited dredged material may provide an enhanced food supply, particularly if the material is organically enriched (Rees *et al.*, 1992; Harvey *et al.*, 1998).

Rhoads *et al.* (1978) discussed the potential benefits of managing dredged material disposal in such a way as to maximise benthic productivity in order to improve local fisheries. Although dredgings disposal appears to have increased the numbers of macrofaunal taxa at one of the stations within the disposal site, there are also marginally elevated levels of some trace metals in sediments at this station. Thus, even if benthic productivity could be improved to the benefit of local fisheries, through effective management of the disposal site, there is also increased potential for the uptake of contaminants by local fish populations. Indeed, during dredging operations sediments can become oxidised (Fernandes *et al.*, 1994) which may lead to the release and subsequent increase in the bioavailability of trace metals in recently deposited sediments (Miller, 1986; Darby *et al.*, 1986), but there is no evidence from this study that this is occurring at the Lune Deep site.

Effects on the macrofaunal community that could be attributed to the disposal of dredged material were localised and only discernible with multivariate species analyses. In contrast, effects of disposal on nematode populations were evident up to several km beyond the disposal site. Dredged material disposed of at the Lune Deep site is only slightly contaminated and therefore it is likely that the most significant impact of dredgings disposal within the disposal site is a result of physical disturbance rather than contaminant effects. The disparity in responses of benthic macro- and meio-fauna to physical disturbance has previously been reported by Warwick *et al.* (1990), where the meiofauna appeared less sensitive than the macrofauna to sediment movement caused by the passage of large cruise liners. This is probably as a result of the greater mobility of meiofaunal organisms, which serves to lessen the impact of sediment instability. Thus an emerging view from such studies is that macrofaunal communities may be more sensitive to the effects of physical disturbance than meiofaunal communities. However, meiofaunal communities may respond with greater sensitivity to subtle impacts, e.g. dispersing material from disposal sites, which alter the sedimentary environment in some way. Thus it appears that meiofauna and macrofauna respond differently to perturbation, and furthermore when examined in parallel they may provide complementary information. Hence, where resources permit it would seem advantageous to use both in marine pollution monitoring programmes.

Evidence for the existence of metals accumulation within sediments sampled from the disposal site was slight. Furthermore, gross effects of dredged material disposal on faunal assemblages were not apparent. This is probably a consequence of the strong tidal currents in the area, which rapidly disperse the dredgings. Overall, these findings point to the continued acceptability of this disposal operation at current levels of input, although far-field effects of disposal are discernible and should be monitored periodically.

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# DISPOSAL AT SEA

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## 11. METAL CONCENTRATIONS IN DREDGED SEDIMENT

### 11.1 Introduction

An important consideration before licensing a deposit to sea is the level of both organic and inorganic contaminants in dredged material. The UK Government is assigned to legislation (the Food and Environment Protection Act Part II (1985)) preventing the disposal of hazardous materials to sea. The main conditions are to prevent the pollution of the sea by substances that are liable to create hazards to human health, to harm living resources and marine life, to damage amenities or to interfere with other legitimate uses of the sea.

In the UK there are approximately 150 applications each year for licences to deposit material to sea. If successful, the licences are generally valid for one year under FEPA Part II (1985) although some longer term licenses have been issued. The total quantity of material licensed for disposal is about 50-80 million tonnes (MAFF, 1991, 1992, 1993, 1994; CEFAS, 1997, 1998). The granting of a licence to dispose of material at designated sites around the UK depends on the nature of the material to be deposited and the concentration of contaminants in dredged spoils.

### 11.2 Sampling dredged material

There are two classifications for dredged material; capital and maintenance. This classification depends on the geological origin, hydrological cycling, sedimentation patterns, contamination and frequency of channel or harbour clearance requirements. Capital dredged material is previously undisturbed sediment (clay, chalk, boulder and rocks) and this is material that is not affected by chemicals from anthropogenic activities. The maintenance dredged material is typically fine-grained silts requiring removal and disposal in approach channels to harbours and docks. These materials are often sinks for pollutants and are influenced by compounds such as trace metals, petroleum hydrocarbons or persistent organochlorine compounds.

Both the physical and chemical impacts are assessed before a licence is approved and the guidelines are set by the Oslo Commission for the disposal of dredged material (Oslo Commission, 1993). A description of the chemical composition and mineralogical composition of sediments are required from the site proposed for

dredging. The Oslo Commission recommends sediment sampling to be conducted to characterise the spatial uniformity of the material in support of the licence application. The concentration of metals in dredged sediment is routinely analysed prior to the approval of the licence.

### 11.3 Methods

Sediment samples are collected using a stainless steel Van veen grab and the surface layer is transferred to hexane rinsed glass jars using a PTFE spoon. If dredging is required at a depth of 2 m or below, samples from cores are required. These are stored at -20°C prior to analysis. Metal compounds are extracted from dredged material by microwave digestion and then analysed by ICP-MS. The suite of eight metals include As, Cd, Cr, Cu, Hg, Ni, Pb, and Zn. The methods employed by CEFAS adequately determine trace quantities of metals in dredged sediment (MAFF, 1994).

### 11.4 Results

A summary of the collected data for 1995 to 1997 is shown in Table 18 and the sampling sites are shown in Figure 33.

Table 18 shows the areas sampled for metals ( $\text{mg kg}^{-1}$  wet weight) each year since 1995 together with the percentage of total solids. The data shown in this table are the mean values of the results of several samples taken at a number of sites for an area.

Figures 34(a-h) are geographical representations of the individual metal concentrations in dredged materials showing the areas sampled for each year.

Table 19 shows the percentage of sites, and the number of sites, within a range of contamination levels for individual metals in dredged materials together with the total number of sites sampled annually since 1995. Table 20 shows the annual mean, minimum, maximum and other summary statistics of metal concentrations between 1995 – 1997.

### 11.5 Discussion

The majority of sites sampled for metal concentrations in dredged materials were within the guidelines set by the OSPAR Commission. A few sites contained levels above these guidelines and these sites were regarded as 'hotspots'.

**Table 18. Summary of metal concentrations in dredged materials sampled between 1995-1997**

Year	Location	n = Number	Concentration (mg kg <sup>-1</sup> wet weight)								Total Solids (%)
			Arsenic	Cadmium	Chromium	Copper	Mercury	Nickel	Lead	Zinc	
1995	River Tyne	4	8.50	0.46	30.25	68.75	0.23	18.00	86.00	192.75	53.83
	Barrow-in-Furness	10	3.94	0.00	9.37	2.02	0.02	5.91	6.73	21.50	77.57
1996	Folkestone	9	8.83	0.24	38.31	42.96	0.47	9.00	67.89	76.00	70.91
	Falmouth	9	39.56	0.38	21.00	153.89	1.00	16.44	65.00	272.33	49.72
	Falmouth	8	18.80	0.17	30.25	92.75	0.48	15.25	42.26	135.00	33.95
	Falmouth	8	44.00	0.49	30.38	220.50	0.75	22.63	109.00	249.88	51.48
	Falmouth	3	27.00	0.46	19.67	157.33	1.10	14.33	99.67	262.67	38.70
	Pencarrow Head	4	N/A	0.63	19.25	31.75	0.07	17.25	24.00	80.50	66.15
	Plymouth Sound	3	N/A	0.60	19.33	116.00	0.37	10.83	107.67	187.00	56.23
	Plymouth Sound	11	N/A	0.21	29.91	92.82	0.51	18.18	97.82	156.18	50.80
	Plymouth Sound	5	29.00	0.26	18.80	68.60	0.76	14.60	69.80	95.40	49.60
	River Tamar	8	N/A	0.61	27.88	70.63	0.27	16.50	62.25	115.88	40.85
	Port Issac		18.00	0.17	21.00	35.00	0.06	13.00	18.00	77.00	43.20
	Teignmouth		17.00	0.17	14.00	3.00	0.00	12.00	18.00	64.00	80.50
	Port Issac	9	19.89	0.16	21.33	47.33	0.06	15.33	24.78	103.00	48.29
	Solent	3	18.67	0.01	24.00	5.23	0.02	12.47	8.80	30.33	76.03
	Wareham	4	5.73	0.23	17.75	12.20	0.10	8.58	20.50	53.75	42.13
	Benbridge	3	11.80	0.07	17.33	16.07	0.29	10.00	36.50	43.67	72.87
	Holes Bay	8	5.46	0.23	14.30	8.56	0.14	7.65	17.14	42.85	60.68
	West Cowes	8	9.12	0.33	26.63	13.64	0.06	10.14	13.79	51.13	44.35
	Solent	4	8.23	0.38	28.75	16.00	0.09	16.50	27.50	71.25	53.20
	Gosport	2	8.60	0.07	24.50	16.00	0.09	9.35	23.50	45.50	38.95
	Littlehampton	3	16.67	0.00	19.00	4.70	0.00	9.63	5.63	23.00	64.83
	Portsmouth Harbour	19	11.20	0.08	24.02	9.32	0.04	13.06	13.86	39.26	62.11
	Langstone Harbour	3	8.13	0.39	29.00	23.33	0.07	12.67	28.00	70.00	43.20
	Lymington	7	8.47	0.09	25.43	32.06	0.10	12.07	15.14	50.71	54.93
	Southampton Water	12	9.39	0.14	32.33	20.38	0.11	16.17	22.30	69.00	48.63
	River Itchen	2	7.65	0.17	25.00	16.00	0.07	12.50	17.00	47.50	44.45
	West Cowes	3	10.23	0.08	24.33	14.33	0.06	12.67	17.00	47.00	49.97
	Bridgwater Bay	3	5.60	0.18	22.33	11.30	0.13	13.33	24.67	68.00	46.27
	Chetney Marshes	3	5.90	0.10	22.67	11.87	0.10	12.73	20.97	46.00	41.97
	River Crouch	1	7.80	0.86	107.00	52.00	0.96	17.00	159.00	180.00	29.40
	River Crouch	1	9.60	0.11	31.00	10.00	0.08	16.00	18.00	48.00	50.20
	River Crouch	2	8.85	0.10	28.00	9.45	0.06	14.50	16.50	44.00	47.90
	Milford Haven Approaches	7	6.20	2.32	18.04	8.41	0.04	11.26	17.34	45.57	61.93
	Pembroke Dock	6	6.97	0.07	26.33	8.17	0.02	19.67	13.73	53.33	70.88
	Walton-on-Naze	4	N/A	0.66	26.75	18.43	0.07	15.25	11.05	49.00	37.48
	Felixstowe	10	9.57	0.07	27.20	9.82	0.04	15.60	17.00	45.30	48.03
	River Orwell	5	N/A	0.07	35.80	17.60	0.11	16.00	19.40	52.60	46.08
	Lowestoft	18	9.18	0.11	28.83	11.13	0.07	11.75	25.89	50.06	49.76
	River Welland	3	3.20	0.80	4.70	1.43	0.00	0.90	4.30	14.00	79.77
	Kings Lynn Dock	6	9.62	0.12	30.00	9.90	0.06	15.47	19.83	61.00	51.17
	River Mersey	10	11.59	0.51	39.50	28.00	0.70	15.10	53.20	187.50	56.92
	River Mersey	1	11.00	0.07	5.00	0.73	0.00	2.20	6.40	35.00	78.60
Liverpool Bay (Bootle)	4	10.50	0.39	44.25	30.75	0.87	17.25	75.25	162.50	46.28	
River Humber	6	13.83	0.12	38.67	17.00	0.09	18.50	40.00	98.33	31.08	
River Humber	22	10.00	0.20	49.23	17.64	0.04	30.95	68.35	68.82	80.13	
Off Lune/Wyre	5	6.12	0.12	15.12	4.52	0.08	8.08	13.20	40.40	75.30	
Heysham	8	6.44	0.16	28.38	12.98	0.26	14.38	20.13	69.25	58.78	
Barrow -in- Furness	3	17.10	0.56	42.00	87.00	2.44	23.00	224.67	370.00	69.17	
St Bees Head	12	17.36	1.76	65.98	56.00	0.34	14.93	109.82	105.83	68.10	
Solway (Parton)	20	17.94	2.13	77.15	31.74	0.23	21.45	51.25	117.45	68.20	
River Tees	8	32.94	3.43	400.75	236.88	4.09	14.75	321.00	680.88	45.76	
River Tees	7	9.67	1.05	146.00	61.14	1.22	18.71	193.86	213.29	42.53	
Solway (Wokington)	7	9.19	0.45	35.86	13.57	0.10	12.16	39.86	82.71	57.31	
River Wear	7	10.57	0.48	32.00	27.14	0.17	17.57	152.00	153.43	55.79	
River Tyne	2	8.10	2.30	33.50	42.00	0.27	20.00	107.00	409.00	41.00	
River Tyne	8	12.63	0.27	36.13	101.13	0.24	21.25	71.88	204.63	51.69	
River Tyne	6	10.93	1.10	44.50	53.50	0.34	21.17	104.67	261.33	45.70	
River Tyne	2	10.00	0.62	36.50	40.50	0.23	20.00	57.00	144.00	45.40	
South Shields	3	10.37	2.10	40.33	66.67	0.73	17.67	223.00	414.00	47.60	
Blyth	6	11.78	0.17	32.67	24.17	0.11	25.33	31.83	72.00	56.73	
Alnmouth Bay	3	7.33	0.09	29.00	16.00	0.06	15.33	25.67	50.33	38.80	

**Table 18. continued**

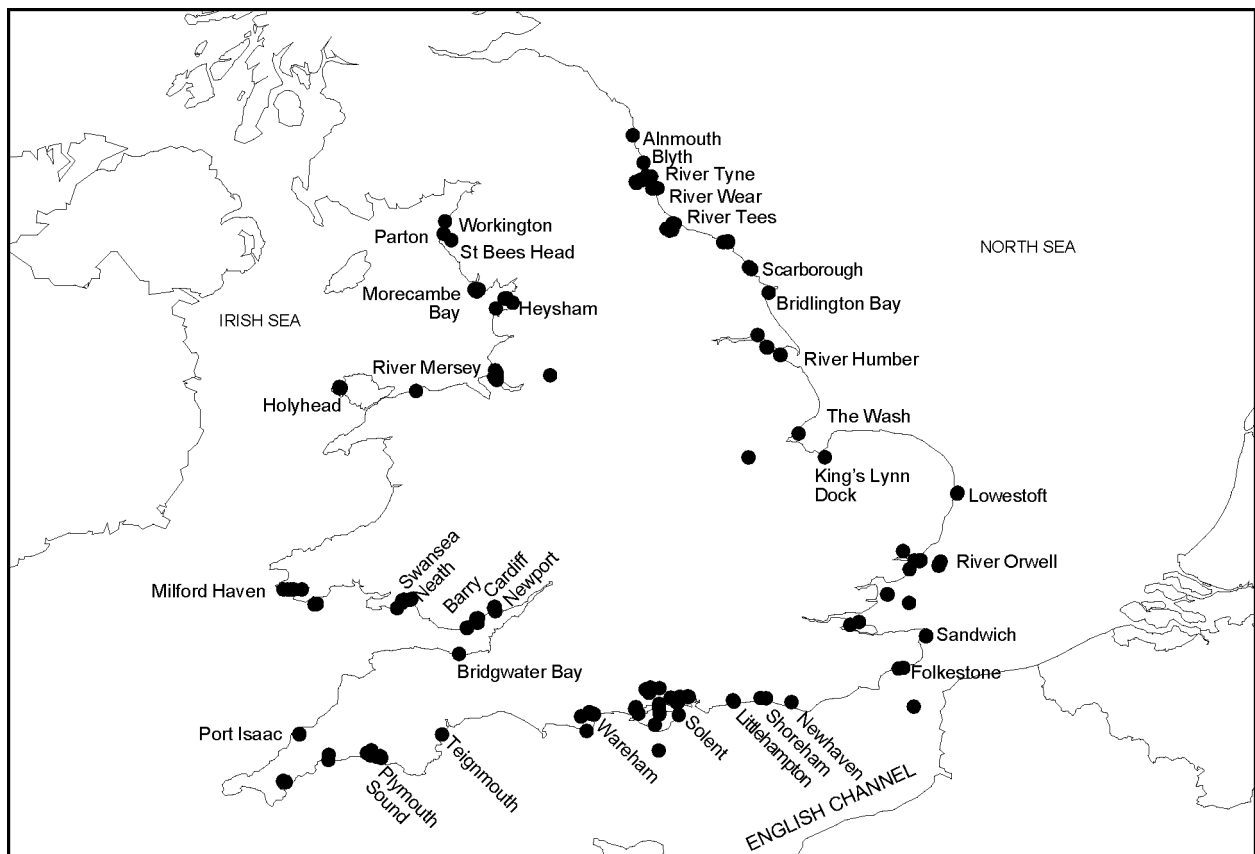
Year	Location	n = Number	Concentration (mg kg <sup>-1</sup> wet weight)							Total Solids (%)	
			Arsenic	Cadmium	Chromium	Copper	Mercury	Nickel	Lead		Zinc
1997	Swanage	3	4.63	0.13	14.03	8.63	0.05	7.00	14.30	39.33	28.80
	Plymouth Sound	5	16.20	0.59	21.60	41.00	0.27	14.52	46.20	85.20	47.44
	River Fowey	6	21.17	0.14	17.83	50.67	0.08	5.73	30.67	99.17	47.02
	River Tamar	20	27.51	0.32	31.34	93.25	0.27	20.69	72.76	134.40	41.60
	Plymouth (St Germans)	1	5.50	0.10	21.00	8.10	0.02	10.00	12.00	30.00	83.10
	Off Isle of Wight	3	6.07	0.59	24.00	36.33	0.22	9.83	27.33	69.67	40.23
	River Tamar	7	38.33	0.70	20.31	89.70	0.27	15.14	59.07	117.39	50.59
	Solent	2	14.00	0.22	24.00	29.50	0.12	13.50	36.50	83.00	45.30
	Solent	10	8.20	0.37	29.70	21.37	0.15	20.30	8.28	42.40	60.72
	Holes Bay	22	5.57	0.47	19.94	13.29	0.15	8.19	19.49	49.73	46.28
	West Cowes	11	9.25	0.11	27.18	16.35	0.05	13.77	12.56	46.45	39.51
	Solent	9	14.03	0.15	23.89	62.27	0.31	11.82	36.64	93.67	43.48
	Solent (Gosport)	12	7.02	0.17	19.15	29.12	0.08	7.13	73.28	121.48	47.48
	Newhaven	3	5.63	0.41	17.67	12.87	0.04	11.47	93.67	44.33	53.90
	Shoreham	4	19.75	0.12	51.00	34.45	0.15	35.23	24.95	90.50	41.60
	Solent	3	24.00	0.18	16.00	7.33	0.01	11.00	11.03	41.33	43.80
	Solent (Langstone Harbour)	5	6.92	0.36	17.00	23.00	0.07	10.88	18.80	55.00	45.70
	Southampton Water	36	7.46	0.18	28.92	18.42	0.16	11.40	26.42	64.42	47.17
	River Itchen	4	6.25	0.54	30.00	24.50	0.13	14.50	57.50	66.75	35.75
	Folkstone	4	5.55	0.63	18.00	7.05	0.02	8.60	22.18	43.75	51.45
	Sandwich Bay	6	8.22	0.14	23.17	9.52	0.05	11.88	17.17	34.17	60.20
	Barry	5	6.62	0.15	25.80	14.40	0.18	14.40	35.60	98.80	47.02
	Cardiff	3	7.07	0.17	33.33	15.33	0.14	18.33	38.00	109.00	40.00
	Medway Approach	8	6.48	0.23	20.50	1.13	0.05	10.60	11.54	34.00	62.86
	Cardiff	17	11.81	0.78	27.53	37.06	0.21	17.18	58.82	132.71	46.13
	Swansea Bay	9	5.27	0.83	26.44	11.49	0.11	13.00	29.00	84.33	32.81
	Newport	7	6.16	0.22	28.14	15.14	0.17	15.69	38.00	107.43	39.41
	Caldey Sound	9	9.89	0.29	12.24	16.32	0.04	12.59	18.19	78.44	72.49
	Swansea	6	12.68	2.54	28.50	50.83	0.50	21.50	148.17	450.33	52.53
	Neath	5	8.12	0.24	4.46	0.45	0.02	4.90	8.46	36.00	83.92
	Felixstowe	6	8.45	0.23	40.77	32.45	0.01	19.00	6.83	57.12	75.97
	Harwich	24	9.92	0.86	36.86	14.57	0.02	20.18	6.98	35.14	76.20
	Felixstowe	1	6.20	0.13	64.00	14.00	0.06	37.00	14.00	74.00	59.70
	Wash	5	9.62	0.56	32.20	10.92	0.05	16.96	23.00	59.40	65.32
	Conwy	6	4.18	0.13	15.67	5.87	0.08	8.70	9.35	54.33	31.55
	Holyhead	5	5.00	0.39	23.80	13.62	0.09	14.20	24.00	57.60	56.94
	River Mersey	4	6.73	0.27	26.25	19.75	0.42	10.63	38.25	135.25	43.40
	Liverpool Bay (Wallasey)	3	10.20	0.37	48.00	31.00	0.66	18.67	51.00	158.33	45.47
	River Mersey	1	5.20	0.40	5.00	1.20	0.01	2.90	6.70	29.00	79.90
	Liverpool Bay (Bootle)	6	8.72	0.34	37.50	33.50	0.56	15.00	60.83	138.33	41.73
	River Humber	6	12.67	0.16	36.50	16.67	0.09	17.17	39.00	83.67	36.25
	River Humber (Immingham)	14	21.88	0.38	59.21	27.71	0.16	28.64	54.93	134.93	59.26
	Heysham	8	4.90	0.28	22.38	6.87	0.14	9.53	16.75	48.75	71.71
	Bridlington Bay	6	7.42	0.14	31.17	24.83	0.05	18.00	25.00	69.67	65.38
	Barrow-in-Furness	5	5.32	0.12	15.08	8.06	0.03	10.46	17.48	38.80	78.36
	Scarborough	14	10.42	0.10	25.99	16.49	0.13	11.61	30.40	60.29	69.74
	Robin Hood's Bay	5	7.84	0.15	22.40	12.90	0.07	16.20	26.20	61.00	58.60
	River Tees	19	14.39	1.30	158.21	100.05	1.54	18.89	215.58	334.16	44.85
	River Tees	14	13.59	0.27	66.57	42.21	0.34	22.93	67.93	118.14	52.93
	River Tyne	1	6.90	1.30	23.00	38.00	0.29	14.00	120.00	282.00	42.50
	River Tyne	4	9.40	0.78	35.50	75.50	0.24	18.50	117.50	333.00	42.75
	River Tyne	2	7.30	0.75	20.50	32.50	0.26	12.00	82.50	173.50	41.65
	River Tyne	2	7.70	0.95	24.00	44.50	0.31	13.50	95.00	210.00	48.45
	River Tyne	1	7.50	0.51	20.00	26.00	0.17	12.00	72.00	136.00	50.10
	River Tyne	3	7.00	0.76	19.67	26.33	0.20	10.97	93.67	195.67	43.27
	River Tyne	15	14.30	0.68	52.80	314.07	0.38	25.35	115.67	434.40	53.98

**Arsenic**

The sites sampled for arsenic were generally found to be between 0–50 mg kg<sup>-1</sup>. A total of six sites were above 50 mg kg<sup>-1</sup> and these included; River Tees (2 sites), Falmouth and River Tamar (3 sites). The sites at Falmouth and River Tamar have high natural background levels of arsenic.

**Cadmium**

The majority (96%) of sites sampled for cadmium contained concentrations in the range 0–2.5 mg kg<sup>-1</sup>. There were 29 sites with cadmium concentrations > 2.5mg kg<sup>-1</sup>. These sites included; NE Irish Sea (3 sites), River Tees (11 sites), Milford Haven (2 sites), Solway (6 sites), River Tamar, Harwich (4 sites) and Swansea (3 sites).



**Figure 33. Location of sampling sites measured for metals in dredged material in the UK, 1995-1997**

### **Chromium**

The concentrations of chromium were mostly in the range 0–200 mg kg<sup>-1</sup> with a total of 12 sites (or 2% of sites) > 200 mg kg<sup>-1</sup>. In 1996, 8 sites in the River Tees were found to have high chromium levels and in 1997, 4 sites in the River Tees had concentrations >200 mg kg<sup>-1</sup>.

### **Copper**

The majority of sites measured for copper were found to be in the range 0 – 200 mg kg<sup>-1</sup>. There were 29 sites (or 4% of sites) with levels >200 mg kg<sup>-1</sup>. In 1996, sites with high copper concentrations included; NE Irish Sea, River Tees (5 sites), River Tyne, Falmouth (8 sites) and Plymouth Sound. In 1997, sites with copper concentrations >200 mg kg<sup>-1</sup> included; River Tyne (10 sites), River Tamar, River Tees, Gosport and Cardiff.

### **Mercury**

A total of 97% of sites sampled for mercury fell between 0–1.5 mg kg<sup>-1</sup>, with 24 sites > 1.5 mg kg<sup>-1</sup>. In 1996, NE Irish Sea, River Tees (10 sites), Morecambe Bay (2 sites), River Mersey and Plymouth Sound had concentrations of mercury >1.5 mg kg<sup>-1</sup>. In 1997, Southampton Water and River Tees (9 sites) had high mercury levels in dredged spoils.

### **Nickel**

The concentrations of nickel in dredged spoils were within the agreed OSPAR guidelines with only one site exceeding >100 mg kg<sup>-1</sup> in 1997. This site was located in the River Tamar.

### **Lead**

The concentrations of lead in dredged spoils were mostly in the range 0 – 250 mg kg<sup>-1</sup>. There were 21 sites with lead concentrations >250 mg kg<sup>-1</sup> and these included; NE Irish Sea, River Tees (7 sites), Morecambe Bay, River Wear and River Humber in 1996, and sites in Southampton Water, River Tees (7 sites), Swansea and Gosport in 1997. The highest level was measured in the River Humber.

### **Zinc**

A total of 96% of all sites sampled for zinc fell within the range 0 – 400 mg kg<sup>-1</sup>. There were 31 sites with zinc concentrations > 400 mg kg<sup>-1</sup> including 4 sites with concentrations >1,000 mg kg<sup>-1</sup>. Sites with high zinc levels included; River Tees (6 sites), Morecambe Bay and River Tyne (2 sites) in 1996, and Southampton Water, River Tyne (5 sites), River Tees (6 sites), River Tamar, Swansea (2 sites), Gosport and Cardiff in 1997. The highest levels of zinc were found in the River Tees in 1996, and the River Tyne and Swansea in 1997.

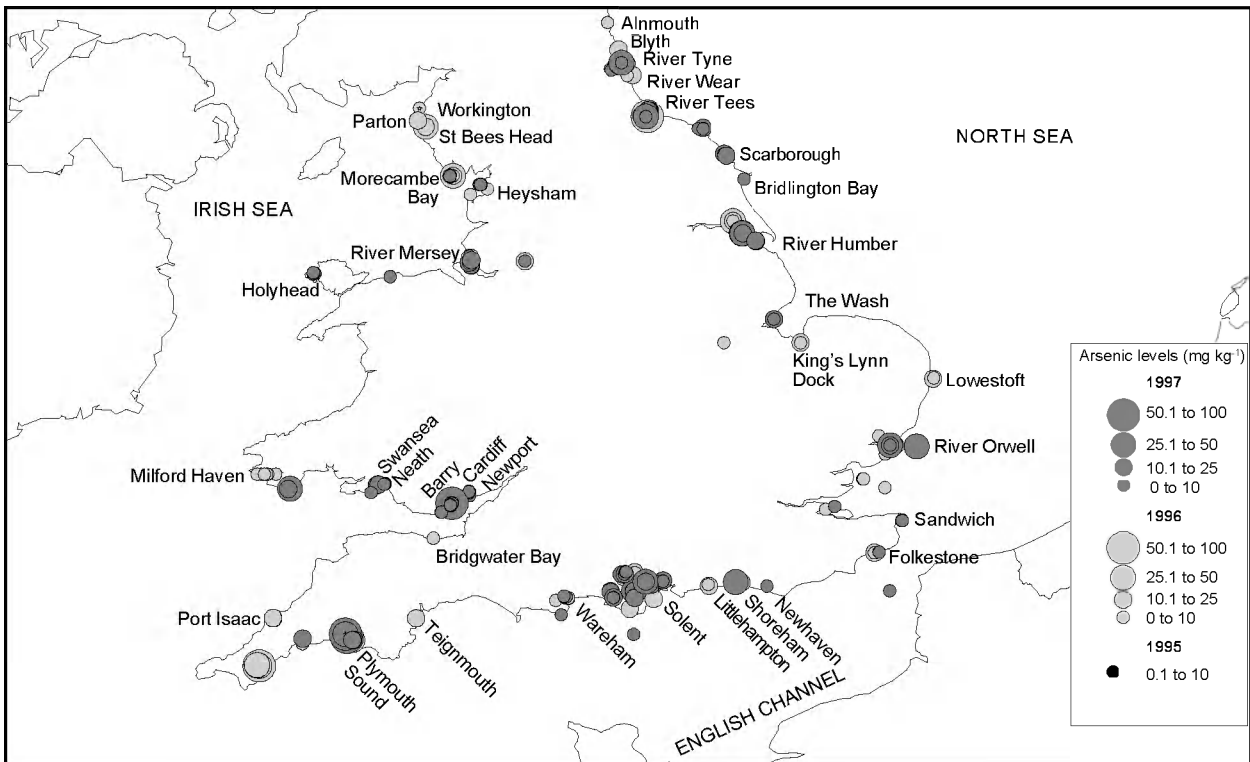


Figure 34(a). Arsenic levels in dredged material licensed for sea disposal in the UK, 1995-1997

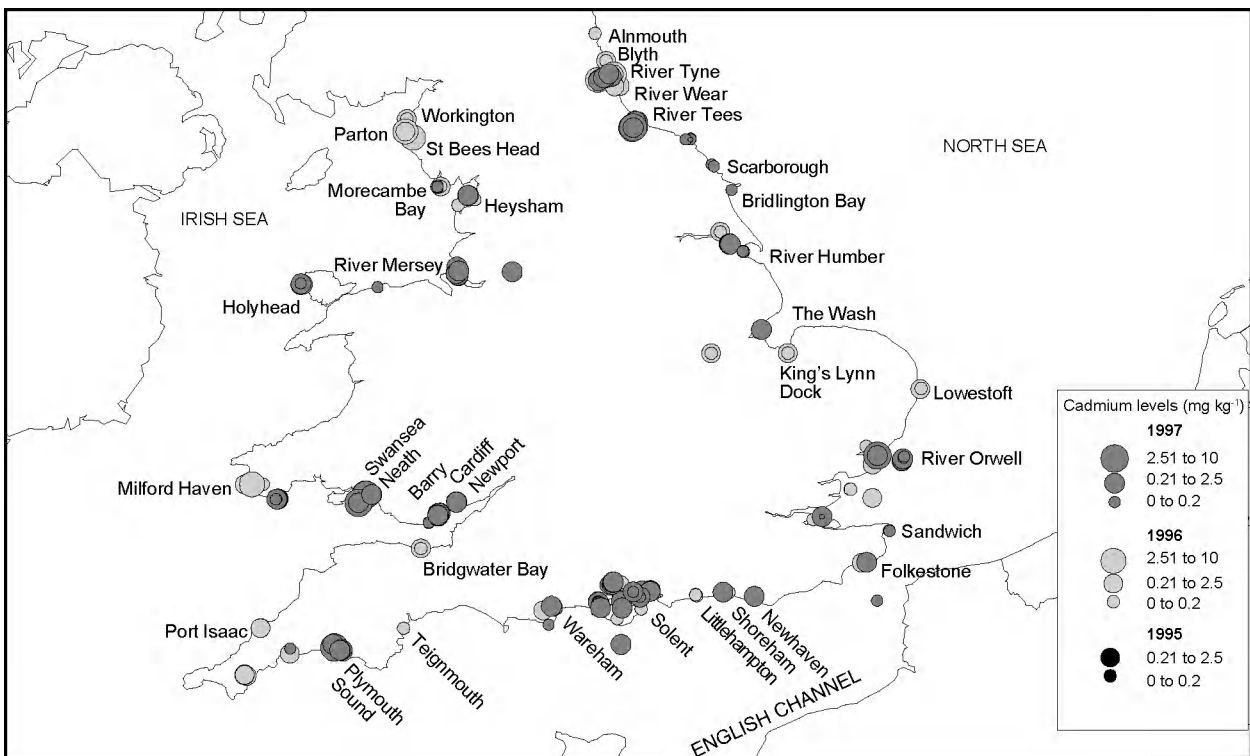


Figure 34(b). Cadmium levels in dredged material licensed for sea disposal in the UK, 1995-1997



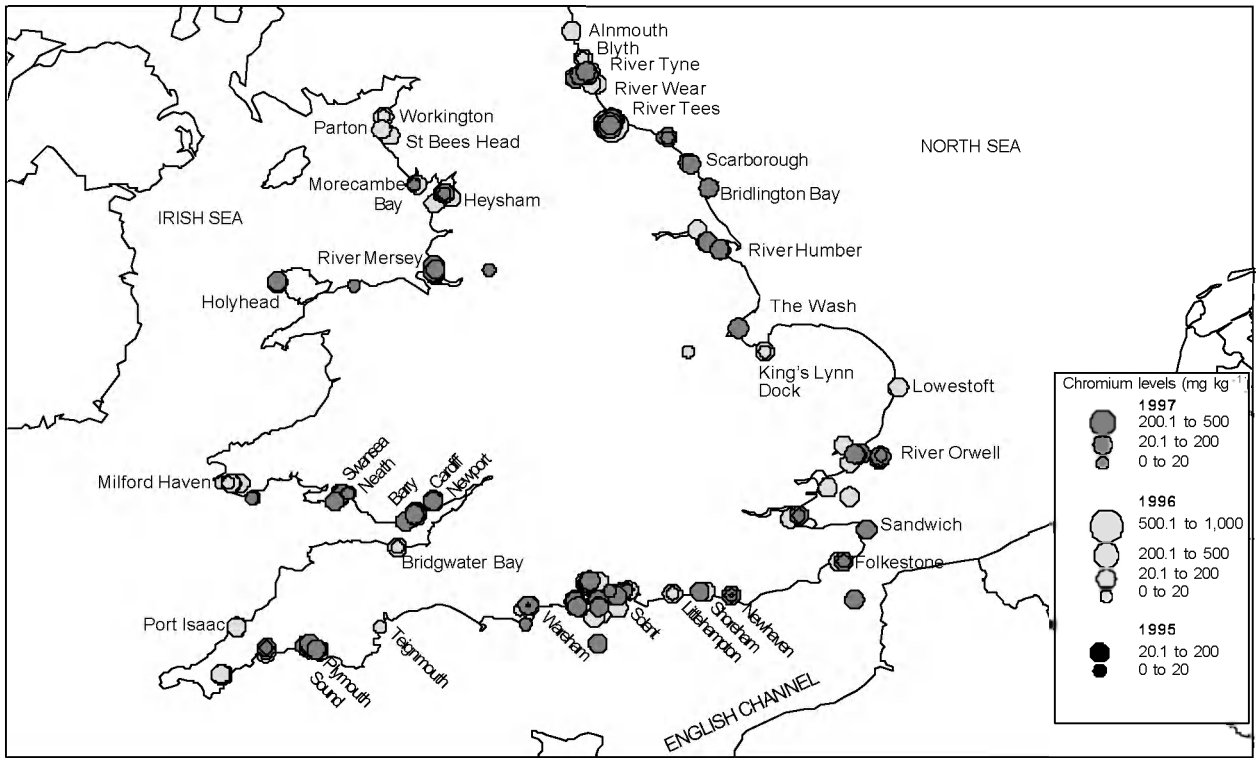


Figure 34(c). Chromium levels in dredged material licensed for sea disposal in the UK, 1995-1997

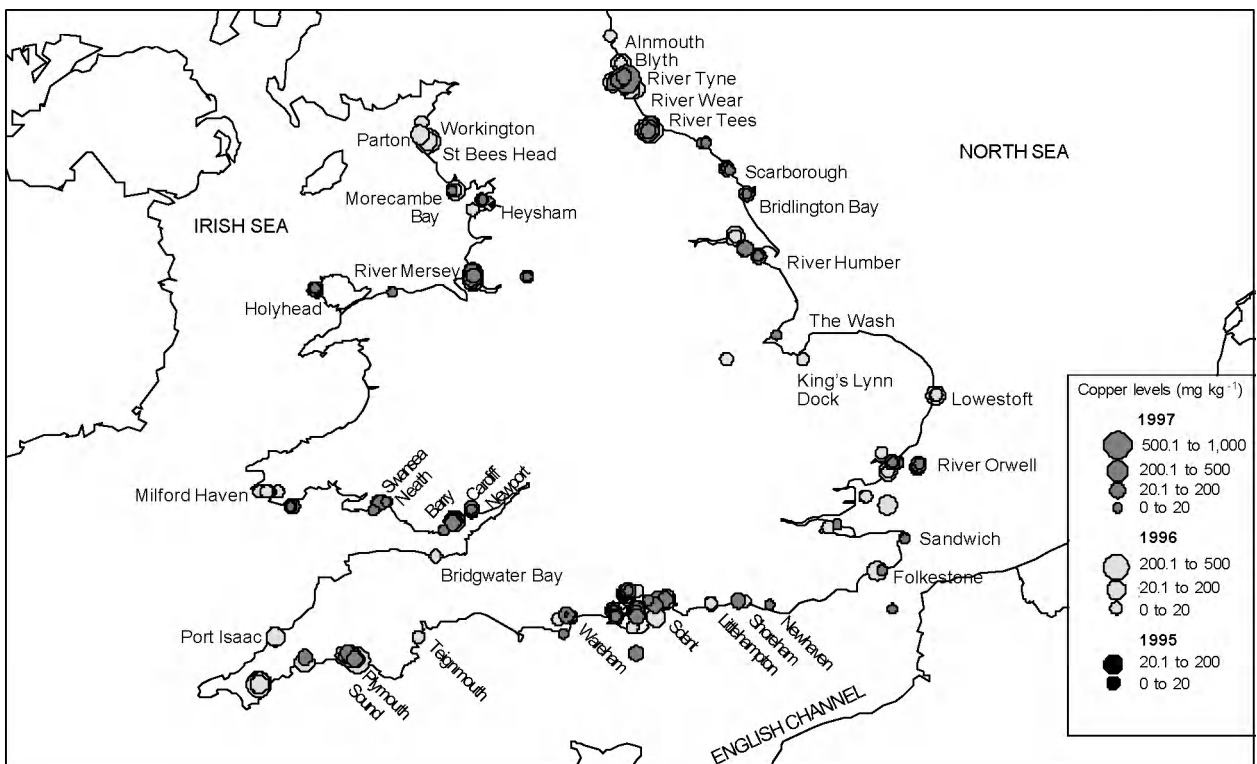


Figure 34(d). Copper levels in dredged material licensed for sea disposal in the UK, 1995-1997

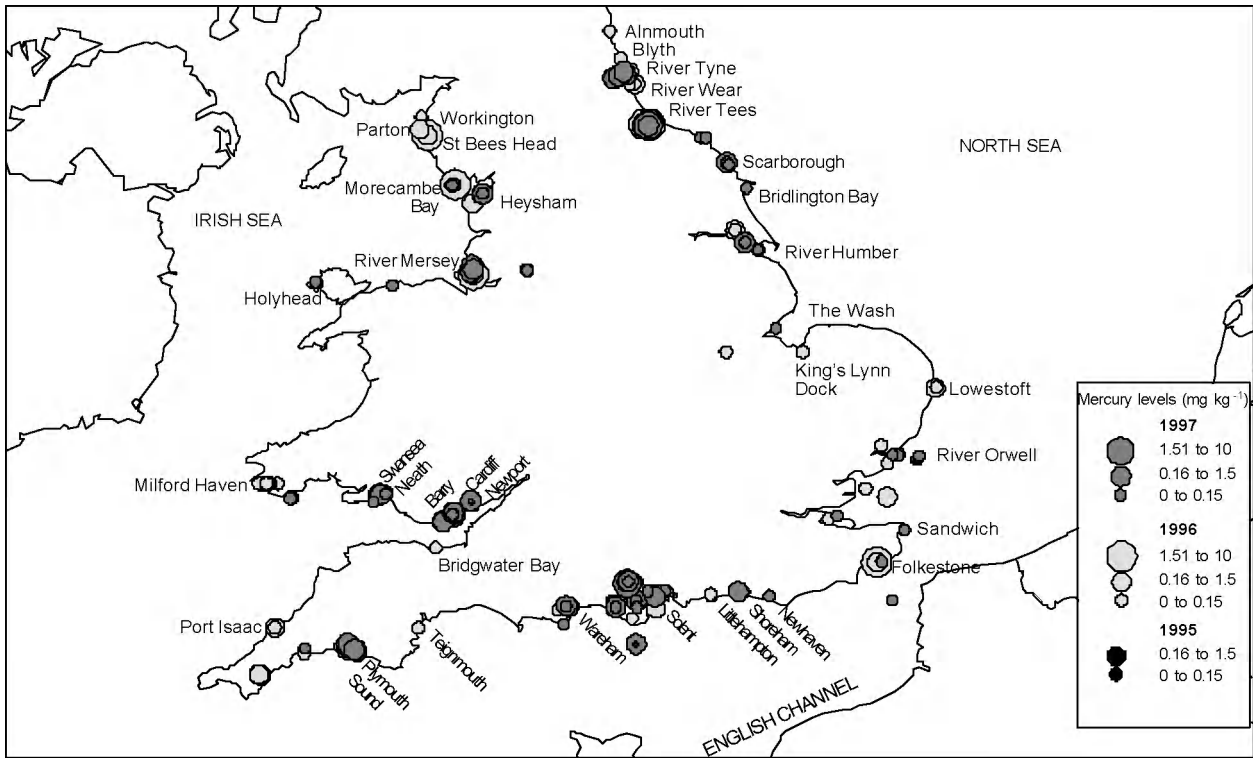


Figure 34(e). Mercury levels in dredged material licensed for sea disposal in the UK, 1995-1997

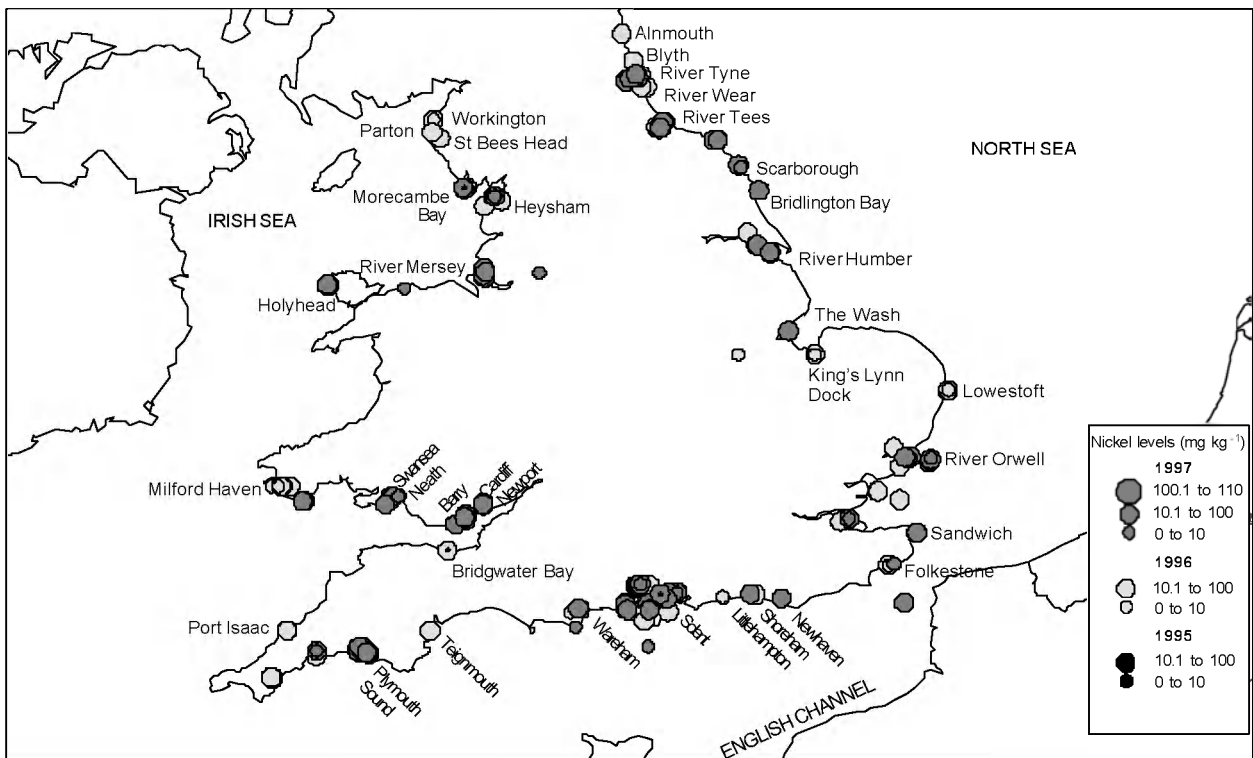


Figure 34(f). Nickel levels in dredged material licensed for sea disposal in the UK, 1995-1997

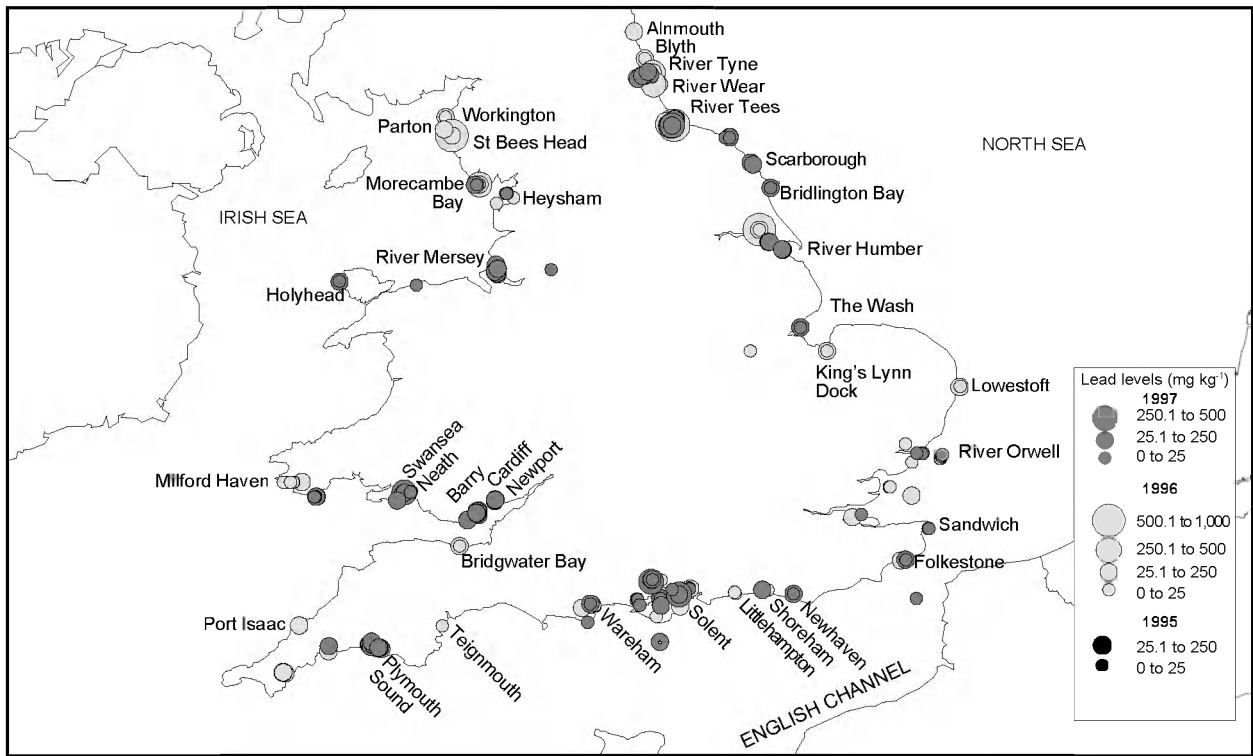


Figure 34(g). Lead levels in dredged material licensed for sea disposal in the UK, 1995-1997

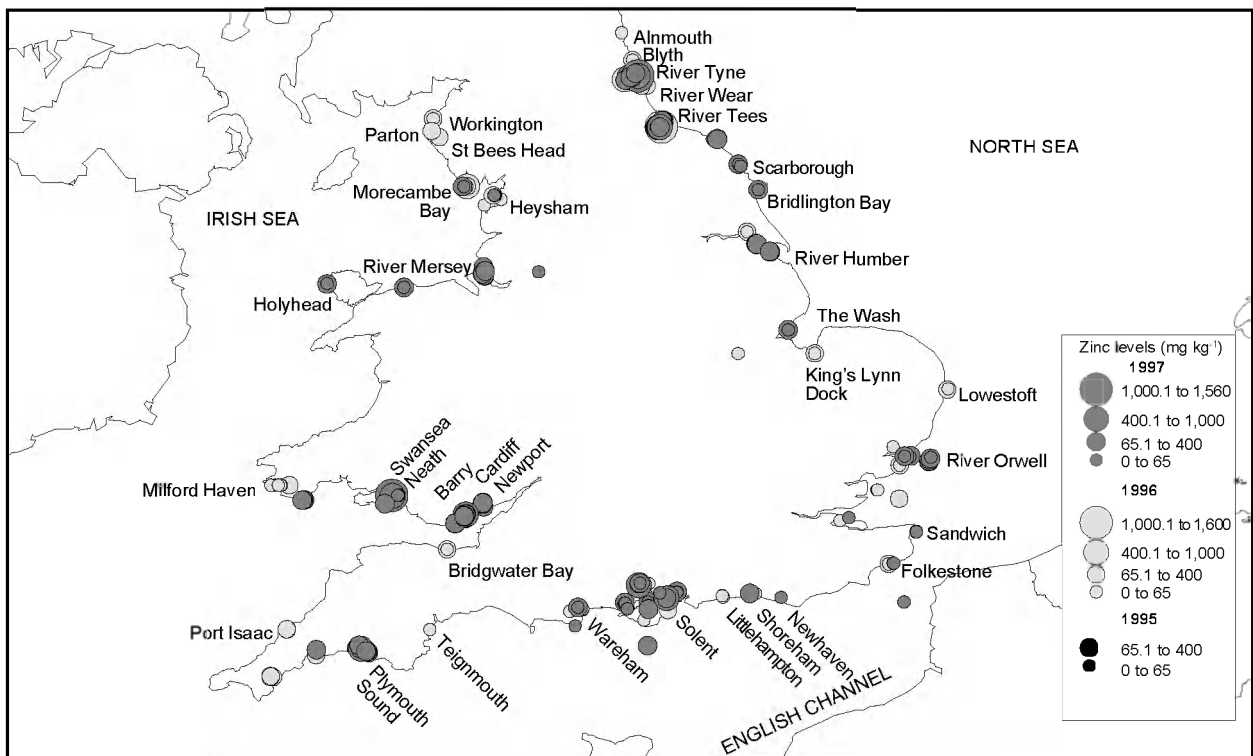


Figure 34(h). Zinc levels in dredged material licensed for sea disposal in the UK, 1995-1997

**Table 19. Metals in dredged material (mg kg<sup>-1</sup> wet weight) between 1995-1997**

Metal	Level (mg kg <sup>-1</sup> )	Year			Total number of sites
		1995	1996	1997	
Arsenic	0 – 10	100 % (14)	60 % (220)	67 % (275)	793
	10.1 – 25	0 %	32 % (117)	27 % (110)	
	25.1 – 50	0 %	7 % (27)	5 % (24)	
	> 50	0 %	1 % (3)	1 % (3)	
Cadmium	0 – 0.2	79 % (11)	47 % (175)	46 % (193)	804
	0.21 – 2.5	21 % (3)	48 % (180)	51 % (213)	
	2.51 - 10	0 %	5 % (18)	3 % (11)	
Chromium	0 – 20	71 % (10)	17 % (66)	26 % (106)	795
	20.1 – 200	29 % (4)	80 % (297)	73 % (300)	
	200.1 – 500	0 %	2 % (6)	1 % (4)	
	> 500	0 %	1 % (2)	0 %	
Copper	0 – 20	71 % (10)	53 % (198)	57 % (239)	814
	20.1 – 200	29 % (4)	43 % (163)	40 % (171)	
	200.1 – 500	0 %	4 % (16)	2 % (10)	
	> 500	0 %	0 %	1 % (3)	
Mercury	0 – 0.15	71 % (10)	58 % (214)	61 % (253)	796
	0.16 – 1.5	29 % (4)	38 % (139)	37 % (152)	
	1.51 - 10	0 %	4 % (14)	2 % (10)	
Nickel	0 – 10	71 % (10)	14 % (52)	26 % (107)	784
	10.1 – 100	29 % (4)	86 % (308)	73 % (302)	
	100.1 - 110	0 %	0 %	1 % (1)	
Lead	0 – 25	71 % (10)	47 % (176)	47 % (195)	806
	25.1 – 250	29 % (4)	50 % (185)	51 % (215)	
	250.1 - 500	0 %	2 % (7)	2 % (10)	
	> 500	0 %	1 % (4)	0 %	
Zinc	0 – 65	71 % (10)	46 % (171)	45 % (192)	810
	65.1 – 400	29 % (4)	51 % (192)	50 % (210)	
	400.1 – 1,000	0 %	2 % (9)	4 % (18)	
	> 1,000	0 %	1 % (2)	1 % (2)	

*N.B. Percentage of sites at a particular concentration range with number of sites in brackets*

**Table 20. Annual summary statistics of metal concentrations measured in dredged materials between 1995-1997**

Year		Arsenic	Cadmium	Chromium	Copper	Mercury	Nickel	Lead	Zinc
1995	Min	2.7	0.15	4.5	0.54	0.07	2.9	2.6	10
	Max	9	0.79	31	121	0.25	20	107	240
	Mean	5.24	0.46	15.51	21.08	0.18	9.36	29.38	70.43
	Stdev	2.25	0.31	10.66	35.51	0.07	6.14	39.12	85.71
	Skew	0.81	0.81	0.59	2.02	-0.90	0.69	1.35	1.37
	N	14							
1996	Min	1.4	0.02	1.2	0.25	0.01	0.34	1.1	2.3
	Max	79	8.4	847	484	6.1	87	957	1591
	Mean	13.00	0.60	43.28	43.58	0.41	16.14	57.45	117.36
	Stdev	9.72	3.54	69.59	0.80	7.23	97.49	136.90	
	Skew	2.77	3.54	7.47	4.50	2.79	5.82	5.12	
	N	336							
1997	Min	0.32	0.02	2.20	0.93	0.01	0.48	0.15	0.76
	Max	92	6	370	866	3	102	467	1370
	Mean	11.14	0.53	35.55	42.15	0.26	15.56	48.91	116.26
	Stdev	9.62	0.77	38.61	80.72	0.42	9.14	62.29	116.26
	Skew	3.88	3.69	5.02	6.48	4.27	3.35	3.09	4.47
	N	432							

## 11.6 Conclusion

The analysis of dredged materials for metals has proven that the majority of sites in the UK are within the guidelines set by OSPAR. A limited number of sites have exceeded these levels and such cases are considered carefully before a licence is granted. The majority of sites with high concentrations of metals are often located in or adjacent to industrial areas which may have influenced contaminant levels locally. Some areas, such as parts of Cornwall, have natural high background levels of metals.

The data provide a record of changes in metal concentrations over time and provides evidence that levels in dredged materials are within an acceptable level for sea disposal. The analysis of metals will routinely be employed in current and future assessments for the licensing of dredged materials to sea.

## 12. TBT CONCENTRATIONS IN DREDGED SEDIMENT

### 12.1 Introduction

When considering the presence of chemical compounds in dredged material, CEFAS follows the guidelines set out by the Oslo Commission for the management of dredged sediment (Oslo Commission, 1993). These guidelines recommend the methodology for sampling and lists the contaminants to be measured in the sediment prior to dredging and sea disposal.

Organotins, particularly tributyltin (TBT) are significant environmental contaminants and are routinely measured in dredged material before issuing a licence to dispose to sea.

### 12.2 Sampling of dredged material for TBT

Oslo Commission Guidelines suggest that each dredged area is sampled every 3 to 5 years or on renewal of an application depending on known concentration levels. Sampling is sometimes requested from the whole of the area to be dredged to ensure that samples are representative (CEFAS, 1997). More frequently, sample locations are selected by CEFAS to reflect areas where sediments are fine enough to retain contaminants. The sampling sites examined are not typical of background UK levels of contaminants but represent areas that are likely to have point source inputs, for example, marinas, docks or harbours.

### 12.3 Methods

Sediment samples are collected using a Van veen grab and the surface layer is transferred to plastic bags.

These are stored at  $-20^{\circ}\text{C}$  prior to analysis. Since TBT is highly toxic to aquatic organisms, methods of analysis must be adequate for the determination of trace quantities (nanograms per gram) of analyte.

Organotin compounds are extracted from dredged material by sodium hydroxide and methanol, converted to hydrides and partitioned into hexane. The derivatives are then analysed by gas chromatography with flame photometric detection (Waldock *et al.*, 1989). The detection limit for the method is  $0.005\ \mu\text{g g}^{-1}$  for TBT,  $0.010\ \mu\text{g g}^{-1}$  for DBT and  $0.02\ \mu\text{g g}^{-1}$  for MBT.

### 12.4 Results

A summary of the data collected from 1992 to 1997 is shown in Table 21 and Figure 35. Table 21 lists the areas sampled since 1992 and the number of samples taken in each area. The data shown in this table are the mean values of the results of several samples taken at a different number of sites for each area. The values are summarised in Table 22. Figure 35 is a geographical representation of the data showing the areas sampled for each year.

Between 1992 and 1997, a total of 1075 sites were sampled. Of these, 46% (492 sites) contained TBT levels in dredged material below  $0.1\ \text{mg kg}^{-1}$ , 41% (439 sites) fell between  $0.1$  and  $1.0\ \text{mg kg}^{-1}$  and in 13% (144 sites) TBT was above  $1\ \text{mg kg}^{-1}$ .

### 12.5 Discussion

Inputs of organotins to the aquatic environment arise from the use of triorganotins as biocides and diorganotins as catalysts, timber preservatives and stabilisers in the plastics industry. Tributyltin (TBT) is widely used as the biocidal component in boat antifouling preparations on vessels over 25 m. The use of TBT in antifouling preparations on vessels less than 25 m was banned in 1987. TBT in antifouling paints has been widely used throughout Europe and TBT is often detected in environmental samples close to harbours, docks and marinas.

The assessment of TBT concentrations in dredged material follows guidelines set out by OSPAR. The assessment considers the nature of the material, the quantity to be disposed to sea, and other physical and chemical analyses. The characteristics of the disposal site are also considered before a licence is approved.

Using the guidelines above, the majority of dredged spoil analysed for TBT, shown in Table 21, are within the levels acceptable for disposal to sea. However, because routine dredging is necessary in ports, harbours and dockyards, some sites assessed between 1992-1997 were considered to be contaminated with TBT to an unacceptable degree. For example, sites within

**Table 21. Summary of mean TBT concentrations in dredged sediment collected between 1992-1997**

Year	Latitude	Longitude	Location	N = number	Concentration TBT (mg kg <sup>-1</sup> wet weight)
<b>1992</b>	50° 45.16' N	1° 50.00' W	Dorset	4	0.54
	55° 0.00' N	1° 26.60' W	River Tyne	3	3.02
<b>1993</b>	50° 10.00' N	5° 4.00' W	Falmouth	1	34.88
	50° 20.50' N	4° 42.20' W	St. Austell Bay	1	0.08
	50° 21.50' N	4° 7.50' W	Plymouth Sound	4	0.07
	50° 43.00' N	1° 30.00' W	Solent	3	0.22
	50° 45.00' N	1° 31.50' W	West Cowes	2	0.26
	50° 45.74' N	1° 17.58' W	Solent	3	0.05
	50° 46.00' N	2° 0.00' W	Dorset	3	0.74
	50° 47.80' N	1° 7.00' W	Solent (Gosport)	1	0.33
	50° 48.45' N	0° 6.30' W	South Coast	2	0.01
	50° 50.92' N	1° 10.53' W	Solent	1	0.38
	50° 52.06' N	1° 23.09' W	Solent (Lymington)	7	0.17
	50° 53.67' N	1° 23.40' W	Southampton Water	8	0.55
	50° 54.80' N	1° 22.80' W	River Itchen	3	1.07
	51° 7.10' N	1° 19.70' W	Dover	3	0.02
	51° 19.75' N	1° 25.45' E	Sandwich	2	0.05
	51° 23.60' N	3° 15.50' W	Barry	5	0.03
	51° 41.90' N	4° 55.00' W	Inner Milford Haven	1	0.02
	51° 59.71' N	0° 16.42' E	Thames	8	0.06
	52° 0.36' N	1° 11.80' W	Southampton	3	0.10
	52° 1.92' N	1° 9.22' E	River Orwell	13	11.82
	52° 28.00' N	1° 45.00' E	Lowestoft	3	0.35
	52° 42.00' N	5° 2.00' W	Irish Sea	3	0.04
	52° 45.65' N	0° 23.60' E	Wash	2	0.18
	52° 56.40' N	1° 29.84' E	Mundesley	1	0.03
	52° 58.00' N	0° 1.00' W	Wash	4	0.08
	53° 34.80' N	0° 3.90' W	River Humber	4	0.14
	53° 38.20' N	0° 11.80' W	River Humber (Immingham)	11	0.14
	53° 42.00' N	0° 52.20' W	River Humber	8	0.56
	53° 43.30' N	0° 18.00' W	River Humber	3	0.05
	54° 0.00' N	2° 51.00' W	Morecambe Bay	8	0.02
	54° 28.80' N	0° 37.80' W	Robin Hood's Bay	3	0.02
	54° 41.00' N	1° 12.00' W	Hartlepool Bay	17	0.33
54° 41.95' N	1° 11.60' W	Hartlepool Bay	2	0.51	
54° 52.00' N	1° 19.50' W	River Tyne	3	0.14	
54° 55.00' N	1° 22.00' W	River Wear	7	0.14	
54° 59.80' N	1° 27.00' W	River Tyne	18	0.80	
55° 1.00' N	1° 25.00' W	River Tyne	9	1.29	
55° 54.40' N	1° 23.25' W	River Tweed	1	0.13	
<b>1994</b>	50° 3.00' N	5° 3.00' W	Falmouth	5	15.38
	50° 20.21' N	4° 8.44' W	Plymouth Sound	8	0.03
	50° 35.93' N	1° 18.98' W	Solent	1	0.32
	50° 40.00' N	3° 27.00' W	Lyme Bay	2	6.32
	50° 43.25' N	2° 0.02' W	Holes Bay	6	0.80
	50° 47.08' N	1° 7.00' W	Solent (Gosport)	8	0.25
	50° 48.75' N	1° 50.50' W	Bournemouth	1	0.01
	50° 49.00' N	1° 5.80' W	Portsmouth Harbour	2	0.28
	50° 51.58' N	1° 23.18' W	Solent (Lymington)	6	0.13
	50° 52.54' N	1° 17.57' E	Dover Strait	6	0.34
	50° 53.65' N	1° 25.10' W	Southampton Water	16	0.37
	50° 54.20' N	1° 27.28' W	Solent	3	0.48
	51° 6.70' N	1° 19.32' E	Dover	6	0.03
	51° 21.70' N	1° 1.60' E	Isle of Sheppey	2	0.07
	51° 27.00' N	3° 10.00' W	Cardiff	6	0.01
	51° 29.00' N	3° 11.00' W	Outer Severn Estuary	2	0.01
	51° 37.85' N	0° 48.80' E	Crouch	12	0.16
	51° 43.80' N	0° 41.30' E	Bradwell	1	0.17

**Table 21. continued**

Year	Latitude	Longitude	Location	N = number	Concentration TBT (mg kg <sup>-1</sup> wet weight)	
<b>1994 cont:</b>	51° 44.00' N	0° 43.00' E	Crouch	3	0.11	
	51° 56.35' N	1° 19.13' E	Harwich	36	0.08	
	52° 28.34' N	1° 44.82' E	Lowestoft	5	0.14	
	52° 34.35' N	1° 44.45' E	Great Yarmouth	4	0.06	
	52° 58.00' N	0° 1.00' W	Humber	6	0.03	
	53° 18.54' N	4° 37.70' W	Holyhead	10	0.24	
	53° 19.60' N	2° 57.00' W	Manchester Ship Canal	3	0.02	
	53° 23.17' N	3° 0.32' W	River Mersey	3	0.21	
	53° 24.18' N	3° 2.25' W	River Mersey	8	2.06	
	53° 26.00' N	3° 0.00' W	River Mersey	14	0.31	
	53° 34.53' N	0° 4.90' W	River Humber	15	0.09	
	53° 37.65' N	0° 11.40' W	River Humber (Immingham)	6	0.11	
	53° 41.77' N	0° 52.58' E	River Humber (Outer Estuary)	6	0.97	
	54° 4.48' N	0° 11.07' W	Bridlington Bay	2	0.15	
	54° 4.77' N	0° 11.12' E	River Humber	2	5.09	
	54° 5.00' N	3° 13.00' W	Morecambe Bay	2	0.02	
	54° 17.00' N	0° 23.00' W	Scarborough	9	1.31	
	54° 41.29' N	1° 11.49' W	Hartlepool Bay	1	0.04	
	54° 42.80' N	3° 30.20' W	Solway (Maryport)	4	0.03	
	54° 54.61' N	1° 22.09' W	River Wear	5	0.21	
	54° 59.32' N	1° 27.88' W	River Tyne	2	0.13	
	<b>1995</b>	50° 21.47' N	4° 9.18' W	River Tamar	3	0.34
		50° 24.66' N	4° 12.07' W	River Tamar	1	0.05
		50° 36.48' N	2° 7.45' W	Lulworth	10	0.77
		50° 41.80' N	0° 56.96' W	Solent	1	0.01
		50° 42.25' N	1° 56.90' W	Holes Bay	10	1.35
		50° 43.50' N	2° 58.50' W	Lyme Bay	3	0.26
50° 45.60' N		1° 17.70' W	Solent	3	0.08	
50° 46.91' N		0° 3.53' W	Newhaven	4	0.59	
50° 48.18' N		0° 49.32' W	Solent	3	0.07	
50° 48.20' N		1° 6.60' W	Portsmouth Harbour	31	0.22	
50° 49.27' N		0° 14.47' W	Shoreham	6	0.10	
50° 49.46' N		1° 2.40' W	Solent (Langstone Harbour)	3	0.10	
50° 50.00' N		1° 19.00' W	Solent	9	0.13	
50° 50.30' N		1° 19.71' W	Southampton Water	6	0.10	
50° 51.10' N		1° 18.24' W	River Hamble	6	0.57	
50° 53.39' N		1° 23.23' W	Southampton Water	2	0.25	
50° 54.47' N		1° 22.50' W	River Itchen	6	0.66	
51° 7.07' N		1° 18.68' E	Dover	4	0.23	
51° 23.90' N		3° 15.60' W	Barry	5	0.44	
51° 29.80' N		2° 43.50' W	Portishead	1	0.03	
51° 31.65' N		2° 58.35' W	Newport	1	0.04	
51° 34.50' N		4° 48.20' W	Caldey Sound	2	0.02	
51° 36.93' N		3° 55.00' W	Swansea	4	1.04	
51° 41.25' N		4° 58.50' W	Inner Milford Haven	6	0.03	
51° 41.75' N		5° 0.35' W	Milford Haven	1	0.46	
52° 2.50' N		1° 9.75' E	Thames	5	0.15	
53° 19.60' N		2° 57.00' W	Manchester Ship Canal Approaches	3	0.78	
53° 24.62' N		3° 3.38' W	River Mersey	3	8.84	
53° 24.90' N		2° 59.53' W	River Mersey	12	0.17	
53° 26.95' N		3° 0.87' W	River Mersey	3	0.14	
53° 41.82' N	0° 52.50' W	River Humber	6	0.56		
54° 34.42' N	1° 13.20' W	River Tees	6	0.22		
54° 57.74' N	1° 32.30' W	River Tyne	33	1.74		
55° 0.66' N	1° 24.66' W	River Tyne	1	0.01		
55° 7.50' N	1° 30.00' W	Blyth	2	0.04		
<b>1996</b>	50° 9.30' N	5° 3.60' W	Hand Deep	20	2.16	
	50° 20.00' N	4° 38.00' W	Pencarrow Head	4	0.08	
	50° 21.00' N	4° 6.00' W	Plymouth Sound	12	0.05	
	50° 22.89' N	4° 11.15' W	River Tamar	2	0.38	
	50° 32.32' N	4° 56.08' W	Port Issac	10	0.67	
	50° 36.90' N	1° 20.00' W	Solent	1	0.01	
	50° 41.00' N	2° 5.00' W	Wareham	4	0.13	
	50° 41.60' N	1° 5.55' W	Solent (Benbridge)	3	0.44	
	50° 42.70' N	1° 59.50' W	Holes Bay	5	0.19	



**Table 21. continued**

Year	Latitude	Longitude	Location	N = number	Concentration TBT (mg kg <sup>-1</sup> wet weight)	
<b>1996 cont:</b>	50° 45.00' N	1° 31.50' W	West Cowes	9	0.12	
	50° 47.00' N	1° 17.50' W	Solent	4	0.05	
	50° 47.98' N	1° 7.20' W	Solent (Gosport)	2	0.08	
	50° 48.66' N	0° 32.59' W	Solent	2	0.02	
	50° 48.73' N	1° 5.54' W	Portsmouth Harbour	2	0.08	
	50° 50.37' N	0° 59.57' W	Langstone Harbour	3	0.05	
	50° 52.25' N	1° 23.70' W	Lymington	6	0.14	
	50° 53.00' N	1° 24.00' W	Southampton Water	9	0.18	
	50° 54.50' N	1° 22.60' W	River Itchen	2	0.15	
	51° 39.68' N	1° 2.13' E	Thames	7	0.26	
	51° 56.00' N	1° 21.50' E	Felixstowe	5	0.04	
	52° 0.50' N	1° 11.70' E	River Orwell	3	0.08	
	52° 28.15' N	1° 45.29' E	Lowestoft	12	0.08	
	52° 45.55' N	0° 23.40' W	King's Lynn Dock	6	0.03	
	53° 23.18' N	3° 0.32' W	River Mersey	7	0.44	
	53° 25.82' N	3° 0.22' W	River Mersey	2	0.15	
	53° 34.80' N	0° 3.70' W	River Humber	6	0.28	
	53° 59.93' N	2° 50.84' W	Heysham	8	0.09	
	54° 6.28' N	3° 12.30' W	Morecambe Bay	1	0.10	
	54° 30.00' N	3° 30.00' W	St. Bee's Head	12	0.06	
	54° 35.00' N	1° 11.50' W	River Tees	8	0.44	
	54° 39.00' N	3° 34.20' W	Solway (Wokington)	6	0.03	
	54° 54.80' N	1° 24.35' W	River Wear	7	0.26	
	54° 59.00' N	1° 26.95' W	River Tyne	8	8.24	
	54° 59.09' N	1° 31.30' W	River Tyne	4	0.55	
	54° 59.10' N	1° 31.58' W	River Tyne	2	2.95	
	55° 0.00' N	1° 27.00' W	River Tyne	4	2.28	
	55° 0.60' N	1° 25.00' W	River Tyne	3	0.89	
	55° 7.50' N	1° 30.00' W	Blyth	6	0.03	
	55° 21.00' N	1° 37.00' W	Alnmouth Bay	3	0.15	
	<b>1997</b>	50 21.60' N	4° 7.35' W	Plymouth Sound	5	0.39
		50° 22.50' N	4° 37.80' W	Fowey	6	0.05
		50° 24.54' N	1° 17.57' W	English Channel	3	0.37
50° 42.10' N		1° 17.20' W	Solent	17	0.99	
50° 42.62' N		1° 58.69' W	Holes Bay	21	0.15	
50° 44.40' N		1° 31.90' W	West Cowes	7	0.50	
50° 47.50' N		1° 6.30' W	Solent (Gosport)	8	0.76	
50° 49.80' N		0° 15.85' W	Shoreham	4	0.02	
50° 53.30' N		1° 24.50' W	Southampton Water	18	0.12	
50° 54.70' N		1° 22.50' W	River Itchen	4	0.30	
51° 4.33' N		1° 11.44' E	Folkstone	4	0.07	
51° 23.46' N		3° 15.42' W	Barry	5	0.02	
51° 26.15' N		3° 9.41' W	Inner Severn Estuary	3	0.01	
51° 27.03' N		3° 10.03' W	Outer Severn Estuary	1	0.01	
51° 27.07' N		3° 9.63' W	Cardiff	12	0.32	
51° 32.19' N		2° 58.33' W	Newport	5	0.56	
51° 33.10' N		3° 58.60' W	Swansea Bay	4	0.03	
51° 36.08' N		3° 55.73' W	Swansea	3	2.37	
51° 37.30' N		3° 50.20' W	Neath	1	0.00	
51° 56.00' N		1° 18.60' E	Harwich	1	0.02	
52° 57.00' N		0° 7.50' E	Wash	4	0.04	
53° 18.71' N		4° 37.64' W	Holyhead	5	0.01	
53° 23.15' N		3° 0.35' W	River Mersey	4	2.45	
53° 24.05' N		3° 1.45' W	River Mersey	3	0.17	
53° 25.00' N		2° 26.80' W	River Mersey	1	0.01	
53° 25.46' N		3° 0.00' W	River Mersey	6	0.50	
53° 34.71' N		0° 3.53' W	River Humber	6	0.13	
53° 38.40' N		0° 11.80' W	River Humber (Immingham)	9	0.02	
54° 4.69' N		0° 11.00' W	Bridlington Bay	11	0.43	
54° 17.00' N		0° 23.50' W	Scarborough	8	1.12	
54° 28.97' N		0° 36.58' W	Robin Hood's Bay	5	0.03	
54° 34.65' N		1° 13.40' W	River Tees	13	0.18	
54° 37.86' N		1° 10.01' W	River Tees	7	0.02	
54° 57.55' N	1° 34.39' W	River Tyne	14	4.47		
54° 59.52' N	1° 26.60' W	River Tyne	12	65.25		

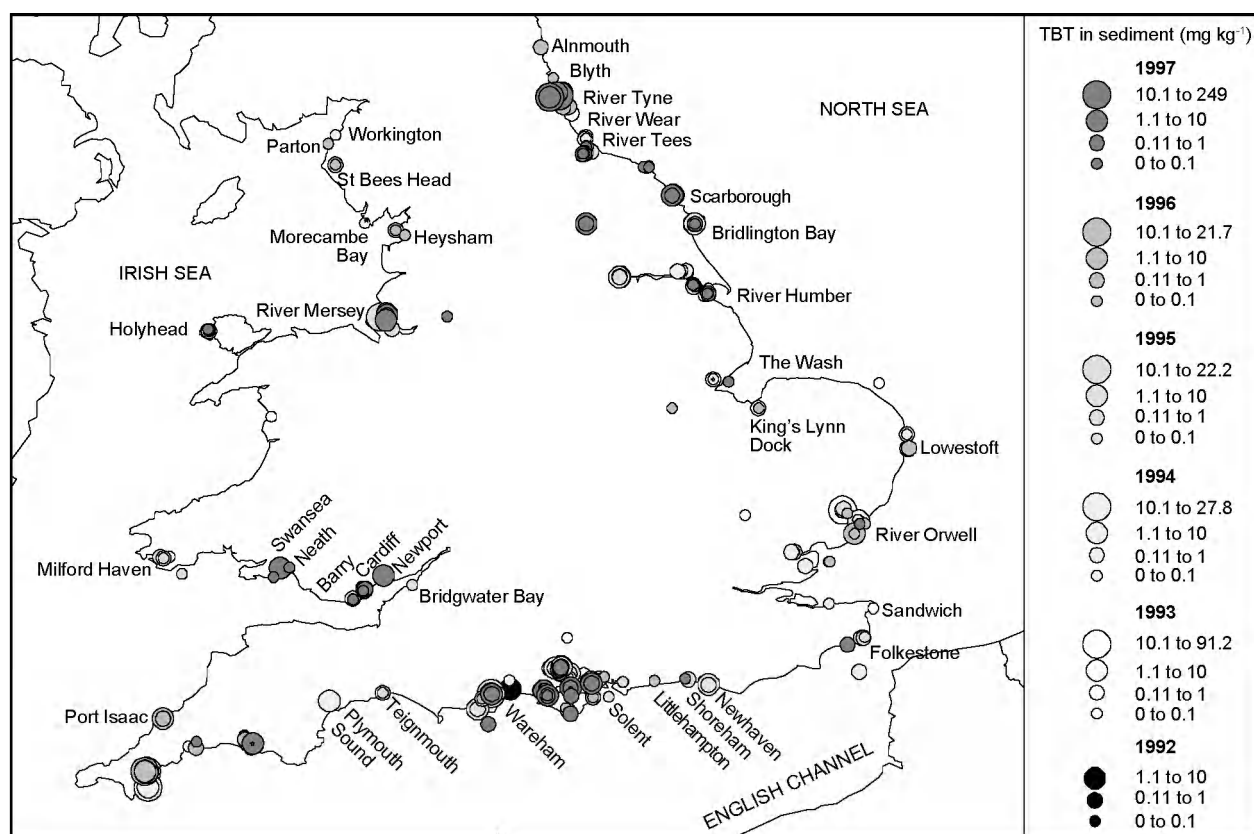


Figure 35. TBT concentrations in dredged material licensed for sea disposal in the UK, 1992-1997

Table 22. TBT in Sediments ( $\text{mg kg}^{-1}$  wet weight) between 1992-1997

TBT levels ( $\text{mg kg}^{-1}$ )	1992	1993	1994	1995	1996	1997	Total levels 1992-1997
0 - 0.1	14% (1)	47% (81)	50% (116)	38% (78)	51% (110)	44% (106)	46% (492)
0.11 - 1	43% (3)	43% (76)	42% (95)	51% (104)	32% (69)	38% (92)	41% (439)
1.1 - 10	43% (3)	8% (14)	7% (17)	10% (20)	15% (33)	12% (29)	10.5% (116)
>10.1	0% (0)	2% (4)	1% (3)	1% (3)	2% (4)	6% (14)	2.5% (28)
							<b>Total No. of sites</b>
							<b>1075</b>

N.B. (Number of sites in brackets)

Falmouth and River Orwell in 1993, Lyme Bay, River Humber and River Mersey in 1994, River Mersey in 1995, River Tyne in 1996 and River Mersey, Swansea and River Tyne in 1997.

Where TBT concentrations were considered to be above an acceptable level for sea disposal, additional sampling was undertaken to investigate possible local sources of contamination. An assessment of the extent of the contamination was completed and either the licence was refused or conditions applied to prevent high levels of TBT entering the marine environment.

## 12.6 Conclusion

The concentrations of TBT measured in these dredged materials indicate a substantial reservoir of TBT in estuarine sediments in the UK. These sediments cannot be regarded as a sink for TBT because the resuspension of sediments through storm-flows together with bioturbation will re-mobilise TBT. Resuspended sediments comprise of very fine particles and are an important route in the transport and dispersion of TBT which becomes available to filter feeders and organisms living and feeding within contaminated sediments (Waldock and Reed, 1996).

Further work is necessary to determine more accurately the environmental fate of the material and its potential impact. The possibility of remediation should also be explored to allow disposal to sea or beneficial uses such as beach nourishment schemes, saltmarsh rehabilitation and other coastal zone management programmes, which are increasingly used as alternatives to the sea disposal of dredged sediments.

In the future, the use of sediment bioassays may be employed to assist the current methods for licensing dredged material for disposal to sea and protocols presently under development confirm the toxic nature of TBT-contaminated sediments.

### 13. ADVICE ON FISHERY IMPLICATIONS OF PIPELINE DISCHARGES

This section gives a brief summary of activities carried out during 1997 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 and 1995(b) and CEFAS, 1997).

A total of 316 applications were sent to CEFAS for assessment during 1997. Table 23 shows how this compares with numbers assessed in previous years.

**Table 23. Applications for consent to discharge assessed by CEFAS 1994-1997**

Year	Sewage	Trade effluent (including surface water)	Total
1994	168	38	206
1995	138	64	202
1996	136	64	200
1997	241	75	316

The increase in numbers of applications is mainly due to an increase in the number of major re-sewerage schemes aimed at meeting the Water Companies' commitments under 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, 1991).

As in previous years, the majority of the applications were for discharge of domestic sewage, of which the main risk to fisheries is the contamination of bivalve shellfish. The Water Companies are under no statutory obligation to design or modify a sewage disposal scheme for the sole purpose of reducing contamination of shellfish by sewage micro-organisms. Although their other commitments generally require improved levels of sewage treatment, this does not automatically reduce contamination of any shellfish in the area. For instance, in order to comply with bathing water standards, a Water Company may decide to treat the sewage by UV disinfection during the bathing season. However, the main harvesting season for shellfish is during the winter months so protection of shellfisheries is likely to require UV treatment to be provided all year round. Where an outfall is relocated in order to move a discharge away from a bathing beach this could bring it closer to a shellfish area. The role of MAFF/CEFAS in the statutory consultation process therefore has a valuable role to play in attempting to negotiate the best possible deal for shellfisheries in the design of such schemes.

Even when upgrading of the main sewage discharge does reduce contamination of shellfisheries, there may remain a risk from the associated storm or emergency overflows. Storm overflows, which are required to minimise the risk of flooding, allow untreated sewage to by-pass the treatment works during times of heavy rainfall. For overflows which impact on designated bathing waters, there is a statutory requirement to restrict overflow operation to a maximum of three spills per bathing season. However, there is no such requirement for shellfish waters so MAFF/CEFAS had to negotiate conditions for each outfall on a case by case basis. The aim has been to get overflows sited in a location where their operation will not impact on the shellfish water or, if that is not possible, to ensure that the spill frequency is as low as possible.

Emergency overflows only come into operation when there is a major failure at the sewage treatment works or pumping station. Although this should not occur, if it does, it may cause severe contamination of shellfish in the area. In advising on such applications we therefore press 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.

Discharges of domestic sewage to coastal waters pose few problems for species other than bivalve shellfish unless they contain persistent plastics or other material that could foul fishing gear. These are being gradually reduced as all major discharges are upgraded in order to comply with the requirements of Directive 91/271 concerning urban waste water treatment (European Communities, 1991).

As noted in an earlier report in this series (CEFAS, 1997), trade effluent discharges from the most potentially polluting or technologically complex industrial processes in England and Wales have, since 1 April 1991, been subject to Integrated Pollution Control (IPC) under Part I of the Environmental Protection Act 1990 (Great Britain - Parliament, 1990). New processes came under immediate control, with controls on existing processes being phased in between 1 April 1991 and 31 January 1996. The statutory consultation with MAFF for such processes is lead by Food Contaminants Division. However, all applications which include a discharge to tidal water are sent to CEFAS at Burnham for assessment of fishery implications in the same way as those covered under the Water Resources Act.

A total of 37 applications for IPC authorisation were received during 1997. A small number of these were for new processes - mainly power stations. 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. However, the majority of applications received during the year were part of a four-yearly review of existing processes. Most of these were not previously assessed by CEFAS so the review process provides a valuable opportunity to advise on fishery implications of these processes.

Two of the applications received during 1997 were for processes involving tributyl tin (TBT) removal and re-application, at dockyards. CEFAS monitoring data indicated that levels of TBT, and in some cases other contaminants such as copper, were highly elevated in sediments adjacent to the process. This information was passed on to the Environment Agency so that they could take it into account in formulating conditions for the IPC authorisation. All such processes have now been issued with interim authorisations which require them to achieve BPEO by October 1998. This will significantly reduce emissions to water, leaving historically contaminated sediments as the only remaining source of major TBT contamination.

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.

## **14. LICENSING OF DEPOSITS IN THE SEA**

### **14.1 Introduction**

This section gives information about the licensing of deposits in the sea during 1997 under of the Food and

Environment Protection Act 1985, Part II, (FEPA) (Great Britain Parliament, 1985(a)). For convenience, licensing statistics for Scotland and Northern Ireland are included in this Section to provide data for the UK as a whole.

### **14.2 Legislation and licensing authorities**

The deposit of articles and substances at sea, principally the dumping of waste (as opposed to discharge into the sea via pipelines) and the use of materials during construction works and coast defence works (including beach recharge), is controlled by means 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 1985(b)) and under the Deposits in the Sea (Exemptions) Order (Northern Ireland) 1995. During the period covered by this report, the licensing function in England and Wales rested with MAFF and in Scotland with the Scottish Office Agriculture, Environment and Fisheries Department (SOAEFD). 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 round the Welsh coast on behalf of the Welsh Assembly. In Scotland the licensing function became the responsibility of the Scottish Executive Rural Affairs Department. In Northern Ireland, the Environment and Heritage Service of the Department of the Environment continued to licence deposits in the sea around Northern Ireland

Information about licence applications, breaches of the legislation and any enforcement action is held on a Public Register by each Licensing Authority.

### **14.3 Enforcement**

Scientists from the CEFAS Burnham-on-Crouch Laboratory have powers to enforce licence conditions. Visits are made to construction sites and treatment works, disposal vessels and storage facilities. Records, including logbooks, are checked and samples are taken. Scientific staff carried out 15 inspections in 1997. The Sea Fisheries Inspectorate, with staff based on the coast, reports unlicensed activities and enforces licence conditions relating to construction and the disposal of wastes at designated disposal sites. They made 513 inspections in 1997.

In Scotland, similar enforcement powers are held by staff of the SOAEFD Marine Laboratory. Aberdeen and

by the Scottish Fisheries Protection Agency (SFPA). The Marine Laboratory made 8 inspections in 1997 and the SFPA made a further 20 enforcement visits. In Northern Ireland, 1 inspection visit was made during 1997.

In England and Wales, relatively few cases of apparent infringement of the Act required investigation during 1997 and primarily involved activities for which no licence had been obtained.

Following a routine inspection of the disposal vessel *W D SEVERN* working under contract to Associated British Ports, Fleetwood, the local District Inspector of Fisheries advised the Licensing Authority that over half of the 40 disposal operations undertaken during the early part of December 1996 had apparently taken place outside the designated disposal site. A formal investigation was instigated in March 1997. This concluded that, having regard to the navigational risks involved, it was unlikely that disposal had in fact been effected at the locations recorded in the vessel's log. Nevertheless, there was reasonable cause to believe that the log had not been properly maintained in accordance with the conditions attached to the licence. A formal warning was given to the licensee and the contractor, Westminster Dredging. The licence was also varied to include a requirement for electronic position recording during each disposal operation.

In June 1997, the local Fisheries Officer inspected a site at Pwllheli where contractors working for Gwynedd County Council were constructing a concrete slipway. Enquiries established that no licence had been applied for. The Council accepted that there had been an oversight and a licence was subsequently granted.

Also during June and following an exchange of correspondence earlier in the year, representatives of the Licensing Authority held a meeting with the owner of a boat yard at Penpol, Cornwall, attended also by the Planning Authority, Environment Agency and English Nature to discuss the unlicensed construction of a jetty. It was agreed that formal enforcement action would be suspended to allow the owner to submit a licence application accompanied by an assessment of the potential hydrological effects which would also take into account certain constructional modifications. After further discussions and a new planning application, an application for a FEPA licence is anticipated.

In November 1997, the Environment Agency drew to the attention of the Ministry's Fisheries Officer, a fisherman who had been disposing of crab shells by tipping over the cliff into the sea. A warning letter was sent advising the fisherman that such unlicensed activity was as an offence under the Act.

In Scotland, 17 investigations were conducted during 1997 by the Marine Laboratory, Aberdeen; a further

five being undertaken by the Fisheries Protection Agency. As a result, deposit activity was halted in 5 cases and in three cases materials were required to be removed. A total of 12 licences were issued retrospectively. No investigations were carried out in Northern Ireland during this period.

#### **14.4 Report on licensing activities**

Tables 24 to 28 give details, for the period 1993-1997 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 (Great Britain-Parliament, 1972 (a-b)).

#### **14.5 Licensing of minestone disposal**

Licences are no longer issued in respect of the disposal of colliery waste at sea and onto beaches. One licence however was in force during 1997 which authorised RJB Mining to deposit minestone on the foreshore at Lynemouth as an interim measure to sustain coastal defences, pending agreement on the basis of a shoreline management plan to a longer-term strategy for the area.

#### **14.6 Licensing of sewage sludge disposal**

Tables 24(a) and 24(b) give details of licences issued for the disposal of sewage sludge at sea. Total quantities of key metallic contaminants in sewage sludge actually disposed of at sea are shown in Table 24(b). Figure 36 shows the location of the disposal sites for sewage sludge and the quantities deposited at each site in 1997.

At the 1987 Second International Conference on the Protection of the North Sea, (Department of Environment, 1987), the Government indicated it was taking urgent action to reduce the contamination by persistent, toxic or bioaccumulable materials present in sewage sludge deposited in the North Sea and to ensure that the quantities of such contaminants deposited in the North Sea did not increase above 1987 levels. Previous reports have explained that to apply this control, limits were set for a series of key contaminants deposited under each licence. Since that time reductions have been imposed on the quantities for these metals permitted for disposal and these were further tightened in 1997. Table 25 shows how the aggregate figures authorised for disposal in the North Sea in 1997, compared with the estimated quantity at 1987 licensed levels. These are now near background levels, with most of these contaminants coming from general domestic sources rather than from industry.



**Table 24(a). Sewage sludge licensed for disposal at sea in 1997**

Country	Licensed quantity (Tonnes) <sup>(1)</sup>	Company and source of waste	Disposal sites	Quantity deposited (Tonnes) <sup>(1)</sup>
England and Wales	177,000	Anglian Water (Tilbury STW)	Barrow Deep	195,001
	499,000	Northumbrian Water (Howdon, Chester-le-Street, Cramlington, Washington STWs)	Tyne/Spurn Head	524,107
	105,000	Northumbrian Water (Portrack, Billingham, Guisborough, Ayton STWs)	Tyne/Spurn Head	81,880
	1,965,000	North West Water (Davyhulme, Liverpool, Warrington STWs)	Liverpool Bay	1,874,975
	330,000	Southern Water (Woolston, Portswood, Millbrook, Slowhill Copse STWs)	Nab Tower	336,683
	50,000	South West Water (Countess Wear STW)	Lyme Bay	45,327
	55,000	South West Water (Plympton, Radford, Camel's Head, Ernesettle, Ivybridge, Saltash, Newton Ferrers STWs)	Plymouth	51,112
	1,400,000	Thames Water (Crossness STW)	Barrow Deep	1,178,819
	3,100,001	Thames Water (Beckton, Riverside and Deephams STWs)	Barrow Deep	3,021,182
	0	Yorkshire Water (Knostrup STW)	Spurn Head	25,913
Scotland	500,000	Lothian Regional Council	St Abb's Head/ Bell Rock	375,079
	2,500,000	Strathclyde Regional Council	Garroch Head	1,703,500
Northern Ireland	0	Dept. Environment (Northern Ireland)	Belfast Sludge	241,920 <sup>(2)</sup>

Notes : <sup>(1)</sup> All figures are for tonnage in wet weight unless indicated otherwise

<sup>(2)</sup> 241,920 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 1997, which may be covered by 2 or more licences, including one or more issued in 1996

**Table 24(b). Summary of sewage sludge licensed and disposed of at sea in 1997**

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	1993	13	7,884,000	7,733,656	203,517	1.09	49	112	0.65	15	74	216
	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
Scotland	1993	2	3,000,000	1,946,340	60,470	0.19	18	26	0.07	3	18	35
	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
Northern Ireland	1993	0	0	243,200 @	10,141	0.04	2	3	0.02	0	3	9
	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
UK Total	1993	15	10,884,000	9,923,196 @	274,128	1.31	69	141	0.75	18	95	260
	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 \$	253,307	0.70	57	121	0.51	13	76	270

Notes : @ Includes 243,200 t disposed of by DOE(NI) Water Services under an administrative authorisation

% 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

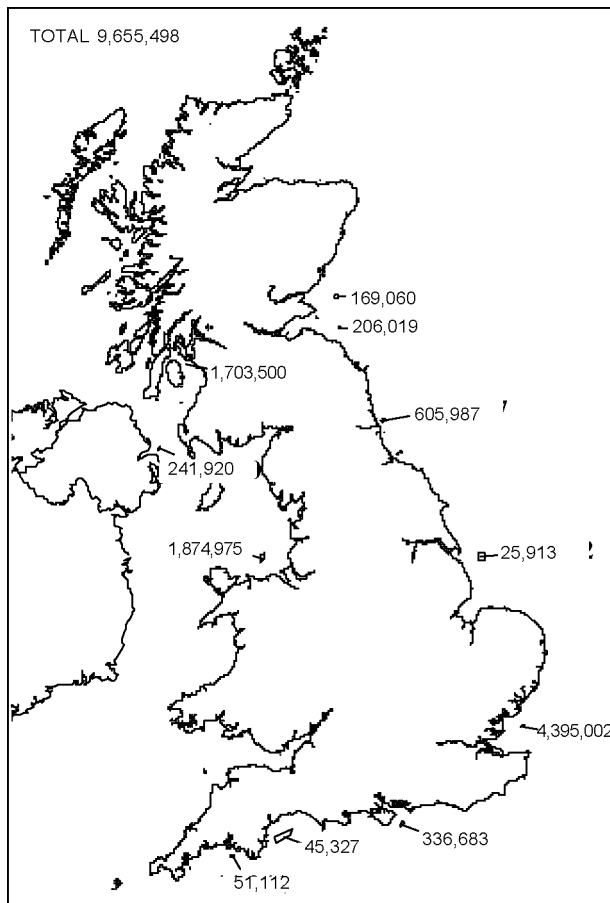
# Includes 329,015 t disposed of by DOE(NI) Water Services under an administrative authorisation

\$ Includes 241,920 t disposed of by DOE(NI) Water Services under an administrative authorisation

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

**Table 25. Contaminants in sewage sludge authorised for disposal in the North Sea in 1997 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
1997	0.6	20.5	95.9	0.5	10.5	73.4	162.8



**Figure 36. UK sewage-sludge disposal sites and amounts deposited in tonnes for 1997**

## 14.7 Licensing of dredged material disposal

The bulk of the dredged material licensed for disposal at sea is silt and sand, but coarse sand and shingle can occur in 'maintenance' dredging and shingle, cobbles, rocks and heavy clay can be present in 'capital' material arising, for example from channel development and deepening. Table 26 shows the numbers of licences issued, the quantity licensed, and the quantity deposited, together with figures for the quantity of a range of trace metals which enter the sea in the dredged materials. As noted in previous reports, a proportion of the trace metals in dredged material is natural and occurs within the mineral structure or is otherwise tightly bound, such that it will not be available to marine organisms. Figure 37 shows the main disposal sites used in 1997 and the quantities deposited at each site. However all applicants for licences are now required to show evidence that they have considered alternative disposal options including beneficial uses for dredged material and to explain why such alternatives are not considered practical.

## 14.8 Other licensed activity

Under Part II of FEPA, licences are also required for certain other activities which involve the deliberate deposit of articles or substances in the sea below the mean high water spring tide mark. Each licence application is carefully considered, in particular, to

**Table 26. Summary of dredged material licensed and disposed of at sea in 1997**

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	1993	110	66,074,966	23,068,903	12,793,107	5.8	790	521	4.9	367	884	2,194
	1994	106	53,187,009	37,219,028	21,395,174	9.6	1,385	823	6.2	683	1,502	3,655
	1995	109	54,300,948	35,215,611	17,941,131	5.8	1,298	625	5.2	548	1,380	3,161
	1996	120	82,395,490	48,513,953	25,953,191	8.8	1,556	743	6.9	673	1,731	3,991
	1997	113	56,536,922	38,627,660	21,165,143	6.5	1,182	574	5.5	471	1,242	2,941
Scotland	1993	26	3,174,050	2,025,525	1,117,770	2.4	50	44	0.8	21	63	132
	1994	23	3,643,250	1,822,053	820,368	0.9	42	36	0.5	20	56	122
	1995	32	6,186,600	4,782,421	2,204,223	1.1	155	120	3.5	66	153	349
	1996	30	3,971,045	2,601,864	1,174,999	0.4	56	89	0.7	26	81	155
	1997	29	3,910,900	2,436,745	1,045,762	0.2	46	50	0.7	25	69	153
Northern Ireland	1993	7	996,500	3,392,994	2,261,930	1.8	11	26	1.1	13	23	70
	1994	5	113,200	91,314	59,067	0.0	0	0	0.0	0	0	1
	1995	9	335,280	249,593	170,297	0.2	2	1	0.1	2	2	8
	1996	6	166,000	135,550	106,768	0.0	2	2	0.0	3	2	4
	1997	7	206,000	176,919	122,289	0.2	1	1	0.0	1	1	5
UK Total	1993	143	70,245,516	28,487,422	16,172,807	10.0	852	591	6.8	401	969	2,396
	1994	134	56,943,459	39,132,395	22,274,610	10.4	1,427	860	6.7	703	1,558	3,777
	1995	150	60,822,828	40,247,625	20,315,652	7.2	1,455	746	8.7	616	1,535	3,517
	1996	156	86,532,535	51,251,367	27,234,957	9.2	1,613	835	7.6	701	1,814	4,149
	1997	149	60,653,822	41,241,324	22,333,194	6.9	1,230	624	6.2	497	1,312	3,100

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



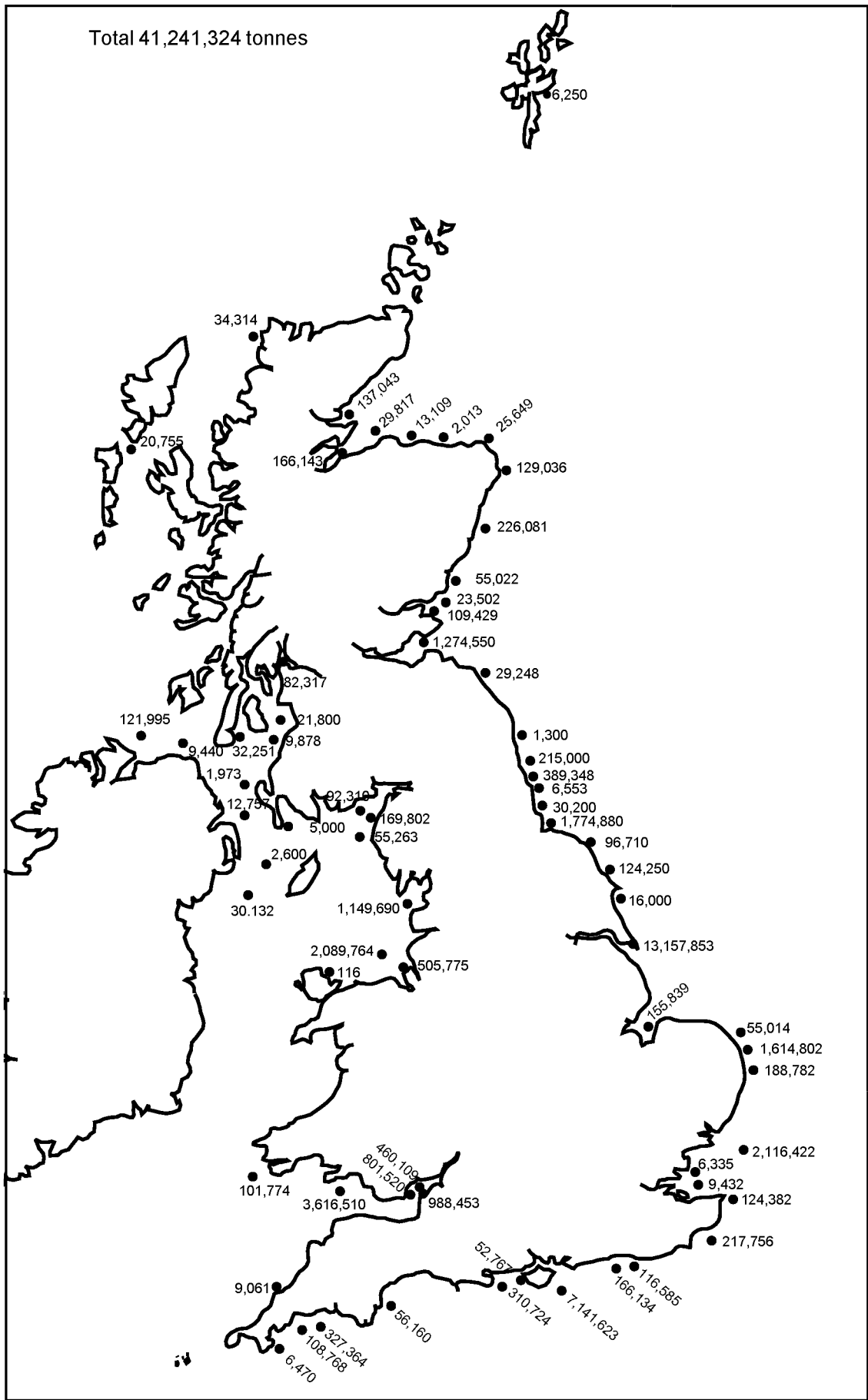


Figure 37. UK dredged material disposal sites and amounts deposited in tonnes for 1997

assess the impact upon 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 at sea in 1997 of small quantities of fish waste; details of which

are included in Tables 27(a) and (b). Further activities involved 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 numbers of such licences issued in 1997.

**Table 27(a). Fish waste licensed for disposal at sea in 1997<sup>(1)</sup>**

Country	Licensed Quantity (Tonnes) <sup>(1)</sup>	Company and source of waste	Disposal sites	Quantity Deposited (Tonnes) <sup>(2)</sup>
England and Wales	750	Quay Fresh & Frozen Foods Ltd, New Quay	New Quay	747
Scotland	250	Orkney Fishermen's Association, Stromness	Stromness C Stromness B	51 0
	12	Orkney Fishermen's Association, Stromness	Stromness C Stromness B	0 0

Notes : <sup>(1)</sup> No Fish Wastes were licensed or disposed of in Northern Ireland during the period covered by this report

<sup>(2)</sup> All figures are for tonnage in wet weight

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 1997**

Country	Year	Licences issued	Licensed quantity (tonnes)	Wet tonnage deposited	Dry tonnage deposited
England and Wales	1993	0	0	0	0
	1994	0	0	0	0
	1995	0	0	0	0
	1996	1	750	16	16
	1997	1	750	747	747
Scotland	1993	0	0	0	0
	1994	0	0	0	0
	1995	0	0	0	0
	1996	0	0	0	0
	1997	2	262	51	41
Northern Ireland	1993	0	0	0	0
	1994	0	0	0	0
	1995	0	0	0	0
	1996	0	0	0	0
	1997	0	0	0	0
UK Total	1993	0	0	0	0
	1994	0	0	0	0
	1995	0	0	0	0
	1996	1	750	16	16
	1997	3	1012	798	788

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

**Table 28. Other categories of licences issued in 1997**

Licence category	Year	England and Wales	Scotland	Northern Ireland	Total
Construction - new and renewal	1997	273	73	3	349
Tracers, biocides etc.	1997	17	0	0	17
Burial at Sea	1997	14	0	0	14

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

### **SEA WATER**

Winter nutrients in coastal waters S. Malcolm

### **BIOTA**

Heavy metals in bivalve molluscs J. Jones

Arsenic speciation in fish muscle B. Jones

PAH levels in fish and shellfish R. J. Law

### **SEDIMENTS**

Arsenic in North Sea sediments C. Whalley

### **BIOLOGICAL EFFECTS**

The use of enzyme biomarkers M. Kirby  
S. Morris

Oestrogenic endocrine disruption of flounder Y. Allen

Marine fish diseases S. Feist

### **BENTHOS**

Epifaunal studies H. Rees

Meiofauna research S. Boyd

### **DISPOSAL AT SEA**

Metal concentrations in dredged sediment J. Reed

TBT concentrations in dredged sediment J. Reed

Advice on fishery implications of pipeline discharges F. Franklin

Licensing of deposits in the sea G. Boyes  
C. Vivian

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## ANNEX 2. Standards/guidelines for contaminants in fish and shellfish

### A2.1 Metals

#### (a) Mercury

The European and Paris Commissions have adopted an Environmental Quality Standard (EQS) for mercury, which requires that the mean concentration of mercury in the flesh of a representative sample of fish, locally caught from areas receiving significant inputs of mercury, shall not exceed  $0.3 \text{ mg kg}^{-1}$  on a wet weight basis (EC Directive Nos. 82/176 and 84/156 - European Communities, 1982 and 1984).

Community Decision 93/351 EEC (European Communities, 1993) applies to samples of fishery products. This states that the mean total mercury content of the edible parts of fishery products must not exceed  $0.5 \text{ mg kg}^{-1}$  fresh weight, increased to  $1.0 \text{ mg kg}^{-1}$  fresh weight for some species listed in an annex.

For the purposes of the Joint Monitoring Programme (JMP) of the Oslo and Paris Commissions, the following arbitrary, purely descriptive, guidelines have been adopted.

Level	Fish flesh and crustaceans	Molluscs
Lower	$<0.1 \text{ mg kg}^{-1}$ wet weight	$<0.6 \text{ mg kg}^{-1}$ <u>dry</u> weight
Medium	$0.1\text{-}0.3 \text{ mg kg}^{-1}$ wet weight	$0.6\text{-}1.0 \text{ mg kg}^{-1}$ <u>dry</u> weight
Upper	$>0.3 \text{ mg kg}^{-1}$ wet weight	$>1.0 \text{ mg kg}^{-1}$ <u>dry</u> weight

#### (b) Cadmium

There are no standards or guidelines in England and Wales for fish flesh. The expected values are  $<0.2 \text{ mg kg}^{-1}$  wet weight.

The JMP guidelines for cadmium in mussels are as follows:

Level	Mussel tissue	<u>Approximate</u> equivalent
Lower	$<2 \text{ mg kg}^{-1}$ <u>dry</u> weight	( $<0.4 \text{ mg kg}^{-1}$ wet weight)
Medium	$2\text{-}5 \text{ mg kg}^{-1}$ <u>dry</u> weight	( $0.4\text{-}1.0 \text{ mg kg}^{-1}$ wet weight)
Upper	$>5 \text{ mg kg}^{-1}$ <u>dry</u> weight	( $>1.0 \text{ mg kg}^{-1}$ wet weight)

From past CEFAS work, 'expected' values (i.e. using data from estuaries not known to be severely contaminated) would be up to  $0.3 \text{ mg kg}^{-1}$  wet weight for crustaceans but up to  $10 \text{ mg kg}^{-1}$  wet weight for crab 'brown' meat.

#### (c) Lead

From the Lead in Food Regulations 1979 (Great Britain - Parliament, 1979): lead in fish should not exceed  $2.0 \text{ mg kg}^{-1}$  wet weight, and lead in shellfish  $10.0 \text{ mg kg}^{-1}$  wet weight.

From past work, 'expected' values are  $0.2\text{-}0.3 \text{ mg kg}^{-1}$  wet weight in fish, up to  $1.0 \text{ mg kg}^{-1}$  wet weight in crustaceans, and up to  $5.0 \text{ mg kg}^{-1}$  wet weight in some molluscs.

#### (d) Copper

From the Food Standards Committee's Report on Copper (MAFF, 1956), revised recommendations for limits for copper content of food are as follows:

'levels of copper in food should not exceed  $20 \text{ mg kg}^{-1}$  wet weight (but higher levels in shellfish are permitted if copper is of natural occurrence).'



From past CEFAS work, 'expected' levels in fish are up to 0.6 mg kg<sup>-1</sup> wet weight (in excess of 1.0 mg kg<sup>-1</sup> wet weight in fatty fish such as herring), 20-30 mg kg<sup>-1</sup> wet weight for crustaceans and up to 500 mg kg<sup>-1</sup> wet weight for molluscs.

*e) Zinc*

From the Food Standards Committee's Report on Zinc (Ministry of Food, 1953), as a guideline:

'levels of zinc in food should not exceed 50 mg kg<sup>-1</sup> wet weight (but higher levels are permitted in food which naturally contain more than 50 mg kg<sup>-1</sup>, such as herring and shellfish).'

'Expected' values commonly found are up to 6.0 mg kg<sup>-1</sup> wet weight in most fish flesh, (though up to 10 mg kg<sup>-1</sup> in flounder and considerably more in fatty fish), up to 100 mg kg<sup>-1</sup> wet weight in crustaceans and well in excess of 100 mg kg<sup>-1</sup> wet weight for some molluscs.

## A2.2 Pesticides/PCBs

There are no standards in fish and shellfish from England and Wales.

*(a) HCB*

The 'expected' value is up to 0.10 mg kg<sup>-1</sup> wet weight in fish liver.

*(b) HCH*

Codex Alimentarius Commission's maximum residue limit (MRL) (FAO/WHO, 1987) is 2 mg kg<sup>-1</sup> in meat fat for  $\gamma$ -HCH. The 'expected' values are up to 0.05 mg kg<sup>-1</sup> wet weight for each of  $\alpha$ - and  $\gamma$ -HCH in fish liver.

*(c) Dieldrin*

Codex Alimentarius Commission's MRL is 0.2 mg kg<sup>-1</sup> in meat fat. The 'expected' values are 0.2-0.3 mg kg<sup>-1</sup> wet weight in fish liver.

*(d) Total DDT*

Codex Alimentarius Commission's MRL is 5 mg kg<sup>-1</sup> in meat fat. The 'expected' values are up to 0.5 mg kg<sup>-1</sup> wet weight for each of DDE, TDE and pp DDT in fish liver.

*(e) PCBs*

JMP guidelines are as follows (all mg kg<sup>-1</sup> wet weight):

Level	Fish muscle	Cod <sup>1</sup> liver	Flounder <sup>2</sup> liver	Molluscs	Crustaceans
Lower	<0.01	<2.0	<0.50	<0.02	<0.01
Medium	0.01-0.05	2.0-5.0	0.50-1.0	0.02-0.10	0.01-0.05
Upper	>0.05	>5.0	>1.0	>0.10	>0.05

<sup>1</sup> Values used for all roundfish in this report

<sup>2</sup> Values used for all flatfish in this report

## A2.3 References

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