# Monitoring of the quality of the marine environment, 2003-2004



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#### **Foreword**

Aquatic Environment Monitoring Report No. 58 collects together work carried out in 2003-04 by Cefas scientists in support of our monitoring and surveillance duties (see overleaf). The information presented covers both environmental surveillance at offshore and coastal sites and site-specific work carried out in support of risk assessments and regulatory procedures. Some of the science reported here forms part of wider efforts to integrate data from Departments and Agencies in the UK to provide a comprehensive picture of the quality of the marine environment via the UK National Marine Monitoring Programme (NMMP). Other components are unique to Cefas due to our requirement to understand ecosystem response resulting from potential pressures from deposit, extraction and discharge activities.

The strategy for the NMMP is described in publications commissioned by the Marine Environment Monitoring Group (MEMG). The programme manual, known as the Green Book, is available in downloadable format from the Scottish Environmental Protection Agency's website at:

www.sepa.org.uk/marine/

The programme seeks to develop time trend data for a limited number of sites around the UK and this work is augmented by special surveys of compounds likely to pose specific risks, or for which few data exist.

During 2005, Cefas has developed a new framework within which our science is organised. In this report, the various chapters are organised under headings relating to the three new science themes, Ecosystem Interactions, Organism Health and Resource Management.

Within the theme of Ecosystem Interactions, studies have shown that mapping the distribution of sediments and the biota they support at different spatial scales will support marine spatial planning (Chapter 1). For the first time, extensive regional sampling of benthos in the North Sea has allowed a critical evaluation of the relationship between the nematode fauna and their sedimentary habitat and other , larger-sized, faunal groups, including macrobenthic infauna, invertebrate epifauna and fish (Chapter 2).

Within the theme of Organism Health, we have been able to quantify the additional risk of death from infectious disease of increased blubber levels of chlorobiphenyls in

porpoises (Chapter 3), and observed that plasma vitellogenin levels in male flounder, probably linked to contamination by oestrogenic compounds, remain elevated in several UK estuaries. However, downward trends are evident in several key estuaries and these are linked to improved sewage treatment facilities (Chapter 5).

Within the theme of Resource Management, results from a radiological assessment of the disposal of dredged material from Whitehaven Harbour indicated that radiation doses associated with the operation were likely to be low and below legislative "de minimis" levels of exposure (Chapter 9). Also, levels of <sup>137</sup>Cs in UK coastal waters continue to exhibit a slow downward trend. In contrast, the influence of <sup>3</sup>H discharges from nuclear establishments in the Bristol Channel and Irish Sea remains apparent (Chapter 11). A small-scale survey suggested that levels of a range of volatile organic compounds in the River Tees have declined since 1992 (Chapter 12). We explored a variety of biological indicators from the standpoint of their utility in meeting both scientific and management criteria governing effectiveness (Chapter 14). The first survey undertaken for one of the chemicals on the OSPAR Priority List for Action, 2,4,6-tri-tert-butylphenol, has found it to be present in some estuarine sediments at low concentrations (Chapter 16). Acoustic surveys undertaken at the Inner Gabbard dredged material disposal site suggests that fine dredged material is accumulating within the site (Chapter 18). A review of seabed indicators derived from acoustic outputs resulted in an assessment of the potential of each technique evaluated against agreed criteria governing their utility (Chapter 21).

During the period under report, Cefas has also assisted Defra in the preparation of their state of the seas report "Charting Progress", which is available for downloading from

www.defra.gov.uk/environment/water/marine/ uk/stateofsea/index.htm

This report, earlier reports in the AEMR series and other publications are also available in downloadable format from the Cefas website

www.cefas.co.uk

Robin Law Lindsay Murray

## Background to the work

As an Executive Agency of the Department for Environment, Food and Rural Affairs (Defra), Cefas carries out work in support of Defra's five strategic priorities, all of which underpin the overarching aim of promoting sustainable development:

- Climate change and energy
- Sustainable consumption and production
- Natural resource protection
- Sustainable rural communities
- A sustainable farming and food sector, including animal health and welfare.

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

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

The Cefas Inspectorate evaluates scientific and technical aspects of licence applications and makes regular visits to licence holders to ensure that any stipulated conditions are being met. Conducting monitoring programmes in support of risk assessments enables Defra to ensure the effectiveness of the assessment process and provides a basis for decisions on future policy for the management of marine resources. Cefas scientists monitor the environmental conditions at marine disposal sites and compare the results with those obtained during more general monitoring studies, allowing action to be taken if unexpected impacts should occur. This, together with underpinning R & D, also provides a feedback loop which ensures that risk assessments undertaken within the

licensing process incorporate the most recent research findings.

Sewage discharges are consented under the Water Resources Act (1991), Great Britain Parliament, 1991) as amended by the Environment Act (1995), (Great Britain Parliament, 1995). Under the Control of Pollution (Applications, Appeals and Registers) Regulations 1996 regulation 5(1)(b), (Great Britain Parliament, 1996), the Environment Agency is required to consult the Secretary of State for the Environment. Defra Marine & Environment Division (MED) is a statutory consultee for all discharges to controlled (tidal) waters. Cefas scientists assess the fishery implications of applications for consent to discharge permits on behalf of Defra MED, and make direct comment to the Environment Agency.

We also provide advice to the Department of Trade and Industry (DTI) and the Office of the Deputy Prime Minister (ODPM) concerning the control of pollution in other areas affecting the marine environment including the extraction of offshore oil and gas and marine aggregate. The statutory Offshore Chemical Notification Scheme and the Government View on the winning of aggregates, respectively, control these activities, and the regulatory regime for aggregates is presently also changing to a statutory scheme.

On Defra's behalf, Cefas is responsible for monitoring intermediate and offshore stations within the UK National Marine Monitoring Programme (NMMP), which seeks to integrate national and international monitoring programmes for all UK agencies. Each year, we collect samples of seawater, sediment and biota for chemical analysis and deploy a number of biological effects techniques, including water and sediment bioassays and fish disease surveys. The current phase of the NMMP (phase II) is focused on the detection of long-term temporal trends in contaminant concentrations and the development and deployment of a wider range of biological effects techniques studying organism response at a variety of cellular and sub-cellular levels. The NMMP allows us to ascertain the effectiveness of regulatory measures taken 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 EU 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); the Commission Decision 93/351/EEC concerning maximum mercury limits in fishery products, and similar requirements under OSPAR. Currently, a group led from within Cefas is working to redesign the NMMP so as to ensure that it meets current requirements and, as far as possible, to dovetail with proposed monitoring to be undertaken under the EU Water Framework Directive (2000/60/EC) in rivers, estuaries and coastal waters.

In order to ensure that the advice provided to Defra and other regulators is always based on the most up-to-date knowledge and techniques, Cefas carries out a wide range of research and development to provide for the future needs of monitoring and surveillance programmes. For example,

we have developed new and more sensitive bioassay techniques, analytical methods and unattended sampling and monitoring devices. Within these programmes we have made a number of significant contributions to environmental protection and as a consequence of our work have established a worldwide reputation in the field of aquatic environmental research. More information on our research programmes is available on the Cefas website: www.cefas.co.uk.

## Glossary of terms

ANOSIM Analysis of similarities
ANOVA Analysis of variance

ASCOBANS Agreement on the Conservation of Small Cetaceans of the Baltic and North Seas

ASG Ammonium duodeca-molybdophosphate on silica gel

BDE Brominated diphenyl ether

BEQUALM Biological Effects Quality Assurance in Monitoring programme

BFR Brominated flame retardant
BNFL British Nuclear Fuels Limited

BTEX Benzene, Toluene, Ethylbenzene and Xylenes

CEMP Co-coordinated Environmental Monitoring Programme

CYP1A1 Cytochrome P4501A1

DAMOS Disposal Area Monitoring System

DMECS Development of a National Marine Ecotoxicological Analytical Control Scheme

DNA Deoxyribose Nucleic acid

DR-CALUX Dioxin-responsive chemically-activated luciferase expression assay

DRZs Diagonal Radioactive Zones

EARP Enhanced Actinide Removal Plant (at BNFL Sellafield)

EDCs Endocrine Disrupting Chemicals

EDMAR Endocrine Disruption in the Marine Environment
ELISA Enzyme Linked Immuno-Sorbent Assays
EPA Environmental Protection Agency (of the USA)

EROD Ethoxyresorufin-O-deethylase

EU European Union

FCA Foci of Cellular Alteration

FEPA Food and Environment Protection Act 1985

FRS MLA Fisheries Research Services Marine Laboratory, Aberdeen

GOV Grande Ouverture Verticale
GPS Global Positioning System
GSI Gonado-somatic index
H & E Haematoxylin and Eosin
HBCD Hexabromocyclododecane

IAEA International Atomic Energy Agency

ICES 7 The sum of concentrations of PCB congeners CB28, CB52, CB101, CB118, CB138, CB153 and CB180,

developed by ICES as a comparative measure

IMS Industrial Methylated Spirit

JMP Joint Monitoring Programme (of OSPAR)

MARPOL International Convention for the Prevention of Pollution from Ships, 1973

MDS Multi-dimensional scaling

MEMG Marine Environment Monitoring Group

MFO Mixed-function oxidase

MPMMG Marine Pollution Monitoring Management Group NADPH β-Nicotinamide Adenine Dinucleotide Phosphate

NAO North Atlantic Oscillation
NBF Neutral Buffered Formalin

NMMP UK National Marine Monitoring Programme

OSPAR Oslo and Paris Commission
PACs Polycyclic Aromatic Compounds
PAH Polycyclic aromatic hydrocarbon
PBDE Polybrominated diphenyl ether
PCB Polychlorinated biphenyl

POPs Persistant Organic Pollutants

PRIMER Plymouth Routines in Multivariate Ecological Research

PSA Particle Size Analysis

PTFE Polytetrafluoroethylene (also known as Teflon)

RAP Registry of Aquatic Pathology

RELATE a multivariate data analysis routine in PRIMER

SIMPER Similarity Percentages Routine

SIXEP Site Ion Exchange Effluent Plant (at BNFL Sellafield)

SPI Sediment Profile Images SRD Sewage Related Debris

TEQ Toxic equivalent

THORP Thermal Oxide Reprocessing Plant (at BNFL Sellafield)

TIE Toxicity Identification and Evaluation (or bioassay-directed fractionation)

UK United Kingdom

UNEP United Nations Environment Programme

VOC Volatile Organic Compounds

VTG Vitellogenin

YES Yeast Oestrogen Screening Assay

### **Ecosystem interactions**

# Developing a strategy for seabed mapping at different spatial scales

#### 1.1 Introduction

The ability of acoustic techniques to detect seabed features and discriminate different types of sediment presents the prospect of making accurate and reliable seabed maps that are of great value in the conservation, management and monitoring of the marine environment (Brown et al., 2001, 2002, 2004). However, their limited spatial coverage and inability to detect benthic fauna means their application in mapping seabed habitats requires a structured approach to survey design that can be adapted to suit the requirements for mapping at different spatial scales. There are two main steps in this structured approach, the first being to map acoustically distinct areas of seabed, and the second to ground-truth that map to determine the nature of the sediments and fauna in each area. If it can be demonstrated that different benthic communities are associated with different sediment types, then the distribution of those sediments can be used as a proxy to map benthic habitats and biotopes (Roff et al., 2003).

Different species are adapted to live in different sediments, but gross sediment type is not the only determinand of habitat suitability. Other physical factors such as depth of water and current velocity are also influential in structuring the seabed (eg different bedforms such as sand waves or megaripples) and their associated communities (Warwick and Uncles, 1980; Holme and Wilson, 1985; Barros et al., 2004). Consequently, seabed facies (distinct combinations of sediment type and bedform) may prove better proxies for mapping benthic habitats and biotopes than gross sediment type.

The swathe width of sidescan and multibeam sonar systems is typically limited to a few hundred metres, so to achieve an acoustic image of a wider area requires several parallel swathe tracks to be pasted together in an acoustic 'mosaic'. Areas up to ~ 50 sq km can be surveyed in a single day of vessel time and this is usually sufficient to cover discrete sites of specific interest, such as those licensed for aggregate extraction or dredge material disposal. Considerably greater time and effort would be required to cover broadscale areas (~ 500 sq km) or regional seas where there is currently great demand for maps to underpin marine spatial planning initiatives, but the cost of doing so may become prohibitive.

The aim of this study was to develop a strategy for investigating seabed conditions over different spatial scales. To this end we adopted a nested survey design, expanding and developing surveys of the Hastings Shingle Bank undertaken by Brown *et al.*, (2001, 2004), and of the

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broader area between Hastings and Dungeness (Foster-Smith et al., 2004). These earlier studies employed a relatively coarse interpretation of acoustic data to classify seabed sediments, but the present study focused on a more detailed interpretation, supported by existing geological data sets and complemented by ground-truthing techniques, to investigate the feasibility and utility of mapping seabed facies. With a further view to developing a cost-effective strategy for surveying larger spatial areas, we investigated the density of sidescan coverage required to produce broadscale facies maps that were fit for purpose.

#### 1.2 Background

The study site comprised a broadscale area (40 x 15 km) within which was nested the Hastings site (4 x 12 km) containing an area of the Hastings Shingle Bank that is licensed for aggregate extraction (Figure 1.1). Brown et al. (2001, 2004) had acquired 100% sidescan coverage of the Hastings site, and recognised four acoustically distinct regions (Figure 1.2) that were ground-truthed by random stratified sampling. The nature of the sediments and their associated infaunal communities were determined from sediment samples collected by a 0.1 m<sup>2</sup> Hamon grab, while epifaunal communities were sampled by a 2metre wide Jennings beam trawl (Jennings et al., 1999). Statistical analyses of the ground-truth data showed the four acoustic regions differed in both their physical and biological characteristics, allowing each to be described as a different biotope (Figure 1.2, A = shallow water polychaete dominated fine sand, B = coarse gravel with attached epifauna, C = disturbed (dredged) sandy gravel, D = deeper water coarse sand). Although the biotopes were clearly distinct from one another, Brown et al. (2001, 2004) noted that replicate samples from within each acoustic region could be guite dissimilar.

#### 1.3 Methods

#### 1.3.1 Hasting study area

The present study made a more detailed interpretation of the original sidescan sonar mosaic of the Hastings site, taking into consideration the bedforms and transport indicators and further geophysical data available in the archives of the British Geological Survey. This produced a seabed facies map (Figure 1.3), recognising nine different facies and mapping the area into > 20 regions, as opposed to the original four. Overlaying the original ground-truth

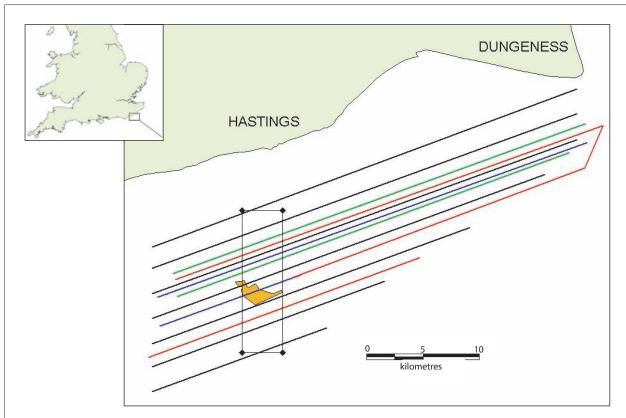


Figure 1.1. Location of the study area off the SE coast of England. The rectangle indicates the fine scale study site off Hastings, containing an area licensed for aggregate extraction (orange polygon). Lines indicate sidescan tracks for the broadscale study area, the seven lines of the original survey (2000) shown in black and 'in-fill' lines, collected in subsequent surveys shown in green, blue and red

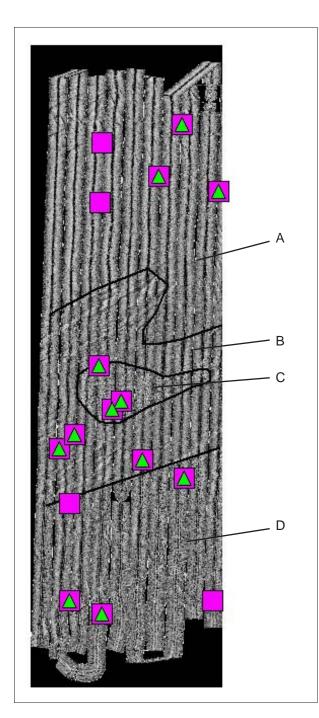
positions on the new facies map showed that a number of different facies had been sampled within each acoustically distinct area (Figure 1.4), which may account for the variability within replicates noted by Brown *et al.* (2001, 2004). The site was re-visited to ground-truth the facies map using a suite of sampling techniques.

The accuracy of the facies descriptions and the distinctness of their boundaries was assessed from video recordings of seven camera sledge tows (Shand and Priestly, 1999) made at strategic points throughout the site (Figure 1.5, CS1 - 7). The entire video record for each tow was analysed, noting sediment types and topographic features, and comparing these observations with what was expected from the facies map.

Epifaunal samples were collected from ten of the facies using the standard 2-metre Jennings beam trawl towed over a nominal distance of 200 metres (Figure 1.5, JB1 - 10). Samples were washed over a 5 mm square mesh sieve prior to identification and enumeration, with colonial species being recorded as 'present'. Associations between epifaunal assemblages and seabed facies were examined by univariate and multivariate analyses following the general methodology of Clarke and Warwick (1994), using the PRIMER software package (v5, Clarke and Gorley,

2001). Sample similarities were calculated using the Brav-Curtis similarity index based on square root transformed abundance data and represented by dendrograms and non metric multi-dimensional scaling (MDS) ordination to determine cluster groups. The significance of differences between cluster groups was tested by an analysis of similarities (ANOSIM, Clarke and Green, 1988) and the taxa primarily accounting for their similarities/differences determined using the similarity percentages routine (SIMPER, Clarke, 1993). Analyses were based on numeric (abundance) data, so colonial taxa were excluded. A comparison between the two studies of the Hastings site (Brown et al., 2004 and the present study) was made using similar analyses on a pooled data set. To lessen the influence of rare taxa, those with an occurrence of n < 10 in the pooled data set were excluded from the analysis.

Sediment and infaunal samples were collected from four of the facies using a 0.1 m<sup>2</sup> Hamon grab, with a novel sampling design being tested to investigate variability within each facies (Figure 1.6). The sampling pattern described an angular spiral with sides of relative length 1, 2, 3, 5 and 8, based on the Fibonacci series of numbers (add the previous two to get the next). With just six sampling points, 15 different pair-wise comparisons can be made



(5 + 4 + 3 + 2 + 1), each having a different 'lag' distance (separation between points). Using this pattern, spatial variability within a facies can be assessed graphically, plotting some measure of similarity (or dissimilarity) between sample pairs against lag distance in a simple Cartesian (x,y) chart. The sampling pattern provided a more structured approach to assessing variability than would a random stratified sampling design. In practice the distances between sampling points were 100, 200, 300 etc metres. Each sample was treated in the same manner, with a sub-sample used for particle size analysis, and the remaining material washed over a 1mm sieve to collect infauna for identification and enumeration.

Figure 1.2. Sidescan mosaic of the Hastings site, showing the four acoustically distinct areas determined by Brown *et al.* (2001, 2004) and the position of their ground-truth sampling with Hamon grabs (purple squares) and 2-metre beam trawls (green triangles).

Sediment grain size was expressed in whole phi (Ø) units and samples compared by graphical analysis of the size-frequency distributions. Spatial variability among samples was examined as described above, plotting Euclidean dissimilarity for sediment samples (based on square root transformation of % frequency in each grain size class) and Bray-Curtis dissimilarity for biotic samples (100 - Bray-Curtis similarity, based on 4th root transformed abundance data, colonial organisms excluded). Further community analyses were undertaken using the PRIMER package to investigate the linkage between different infaunal assemblages and different sediment types, and to determine the species that typify or discriminate between the four facies that had been sampled.

#### 1.3.2 Broadscale study area

In the broadscale study area between Hastings and Dungeness, the earlier studies had conducted a sidescan survey comprising seven parallel lines at a 2 km track spacing (Figure 1.1) using a digital chirps sidescan system with a 400 m total swathe width (Brown et al., 2004, Foster-Smith et al., 2004). To obtain some indication of the density of sidescan coverage required to produce a reliable broadscale facies map, the current project surveyed a series of 'in-fill' lines to provide progressively greater density coverage, reaching 100% along a central SW-NE corridor. Each of the sidescan lines was individually interpreted to mark the facies boundaries, following which four interpolated facies maps were made representing progressively greater density coverage, three based on subsets of the data (namely 4 km, 2 km and 1 km line spacing) and the fourth using all available lines.

Ground-truth sampling was undertaken concurrently with the sidescan surveys (Figure 1.7). In the initial survey, a series of 18 Shipek grabs (denoted SG1-18) were taken for particle size analysis only. In the current project, a further 20 stations were sampled by Hamon grab (denoted HG11-30) for PSA and infaunal analysis following standard procedures. The same locations were sampled for epifauna using a 2-metre Jennings beam trawl (denoted JB11-30) towed for a nominal distance of 200 m; again samples were processed following standard procedure. The array of sampling sites was selected to give a broad coverage of the survey area, with each site lying on (or close to) one of the sidescan lines. The interpretation of the ground-truth data was used to make inferences about the suitability of the broadscale facies map and to examine and compare multivariate patterns in the physical and biological data.

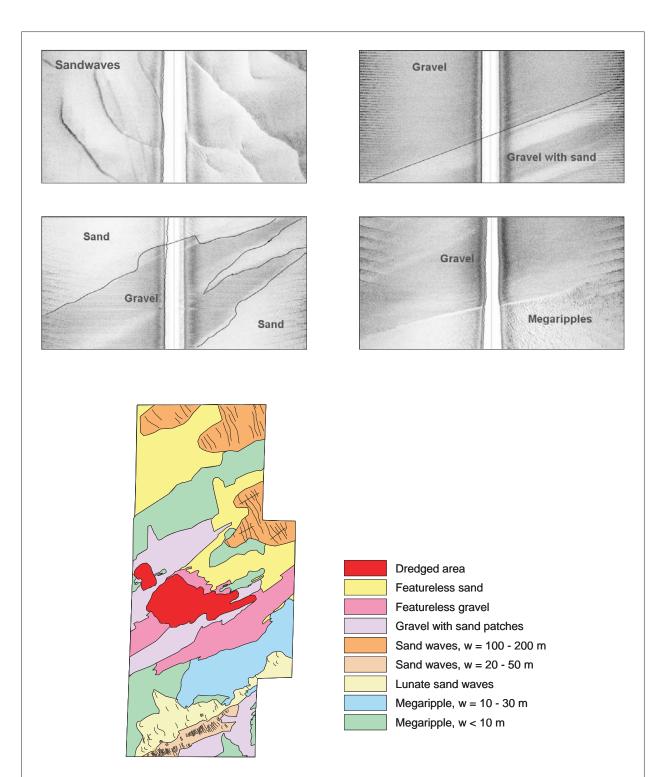
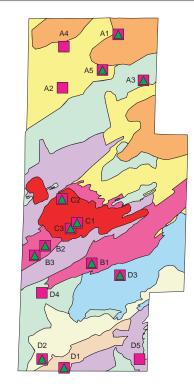


Figure 1.3. Four examples of facies interpretations from sidescan sonar images (400 m swathe width) and the seabed facies map of the Hastings study site derived by interpretation of the sidescan sonar mosaic shown in Figure 1.2. Coloured polygons delineate individual facies, and lines within them mark the crests of individual sand waves (w = wavelength).



**Figure 1.4.** Location of ground-truth sampling stations taken by the prior study (Brown *et al.*, 2001, 2004) overlain on the seabed facies map for Hastings. Symbols as in Figure 1.2.

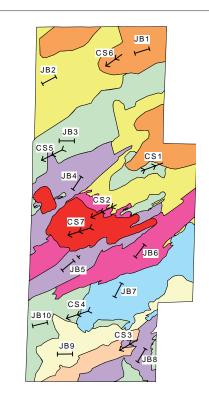


Figure 1.5. Location of ground-truth sampling stations for the present study. Bars indicate beam trawls (JB1 - 10), arrows indicate camera sledge tows (CS1 - 7).

Figure 1.6. Location of Hamon grab samples used for ground-truthing the Hastings facies map, and the 'Fibonacci' sampling pattern designed to test variability within each facies. The angular spiral has six sampling points (numbered boxes) separated by increasing distances (italic numerals), providing 15 lag distances in all.

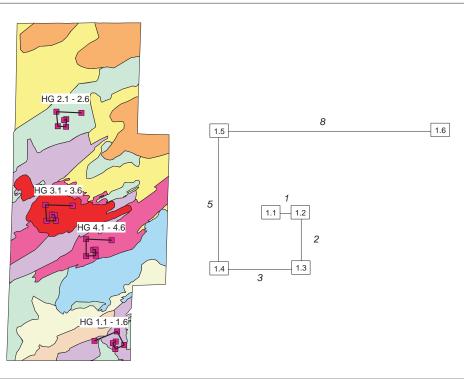
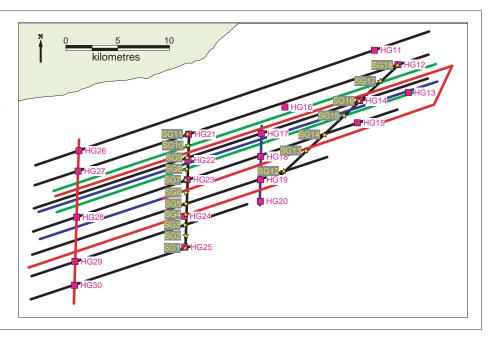


Figure 1.7. Positions and numbering of ground-truth sampling sites in relation to sidescan survey lines. SG = Shipek Grabs (yellow stars), HG = Hamon Grabs (purple squares). Beam trawl samples were also taken at each of the HG locations.



#### 1.4 Results

#### 1.4.1 Hastings study area

#### Facies map

The facies interpretation provided a higher resolution map than the coarser interpretation of the previous study (Brown et al., 2001, 2004). The two interpretations were consistent, showing shallow sand substrates to the north, dredged and undisturbed gravel areas in the centre and deeper sand substrates to the south, but each of the nondredged areas identified by Brown et al. (2001, 2004) was seen to comprise several different facies (Figure 1.3). The shallow sand area contained long wavelength sand waves, featureless sand and short wavelength megaripples. The central area contained both featureless gravel and gravel with sand patches, and two areas of dredged gravel. The deeper sands were separated into two megaripple facies (short and long wavelength), two sand wave facies (lunate and short wavelength) and an area of gravel (with sand patches) at the extreme south east of the site, which had not previously been noted. Considerable detail was evident in the delineation of boundaries between facies, which appear well defined.

#### Video assessment

There was a high degree of agreement between the sediment types observed on the video record derived from the camera sledge tows and those expected from the seabed facies map. Boundaries between facies appeared well defined on the video, generally changing from one type to another in a brief transitional zone over an estimated distance of 10 to 20 metres. Occasionally, boundaries were extremely abrupt, changing in < 5 m distance. The presence/absence of motile and attached epifauna was noted and proved useful in characterising the different facies.

#### Epifaunal analysis

A total of 70 epifaunal taxa were identified in the 10 trawls, with samples from gravel substrates having notably more specious and abundant epifauna, leading to higher indices of richness (Table 1.1). Cluster analysis and multi-dimensional scaling (MDS) ordination identified three major clusters among the samples (Figure 1.8) which were significantly different (ANOSIM, p = 0.1%). These three clusters map to three general sediment types, namely the shallow sands to the north of the dredge site (trawls 1, 2 and 3), deeper sands to the south (trawls 7, 9 and 10) and gravel substrates (both around the dredge site and in deeper water; trawls 4, 5, 6 and 8). Samples from deep sands proved most similar (61.5%; Table 1.2), while the greatest dissimilarity was between the shallow sands and the gravel clusters (72.2%; Table 1.2). The main characterising taxa from each cluster were determined by SIMPER analysis (Table 1.3). Juvenile swimming crabs (Liocarcinus juv.) and shrimps (Crangon sp. and Pontophilus sp.) characterised both shallow and deep sand substrates, but not gravel. Hermit crabs characterised all three clusters, though Pagarus bernhardus was a main species on shallow sands and Anapagarus laevis on deep sands. Both species were also important on gravel substrates, along with another hermit crab, Pagarus prideauxi and its commensal anemone, Adamsia carciniopados. As their common name suggests, two stone crab genera, Macropodia sp. and Hyas sp. (family Majidae) also characterised the gravel substrates, as did the urchin Psammechinus milaris. Two other echinoderms also featured, but were less discriminant; the brittle star, Ophiura albida, was the foremost characterising species of gravels, but was also a major species on deeper sand substrates, while the starfish, Asterias rubens, was the second most characteristic species for shallow sands, and the 8th for gravels. Trawl sample and video records also indicated that attached epifauna (particularly hydroids) were characteristic of gravel substrates.

Table 1.1. Summary statistics for the ten beam trawl epifauna samples (JB1-10) collected in the present study, giving the depth zone of the station, the seabed facies, number of taxa (S), number of individuals (N) and Margalef's index of richness (d).

Sample	Depth zone (m)	Seabed facies	S	N	d
JB1	10-15	sand waves	18	125	3.52
JB2	10-15	featureless sand	9	58	1.97
JB3	10-15	short megaripples	12	24	3.46
JB4	15-20	gravel with sand patches	34	746	4.99
JB5	15-20	gravel with sand patches	32	238	5.66
JB6	15-20	featureless gravel	29	292	4.93
JB7	25-30	long megaripples	21	153	3.98
JB8	40-45	gravel with sand patches	29	446	4.59
JB9	35-40	lunate sand waves	14	161	2.56
JB10	30-35	short megaripples	24	221	4.26

Table 1.2. Results from SIMPER analyses for the beam trawl samples from the Hastings site, showing the average % similarity within cluster groups (shaded cells) and the average % dissimilarity between them.

	Shallow Sands	Gravels	Deep Sand
Shallow Sands	56.2	-	-
Gravels	72.2	55.4	-
Deep Sands	63.3	65.0	61.5

Analysis of the available data gave a similar overall interpretation of the Hastings site as the initial study (Brown et al., 2004), namely that there were three main epifaunal assemblages associated with shallow sands, gravel and deeper sand. A more formal comparison was made between the two studies by analysing a pooled data set, with colonial and rare taxa (total n < 10) excluded. The distinction between the shallow sands, gravel and deep sand substrates persisted, showing a consistent pattern over time, though there was some separation of the samples from the different studies (Figure 1.9). While depth appears to be an important factor in discriminating epifaunal communities of sandy substrates, the importance of sediment type in structuring the communities is highlighted by the association of the deeper gravel site (trawl 8) with the cluster of other gravel sites rather than other deep sites (Figures 1.8 and 1.9) and is further reflected by comparison of the univariate indices among the various sites (Table 1.1)

#### Sediment and infaunal analysis

The composition and variability of sediments and their associated infauna was examined in four of the seabed facies, sampled by 0.1m<sup>2</sup> Hamon grab following the spiral sampling pattern described above (Figure 1.6). The frequency distribution plots of grain size presented in Figure 1.10 provide an overview of sediment variability within each of the facies. Samples from the deep area of gravel with sand patches (HG1) showed least variability, each containing some gravel (-1 to -5 phi) but being dominated by a single mode of medium sand grains (2 phi). Variability was greatest in the dredged gravel area (HG3), some samples having a high gravel content while others (HG3.6) were mostly fine sand (3 phi). In the shallow water megaripple field (HG2) one sample had a far higher gravel content than all others (HG2.6), and in the undredged gravel area (HG4) there was an appreciable difference in gravel particle size between samples (-3, -4, -5 phi). Principal component analysis showed that three grain size categories, namely 3, 2 and -5 phi contributed 94% of total variability between sediment samples.

The spiral sampling pattern revealed notable spatial trends in two of the four seabed facies (Figure 1.10). In the shallow water megaripple field, the composition of sediments and infaunal communities became more dissimilar as distance between samples (lag distance) increased, while in the dredged gravel area, sediment dissimilarity increased with lag distance, but faunal dissimilarity remained consistently high, indicating a 'patchiness' in the faunal distribution. These two facies were therefore considered to be quite heterogeneous. There were no marked spatial trends in the two remaining facies, but a consistently low dissimilarity between sediment samples from the gravel with sand facies (HG1) indicated this area was notably homogeneous.

**Table 1.3.** Results of SIMPER analysis for epifaunal samples from the Hastings site (excluding colonial species) giving the average abundance and contribution to similarity (as % and cumulative %) for the main characterising taxa in clusters representing three general sediment types at the Hastings site.

Sediment type	Taxon	Average abundance	% Contribution	<b>Cumulative</b> %
Shallow water sands	Pagurus bernhardus	19.3	21.8	21.8
	Asterias rubens	8.3	18.0	39.8
	Hinia sp.	7.7	16.2	55.9
	Liocarcinus juv.	3.3	11.2	67.1
	Pontophilus sp.	4.3	10.6	77.7
Gravels	Ophiura albida	85.3	11.2	11.2
	Pagurus bernhardus	24.8	8.9	20.1
	Macropodia sp.	23.8	8.2	28.3
	Pagurus prideauxi	16.0	7.5	35.8
	Psammechinus miliaris	40.5	7.2	42.9
	Adamsia carciniopados	11.8	6.6	49.5
	Anapagurus laevis	20.5	5.3	54.8
	Asterias rubens	17.5	4.8	59.6
	Gobiidae	12.5	4.7	64.3
	Buccinum undatum	9.8	4.2	68.5
	Aequipecten opercularis	8.0	3.9	72.4
	Hyas sp.	4.3	3.3	75.6
Deeper water sands	Liocarcinus juv.	42.0	19.4	19.4
	Crangon sp.	41.3	18.3	37.8
	Ophiura albida	14.7	10.7	48.5
	Echiichthys vipera	13.0	10.3	58.8
	Anapagurus laevis	12.3	9.3	68.1
	Pontophilus sp.	9.3	8.8	76.9

Figure 1.8. Dendrogram and MDS ordination of similarities between epifaunal samples collected in 10 beam trawl tows (JB1-10) from the Hastings site. Contours on the MDS plot represent the 45% similarity line indicated on the dendrogram. Analysis based on Bray-Curtis similarity index of square root transformed abundance data (colonial taxa excluded).

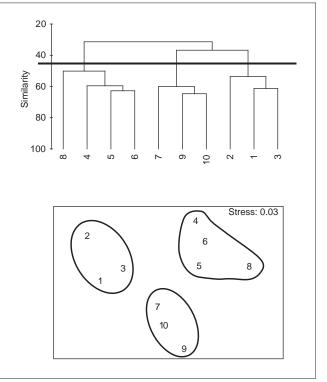
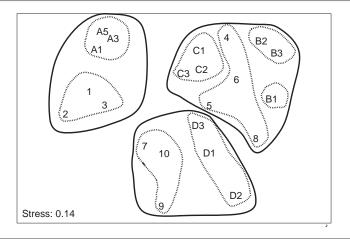


Figure 1.9. MDS plot for the pooled epifauna data from the present study and that of Brown et al. (2004), based on Bray-Curtis similarity index for square root transformed abundance data. Contours represent similarities of 45% (solid line) and 55% (dashed line). Major clusters form for samples from shallow sands (upper left), gravels (upper right) and deeper sands (lower centre), while minor clusters tend to separate samples from the two studies. Site labels are given in Figures 1.4 and 1.5.



A total of 198 taxa were identified from the Hamon grab samples (excluding colonial species). indices highlighted the gross differences in populations between facies (Figure 1.11) indicating an impoverished fauna (low abundance, few species) in both the shallow megaripple field (HG2) and dredged gravel area (HG3), but a rich and diverse community in the undredged gravel (HG4). Multivariate analyses gave a more detailed insight into these differences and how they relate to sediment structure. Figure 1.12 compares the outputs from nonmetric multi-dimensional scaling (MDS) ordination for the sediment grain structure (PSA data) and infaunal community structure (abundance data), showing three clusters among the sediments, but four clusters among the infauna. Both the shallow megaripple field (HG2) and the deeper gravel / patchy sand facies (HG1) had distinct sediment types and distinct communities, while the dredged and undredged gravel facies (HG3 and HG4) had a similar sediment structure but supported distinctly different communities. The two ordinations have a similar spatial distribution of points, and a strong correlation between their underlying (dis)similarity matrices (RELATE routine in PRIMER, rho = 0.434, p = 0.1%) indicated a significant relationship between sediment type and community structure. Analysis of similarities confirmed a highly significant difference among the infaunal communities of the four facies (ANOSIM, global p = 0.1%) and that each had a recognisably different infaunal community (all pair-wise comparisons having R > 0.75).

Species that typify and/or discriminate the different facies were determined using the PRIMER routine SIMPER, which identifies taxa that contribute most to the similarity within and dissimilarity between sample groups (Tables 1.4 and 1.5). Relatively few species characterised the shallow megaripple field and the dredged gravel area, consistent with earlier observation based on univariate indices that these communities were impoverished. The other facies supported more diverse communities, and consequently there are more characterising species and a more even spread in their contribution to the overall similarity. For species that best discriminate between facies, there are numerous examples of highly specific species that occur in one facies but not another (eg Magelona johnstoni present in HG2 but not HG3), and more generalist species whose relative abundance is markedly different between facies (eg Scalibregma inflatum in HG4 vs HG3 and HG4 vs HG2). Sample similarity was greatest in the (undredged) featureless gravel (HG4) and least in the dredged gravel (HG3), indicating respectively the most homogeneous and heterogeneous areas (Table 1.6). The greatest difference in infaunal communities was between the sandy megaripple field (HG2) and the undredged gravel area (HG4) with a dissimilarity of 88.6%.

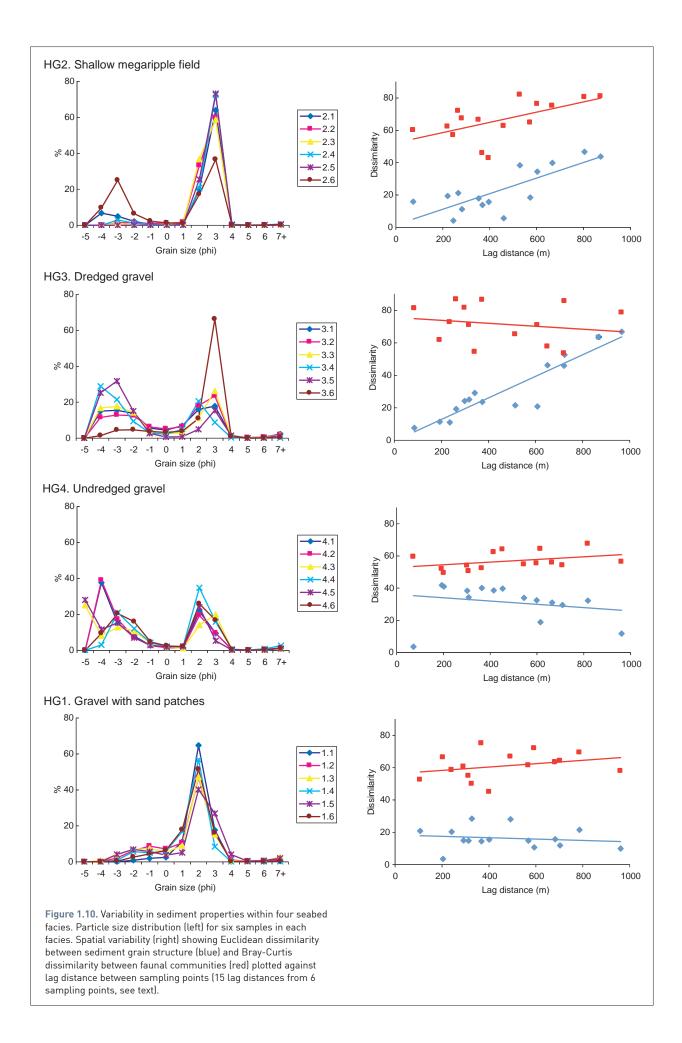
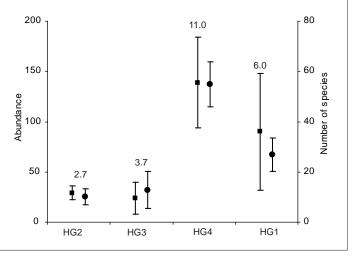
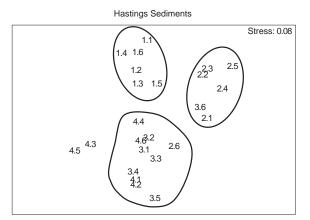
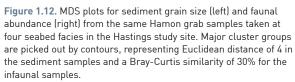
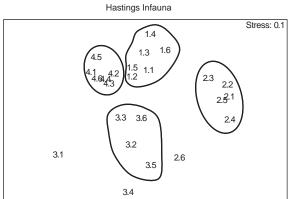


Figure 1.11. Univariate indices of infaunal community structure for four seabed facies in the Hastings study site (as indicated in Figure 1.6), showing mean and 95% confidence intervals for the number of individuals per 0.1 m<sup>2</sup> (squares) and the number of species (circles). Figures indicate the mean Margalef's richness









**Table 1.4.** Results of SIMPER analysis for infaunal samples (excluding colonial species) indicating taxa that most typify four seabed facies at the Hastings site, giving their average abundance and contribution (as % and cumulative %) to the overall similarity between replicates.

Seabed facies	Taxon	Average abundance	% Contribution	Cumulative %
Shallow Megaripple field	Magelona johnstoni	13.2	40.0	40.0
(HG2)	Nephtys cirrosa	2.3	25.7	65.7
	Bathyporeia elegans	2.0	13.3	79.0
	Spiophanes bombyx	2.3	10.0	89.0
	Travisia forbesii	1.2	4.3	93.3
Dredged gravel	Balanus crenatus	63.0	52.0	52.0
(HG3)	Lagis koreni	2.3	10.4	62.4
	Spiophanes bombyx	5.5	10.3	72.7
	Urothoe elegans	1.2	9.2	82.0
	Ampelisca spinipes	1.0	3.8	85.8
	Lanice conchilega	0.7	3.2	89.0
	Cerianthus Iloydii	0.7	2.9	91.9
Featureless gravel	Scalibregma inflatum	10.3	10.8	10.8
(undredged)	Echinocyamus pusillus	8.8	10.1	20.9
(HG4)	Nemertea	4.5	8.0	28.9
	Lumbrineris gracilis	4.2	7.8	36.8
	Timoclea ovata	6.0	7.8	44.6
	Notomastus sp.	4.0	5.8	50.4
	Polycirrus sp.	2.7	5.4	55.8
	Pisidia longicornis	2.7	5.2	61.0
	Lagis koreni	2.7	4.9	65.8
	Ophiura juv.	3.2	4.7	70.6
	Balanus crenatus	5.3	3.5	74.1
	Ampelisca spinipes	3.3	3.4	77.5
Gravel with sand patches	Nemertea	3.0	12.7	12.7
(HG1)	Echinocyamus pusillus	17.5	12.2	24.8
	Spiophanes bombyx	5.2	12.2	37.0
	Lagis koreni	19.8	10.6	47.6
	Scalibregma inflatum	7.5	8.7	56.3
	Spisula juv.	2.3	7.9	64.2
	Ophelia borealis	1.8	7.1	71.3
	Polycirrus sp.	1.2	7.0	78.3

Table 1.5. Results of SIMPER analysis for infaunal samples (excluding colonial species) indicating taxa that most discriminate between four seabed facies at the Hastings site, giving their average abundance and contribution (as % and cumulative %) to the overall dissimilarity between samples.

Seabed facies	Taxon	Average abundance	Average abundance	% Contribution	Cumulative %
		——————————————————————————————————————	- ———— НG3		
HG2 vs HG3	Magelona johnstoni	13.2	0.0	11.7	11.7
1102 13 1100	Balanus crenatus	87.2	63.0	11.5	23.2
	Nephtys cirrosa	2.3	0.3	6.8	30.0
		2.0			
	Bathyporeia elegans		0.0	5.9	35.9
	Urothoe elegans	0.0	1.2	5.3	41.1
	Spiophanes bombyx	2.3	5.5	4.9	46.0
	Lagis koreni	0.2	2.3	4.4	50.5
		HG2	HG4		
HG2 vs HG4	Magelona johnstoni	13.2	0.0	7.1	7.1
	Echinocyamus pusillus	0.0	8.8	5.5	12.5
	Scalibregma inflatum	0.2	10.3	5.1	17.6
	Nephtys cirrosa	2.3	0.0	4.6	22.2
	Timoclea ovata	0.0	6.0	4.5	26.7
	Lumbrineris gracilis	0.0	4.2	4.3	31.0
	Notomastus sp.	0.0	4.0	4.1	35.1
	Balanus crenatus	87.2	5.3	3.9	39.0
	Pisidia longicornis	0.0	2.7	3.5	42.6
	Polycirrus sp.	0.3	2.7	3.4	46.0
	Ophiura juv.	0.0	3.2	3.4	49.4
			0.2	3.4	
	Bathyporeia elegans	2.0		3.4	52.7
		HG2	HG1		
HG2 vs HG1	Magelona johnstoni	13.2	0.0	9.8	9.8
	Echinocyamus pusillus	0.0	17.5	8.0	17.8
	Lagis koreni	0.2	19.8	6.4	24.2
	<i>Spisula</i> juv.	0.0	2.3	5.7	29.9
	Scalibregma inflatum	0.2	7.5	5.3	35.2
	Bathyporeia elegans	2.0	0.0	4.9	40.1
	Nemertea	0.3	3.0	4.5	44.6
	Polycirrus sp.	0.3	1.2	4.4	49.0
	Ophelia borealis	0.5	1.8	4.3	53.3
		HG3	HG4		
HG3 vs HG4	Echinocyamus pusillus	0.0	8.8	6.5	6.5
	Balanus crenatus	63.0	5.3	5.7	12.2
	Timoclea ovata	0.0	6.0	5.4	17.6
	Scalibregma inflatum	1.3	10.3	5.2	22.8
	Polycirrus sp.	0.0	2.7	4.4	27.2
	Pisidia longicornis	0.0	2.7	4.2	31.4
	Nemertea	0.3	4.5	4.1	35.6
	Notomastus sp.	0.3	4.0	4.1	39.6
	Ophiura juv.	0.0	3.2	4.1	43.7
	Lumbrineris gracilis	0.5	4.2	4.0	47.7
	Mysella bidentata	0.2	9.8	3.6	51.3
1100 1104	Delemon o	HG3	HG1	11.1	11 1
HG3 vs HG1	Balanus crenatus	63.0	0.0	11.1	11.1
	Echinocyamus pusillus	0.0	17.5	7.9	19.0
	Spisula juv.	0.2	2.3	5.2	24.2
	Ophelia borealis	0.0	1.8	5.1	29.3
	Nemertea	0.3	3.0	4.9	34.1
	Scalibregma inflatum	1.3	7.5	4.8	38.9
	Polycirrus sp.	0.0	1.2	4.5	43.4
	Lagis koreni	2.3	19.8	4.5	47.9
	Urothoe elegans	1.2	0.0	4.3	52.2

**Table 1.5. continued.** Results of SIMPER analysis for infaunal samples (excluding colonial species) indicating taxa that most discriminate between four seabed facies at the Hastings site, giving their average abundance and contribution (as % and cumulative %) to the overall dissimilarity between samples.

Seabed facies	Taxon	Average abundance	Average abundance	% Contribution	Cumulative %
		HG4	HG1		
HG4 vs HG1	Timoclea ovata	6.0	0.0	6.2	6.2
	Ophelia borealis	0.0	1.8	5.1	11.3
	Pisidia longicornis	2.7	0.0	4.9	16.2
	Balanus crenatus	5.3	0.0	4.7	20.9
	<i>Spisula</i> juv.	0.5	2.3	4.6	25.5
	Notomastus sp.	4.0	0.5	4.5	30.0
	Ampelisca spinipes	3.3	0.0	4.2	34.2
	Lagis koreni	2.7	19.8	4.1	38.2
	Gibbula tumida	3.2	0.0	4.0	42.3
	Galathea intermedia	3.0	0.2	3.7	46.0
	Ophiura juv.	3.2	1.8	3.7	49.7
	Mysella bidentata	9.8	0.8	3.5	53.1

**Table 1.6.** Results from SIMPER analyses for the infaunal samples from four facies at the Hastings site, showing the average % similarity for six replicates within each facies (shaded cells) and the average % dissimilarity between facies.

	Shallow megaripple	Dredged gravel	Featureless gravel	Gravel with sand patches
Shallow megaripple	42.0			
Dredged gravel	84.9	37.3		
Featureless gravel	88.6	70.8	62.4	
Gravel/sand patches	79.3	77.4	53.4	59.5

#### 1.4.2 Broadscale study area

#### Interpolated facies maps

The broadscale study covered an area of ~400 sq km, with the Hastings study site lying in the westernmost third of the area (Figure 1.1). A pattern of 13 parallel sidescan lines were surveyed in a SW-NE orientation over a maximum distance of 40 km, though some were foreshortened due to navigation hazards (static fishing gear) being encountered due south of Dungeness. Figure 1.13 presents the results of the individual interpretations for each of the sidescan sonar tracks, showing the subsets of data used to derive the four corresponding interpolated facies maps. With a total swathe width of 400 m for each track, line spacings of 4, 2 and 1 km represented 18, 33 and 57% density coverage of the area respectively, leaving 82, 66 and 43% of the facies map to be completed by interpolation. At 4 km spacing, few of the facies in adjacent lines appeared to match and the resulting map was considered to be highly speculative. At 2 km spacing, matching between adjacent lines improved, but remained poor in some areas. At 1 km line spacing, data density had improved to the point where the underlying facies pattern could be recognised allowing the majority of interpolation between lines to be made with a high degree of confidence. In the final interpolation based on all the available lines, a coverage of nearly 100% had been achieved along a central corridor having five lines spaced at 0.5 km, but this additional coverage gave only marginal improvements to the interpolation for that area achieved with 1 km line spacing. Two cross lines running N-S were also available and proved valuable in confirming some of the interpolated areas.

#### Ground-truthing – sediment samples

The physical nature of the sediments was determined by particle size analysis (PSA) on the samples collected by Hamon and Shipek grabs (Figure 1.7). A rapid analysis technique was used (no laser sizing), giving the sample composition classified by five particle size groups, namely gravel (G), coarse sand (cS), medium sand (mS), fine sand (fS) and mud (M). There were notable differences in

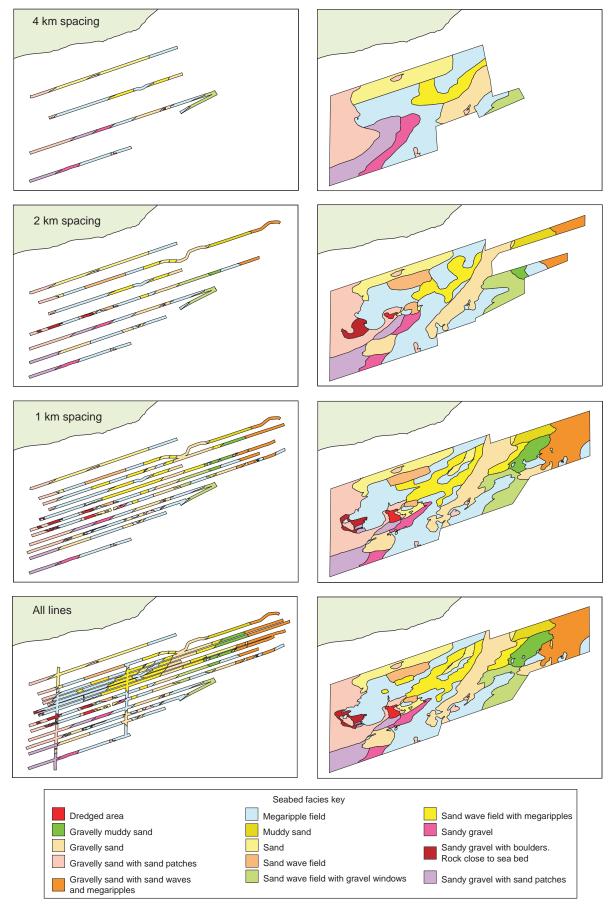


Figure 1.13. Sequence of seabed facies maps (right column) derived by interpolation of interpreted sidescan lines (left column), showing the effect of progressively closer line spacing (top to bottom).

Figure 1.14. Particle size distribution of sediments 100 from Shipek grab (top) and Hamon grab (bottom) sampling stations (as in Figure 1.7). Sediment types 80 are gravel (G), coarse sand (cS), medium sand (mS), fine sand (fS) and mud (M). 60 Percent 10 40 12 20 15 16 18 cS mS Sediment Type 100 80 60 Percent 40 20 21 20 24 26 fS

composition of samples collected by the different gears (Figure 1.14), those from Hamon grabs showing three major sediment forms (well sorted fine sands, moderately sorted medium sands and poorly sorted medium/fine sands) while those from Shipek grabs showed only one (well sorted fine sands). This was evident even between paired Hamon and Shipek samples (ie taken from the same site; not illustrated here) and is attributed to the different penetration depth of the two sampling devices, being ~10 cm for Shipek grabs and ~30 cm for the 0.1m² Hamon Grab. These discrepancies indicate some vertical stratification of sediments that may be important in ground-truthing the seabed facies map and highlights

the importance of understanding the characteristics of different sampling devices.

29 30

Sediment Type

PSA data were re-classified according to the Folk classification (gravel, sand, mud) to enable a more equitable comparison with the descriptions of the seabed facies, which use this same terminology (Figure 1.15). Generally, there was good agreement, apart from a central band running SSW-NNE which was shown on the facies map as gravelly-sand, but samples indicated was muddy-sand (mS at HG16 and 17) and slightly gravelly, sandy-mud ((g)sM at HG24). The original PSA data for these stations show the sand component was almost exclusively fine sand, and the noted discrepancy

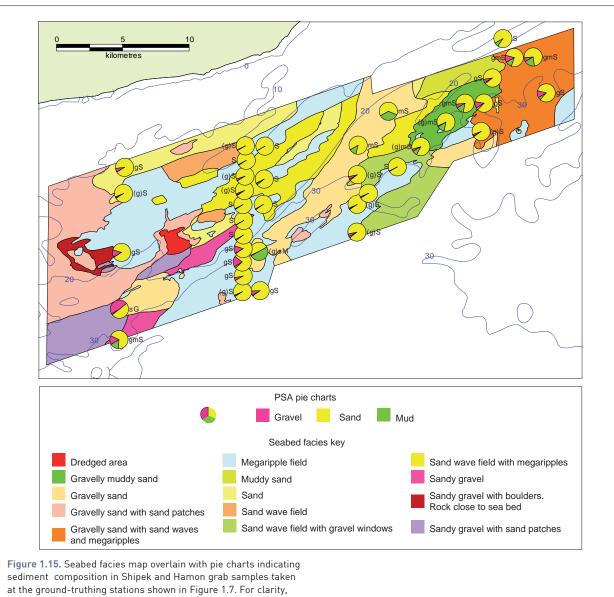


Figure 1.15. Seabed facies map overlain with pie charts indicating sediment composition in Shipek and Hamon grab samples taken at the ground-truthing stations shown in Figure 1.7. For clarity, pie charts are offset from the sampling points (Shipek grabs to the west, Hamon grabs to the east). Letters indicate standard nomenclature for Folk classification of the sediment (eg gmS = gravelly muddy sand, (g)mS = slightly gravelly muddy sand). 10 metre bathymetric contours are indicate in blue.

would be consistent with the presence of a veneer of sand. Alternatively, gravelly-sands and muddy-sands may have a very similar acoustic reflectance, so would be difficult to differentiated on the sidescan image. The facies descriptor 'megaripple field' does not give any reference to sediment type, but infers a predominantly sandy substrate. Figure 1.15 indicates the presence of some gravel in this facies. Video observations of similar facies in the Hastings area showed that gravel particles may be scattered among the sand and can accumulate in the troughs of the megaripples, so the facies map and the PSA analyses are consistent in this respect. The two samples in the NW area (HG 26 and 27) indicate a gravel

component to the sediment not suggested by the facies map; again this could be due to some degree of patchiness which seems likely as a patchy, gravelly-sand facies lies just to the west of the two sample points. At HG 28 it took five attempts to obtain even a small sample (0.5 I), indicating hard ground, which is again consistent with the facies description (sandy-gravel with boulders, rock close to seabed). The sandy-gravel at HG29 is consistent with the facies description, but at HG30 the sample comprised less gravel and a greater amount of sand than indicated by the facies map. This is most likely due to local patchiness and indicates that for optimal analysis several replicates per facies are required.

#### Ground-truthing - infaunal samples

A total of 236 taxa were recorded from the Hamon grab samples taken in the broadscale study area, including 16 colonial taxa that were recorded as 'present' but excluded from the analysis. Many of the taxa were present in low frequencies, so a data subset was selected using the criteria that the total number of individuals across all 20 samples was >= 10; this subset comprised 52 taxa. Station HG28 was omitted from the analysis due to the low sample volume (0.5 l). Similarities between samples were investigated in PRIMER, using cluster analysis and MDS ordination (Figure 1.16) based on the Bray-Curtis similarity index applied to square root transformed abundance data. There were three major clusters (i-iii below), two from shallow waters (<20 m) and one from deeper water (>20 m).

- i) Fine shallow water sands, comprising muddy fine sands (samples HG11, 16 and 17), a fine sand (HG21) and a slightly muddy medium sand (HG22). Faunal density was typically high (a mean of 2874 individuals m<sup>-2</sup>; SD = 197.5), the samples having an average similarity of 39.5% (SIMPER analysis in PRIMER) and were characterised by polychaetes, typically having some form of sand or mucous tube (*Lagis koreni*, *Magelon johnstoni*, *Poecilochaetus serpens*, *Spiophanes bombyx* and *Euclymene oerstedii*), and the small bivalves *Mysella bidentata* (~3 mm long, frequently commensal in tubes and burrows), *Abra alba* and *Phaxas pellucidus*.
- ii) Shallow medium sands, two with approximately equal proportions of medium and fine sand (HG23 and 26) and the other (HG27) with > 80% medium sand. Faunal density was notably low (mean 230 individuals m<sup>-2</sup>; SD = 55.7), the samples having an average similarity of 43.9%, with two active errant species (the amphipod *Bathyporeia elegans* and the polychaete *Nephtys cirrosa*) contributing >20% each to this similarity.
- iii) Deeper water sands and coarse sediments, with some sub-clusters formed by the gravelly muddy sands (HG12, 13, 14 and 24) and slightly gravelly sands (HG15, 18 and 19) and the deepest sandy gravel / gravelly sands (HG20, 25 and 29). Fauna density was typically moderate (mean 1,331 individuals m<sup>-2</sup>; SD = 621.5), with the gravelly sands/sandy gravels showing the greatest richness. Samples had an average similarity of 47.0%, with the first four characterising species being polychaetes, including the deep-burrowing species, *Scalibregma inflatum*, which was common at all but the deepest sites (HG20, 25, 29) and *Lumbrineris gracilis* which is known to be characteristic of muddy sands or gravels. The fifth characterising species was the small

urchin *Echinocyamus pusillus* which is typically found in coarse sands/fine gravels in deep water.

Dissimilarities between these three main clusters were of the order of 70%. The fauna at station HG30 appeared markedly dissimilar to that at other stations (range 84 to 90%), with 93% of individuals being barnacles (*Balanus crenatus*) attached to cobbles.

#### Ground-truthing - epifaunal samples

Beam trawl samples were collected at all stations except 25 and 28, and a total of 112 epifaunal taxa were recorded, including 17 colonial organisms. Data analysis followed the same procedures used for the Hamon grab samples, with a data subset of 51 taxa (having n across all samples >= 10), but employing a 4th root transformation of abundance data, as some taxa were highly numerous (maximum 9,104 *Ophiura albida* in JB14).

The MDS ordination (Figure 1.17) showed two major clusters. The first contained four stations (11, 17, 21 and 14) where epifauna were typically very abundant (max. 11,204 individuals in sample JB17) being dominated by the gastropod Hinia sp., the hermit crab Pagarus bernhardus and two species of brittle star, Ophiura ophiura and O. albida, which accounted for 39% of the similarity between the samples (Table 1.7). Three of these stations (11, 17 and 21) were relatively shallow and had strikingly similar sediments, comprising ~80% or more fine sand (Figure 1.14). The second cluster contained 13 stations where the epifaunal community appeared to be far less abundant (normally in the order of 10<sup>2</sup> individuals per sample) but was characterised by many of the same species as the first cluster, with the notable addition of the small hermit crab Anapagarus laevis in place of the brittle star O. ophiura (Table 1.7). Sediments at these stations were typically the coarser or mixed substrates and three minor clusters were evident (Figure 1.17 - 60% similarity contour), one containing the three sites that had >10% gravel content (13, 29 and 30). Epifauna at station 16 were quite dissimilar from other stations, having the lowest number of taxa (12) but the fourth highest total abundance (N = 2,322), 78% of these being the tube-building polychaete Lagis koreni. This was the only station where the ophiuroid Amphiura filiformis was recorded (n=308).

SIMPER analysis indicated the two main clusters were separated largely on the basis of the relative abundance of the same set of dominant species (as listed above), which tend to be ecological generalists, capable of living in many types of habitat. Such species are not particularly helpful in mapping habitats, and it is more useful to identify

Figure 1.16. Multi-dimensional scaling (MDS) ordination of similarities between macro-infaunal assemblages at ground-truth stations sampled by Hamon grab (HG11 – 30 as in Figure 1.7), with similarity contours at ~30% (solid line) and 50% (dotted line). Bray-Curtis similarity index on square root abundance data, where n >= 10 across all samples, excluding colonial taxa.

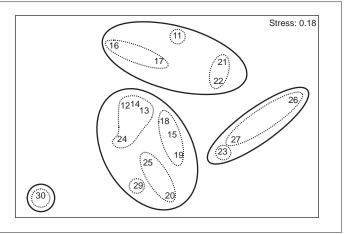
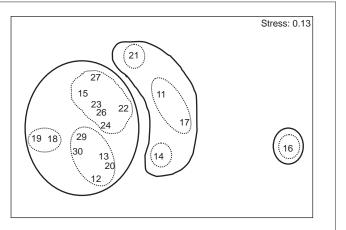


Figure 1.17. Multi-dimensional scaling (MDS) ordination of similarities between macroepifaunal assemblages at ground-truth stations sampled by beam trawls at Hamon grab stations (HG11 – 30, as in Figure 1.7), with similarity contours at ~50% (solid line) and 60% (dotted line). Bray-Curtis similarity index on 4th root abundance data, where n >= 10 across all samples, excluding colonial taxa.



**Table 1.7.** Results of SIMPER analysis for epifauna from beam trawl samples in the broadscale study, grouped as per the two major clusters indicated in Figure 1.17, listing the top ten taxa, their average abundance, the % and cumulative % contribution to average similarity, and the average similarity between samples in a cluster.

Cluster	Taxon	Average abundance	% Contribution	Cumulative %	Average similarity
1	Hinia sp.	1071.00	14.57	14.57	
	Pagurus bernhardus	256.25	11.11	25.68	
	Ophiura ophiura	214.25	7.63	33.31	
	Ophiura albida	3137.50	5.97	39.28	51.48%
	Callionymus sp.	9.00	4.86	44.13	
	Macropodia sp.	16.75	4.63	48.76	
	Pagurus prideauxi	5.50	4.30	53.06	
	Aphrodita aculeata	8.50	4.13	57.19	
	Corbula gibba	71.25	4.10	61.29	
	Gobiidae	25.25	3.96	65.24	
2	Pagurus bernhardus	92.54	12.50	12.50	
	Anapagurus laevis	31.85	8.90	21.40	
	Ophiura albida	123.92	7.74	29.14	
	Hinia sp.	32.00	6.77	35.91	58.16%
	Callionymus sp.	5.92	5.47	41.38	
	Macropodia sp.	10.69	4.76	46.14	
	Aequipecten opercularis	7.38	4.60	50.74	
	Pagurus prideauxi	6.69	4.49	55.23	
	Liocarcinus holsatus	3.92	4.25	59.48	
	Gobiidae	4.00	3.99	63.47	

Table 1.8. Results of SIMPER analysis showing dissimilarity between epifaunal samples in the two major clusters indicated in Figure 1.17, using a presence/absence transformation of abundance data (see text). Listing taxa contributing to the first 40% of the overall dissimilarity (42.21%), their average abundance in each of the sample clusters and the % and cumulative % contribution to the overall dissimilarity between clusters.

Taxon	Cluster 1 Average abundance	Cluster 2 Average abundance	% Contribution	Cumulative %
Aphrodita aculeata	8.50	0.31	4.19	4.19
Phaxus pellucidus	4.00	0.00	3.61	7.80
Acanthocardia sp.	7.00	0.00	3.61	11.41
Limanda limanda	7.50	0.08	3.53	14.94
Abra sp.	113.25	0.08	3.41	18.35
Echinocardium cordatum	9.75	0.15	3.22	21.57
Liocarcinus pusillus	0.75	2.08	3.10	24.68
Buglossidium luteum	5.75	0.92	2.78	27.46
Echiichthys vipera	0.25	1.15	2.72	30.18
Ophiura ophiura	214.25	2.31	2.68	32.86
Aequipecten opercularis	1.50	7.38	2.53	35.39
Crangon allmanni	0.50	3.46	2.51	37.91
Psammechinus miliaris	0.00	5.15	2.45	40.36

species that have greater habitat fidelity or specificity, high fidelity meaning a species is always present in a particular habitat and high specificity meaning it is only found there. Consequently, a second SIMPER analysis was performed using presence/absence data, which removed the influence of abundant species and highlighting those showing high specificity and/or fidelity to the two main clusters (Table 1.8). Some taxa were unique to each cluster; the bivalves *Phaxus pellucidus* and *Acanthocardia* sp. in the first cluster and the urchin *Psammechinus miliaris* in the second.

Two further analytical steps were informative. similarity matrices underlying the MDS plots for infauna and epifauna (Figures 1.16 and 1.17) were compared to see if their patterns were similar, but no significant correlation was found (RELATE routine in PRIMER, Spearman's rank correlation, rho = 0.18, p = 11.9%). However, there were significant correlations between the pattern in each of these faunal elements and the measured environmental variables of sediment type and water depth (Infauna: rho = 0.438, p = 0.4%; Epifauna: rho = 0.457, p = 0.1%). This suggests little linkage between the distribution of infaunal and epifaunal taxa, but that both are significantly influence by environmental factors. The particular suite of environmental variables that best explained the biotic pattern for infauna was the combination of water depth, % gravel, % fine sand and % mud (BIO-ENV routine in PRIMER, Spearman's rank correlation, rho = 0.502). Similar analysis was not considered valid for epifauna, as the beam trawl integrates samples over a strip of seabed where as the environmental data refer to point samples.

#### 1.5 Discussion

#### 1.5.1 Hastings site

Results of the studies in the Hastings site show that seabed facies maps are a reliable spatial representation of the physical nature of the seabed. Video observations confirmed the distinctness of boundaries between facies and justified their depiction as discrete polygons. They also confirmed the accuracy of the facies descriptions and the underlying interpretation of sidescan sonar mosaics. A reference catalogue of facies interpretations from different remote sampling techniques (eg sidescan sonar, multibeam sonar and video observations) would provide a valuable asset in standardising this process and training others in rendering facies maps. The map produced in this study represents a significant advance over the coarser interpretation of the earlier studies (Brown et al., 2001, 2004), providing far greater detail and resolution yet retaining clarity of presentation at this spatial scale.

Epifaunal communities differed between major sediment types in a manner that was consistent with the earlier study of Brown *et al.* (2001, 2004). Greater sample replication is required to fully test the difference in epifaunal communities between facies, but the similarity between three samples collected from sandy gravel facies at different depths (JB4, 5 and 8 in Figure 1.5, 1.11 and 1.12) indicates that substrate type is an important controlling factor in structuring epifaunal communities.

The novel spiral sampling pattern based on the Fibonacci number series also proved successful, proving a structured

approach to assessing physical and biotic variability within a facies and sufficient replication to enable robust comparison between them. Spatial analysis identified facies having distinct spatial trends in grain size and infaunal community structure and provided a means to assess the degree of heterogeneity and/or patchiness within facies. sampling pattern can easily be extended to cover larger spatial areas, and further trials would be valuable to investigate the properties of this sampling design. The analysis of particle size and infaunal abundance data showed a clear relationship between different sediment types (grain structure) and their associated infaunal communities. It was further established that infaunal assemblages were significantly different between each of the four facies sampled. Together, these results demonstrate that seabed facies are ecologically significant units and validate their use in mapping seabed habitats.

#### 1.5.2 Broadscale study

In the series of interpolated facies maps, low density coverage produced some distinctly linear features running parallel with the survey track (eg the most northerly sand facies in the central sector, Figure 1.13). The resolution of the facies map increased with density of sidescan sonar coverage, identifying more discrete facies and improving confidence in boundary definition. The dredged area due south of Hastings first appeared at 2 km line spacing and was more accurately represented at 1 km spacing, while a significant area of sand lying 12 km NE of the dredged area only became apparent at 1 km line spacing. Confidence in the interpolation therefore increases from low, to moderate, to high for the 4, 2 and 1 km interpretations. The latter of these represents approximately 50% density coverage. Further 'in-fill' lines giving 100% coverage resulted in only marginal increase in resolution that would not appear to justify the doubling of survey time and cost when mapping over this broad spatial scale. At this site, a 50% density coverage appears optimal, which equates to 1 km line spacing for a sidescan system with 400 m total swathe width.

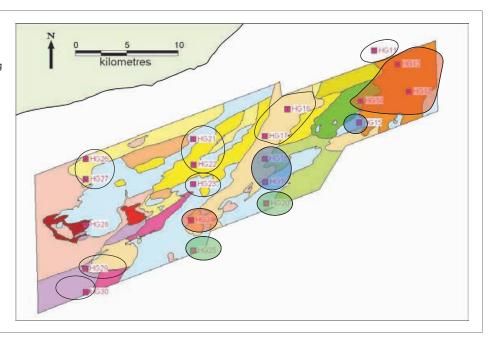
Particle size analysis from sediment samples provided a valuable means of cross-checking the broadscale facies map and confirming that the sediment type had been correctly assigned. A few samples contained more gravel or mud than was anticipated from the facies map, and served to highlight specific areas that might need more detailed investigation, possibly using a complementary method such as video observation to verify the true nature of the sediment. The different sampling characteristics of the Shipek and Hamon grabs led to some disparity in

granulometric analysis, and it appeared that Hamon grab samples were generally more representative as the Shipek grab tended to under-sample the larger size fractions of the sediment (ie the gravel).

The pattern of grab and beam trawl sampling used for the broadscale study followed a strategy that would normally be employed in a pilot survey, selecting a regular grid of sites to cover the survey area with a view to providing preliminary information on the nature of the site. It would not be valid to rely solely on these samples to provide sufficient data for a habitat map, due to the wide spatial separation of the sampling sites and the lack of replication. However, the knowledge gained from this 'pilot' sampling is invaluable in planning and designing the full ground-truth survey, the purpose of which is to target and characterise the distinct areas identified by the facies map (as per the current Hastings study). The pilot survey revealed a range of sediment types over the broadscale area that require different sampling techniques. Most of the area would appear to be amenable to sampling by Hamon grabs, but Day grabs or corers may be more suitable for the areas of sandy mud and drop-down video for the rock outcrops.

Faunal analysis identified some species that are common to a variety of habitats and others that show a higher degree of specificity and fidelity and will therefore be more important in discriminating between habitats. Three major cluster groups were evident for the infauna, separating shallow from deep sites and splitting the shallow sites into fine sands and medium sands. There was clear evidence of further grouping within these clusters, particularly where there was a mud or gravel component in the sediment and mapping these minor clusters onto the facies map gives a first indication of how the spatial relationships between habitats might appear once the full ground-truthing data are available (Figure 1.18). As there was not a close linkage between the distribution patterns of infaunal and epifaunal assemblages, the two elements of the biota will require separate consideration when constructing a habitat map. Epifaunal assemblages were characterised by motile carnivorous species whose habit has little dependence on sediment properties (eg hermit crabs, shrimps, starfish) and so show very low specificity to sediment type. Other environmental variables such as depth and current speed will have a more dominant influence in shaping their distribution, along with the basic habitat requirement for provision of food and shelter. As infaunal species live within the sediments, their physical characteristics are likely to be the principal environmental factor in structuring infaunal communities.

Figure 1.18. Preliminary differentiation of infaunal habitats for the broadscale study site, grouping sampling sites that lay within the 50% similarity contours of Figure 1.16. Where stations are not adjacent to each other, similarity is also indicated by colour coding (eg stations 15, 18 and 19 all lie within the same 50% similarity contour).



This structured approach to surveying the physical and biological conditions of the seabed can be adapted to suit a range of spatial scales and is suitable for both local and regional application. It is recommended that facies maps are constructed using acoustic and sediment data prior to undertaking a directed ground-truth sampling programme which is targeted at each of the apparent seabed facies. Existing acoustic data may be suitable for the basis of the facies map, but if it is not available a dedicated acoustic survey will be required. Directed ground-truth sampling provides more efficient use of available resources, leads to higher confidence in the resulting maps and ensures they

are fit for the purpose of marine monitoring and spatial planning. It is recognised that logistical constraints can influence the timing of surveys and it may be impractical to undertake the acoustic survey prior to faunal and sediment sampling. Under these circumstances, a more intensive sampling programme is recommended to ensure adequate density and replication of samples. In the absence of any knowledge about the distribution of sediments within the area of interest, the sampling programme should be based in a conventional grid design, with a maximum spacing of 1 km between sampling points.

# 2. Patterns of benthic communities in the south western North Sea and their link to environmental parameters and anthropogenic activities

#### 2.1 Introduction

During the past decade, considerable progress has been made in describing structural aspects of the biological communities of the offshore demersal ecosystem, and in particular the determinants of the distribution and abundance of larger-sized, commercial species and the assemblages in which they occur (Dyer et al., 1983; Frauenheim et al., 1989; Rogers et al., 1998; Jennings et al., 1999; Rees et al., 1999; Callaway et al., 2002). There are fewer data sets available for the smaller-sized macrobenthic infauna and meiofauna, and no comparable assessments of community structure of these components on regional scales, despite the important contribution of these taxa to benthic biomass and productivity (Heip et al., 1992; Huys et al., 1992; Künitzer et al., 1992; Heip and Craeymeersch, 1995).

The nematodes are numerically the dominant animal group in most marine meiobenthic habitats. Their ubiquitous distribution as well as their high abundance and diversity often provide more robust data sets than can be obtained from most larger-sized organisms. The ecological and practical advantages associated with using nematodes in benthic biological studies (summarised by Schratzberger et al., 2000) provide good reasons to study nematode communities in the Southwestern North Sea where this important ecosystem component has not been previously studied in detail. The objective of this investigation was therefore to evaluate the contribution made by this group to the benthic community on a regional scale. Specific aims were:

To provide novel information on the species composition and abundance of North Sea meiobenthic nematode assemblages and identify the main environmental variables, biogeographical factors and potential anthropogenic impacts that determine the distribution of community types.

To compare biotic data obtained from this survey with data from the study of other faunal groups in the study area (including macrobenthic infauna, invertebrate epifauna and fish) to provide a more complete assessment of the ecology of the southwestern North Sea.

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This study is part of a larger programme to examine the feasibility of sampling a broad range of ecosystem components in contrasting benthic habitats. The programme is aimed at advising UK Government on the most appropriate components of benthic fauna that could be sampled under long-term monitoring programmes for habitat quality, based on an assessment of their sampling efficiency, biomass and functional contribution to the benthic community.

#### 2.2 Methods

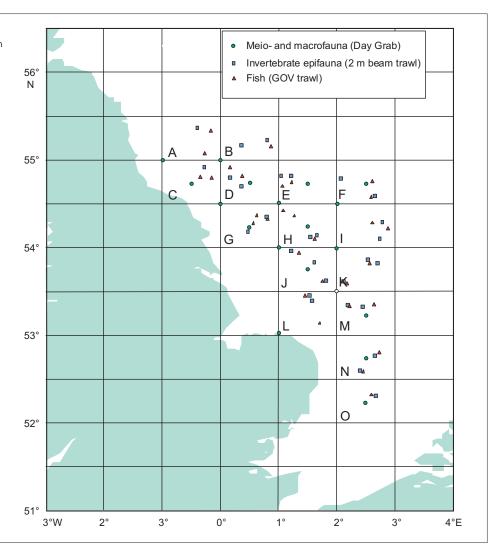
In 2000/2001, nematode assemblages were collected at 19 stations in the southwestern North Sea (Figure 2.1). In order to minimise the potential effect of anthropogenic activities on nematode communities, most stations studied were located away from point-source impacts. At each station, replicate sediment samples were collected from within a 100 m range ring by means of a 0.1 m² Day Grab. From each Day Grab, two sub-samples, one for particle size and organic carbon content analysis and one for the study of meiofauna, were collected with a perspex corer (7.1 cm² surface area) to a depth of 5 cm. The remainder of the sediment was retained for analysis of macrobenthic infauna.

Meiofauna samples were washed onto a 63  $\mu$ m sieve and processed following the extraction protocol described by Somerfield and Warwick (1994). All nematodes were counted and identified to genus or species level. Macrofauna samples were washed through a 1000  $\mu$ m sieve and retained individuals were counted and identified to family or species level.

Patterns observed for meiobenthic nematodes and macrobenthic infauna were compared with those from larger-sized fauna, including invertebrate epifauna and fish. Invertebrate epifauna in the study area was collected in 2000 by means of a 2 m beam trawl, fitted with a chain mat. The trawl was towed for 5 minutes at approximately 1 knot. Sampling of fish communities consisted of 30-minute tows at 4 knots with a Grande Ouverture Verticale (GOV) otter trawl (Figure 2.1). Trawl samples were washed through a 5 mm sieve and fauna was identified to family or species level.

The comparison of nematode records with those of the above mentioned faunal groups was based on a grid

Figure 2.1. Location of stations in the southwestern North Sea sampled for meiobenthic nematodes, macrobenthic infauna, invertebrate epifauna and fish in 2000/01.



of ICES rectangles as not all sampling positions coincided. Nematode and macrofauna sampling stations located at the corner of a rectangle were assigned to the rectangle to the northeast of the original sampling position (Figure 2.1).

A combination of uni- and multivariate statistical analyses were performed, based on species abundance data. For each of these, appropriate statistical tests (eg one-way analysis of variance for univariate indices or one-way analysis of similarities for multivariate data) were applied to determine the significance of differences between replicated samples. The nature of the relationship between environmental variables and univariate community attributes was investigated using simple regression analyses. The relationship between environmental parameters and community structure was assessed by calculating rank correlations between similarity matrices derived from the biotic data and matrices derived from various environmental data (Clarke and Warwick, 1994).

#### 2.3 Results

The study area comprised a temporally stable sedimentary environment decreasing significantly in depth towards the south (F = 4.74, p = 0.04) and the east (F = 9.89, p = 0.04)

p = 0.01). Comparatively lower tidal currents at higher latitudes resulted in increasingly finer sediments (F = 23.00, p < 0.01). Most of the sediment studied consisted of moderately to poorly sorted muddy sands with a generally low silt-clay fraction and low concentrations of trace metals.

Nematode density and species richness increased significantly with decreasing median particle diameter of the sediment. Species richness, diversity and dominance of resident biota were significantly correlated with water depth. High diversity assemblages with a low degree of dominance were typical for deeper waters (> 60 m) in the northern part of the study area. Spatial differences in environmental parameters and biogeographical factors were also highly influential in determining species distribution patterns. The most important environmental measures were water depth, median particle diameter as well as the composition (% sand and silt fraction) and sorting of the substrate. The concentration of most trace metals and carbon content in the sediment bore little relation to univariate community attributes and observed species distribution patterns.

Nematode, macrofauna, epifauna and fish data sets differed in terms of number of taxa and species (Table 2.1).

Table 2.1. Number of taxa, orders and species in the data sets used.

	Size range [µm]	Taxa	Orders	Species
Meiobenthic nematodes	63 – 1000	1	4	169
Macrobenthic infauna	> 1000	10	33	179
Invertebrate epifauna	> 5000	5	23	81
Fish	n/a	1	11	39

Results from the multivariate analyses including all benthic components showed the following characteristics in the multi-dimensional scaling (MDS) ordinations (Figure 2.2):

- For all invertebrate data sets, a clear separation occurred
  for assemblages collected in the northern part of the
  study area (area A to I, positioned on the left hand side
  of the plots) from those sampled in the southern part
  (area J to O, located at the right hand side of the plots).
  Assemblages collected in the southern part of the study
  area displayed a more variable species composition
  compared to those sampled in the northern part.
- Meiobenthic nematode, macrobenthic infauna and invertebrate epifauna communities collected in area A and I, where the finest sediments with highest silt content were found, clustered separately from other more sandy stations in the northern part of the study area
- Fish assemblages sampled in area F were separated from other communities at the 60% Bray-Curtis similarity level. This sampling area was shallower than other locations in the northern part of the study area.

Geographic location of the sampling area and associated differences in water depth were the main factors affecting

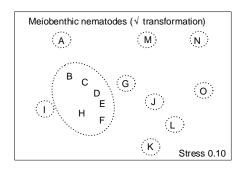
assemblage structure. The influence of factors related to sediment granulometry (eg median particle diameter, silt content etc.) decreased with increasing size and mobility of the faunal group.

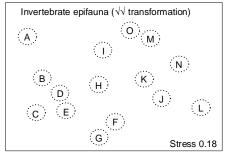
#### 2.4 Discussion

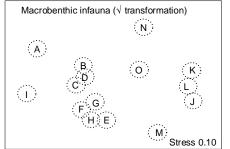
For the first time, extensive regional sampling of benthos in the North Sea has allowed a critical evaluation of the relationship between the nematode fauna and their sedimentary habitat and other, larger-sized faunal groups including macrobenthic infauna, invertebrate epifauna and fish.

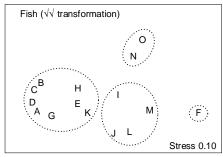
In terms of diversity and species composition, nematode communities encountered were similar to those occurring in comparable environments world-wide. Correlation analyses of nematode populations with the varied substrate occurring in the southwestern North Sea revealed that locations with similar sediment type and water depth were also most similar faunistically. This close association confirms previous studies showing that the median grain size and the silt content of the sediment are often dominant factors that explain a significant part of the variance in species abundance and diversity (review by Heip *et al.*, 1985). In addition, water depth was potentially

Figure 2.2. Non-parametric multi-dimensional scaling (MDS) ordination based on transformed mean abundance of meiobenthic nematodes, macrobenthic infauna, invertebrate epifauna and fish. Lines indicate 60% Bray-Curtis similarity.









important in affecting nematode assemblage structure, most likely because it determined other factors such as the amount and nature of phytoplankton-derived food reaching the seabed and the stability of physico-chemical factors.

Results from the combined analysis of different faunal groups in the southwestern North Sea revealed a notable similarity between species distribution patterns, partly based on common affinities for particular habitat conditions. The topography of the North Sea is an important factor in determining the pattern of water movements and thus the environmental conditions to which fauna and flora are subjected.

Of all faunal groups investigated, patterns in the assemblage structure of benthic invertebrates coincided most closely with sedimentary conditions at the sampling stations. This is partly because the morphology, physiology and life-history characteristics of the benthic in- and epifauna are also strongly influenced by the substrate. In contrast to these smaller-sized, less mobile organisms, fish display a wider range of life histories. In addition to the sedimentary environment, factors related to habitat topography, water flow, proximity to source populations and length of larval

life are important in structuring these assemblages. Whilst benthic infauna and sediment samples were collected at comparable spatial scales, the trawl samples represented organisms from a wider range of substrates. Thus, in view of the relatively small surface area sampled for sediment analyses (ie 0.1 m² Day Grab), results are not fully representative of the variety of habitats and environmental conditions prevailing in the trawled areas.

As evident from sediment, contaminant and faunal analyses, acute effects of human activities on nematode populations seem unlikely, although an effect of fishing on the nematode fauna of the southwestern North Sea cannot be ruled out. The North Sea is one of the most environmentally diverse regions in the northeast Atlantic and this generally precludes a separation of the combined influence of natural environmental parameters from artificial effects of human activities on a North Sea-wide scale. Assessing the relative roles of environmental variables and various anthropogenic activities, all operating on different spatial and temporal scales, in contributing to differences in biological diversity and the structure of benthic communities offers interesting and challenging avenues for future research.

#### Organism health

## 3. Contaminants in marine mammals

#### 3.1 Introduction

Polybrominated diphenyl ethers are a group of flame retardant compounds which have been widely used in recent years. Increasing evidence of their environmental persistence and potential for effects has led to the production and use of the most environmentally mobile polybrominated diphenyl ether (PBDE) formulations being curtailed within the EU and to a call for them to be added to the list of persistent organic pollutants (POPs) listed within the Stockholm Convention which came into force during 2004 (Tanabe, 2004).

Marine mammal blubber samples were collected within the UK Marine Mammal Strandings Programme and derive from 34 animals of twelve species stranded between 1992 and 2002. Post-mortem studies followed strict protocols and tissues selected for analysis came from animals assessed as freshly dead, slightly or moderately decomposed (Law, 1994). Details are given in Table 3.1.

Most of the bottlenose dolphins came from the coastal Moray Firth population, and would have fed on a wide variety of fishes, squid and octopi. Cuvier's and Sowerby's beaked whales feed in deep offshore waters, taking fish and squid. Risso's dolphins feed on the steep upper continental slope and on the shelf, presumably in response to the availability of their preferred prey, squid. The northern bottlenose whale feeds in deep water, eating squid (particularly Gonatus fabricii). Pygmy sperm whales are thought to reside primarily seaward of the continental shelf and prey on cephalopods, crustaceans and fish. Fin and sei whales are pelagic species feeding on euphausiids and fish. Minke whales are mainly pelagic, but will approach coasts. They eat euphausiids and a variety of small schooling fishes, including herring, capelin and sandlance. Humpback whales spend much of the year feeding in shallower waters than other balanopterids, usually feeding and breeding on offshore banks. They mostly feed within 50 m of the surface, eating krill and shoaling fish. Killer whales occur in all oceans and most seas, and in both coastal and oceanic waters. They are the largest of the delphinidae, and have the most diverse diet of all cetaceans. As opportunistic top predators they take a variety of large living prey, including other marine mammals. Hooded seals breed in their primary home range in the western and central North Atlantic Ocean and are rare visitors to the UK. They feed on fish and squid. All of the information on feeding habits and distribution is drawn from Martin (1990) and Reeves et al. (2002).

#### Author: Robin Law

BDE analysis was conducted using established methodology which has also been validated within an international intercomparison programme (de Boer *et al.*, 2001a). Briefly, this involves homogenisation of tissue samples, followed by Soxhlet extraction with n-hexane, clean-up on alumina and silica columns, and determination of BDEs (congeners BDE28, BDE47, BDE66, BDE85, BDE99, BDE100, BDE138, BDE153, BDE154 and BDE183) using coupled gas chromatography-mass spectrometry operating in the electron capture negative ionisation mode. This was conducted using an HP5973 quadrupole instrument, monitoring bromine at 79 and 81 Da. Full analytical quality control protocols involving the use of internal standards and the analysis of blanks and laboratory reference materials within each batch were utilized.

The BDE concentrations determined are given in Table 3.2. BDE209 was not determined, and BDE183 (often considered a marker for the octa-mix polybrominated diphenyl ether commercial formulation) was not detected in any samples. The highest  $\Sigma$ BDE concentration (the sum of the BDE congeners determined) was seen in one of the five killer whales, 16.2 mg kg-1 wet weight (23.8 mg kg<sup>-1</sup> on a lipid basis). The other four killer whales showed much lower  $\Sigma$ BDE concentrations, ranging from 0.49 to 2.0 mg kg<sup>-1</sup> wet weight. In studies undertaken in Canadian and US waters from Washington State to Alaska, two different eco-types of killer whale, "transient" and "resident", were found to occur in all areas (Ylitalo et al., 2001). Concentrations of organochlorine contaminants in blubber biopsy samples were found to be much higher in transient killer whales (marine mammal eating) than in resident animals (fish-eating), apparently due to their differences in diet.  $\Sigma$ BDE concentrations in the bottlenose dolphins ranged from 0.85 to 11.6 mg kg<sup>-1</sup> wet weight (1.1 to 15.6 mg kg<sup>-1</sup> on a lipid basis), the highest value occurring in a female animal (SW1999/136A) found off Findochty, Grampian, in 1999. Lower  $\Sigma$ BDE concentrations were observed in the Sowerby's beaked whales (0.11 to 0.85 mg kg-1 wet weight), and all the other species showed  $\Sigma$ BDE concentrations <0.5 mg kg<sup>-1</sup> wet weight. BDE47 was the dominant congener, followed by BDE99 and BDE100, and contributed 23 – 100% of  $\Sigma$ BDE (Table 3.2; Law et al., 2005).

One of the aims of the UK Marine Mammals Strandings Programme is to investigate links between contaminant levels in tissues and infectious disease mortality. Recent work has confirmed an association between PCB concentrations in blubber and death due to infectious disease, probably

**Table 3.1.** Sampling location and biological information for the marine mammals studied.

Reference no.	Species	Sex	Length (cm)	Date	Location
SW1995/145a	Bottlenose dolphin	М	289	31/12/1995	Hilton of Cadboll, Highland
SW1996/84a	Bottlenose dolphin	Μ	274	06/05/1996	Findochty Harbour, Grampian
SW1996/103c	Bottlenose dolphin	M	270	17/06/1996	Munlochy Bay, Highland
SW1998/18a	Bottlenose dolphin	M	290	25/01/1998	Torrin, Isle of Skye
SW1999/66b	Bottlenose dolphin	F	230	30/03/1999	Shandwick, Highland
SW1999/136A	Bottlenose dolphin	F	260	12/07/1999	off Findochty, Grampian
SW1999/206d	Bottlenose dolphin	M	267	21/12/1999	Pettens Links, Aberdeen, Grampian
SW2001/75a	Bottlenose dolphin	M	314	02/04/2001	Gedin Tailor, Highland
SW2001/111a	Bottlenose dolphin	F	330	23/05/2001	Whiteness Sands, Ardesier, Highland
SW1992/168c	Sowerby's beaked whale	F	490	28/07/1992	Dunvegan, Isle of Skye
SW1992/180a	Sowerby's beaked whale	F	504	19/08/1992	Balintore, Easter Ross
SW1994/136b	Sowerby's beaked whale	M	472	05/08/1994	Burghead Bay, Highland
SW1996/153b	Sowerby's beaked whale	F	459	05/10/1996	Burntisland, Fife
SW1996/153c	Sowerby's beaked whale	M	269	06/10/1996	Hound Point, South Queensferry, Lothian
SW2000/2b	Sowerby's beaked whale	F	414	07/01/2000	Kirkcaldy, Fife
SW2001/81e	Sowerby's beaked whale	F	450	14/04/2001	Brora Beach, Highland
SW2001/210b	Sowerby's beaked whale	M	459	18/09/2001	Rosemarkie, Highland
SW2002/201	Sowerby's beaked whale	F	444	07/06/2002	Praa Sands, Cornwall
SW1994/56a	Killer whale	F	550	15/04/1994	Sandside Bay, Reay, Highland
SW1994/169a	Killer whale	M	610	16/11/1994	Catfirth Voe, Shetland
SW1994/177b4	Killer whale	-	-	07/12/1994	Uyeasound, Unst, Shetland
SW1997/135c	Killer whale	F	610	16/08/1997	Northton, Harris, Western Isles
SW2001/234	Killer whale	M	590	09/10/2001	River Mersey, NW England
SW1999/185d2	Pygmy sperm whale	-	208	18/10/1999	Stranraer, Dumfries and Galloway
SW2002/2	Pygmy sperm whale	F	288	03/01/2002	Thurlestone, Devon
SS2000/105	Hooded seal	F	119	24/10/2000	Skegness, Lincolnshire
SS2000/106	Hooded seal	M	118	30/10/2000	Sea Palling, Norfolk
SW2002/222	Cuvier's beaked whale	M	565	20/06/2002	Walcott, Norfolk
SW2000/200	Fin whale	F	1110	27/11/2000	Morecambe Bay, Lancashire
SW2001/219	Sei whale	-	-	28/09/2001	Cumbria
SW2001/120	Minke whale	Μ	520	10/06/2001	Holy Island, Northumberland
SW2001/204c	Northern bottlenose whale	F	580	04/09/2001	Scallastie Bay, Mull, Strathclyde
SW2001/60	Humpback whale	Μ	1066	21/03/2001	Sandwich Bay, Kent
SW2000/179	Risso's dolphin	F	187	25/10/2000	Morfa Nefyn, Gwynedd

due to immunosuppression although no causal link can be demonstrated (Jepson *et al.*, 2005). Subsequently, we have tried to make a quantitative estimate of the increased risk posed to porpoises of mortality from infectious disease (Hall et *al.*, in press). This is the first time that data from a long-

term marine mammal strandings scheme have been used to estimate any increase in risk. For each 1 mg kg<sup>-1</sup> increase in blubber PCB concentrations (expressed on a lipid basis) the average increase in risk was 2%, with the risk doubling at a blubber PCB concentration of 45 mg kg<sup>-1</sup> lipid.

 $\begin{tabular}{ll} \textbf{Table 3.2.} & \textbf{Concentrations of brominated diphenyl ether congeners in marine mammal blubber samples (mg kg^{-1} wet weight).} \end{tabular}$ 

Species	% lipid	BDE28	BDE47	BDE66	BDE85	BDE99	BDE100
Bottlenose dolphin	74	0.01	2.7	0.01	< 0.02	0.61	0.75
Bottlenose dolphin	58	0.07	5.7	0.05	< 0.03	1.6	1.3
Bottlenose dolphin	77	0.04	3.3	< 0.02	< 0.02	0.46	0.68
Bottlenose dolphin	76	0.04	0.46	0.03	< 0.02	0.1	0.08
Bottlenose dolphin	85	0.05	2.5	0.04	< 0.02	0.67	0.64
Bottlenose dolphin	75	< 0.02	7.5	< 0.02	< 0.02	0.84	2.6
Bottlenose dolphin	85	0.04	5.9	< 0.02	< 0.02	0.92	2.2
Bottlenose dolphin	66	< 0.02	0.84	< 0.02	< 0.02	0.14	0.21
Bottlenose dolphin	66	0.05	2.6	0.03	< 0.02	0.46	0.78
Sowerby's beaked whale	72	< 0.02	0.088	< 0.02	< 0.02	0.034	< 0.02
Sowerby's beaked whale	83	< 0.005	0.08	< 0.005	< 0.02	0.02	0.009
Sowerby's beaked whale	89	< 0.005	0.16	< 0.005	< 0.02	0.09	0.03
Sowerby's beaked whale	90	< 0.02	0.13	< 0.02	< 0.02	0.06	0.03
Sowerby's beaked whale	90	0.03	0.36	0.02	< 0.02	0.14	0.05
Sowerby's beaked whale	87	< 0.02	0.1	< 0.02	< 0.02	0.05	0.02
Sowerby's beaked whale	87	< 0.02	0.17	< 0.02	< 0.02	0.08	0.02
Sowerby's beaked whale	91	0.02	0.4	0.02	< 0.02	0.23	0.08
Sowerby's beaked whale	81	< 0.005	0.13	< 0.005	< 0.02	0.03	0.01
Killer whale	65	0.03	0.33	0.09	< 0.02	0.12	0.13
Killer whale	71	< 0.005	0.29	0.005	< 0.02	0.14	0.05
Killer whale	44	0.02	0.29	0.02	< 0.02	0.14	0.05
Killer whale	68	< 0.02	9.5	< 0.02	< 0.02	3.7	2.0
Killer whale	46	< 0.005	0.47	< 0.005	0.006	0.64	0.47
Pygmy sperm whale	72	< 0.02	0.03	< 0.02	< 0.02	< 0.02	< 0.02
Pygmy sperm whale	78	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	0.011
Hooded seal	91	< 0.005	0.005	< 0.005	< 0.005	< 0.005	< 0.005
Hooded seal	90	< 0.005	0.007	< 0.005	< 0.005	< 0.005	< 0.005
Hooded seal  Species	90 % lipid					< 0.005 <b>∑BDE</b>	< 0.005 BDE47/∑BDE
Species		< 0.005	0.007	< 0.005	< 0.005		
Species Bottlenose dolphin	% lipid	< 0.005 BDE138	0.007 BDE153	< 0.005 BDE154	< 0.005 <b>BDE183</b>	ΣBDE	BDE47/∑BDE
Species  Bottlenose dolphin Bottlenose dolphin	% lipid 74	< 0.005  BDE138  < 0.02	0.007  BDE153  0.06	< 0.005  BDE154  0.13	< 0.005 <b>BDE183</b> < 0.02	<b>∑BDE</b> 4.27	BDE47/∑BDB 0.63
Species  Bottlenose dolphin Bottlenose dolphin Bottlenose dolphin	% lipid 74 58	< 0.005 <b>BDE138</b> < 0.02 < 0.03	0.007  BDE153  0.06 0.12	< 0.005  BDE154  0.13 0.21	< 0.005  BDE183  < 0.02 < 0.03	ΣBDE 4.27 9.05	BDE47/∑BDI 0.63 0.63
Species  Bottlenose dolphin  Bottlenose dolphin  Bottlenose dolphin  Bottlenose dolphin	% <b>lipid</b> 74 58 77	< 0.005  BDE138  < 0.02 < 0.03 < 0.02	0.007  BDE153  0.06 0.12 < 0.02	< 0.005  BDE154  0.13 0.21 0.12	< 0.005  BDE183  < 0.02 < 0.03 < 0.05	ΣBDE  4.27  9.05  4.6	BDE47/∑BDE 0.63 0.63 0.72
Species  Bottlenose dolphin Bottlenose dolphin Bottlenose dolphin Bottlenose dolphin Bottlenose dolphin	% lipid 74 58 77 76	< 0.005  BDE138  < 0.02 < 0.03 < 0.02 0.05	0.007  BDE153  0.06 0.12 < 0.02 0.03	< 0.005  BDE154  0.13 0.21 0.12 0.06	< 0.005  BDE183  < 0.02 < 0.03 < 0.05 < 0.05	ΣBDE  4.27 9.05 4.6 0.85 4.13	BDE47/∑BDE 0.63 0.63 0.72 0.54 0.61
Species  Bottlenose dolphin Bottlenose dolphin Bottlenose dolphin Bottlenose dolphin Bottlenose dolphin Bottlenose dolphin	% lipid 74 58 77 76 85	< 0.005  BDE138  < 0.02 < 0.03 < 0.02 0.05 < 0.02	0.007  BDE153  0.06 0.12 < 0.02 0.03 0.09	< 0.005  BDE154  0.13 0.21 0.12 0.06 0.14	< 0.005  BDE183  < 0.02 < 0.03 < 0.05 < 0.05 < 0.05	ΣBDE  4.27 9.05 4.6 0.85	BDE47/∑BDI 0.63 0.63 0.72 0.54
Species  Bottlenose dolphin	% lipid  74  58  77  76  85  75  85	< 0.005  BDE138  < 0.02 < 0.03 < 0.02 0.05 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02	0.007  BDE153  0.06 0.12 < 0.02 0.03 0.09 0.17 0.17	< 0.005  BDE154  0.13 0.21 0.12 0.06 0.14 0.45 0.45	< 0.005  BDE183  < 0.02 < 0.03 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05	<b>ΣBDE</b> 4.27 9.05 4.6 0.85 4.13 11.56 9.68	BDE47/∑BDI  0.63 0.63 0.72 0.54 0.61 0.65 0.61
Species  Bottlenose dolphin	% lipid  74  58  77  76  85  75  85  66	< 0.005  BDE138  < 0.02 < 0.03 < 0.02 0.05 < 0.02 < 0.02 < 0.02 < 0.02	0.007  BDE153  0.06 0.12 < 0.02 0.03 0.09 0.17 0.17 < 0.02	< 0.005  BDE154  0.13 0.21 0.12 0.06 0.14 0.45	< 0.005  BDE183  < 0.02 < 0.03 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05	ΣBDE  4.27 9.05 4.6 0.85 4.13 11.56 9.68 1.59	BDE47/∑BDE 0.63 0.63 0.72 0.54 0.61 0.65 0.61 0.53
Bottlenose dolphin	% lipid  74  58  77  76  85  75  85	< 0.005  BDE138  < 0.02 < 0.03 < 0.02 0.05 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02	0.007  BDE153  0.06 0.12 < 0.02 0.03 0.09 0.17 0.17	< 0.005  BDE154  0.13 0.21 0.12 0.06 0.14 0.45 0.45 0.14	< 0.005  BDE183  < 0.02 < 0.03 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05	<b>ΣBDE</b> 4.27 9.05 4.6 0.85 4.13 11.56 9.68	BDE47/∑BDE 0.63 0.63 0.72 0.54 0.61 0.65 0.61
Species  Bottlenose dolphin	% lipid  74 58 77 76 85 75 85 66 66 72	< 0.005  BDE138  < 0.02 < 0.03 < 0.02 0.05 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02	0.007  BDE153  0.06 0.12 < 0.02 0.03 0.09 0.17 0.17 < 0.02 0.1 < 0.02	< 0.005  BDE154  0.13 0.21 0.12 0.06 0.14 0.45 0.45 0.14 0.19 < 0.02	< 0.005  BDE183  < 0.02 < 0.03 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05	ΣBDE  4.27 9.05 4.6 0.85 4.13 11.56 9.68 1.59 4.21 0.122	BDE47/∑BDI  0.63 0.63 0.72 0.54 0.61 0.65 0.61 0.53 0.62 0.72
Bottlenose dolphin	% lipid  74  58  77  76  85  75  85  66  66  72  83	< 0.005  BDE138  < 0.02 < 0.03 < 0.02 0.05 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02	0.007  BDE153  0.06 0.12 < 0.02 0.03 0.09 0.17 0.17 < 0.02 0.1 < 0.02 < 0.005	< 0.005  BDE154  0.13 0.21 0.12 0.06 0.14 0.45 0.45 0.14 0.19	< 0.005  BDE183  < 0.02 < 0.03 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05	ΣBDE  4.27 9.05 4.6 0.85 4.13 11.56 9.68 1.59 4.21 0.122 0.109	BDE47/∑BDI  0.63 0.63 0.72 0.54 0.61 0.65 0.61 0.53 0.62 0.72 0.73
Bottlenose dolphin Sowerby's beaked whale Sowerby's beaked whale	% lipid  74  58  77  76  85  75  85  66  66  72  83  89	< 0.005  BDE138  < 0.02 < 0.03 < 0.02 0.05 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.002 < 0.002 < 0.002 < 0.002 < 0.002	0.007  BDE153  0.06 0.12 < 0.02 0.03 0.09 0.17 0.17 < 0.02 0.1 < 0.02 < 0.005 < 0.005	< 0.005  BDE154  0.13 0.21 0.12 0.06 0.14 0.45 0.45 0.14 0.19 < 0.02 < 0.005 0.007	< 0.005  BDE183  < 0.02 < 0.03 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05	ΣBDE  4.27 9.05 4.6 0.85 4.13 11.56 9.68 1.59 4.21 0.122 0.109 0.287	BDE47/∑BDI  0.63 0.63 0.72 0.54 0.61 0.65 0.61 0.53 0.62 0.72 0.73 0.56
Bottlenose dolphin Sowerby's beaked whale Sowerby's beaked whale Sowerby's beaked whale	% lipid  74  58  77  76  85  75  85  66  66  72  83  89  90	< 0.005  BDE138  < 0.02 < 0.03 < 0.02 0.05 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02	0.007  BDE153  0.06 0.12 < 0.02 0.03 0.09 0.17 0.17 < 0.02 0.1 < 0.02 < 0.005 < 0.005 < 0.002	< 0.005  BDE154  0.13 0.21 0.12 0.06 0.14 0.45 0.45 0.14 0.19 < 0.02 < 0.005 0.007 0.03	< 0.005  BDE183  < 0.02 < 0.03 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05	ΣBDE  4.27 9.05 4.6 0.85 4.13 11.56 9.68 1.59 4.21 0.122 0.109 0.287 0.25	BDE47/∑BDI  0.63 0.63 0.72 0.54 0.61 0.65 0.61 0.53 0.62 0.72 0.73 0.56 0.52
Species  Bottlenose dolphin Bowerby's beaked whale Sowerby's beaked whale Sowerby's beaked whale Sowerby's beaked whale Sowerby's beaked whale	% lipid  74  58  77  76  85  75  85  66  66  72  83  89  90  90	< 0.005  BDE138  < 0.02 < 0.03 < 0.02 0.05 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02	0.007  BDE153  0.06 0.12 < 0.02 0.03 0.09 0.17 0.17 < 0.02 0.1 < 0.02 < 0.005 < 0.005	< 0.005  BDE154  0.13 0.21 0.12 0.06 0.14 0.45 0.14 0.19 < 0.02 < 0.005 0.007 0.03 0.03	< 0.005  BDE183  < 0.02 < 0.03 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05	ΣBDE  4.27 9.05 4.6 0.85 4.13 11.56 9.68 1.59 4.21 0.122 0.109 0.287 0.25 0.65	BDE47/∑BDI  0.63 0.63 0.72 0.54 0.61 0.65 0.61 0.53 0.62 0.72 0.73 0.56 0.52 0.55
Bottlenose dolphin	% lipid  74 58 77 76 85 75 85 66 66 72 83 89 90 90 87	< 0.005  BDE138  < 0.02 < 0.03 < 0.02 0.05 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02	0.007  BDE153  0.06 0.12 < 0.02 0.03 0.09 0.17 0.17 < 0.02 < 0.002 < 0.005 < 0.005 < 0.005 < 0.02 0.02 < 0.02	< 0.005  BDE154  0.13 0.21 0.12 0.06 0.14 0.45 0.45 0.14 0.19 < 0.02 < 0.005 0.007 0.03 0.03 0.03	< 0.005  BDE183  < 0.02 < 0.03 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05	ΣBDE  4.27 9.05 4.6 0.85 4.13 11.56 9.68 1.59 4.21 0.122 0.109 0.287 0.25 0.65 0.2	BDE47/∑BDI  0.63 0.63 0.72 0.54 0.61 0.65 0.61 0.53 0.62 0.72 0.73 0.56 0.52 0.55 0.50
Bottlenose dolphin	% lipid  74 58 77 76 85 75 85 66 66 72 83 89 90 90 87 87	< 0.005  BDE138  < 0.02 < 0.03 < 0.02 0.05 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02	0.007  BDE153  0.06 0.12 < 0.02 0.03 0.09 0.17 0.17 < 0.02 0.01 < 0.02 < 0.005 < 0.005 < 0.002 0.02 0.03	< 0.005  BDE154  0.13 0.21 0.12 0.06 0.14 0.45 0.45 0.14 0.19 < 0.02 < 0.005 0.007 0.03 0.03 0.03 0.03	< 0.005  BDE183  < 0.02 < 0.03 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05	ΣBDE  4.27 9.05 4.6 0.85 4.13 11.56 9.68 1.59 4.21 0.122 0.109 0.287 0.25 0.65 0.2 0.33	BDE47/∑BDE  0.63 0.63 0.72 0.54 0.61 0.65 0.61 0.53 0.62 0.72 0.73 0.56 0.52 0.55 0.50 0.52
Bottlenose dolphin Bottlenose do	% lipid  74  58  77  76  85  75  85  66  66  72  83  89  90  90  87  87  91	< 0.005  BDE138  < 0.02 < 0.03 < 0.02 0.05 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02	0.007  BDE153  0.06 0.12 < 0.02 0.03 0.09 0.17 0.17 < 0.02 0.1 < 0.02 < 0.005 < 0.005 < 0.02 0.03 0.05	< 0.005  BDE154  0.13 0.21 0.12 0.06 0.14 0.45 0.45 0.14 0.19 < 0.02 < 0.005 0.007 0.03 0.03 0.03 0.03 0.05	< 0.005  BDE183  < 0.02 < 0.03 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.02 < 0.02 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05	ΣBDE  4.27 9.05 4.6 0.85 4.13 11.56 9.68 1.59 4.21 0.122 0.109 0.287 0.25 0.65 0.2 0.33 0.85	BDE47/∑BDE  0.63 0.63 0.72 0.54 0.61 0.65 0.61 0.53 0.62 0.72 0.73 0.56 0.52 0.55 0.50 0.52 0.47
Bottlenose dolphin Bottlenose do	% lipid  74 58 77 76 85 75 85 66 66 72 83 89 90 90 87 87 87 91 81	< 0.005  BDE138  < 0.02 < 0.03 < 0.02 0.05 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02	0.007  BDE153  0.06 0.12 < 0.02 0.03 0.09 0.17 0.17 < 0.02 0.01 < 0.005 < 0.005 < 0.002 0.03 0.05 < 0.005	< 0.005  BDE154  0.13 0.21 0.12 0.06 0.14 0.45 0.45 0.14 0.19 < 0.02 < 0.005 0.007 0.03 0.03 0.03 0.03 0.03 0.05 < 0.005	< 0.005  BDE183  < 0.02 < 0.03 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.02 < 0.02 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.005 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05	ΣBDE  4.27 9.05 4.6 0.85 4.13 11.56 9.68 1.59 4.21 0.122 0.109 0.287 0.25 0.65 0.2 0.33 0.85 0.17	BDE47/∑BDE  0.63 0.63 0.72 0.54 0.61 0.65 0.61 0.53 0.62 0.72 0.73 0.56 0.52 0.55 0.50 0.52 0.47 0.76
Bottlenose dolphin Bottlenose do	% lipid  74  58  77  76  85  75  85  66  66  72  83  89  90  90  87  87  91	< 0.005  BDE138  < 0.02 < 0.03 < 0.02 0.05 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.01 < 0.02 < 0.02 < 0.01 < 0.02 < 0.02 < 0.01 < 0.01 < 0.02 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01	0.007  BDE153  0.06 0.12 < 0.02 0.03 0.09 0.17 0.17 < 0.02 0.1 < 0.02 < 0.005 < 0.005 < 0.02 0.03 0.05 < 0.005 0.03	< 0.005  BDE154  0.13 0.21 0.12 0.06 0.14 0.45 0.45 0.14 0.19 < 0.02 < 0.005 0.007 0.03 0.03 0.03 0.03 0.03 0.05 < 0.005 0.12	< 0.005  BDE183  < 0.02 < 0.03 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.02 < 0.02 < 0.02 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.005 < 0.005 < 0.005 < 0.005 < 0.005 < 0.005 < 0.005 < 0.005 < 0.005 < 0.005 < 0.005 < 0.005 < 0.005 < 0.005 < 0.005 < 0.005 < 0.005 < 0.005 < 0.005 < 0.005 < 0.005 < 0.005 < 0.005 < 0.005 < 0.002 < 0.002	ΣBDE  4.27 9.05 4.6 0.85 4.13 11.56 9.68 1.59 4.21 0.122 0.109 0.287 0.25 0.65 0.2 0.33 0.85 0.17 0.96	BDE47/∑BDE  0.63 0.63 0.72 0.54 0.61 0.65 0.61 0.53 0.62 0.72 0.73 0.56 0.52 0.55 0.50 0.52 0.47 0.76 0.34
Bottlenose dolphin Bottlenose do	% lipid  74 58 77 76 85 75 85 66 66 72 83 89 90 90 87 87 87 91 81 65 71	< 0.005  BDE138  < 0.02 < 0.03 < 0.02 0.05 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.01 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02	0.007  BDE153  0.06 0.12 < 0.02 0.03 0.09 0.17 0.17 < 0.02 < 0.005 < 0.005 < 0.005 < 0.02 0.03 0.05 < 0.005 < 0.005 0.03 < 0.005	< 0.005  BDE154  0.13 0.21 0.12 0.06 0.14 0.45 0.45 0.14 0.19 < 0.02 < 0.005 0.007 0.03 0.03 0.03 0.03 0.03 0.05 < 0.005 0.12 < 0.005	< 0.005  BDE183  < 0.02 < 0.03 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.02 < 0.02 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.02 < 0.02	ΣBDE  4.27 9.05 4.6 0.85 4.13 11.56 9.68 1.59 4.21 0.122 0.109 0.287 0.25 0.65 0.2 0.33 0.85 0.17 0.96 0.485	BDE47/∑BDE  0.63 0.63 0.72 0.54 0.61 0.65 0.61 0.53 0.62 0.72 0.73 0.56 0.52 0.55 0.50 0.52 0.47 0.76 0.34 0.60
Bottlenose dolphin Bottlenose do	% lipid  74 58 77 76 85 75 85 66 66 72 83 89 90 90 87 87 91 81 65 71 44	< 0.005  BDE138  < 0.02 < 0.03 < 0.02 0.05 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.002 < 0.002 < 0.002 < 0.002 < 0.002 < 0.002 < 0.002 < 0.002 < 0.002 < 0.002 < 0.002 < 0.002 < 0.002 < 0.002 < 0.002 < 0.002 < 0.002 < 0.002 < 0.002 < 0.002 < 0.002 < 0.002 < 0.002 < 0.002 < 0.002 < 0.002 < 0.002 < 0.002 < 0.002 < 0.002 < 0.002 < 0.002	0.007  BDE153  0.06 0.12 < 0.02 0.03 0.09 0.17 0.17 < 0.02 0.1 < 0.02 < 0.005 < 0.005 < 0.02 0.03 0.05 < 0.005 0.03 < 0.005 0.03	< 0.005  BDE154  0.13 0.21 0.12 0.06 0.14 0.45 0.45 0.14 0.19 < 0.02 < 0.005 0.007 0.03 0.03 0.03 0.03 0.03 0.05 < 0.005 0.12 < 0.005 0.03	< 0.005  BDE183  < 0.02 < 0.03 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.02 < 0.02 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05	ΣBDE  4.27 9.05 4.6 0.85 4.13 11.56 9.68 1.59 4.21 0.122 0.109 0.287 0.25 0.65 0.2 0.33 0.85 0.17 0.96 0.485 0.61	BDE47/∑BDI  0.63 0.63 0.72 0.54 0.61 0.65 0.61 0.53 0.62 0.72 0.73 0.56 0.52 0.55 0.50 0.52 0.47 0.76 0.34 0.60 0.48
Bottlenose dolphin Bottlenose do	% lipid  74 58 77 76 85 75 85 66 66 72 83 89 90 90 87 87 91 81 65 71 44 68	< 0.005  BDE138  < 0.02 < 0.03 < 0.02 0.05 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.03 < 0.02 < 0.002 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02	0.007  BDE153  0.06 0.12 < 0.02 0.03 0.09 0.17 0.17 < 0.02 0.1 < 0.02 < 0.005 < 0.005 < 0.02 0.03 0.05 < 0.005 0.03 < 0.005 0.03 0.047	< 0.005  BDE154  0.13 0.21 0.12 0.06 0.14 0.45 0.45 0.14 0.19 < 0.02 < 0.005 0.007 0.03 0.03 0.03 0.03 0.05 < 0.005 0.12 < 0.005 0.03 0.03 0.05 < 0.005 0.12	< 0.005  BDE183  < 0.02 < 0.03 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.02 < 0.02 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.005 < 0.005 < 0.005 < 0.005 < 0.005 < 0.005 < 0.005 < 0.005 < 0.005	2 BDE  4.27 9.05 4.6 0.85 4.13 11.56 9.68 1.59 4.21 0.122 0.109 0.287 0.25 0.65 0.2 0.33 0.85 0.17 0.96 0.485 0.61 16.19	BDE47/∑BDE  0.63 0.63 0.72 0.54 0.61 0.65 0.61 0.53 0.62 0.72 0.73 0.56 0.52 0.55 0.50 0.52 0.47 0.76 0.34 0.60 0.48 0.59
Bottlenose dolphin Bottlenose do	% lipid  74 58 77 76 85 75 85 66 66 72 83 89 90 90 87 87 91 81 65 71 44 68 46	< 0.005  BDE138  < 0.02 < 0.03 < 0.02 0.05 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02	0.007  BDE153  0.06 0.12 < 0.02 0.03 0.09 0.17 0.17 < 0.02 0.01 < 0.02 < 0.005 < 0.005 < 0.02 0.03 0.05 < 0.005 0.03 < 0.005 0.03 0.47 0.064	< 0.005  BDE154  0.13 0.21 0.12 0.06 0.14 0.45 0.45 0.14 0.19 < 0.02 < 0.005 0.007 0.03 0.03 0.03 0.03 0.03 0.05 < 0.005 0.12 < 0.005 0.03 0.52 0.12	< 0.005  BDE183  < 0.02 < 0.03 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.02 < 0.02 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.005 < 0.005 < 0.005 < 0.005 < 0.005 < 0.005 < 0.005	ΣBDE  4.27 9.05 4.6 0.85 4.13 11.56 9.68 1.59 4.21 0.122 0.109 0.287 0.25 0.65 0.2 0.33 0.85 0.17 0.96 0.485 0.61 16.19 2.01	BDE47/∑BDI  0.63 0.63 0.72 0.54 0.61 0.65 0.61 0.53 0.62 0.72 0.73 0.56 0.52 0.55 0.50 0.52 0.47 0.76 0.34 0.60 0.48 0.59 0.23
Bottlenose dolphin Bottlenose do	% lipid  74 58 77 76 85 75 85 66 66 72 83 89 90 90 87 87 91 81 65 71 44 68 46 72	< 0.005  BDE138  < 0.02 < 0.03 < 0.02 0.05 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.03 < 0.02 0.24 < 0.05	0.007  BDE153  0.06 0.12 < 0.02 0.03 0.09 0.17 0.17 < 0.02 0.01 < 0.005 < 0.005 < 0.002 0.03 0.05 < 0.005 0.03 < 0.005 0.03 < 0.005 0.03 < 0.005 0.03 < 0.005 0.03 < 0.005 0.03 0.47 0.064 < 0.02	< 0.005  BDE154  0.13 0.21 0.12 0.06 0.14 0.45 0.45 0.14 0.19 < 0.002 < 0.005 0.007 0.03 0.03 0.03 0.03 0.05 < 0.005 0.12 < 0.005 0.03 0.52 0.12 < 0.02 < 0.02	< 0.005  BDE183  < 0.02 < 0.03 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.005 < 0.02 < 0.02 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.005 < 0.005 < 0.005 < 0.005 < 0.005 < 0.005 < 0.005 < 0.005 < 0.005 < 0.005 < 0.005 < 0.005	2BDE  4.27 9.05 4.6 0.85 4.13 11.56 9.68 1.59 4.21 0.122 0.109 0.287 0.25 0.65 0.2 0.33 0.85 0.17 0.96 0.485 0.61 16.19 2.01 0.03	BDE47/∑BDE  0.63 0.63 0.72 0.54 0.61 0.65 0.61 0.53 0.62 0.72 0.73 0.56 0.52 0.55 0.50 0.52 0.47 0.76 0.34 0.60 0.48 0.59
	% lipid  74 58 77 76 85 75 85 66 66 72 83 89 90 90 87 87 91 81 65 71 44 68 46	< 0.005  BDE138  < 0.02 < 0.03 < 0.02 0.05 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02 < 0.02	0.007  BDE153  0.06 0.12 < 0.02 0.03 0.09 0.17 0.17 < 0.02 0.01 < 0.02 < 0.005 < 0.005 < 0.02 0.03 0.05 < 0.005 0.03 < 0.005 0.03 0.47 0.064	< 0.005  BDE154  0.13 0.21 0.12 0.06 0.14 0.45 0.45 0.14 0.19 < 0.02 < 0.005 0.007 0.03 0.03 0.03 0.03 0.03 0.05 < 0.005 0.12 < 0.005 0.03 0.52 0.12	< 0.005  BDE183  < 0.02 < 0.03 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.02 < 0.02 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.005 < 0.005 < 0.005 < 0.005 < 0.005 < 0.005 < 0.005	ΣBDE  4.27 9.05 4.6 0.85 4.13 11.56 9.68 1.59 4.21 0.122 0.109 0.287 0.25 0.65 0.2 0.33 0.85 0.17 0.96 0.485 0.61 16.19 2.01	BDE47/∑BDE  0.63 0.63 0.72 0.54 0.61 0.65 0.61 0.53 0.62 0.72 0.73 0.56 0.52 0.55 0.50 0.52 0.47 0.76 0.34 0.60 0.48 0.59 0.23

# 4. DNA adducts analysis and histopathological biomarkers in European flounder (*Platichthys flesus*) sampled from UK estuaries

Authors: Brett Lyons, Steve Feist and Grant Stentiford

#### 4.1 Introduction

The increasing emphasis on the assessment and monitoring of estuarine ecosystems has highlighted the need to deploy appropriate biological indices and measurements for these locations. Due to their close association with contaminated sediments, benthic flatfish species have been commonly used as bioindicator organisms in monitoring programmes (Stein et al., 1990; Myers et al., 1998; Kirby et al., 1999). The European flounder (Platichthys flesus) is one such benthic marine flatfish common to UK coastal waterways, and consequently it is routinely used as a model species for biological effects monitoring in European estuarine systems (Kirby et al., 1999; Lyons et al., 1999; Stentiford et al., 2003). The formation of DNA adducts in selected aquatic bioindicator species has been extensively used as a biomarker of genotoxin exposure (Varanasi et al., 1989; Ericson et al., 1998; Lyons et al., 1999). The majority of studies to date have used the 32P-postlabelling technique for detecting DNA adducts and studies with fish have shown that they persist for many months once formed and are therefore particularly suited to monitoring chronic exposure to genotoxic contaminants (Gupta et al., 1982; Sikka et al., 1990; French et al., 1996).

Fish diseases and pathologies, with a broad range of aetiologies, are increasingly being used as indicators of environmental stress (Stentiford *et al.*, 2003). Additionally, histological biomarkers provide a powerful tool to detect and characterise the biological endpoints of toxicant and carcinogen exposure (Myers *et al.*, 1991; Hinton *et al.*, 1992). As such, histological lesions in European flounder are increasingly being used as indicators of environmental contamination since they provide a definitive biological endpoint of historical exposure (Stentiford *et al.*, 2003).

As part of the DMECS (Development of a National Marine Ecotoxicological Analytical Control Scheme) programme samples of European flounder (*Platichthys flesus*) were collected in support of the UK's on going biological effects monitoring requirements as stipulated under the Cocoordinated Environmental Monitoring Programme (CEMP) for coastal and estuarine waters (OSPAR, 1995). During the autumn of 2002, fish from 8 UK estuaries were surveyed and samples taken for evaluating biological effect markers and disease prevalence. The work presented in this study details the analysis of hepatic DNA adducts and liver pathology.

#### 4.2 Material and Methods

#### 4.2.1 Sampling strategy

Fish from 8 inshore areas around the UK were surveyed and samples of biota for biological effects measurements taken. These areas were the Alde considered a clean reference site, and the industrialized Thames, Southampton, Mersey, Tyne, Forth, Clyde and Belfast (see Figure 4.1). These sites were sampled between September and December of 2002 using a 2 m beam trawl. Due to a limited number of fish being obtained from Belfast only DNA adduct data for this site is presented here.



**Figure 4.1.** Map of the UK showing estuarine sites sampled during this study.

### <sup>32</sup>P-postlabelling for the detection of hepatic DNA adducts

Samples of liver (up to 10 per site, 5 males 5 females, size range: > 15 cm) were dissected out and immediately snap frozen in liquid nitrogen. Hepatic DNA was prepared

from crude nuclei homogenates using a phenol/chloroform extraction method essentially as described by (Phillips and Castegnnaro, 1999). The DNA samples from individual liver samples were then adjusted to a final concentration of 1 μg μl<sup>-1</sup> and stored at -80°C prior to <sup>32</sup>P-postlabelling analysis. DNA adducts were determined using the standardised nuclease P1 version of the <sup>32</sup>P-postlabelling assay. Appropriate negative and positive DNA controls were analysed throughout the studies as described by Harvey and Parry, (1998). Dr Alan Hewer and Prof. David Phillips (Institute of Cancer Research, Sutton, UK) kindly provided the positive control, which consisted of BaP labelled DNA. Statistical analyses conducted were Mann Whitney or one-way ANOVA according to normality using the Minitab™ software package.

#### 4.2.2 Disease assessment and histology

Liver pathology criteria in European flounder followed guidelines developed by the International Council for the Exploration of the Sea (ICES) (Feist *et al.*, 2004). The liver tissue from up to 50 fish per site (>15 cm) were removed and prepared for histology.

Following euthanasia, samples of liver from European flounder were placed directly into pre-labelled histological cassettes and then into 10% neutral buffered formalin (NBF). In all cases, fixation was allowed to proceed for 24 h with occasional agitation to ensure even fixation of specimens. Specimens were then transferred to 70% industrial methylated spirit (IMS) for storage and transportation to the Cefas Weymouth Laboratory.

Fixed samples were processed to wax in a vacuum infiltration processor using standard protocols. Sections were cut at 3-5  $\mu m$  on a rotary microtome and resulting tissue sections were mounted onto glass slides before staining with haematoxylin and eosin (H & E). Stained sections were analysed by light microscopy (Nikon Eclipse

E800) and digital images were taken using the Lucia™ Screen Measurement System (Nikon).

#### 4.3 Results

#### 4.3.1 Hepatic DNA adduct analysis

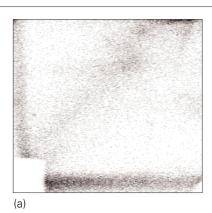
The total hepatic DNA adduct levels (adducted nucleotides per  $10^8$  normal nucleotides) along with representative chromatograms of  $^{32}$ P-postlabelled hepatic DNA from individual samples of European flounder are displayed in Table 4.1 and Figure 4.2 respectively. Overall the level of DNA adducts detected were similar to those reported previously for European flounder collected from UK estuaries (Lyons  $et\ al.$ , 1999). DNA adduct levels varied between the sample sites with the English control site (Alde) and Belfast displaying the lowest levels of genetic damage. However, the results from Belfast should be treated with caution as only a limited number (n = 5) of fish were obtained from this site.

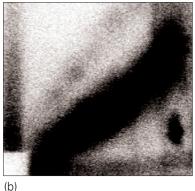
At the majority of contaminated sites (Southampton, Thames, Clyde, Tyne and Mersey) the predominant DNA adduct profile consisted of diagonal radioactive zones (DRZs), which is the characteristic profile obtained following exposure to complex mixtures of aromatic and/or hydrophobic genotoxins, such as those formed by PAHs (Figure 4.2(b)). In contrast, flounder collected from the Forth, Alde and Belfast lacked DRZs with only background levels of DNA damage being observed (Figure 4.2(a)). European flounder samples collected from Southampton contained the highest levels of DNA adducts (Figure 4.2(b)). Statistical differences were observed between several of the sites sampled with the levels (mean adducted nucleotides per  $10^8$  undamaged nucleotides  $\pm$  SE) detected in Southampton (93.9  $\pm$  37.0), Thames (51.1  $\pm$  19.2) and Clyde sites (36.2  $\pm$  15.8) statistically elevated (p<0.05) over those levels detected at the Tyne (Southampton and

Table 4.1. DNA adduct levels (mean adducted nucleotides per  $10^8$  undamaged nucleotides  $\pm$  SE, n=10, 5 males and 5 females) detected in European flounder sampled from UK estuaries in 2002. 
aStatistically elevated (p<0.05) when compared with DNA adduct levels detected in the Tyne, Forth, Alde and Belfast. 
bStatistically elevated (p<0.05) when compared with DNA adduct levels detected in the Forth, Alde and Belfast. Statistical significance determined using Mann Whitey and one way ANOVA. 
cOnly 5 fish available for analysis.

#### Estuarine site

Alde	Southampton	Thames	Forth	Clyde	Tyne	Mersey	Belfast
5.2 ± 1.4	93.9 ± 37.0°	51.1 ± 19.2°	9.3 ± 2.7	36.2 ± 15.8 <sup>b</sup>	13.7 ± 4.9	17.4 ± 5.9	4.2 ± 0.5°





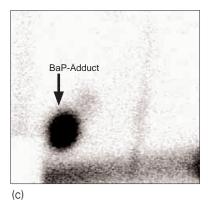


Figure 4.2. Representative hepatic DNA adducts profiles produced following <sup>32</sup>P-postlabelling. (a) Liver profile from the Alde, indicating a low level of DNA adducts representative of a clean reference location. (b) Individual flounder hepatic DNA sample collected from Southampton displaying a clear DRZ of <sup>32</sup>P-labelled DNA adducts indicating the fish has been exposed to a complex mixture of genotoxins. (c) Positive control consisting of BaP labelled DNA (115 nucleotides per 10<sup>8</sup> undamaged nucleotides) run with each batch. Kindly provided by Prof. David Phillips and Dr Alan Hewer (Cancer Research Institute, Sutton UK).

Thames only), Forth, Alde and Belfast sites. No intra-site related differences in DNA adduct level were detected between male and female fish in this study.

#### 4.3.2 Flounder histopathology

Livers were assessed according to thirty-one lesion categories, ranging from non-toxicopathic cellular changes through foci of cellular alteration to benign and malignant neoplasia, of which eleven were observed in this present study (Feist *et al.*, 2004). Significantly, the sites with the largest proportion of flounder containing at least one of these lesions types were the Mersey, Tyne, Clyde and

Forth estuaries (over 80%). Lower proportions were recorded in the Thames and Southampton (>70%), while the site with the lowest proportion of flounder with at least one lesion was the Alde estuary (below 25%).

More detailed analysis of specific lesions types revealed that non-toxicopathic lesions such as those associated with cell turnover (apoptosis, necrosis, regeneration) and immune-related functions (inflammation and melanomacrophage aggregates containing effete material, probably within lysosomes) were seen in European flounder from all sites (Table 4.2). Although it is difficult to associate higher prevalence of these lesion types with

Table 4.2. Major hepatic pathologies (%) detected in European flounder sampled from UK estuaries in 2002. <sup>a</sup>Lesions associated with cell turnover and immune related functions. Foci of cellular alteration (FCA); basophilic (b), eosinophilic (e), vacuolated (v) and clear cell (cc).

Lesion classification Estuary sampled

	,						
	Alde	Southampton	Thames	Forth	Clyde	Tyne	Mersey
Coagulative necrosis <sup>a</sup>	0	7.0	2.0	11	2.0	10	6.0
Apoptosisa	0	0	8.0	7.0	0.0	2.0	2.0
MMA <sup>a</sup>	6.0	36	48	16	0	65	27
Inflammationa	6.0	14	16	18	8.0	22	5.0
Regenerationa	0.0	0.0	4.0	0.0	0.0	2.0	0.0
Hepatocellular fibriller inclusions	0	36	16	56	84	30	63
Hepatocellular nuclear pleomorphisms	0	7.0	0.0	27	70	15	27
ccFCA	0.0	0.0	0.0	0.0	0.0	2.0	4.1
vFCA	6.0	2.3	4.0	2.3	2.0	8.2	6.1
eFCA	0	0.0	4.0	0.0	0.0	2.0	2.0
bFCA	2.0	0.0	8.0	6.8	2.0	0.0	8.1

specific sites, generally, the lowest prevalence was seen in flounder captured from the Alde estuary, with higher prevalence (particularly of melanomacrophage aggregates, inflammation and necrotic foci) seen in fish from the contaminated sites. Since at least some of these lesion types are known to be associated with pathogen and parasite infections, care must be taken when associating their presence with site-specific factors (such as genotoxic contamination).

In addition to pathologies associated with cell turnover and the immune system, two other significant cellular alterations of unknown aetiology were also seen in European flounder captured from several sites (Table 4.2). As previously reported by Stentiford *et al.*, (2003) hepatocellular fibrillar inclusions were seen in flounder captured from all sites apart from the Alde estuary, with the highest prevalence of this condition in flounders captured from the Mersey, Clyde and Forth sites. Associated with the hepatocellular fibrillar inclusions was a high prevalence of nuclear pleomorphism. The prevalence of this lesion was once again highest in flounder captured from the Mersey, Forth and Clyde estuaries and was not seen in those captured from the Alde or Thames estuary.

All four types of foci of cellular alteration (FCA); basophilic (b), eosinophilic (e), vacuolated (v) and clear cell (cc) were observed in flounders captured during the current study (Table 4.2). The prevalence of bFCA was highest in flounder captured from the Mersey, Forth and Thames estuaries (though this lesion was also recorded in low prevalence in fish captured from the Alde). The prevalence of eFCA was highest in the Thames, Tyne and Mersey estuaries. Vacuolated FCA were seen at all sites and were even see at relatively high prevalence (6%) in flounder from the Alde estuary while ccFCA was only detected in flounder from the Tyne and Mersey estuaries. The Mersey was the only estuary that contained flounder that exhibited all four categories of FCA.

Benign and malignant neoplasms of the liver were rarely observed in the current study. Basophilic adenoma was diagnosed in one flounder captured from the Thames estuary while another fish, also captured from the Thames, exhibited a putative hepatocellular carcinoma.

#### 4.4 Discussion

The results presented in this report further highlight the fact that fish populations in certain industrialised UK estuaries are being exposed to complex mixtures of genotoxic and potentially carcinogenic contaminants. Hepatic DNA adduct profiles characteristic of exposure to complex mixtures of aromatic/hydrophobic genotoxins were detected in European flounder collected from the Southampton, Thames, Clyde, Mersey and Tyne estuaries. These findings support previous studies, which have demonstrated that European flounder populations inhabiting industrialised UK estuaries are exposed to high levels of sediment PAH, and that a proportion of the bioavailable PAHs are being metabolised to carcinogenic metabolites (Lyons et al., 1999). The links between detecting DNA adduct profiles, characterized by DRZs, and PAH exposure has been supported by other studies utilizing benthic fish species. For example, similar DNA adduct profiles have been observed in experimental studies exposing English sole (Pleuronectes vetulus) to PAH contaminated sediment (French et al., 1996). Of significance to this current study, Bann et al., (1994) demonstrated the formation of hepatic DNA adducts in European flounder kept in mesocosms containing PAH spiked sediment. Furthermore, their results correlated the levels of DNA adducts with the degree of PAH contamination within each mesocosm and reflected the profile of hepatic pathology reported by Vethaak et al. (1996).

European flounder collected from Southampton contained the highest levels of DNA adducts. The reasons behind the elevated level of DNA adducts at this site are not immediately clear. Previous surveys of sediment PAH contamination (∑15 PAH dry weight) in UK estuaries have ranked Southampton water (750 µg kg<sup>-1</sup>), behind the Tyne  $(10,790 - 43,470 \, \mu g \, kg^{-1})$  Mersey (1,811 - 5,740) $\mu g \ kg^{-1}$ ) and Thames (597 - 5,350  $\mu g \ kg^{-1}$ ) (Woodhead et al., 1999). Furthermore, 1-OH pyrene bile metabolite data (Gubbins, unpublished data) collected from the fish used in this current study ranks Southampton behind the Mersey, Tyne and Thames in terms of PAH contamination. However, one must consider that in this case the ranking was only based on pyrene metabolites and it is possible that other carcinogenic PAHs (along with other aromatic non-PAH genotoxins) were present at elevated levels at Southampton during this current survey.

### 4.5 Possible relationship between DNA adducts and histopathological lesions

The results presented in this study are part of a larger programme of work that has attempted to investigate the links between the identification of contaminant related pathologies and genotoxic biomarkers of exposure in fish from UK estuaries. Numerous studies, as reviewed by Stein *et al.* (1990), have established correlations between the presence of genotoxins, including polycyclic aromatic

hydrocarbons (PAHs) in the sediment, the formation of DNA adducts and the incidences of hepatic neoplasia in fish populations.

While higher level malignant neoplastic lesions (eg hepatocellular carcinoma) have been noted in previous surveys of European flounder collected from estuarine and coastal waters they were not seen to any significant degree in this or previous surveys of UK estuaries (Stentiford et al., 2003). However, hepatocellular FCAs are regarded as early stage pathologies in the stepwise formation of hepatic neoplasia in fish and consequently provide a histopathological biomarker of carcinogen exposure (Hinton et al., 1992). The results of this current survey support the findings of our previous research that detected elevated levels of FCAs at those sites deemed to be the most contaminated by PAH (Tyne, Meresy and Thames). Other non-specific lesion types, including hepatocellular nuclear pleomorphism, which are regarded as an early stage lesion resulting from exposure to toxic and carcinogenic chemicals were again recorded at their highest levels in European flounder collected from contaminated sites. Such lesions have previously been associated with carcinogenic exposure in North American flatfish species (Myers et al., 1998). Samples of European flounder and viviparous blenny (Zoarces viviparus) have previously displayed this specific pathology at contaminated UK estuaries and this has been linked to PAH exposure (Stentiford et al., 2003; Lyons et al., 2004).

Although it is difficult to unequivocally associate the higher prevalence of toxicopathic lesions with the observation of DNA adducts in this study the data would suggest that while exposure to PAHs is a possible factor in the induction of certain lesions in the Thames and Clyde it does not appear to significantly implicated in their prevalence in fish from Southampton, Mersey, Forth or Tyne. Indeed, while the present study has focused on the types of DNA damage typically caused by PAHs, they are far from being the only source of carcinogenic exposure in estuaries. Monitoring surveys have shown that several classes of ICES ranked priority pollutants, including PCBs and heavy metals, are elevated in a number of contaminated sites around the UK

(Cefas, 1998). It is clear from the results presented in this study that most of the biological effects seen in estuarine and coastal environments around the UK are not generally being caused by single class of contaminant. supported by previous surveys that have identified over 70 anthropogenic organic substances present in surface waters collected from UK estuaries (Matthiessen et al., 1993). Additional studies have tried to characterize the classes of genotoxic contaminants present in estuarine ecosystems. Hendriks and co workers utilised the Salmonella (Ames) assay on water extracts from the Rhine estuary in Germany and demonstrated the majority of the mutagenicity detected was recovered in the moderately hydrophilic fractions of the water extracts that would not contain PAH residues (Hendriks et al., 1994). Similarly, Thomas and co workers used the mutagenic screening assay Mutatox™ to assess the genotoxic activity associated with sediment samples collected from five UK estuaries, including the Tyne, Thames, Mersey and Southampton (Thomas et al., 2002). In this study they identified at least one sediment sample (organic extract) from each estuary that contained potential genotoxins. A bioassay directed fractionation procedure was then used to identify genotoxins including, PAHs, alkyl substituted PAH, nitro-polycyclic aromatic compounds (PACs), polycyclic aromatic ketones and oxygenated-PACs. A remaining proportion the potentially genotoxic contaminants could not be identified.

#### 4.6 Summary

In summary, this study investigated the links between the identification of contaminant related pathologies and genotoxic biomarkers of exposure in fish in order to establish the health status of UK estuaries. While links between such pathologies and genotoxic biomarkers are not definitive the research adds to the growing database of biological effects parameters for UK estuaries. Continuing research is required if we are to establish the significance of these findings, and determine in full the potential ecosystem effects of chronic contaminant exposure in the UK marine environment.

## 5. Plasma vitellogenin and intersex in male flounder from UK estuaries

#### 5.1 Introduction

In recent years a great deal of research effort has been expended on the investigation of endocrine disruption in fish. Evidence of the impact of endocrine disrupters on this important group has been found in lakes, rivers, estuaries, coastal regions and offshore (Jobling et al., 1998; Allen et al., 1999; Matthiessen et al., 1998) and in many areas of the developed and developing world. This effort reflects the importance of fish, both as a resource and as pivotal components of the ecosystem of most aquatic environments. While more recent concern has been raised as to the potential effects of androgenic, anti-androgenic and anti-oestrogenic (Katsiadaki et al., 2001) endocrine disrupters (including effects attributable to pulpmill discharges) the most widespread evidence of environmental effects remains with the feminisation of males thought to be caused by oestrogenic exposure.

The perceived dominance of oestrogenic endocrine disruption in the environment is indicative of the many classes of ubiquitous contaminants that have been shown to demonstrate an oestrogenic capacity including; alkylphenols, phthalates, some pesticides, PCBs, certain brominated flame retardants and, of course, synthetic oestrogens being released via sewage outfalls as a consequence of normal human excretion and the use of the contraceptive pill. A number of feminising effects have been noted in male fish in the environment as a potential consequence of exposure to these substances including; the production of the female specific volk pre-cursor protein vitellogenin (VTG) (Allen et al., 1998), the presence of the ovotestes and certain morphological changes (Kirby et al., 2003). The most widely used of these feminisation biomarkers is the presence of VTG in male blood plasma which has been shown to be sensitive and measured relatively easily used enzyme linked immuno-sorbent assays (ELISA) or radio-immuno assay (RIA) techniques.

In the United Kingdom, the earliest examples of oestrogenic endocrine disruption, both in terms of VTG induction and the presence of ovotestis, was discovered in certain freshwater based studies of roach (*Rutilus rutilus*) (Jobling *et al.*,1998). Surveys sampling flounder (*Platichthys flesus*) (Matthiessen *et al.*, 1998), conducted by Cefas, showed that these effects of oestrogenic exposure were also present in the estuarine environment. Furthermore, the levels of plasma VTG in male flounder were highest in those estuaries most associated with industrial activity or high inputs of domestic sewage effluent.

In response to the growing evidence that species inhabiting estuarine and coastal waters were being exposed

#### Author: Mark Kirby

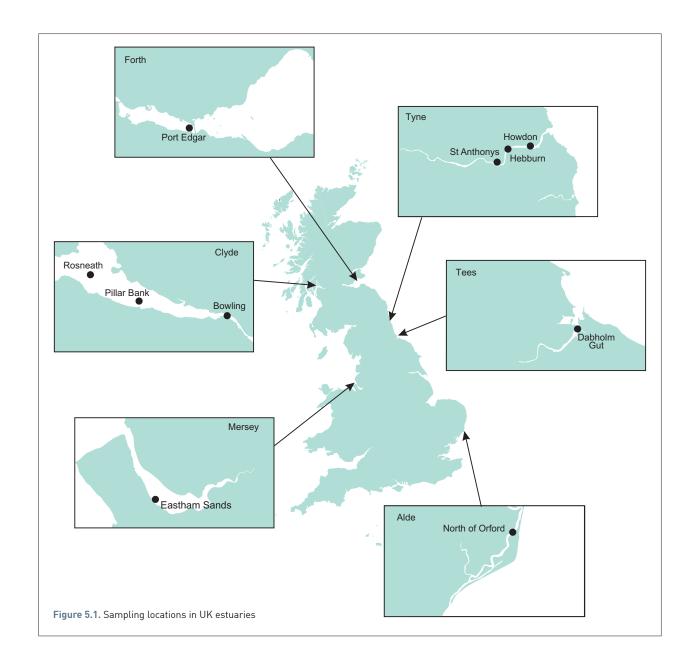
to sufficiently high concentrations of oestrogenic substances to elicit significant responses, the 'Endocrine Disruption in the Marine Environment' (EDMAR) programme was initiated in 1998. The EDMAR programme had a wide remit to investigate the effects of endocrine disrupting chemicals (EDCs) in the UK marine environment and to develop new techniques and biomarkers for fish and invertebrates. A significant part of the project allowed for the continued sampling of flounder from several UK estuaries to follow up on the results reported in previous studies. Concentrations of plasma VTG in male flounder and the occurrence of ovotestis were investigated from 1996 to 2001 and have formed a short time series which is collectively presented here. Positions of sampling sites are shown in Figure 5.1.

#### 5.2 Results and discussion

These surveys have demonstrated that male flounder in many UK estuaries are showing elevated plasma VTG concentrations as a result of oestogenic exposure (Kirby *et al.*, 2004). Results are shown in Table 5.1. However, in most estuaries, the degree of elevation does not appear to be as high as when sampling was first carried out in 1996. This is particularly apparent at the Dabholm Gut site on the Tees, but is also true of sites on the Mersey and Tyne. However, the temporal dataset also provides good evidence of a decline in VTG concentrations at other sites in the Mersey and Tyne. (Figure 5.2). The temporal trend for VTG in fish from Eastham Sands on the Mersey is a good example, with a reduction in mean concentration from approximately 10<sup>7</sup> to  $10^4$  ng ml<sup>-1</sup> over the 5 year study period.

The apparent downward trend in the mean VTG levels is supported by a general increase in the proportions of fish showing no induction and a reduction in highly induced specimens. Of particular significance are the changes seen during 2000 which show large reductions in plasma VTG concentrations in samples from the Tyne and Clyde and the survey results from 2001 show little evidence of any significant VTG response in male flounder from the Forth and Clyde. Furthermore, nearly 60% of the specimens at the once 'hot' site of Howdon on the Tyne showed no evidence of induction in 2001.

The sites that demonstrated the smoothest downward VTG trend over time are Eastham Sands on the Mersey and Hebburn on the Tyne (Figure 5.2). These sites are associated with contamination from several diffuse sources and therefore may act as a monitor of general oestrogenic contamination of the estuary. As such the downward trend may reflect an overall improvement in contaminant levels in these rivers.



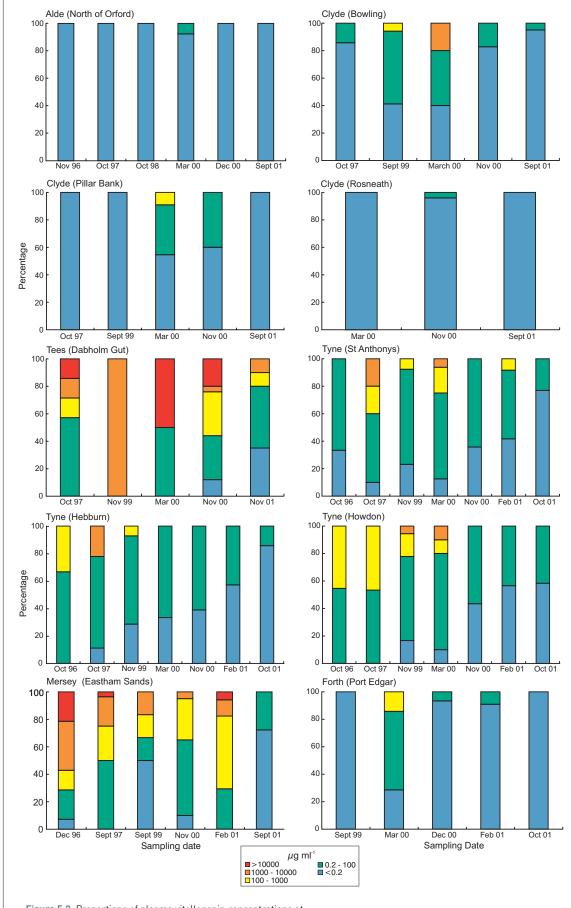
One of the reasons it has been difficult to reach any conclusion on downward trends in other sites is our discovery of short-term variation in VTG concentrations in most of the estuaries (including even the control estuary the Alde). Examination of the 2000 and 2001 data indicates that males caught in September have uniformly lower concentrations of VTG than in the succeeding months. With the progress of autumn and then winter, average concentrations become higher and higher, reaching their peak in March. Such a pattern is consistent with fish entering the contaminated estuaries in summer and leaving again in the spring - with the assumption that, in September, they are relatively free of contamination, but by March they have had over six months of exposure to oestrogenic compounds within the estuaries (whether directly from the water or through their diet).

The two sites (Howdon and Dabholm Gut) specifically associated with previously identified (Thomas *et al.*, 2001) point sources of oestrogenic contamination show different

trend characteristics. The Howdon site on the Tyne is associated with a major sewage effluent discharge. The plasma VTG concentrations in male flounder remained high and consistent from 1996 to March 2000 and then showed a clear reduction but once again remained very consistent from November 2000 to September 2001. At approximately the end of 2000 the Howdon sewage treatment plant brought secondary treatment processes on line. Work commissioned by Northumbrian Water (Thomas et al., 2002a), the owners of the treatment plant, measured the levels of the main oestrogen receptor agonists (17βoestradiol (E2), oestrone (E1) and  $17\alpha$ -ethynylestradiol (EE2)) in the Howdon STW effluent during 2000/01. As part of the same study, oestrogenic potency (as E2 equivalents) of the effluent was also determined using the in vitro yeast oestrogen screen (YES). The analytical data show that the secondary treatment of the effluent was able to successfully reduce mean concentrations of E2, E1 and EE2 from 25 ng  $l^{-1}$  to 5 ng  $l^{-1}$ , 55 ng  $l^{-1}$  to 7 ng  $l^{-1}$  and

**Table 5.1.** Numbers, mean lengths and mean plasma VTG concentrations of male flounder sampled from each site between 1996 and 2001.

Estuary	Site	Date	No.	Mean plasma VTG concentration (µg ml <sup>-1</sup> )	Mean length cm (S.E.M.)
Alde	North of Orford	26/11/96	10	0.2	24.7 (2.5)
		28/10/97	23	0.2	25.9 (2.0)
		08/10/99	10	0.2	17.0 (3.8)
		27/03/00	13	0.8	18.9 (2.5)
		19/12/00	25	0.2	24.7 (3.4)
		17/09/01	15	0.2	23.4 (3.0)
Clyde	Rosneath	15/03/00	2	0.2	21.6 (1.8)
,		28/11/00	25	0.2	21.3 (2.5)
		11/09/01	5	0.2	21.1 (2.8)
	Pillar Bank	02/10/97	19	0.2	22.1 (1.2)
	T IIIdi Barik	30/09/99	16	0.2	20.4 (1.3)
		14/03/00	11	15.3	17.5 (2.5)
		28/11/00	5	3.6	20.0 (0.9)
			18	0.2	
	Davelina	10/09/01			20.9 (1.5)
	Bowling	02/10/97	14	0.2	20.6 (1.1)
		29/09/99	17	53.9	22.1 (1.4)
		14/03/00	5	275.9	16.9 (1.9)
		27/11/00	29	1.0	19.9 (1.8)
<del>-</del>	D 11 1 C	10/09/01	20	0.2	21.5 (2.1)
Tees	Dabholm Gut	14/10/97	7	4265.5	23.7 (5.8)
		09/11/99	2	1318.2	17.0 (3.6)
		20/03/00	2	12987.5	17.1 (3.7)
		20/11/00	25	3355.2	22.6 (1.7)
		19/11/01	20	597.0	22.3 (2.7)
Tyne	St Anthonys	16/10/96	3	1.5	19.8 (0.9)
		21/10/97	10	286.3	23.4 (5.0)
		10/11/99	13	26.4	23.7 (4.5)
		23/03/00	16	166.7	22.2 (1.6)
		23/11/00	14	1.8	23.1 (2.6)
		13/02/01	12	66.3	23.3 (1.6)
		09/10/01	13	0.2	23.1 (3.5)
	Hebburn	16/10/96	9	113.0	23.4 (4.9)
		16/10/97	9	448.3	24.6 (5.2)
		10/11/99	14	39.7	21.1 (4.3)
		22/03/00	12	6.9	20.6 (1.9)
		22/11/00	18	6.3	24.4 (1.3)
		13/02/01	21	0.9	23.3 (1.8)
		09/10/01	21	0.5	23.4 (2.9)
	Howdon	16/10/96	11	116.2	23.4 (4.6)
		16/10/97	15	90.3	19.7 (2.7)
		11/11/99	18	150.7	24.7 (4.7)
		22/03/00	20	238.8	20.2 (1.7)
		22/11/00	23	2.1	24.6 (1.3)
		12/02/01	23	2.8	24.0 (2.8)
			24	1.9	
Mercov	Eastham Sands	09/10/01	24 14	19226.2	24.5 (2.4)
Mersey	Lasulatti SätiüS	02/12/96			26.3 (3.1)
		02/09/97	28	1239.5	26.1 (5.0)
		06/09/99	6	307.5	18.5 (3.4)
		01/11/00	20	194.0	23.8 (3.9)
		20/02/01	17	1268.5	22.8 (2.8)
		25/09/01	18	3.5	20.8 (3.2)
Forth	Port Edgar	16/03/00	7	55.6	20.6 (2.3)
		01/12/00	15	0.2	22.6 (3.4)
		28/02/01	10	2.0	22.6 (1.8)
		22/10/01	10	0.2	17.9 (2.2)



**Figure 5.2.** Proportions of plasma vitellogenin concentrations at a range of induction levels in male flounder from UK estuaries – 1996 to 2001 trends.

6 ng l<sup>-1</sup> to 2 ng l<sup>-1</sup>, respectively. Furthermore the oestrogenic potency (E2 equivalents) was greatly reduced from a mean activity of approximately 80 ng E2 l<sup>-1</sup> to <4 ng E2 l<sup>-1</sup>. The close relationship between the introduction of secondary treatment, the reduction in oestrogen receptor agonist concentrations and the reduction in plasma VTG in male flounder is striking.

The survey site at Dabholm Gut on the Tees is associated with multiple discharges containing significant quantities of both industrial and domestic effluents. The high oestrogenicity of the Dabholm Gut effluent has been associated with high levels of industrial contaminants such as nonylphenol as well as the steroidal agents (ie ethynylestradiol, estrone, oestradiol etc) that have been shown to be the prime cause of oestrogenicity in domestic effluents such as at Howdon (Thomas et al., 2001). There have been consistently high plasma VTG response in male flounder from the Dabholm Gut site between 1997 and 2000. In the most recent sampling, November 2001, the mean plasma VTG level was the lowest so far recorded at >20x less than the peak levels seen earlier. However, there is not yet any firm evidence for a sustained reduction in the VTG levels in fish from this site. This is slightly surprising as it is known that the main industrial effluent containing alkylphenols and their ethoxylates, has had its contents of nonylphenol, octylphenol and related ethoxylates significantly reduced over the study period. Furthermore, the large municipal sewage effluent discharging to Dabholm Gut has, like the effluent at Howdon on the Tyne, undergone secondary treatment since late 2000. The fact that a more significant reduction in male plasma VTG, such as that observed at Howdon, has not occurred may be because; there are other, unknown, oestrogenic chemicals causing much of the VTG induction; the effluent treatment regimes are not reducing the load of oestrogenic chemicals sufficiently; or, the historic oestrogenic contamination at this site, perhaps associated with the sediments, is much more persistent than in other sampled areas

The earlier studies of plasma VTG and the occurrence of ovotestis in flounder revealed no close relationship between the two. Studies from 1996 (Allen *et al.*, 1998) demonstrated that this intersex condition occurred solely in flounder from the Mersey (5/30 = 17%) and further surveys in 1997 revealed the condition in both the Mersey (6/62 = 9.2%) and Tyne (5/65 = 7.5%). The results presented here (Figure 5.2) show that these estuaries continue to be the 'hotspots' even though, in some areas, the VTG response in male fish appears to be decreasing. It remains difficult

to draw any firm conclusions with respect to the presence of ovotestis and contamination by EDCs. However, the consistent, if relatively low, prevalence of the ovotestis condition in the Tyne and Mersey coincide with areas of known historical oestrogenic contamination as shown by the plasma VTG data presented here. What is more difficult to explain is the apparent lack of ovotestis in fish taken from the Tees. This suggests that, if the condition is caused by exposure to EDCs (a highly likely supposition), it may not simply be due to oestrogenically active compounds in general but rather to specific classes of substances that have been more prevalent in the Tyne and Mersey. It might be significant that the discharge at Dabholm Gut has been more readily associated with the oestrogenicity of alkyphenolic compounds than at the other sites. It is also recognized that because the numbers of specimens caught in the Tees is lower than in the other estuaries the apparent lack of ovotestis may be an artefact of the sampling success.

#### 5.3 Conclusions

Plasma VTG concentrations in male flounder have remained elevated over the period 1996-2001 at some sites in several industrial estuaries in the UK (eg Tees, Mersey and Tyne).

Time series data collected since 1996 strongly suggests, however, that there has been a long-term reduction in oestrogenic contamination (measured by VTG induction in male flounder) at sites in the Tyne and Mersey.

Analysis of data from 2000 and 2001 indicates that, for most estuaries, VTG concentrations from fish caught in the winter months are invariably higher than those from fish caught in the autumn months. This is presumed to be due to migratory behaviour of the flounder.

Oestrogenic contamination (again measured by VTG induction) in the Clyde and Forth has reduced to near baseline levels in the latest surveys by comparison with 2000.

The reducing VTG trends in fish caught near Howdon STW are associated with reduced levels of oestrogenic contamination in the Howdon discharge which appear related to the introduction of secondary treatment at the plant. No major change in mean plasma VTG concentrations in male fish from near the Dabholm Gut discharges was noted over the study period.

Occurrence of ovotestis in male flounder continues at a relatively low but consistent level. Fish from the Tyne and Mersey continue to provide the bulk of cases in this species although a few examples have been observed elsewhere.

#### Resource management

# 6. Seabed habitat mapping: a useful tool for monitoring and management of a dredged material disposal site

#### Author: Silvana Birchenough

#### 6.1 Introduction

Following the Bergen declaration (20-21 March, 2002) at the 5th North Sea Conference, it was recognised that all anthropogenic activities that can potentially adversely affect the North Sea should be strictly managed to ensure conservation of biological biodiversity and environmental sustainability (MEMG, 2003).

Dredged material is sediment excavated by mechanical or hydraulic means from a sea, river or lakebed. This process allows the maintainance of navigable waterways in coastal areas. Processes such as dredging and the aquatic disposal of dredged material can have direct impacts on the environment, primarily on the seafloor and benthic habitats (Valente, 2004).

Worldwide, dredged material disposal at sea is an activity which is increasing as a consequence of economic development in coastal areas. As a consequence, this has stimulated a number of studies, which have focused on the effects resulting from dredged material disposal on benthic communities (Fredette and French, 2004; Cruz-Motta and Collins, 2004; Valente, 2004; Zimmerman et al., 2003; Smith and Rule, 2001 among others). More recently, the need for seafloor mapping facilitating a more effective dredged material monitoring and management strategy has emerged (Preston et al., 2003; Rhoads and Germano, 1990; DAMOS://www.nae.usace.army.mil/environm/damos/damos\_contributors.asp:).

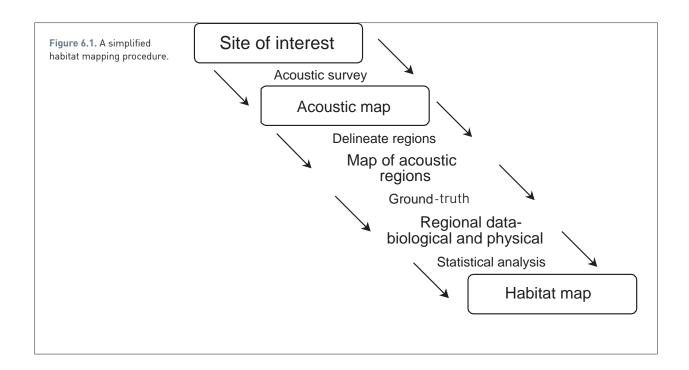
In England and Wales, the disposal of dredged material is licensed under the Food and Protection (FEPA) act 1985a Part 2, following guidelines laid down by International Convention, (Great Britain - Parliament 1972a, b; 1985a). This act controls deposits in the sea below the mean high water spring tide level in order to protect human health, the marine environment and legitimate uses of the sea and is regulated by the Department for Environment, Food and Rural Affairs (Defra). Scientific assessment of each dredge material licence is undertaken on behalf of Defra by Cefas in accordance with the requirement of the OSPAR Commission (1988). Prior to issuing a disposal licence, alternative disposal options must be explored by the applicant (Murray, 1994). This includes beneficial uses of the dredged material, such as for beach recharge,

coastal defence and habitat enhancement (Waldock *et al.*, 2002). However, in many cases, open water disposal in designated sites is the best practicable environmental option and also the only economically viable one. Criteria which must be satisfied before a licence is granted to dredge and dispose material at sea include the chemical quality of the material, the quantity to be disposed of, its nature and origin and its predicted impacts at the area of disposal (Rees *et al.*, 2002).

In the past, scientists have relied on traditional sampling tools (eg grabs, corers and dredges) to quantify sediments and their associated benthic fauna (macrofauna and meiofauna) (eg Boyd et al., 2002; Eleftheriou and McIntyre, 2005). However, recent developments in acoustic technology have, together with traditional survey techniques, allowed scientists to map the seafloor at high resolution (Pickrill and Todd, 2003). The habitat maps generated in this way provide essential information for the sustainable management of offshore resources (Kenny et al., 2003; Pickrill and Todd, 2003). The main benefits of seabed mapping techniques are the opportunity to conduct cost-effective, wide-scale reconnaissance surveys, which may serve a number of important purposes (ie identification of resources, site features and conservation areas; sampling designs and multiple uses of the seafloor).

A number of acoustic techniques (especially sidescan sonar and, more recently, multibeam echosounders) have been widely employed to monitor physical disturbance of seabed habitats (eg Brown et al., 2004, 2001, 2002; Todd et al., 1999, 2000; Limpenny et al., 2002). Kenny et al. (2003) provided a clear overview of technologies for accurately described the characteristics of seabed mapping technologies for application in marine habitat classification.

The increasing accessibility of these acoustic techniques has stimulated large-scale habitat mapping projects. These include international initiatives such as the Irish National Seabed Mapping project (Parsons *et al.*, 2004) and Canadian Geoscience for Oceans Management programme. At a national level, the U.K has also learned from overseas research. Currently, two large-scale habitat-mapping programmes in the Outer Bristol Channel and the Eastern English Channel are being developed. The outputs from



these two programmes will deliver the first regional-scale, high-resolution benthic habitat maps for the UK (James *et al.*, 2005; Philpott *et al.*, 2005).

Habitat mapping can be considered as the preferred tool to provide spatial representation of described and classified habitat units (Valente *et al.*, 2004). Several researchers have adopted these tools (Kostylev *et al.*, 2001, 2005; Brown *et al.* 2001) for generating habitat maps. There are several steps in the production of a habitat map. Figure 1 presents a summarised version of this process. For a more comprehensive description of this procedure see "A1033 approach for producing a seabed habitat map" in Boyd *et al.* 2006.

Once a site of interest has been identified, an acoustic survey should be undertaken. The first stage involves the production of an initial acoustic basemap. This basemap is used to define acoustically-distinct areas of the seabed based on the physical properties of the sediments and the topographic features characterising each distinct area. Once the acoustically distinct areas are delimited, the use of the acoustic basemap is vital to plan the 'ground-truth' survey design (collection of samples) to aid a robust characterisation of the acoustically-distinct areas previously delineated. The ground-truth survey is an important spatial validation of the biological and physical characteristics within each of the acoustically-distinct areas identified from the basemap.

There is a wide spectrum of techniques available for ground-truth surveys. These range from point samples (ie grab, drop down camera, sediment profile camera) to towed gear, which provide coverage of sections of the seabed (ie trawls, camera sledge). The aims of the project and budget quite often dictate the suite of techniques available to ground-truth the acoustic basemap. Currently, there are a limited number of examples where a combination of more than 2 ground-truth techniques have been used to validate acoustic information (Zajac et al., 2003; DAMOS, 2004). For example, the use of optical techniques (such as video images or sediment profile images (SPI)) enable the capture of in-situ images of the seabed habitats which show the relationship of surface sediments and inhabiting communities (epifauna) directly (Kostylev et al., 2001).

The major benefits of adopting a combination of ground-truth techniques are the clear evidence provided (from a combination of collective data sets), corroboration (one technique confirming features detected by another) and clarification (the exposure of data artefacts during composite interpretation). Results from an integration of approaches to characterise an area licensed for dredged material disposal were combined providing clear benefits for the monitoring and potential management of an area licensed for dredged material. The case study is presented in Chapter 17 of this report.

## 7. Licensing of deposits in the sea

#### Authors: Andrew Dixon and Chris Vivian

#### 7.1 Introduction

This section gives information about the licensing of deposits in the sea around the coasts of England and Wales in 2004 under Part II of the Food and Environment Protection Act 1985 (as amended) (FEPA) (Great Britain - Parliament, 1985a). In order to provide a complete picture for the UK as a whole, licensing statistics for Scotland and Northern Ireland are also included in this section.

#### 7.2 Legislation and licensing authorities

The deposit of substances and articles in the sea, principally the disposal of dredged material (as opposed to discharge into the sea via pipelines) and the use of material during marine construction and coastal defence works, is controlled by a system of licences issued under Part II of FEPA. Certain operations (eg the deposit of scientific equipment or navigation aids) are exempt from licensing under the Deposit in the Sea (Exemptions) Order 1985 (Great Britain - Parliament, 1985b).

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

#### 7.3 Enforcement

Scientists from the Cefas Burnham Laboratory have the powers to enforce Licence provisions. Visits are made to construction sites and disposal vessels. Samples are taken and records, including logbooks, are checked. Scientific staff carried out 6 inspections in 2004.

Officers of the Department's Marine Fisheries Agency (MFA) are charged with enforcing the provisions of FEPA (Part II) and undertake regular inspections from a network of port offices in England and Wales. The MFA (then the Sea Fisheries Inspectorate) carried out 128 inspections in 2004 in relation to construction works and the disposal of waste materials (dredged materials and a small amount of shellfish waste) at designated disposal areas. Further details are given in Table 7.1.

Table 7.1. Inspection activity by the SFI during 2004.

District	No. of inspect	ions	No. of
	Construction	Disposal	infringements
Eastern	6	4	1
Humber	7	2	0
London	3	0	1
North Eastern	9	1	1
North Western	2	1	0
South Eastern	34	5	4
South Western	9	16	5
Wales	26	3	4
Total	96	32	16

In England and Wales 2 written warning letters were issued for apparent breeches of licensing controls in 2004. Details are as follows:

- Investigations into unlicensed construction works in Helford Creek Cornwall resulted in an official warning being issued in November 2004.
- Investigations into unlicensed construction works on the Penryn River resulted in an official warning being issued in December 2004.

In England and Wales in 2004 there were no successful prosecutions for illegal marine works with one case being dropped and a further case being adjourned until 2005. The details are as follows:

- Investigations into unlicensed disposal and construction works at Mostyn, Flintshire resulted in the case being adjourned until 2005.
- Investigations into unlicensed construction works at Pwhelli, North Wales resulted in the case being dropped.

In Scotland, certain authorised staff of the Fisheries Research Services (FRS) Marine Laboratory, Aberdeen and the Scottish Fisheries Protection Agency (SFPA) hold similar enforcement powers. The FRS made 9 enforcement visits in 2004. The SFPA made 2 enforcement visits in 2004.

In Northern Ireland the Environment and Heritage Service (EHS) made 11 enforcement visits in 2004. EHS also carried out 14 investigation visits in 2004 which resulted in 6 warning letters being issued to terminate the unlicensed activities.

**Table 7.2.** Summary of dredged material licensed and disposed of at sea, 2000-2004.

Country	Year	Licences issued	Licensed quantity (tonnes)	Wet tonnage deposited	Dry tonnage deposited	Quantities of metal contaminants in wastes deposited (tonnes)						
			(tolliles)	uepositeu	uepositeu	Cd	Cr	Cu	Hg	Ni	Pb	Zn
England	2000	119	55,902,025	28,257,192	14,077,169	8.76	1,043	663	4.78	485	1,099	2,948
and Wales	2001	124	39,297,549	29,660,448	14,881,254	7.61	1,040	731	5.84	478	1,099	3,310
	2002	124	72,851,190	27,884,495	14,725,603	5.53	912	457	4.67	409	1,166	2,664
	2003	97	31,836,123	29,544,681	15,812,482	5.42	929	498	12.73	443	1,183	2,694
	2004	80	21,268,369	28,516,645	14,949,123	5.27	886	513	4.35	412	1,190	2,648
Scotland	2000	30	6,135,400	4,155,018	2,034,213	0.51	87	80	1.79	73	139	298
	2001	29	3,307,800	2,217,981	1,162,856	0.36	79	48	0.74	36	77	165
	2002	21	2,959,045	2,203,016	1,188,129	0.33	59	46	0.85	29	69	134
	2003	29	3,573,981	2,764,020	1,647,881	0.61	70	57	1.40	41	101	175
	2004	23	2,412,670	1,484,408	742,204	0.19	27	19	0.51	14	31	54
Northern	2000	3	3,950,000	640,815	455,222	0.13	45	7	0.05	13	14	42
Ireland	2001	3	183,000	3,420,411	2,495,714	0.72	246	37	0.42	66	76	226
	2002	8	1,161,500	976,102	458,108	0.46	31	19	0.19	19	26	86
	2003	2	189,900	115,404	73,382	1.47	8	4	0.06	3	2	12
	2004	4	432,904	111,208	79,135	0.04	3	1	0.06	1	1	7
UK Total	2000	152	65,987,425	33,053,025	16,566,605	9.39	1,176	750	6.63	571	1,252	3,289
	2001	156	42,788,349	35,298,840	18,539,824	8.69	1,365	816	7.01	579	1,251	3,701
	2002	153	76,971,735	31,063,613	16,371,841	6.31	1,003	522	5.70	457	1,261	2,884
	2003	128	35,600,004	32,424,105	17,533,744	7.50	1,006	540	5.75	487	1,270	2,882
	2004	107	24,113,943	30,112,261	15,770,462	5.50	917	533	4.92	427	1,223	2,709

Notes: Tonnages deposited relate to quantities in the calendar year 2003, which may be covered by 2 or more licences, including one or more issued in previous years.

#### 7.4 Licensing of dredged material

Table 7.2 give details for the period 2000 to 2004 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. A proportion of the trace metals in this dredged material is natural, but the mineral structure is such that it will not be available to marine organisms.

Figure 7.1 shows the main disposal sites used in 2004 and the quantities used at each site. Although applications for licences are required to show evidence that they have considered alternative disposal options including beneficial use, the problems of having silty materials, and matching the timing of dredging campaigns and the demand for sediments, have meant that most of the finer materials, in particular, are deposited at sea.

#### 7.5 Other licensed activity

Under Part II of FEPA, licences are also required for certain other activities or deposits made below the mean high water springs mark for construction purposes. Each licence application is carefully considered, in particular, to assess the impact on the tidal and intertidal habitat, hydrological effects, potential interference to other users of the sea and risk to human health. Details of these licences issued in 2004 are shown in Table 7.3.

Further activities involve the use of tracers, the application of biocides, and burial at sea. Generally the anticipated environmental impact from these deposits is minimal and little or no monitoring is required. Details of these licences issued in 2004 are also shown in Table 7.3.

Such licences have also authorised the disposal of a small amount of fish waste, details given in Table 7.4.

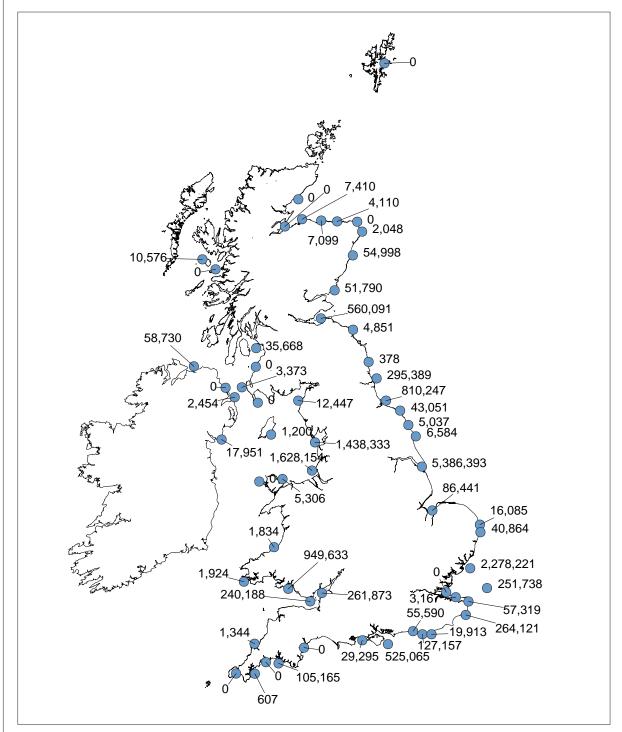


Figure 7.1. Amounts of dredged material disposed at sea in 2004, in dry tonnes  $\,$ 

Table 7.3. Other categories of licences issued in 2004.

Licence category	<b>England and Wales</b>	Scotland	Northern Ireland	Total
Construction - new and renewal	180	110*	6	296
Tracers, biocides etc	4	0	0	4
Burial at Sea	13	0	0	13

<sup>\*</sup> Includes 70 Outfall licences

**Table 7.4.** Summary of fish waste licensed and disposed of at sea 2000-2004.

Country	Year	Licences issued	Licensed quantity (tonnes)	Wet tonnage deposited	Dry tonnage deposited
England	2000	1	1,000	1,559	1,559
and Wales	2001	3	938	687	687
	2002	2	2,200	808	808
	2003	1	6,000	953	953
	2004	0	0	1,834	1,834
Scotland	2000	1	200	45	36
	2001	0	0	66	53
	2002	0	0	0	0
	2003	0	0	0	0
	2004	0	0	0	0
Northern	2000	0	0	0	0
Ireland	2001	0	0	0	0
	2002	0	0	0	0
	2003	0	0	0	0
	2004	0	0	0	0
UK Total	2000	2	1,200	1,604	1,595
	2001	3	938	753	740
	2002	2	2,200	808	808
	2003	1	6,000	953	953
	2004	0	0	1,834	1,834

Notes: For information on licensed quantities and tonnages deposited see footnote to Table 7.2.

## 8. Swanage Bay dredgings disposal ground survey 2004 – a preliminary assessment of status

#### Authors: Mathew Curtis, Hubert Rees, Dave Limpenny, Becky Smith, Sarah Campbell, Marie Pendle and Koen Vanstaen

#### 8.1 Introduction

The Swanage Bay dredgings disposal ground is located some 5 km east of Swanage at a depth of about 20 m and has been used for the disposal of dredged material from Poole harbour for more than 50 years. In recent years, an average of about 250,000 wet tonnes per annum has been deposited within the disposal ground (Table 8.1). The aim of the 2004 survey was to collect baseline information on the status of the area, both within and around the disposal ground, prior to the proposed deposit of 2 million tonnes of capital dredged material at the site over the 2005-2006 winter period. This is to accommodate the deepening of Poole harbour to allow larger shipping into the port (Royal Haskoning, 2004). The area was surveyed on 13 June 2004 using the RV Cefas Endeavour (Figure 8.1). Survey effort included conventional grab-sampling techniques along with acoustic methods to assist in identifying the presence and distributional boundaries of ecologically important species that could be affected by the increased disposal. It was expected that these would include maerl (Phymatolithon calcareum), a coralline red alga, and the reef-building worm, Sabellaria spinulosa, which have previously been recorded from this area (Collins, 2003).

#### 8.2 Methods

#### 8.2.1 Survey design

The grab-sampling survey was designed to take into account the most likely route of any dispersing material away from the disposal ground, due principally to tidal action. The stations were positioned in areas of probable sediment deposition derived from dispersion modelling data of the area (Royal Haskoning, 2004). Four stations were sampled in triplicate for benthic macrofauna and sediments to provide a preliminary assessment of the extent of any effects of disposal activity.

The acoustic survey was designed to acquire information on the bathymetry and sedimentary features of the area both within and around the disposal ground (Figure 8.1). Sediment samples and video footage of the seabed were collected at six stations to aid in ground-truthing the acoustic data. The aims were to identify any evidence of deposited dredged material within or outside the disposal ground and to characterise acoustically distinct regions within the survey area.

**Table 8.1.** Recent dredge disposings history of Swanage Bay disposal ground.

Year	Total tonnage disposed					
1990	293,291					
1991	535,937					
1992	1,103,379					
1993	151,353					
1994	104,751					
1995	224,091					
1996	167,575					
1997	310,724					
1998	87,072					
1999	158,994					
2000	76,927					
2001	250,755					
2002	40,941					
2003	62,966					

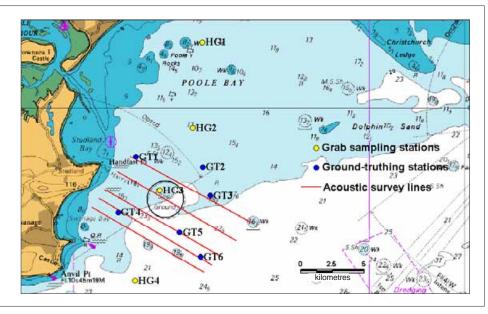
#### 8.2.2 Field sampling procedure

Replicate samples of sediments for the later analysis of benthic macrofauna and sediment particle size distribution were collected using a  $0.1~\text{m}^2$  Hamon grab. Following estimation of sample volume, a sub-sample was removed for particle size analysis. The whole sample was then washed over 5 mm and 1 mm sieve meshes to remove fine sediment. The two fractions were combined and back-washed into an appropriate container and fixed in 4 - 6% buffered formaldehyde solution.

Sediment from the ground-truthing stations were collected with a  $0.04~\text{m}^2$  Shipek grab. A sub-sample from the grab was removed for particle size analysis.

The acoustic system used for this study was a 300kHz, Kongsberg Simrad™ EM3000D dual-head "true" multibeam system, which used transducers mounted in 'V' formation on the drop keel of the *Cefas Endeavour*. Positioning of the data was achieved using a Thales 3011™/ Fugro SeaStar™ satellite differential GPS system. Vessel, pitch, roll and heave were measured with a Kongsberg/Seatech™ MRU5 motion reference unit. These parameters were corrected in real time within the EM3000D processing unit, which then output the data to a Triton Isis™ logging

Figure 8.1. Swanage Bay survey 2004. Reproduced from Admiralty Chart 2454 by permission of the Controller of Her Majesty's Stationery Office and the UK Hydrographic Office (www. ukho.gov.uk). Not to be used for navigation.



package. Sound velocity at the sonar head was measured with a Reson™ sound velocity probe. The data were then imported into CARIS™ HIPS where further corrections were made using predicted tidal data and sound velocity profiles. Erroneous soundings were filtered and a corrected XYZ dataset was produced for the survey. These XYZ grids were then imported into the 3D visualisation software package Fledermaus™, where coloured, sun-illuminated topographic images depicting the surface of the seabed were produced.

Video footage of the seabed was collected using a 0.1 m<sup>2</sup> Hamon grab fitted with a video camera and light. The camera was lowered close to the seabed as the vessel drifted with the tide. Video images were recorded onto both high-resolution SVHS and digital tapes.

#### 8.2.3 Laboratory procedure

In the laboratory, macrofauna samples from each Hamon grab were first washed with freshwater over a 1 mm sieve in a fume cupboard to remove excess formaldehyde solution and then placed on plastic trays and examined under an illuminated magnifier. Specimens were picked from the trays and placed in labelled Petri dishes containing a preservative mixture of 70% IMS for identification and enumeration.

The sediment sub-samples from each grab were analysed for their particle size distributions. Samples were first wet-sieved on a 500 µm stainless steel test sieve using a sieve shaker. The <500 µm sediment fraction passing through the sieve, was allowed to settle from suspension in a container for 48 hours. The supernatant was then removed using a vacuum pump and the remaining <500 µm sediment fraction was washed into a Petri dish, frozen for 12 hours and freeze-dried. The total weight of the freeze-dried fraction was recorded. A sub-sample of the <500 µm fraction was then analysed using a laser sizer. The >500 µm fraction was washed from the test sieve into a foil tray and oven dried at ~90°C for 24 hours. It was then dry sieved on a range of stainless steel test sieves, placed at 0.5-phi intervals, down to 1 phi (500 µm). The sediment on each sieve was weighed to 0.01 g and the values recorded. The results from these analyses were combined to give full particle size distributions for each sample.

#### 8.3 Results

#### 8.3.1 Sediments

Sediment particle size characteristics for the macrofauna and ground-truthing stations are summarised in Tables 8.2 and 8.3, summary description from the camera stills and

**Table 8.2.** Mean values (±SD) of sediment particle size characteristics at stations sampled for the benthic macrofauna.

Station code	Mean particle size (mm)	Sorting	Skewness	Kurtosis	Sand %	Silt/clay %
HG1	0.17 (±0.03)	2.25 (±2.67)	-0.07 (±0.7)	5.35 (±0.81)	79.47 (±0.72)	15.31 (±2.02)
HG2	0.39 (±0.12)	1.36 (±0.31)	-1.83 (±0.63)	7.10 (±3.0)	91.85 (±5.72)	0.00 (±0.0)
HG3	0.59 (±0.4)	3.08 (±0.58)	-0.18 (±0.3)	2.96 (±0.9)	54.89 (±14.23)	23.35 (±22.76)
HG4	1.27 (±0.05)	2.10 (±0.18)	0.74 (±0.09)	5.23 (±0.4)	51.94 (±0.31)	3.35 (±0.89)

**Table 8.3.** Mean value of sediment particle size characteristics at ground-truthing stations.

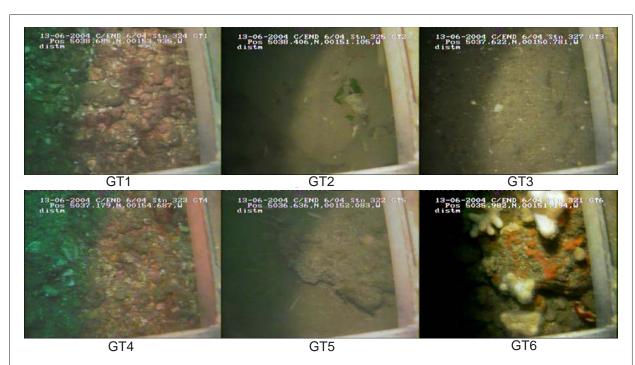
Station code	Mean particle size (mm)	Sorting	Skewness	Kurtosis	Gravel %	Sand %	Silt/clay %
GT1	1.49	2.67	0.39	3.18	43.94	52.02	4.03
GT2	0.34	0.97	-2.71	13.16	3.55	96.45	0.00
GT3	0.65	0.88	-0.72	3.36	5.46	94.54	0.00
GT5	0.55	0.86	-0.73	3.80	3.45	96.55	0.00

video images at the ground-truthing stations are given in Table 8.4 and Figure 8.2. These show that the sediment becomes coarser from north to south with gravel and cobbles overlying sand at the western stations.

Station HG3 was characterised by a poorly-sorted muddy gravelly sand, suggesting evidence of dredged material disposal activity at this station. Station HG2 consisted of slightly gravelly medium sand with no clay/silt component, which suggests the

**Table 8.4.** Visual descriptions taken from the drop camera at ground-truthing stations.

Station	Visual description	Station	Visual description
GT1	Gravel and cobbles interspersed with maerl and shell fragments. Red algae, <i>Flustra</i> sp. and red encrusting sponge attached to cobbles.	GT4	Gravel and cobbles interspersed with maerl and shell fragments. Red algae, <i>Flustra</i> sp. and red encrusting sponge attached to cobbles.
GT2	Rippled sand with shell fragments in the trough and occasional cobble. Sparse algae.	GT5	Rippled sand with shell fragments on surface and in troughs. Occasional cobble or boulder with <i>Flustra</i> sp. attached.
GT3	Clean coarse shelly sand. Rippled fine sand overlying coarse sand in patches.	GT6	Large boulders heavily encrusted with deadman's fingers, <i>Alcyonium digitatum</i> , and sponges.



**Figure 8.2.** Images taken from the drop camera at the ground-truthing stations.

absence of any influence of the fine component of dispersing dredge material. The sediments at stations GT2, GT3 and GT5 comprised medium sands with no silt/clay component, indicating the absence of any accumulation of fine particulates, probably as a consequence of current action.

#### 8.3.2 Acoustics survey

The multibeam survey provided both a bathymetry and backscatter map of the seafloor around the Swanage Bay disposal ground (Figures 8.3 and 8.4).

Figure 8.3. Bathymetry mosaic of Swanage Bay 2004. Reproduced from Admiralty Chart 2615 by permission of the Controller of Her Majesty's Stationery Office and the UK Hydrographic Office (www.ukho.gov.uk). Not to be used for navigation.

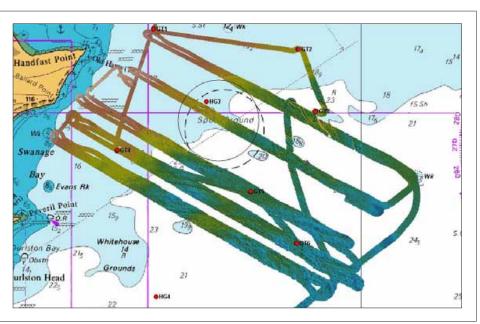


Figure 8.4. Backscatter mosaic of Swanage Bay 2004. Reproduced from Admiralty Chart 2615 by permission of the Controller of Her Majesty's Stationery Office and the UK Hydrographic Office (www.ukho.gov.uk). Not to be used for navigation.

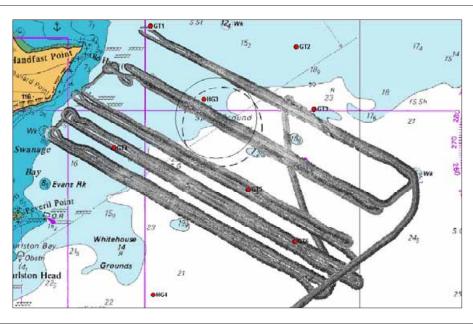
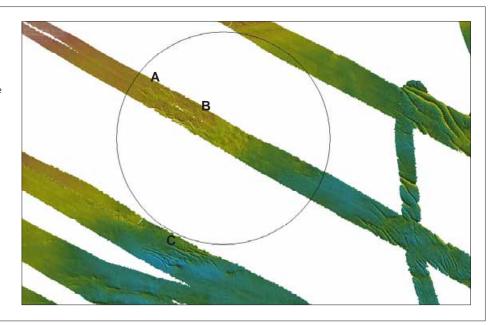


Figure 8.5. Bathymetry image showing mobile sediment (A) and uneven topography (B) within the disposal ground and mobile sediment (C) just outside the south-eastern margin of the disposal ground.



A discrete patch of mobile sediment is apparent within the central and north-eastern part of the disposal ground (Figure 8.5: A). These sand waves indicate a net north-easterly transport regime that is consistent with local bed-sediment pathways. It is possible that these sediments are present as a result of disposal activities. This region is also characterised by an area of uneven topography that may reflect the presence of residual dredged material at the seabed (Figure 8.5: B). Mobile sediments are present just outside the south-eastern margin of the disposal ground (Figure 8.5: C) and may be anthropogenic or natural

in origin. Their asymmetric structure indicates a northeasterly transport direction, again consistent with known sediment transport pathways.

In the eastern part of the disposal site, multibeam bathymetry indicates the presence of a number of "doughnut-shaped" features at the seabed (Figure 8.6). The "doughnuts" are approximately 30 m in diameter and the outer ring is approximately 1 m in height. Features such as these are commonly observed at other UK dredged material disposal sites and are a characteristic result of individual disposal events.

Figure 8.6. Bathymetry image showing doughnut shaped features caused by individual dredging disposal events within the disposal ground.

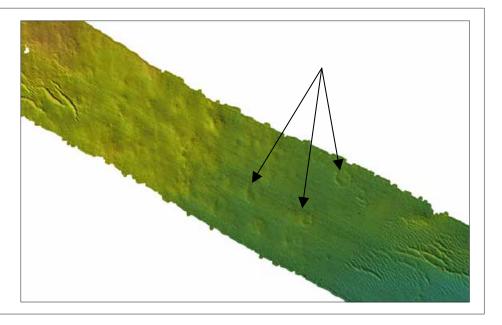
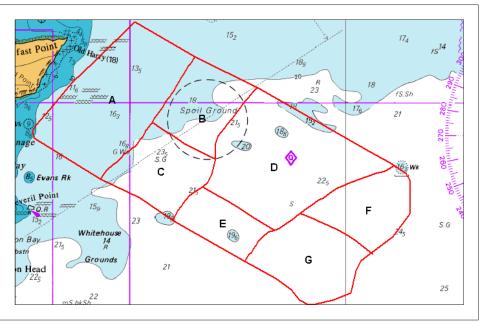


Figure 8.7. Map showing the seven acoustically distinct regions determined from the multibeam data. Reproduced from Admiralty Chart 2615 by permission of the Controller of Her Majesty's Stationery Office and the UK Hydrograpic Office (www. ukho.gov.uk). Not to be used for navigation.



From the multibeam data seven acoustically distinct regions were differentiated and described (Figure 8.7).

Region A consists of a hard flat substratum, which, at station GT1, was shown to be slightly muddy gravelly sand (Table 8.3). Ground-truthing video footage taken at stations GT1 and GT4 showed gravel, cobbles and shell fragments overlying the finer sediment (Table 8.4). The cobbles were seen to be heavily encrusted with algae, bryozoans and sponges. The video footage also showed the presence of maerl in the area.

Region B contains a large part of the Swanage disposal ground including the northwest quadrant where a large proportion of the maintenance dredged material disposal takes place. The area consists predominantly of mobile sand waves and ripples orientated at right angles to the tidal vector and an area of sand ribbons, which lie parallel to the tidal vector; these overly a generally coarser substratum. An area of uneven bathymetry was seen within the disposal ground, which appears to reflect previous disposal activity. At station HG3, in the northwest corner of the disposal ground, the sediment was shown to consist of gravelly muddy sand (Table 8.2). No overall shallowing of the area was seen due to the disposal activity.

Region C is an area of uneven seafloor where the underlying bedrock controls the topography of the surface sediments to a large extent. A slightly deeper channel (~2 m) runs through the middle of the region in a roughly NE/SW direction. The area contains areas of sand ribbons parallel with the tidal vector, patches of mobile sand waves and patches of sand ripples orientated at right angles to the

tidal vector; these overly a generally coarser substratum. The backscatter and bathymetric data show structures that may be reef-forming *Sabellaria* in this area, but our knowledge of the nature and form of the acoustic signature of these structures is limited, and further ground-truthing would be required to confirm this possibility.

Region D consists of an extensive area of mobile sand waves and sand ripples orientated at right angles to the tidal vector (Figure 8.8); these overly a generally coarser substratum. At both stations GT3 and GT5 the sediment was seen to consist of coarse sand with ripples of fine sand overlying the coarse sand in patches at station GT3 and occasional cobbles and boulders at station GT5 (Table 8.4); here the underlying bedrock begins to control the topography of the surface sediments.

Region E consists of an area of uneven seafloor with raised strata of underlying bedrock that outcrop occasionally with a few patches of sand ripples and possible isolated sand waves. The bathymetry also suggests the presence of boulder reefs in this area.

Region F consists of a hard flat substratum with patches of softer sediment.

Region G consists of an uneven area of exposed bedrock and boulder reefs with softer sediment covering the rock in places (Figure 8.9). The ground-truthing video taken at station GT6 showed the presence of bedrock heavily encrusted with dead-man's fingers, *Alcyonium digitatum*, and sponges (Table 8.4).

Figure 8.8. Example of a bathymetry image from Region D showing an area of mobile sand waves and ripples.

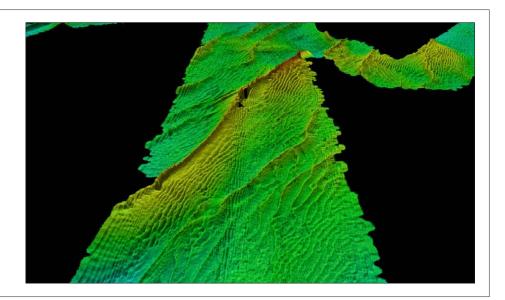
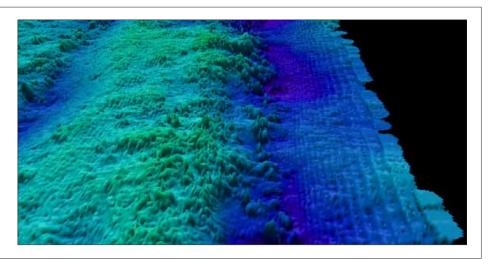


Figure 8.9. Example of a bathymetry image from Region G showing an area of boulder reef.



#### 8.3.3 Benthic macrofauna

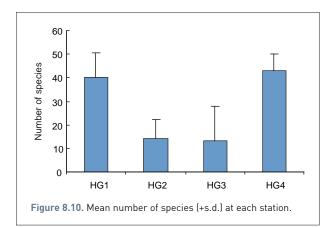
A total of 128 taxa were identified from stations HG1, HG2, HG3 and HG4 of which 43 occurred only once. Polychaetes were the dominant species of which *Harmothoe* spp., *Nephtys* spp., *Spiophanes bombyx*, *Notomastus latericeus*, *Lanice conchilega* and *Thelepus cincinnatus* were the most abundant. The slipper limpet, *Crepidula fornicata*, and the amphipod, *Ampelisca* spp., were both present in high numbers at station HG1 with, respectively, 519 and 94 individuals encountered.

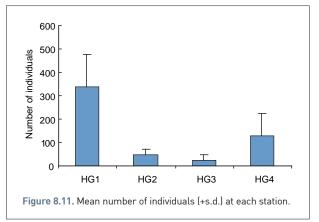
Stations HG2 and HG3 had reduced average numbers of species and individuals compared with stations HG1 and HG4 (Figures 8.10 and 8.11); one-way ANOVA of the data showed these differences to be significant (p < 0.05).

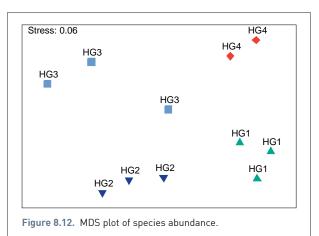
Multi-dimensional scaling analysis was performed using PRIMER v.5 (Clarke and Gorley, 2001) (Figure 8.12). This showed that the replicates from each station clustered together relatively closely but were separated from the other stations. Station HG3 showed a high degree of

variability between replicates, which can be an indicator of disturbance (Warwick and Clarke, 1993). Analysis of similarities (ANOSIM) (Clarke, 1993) was performed on the data and this confirmed the pattern seen in the MDS plot, namely that all stations were significantly different from each other (p < 0.05) in terms of species-abundance relationships.

The similarity percentages program (SIMPER) was used to indicate which taxa contributed the most towards similarity between replicates from within each station (Table 8.5). Station HG3 was dominated by cirratulids and capitellids, which are commonly-cited indicators of organic enrichment and disturbance (Pearson and Rosenberg, 1978). The suspended detritus feeders, *Spiophanes bombyx* and *Lanice conchilega* dominated station HG2; these and other characterising species are typical of exposed inshore sandy environments. The reduced dominance/absence of enrichment/disturbance indicators suggest that natural influences prevail at this location.







**Table 8.5.** Results of SIMPER analysis of the benthic data showing average abundance, average similarity, and % contribution, both individually and accumulatively.

Station	Taxonomic group	Average abundance	Average similarity	% contribution	Cumulative % 18.44	
HG1	Crepidula fornicata	12.16	10.29	18.44		
	Nucula	3.83	4.68	8.39	26.82	
	Maldanidae	3.33	3.66	6.56	33.39	
	Poecilochaetus serpens	3.18	3.51	6.28	39.67	
	Notomastus latericeus	3.23	3.14	5.62	45.29	
	Abra	2.61	2.36	4.22	49.51	
	Nephtys	2.3	2.27	4.07	53.58	
HG2	Spiophanes bombyx	4.88	20.66	42.25	42.25	
	Lanice conchilega	2.29	8.08	16.53	58.79	
	Nephtys	1.63	7.44	15.23	74.01	
	Spio	1.47	5.65	11.56	85.57	
	Ophelia	0.67	2.06	4.21	89.78	
	Eumida	0.8	1.25	2.55	92.34	
HG3	Caulleriella	1.67	13.81	47.88	47.88	
	Spio	1.14	8.44	29.27	77.15	
	Notomastus latericeus	1.14	2.06	7.15	84.3	
	Ophelia	0.67	1.61	5.59	89.89	
	Glycera	0.67	1.46	5.05	94.95	
HG4	Thelepus cincinnatus	3.66	5.16	13.33	13.33	
	Kefersteinia	2	3.11	8.04	21.37	
	Demonax	3.05	2.69	6.96	28.33	
	Maldanidae	1.57	2.2	5.68	34.02	
	Amphiura filiformis	1.57	2.2	5.68	39.7	

#### 8.4 Discussion

The objective of this account was to provide a preliminary assessment of the nature and extent of any effects of disposal activity in advance of a planned increase in the amount of dredged material to be deposited at the site. Further information on trace contaminants and additional acoustic and ground-truthing data will be reported at a later date.

The acoustic survey identified large and small-scale features caused by both anthropogenic and natural processes. The results of the survey suggest that there is some evidence of dredged material disposal activity within the confines of the site. These include areas of uneven topography, discrete patches of mobile sediment, "doughnut-shaped" features caused by individual disposal events (Figures 8.5 and 8.6) and poorly sorted mixed sediment (Table 8.2). Little evidence of sediment dispersal from the disposal site was observed from the acoustic survey with mobile sediment seen only to the southeastern margin of the disposal ground (Figure 8.5: C).

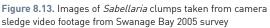
Seven acoustically distinct regions were identified and described from the multibeam data. Ground-truthing at Region A, west of the disposal ground, identified the presence of maerl. No evidence of Sabellaria reefs was obtained from this survey but our knowledge of the nature and form of the acoustic signature of these structures is limited at present. Preliminary observations from towed camera footage taken in summer 2005 did identify aggregations of Sabellaria tubes south-west of the disposal ground (Figure 8.13).

The grab-sampling survey showed that station HG3, situated within the disposal ground, exhibited a large between-sample variability (Figure 8.12), a fauna dominated by cirratulids and capitellids (Table 8.5), and a reduced number of species and individuals (Figures 8.10 and 8.11), all of which are indicators of disturbance (Warwick and Clarke, 1993; Pearson and Rosenberg, 1978). Such indicators provide clear evidence of the effects of disposal activity at this location. Station HG2 also showed reduced numbers of species and individuals but no influence of deposited material was evident from the particle size distributions. Natural influences appeared to prevail at this location, where the sediment consisted of medium sand, which provided a less diverse and probably more mobile habitat compared to the other stations. Stations HG1 and HG4 showed no discernable effects of disposal activity indicating the absence of farfield consequences of the dispersal of fine particulates under tidal influence.

#### 8.5 Conclusion

Survey work to date has therefore identified localised impacts on the benthic macrofauna and sediments arising from dredged material disposal, but there was no evidence of adverse effects arising from the tidally induced dispersal of finer particulates away from the disposal site. We aim to conduct a more extensive survey of the area in 2006 to test predictions concerning the limitation of effects of the planned increase in quantities disposed of to the site.







## 9. Radionuclide concentrations in dredged sediment

#### Author: David McCubbin

#### 9.1 Introduction

In England and Wales, Defra issues licences to operators for the disposal of dredged material under the Food and Environment Protection Act, 1985 (Great Britain - Parliament, 1985a). The protection of the marine environment is considered before a licence is issued. Since dredge material may contain radioactivity, assessments are undertaken, where appropriate, for assurance that there is no significant foodchain or other risk from the disposal. In 2004, a specific assessment was carried out for the disposal of sediment from Whitehaven Harbour in Cumbria. Whitehaven Harbour is known to contain significantly enhanced quantities of artificial and natural radionuclides due to discharges from BNFL Sellafield and the former Marchon Products Ltd. phosphoric acid production plant at Saltom Bay, respectively. Another source of radioactivity was spillages of phosphate ore whilst unloading ships.

#### 9.2 Materials and methods

Sediments cores were collected from a variety of locations to ensure the data provided representative information. Radionuclide assay was achieved using gamma-ray spectrometry by which it is possible to simultaneously measure a wide range of radionuclides commonly found in radioactive wastes. Additional analyses for supported <sup>210</sup>Pb were carried out by radiochemical separation and alpha counting the <sup>210</sup>Po daughter product.

#### 9.3 Results and discussion

Results from the sediment analyses are provided in Table 9.1 The assessment showed that the impact of the radioactivity associated with the disposal operation was low and below 'de minimis' levels of exposure. 'De minimis' relates to doses of

minimis' levels of exposure. 'De minimis' relates to doses of the order of 0.010 mSv or less, and guidance on exemption criteria for radioactivity in relation to sea disposal is available from the International Atomic Energy Agency (International Atomic Energy Agency, 1999, 2003).

**Table 9.1.** Radioactivity in sediment dredged from Whitehaven Harbour. Cumbria. 2004

Area Core No. Depth (m) Radioactivity concentration (dry), Bq kg-1

		•							
			<sup>60</sup> Co	<sup>137</sup> Cs	<sup>154</sup> Eu	<sup>226</sup> Ra (via <sup>214</sup> Pb) <sup>(1)</sup>	<sup>232</sup> Th (via <sup>228</sup> Ac) <sup>(1)</sup>	<sup>238</sup> U (via <sup>234</sup> Th) <sup>(1)</sup>	<sup>241</sup> Am
A	1	Surface	4.4	640	5.6	110	36	180	660
Α	1	1	<1.0	8.6	<2.0	29	31	48	9.0
Α	1	2.4	<1.0	<1.0	<2.0	16	17	26	<3.0
Α	2	Surface	2.4	5400	20	210	42	380	2400
Α	2	1	<1.0	55	<2.0	45	36	91	21
Α	2	2	<1.0	7.4	<2.0	31	37	58	7.8
Α	3	Surface	5.0	1300	2.9	120	33	360	960
Α	3	1	<1.0	110	<2.0	120	36	240	180
Α	3	2.3	<1.0	7.5	<2.0	31	35	62	8.0
Α	4	Surface	9.0	870	5.9	90	27	190	1200
Α	4	1	1.7	5000	14	200	39	480	2100
Α	4	2.5	<1.0	970	4.7	170	44	360	1700
В	5	Surface	11	590	6.9	50	30	86	880
В	5	1	2.0	1500	12	110	35	340	1400
В	6	Surface	11	720	7.1	55	35	93	1000
В	6	1	2.3	3700	15	240	43	490	2700

<sup>🕅</sup> Parent nuclides not directly detected by the method used. Instead, concentrations were estimated from levels of their daughter products.

## 10. Distribution of seabed litter at coastal and offshore sites around England and Wales

#### 10.1 Introduction

Marine litter originates from a variety of sources, ie shipping, tourism, the fishing industry, sewage treatment works, urban run-off and oil installations. It varies in type, but plastic is generally predominant (~60%–90%, (MPMMG, 2002)) because of its globally extensive use and slow degradability. Litter can circulate in the sea for long periods of time, depending on the nature of the item, eg its buoyancy. It is eventually deposited and accumulates in areas of seabed and beaches known as *litter sinks* (Williams *et al.*, 1993), which may be a considerable distance from the source of an item. Litter generally disintegrates over time but some items such as metal and plastics may persist for many years and this influences their impact (UNEP, 1990). Plastics may also be degraded into alternative substances some of which may add to the concerns.

Marine litter can give rise to numerous adverse effects including; entanglement, ingestion, smothering, transport of invasive species, habitat removal and poisoning by breakdown products (MEMG, 2002). Consequently, marine litter has been monitored for many years and there is an abundance of literature on the topic. However, the majority of monitoring undertaken consists of beach and estuarine surveys or floating debris but the availability of data on submerged marine litter is very limited.

There are national and international regulatory controls on litter in the marine environment. Litter from shipping is controlled under Annex V of the International Convention for the Prevention of Pollution from Ships 1973 (MARPOL), whilst land sourced litter is controlled mainly by the Environment Act 1990, covering England, Scotland and Wales and the 1994 Northern Ireland Litter order.

Cefas is a Competent Monitoring Authority for the UK National Marine Monitoring Programme (NMMP). Fish are collected annually under this programme for contaminants and biological effects monitoring and in recent years, the litter from each trawl has been identified and enumerated as part of the sample collection process. Data for 2003 and 2004 are reported here.

#### 10.2 Methods

NMMP surveys were undertaken at intermediate and offshore sites around England and Wales, in June/July 2003 and 2004, using the Cefas research vessels *Cirolana* and *Cefas Endeavour*. Fish were collected using a Granton trawl (~25 m spread and 75 mm mesh with a lining in the

### Authors: John Thain and Jacqueline Jones

codend). At each station, the trawl was towed for 30 min at a speed of 2 knots covering a distance of 1 nautical mile (1,848 m). Litter was removed from the catch during the sorting process and all items were recorded. The number of tows at each station was variable. Litter was recorded for all tows, however, for comparative purposes, only data from the first 2 tows have been assessed.

Data was classified into 12 categories, polythene sheeting/bags, plastic, rope and twine, metal, glass, rubber, cloth, paper/cardboard, polystyrene, wood, sanitary/sewage related debris (SRD) and other, which included items such as shoes and a television set. The litter was quantified by both the number of items in each category and the total number of items per station. The density of litter (no. items/ha) was also calculated for each station

#### 10.3 Results

Litter was collected from 31 NMMP stations in 2003 and 26 in 2004. The total number of litter items collected per station ranged from 0 to 161.

#### 10.3.1 Density of litter

The density of litter (number of items per hectare), was determined for each station by dividing the total number of litter items per station by the area trawled:

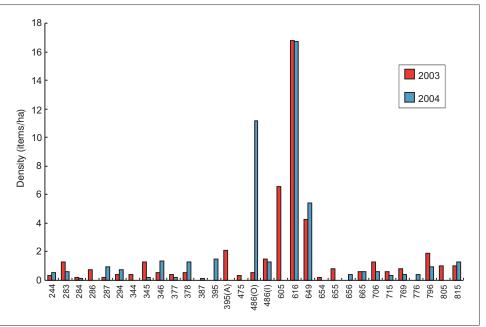
Area trawled per tow = Spread of net x distance travelled =  $25.91 \text{ m} \times 1,848 \text{ m}$ = 4.79 ha

The density of litter ranged from 0 – 16.81 items/ha (Figure 10.1). Litter densities of <2 items/ha were found at the majority of stations. However, significantly higher densities occurred (maximum values) in Carmarthen Bay at NMMP station 616 (16.81 items/ha), Rye Bay (Outer) NMMP 486 (11.17 items/ha), Celtic Deep NMMP 605 (6.58 items/ha) and North Cardigan Bay NMMP 649 (4.81 items/ha).

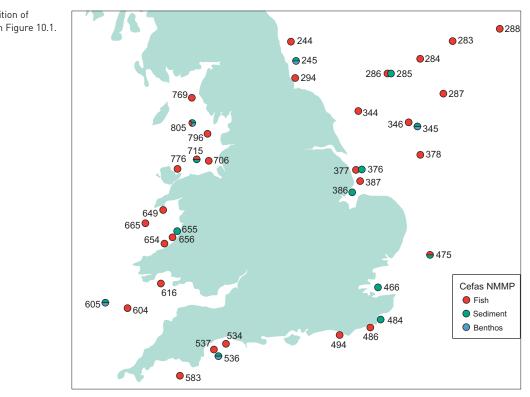
There was generally little year-to-year change in the density of litter, with the exception of Rye Bay (Outer), where the density rose from 0.52 items/ha in 2003 to 11.17 items/ha in 2004.

Table 10.1 shows the mean densities of litter by region. Lowest mean values were found in the North Sea and the highest in the Celtic Seas. (Data for the Channel is inconclusive as it is only for two stations in Rye Bay and the between year variation at Rye Bay Outer is very high).

Figure 10.1. Density of Litter at NMMP sites, June/July 2003 and 2004. Station positions are shown in Figure 10.2.



**Figure 10.2.** Position of stations shown in Figure 10.1.



**Table 10.1.** Mean densities of litter in different regions around England and Wales.

Region	Nos. of stations	Mean no: items/ha			
		2003	2004		
North Sea	15	0.579	0.620		
English Channel	2	6.16	0.992		
Celtic Seas	14	2.47	2.57		

#### 10.3.2 Typological analysis

Table 10.2 shows the breakdown of litter items by category. In both years, plastic (mainly polythene sheet/bags) was the predominant litter type, contributing 72% and 62% of the total items, in respective years and was present at 70%-80% of all stations. Rope and twine (12.8% and 15.9%) and metal/foil (8.4% and 13.4%) were the second most prevalent types and were present at  $\sim$ 60% and  $\sim$ 30% of all stations, respectively. (Note: the quantities of rope/twine for 2003 are underestimated as

**Table 10.2.** Quantities of litter by category, collected at NMMP sites in 2003 and 2004.

	Litter type	Poly sheet /bag	Plastic	Rope/ twine	Metal/ foil	Glass	Sanitary /SRD	Rubber	Cloth	Wood	Card/ paper	Poly- styrene	Other (e.g shoes)
2003	No: items	254	37	52	34	0	1	13	11	2	3	1	2
	%	62.7	9.1	12.8	8.4	0	0.25	3.2	2.7	0.5	0.74	0.25	0.5
2004	No: items	211	39	64	54	12	9	8	0	0	0	5	4
	%	52.5	9.5	15.9	13.4	3	2	2	0	0	0	0.7	1

an additional 5 buckets was collected at the Camarthen Bay station, but not quantified) The quantities of litter in the remaining categories, including sewage related debris, were all relatively low (0%–4%) and were present at a maximum of 28% of stations.

#### 10.4 Conclusions/discussion

This is one of a limited number of studies of seabed litter in UK waters and the first study of the distribution and abundance of seabed litter at NMMP stations around England and Wales. The results indicate that litter is ubiquitous in UK waters, but at generally low abundances. This would suggest that the majority of the litter was collected at intermediate points along litter pathways (MPMMG 2002). The significantly higher densities of litter found at Carmarthen Bay, North Cardigan Bay, Celtic Deep and Rye Bay, would suggest that these are areas

of accumulation, ie litter sinks. This seems to support the findings of Galgani *et al.*, 2000, who also reported similar zones of accumulation in the Celtic Sea and NE English Channel.

Work at HR Wallingford on coastal management suggests that the coastline of England and Wales can be divided into 11 major sediment cells and 47 sub-cells. These cells are defined as lengths of coastline, which are relatively self-contained as far as the movement of sand and shingle is concerned. MPMMG, 2002 suggests that litter may tend to circulate in these cells. The sites at Carmarthen Bay, North Cardigan Bay and Rye Bay are all located within sediment sub-cells, which would seem to support this hypothesis.

The current dataset now covers a period of 5 years. The additional data should enable us to validate some of the conclusions and hypothesis and also to explore trends in litter densities.

## 11. Radioactivity in UK coastal waters

#### Author: David McCubbin

#### 11.1 Introduction

The UK government is committed to preventing pollution of the marine environment from ionising radiation, with the ultimate aim of reducing concentrations in the environment to near background values for naturally occurring radioactive substances, and close to zero for artificial radioactive substances (Defra, 2002). Therefore a programme of surveillance into the distribution of selected radionuclides is maintained using research vessels and other means of sampling. Evidence to help gauge progress towards achievement of the Government's vision for radionuclides and other hazardous substances is set out in a recent report (MEMG, 2005). The seawater surveys reported here also support international studies concerned with the quality status of coastal seas (eg OSPAR, 2000) and provide information that can be used to distinguish different sources of man-made radioactivity (eg Kershaw and Baxter, 1995).

Detailed historical data for <sup>134</sup>Cs and <sup>137</sup>Cs in seawater have been published in a series of reports so as to aid model development (Camplin and Steele, 1991; Baxter *et al.*, 1992; Baxter and Camplin, 1993a–c) and have been used to derive dispersion factors for nuclear sites (Baxter and Camplin, 1994). The data have also been used to examine the long distance transport of activity to the Arctic (Kershaw *et al.*, 1999) and long-term trends in Northern European seas (Povinec *et al.*, 2003).

Discharges from Sellafield peaked in the mid 1970s. A number of counter-measures were introduced, including the Site Ion Exchange Effluent Plant (SIXEP, in 1986), which controlled Cs discharges, and the Enhanced Actinide Removal Plant (EARP, in 1994). EARP allowed the treatment of medium-active, stored liquors, which also contained <sup>99</sup>Tc - not treated by EARP- and consequently these discharges (which are of limited radiological significance) rose in 1994. However, following a successful trial of new abatement technology, discharges once again decreased in 2003 (Mayall, 2005). Discharges of <sup>129</sup>I, <sup>90</sup>Sr, <sup>14</sup>C, <sup>60</sup>Co, and <sup>3</sup>H also increased in the mid 1990s, as a result of operational changes at the site, including the starting up of the Thermal Oxide Reprocessing Plant (THORP) in 1995.

Studies of the migration behaviour of <sup>99</sup>Tc have afforded opportunities to substantiate and extend the information obtained from earlier similar studies of <sup>137</sup>Cs. The distribution of <sup>99</sup>Tc in waters around the British Isles prior to, and immediately after, the increased <sup>99</sup>Tc discharges (in 1994) indicated a rapid advection of <sup>99</sup>Tc within and from the Irish Sea to the north of Scotland as compared to previous estimates (Leonard *et al.*, 1997a,b; McCubbin

et al., 2002). The subsequent transport rate out of the North Sea and northwards with the Norwegian Coastal Current and West Spitsbergen Current slowed markedly, in apparent correspondence with variations in the North Atlantic Oscillation (NAO) winter index (Kershaw et al., 2004).

#### 11.2 Sampling

The research vessel programme on radionuclide distribution currently comprises an annual survey of the Bristol Channel together with biennial surveys of the Irish Sea and the North Sea. Large volume surface seawater samples (50 litres) are collected, using the ships pumped supply, during cruises of the Cefas research vessels, *Cefas Endeavour* and *Corystes*. Surveys of the Bristol Channel, Irish Sea and the western English Channel were carried out in September/October 2003 and of the Bristol Channel, North Sea and western English Channel between August-October 2004.

#### 11.3 Sample analysis

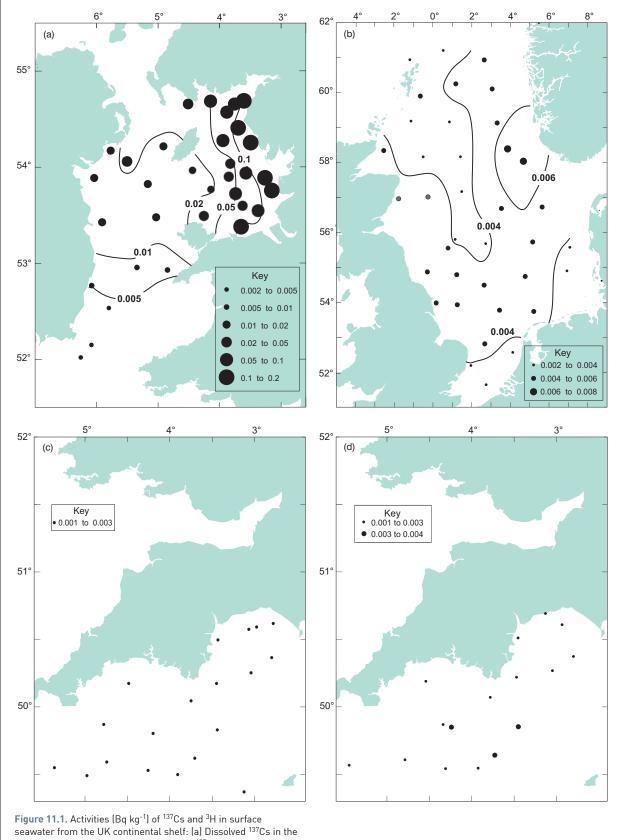
Samples were filtered (0.45 µm) to separate dissolved and particulate phases. Analyses of dissolved <sup>137</sup>Cs involved pumping filtered seawater, acidified with nitric acid, through cartridges filled with ASG resin (ammonium duodecamolybdophosphate on silica gel) to extract caesium. Analyses of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

#### 11.4 Results and discussion

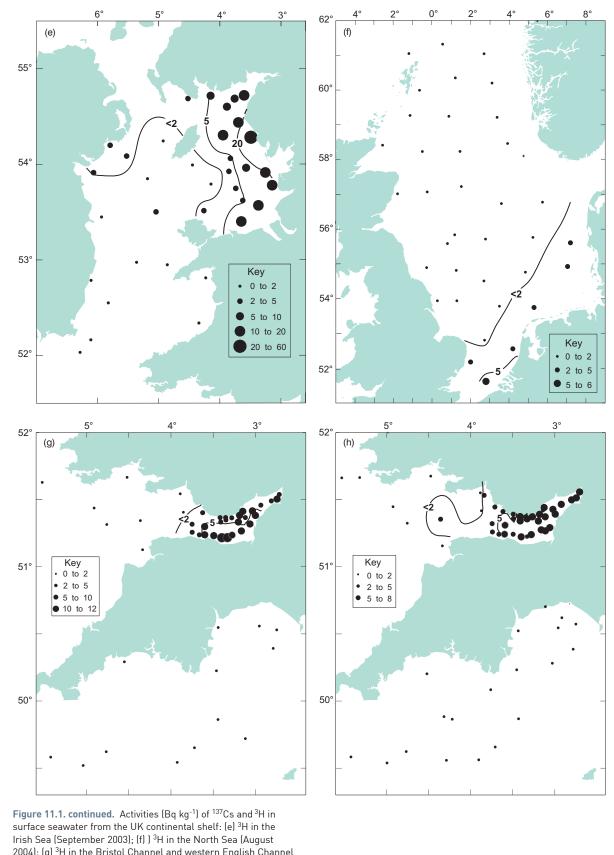
The results of the seawater surveys are given in Figures 11.1(a)–(h).

#### 11.5 137Cs distribution

The Irish Sea  $^{137}$ Cs data (Figure 11.1(a)) indicate that the concentrations observed along a large section of the British coastline, extending from Liverpool Bay in the south to the Mull of Galloway in the north (typically 0.05–0.1 Bq kg<sup>-1</sup>), were significantly greater than those observed along the Irish coastline (typically 0.005–0.02 Bq kg<sup>-1</sup>). The  $^{137}$ Cs contours extend parallel to the Cumbrian coastline. The overall distribution of  $^{137}$ Cs is in line with that expected



**Figure 11.1.** Activities (Bq kg $^{-1}$ ) of  $^{137}$ Cs and  $^{3}$ H in surface seawater from the UK continental shelf: (a) Dissolved  $^{137}$ Cs in the Irish Sea (September 2003); (b) Dissolved  $^{137}$ Cs in the North Sea (August 2004); (c) Dissolved  $^{137}$ Cs in the western English Channel (September - October 2003) and (d) Dissolved  $^{137}$ Cs in the western English Channel (September - October 2004).



2004); (g) <sup>3</sup>H in the Bristol Channel and western English Channel (September - October 2003) and (h) <sup>3</sup>H in the Bristol Channel and western English Channel (September - October 2004).

from our knowledge of mean surface water circulation in the Irish Sea (Dickson, 1987). The predominant flow of water is northward via input of Atlantic water from St. George's Channel, passing to the west of the Isle of Man. A minor component of the flow enters the eastern Irish Sea to the north of Anglesey and moves anti-clockwise round the Isle of Man before rejoining the main flow to exit through the North Channel. The <sup>137</sup>Cs activities observed here are only a small percentage of those prevailing in the late 1970s. Levels as high as 30 Bq kg-1 have been observed in the vicinity of the Sellafield outfall (Baxter et al., 1992) during the period when discharges from Sellafield were substantially greater. Indeed, differences between the <sup>137</sup>Cs/<sup>99</sup>Tc ratio in Sellafield discharges and seawater indicate that <sup>137</sup>Cs remobilisation, from sediments contaminated by large discharges in the 1970s, is presently the predominant (~90%) source term to the water column (McCubbin et al., 2002).

The 2004 <sup>137</sup>Cs data for the North Sea (Figure 11.1(b)) show very low concentrations (<0.01 Bg kg<sup>-1</sup>) throughout the survey area that are only slightly above the global fallout levels in North Atlantic surface waters (~0.0012 Bq kg-1 in 2002, Bailly du Bois pers. comm.). The distribution in the North Sea is typical of that observed in the last 5 years. The highest concentrations were observed at two stations sites close to the Norwegian coast, due to the input of Chernobyl-derived <sup>137</sup>Cs from the Baltic via the Skaggerak. In the previous three decades, the impact of discharges from the reprocessing plants at Sellafield and La Hague has been readily apparent, carried by the prevailing residual currents from the Irish Sea and the Channel, respectively (Povinec et al., 2003). The concentrations of <sup>137</sup>Cs in the North Sea have tended to follow the temporal trends of the aforementioned discharges, albeit with a time lag. The maximum discharge of <sup>137</sup>Cs occurred at Sellafield in 1975 and <sup>137</sup>Cs concentrations of up to 0.5 Bg kg<sup>-1</sup> were measured in the late 1970s. Due to significantly decreasing discharges after 1978, remobilisation of <sup>137</sup>Cs from contaminated sediments in the Irish Sea appears to be the dominant source of water contamination for much of the North Sea (McCubbin et al., 2002).

Concentrations in the western English Channel (average activity 0.002 Bq kg<sup>-1</sup>) were only slightly enhanced compared with the background level resulting from global fallout (Figures 11.1(c) and (d)).

#### 11.6 <sup>3</sup>H distributions

Levels of <sup>3</sup>H in the Irish Sea (Figure 11(e)) were below the limit of detection (~2 Bq kg<sup>-1</sup>) over a large proportion of the survey area. However, the impact of discharges from Sellafield and the Heysham nuclear power plant was apparent along the Cumbrian and southern Scottish coastline, extending from Morecambe Bay in the south to Luce Bay in the north. Along this section, <sup>3</sup>H activities were in the range 5 - 55 Bq kg<sup>-1</sup>.

The concentrations of tritium observed in the North Sea (Figure 11.1(f)) were also below the limit of detection (~2 Bq kg-1) over most of the survey area. However, slightly enhanced levels were apparent along a part of the European coastline. These were likely to be a result of discharges from the La Hague nuclear fuel reprocessing plant in France.

In the Bristol Channel (Figure 11.1(h)), the greatest <sup>3</sup>H concentrations in 2004 (~5 - 8 Bg kg-1) were observed in the Severn estuary towards the eastern limit of the survey area. <sup>3</sup>H concentrations decreased rapidly with distance downstream of the points of discharge (ie in a westerly direction). Concentrations at the mouth of the Bristol Channel were below the limit of detection (2 Bq kg-1). There was measureable elevation in the vicinity of the Hinkley Point nuclear power plant and the Amersham radiopharmaceutical plant at Cardiff. The spatial distribution is consistent with conservative dispersion behaviour in the macrotidal Severn estuary. Tidal current speeds generally exceed 1.5 ms<sup>-1</sup> at springs and 0.75 ms<sup>-1</sup> at neaps, meaning that water parcels can move up to 25 km during a single flood or ebb tide (Uncles, 1984). Similar trends were apparent in data for 2003 (Figure 11.1(g)), albeit that levels were slightly enhanced on the English side close to the Hinkley Point power station.

#### 11.7 Other radionuclides

Concentrations of <sup>99</sup>Tc in seawater are now decreasing, following the installation of new effluent treatment procedures at Sellafield. The results of research cruises involving studies of this radionuclide have been published by Leonard *et al.* (1997a and b, 2001, 2004) and McCubbin *et al.* (2002). Trends in plutonium and americium concentrations in the seawater of the Irish Sea have been considered by Leonard *et al.* (1999). A full review of the quality status of the north Atlantic has been published by OSPAR (2000).

## 12. Determination of volatile organic compounds in seawater

#### Authors: Carole Kelly, Katherine Langford and Lisa Johnsey

#### 12.1 Introduction

Volatile organic compounds (VOCs) are frequently found in aquatic systems as a result of industrial discharges and fuel production, storage and use. VOCs are released from a variety of industrial sources, including the production and use of solvents, adhesives and refrigerants (Nikolaou et al., 2002). For example, chloroform and 1,2-dichloroethane are used as fumigants, xylenes are used as solvents for pesticides, trichloroethene is used as a degreaser and tetrachloroethene as an industrial solvent. halogenated VOCs can be formed by water treatment processes that use chlorine. Specific non-halogenated VOCs derive from fuel usage, which includes spills, leaks from storage tanks, general handling and the use of petrol in vehicles. All of these sources allow VOCs entry into rivers, estuaries and the sea. Concentrations detected in UK environmental waters can be as low as 10 ng l-1 and range to the µgl l-1 level, however, after a fuel spill levels of the volatile, one-ring aromatic compounds collectively known as BTEX (benzene, toluene, ethylbenzene and xylenes) could be expected to be much higher and may be acutely toxic to marine organisms.

#### 12.2 Methods

An analytical method has been developed for the detection of VOCs, primarily to enable the determination of BTEX compounds following maritime oil spills. The method used a purge and trap system which consisted of a Tekmar 2016 autosampler with a Tekmar 3010 concentrator coupled to a gas chromatography-mass spectrometry (GC-MS) instrument (Polaris Q Thermo Finnigan UK). Known amounts of internal standards (dichlorobenzene-d<sub>4</sub>; dichlorethane-d<sub>4</sub>. toluene-d<sub>8</sub>) were added to the seawater sample aliquots (25 ml) and the samples injected into the purge vessel. Analytes were purged from the purge vessel for 11 mins with helium at a flow rate of 38 ml min<sup>-1</sup> before a 2 min dry purge. The trap used was Vocarb 2000 (Supelco, UK) with a desorption time of 4 mins at 250°C. The analytical conditions of the GC-MS were as follows: A 60 m x 0.25 mm I.D. x 1.4 µm film thickness DB624 column was used (J&W Scientific, UK). An initial temperature of 35°C was held for 3 mins, increased to 90°C at 8°C min-1, held for 4 mins, increased to 200°C at 6°C min-1 and held for 10 mins. The MS was operated in full scan mode from 50-180 Daltons with a 0.85 second cycle time.

#### 12.3 Sampling sites

Following validation of this method in the laboratory, a small survey of VOCs in the River Tees was undertaken, with seawater samples being collected from sites previously sampled in 1992 (Dawes and Waldock, 1994). The locations as shown in Figure 12.1.

Samples (2.5 I) were taken at a depth of 0.5 m using a weighted Winchester sampler. They were then decanted into amber EPA vials (40 ml) without headspace or air bubbles, sealed with PTFE lined septa, kept in a cool box for transit and refrigerated until required for analysis, which was conducted within 48 hours of collection.

#### 12.4 Results

The concentrations of the selected VOCs determined in this and the 1992 study are presented in Table 12.1.

#### 12.5 Discussion

The Tees Estuary, as well as supporting a busy port, is one of the UK's most significant industrial areas with chemical and petroleum industries on both banks. In North Teeside there is a plant which which manufactures BTEX compounds, while on the south side there is one of the largest petrochemical plants in Europe manufacturing ethylene, butadiene, propylene and *p*-xylene. Both these sites are situated between the inner and mid sampling points. Numerous other potential VOC sources are situated along the Tees Estuary.

Over the last 10 years, clean up of the estuary has become a high priority. As a result, direct discharge from industry without prior treatment has ceased and generally the VOC concentrations found were lower than in our previous study. Concentrations determined during the 1992 survey ranged from 11,500 ng l<sup>-1</sup> for chloroform in the mid estuary to below the limit of detection for benzene at all sites. In the 2004 study, most compounds were below the limit of detection of <10 ng l<sup>-1</sup>, with the exception of BTEX, chloroform and bromoform, the latter of which reached 634 ng l<sup>-1</sup> at the inshore Tees site.

In 1992 Dabholm Gut was receiving both treated and untreated primary sewage effluent. Following improvements it currently receives secondary treated effluent from Bran Sands wastewater treatment works.

Figure 12.1. Tees sample locations.

Inshore

Estuary

Mid estuary

Inner estuary

O

2
4
6
Kilometres

Table 12.1. Concentrations of VOCs  $\{ng l^{-1}\}\$  in the Tees Estuary; 1992 sample results compared to 2004 sample results  $\{*nr = not \text{ reported}\}\$ .

Compound	Inner T Estuar		Mid Tee Estuary		Lower Estuar		Estuar Mouth	у	Inshore Tees	9
	1992	2004	1992	2004	1992	2004	1992	2004	1992	2004
1,1-dichloroethane	1150	<10	4020	<10	1410	<10	1210	<10	720	<10
chloroform	904	210	11500	186	1490	295	286	289	<10	140
1,1,1-trichloroethane	115	<10	602	<10	94	<10	30	<10	<10	<10
carbon tetrachloride	<25	<10	29	<10	<25	<10	<25	<10	<25	<10
benzene	<10	67	<10	34	<10	465	<10	<10	<10	<10
trichloroethene	55	<10	269	<10	33	<10	<10	<10	<10	<10
cis-1,3-dichloropropene	nr	<10	nr	<10	nr	<10	nr	<10	nr	<10
toluene	<10	45	61	17	<10	37	<10	<10	<10	<10
trans-1,3-dichloropropene	nr	<10	nr	<10	nr	<10	nr	<10	nr	<10
1,1,2-trichloroethane	nr	<10	nr	<10	nr	<10	nr	<10	nr	<10
tetrachloroethene	67	<10	185	<10	34	<10	<10	<10	<10	<10
ethylbenzene	<10	<10	46	<10	<10	<10	<10	<10	<10	<10
<i>p</i> -xylene	nr	230	nr	99	nr	249	nr	<10	nr	<10
o-xylene	<10	12	1340	<10	<10	38	<10	<10	<10	<10
bromoform	78	122	101	279	113	243	71	410	63	634

The provision of this additional treatment may have contributed to the reduction in short chain chlorinated compound concentrations observed in the 2004 survey.

Concentrations of BTEX compounds were highest at the inner Tees and lower Tees sampling points. The increase in BTEX compounds detected may be attributed in part to the increased shipping activity in the estuary, as BTEX compounds may be released during various shipping activities, such as transhipment of oil and fuel transfer.

Bromoform was detected in higher concentrations than other compounds, at the Tees mouth and the

inshore sampling point. This may be due to its lower volatility, enabling it to travel further down the estuary before volatilisation into the atmosphere. It has also been suggested that high concentrations may result from diffuse bromination reactions rather than direct discharge, which would favour bromoform formation over chloroform.

This study has enabled us to develop and validate a method of determining VOC concentrations in water, which will prove useful in contributing to the advice we provide in emergency response situations.

# 13. The detection of HBCD and TBBPA brominated flame retardants in estuaries and North Sea food webs

#### Author: Steven Morris

#### 13.1 Introduction

Brominated flame retardants (BFRs) are used to inhibit combustion processes and are found in electronic equipment, polystyrene foam, textiles and building materials. Three BFRs, decabromodiphenyl ether (deca-BDE), tetrabromobisphenol A (TBBPA), and hexabromocyclododecane (HBCD) account for approximately 50% of the world's usage of BFRs. In 1999, 9,200 t of HBCD (OSPAR, 2001) and 13,800 t of TBBPA (BSEF, 2000) were used in the European Union.

The environmental presence of brominated flame retardants is already well known, although environmental data are mainly restricted to the polybrominated diphenylethers. Where information relating to the distribution of HBCD exists, its detection is similar to that observed for other BFR formulations such as pentabromodiphenyl ether (penta-BDE) (de Boer et al., 1998), and this alerted us

to a potential environmental concern. We developed analytical methods for the detection and quantification of individual ( $\alpha$ -,  $\beta$ - and  $\gamma$ -) HBCD diastereoisomers as well as TBBPA by liquid chromatography coupled to electrospray ionization, mass spectrometry (LC-ESI-MS). This study is the first of its kind to apply LC-MS to their detection and quantitation, and presents data describing their distribution amongst biotic and abiotic aquatic compartments. Further information can be found in Morris *et al.* (2004).

#### 13.2 Methods

A sample location map of sediment and biota taken from the rivers and estuaries of the North and Irish Seas, and Scheldt basin is presented in Figure 13.1. Sediments were either collected by a van Veen grab or by a hand-held Day grab. Yellow eels (*Anguilla anguilla*) were acquired by either electro- or fyke fishing techniques. Eggs (n =

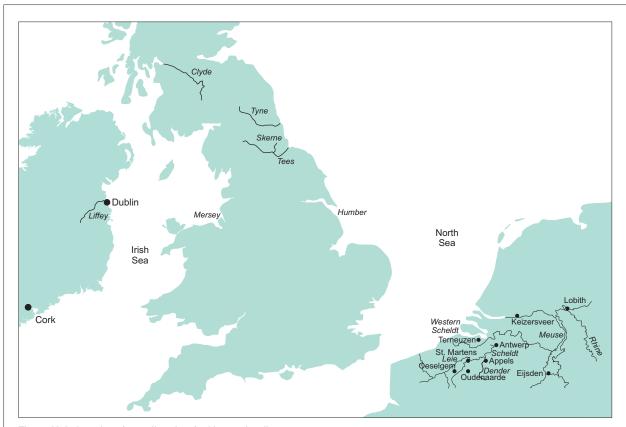


Figure 13.1. Location of sampling sites for biota and sediments.

10) from the common tern (Sterna hirundo), one sample of mysid shrimp (Crangon crangon), and one of sand goby (Pomatoschistus minutus) were taken in 2001. Invertebrates and fish were taken during a research vessel (RV Pelagia) cruise (1999). Three whiting and a single seastar (Asterias rubens) were taken within the mouth of the Tees estuary (RV Cirolana; 1999). Samples of marine mammals were taken from stranded or by-caught individuals. Cormorant (Phalacrocorax carbo) livers were obtained between 1999 and 2000, and acquired under the UK's Home Office License. Influents, effluents and sewage sludges were taken in 2002, from selected sewage treatment works (STWs) in the Netherlands, UK, and Eire. Influents and final effluents were filtered using 0.45 µm polyvinyl disc filters (BDH, Dorset, UK) to obtain the dissolved and particulate phases. Leachates from landfill sites in receipt of domestic wastes were sampled in 2002, and from nine locations in The Netherlands, three from southeast England, and three from Eire.

Liquid-solid extractions using acetone: n-hexane mixtures were performed on sediments and biota. An aliquot of the crude extract was then shaken with concentrated sulphuric acid to degrade co-extracted lipid material and this extract was added to a gel permeation chromatography system. Further clean up was applied using silica gel column chromatography.

Analysis involved liquid chromatography-mass spectrometry (LC-MS). A  $C_{18}$  LC column was used to separate the TBBPA and HBCD analytes. Selected ion recording of the [M-H]- ions was then performed. Concentrations of these BFRs were determined by external and internal quantification, respectively, and total ( $\Sigma$ )HBCD values were obtained by the summation. The lowest limits of quantitation for the HBCDs and TBBPA were 0.15 and 0.05 ng on column, respectively.

#### 13.3 Results and discussion

Possible sources of HBCD and TBBPA released to the aquatic environment include effluent discharges from STWs and leachates from landfill sites. For UK STW influents, effluents and sludge samples and, where detectable, both  $\Sigma$ HBCD and TBBPA were present in influents and, for HBCD, levels were measured in the dissolved phase (up to 24 ng l $^{-1}$ ) as well as the particulate phase [<4-29  $\mu g \ kg^{-1}$  dry weight (d.w.)]. As a product of its hydrophilic nature, TBBPA could also be quantified (maximum 85 ng l $^{-1}$ ) in the influent dissolved phase. Levels of both  $\Sigma$ HBCD and TBBPA in the dissolved and particulate phases of the final effluents were below the limit of detection and, as

expected, these compounds were enriched in settled sewage sludges. A maximum concentration of  $\Sigma HBCD$  of 8.3 mg kg-1 (d.w.) was quantified in a secondary treated sludge sample from Cork, Eire and levels of TBBPA in sludges were similar to those previously found in Swedish sludges (Öberg et al., 2002). Release of HBCD from dust (Leonards et al., 2001) during domestic washing processes may account, in part, for their presence in influents and STW sludges. The accumulation of TBBPA in sewage sludges may also be explained by the use of recycled thermal paper used in the production of toilet paper in which the former has been shown to contain free TBBPA (Kuch et al., 2001). Landfill sites in receipt of waste, including dust material, also represent point sources for the dissemination of these chemicals. However, the leachates from three UK repositories showed no evidence of either HBCD or TBBPA. It was only in the particulate phase of Dutch leachates that  $\Sigma HBCD$  and TBBPA were found, and at concentrations of 110 and <25 µg kg<sup>-1</sup> d.w., respectively. One leachate sample yielded a maximum of 76 mg( $\Sigma$ HBCD) kg<sup>-1</sup>, and both  $\alpha$ - and  $\beta$ -isomers were present in all Dutch landfill samples.

### 13.3.1 HBCD and TBBPA concentrations in sediments

It was found that  $\Sigma$ HBCD was present in all river and estuarine sediments sampled from the UK, Belgium and the Netherlands (Table 13.1). The highest level was detected in sediments from the River Skerne, N.E. England (1.7 mg kg-1 d.w.), and in the vicinity of a site of manufacture at Newton Aycliffe, County Durham. Levels in sediments from the R. Rhine were higher than those found in the R. Meuse, and a range of concentrations from 2.3 to 34 µg kg<sup>-1</sup> d.w. reflected the industrial activity that is present in the Rhine basin. The impact from textile industries possibly utilising HBCD in their products, as well as HBCD production or processing facilities in the vicinity, was evident in sediments from the Western Scheldt, Antwerp harbour, and at several locations along the Scheldt basin in Belgium. The 'stereoisomeric' distribution in sediments was often similar to the pattern of the commercial formulation (Figure 13.2(c) and (d)) in that  $\gamma$ -HBCD predominated and  $\alpha$ -HBCD was <10% of the sum of the three isomers. The  $\beta$ -isomer was present in the lowest quantities.

During the treatment of products and materials with HBCD, temperatures of >160°C are often applied and this can cause a thermal rearrangement and conversion of  $\gamma$ -HBCD, resulting in a considerably higher percentage of  $\alpha$ -HBCD (Peled *et al.*, 1995). At some locations, close to

Table 13.1. Range and mean concentrations including one standard deviation (1s.d.) of total ( $\Sigma$ )HBCD and TBBPA in aquatic biota ( $\mu$ g kg<sup>-1</sup>; lipid weight), and sewage, landfill leachates and sediments ( $\mu$ g kg<sup>-1</sup>; dry weight).

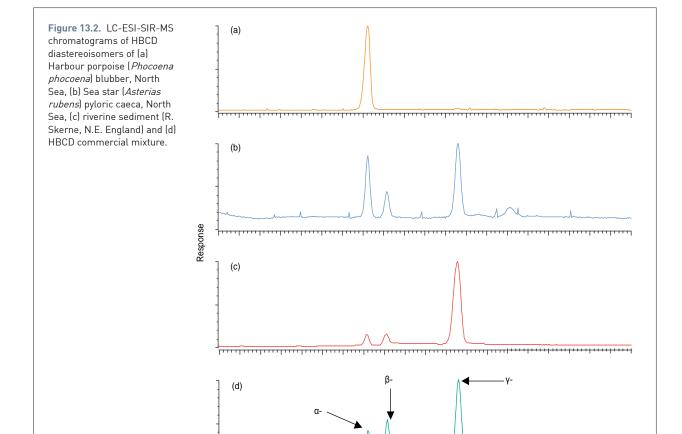
Sample type	Location	n	∑HBCD		ТВВРА		
			Range	Mean (1s.d.)	Range	Mean (1s.d.)	
Biota		-					
Common whelk (whole)	North Sea	3	29-47	35 (10)	5.0-96	45 (46)	
Sea star (digestive system)	Western Scheldt	3	<30-84	44 (42)	<1-2	4 (5)	
Hermit crab (abdomen)	North Sea	9	<30	-	<1-35	11 (15)	
Whiting (muscle)	North Sea	3	<73	-	<97-245	136 (125)	
Cod (liver)	North Sea	2	<0.7-50	-	<0.3-1.8	-	
Hake (liver)	Atlantic - S. Ireland	1	<0.6	-	<0.2	-	
Eel	Scheldt basin, Belgium	18	<1.7-33000	2705 (7793)	<0.1-13	1.6 (3.2)	
Eel	Rivers - Netherlands	11	12-850	293 (269)	<0.1-1.3	0.3 (0.5)	
Cormorant (liver)	England	5	138-1320	796 (482)	2.5-14	7.1 (4.5)	
Common Tern (eggs)	Western Scheldt	10	330-7100	1501 (1997)	<0.9	-	
Harbour seal (blubber)	W. Wadden Sea	2	63-2055	-	<14	-	
Harbour porpoise (blubber)	North Sea	4	440-6800	2945 (2920)	<11	_	
Harbour porpoise (blubber)	N. Sea - E. England	5	<5-1019	312 (422)	0.1-418	83 (187)	
Sewage							
Influent	Netherlands	5	<3-570	114 (255)	<6.9	-	
Effluent	Netherlands	5	< 0.4-140	48 (67)	3.1-63	42 (24)	
Sludge	Netherlands	9	<0.4-93	35 (29)	2-600	79 (196)	
Influent	S.E. England	5	0-29.4	6.3 (13)	<3.9-21.7	7.5 (8)	
Effluent	S.E. England	5	<3.9	-	<3.9	-	
Sludge	S.E. England	5	531-2683	1401 (814)	15.9-112	59 (41)	
Sludge	Cork, Ireland	6	153-9120	3322 (3942)	<2.4-192	95 (83)	
Landfill							
Leachate water	Netherlands	9	<29-67700	10074 (22788)	<6-320	54 (108)	
Sediments							
	Scheldt basin	19	<0.2-260	29 (69)	<0.1-67	5.4 (16)	
	Western Scheldt	19	<0.1-128	18 (38)	<0.1-3.2	1 (1)	
Estuarine + riverine	Netherlands	9	<0.5-34	10 (13)	<0.1-6.9	2.2 (2.2)	
Estuarine + riverine	England	22	<2.4-1680	199 (364)	<2.4-9750	451 (2077)	
	Dublin Bay, Ireland	9	<1.7-12	2.9 (5)	-	-	

textile industries (R. Scheldt near Oudenaarde, Belgium), the detection of higher percentages of both the  $\alpha\text{-}$  and  $\beta\text{-}$  isomers may have been related to use of these flame inhibitors in textile materials. Concentrations of HBCD have been reported previously (Sellström and Jansson, 1995), and its detection in fish from the River Viskan (Sweden) was also related to the presence of textile industry upstream of the sampling location.

Despite the higher production volume of TBBPA, this compound was found in some but not all samples (Table 13.1), and at substantially lower levels than  $\Sigma \text{HBCD}.$  Concentrations of TBBPA in Dutch and UK sediments ranged from 2 to 9 750  $\mu g \ kg^{\text{-}1}$  (d.w.).

## 13.3.2 Evidence of HBCD bioaccumulation and biomagnification in North Sea food webs

There is evidence to suggest that biomagnification of HBCDs is occurring in some components of the North Sea food web. Concentrations reported here (Table 13.1) have been normalised to the lipid weight (l.w.) of the organism. The bioaccumulative potential of  $\Sigma$ HBCD was supported by evidence of biomagnification from mysid shrimp, via the gobiid fish species, to eggs of the common tern in the Western Scheldt estuary. Tern eggs showed  $\Sigma$ HBCD residues ranging from 0.3-7.1 mg kg<sup>-1</sup> l.w. The biomagnification of  $\Sigma$ HBCD is not a simple process to explain since, at the trophic levels of fish and top-predators,



 $\alpha\text{-HBCD}$  was observed to dominate the stereoisomeric profile. This can be caused either by a preferential uptake of the  $\alpha\text{-isomer},$  or a much more rapid elimination of the  $\beta\text{-}$  and  $\gamma\text{-}$  isomers.

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Time (min)

Eels taken at the Rhine and Scheldt basin sediment sampling locations also showed high HBCD levels, and a maximum concentration of 33 mg kg $^{-1}$  l.w. was found in eels from the Scheldt basin. The stereoisomeric profiles in these organisms mirrored the observations from other biota whereby  $\alpha\textsc{-HBCD}$  dominated. Liver samples of a top-predator seabird, the cormorant obtained from the Tees estuary in northeast England, contained  $\Sigma\textsc{HBCD}$  in the range of 0.1-1.3 mg kg $^{-1}$  l.w., and there was a strong prevalence of the  $\alpha\textsc{-isomer}$  (>85% of  $\Sigma\textsc{HBCD}$ ).

North Sea macro invertebrates yielded detectable levels of  $\Sigma$ HBCD, albeit two orders of magnitude lower than those in top-predators. A range of <30-84  $\mu$ g kg<sup>-1</sup> l.w. was found in the sea star and of 29-47  $\mu$ g kg<sup>-1</sup> in the common whelk (*Buccinum undatum*). Sea stars were also acquired close to sites where HBCD production currently takes place (ie in the Tees estuary as well as in the Western Scheldt). The observed levels and isomeric profiles

(Figure 13.2(b)) in these sentinel organisms are possibly indicative of dispersion processes from localised industrial manufacture and usage.

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The highest levels of  $\Sigma$ HBCD (2.1-6.3 mg kg<sup>-1</sup>) were found in the liver and blubber of lung-breathing, top-predators that cannot eliminate their contaminant load to the ambient seawater as efficiently as gill-breathing aquatic organisms. Still, these levels were approximately five times lower than HBCD residues measured in eels from the Scheldt basin. The stereoisomeric profiles in harbour porpoise (Phocoena phocoena) and harbour seal (Phoca vitulina) were also strongly dominated by  $\alpha$ -HBCD (>80% of  $\Sigma$ HBCD; Figure 13.2(a)). It is interesting to note that at temperatures of 160 to 200°C, the three HBCD stereoisomers can thermally rearrange into each other (Peled et al., 1995). Independent of the initial isomeric composition, the final distribution is the same with the  $\alpha$ -,  $\beta$ -, and  $\gamma$ - stereoisomers accounting for 79  $\pm$  2, 13  $\pm$  1, and 9  $\pm$  1% of the mixture, respectively. Thus, the most abundant isomer detected in aqueous biota is also the most thermodynamically stable one found at highest concentration after an HBCD mixture has been subjected to high temperatures.

#### 13.3.3 Levels of TBBPA in aquatic organisms

Eels from the Scheldt showed TBBPA values of up to 13 µg kg-1 l.w., which is more than three orders of magnitude lower than the  $\Sigma$ HBCD level (33 mg kg<sup>-1</sup>). TBBPA was also detected in both cormorants and porpoises (R. Tees, UK) although levels were again up to two orders lower than those of HBCD. Thus, the relatively low environmental concentrations of TBBPA compared to HBCD might be related to the fact that TBBPA is chemically bound to the polymer matrix of the product into which it is applied (Sellström and Jansson, 1995). In this case, potential emissions to the environment of TBBPA from products are likely to be limited in comparison to other BFR compounds such as HBCD and the PBDEs, which are 'mixed' with the polymer matrix. Alternatively, the more polar and reactive molecular properties of TBBPA might result in a lower degree of bioaccumulation. For instance, the presence of the phenolic groups allows direct phase-II biotransformation processes via conjugation to glucuronic acid or sulphate (van Leeuwen and Hermens, 1995). In addition to this, TBBPA may bind to other endogenous compounds such as proteins; and these complexes might not be extractable with the applied methodologies.

#### 13.4 Conclusions

This study has generated new analytical data describing the presence of individual HBCD stereoisomers and TBBPA in STW influents and effluents and a number of estuarine, freshwater and seawater organisms. In contrast to TBBPA, considerably higher concentrations of  $\Sigma$ HBCD were detected in the samples. Losses during HBCD production or processing and, in particular, during application in the textile industry and residue discharge to local water treatment facilities, may cause a local elevation in HBCD concentrations. The retention and accumulation of HBCD by river and estuarine sediments as abiotic sinks, and the uptake by benthic invertebrates has resulted in evidence of bioaccumulation at that trophic level, and biomagnification in the ascending aquatic food chain, especially from fish to marine mammals and fish-eating birds. This study draws similar conclusions to those described in (Boon et al., 2002). There appears to be a preferential accumulation of  $\alpha ext{-HBCD}$  in biota, whereas the  $\gamma ext{-}$  stereoisomer dominates in sediments, in which the isomer pattern resembles that of the HBCD technical formulation.

## 14. Comparative evaluation of biological indicators of change in response to human activities at sea

#### Authors: Jenny Sneddon, Hubert Rees, Siân Boyd and Suzanne Ware

#### 14.1 Introduction

Marine environmental management decisions are strongly influenced by scientific advice. To assist in the interpretation and communication of findings from benthic ecological surveys, indicators have been developed that utilise aspects of community structure, such as species richness and the distribution of abundances among species, in order to summarise changes following anthropogenic disturbances.

It is important that such measures should convey the necessary information in a concise and objective way to enable reliable decisions to be made concerning the protection of the environment. It is accepted that, in principle, environmental indicators should be accurate, sensitive, easily understood and cost effective (Hanson, 2003; Rees *et al.*, 2003). In practice, however, achieving these criteria presents a considerable challenge and also carries the risk of oversimplifying complex environmental situations (Rees *et al.*, 2003).

When interpreting the results of a diversity measure it is important to recognise that it is unlikely that only anthropogenic disturbances are expressed in the index. The benthic fauna are exposed to a variety of spatially and temporally diverse natural environmental influences such as current velocity, water depth and sediment particle size (Stark, 1993). The effects of these will typically be reflected in the results of the diversity measure, which may complicate the interpretation of the anthropogenic disturbance under investigation. In indicator applications employing biological measures, it is therefore essential to have a thorough understanding of the prevailing environmental conditions in order to compensate for the influence of natural variation, and for this to be accompanied by sound sampling design.

The European 'Water Framework' Directive (European Communities, 2000) identified the requirement for new methods to assess the effects of anthropogenic disturbances on the marine biota. In response, Borja *et al.* (2000) formulated the Biotic Coefficient (BC), which is a measure of the ecological quality of soft-bottom benthos for European estuarine and coastal environments (Borja *et al.*, 2003). This measure can be related to the Biotic Index (BI) which measures the quality of the benthic habitat from a scale of 0 (unpolluted) to 7 (extremely polluted) using the value generated by the BC.

An alternative diversity measure was recently formulated by Warwick and Clarke (1995) based on the branch length of the taxonomic tree. This measure assesses the level of relatedness of taxa within a community in such a way that the higher the value obtained, the greater the average evolutionary distance between taxa, and hence the greater the level of intrinsic biodiversity.

Basic information such as the number of species and individuals can also be an important way of identifying the effects of anthropogenic activities on the benthos. Pearson and Rosenberg (1978) noted that "the basic quantitative parameters in almost all benthic ecological investigations are the number of species, their abundance, and biomass". Within a stable benthic community, an equilibrium is reached and only small-scale quantitative and qualitative temporal changes occur (Pearson and Rosenberg, 1978). It follows that anthropogenic disturbances exceeding natural variability and causing detrimental effects may be detectable by assessing the primary statistics of species number, densities and/or biomass.

The Shannon-Weiner Diversity Index, originally developed from information theory (Shannon and Weaver, 1949) has been widely used in marine benthic studies. It is a quantitative measure of diversity, combining the attributes of species richness and evenness (ie the apportioning of individuals among the species) and remains an appropriate choice in any comparative evaluations of indicator performance.

Many other univariate measures of data structure are available for use in aquatic monitoring programmes (eg Washington 1984), but a general consensus on their usefulness is lacking. Hence, there is a degree of uncertainty when deciding on the most effective diversity measure to enable accurate interpretation of environmental disturbances (Danilov and Ekelund, 1999). It is not possible to express every element of biodiversity within one index (Warwick and Clarke, 1995) and differences can occur in the responses of environmental indicators when applied to different anthropogenic disturbances.

This paper presents a comparative analysis of selected summary measures of benthic community structure applied to four sets of field data in order to assess their relative success in the identification of biological gradients. Evaluation of indicator utility is therefore not exhaustive, given the range of available measures, but an important goal of the present work was to evolve an appraisal system combining scientific and management criteria governing effectiveness. Three dredged material disposal sites around the England and Wales coastline were investigated, along with an aggregate extraction site in the English Channel (Figures 14.1-4), using nine different biological measures. The disposal sites have received material of varying quantity, quality and frequency of input. At the aggregate extraction site located within the central English

Channel about 2-3 Mt of sand and gravel are removed annually (Boyd and Rees, 2002).

#### 14.2 Methods

Nine measures of data structure were evaluated, a number of which were permutations of the same overall indicator type. The four test data sets were chosen in the knowledge that moderate to strong biological gradients in response to anthropogenic activities were to be expected. The measures were:

- 1. Number of species (S)
- 2. Number of individuals (N)
- 3. Shannon-Weiner Diversity Index H'(log<sub>a</sub>):

$$H' = -\sum_{i=1}^{k} \mathbf{p}_{i} \log_{e} p_{i}$$

Where pi is the proportion of the total count arising from the ith species.

4. Average Taxonomic Diversity  $\Delta$  (Delta):

$$\Delta = [$$
  $\sum \!\!\sum_{i < j} \; \omega_{ij} \; \chi_{\iota} \chi_{j} \; ] \; / \; [ \; N(N \, \text{--}1) \; / \; 2 \; ]$ 

Where the double summation is over all pairs of species *i* and *j* and N equals the total number of individuals in the sample.

5. Average Taxonomic Breadth  $\Delta^+$  (Delta+):

$$\Delta^{+} = [\sum \sum_{i < i} \omega_{ii}] / [S(S-1) / 2]$$

Where S is the observed number of species in the sample and the double summation ranges over all pairs i and j of these species.

6. Total Phylogenetic Diversity S $\Phi$ <sup>+</sup> (Total PD):

Cumulative branch length of the phylogenetic tree linking all species in a given sample.

7. Average Phylogenetic Diversity  $\Phi^+$  (Average PD):

Total Phylogenetic Diversity averaged over the number of species in the sample.

8. Biotic Coefficient (BC):

 $\{(0 \times \%GI) + (1.5 \times \%GII) + (3 \times \%GIII) + (4.5 \times \%GIV) + (6 \times \%GV)\}/100$ 

Where species are assigned to ecological groups (GI-GV) according to their perceived sensitivity to organic enrichment (ie GI=Species indifferent to enrichment, GV=First order opportunistic species).

9. Biotic Index (BI) (Borja et al., 2000):

Site Pollution Classification	Biotic Coefficient	Biotic Index	Dominating Ecological Group	Benthic Community health
Unpolluted	0.0 <bc≤0.2< td=""><td>0</td><td>1</td><td>Normal</td></bc≤0.2<>	0	1	Normal
Unpolluted	0.2 <bc≤1.2< td=""><td>1</td><td></td><td>Impoverished</td></bc≤1.2<>	1		Impoverished
Slightly Polluted	1.2 <bc≤3.3< td=""><td>2</td><td>III</td><td>Unbalanced</td></bc≤3.3<>	2	III	Unbalanced
Meanly Polluted	3.3 <bc≤4.3< td=""><td>3</td><td></td><td>Transitional to pollution</td></bc≤4.3<>	3		Transitional to pollution
Meanly Polluted	4.3 <bc≤5.0< td=""><td>4</td><td>IV-V</td><td>Polluted</td></bc≤5.0<>	4	IV-V	Polluted
Heavily Polluted	5.0 <bc≤5.5< td=""><td>5</td><td></td><td>Transitional to heavy pollution</td></bc≤5.5<>	5		Transitional to heavy pollution
Heavily Polluted	5.5 <bc≤6.0< td=""><td>6</td><td>V</td><td>Heavily Polluted</td></bc≤6.0<>	6	V	Heavily Polluted
Extremely Polluted	Azoic	7	Azoic	Azoic

#### 14.2.1 Rationale for choice of index

The number of species (S) and individuals (N) in a sample represent the simplest and most widely used univariate expressions of data structure, while the Shannon-Weiner Diversity Index (Shannon and Weaver, 1949) combines the species richness and dominance components of diversity. All three were therefore appropriate choices for the comparative assessment of indicator utility. Taxonomic Distinctness and Taxonomic Diversity are recently evolved indices developed by Warwick and Clarke (1995) that measure the relatedness of species within a benthic community and have the desirable property that they are relatively uninfluenced by sampling effort. Phylogenetic Diversity, a measure based on total path length constituting the full taxonomic tree, is not independent of sampling effort. These novel biodiversity measures appear to have

significant merit in evaluations of marine environmental quality status.

The AMBI (AZTI Marine Biotic index), developed by Borja et al. (2003), was chosen as it is the most recent manifestation of a class of measures which draw heavily from empirical evidence of species responses to human impacts, and has the potential for wider application in environmental quality assessment. Species are classified into one of five ecological groups, principally according to their tolerance to organic enrichment (Pearson and Rosenberg, 1978). The resulting index is scaled from 0 (unpolluted) to 7 (extremely polluted) (Borja et al., 2003). The classification of species by the AMBI in its present form, ie depending mainly upon their tolerance to varying degrees of organic enrichment, clearly has the potential to misrepresent anthropogenic impacts arising from other or confounding activities, and is therefore appropriate for performance evaluation in the present study.

#### 14.2.2 Data analysis

Total number of species (S), total number of individuals (N) and the Shannon-Weiner diversity index (H'log<sub>e</sub>) were calculated from the species abundance matrices. Aggregation files were compiled for each site to enable the analysis of the benthic macrofauna using Taxonomic Distinctness methods developed by Warwick and Clarke (1995). For the marine Biotic Index, species were allocated to each of five ecological groups employing a list compiled by Borja et al. (2003); the % abundances of these groups were then determined to permit index calculation. The AMBI values for all replicates were calculated, and then a mean value for each station was obtained in order to avoid an ambiguous result (Borja et al., 2003). The BC value was then used to derive the Biotic Index value, which may range from 0 to 7.

The Anderson-Darling statistic was used to determine normality of the residuals and Bartlett's and Levene's test was used to test for homogeneity of variance. Analysis of Variance (ANOVA) was used to test for significant differences between stations. Tukey's multiple comparisons test was used in pair-wise comparisons of stations. All univariate analyses were carried out using the software package Minitab.

To complement interpretations of the data based upon univariate measures, non-metric multi-dimensional scaling ordinations of the inter-sample relationships were conducted, using the Bray-Curtis similarity measure applied to fourth-root transformed abundance data. All multivariate analyses were performed using the software package PRIMER.

#### 14.2.3 Scoring of indicator attributes

A score of 1 (poor) - 5 (good) was allocated to each measure according to its performance against criteria for a good indicator as identified by ICES (2001), namely that it should be:

- relatively easy to understand by non-scientists and other users;
- sensitive to a manageable human activity;
- relatively tightly linked in space and time to that activity;
- responsive primarily to a human activity, with low responsiveness to other causes of change;
- easily and accurately measured, with a low error rate.

Although ultimately a subjective exercise, these scores were allocated following a critical evaluation of the outcome of ANOVA and multivariate (MDS) analysis for each location. For visual comparisons, the data were expressed as the combined output from cluster analyses by diversity measure and by attribute.

#### 14.2.4 Site description

Four data sets from Cefas surveys at dredged material disposal and aggregate extraction sites around the UK were analysed using a selection of different measures.

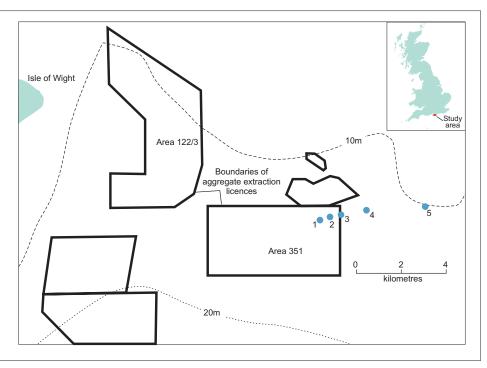
#### Aggregate extraction: English Channel

Data from a survey conducted in 2000 at an extraction site (area 351) off the Isle of Wight, southern England, were employed. About 2 - 3 Mt of marine sand and gravel had been extracted annually in the period prior to the survey (Boyd and Rees, 2002). The site extends to 10 - 20 m depth and tidal current velocities are moderately strong (in excess of 1 ms<sup>-1</sup>; see Boyd and Rees, 2002 for further details).

## Dredged material/solid industrial waste disposal off the Tyne estuary

A 1992 data set from the north-east coast of England encompassed two sites, licensed under the UK Food and Environment Protection Act (Part II, 1985), for the disposal of solid wastes. This included (at that time) minestone and tailings from collieries, fly ash from power stations and dredged material from estuaries. The disposal sites in this area were located at approximately 40 m depth and were therefore relatively sheltered from wave action. Tidal current velocities are also relatively low (0.3 ms<sup>-1</sup>) (see Rees and Rowlatt, 1994 for further details).

Figure 14.1. Aggregate extraction area 351 and location of sampling stations (t=1, t500=2, t1000=3, t2000=4, t5000=5).



## Dredged material disposal at Roughs Tower (outer Thames estuary)

Data from a survey conducted in 2001 at the Roughs Tower disposal site were analysed. This site received about 32 million wet tonnes of dredged material within a two-year period prior to its effective closure in March 2000. It is located in shallow waters of 10-20 m depth and experiences moderately strong tidal currents that can reach more than 1 ms<sup>-1</sup> during spring tides (Rees *et al.*, 2002).

#### Dredged material disposal in Liverpool Bay

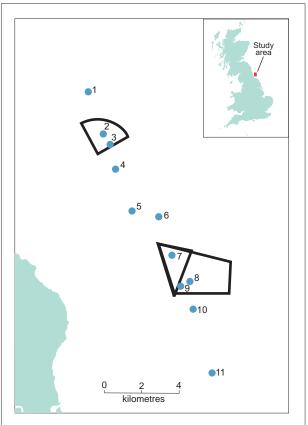
Data from a survey conducted in 1991 at a dredgings disposal site in Liverpool Bay were selected for analysis The site had received 2 - 3 Mt of mud and sand annually since 1982 (Somerfield *et al.*, 1995). This location is shallow (10 m) and is exposed to wave action principally from westerly to northerly winds; tidal current velocities reach up to 0.8 ms<sup>-1</sup> (see Somerfield *et al.*, 1995 for further details).

#### 14.3 Results

## 14.3.1 English Channel - aggregate extraction site

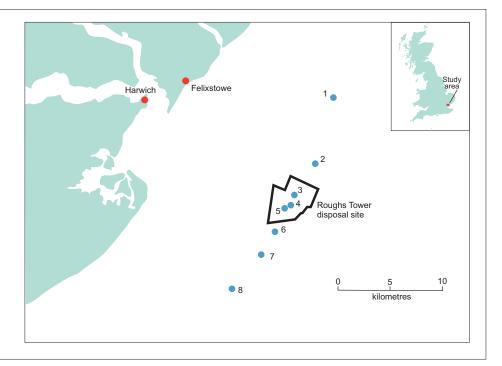
#### Macrofauna assemblage structure

The numerically dominant species at the stations located within the area of intensive extraction activity included the crustaceans *Balanus crenatus* and *Elminius modestus*, the amphipod *Leptocheirus hirsutimanus* and the annelid worm *Polycirrus*. The assemblage structure differed outside the extraction zone, as shown by the separation of sites in



**Figure 14.2.** Disposal sites off the River Tyne and sampling stations.

**Figure 14.3.** Roughs Tower disposal site and location of sampling stations.



the MDS plot of Figure 14.5 and was dominated by the gastropod *Crepidula fornicata*, the bivalve *Nucula nucleus*, the polychaetes *Pomatoceros lamarcki* and *Sabellaria spinulosa* and the crustacean *Pisidea longicornis*. Further details are given in Boyd and Rees (2002).

#### Indicator measures

The selected measures revealed inconsistencies in response to aggregate extraction activities (Table 14.1).

The distribution of numbers of species among the stations supported the separation of the impacted sites from those outside the sphere of impact in the MDS ordination plot (Figure 14.5). Thus numbers of species were significantly greater at the area outside the extraction site than the area of intense dredging activity within it (F=12.69, df=4, p<0.001). This was also the case for the Shannon-Weiner Diversity Index (F=38.45, df=4, p<0.001).

The number of individuals also differed among stations (F=4.47, df=4, p=0.014) with pairwise comparisons indicating significantly greater numbers of individuals at station 3 than at station 5. This was a result of the presence of large numbers of *Balanus crenatus*, which had the effect of masking the otherwise impoverished fauna present within the extraction site compared with elsewhere (see Boyd and Rees, 2002).

Values of Average Taxonomic Breadth ( $\Delta$ +) were significantly different between stations (F=3.75, df=4, p=0.026) with pairwise comparisons indicating significantly higher values at stations 1 and 3 than at station 5. Thus the greater Average Taxonomic Breadth at the inner stations shows that the assemblage consists of more distantly

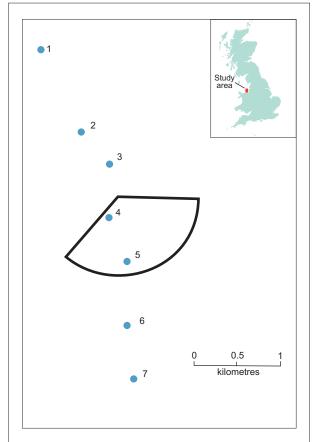


Figure 14.4. Liverpool Bay dredged material disposal site Z and location of sampling stations.

Table 14.1. Mean values for univariate measures of macrofaunal status through an area exposed to trailer suction hopper dredging.

Station	S	N	H'(log <sub>e</sub> )	Delta	Delta+	Av. PD	Total PD	BC	BI
1	20	1593	0.30	7.3	89.4	65.3	1289	1.50	2
2	13	446	0.82	30.8	87.4	72.1	906	1.66	2
3	37	2027	0.44	11.2	89.1	59.7	2161	1.54	2
4	68	433	3.02	75.8	87.0	50.6	3403	1.79	2
5	51	330	2.99	74.0	84.4	49.9	2497	1.40	2

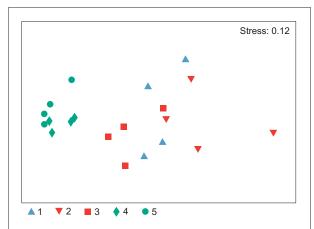


Figure 14.5. Non-parametric multi-dimensional scaling ordination derived from abundance data for macrofaunal species at a site exposed to trailer suction hopper dredging. The key identifies distances in metres from the centre of extraction activity (1=0m, 2=500m, 3=1000m, 4=2000m, 5=5000m).

related species and can be regarded as more biodiverse than stations outside the extraction area. A similar result is obtained from Average Phylogenetic Diversity (F=11.56, df=4, p<0.001), ie the average contribution that each species makes to the total tree length. Values of this diversity measure are significantly greater at stations 1 and 2 than at stations 4 and 5. These measures suggest that the residual benthic assemblage has become intrinsically more biodiverse as a result of the extraction process, perhaps as a result of a change in habitat structure. This may be contrasted with an overall reduction in the numbers of species within the dredging site.

Values of the Biotic Coefficient were not significantly different among stations along the transect. The three sites within the aggregate extraction area have elevated numbers of the group II species *Balanus crenatus*, which are indifferent to enrichment according to the species list developed by Borja *et al.* (2003) and, according to Borja *et al.* (2000), are always present at low densities with nonsignificant variations with time. This is also the case for the Group III species *Leptocheirus hirsutimanus*, which is recorded as being tolerant of excess organic matter enrichment. Such species may occur under normal conditions, but their populations are stimulated by organic enrichment and slightly perturbed environmental conditions (Borja *et al.*, 2000).

Although there were no significant differences in the Biotic Coefficient between stations, station 5 has the lowest mean value. Therefore this station might be interpreted to be the least influenced by extraction processes, as it is furthest away from the activity. However, the second furthest station from the extraction area has the highest mean BC which suggests that this site has a faunal composition indicative of the most polluted site along the transect.

Values of the corresponding BI indicate that there are no detectable differences between stations as all are classified as having an Ecological Group of 2 ('slightly polluted'). This index therefore lacks the ability to extract gradients from the data that are evident when using the number of species and the Shannon-Wiener index which, more obviously, show decreased diversity at stations within the extraction area.

#### Summary

The univariate measures of number of species and the Shannon-Weiner Diversity Index were the most capable of all the indices tested to detect gradients along the transect at the English Channel aggregate extraction site, comparable with those identified by Boyd and Rees (2002). The Average Taxonomic Diversity ( $\Delta$ ) index also showed that an impact had occurred within the extraction site. Average Taxonomic Breadth ( $\Delta$ +), Average Phylogenetic Diversity and the marine Biotic Index either failed to show an impact at the site or showed that a positive (ie apparently beneficial) impact had occurred.

## 14.3.2 North Tyne and Souter Point - dredged material, colliery waste and fly ash disposal sites

#### Macrofauna assemblage structure

The MDS ordination (Figure 14.6) shows that stations 2 and 7 from within the disposal sites are most widely separated from the other stations. This is due principally to colliery waste disposal activities at these stations which has resulted in the depletion of fauna resulting in a high level of biological dissimilarity. Stations 3 and 4 are biologically dissimilar to other stations, as demonstrated by their separation on the MDS plot. These stations are located, respectively, within and just to the south of the North Tyne disposal activities in the area. The third pair of dissimilar stations (1 and 5) are located at either end of the transect through the North Tyne

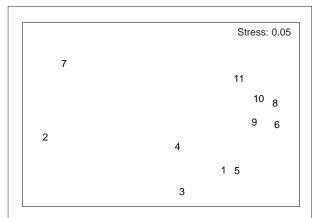


Figure 14.6. Non-parametric multi-dimensional scaling ordination derived from abundance data for macrofaunal species along a transect exposed to dredged material, colliery waste and fly ash disposal off the Tyne estuary, NE England (pooled samples per station).

disposal site (Rees and Rowlatt, 1994). Stations 6, 8, 9, 10 and 11, which form a discrete group to the right of the plot, were dominated by the mollusc *Thyasira flexuosa* and the annelid *Prionospio* spp. The species composition is similar at these stations as shown by the close grouping on the MDS ordination. These stations are located at and to either side of the Souter Point (outer) disposal site and suggest that the macrofauna have not been adversely affected by the disposal of maintenance dredgings and other inputs to this site, which are mainly concentrated at the inshore boundary.

#### Indicator measures

A one-way ANOVA indicated that there were significant differences in species number between certain stations (F=8.01, df=10, p=0.001) with pairwise comparisons indicating significantly lower numbers of species at station 7 than at stations 5, 6, 8, 9 and 10. In addition, the numbers of species at stations 2 and 3 were significantly lower than at stations 6 and 8. Stations 2 and 3 are located within a dredged material and colliery waste disposal site, which also received fly ash until 1990. Station 7 is at the western edge of a dredged material and colliery waste disposal site, which was also a fly ash disposal site until 1990. There is evidence that the majority of disposal activity took place here (Rees and Rowlatt, 1994). The low number of species at these sites can be explained by the waste that these stations have received, though an additional factor was the difficulty of obtaining adequate samples by Day grab, on account of the coarse deposit found here (Rees and Rowlatt, 1994).

Significant differences in densities were also present among stations (F=11.42, df=10, p<0.001) with significantly greater numbers of individuals present at station 6 than at all other stations and significantly greater numbers of individuals at station 9 than at stations 3 and 7 (Table 14.2).

For the Shannon-Weiner Diversity Index, significant differences were also apparent between stations (F=5.70, df=10, p=0.004). Pairwise comparisons indicated that values of the Index were significantly reduced at station 7, within the Souter Point disposal site, compared to stations 1, 4, 6, 8, 9, 10 and 11.

Table 14.2. Mean values for univariate measures of macrofaunal status derived from a transect through two disposal sites off the NE coast of England in receipt of dredged material, colliery waste and fly ash.

Station	S	N	H'(log <sub>e</sub> )	Delta	Delta+	Av. PD	Total PD	BC	BI
1	31	152	2.82	82.2	86.7	62.9	1742	1.36	2
2	15	37	2.38	81.3	89.5	71.8	933	2.23	2
3	19	64	2.46	80.5	85.8	69.0	1039	1.67	2
4	23	75	2.81	82.5	87.3	67.2	1333	1.50	2
5	36	169	2.99	75.1	85.6	58.2	1775	1.66	2
6	48	334	3.27	82.1	87.5	56.5	2483	1.61	2
7	10	45	1.58	65.8	89.0	78.8	667	1.11	1
8	48	172	3.17	81.8	87.1	59.5	2583	1.67	2
9	38	213	2.59	79.5	87.8	59.3	2017	2.25	2
10	43	194	3.15	80.7	88.4	58.4	2275	1.79	2
11	29	96	2.84	77.4	84.1	59.8	1583	2.11	2

Values of Average Taxonomic Diversity ( $\Delta$ ) do not provide evidence of a significant impact at the disposal sites (F=2.46, df=10, p=0.078). This is also the case for measures of Average Taxonomic Breadth (F=0.48, df=10, p=0.870). Although the stations within the disposal sites have been shown to have an impoverished species number and lower Shannon-Weiner diversity compared to other stations, they are not significantly less taxonomically diverse than other stations that do not receive waste material.

The Total Phylogenetic Diversity shows that significant differences exist between stations along the Tyne transect (F=8.65, df=10, p=0.001). These differences correspond with those shown in the MDS ordination plot. Station 6 has a significantly greater total taxonomic tree length than 7 and stations 6 and 8 have a significantly greater Total Phylogenetic Diversity than stations 2 and 3. This measure shows that the disposal activities at stations 2, 3 and 7 have resulted in a faunal composition that is less diverse than the surrounding area.

There are no apparent differences between stations for the Biotic Coefficient (BC). However, stations 2 and 9 had the highest BC values, suggesting that the fauna at these sites might be indicative of the most organically-enriched conditions, employing the criteria of Borja et al. (2003). These stations are located within disposal sites which have received relatively large quantities of maintenance dredgings from the urbanised Tyne estuary, in addition to colliery waste and fly ash. The fauna at these sites are dominated by species classed by Borja et al. (2000) as second order opportunistic species present in slightly perturbed habitats. However, station 8, which is located within the same dredged material disposal site as 9, has a lower BC of 1.67 and a faunal composition dominated by species classed by Borja et al. (2000) as those sensitive to organic enrichment and present under unpolluted conditions. In addition, station 7, located in an area which received significant quantities of colliery waste and fly ash in addition to dredged material, has the lowest BC of 1.11 and is dominated by species classified by Borja et al. (2000) as indicative of an unpolluted habitat.

This result contradicts that from use of the Shannon-Weiner Diversity Index which shows station 7 to have the lowest level of diversity and suggests that the site has been negatively impacted by the disposal operations. This is supported by the observations of Rees and Rowlatt (1994) on the accumulations of solid wastes (especially minestone and tailings) at this location, accompanied by an impoverished benthic assemblage. While this is clearly not reflected in the Biotic Coefficient or the corresponding Biotic Index, which actually suggests that it is the least influenced by disposal practices along the transect, the low

numbers and densities of species present may be a factor, since the robustness of this index may be compromised under such circumstances (Borja *et al.*, 2003).

#### Summary

The indicator measures at the Tyne which best expressed local impacts arising from solid waste disposal were the number of species, the number of individuals, the Shannon-Weiner Index and the Total Phylogenetic Diversity. The other measures did not show any significant differences between stations at and away from the centres of disposal activity.

## 14.3.3 Roughs Tower - dredged material disposal site

#### Macrofauna assemblage structure

There is significant within- and between-station variability in the numbers of taxa resulting primarily from a combination of natural and disposal-induced variability in substratum type (Rees et al., 2002). The relationships between stations along the transect are shown in the MDS ordination of Figure 14.7. Stations within and nearest to the disposal site and those to the south were dominated by the polychaete Lanice conchilega (except station 6, where it was absent). Station 1, which is furthest north from the disposal site, and station 6, were found to support appreciable densities of the molluscs Nucula nucleus and Abra alba. The two southernmost stations, 7 and 8, have a similar benthic assemblage dominated by the annelids Polydora caeca, Pomatoceros lamarcki and Sabellaria spinulosa, the mollusc Abra alba and the cnidarian Actiniaria.

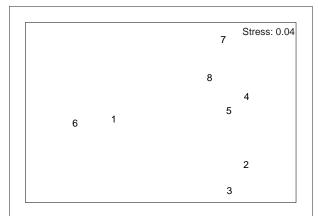


Figure 14.7. Non-parametric multi-dimensional scaling ordination derived from averaged abundance data for macrofaunal species at stations along a transect through the Roughs Tower dredged material disposal site.

#### **Indicator Measures**

A one-way ANOVA detected that there were significant differences in numbers of species between stations (F=4.51, df=7, p=0.006) with subsequent pairwise comparisons indicating greater numbers of species at station 7, the second most southerly station, than at stations 2, 3, 4, 5 and 6. The numbers of species have therefore declined within and surrounding the disposal site as a result of the large quantity of dredged material disposed of there (Table 14.3).

There are significant differences in number of individuals between stations (F=8.00, df=7, p<0.001) with greater numbers at station 4 than at stations 1, 2, 3, 5 and 6, which can be accounted for by the presence of large numbers of juvenile Lanice conchilega (Rees et al., 2002). This species can occur naturally at high densities in relatively exposed shallow waters, and may act as a pioneer coloniser which, through short-term stabilisation of the habitat following the construction of a network of tubes, promotes the survival of other species (Eagle, 1975).

Values of the Shannon-Weiner Index vary significantly among stations (F=7.86, df=7, p<0.001) with pairwise comparisons indicating that diversity is greater at station 1 than at stations 4 and 5, and greater at station 7 than at 3, 4, and 5. This diversity measure therefore indicates the presence of an impact within the disposal area.

Average Taxonomic Diversity ( $\Delta$ ) is also significantly different among stations (F=6.49, df=7, p=0.001) with pairwise comparisons identifying significantly higher values at stations 1 and 7 than at stations 4 and 5. The results employing this measure correspond with those from the

Shannon-Weiner Index, indicating that the assemblage within the disposal area is less biologically diverse than at the stations furthest from the disposal site.

There are no significant differences in Average Taxonomic Breadth ( $\Delta$ +) between stations along the transect (F = 0.94, df=7, p=0.504). The Total Phylogenetic Diversity differs significantly among stations (F=4.10, df=7, p=0.009) with significantly greater values observed at station 7 relative to stations 2, 3, 5 and 6, ie the fauna there exhibits a significantly greater taxonomic tree length.

Values of the Biotic Coefficient do not provide evidence of a 'polluted' habitat at the Roughs Tower disposal site. Stations 2 and 6 at either side of the disposal site have a significantly lower BC than station 7. These stations have a BI of 1, indicative of an unpolluted habitat. However, all other sites including the disposal area have a BI of 2 which, according to Borja *et al.* (2000), reflects a slightly polluted habitat with a species composition tolerant to excess organic matter enrichment.

#### Summary

The number of species, the Shannon-Weiner Diversity Index, Taxonomic Diversity, and Total Phylogenetic Diversity indicate that there is an impact at the disposal site as evidenced by a reduction in values of these measures. However, the Average Taxonomic Distinctness and the Average Phylogenetic Diversity show no significant differences between stations. Values of the BC and BI do not discriminate between impacted and not-so-impacted stations.

Table 14.3. Mean values for univariate measures of macrofaunal status derived from a transect through the Roughs Tower dredged material disposal site.

Station	S	N	H'(log <sub>e</sub> )	Delta	Delta+	Av. PD	Total PD	BC	ВІ
1	18	93	2.02	71.2	87.2	69.9	1133	1.29	2
2	12	103	1.19	48.1	82.6	72.9	761	1.12	1
3	12	276	0.88	41.1	88.8	72.8	811	1.48	2
4	16	836	0.33	10.6	86.7	71.0	950	1.52	2
5	12	210	0.54	19.0	90.5	78.5	761	1.69	2
6	9	56	1.32	52.0	88.3	74.4	644	0.93	1
7	39	499	1.95	63.2	88.1	63.4	2139	1.99	2
8	28	415	1.39	42.9	85.4	65.5	1650	1.33	2

### 14.3.4 Liverpool Bay - dredged material disposal site

#### Macrofauna assemblage structure

The annelid *Lagis koreni* and the mollusc *Mysella bidentata* dominate the benthic communities within and surrounding the disposal site. Other species such as the mollusc *Fabulina fabula* and the annelid species *Spiophanes bombyx* and *Magelona mirabilis*, and the cnidarian *Edwardsia*, are also present at high densities.

The ordination of fourth-root transformed macrofauna data (Figure 14.8) separates stations 4 and 5 taken from within the disposal site from stations 2, 3, 6 and 7. Station 1, to the north of the disposal site, is separated from the other stations, suggesting the influence of some other environmental factor (see Somerfield *et al.*, 1995).

#### Indicator measures

Table 14.4 summarises trends in indicator measures along the transect. A one-way ANOVA detected significant differences between number of species across the transect (F=11.68, df=6, p<0.001). Pairwise comparisons identified significantly lower numbers of species within the disposal site (stations 4 and 5) than elsewhere.

Numbers of individuals were significantly different among stations (F=18.24, DF=6, P<0.001) with values observed to be greater at station 1 than at stations 5 and 7. Also, numbers of individuals were significantly greater at station 4 than at station 2, 3, 5, 6 and 7, on account of high densities of the polychaete *Lagis koreni* (Somerfield *et al.*, 1995).

Values of the Shannon-Weiner Index differed significantly among stations (F=25.43, df=6, p<0.001) with pairwise comparisons showing that the diversity at station 4, within the disposal site, is significantly lower than elsewhere. Also, station 1 has a significantly lower diversity than stations 3 and 7.

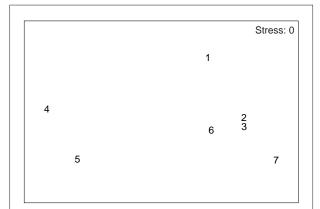


Figure 14.8. Non-parametric multi-dimensional scaling ordination of species abundance data at stations along a transect through the Liverpool Bay dredged material disposal site.

Similarly, the Average Taxonomic Diversity ( $\Delta$ ) differed significantly among stations (F=19.09, df=6, p<0.001) with significantly lower values at station 4, within the disposal site, relative to the surrounding area. Significant differences were detected in Average Taxonomic Breadth among stations (F=8.00, df=6, p=<0.001) with lower values observed at station 5 relative to all others excluding station 4.

No significant differences in Average Phylogenetic Diversity were detected among stations (F=2.25, df=6, p=0.078). However, significant differences in Total Phylogenetic Diversity were apparent among stations (F=16.93, df=6, p<0.001) and the differences observed match trends in the number of species, Shannon-Weiner Diversity and Taxonomic Diversity, showing that the taxonomic tree length is significantly greater at sites outside the disposal site.

**Table 14.4.** Mean values for univariate measures of macrofaunal status derived from a transect through dredged material disposal site Z in Liverpool Bay.

Station	S	N	H'(loge)	Delta	Delta+	Av. PD	Total PD	BC	BI
1	39	885	2.31	70.5	87.3	57.7	2017	0.56	1
2	46	570	2.82	76.0	87.6	56.0	2321	1.12	1
3	46	580	2.92	77.3	88.4	54.8	2313	1.21	2
4	30	1301	1.40	56.5	85.5	58.9	1563	0.22	1
5	30	448	2.15	70.0	83.8	56.8	1533	0.49	1
6	42	686	2.70	73.5	86.8	56.2	2150	1.11	1
7	48	424	3.07	78.9	88.1	55.6	2417	1.51	2

The Biotic Coefficient matches changes in H' but the values are the reverse of expectation. Thus, stations 4 and 5 would appear to be the least polluted as reflected in the values of the derived BI. The main reason for this is that Lagis koreni, which could be classed as an opportunist in the Inner Liverpool Bay area, was (at the time of the present analysis) designated as relatively sensitive to pollution in the species tolerance list underlying the Biotic Coefficient. Thus, the derived Biotic Index does not clearly discriminate between stations at and away from the disposal site. In fact, both stations within the disposal site have an ecological group of 1 that is representative of an assemblage in an unpolluted habitat. This is due to the site supporting large numbers of Lagis koreni and Mysella bidentata which are both classed as group 1 species by Borja et al. (2000), ie they are presumed to be very sensitive to organic enrichment and are generally found under unpolluted conditions. However, a study by Rees et al. (1992b) found Lagis koreni to be capable of successfully colonising disturbed substrata and was therefore a useful indicator of impacts at or near to the dredged material disposal site.

Even though the stations have a similar diversity as shown by the Average Taxonomic Breadth, the stations do not exhibit the same apportioning of individuals among the species groups. Thus differences in the dominance component of diversity exist between the stations, as shown by the Shannon-Weiner Index and the Average Taxonomic Diversity ( $\Delta$ ), that are not evident from the Average Taxonomic Breadth ( $\Delta$ +) alone.

#### Summary

Values of the BI suggest that the species composition at stations 4 and 5 reflects unpolluted conditions. There appear to be no detrimental organic enrichment effects at the site of dredged material disposal. However, other indicator measures demonstrate that there is a significant impact on diversity at these sites, which is likely to be a direct result of the disposal activity. There is some evidence to suggest that the response may reflect a combination of physical disturbance and the presence of elevated organic carbon associated with dispersing particulates (Rees *et al.*, 1992b).

#### 14.3.5 Environmental indicator attributes

The environmental indicators were scored on a scale of 1 (poor) to 5 (good) according to criteria which, in summary, required that an indicator should be: understandable (to non-specialists), sensitive (to manageable human activity), linked (with the effects of specific activities), responsive (largely to human impacts) and subject to low error (along with ease of measurement). Scoring of certain criteria, including sensitivity and responsiveness to a given impact, can be supported by robust statistical analysis resulting in a high level of confidence in the subsequent score assigned to a given metric. However, it is recognised that certain criteria, such as communicability, are more subjective and are, thus, highly dependant on the experience and background of the individual assigning the scores. Refinement of the scoring process for the more subjective criteria is currently receiving attention through wider consultation of relevant parties (ie regulatory advisors as well as ecologists) (Ware et al., in prep.).

Cluster analysis was employed as a convenient way of displaying the outcome (Figure 14.9). Interpretation necessarily depends upon an overall judgement across the sometimes 'competing' demands of the attributes, since no indicator was an unqualified success across all. Although not attempted here, there may be merit in further weighting of these attributes while accepting that, as with the raw scores, this might vary according to the emphasis in study objectives. Given the subjective nature of this exercise, we do not consider such bias to be unreasonable, provided that it is openly stated. Thus, in our scoring of attributes, we placed a conscious premium on ease of understanding (and hence the facility to communicate to non-specialists), in recognition of the need for more effective science/management interactions and public accountability for regulated activities.

The best overall environmental indicators at the English Channel aggregate extraction site (Figure 14.9(a)) appeared to be the number of species and the Shannon-Weiner Index and, to a lesser degree, Average Taxonomic Diversity. Total Phylogenetic Diversity, Biotic Coefficient, Biotic Index, number of individuals, Average Taxonomic Breadth and Average Phylogenetic Diversity were generally deficient in their performance against a number of key attributes and were, accordingly, allocated proportionately lower scores.

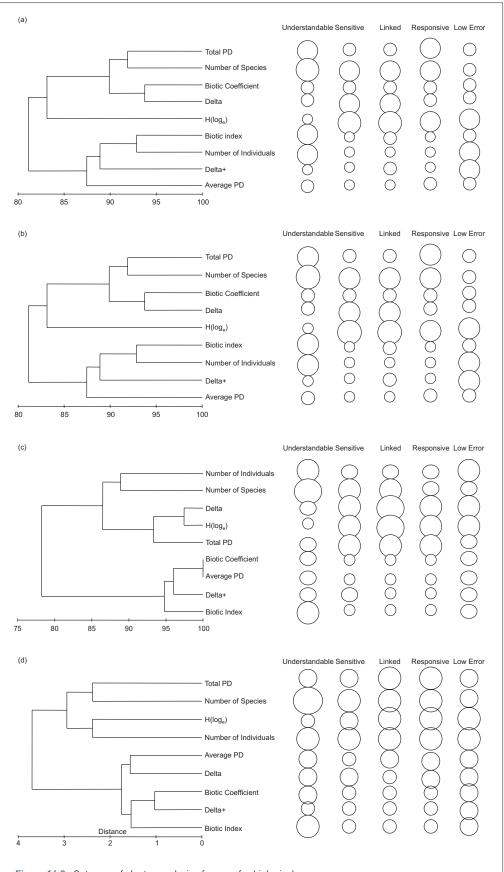


Figure 14.9. Outcome of cluster analysis of scores for biological measures against performance attributes. (a) English Channel aggregate extraction site; (b) Tyne dredged material disposal sites; (c) Roughs Tower dredged material disposal site; (d) Liverpool Bay dredged material disposal site. The size of the circles correspond with the score awarded to the indicator attribute, ie the larger the circle, the better the performance of the indicator for the attribute being considered.

At the Tyne dredged material disposal sites (Figure 14.9(b)), the Total Phylogenetic Diversity, number of species, the Shannon-Weiner Index and the number of individuals were best able to identify impacts within the areas of dredged material disposal activity. The Average Phylogenetic Diversity, Average Taxonomic Diversity, Average Taxonomic Breadth and the AMBI failed to identify significant differences between the impacted sites and the surrounding area.

The outcome for the Roughs Tower dredged material disposal site (Figure 14.9(c)) indicates that the number of species, Average Taxonomic Diversity, the Shannon-Weiner Index and Total Phylogenetic Diversity performed well for sensitivity. The most responsive indicators were Average Taxonomic Diversity, the Shannon-Weiner Index and Total Phylogenetic Diversity. The number of species and the number of individuals were also considered to be responsive to the disturbance. The Biotic Coefficient, Biotic Index, Average Phylogenetic Diversity and Average Taxonomic Breadth were regarded as having poor sensitivity and responsiveness and were not considered to be linked to the anthropogenic disturbance under investigation. This is represented by the smaller circles in the cluster diagram.

The Shannon-Weiner Index and the number of species were most able to detect the occurrence of impacts at the Liverpool Bay dredged material disposal site (Figure 14.9(d)). The Average Taxonomic Diversity, the number of individuals and the Total Phylogenetic Diversity measures also performed relatively well. The Average Phylogenetic Diversity, Average Taxonomic Breadth and the AMBI did not adequately discriminate between stations influenced by dredged material disposal activity and those nearby.

Figure 14.10(a) shows the sum of scores allocated to the indices for all dredged material disposal sites. The number of species received the highest cumulative score, reflecting good performance against criteria important for the communication of findings to non-specialists and for decision-making. Other indices that performed relatively well in identifying impacts at dredged material disposal sites were the Shannon-Weiner Index, the number of individuals, the Total Phylogenetic Diversity and the Average Taxonomic Diversity. The remaining indices received relatively low overall scores on account of poor performance against a number of key indicator attributes. The inclusion of the English Channel aggregate extraction site in the sum of scores (Figure 14.10(b)) does not greatly alter the ranking, with the number of species and the Shannon-Weiner Index again displaying the best overall performance.

The results of a Principal Components Analysis (PCA) of attribute scores are shown in Figure 14.11 as an alternative means of expressing the outcome of the performance assessment. For example, at the Liverpool Bay site (Figure 14.11(d)) 93.7% of the variation is explained by PC1 and The plot illustrates that, although the Shannon-Weiner Index is not regarded as easily understandable compared with other indicators such as the number of species, it scores well in other respects. (The attribute of ease of understanding has an eigenvalue of 0.97 and this is expressed in the disposition of indicators along PC 2). The Total Phylogenetic Diversity, number of individuals and Average Taxonomic Diversity were moderately effective in their ability to detect detrimental effects of anthropogenic activity, while the remaining indicators, although easier to understand than the Shannon-Weiner Index, performed relatively poorly, and this is evidenced by their disposition along PC 1. Plots of the outcome of PCA for the other locations (Figure 14.11(a-c)) show a similar overall pattern in the distribution of indices to that of Liverpool Bay.

#### 14.4 Discussion

Evaluations of the impact of dredged material disposal and aggregate extraction activities on the marine environment are highly dependent on the methods of assessment employed. Measures such as the number of species and the Shannon-Weiner Diversity Index clearly provided strong circumstantial evidence of a negative impact associated with anthropogenic disturbance at all locations. However, Average Taxonomic Breadth, Average Phylogenetic Diversity and the marine Biotic Index generally showed either no significant difference or an increase in biodiversity associated with the activity.

Although the presence of natural as well as anthropogenic variability is likely to be a contributory factor to these inconsistencies, it is clear that some measures yield intuitively more plausible outcomes than others. Nonetheless, an important characteristic of an environmental indicator is that it should possess the ability to indicate the cause of change rather than simply the existence of change (Carignan and Villard, 2002) or, at least, provide insight into its nature. Thus, for example, changes in the total number of species at a site, when examined in isolation, give no immediate indication of the processes which determine the survival of some, but not others. Numbers of species may also be regarded as poor measures of biodiversity as they are heavily dependent on sampling effort (Warwick and Clarke, 1995). However, as no between-site comparisons were made in the present

Figure 14.10. Sum of scores for environmental indicator attributes at dredged material disposal sites (a) and for all sites (b).

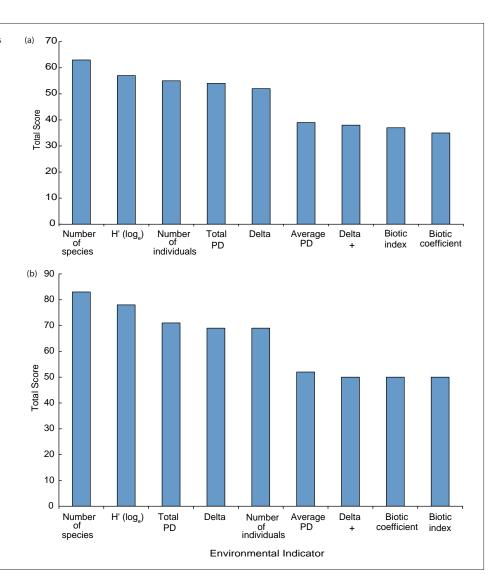
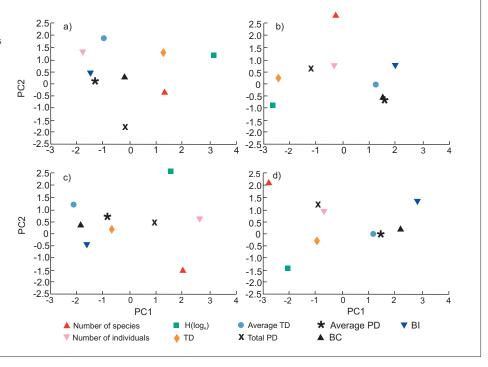


Figure 14.11. Principal
Components Analysis of the
scores for indicator attributes
at dredged material disposal
and aggregate extraction
sites. (a) English Channel
aggregate extraction site;
(b) Roughs Tower dredged
material disposal site; (c)
Tyne dredged material
disposal sites; (d) Liverpool
Bay dredged material
disposal sites.



study, this concern can be disregarded as sampling procedures and effort were internally consistent.

Taxonomic distinctness measures have greater potential to offer a means of identifying response mechanisms occurring at impacted sites (Warwick and Clarke, 1995) but, in practice, may be addressing aspects of evolutionary adaptations to perturbations which are not directly applicable to the immediacy of the effects considered here. It is also evident that, even for indices with greater intrinsic potential for identifying and explaining cause/effect relationships, supporting environmental data, including measures of anthropogenic pressure (disposal/extraction practices in the present context) are essential.

Following statistical analyses of indicator performance against gradients of change in the data, they were subjectively scored according to their ability to fulfil a variety of scientific and management criteria governing their effectiveness in applied studies, in this instance principally relating to the effects of physical perturbations at the sea bed. The indices that achieved the highest overall scores were the number of species, the Shannon-Weiner Index, the Total Phylogenetic Diversity, the number of individuals and the Average Taxonomic Diversity. These indices were the most effective in deducing the presence of biological degradation in response to dredged material (or other solid waste) disposal or aggregate extraction, although not all were the most transparent in terms of communicating findings to non-specialists. The other indices were less successful in identifying impacts at sites of anthropogenic activity. For example, the results obtained with the Biotic Coefficient are not comparable with those obtained using species numbers and the Shannon-Weiner Diversity Index, and are counter-intuitive when considered against other circumstantial evidence for localisation of anthropogenic activities.

These findings contradict the results of Borja et al. (2003) who investigated the response of the BC to different impact sources including drill cuttings at oil platforms employing ester-based mud, submarine outfalls enriched with heavy metals, dredging processes and other industrial and mining waste inputs. Borja et al. (2003) stated that the BC and BI are independent of the pollution source. However, this was clearly not the case when they were tested against the effects of dredgings disposal and aggregate extraction areas in the present study: the BC demonstrated either a positive impact or no significant difference as a result of these activities. The lack of correlation of the AMBI with the indices that were awarded higher scores is likely to be a result of the absence of organic enrichment as a principal source of change at each of the five sites, since

the species' sensitivity scores employed in calculating the index owe much to the work in this area by Pearson and Rosenberg (1978) and others (see Borja et al., 2003). Thus the macrofaunal species present at the dredged material disposal sites were not, in general, representative of the species found following an organic enrichment event and, therefore, the AMBI failed to identify areas exposed to other anthropogenic inputs or activities in this instance.

An important, if unsurprising, conclusion from the present study is that no single biological indicator will be capable of providing all of the information required to interpret the behaviour of an entire ecosystem in response to an anthropogenic impact (see for example, Carignan and Villard, 2002). It is therefore evident that an examination of areas of the sea bed that have undergone alterations resulting from dredged material disposal and aggregate extraction activities requires a combination of appropriate measures of data structure or function in order to improve confidence in deductions concerning cause/ effect relationships. Although the messages conveyed by the recently evolved measures of taxonomic relatedness were disappointing in the present context, their desirable properties and sound theoretical underpinning strongly argue for further exploration and a more wide-ranging testing regime before firm conclusions can be drawn regarding their general utility in routine assessments of environmental quality. Thus, for example, Clarke and Warwick (1994) note that the Taxonomic Distinctness measures are unlikely to be accurate when the dominant taxa re-colonising the area are non-sessile, non-polychaetous taxa, unrepresentative of polluted conditions. The Biotic Index appears to require further adaptation to be effective in the evaluation of impacts additional to those principally arising from organic enrichment and we understand that this work is in progress (A. Borja, pers. comm.).

#### 14.5 Conclusions

Overall, the univariate indices that were considered to express the biological data in a way that best fitted the desired criteria for an environmental indicator were the number of species, the Shannon-Weiner Index, the number of individuals and Average Taxonomic Diversity. It is accepted that the concentration on the effects of dredgings disposal and aggregate extraction, accompanied by the subjective nature of the scoring of indicator attributes, determines that the resulting ranking of selected measures may not necessarily match the outcomes of other studies. Nevertheless, we are encouraged to note that our findings are broadly comparable to an appraisal exercise conducted

by the UK Environment Agency in connection with the needs of the EU 'Water Framework ' Directive (A. Miles, pers. comm.). Further work is in progress to refine the scoring system and to investigate additional indicators at a wider range of study sites (Ware *et al.*, in prep).

For biotic indices such as the AMBI (at least in its present form), it is clear that utility will be influenced by the nature of the anthropogenic disturbance and also the characteristics

of the receiving environment, and much work still remains to be done on identifying the tolerance of individual species to multiple as well as single sources of human-induced pressures upon the environment. Variability in these factors around the UK coastline intuitively suggests the requirement for a suite of environmental indicators, some of which will be more appropriate than others, depending on local circumstances.

## 15. Determination of dioxin-like activity in sediments from the East Shetland Basin

#### 15.1 Introduction

There has been growing environmental concern about dioxins and furans, and other compounds that have dioxin-like properties. The major concerns with dioxin-like compounds are their effects upon wildlife and human health (Van den Berg et al., 1998). Dioxin-like compounds have been shown to be both persistent and able to bioaccumulate (Jones and Voogt, 1999). They have also been shown to produce a number of toxic responses including immunotoxicity, developmental and reproductive effects, neurotoxicity and carcinogenesis (Kerkvliet, 1995; Brouwer, 1991; Giesy et al., 1994; Nebert et al., 1993). Dioxin-like compounds are coplanar and they all produce their toxicity acting through the same aryl hydrocarbon receptor (AhR) mediated mechanism (Safe, 1990; Knutson and Poland, 1984).

Dioxin-like compounds are ubiquitous in the environment (Clapp and Ozonoff, 2000) and their presence is primarily the result of by-products of human activities including industrial (Anderson and Fisher, 2002) and other sources (eg waste incineration; Dyke et al., 2003). They, therefore, enter into the aquatic environment via a number of routes including atmospheric deposition and effluent discharges. Due to their hydrophobic nature, sediments are the eventual sink for dioxin-like compounds in the aquatic environment where they provide a source of potential exposure of dioxins and dioxin-like compounds to aquatic organisms (Lodge, 2002). Once present in the food chain, dioxins and dioxin-like compounds bioaccumulate, thus posing a threat to benthic organisms and their predators (Berggrena et al., 1999).

At present there is no concerted approach for monitoring of dioxin-like compounds in the marine environment. This is due to the extremely low concentrations at which deleterious effects can be observed. In order to monitor these extremely low concentrations, sophisticated analytical techniques such as high-resolution gas chromatography coupled to high-resolution mass spectroscopy (HRGC-HRMS) are required, which makes analysis expensive. An alternative approach is bio-analytical analysis using a receptor-reporter based cell line.

#### 15.2 DR-CALUX assay

The Dioxin Responsive-Chemically Activated Luciferase Expression (DR-CALUX) assay utilises a receptor-reporter gene system. The assay is mechanism specific and is based

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on the interaction of compounds with the arylhydrocarbon receptor (AhR). The assay is not compound specific and produces a response with all compounds capable of interaction with the AhR (Murk *et al.*, 1996; Overmeire *et al.*, 2001). The assay allows the integration of all dioxinlike compounds present and produces toxic equivalent concentrations relative to the most toxic congener 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD-TEQ<sub>CALUX</sub> values) for samples screened.

#### 15.3 Materials and methods

#### 15.3.1 Approach

Single sediment samples (n=108) were collected by means of a Day grab from sites across approximately 3,000 square miles of the East Shetland Basin between 20 July and 3 August 2002. The sampling positions of all the sites are shown in Figure 15.1.

#### 15.3.2 Sediment extraction

Sediment samples were air-dried and solvent extracted with dichloromethane to produce total extracts. The sample extracts were then transferred into hexane before aliquots assigned for assay.

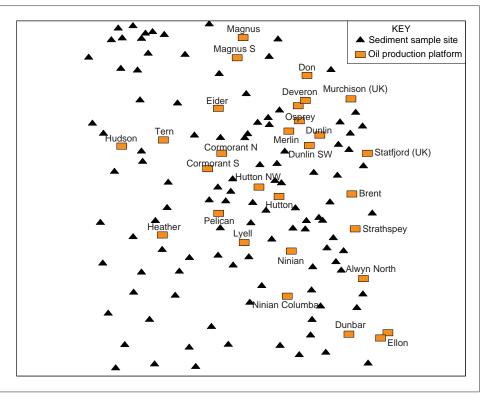
## 15.3.3 Assay for 'Dioxin-like' or Arylhydrocarbon receptor activity (DR-CALUX assay)

The DR-CALUX assay was conducted on both the total and cleaned-up extracts. The total extract was screened to ascertain the contribution from both stable and labile dioxin-like compounds. An aliquot of sample extract was also manipulated using a simple clean-up method to ascertain the contribution from stable dioxin-like compounds only, with the cleaned extract dissolved in dimethyl sulphoxide.

#### 15.3.4 DR-CALUX Assay

The DR-CALUX cell line was provided under licence from *Biodetection Systems Bv.*, Netherlands. The cell line was constructed as described by Garrison *et al.* (1996). These cells consist of the rat hepatoma (H4IIE) cell line that has been stably transfected with the luciferase reporter gene (pGudLuc) from the firefly, *Photinus pyralis*. The luciferase activity was determined using an automated luminometer, and interpolated with a TCDD standard curve to produce the TEQ<sub>CALUX</sub> values for the sample extracts. The DR-CALUX assay was conducted in accordance with a set of strict quality control (QC) criteria.

Figure 15.1. Map showing the positions of all the sampling sites and offshore production platforms within the East Shetland Basin. Sampling area latitude 60.5°– 61.65°N and longitude 0.6°–1.8°E (note one degree of latitude is 60 miles).



#### 15.3.5 Geostatistical interpolation technique

Some of the large spatial data sets produced underwent a geostatistical interpolation technique to aid their interpretation. The Kriging Interpolation method was utilised to produce contour modelling of the data sets.

#### 15.4 Results and discussion

## Arylhydrocarbon receptor activity (DR-CALUX Assay) 15.4.1 Activity of cleaned-up extracts

The results of the DR-CALUX screening of 91 samples collected from the Shetland Basin Area are shown in Table 15.1. The 24 h  $TEQ_{CALUX}$  data for all the samples ranged from 0.1 pg to 34 pg  $TEQ_{CALUX}$   $g^{-1}$  with a mean of 8.5 pg TEQ  $g^{-1}$ . As a comparison, the mean activity for stable compounds in a recent survey of UK estuarine sediments was 24 pg TCDD TEQ  $g^{-1}$ .

To put the values in context is difficult, as the UK does not currently have an Environmental Quality Standard (EQS) or Environmental Quality Guideline (EQG) for dioxin Other countries including compounds in sediments. Canada, the USA and the Netherlands have produced dioxin EQGs. The Canadian, American and Dutch EQGs, quoted as TCDD toxicity equivalents, are 1, 2.5 and 13 pg TEQ g<sup>-1</sup>, respectively (CCME, 2002; US-EPA, 1993; Dutch National Health Council, 1996). Using these guidelines, 89 of the 91 samples are above the most stringent (Canadian) EQG and 17 of the 91 samples are above the least stringent (Dutch) guideline value. For the sites breaching these guidelines, it is likely that some harm would be caused to benthic organisms associated with these sediments and also to predator organisms. The results from the Kriging analysis of the stable dioxin-like activity are shown in Figure 15.2 as a contour map. Areas that breached the least stringent Dutch guideline of 13 pg TEQ  $\rm g^{-1}$  are shown above the labelled 14 pg TEQ  $\rm g^{-1}$  contour. This area covers approximately 500 square miles (one-sixth) of the area sampled. The compounds responsible for the activity are likely to be dioxins, furans and coplanar chlorobiphenyl congeners, although other compounds may also contribute.

#### 15.4.2 Activity of total extracts

Table 15.2 shows the TCDD TEQ<sub>CALUX</sub> values for the total extracts (n=108) with a 6 h exposure period. The values range from 4.4 ng to 122 ng TEQ  $\rm g^{-1}$  with a mean of 36 ng TEQ  $\rm g^{-1}$ . As a comparison, the mean activity of total extracts from a recent survey of UK estuarine sediments was 205  $\rm \mu g$  BaP units  $\rm g^{-1}$ . These values are 3 orders of magnitude higher than those found in the cleaned-up extracts. This is not unusual, and due to the removal of PAHs during clean-up, which show a very potent ArH activity compared to 2,3,7,8 TCDD. The Geostatistical Interpolation technique was again conducted on this dataset with the distribution of the total CALUX activity shown in Figure 15.3.

The vast majority of the dioxin-like activity in the East Shetland sediments is attributable to labile compounds (eg PAHs). PAHs are known AhR agonists due to their coplanar configuration, but only produce dioxin-like toxicity if exposure is sustained in an organism since they are relatively quickly metabolised in the cell. (Poland and Glover, 1974; Machala *et al.*, 2001).

Table 15.1. Results of the CALUX screening of cleaned-up sediment extracts from the East Shetland Basin.

Sample code	Latitude °N	Longitude °E	pg TCDD TEQ g <sup>-1</sup>	Sample code	Latitude °N	Longitude °E	pg TCDD TEQ g <sup>-1</sup>	Sample code	Latitude °N	Longitude °E	pg TCDD TEQ g <sup>-1</sup>
1/11	61.5142	0.6118	1.6	9/9	61.2168	1.7212	1.8	16/1	60.7497	0.9203	7.6
1/12	61.5193	0.6630	2.1	10/1	61.1272	0.9248	10	16/3	60.8295	0.9452	30
1/13	61.5272	0.6867	4.1	10/9	61.0428	0.9120	21	16/4	60.8298	0.7823	13
1/15	61.5040	0.8250	5.0	10/10	61.0005	0.8575	2.4	16/8	60.7637	0.7430	8.7
1/16	61.5365	0.8848	1.8	10/17	60.9993	0.6192	2.4	16/12	60.6958	0.6280	8.8
2/1	61.5517	1.0387	2.3	11/1	61.1223	1.3885	11	16/16	60.6717	0.8030	11
2/3	61.6510	1.1553	1.7	11/2	61.0238	1.3388	7.0	17/4	60.7733	1.2945	34
3/1	61.5362	1.4077	1.1	11/3	61.0512	1.2843	11	17/7	60.7188	1.2378	12
3/2	61.5933	1.4532	15	11/4	61.0595	1.1680	7.6	18/1	60.8305	1.5200	3.1
4/10	61.4043	0.8272	12	11/8	61.0783	1.2345	20	18/3	60.8158	1.6415	2.0
4/13	61.3905	0.9900	3.1	12/1	61.1608	1.7777	5.4	18/4	60.7342	1.7250	2.3
5/4	61.4147	1.2940	3.7	12/2	61.0025	1.7972	3.5	18/5	60.6893	1.6938	2.8
5/10	61.4843	1.1823	12	12/4	61.0445	1.6187	5.7	18/9	60.6990	1.5392	25
6/1	61.4853	1.6742	17	12/7	61.0287	1.4672	11	18/10	60.7533	1.5065	3.8
6/2	61.3400	1.7652	11	12/8	61.1325	1.6610	5.5	19/2	60.5897	0.6902	7.2
6/3	61.3565	1.4335	2.9	12/9	61.1448	1.5595	6.6	19/4	60.5130	0.6450	7.3
7/1	61.3307	0.6118	11	12/12	61.1138	1.4157	8.7	19/6	60.5227	0.8117	5.2
7/2	61.2962	0.6572	1.9	13/1	60.9488	0.7565	2.3	19/13	60.5103	0.9862	7.5
7/4	61.2335	0.8178	2.4	13/5	60.8635	0.6185	2.7	19/15	60.5752	0.9645	5.9
7/6	61.1992	0.8198	5.8	13/9	60.9035	0.8782	1.9	20/4	60.6422	1.0658	9.8
7/7	61.1683	0.6945	2.2	13/13	60.8742	0.9930	20	20/7	60.5810	1.1320	6.8
8/1	61.2747	1.0038	4.0	14/1	60.8790	1.1172	10	20/9	60.5720	1.3523	22
8/5	61.2680	1.2720	5.8	14/2	60.8448	1.1787	5.8	21//3	60.6550	1.4980	4.5
8/6	61.2810	1.2862	5.2	14/3	60.8707	1.2492	19	21//4	60.6033	1.4860	6.1
8/8	61.3327	1.3813	6.6	14/4	60.9700	1.1355	12	21//7	60.5392	1.4308	4.5
8/9	61.3090	1.3837	6.6	14/7	60.9838	1.2692	15	21//9	60.5442	1.5447	4.7
9/1	61.1787	1.4030	4.8	14/11	60.9272	1.3535	14	21//12	60.5032	1.7207	16
9/2	61.2188	1.4415	8.2	15/1	60.9615	1.4488	7.0				
9/3	61.3098	1.6933	0.1	15/3	60.9850	1.5098	18	Blank Set 1			0.63
9/6	61.2935	1.7895	10	15/5	60.9840	1.5802	14	Blank Set 2			1.09
9/7	61.2710	1.7337	0.8	15/6	60.9250	1.6193	24				
9/8	61.2353	1.6772	2.6	15/7	60.8918	1.5885	21				

Figure 15.2. CALUX assay screening: distribution of dioxin-like activity (pg TEQ g⁻¹) produced from stable compounds from East Shetland Basin sediments using a geostatistical interpolation technique.

■ = offshore production platform. Sampling area latitude 60.5°-61.65°N and longitude 0.6°-1.8°E (note one degree of latitude is 60 miles).

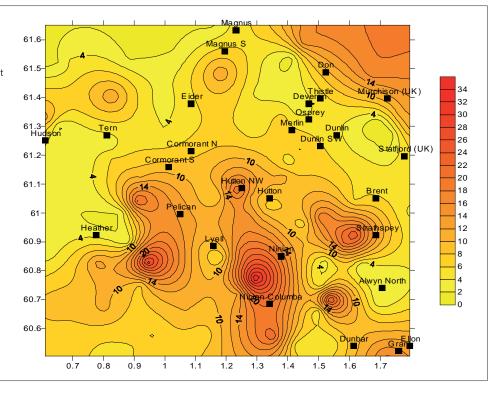
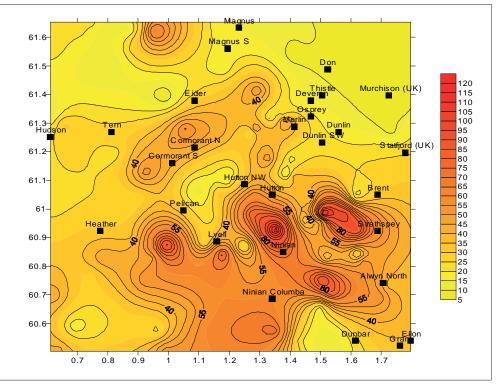


Figure 15.3. CALUX assay screening: distribution of dioxin-like activity (ng TEQ g-1) produced from both labile and stable compounds from East Shetland Basin sediments using a geostatistical interpolation technique.

■ = offshore production platform. Sampling area latitude 60.5°-61.65°N and Long 0.6°-1.8°E (note one degree of latitude is 60 miles).



#### 15.5 Conclusions

The DR-CALUX assay has been shown to be a useful tool for assessing the distribution and level of activity of sediment-associated contaminants in the East Shetland Basin. The stable dioxin-like, or arylhydrocarbon receptor-based activity was shown to occur at potentially harmful levels in some

areas of the East Shetland Basin, according to national guidelines from three countries. Samples, or sites, showing levels of contamination above set action limits would require targeted chemical analyses of a range of known potential candidate compounds to identify the causative compounds. Bioassay-directed fractionation procedures could also be used as the second tier to identify the causative agents.

 Table. 15.2. Results of the CALUX screening of total sediment extracts from the East Shetland Basin.

Sample code	Latitude °N	Longitude °E	ng TCDD TEQ g <sup>-1</sup>	Sample code	Latitude °N	Longitude °E	ng TCDD TEQ g <sup>-1</sup>	Sample code	Latitude °N	Longitude °E	ng TCDD TEQ g <sup>-1</sup>
1/1	61.6522	0.8182	10	9/2	61.2188	1.4415	23	15/4	60.9942	 1.5652	94
1/2	61.6457	0.7527	21	9/3	61.3098	1.6933	8.2	15/5	60.9840	1.5802	99
1/3	61.6073	0.7188	20	9/6	61.2935	1.7895	19	15/6	60.9250	1.6193	95
1/4	61.6125	0.7673	13	9/7	61.2710	1.7337	7.6	15/7	60.8918	1.5885	70
1/5	61.6092	0.8523	18	9/8	61.2353	1.6772	19	15/8	60.8803	1.6587	55
1/6	61.6262	0.8712	13	9/9	61.2168	1.7212	22	15/10	60.8448	1.6217	26
1/7	61.6307	0.9030	27	10/1	61.1272	0.9248	53	16/1	60.7497	0.9203	54
1/8	61.6200	0.9535	65	10/9	61.0428	0.9120	28	16/3	60.8295	0.9452	48
1/9	61.5590	0.8770	26	10/10	61.0005	0.8575	26	16/4	60.8298	0.7823	44
1/10	61.5510	0.6140	22	10/17	60.9993	0.6192	23	16/8	60.7637	0.7430	28
1/14	61.5110	0.7522	11	11/1	61.1223	1.3885	13	16/12	60.6958	0.6280	43
1/15	61.5040	0.8250	12	11/2	61.0238	1.3388	55	16/16	60.6717	0.8030	28
2/1	61.5517	1.0387	31	11/3	61.0512	1.2843	58	17/4	60.7733	1.2945	52
2/3	61.6510	1.1553	17	11/4	61.0595	1.1680	32	17/7	60.7188	1.2378	57
3/1	61.5362	1.4077	6.8	11/5	61.0643	1.0968	10	18/1	60.8305	1.5200	35
3/2	61.5933	1.4532	11	11/6	61.1062	1.1383	19	18/3	60.8158	1.6415	52
4/10	61.4043	0.8272	16	11/7	61.0907	1.1932	17	18/4	60.7342	1.7250	38
4/13	61.3905	0.9900	4.4	11/9	61.1320	1.2055	18	18/5	60.6893	1.6938	54
5/4	61.4147	1.2940	50	12/1	61.1608	1.7777	28	18/9	60.6990	1.5392	79
5/10	61.4843	1.1823	17	12/2	61.0025	1.7972	36	18/10	60.7533	1.5065	94
6/1	61.4853	1.6742	6.2	12/4	61.0445	1.6187	17	19/2	60.5897	0.6902	16
6/2	61.3400	1.7652	6.5	12/7	61.0287	1.4672	37	19/4	60.5130	0.6450	41
6/3	61.3565	1.4335	19	12/8	61.1325	1.6610	27	19/6	60.5227	0.8117	30
7/1	61.3307	0.6118	11	12/9	61.1448	1.5595	28	19/13	60.5103	0.9862	51
7/2	61.2962	0.6572	31	12/12	61.1138	1.4157	39	19/15	60.5752	0.9645	23
7/4	61.2335	0.8178	29	13/5	60.8635	0.6185	31	20/4	60.6422	1.0658	45
7/6	61.1992	0.8198	20	13/9	60.9035	0.8782	34	20/7	60.5810	1.1320	62
7/7	61.1683	0.6945	26	13/13	60.8742	0.9930	97	20/9	60.5720	1.3523	73
8/2	61.2818	1.0538	56	14/1	60.8790	1.1172	27	21/3	60.6550	1.4980	17
8/3	61.2712	1.1662	43	14/2	60.8448	1.1787	79	21/4	60.6033	1.4860	17
8/5	61.2680	1.2720	26	14/3	60.8707	1.2492	22	21/7	60.5392	1.4308	22
8/6	61.2810	1.2862	46	14/4	60.9700	1.1355	21	21/9	60.5442	1.5447	10
8/7	61.3177	1.3337	18	14/7	60.9838	1.2692	78	21/12	60.5032	1.7207	32
8/8	61.3327	1.3813	22	14/11	60.9272	1.3535	122				
8/9	61.3090	1.3837	49	15/1	60.9615	1.4488	28	Blank Set 1			0.036
8/11	61.1765	1.3287	49	15/2	60.9573	1.5018	48	Blank Set 2			0.022
9/1	61.1787	1.4030	18	15/3	60.9850	1.5098	117				

# 16. Preliminary investigations into 2,4,6-tri-*tert*butylphenol (246t*t*BP) in marine sediments

Authors: Carole Kelly, Katherine Langford, Philip Mellor and Stephanie Rowland

#### 16.1 Introduction

The substance 246ttBP is on the OSPAR list of chemicals for priority action. OSPAR invited the working group on concentrations, trends and effects of substances in the marine environment (SIME) to examine their draft background document on 246ttBP and report back their findings regarding monitoring data, quantification of sources and assessment of the extent of problems (OSPAR, 2002). The conclusions drawn were that there are no measured data currently available for 246ttBP in samples from the marine environment and so predicted environmental concentrations (PECs) had been used instead (OSPAR 2002). As the UK leads on this chemical within OSPAR, we were asked to develop a method and analyse a range of previously collected sediment samples to gauge the degree of 246ttBP contamination in marine sediments. OSPAR has identified five potential methods of 246ttBP exposure to the environment from its use:

- as a chemical intermediate in the production of antioxidants,
- as a lubricating agent,
- as a by-product produced during the synthesis of 4-tert butylphenol,
- as an additive for gasoline and fuel oil distillates
- in the offshore oil industry.

With respect to discharges to the marine environment, discharge of wastewater from land based production processes is the most likely exposure route (OSPAR, 2003). There is one company in the UK manufacturing 246ttBP, so investigation of potential inputs from this source may be required. The possible use of 246ttBP in the offshore oil industry was investigated by searching the Offshore Chemicals Notification Scheme database, held by Cefas, which confirmed that it is not currently licensed for use on the UK Continental Shelf.

There is evidence that 246t/BP causes cytotoxic activity (Saito et al., 2001) and also exhibits chronic toxicity (Matsumoto et al., 1991). In Japan, 246t/BP has been detected at trace levels in meat liver and also in fish muscle (Nemoto et al., 2001) highlighting the potential for human exposure. Current data also suggest that 246t/BP may be persistent and bioaccumulative. Despite this, the

risk to the marine environment is considered to be low as 246ttBP production within the EU is reported as being only of the order of 10 tonnes per annum (OSPAR, 2003). Local contamination may be expected to occur via any direct discharges, although there is not any data currently available to support this hypothesis.

#### 16.2 Method

Determination of 246ttBP has been included within a modified version of our standard alkylphenols (AP) method. This involved cold solvent extraction of the compounds of interest from sediment samples by shaking; extracts were then centrifuged, the solvent decanted and passed through a solid phase extraction clean-up. The extracts were concentrated to 300  $\mu l$  by rotary evaporation, placed in an autosampler vial and analysed by coupled gas chromatography - mass spectrometry (GC-MS) in electron impact ionisation mode.

#### 16.3 Results

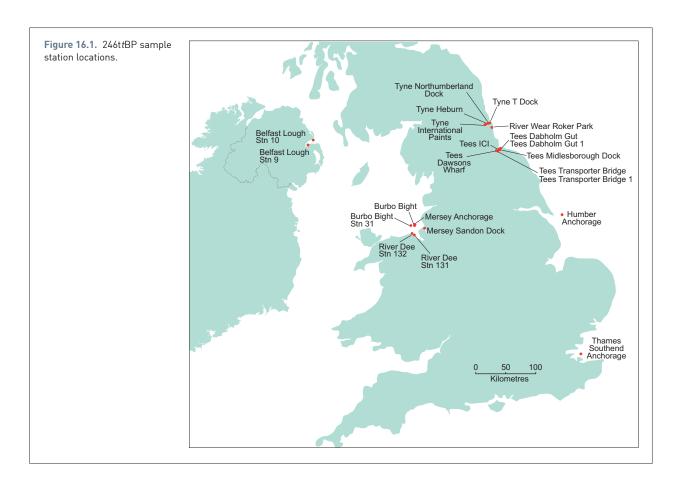
Samples were selected from sediments previously collected as part of national and NMMP surveys. Figure 16.1 shows the locations of the twenty-two samples analysed. They were chosen due to their proximity to urban or industrial land areas, as these were considered the most likely to be contaminated by 246ttBP.

Determinations of octylphenol (OP), nonylphenol (NP) and bisphenol-A (BPA) were made in the same samples and all the results are given in Table 16.1.

#### 16.4 Discussion

Concentrations of 246ttBP found in these sediments ranged from 0.01- 0.09 mg kg<sup>-1</sup> dry weight. Table 16.2 shows how these values relate to the PECs, which were estimated by OSPAR using The European Union System for the Evaluation of Substances (EUSES) modelling protocol. This illustrates that the levels found in the sediments analysed within our study were comparable to the predicted concentrations.

246ttBP has a log K $_{\rm ow}$  value of 6.06 and so is poorly water-soluble. The high log K $_{\rm ow}$  value of 246ttBP indicates that adsorption to organic matter and/or sediments is



**Table 16.1.** Concentrations (mg  $kg^{-1}$  dry weight) of 246ttBP, octylphenol (OP), nonylphenol (NP) and bisphenol-A (BPA) in selected sediment samples.

Sample Site	246t <i>t</i> BP	OP	NP	BPA
Tees, Dawsons Wharf	0.02	0.14	1.28	<0.01
Tees, Transporter Bridge	0.01	0.04	0.44	<0.01
Tees, Transporter Bridge 1	0.09	0.53	3.32	1.14
Tees, Middlesbrough Dock	0.08	0.12	1.12	<0.01
Tees, ICI works	0.01	80.0	0.89	0.46
Tees, Dabholm Gut	0.01	0.2	4.23	1.06
Tees, Dabholm Gut 1	0.01	0.47	5.88	0.16
Tyne, International Paints	< 0.01	< 0.01	< 0.01	<0.01
Tyne, Hebburn	< 0.01	0.01	< 0.01	<0.01
Tyne, Northumberland Dock	0.01	0.01	< 0.01	<0.01
Tyne Dock	0.01	0.02	< 0.01	<0.01
River Wear, Roker Park	0.01	< 0.01	0.07	<0.01
Humber Anchorage	0.01	< 0.01	< 0.01	<0.01
Thames, Southend Anchorage	< 0.01	< 0.01	< 0.01	<0.01
Mersey, Sandon Dock	0.08	0.04	< 0.01	<0.01
Mersey Anchorage	0.02	0.01	0.05	0.03
Burbo Bight	0.01	0.01	0.09	<0.01
Burbo Bight, Station 31	< 0.01	< 0.01	< 0.01	<0.01
River Dee, Stn 131	< 0.01	0.17	0.23	0.02
River Dee, Stn 132	< 0.01	< 0.01	0.04	<0.01
Belfast Lough, Bridge Station 9	0.01	0.01	0.04	<0.01
Belfast Lough, Station 10	0.01	<0.01	<0.01	<0.01

Table 16.2. Predicted environmental concentrations for 246ttBP

	PECs for water (microgram per litre)	PECs for sediment (mg kg <sup>-1</sup> wet wt)	PECs for WWTP organisms (microgram per litre)
Local – Production	0.061	0.136	0.71
Local – Formulation	0.025	0.0547	0.2
Local – Processing	0.022	0.0477	0.25
Regional	0.021	6.88E-04	
Continental	4.95E-05	1.5E-04	

the most likely fate process in aquatic systems. The provisional no-effect concentration  $\mathsf{PNEC}_{\mathsf{sediment}}$  is 0.0334 mg kg-1 wet weight (OSPAR, 2003). Converting the results from this study to wet weight values, Tees Middlesbrough Dock, Transporter bridge 1 and Mersey Sandon dock sites all have concentrations of 0.04 mg kg-1 wet weight indicating that some toxic effects may be observed, in particular for benthic species living in or on the sediments. All other sites sampled had concentrations lower than the  $\mathsf{PNEC}_{\mathsf{sediment}}$  value.

Although data are limited, this survey suggests that, as 246ttBP is manufactured in very small quantities and is not used in all areas (as shown by non-detects in the Tyne samples), it does not pose a significant concern to the open sea. However it may present an issue in certain

areas such as the Tees and Mersey, or within estuaries in other OSPAR regions close to sites of production or use. The levels of alkylphenols found in this study also indicate areas of usage. The concentrations ranged from < 0.01 to 5.88 mg kg<sup>-1</sup> with the Tees area, showing the most consistently high values. Samples from the Mersey, Roker Park, River Dee and at the Belfast Lough Bridge also contain detectable levels of alkylphenols, with all other stations showing results of close to or below the limit of detection. These results are similar to those we found previously in 1999. (Cefas, 2003).

Given that this preliminary survey has produced some positive results, it may prove useful for OSPAR to conduct a wider sampling survey, obtaining sediments from other locations which may have received inputs of this chemical.

### 17. Integration of groundtruthing approaches to characterize an area licensed for dredged material disposal off the north east coast of the UK

#### 17.1 Introduction

Historically, large amounts of industrial wastes (especially colliery waste and fly ash) were disposed of from ships at licensed sites off the northeast coast of England (eg Bustos-Báez, 2003; Herrando-Pérez and Frid, 1998; 2001; Frid et al., 1996; Khan and Garwood, 1995; Bamber, 1984; Eagle et al., 1979). In most cases disposal of these materials started well before statutory controls for the protection of the marine environment were enforced (Eagle et al., 1979). The disposal of industrial wastes and sewage sludge was phased out in 1990.

Presently, sea disposal from ships is largely confined to dredged material, but the environmental consequence of this activity has been the focus of increasing attention from government, the general public and industry (for review see Bolam and Rees, 2003).

TY070 is a licensed dredged material disposal site (DMDS) located 5.7 km off the Northumberland coast receiving maintenance-dredged material from the Tyne Estuary, which sometimes exceeds 150,000 tonnes annually (Figure 17.1). Since the commencement of disposal operations, the Ministry of Agriculture Fisheries and Food (MAFF) (Eagle *et al.*, 1979) and subsequently, the Centre for Environment, Fisheries and Aquaculture Science (Cefas) have intermittently surveyed environmental conditions at this site (eg Cefas, 2003; MAFF, 1994).

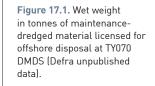
#### Author: Silvana Birchenough

Monitoring at marine disposal sites is undertaken with a view to:

- (i) characterising pre-disposal conditions at new sites;
- (ii) assessing compliance with licence conditions;
- (iii) providing data on environmental conditions to permit informed decisions regarding the continued acceptability of a particular disposal operation; and
- (iv) informing and assisting in the assessment of any future application for disposal licences.

In the past, scientists have relied on traditional sampling tools (eg grabs, corers and dredges) to investigate sediments and their associated benthic fauna (macrofauna and meiofauna) (eg Boyd et al., 2002; Eleftheriou and McIntyre, 2005). This information only relates to a specific point on the seabed from which the sample was collected and, therefore, inferences regarding the wider distribution of substrata and the associated fauna may be unrealistic, especially in patchy environments (Brown et al., 2002). Whilst there are sound reasons for continuing to apply such approaches (eg Rees et al., 1990), with improvements in technology there is increasing interest in using acoustic and optical techniques for assessing anthropogenic effects, particularly in areas of known habitat heterogeneity.

For example, a number of acoustic techniques (especially sidescan sonar and more recently multibeam



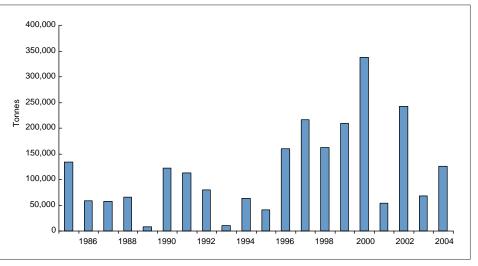
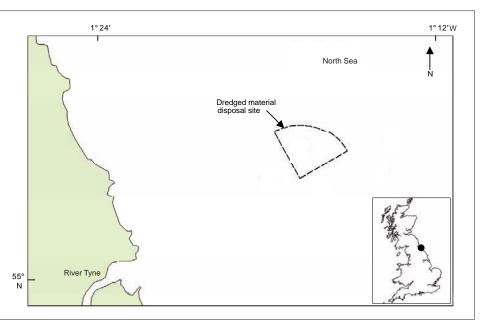


Figure 17.2. Map showing the location of the Tyne dredged material disposal site (TY070) off the northeast coast of the LIK



bathymetry) have been widely employed to monitor physical disturbance of seabed habitats (eg Brown et al., 2004, 2001; 2002; Limpenny et al., 2002). However, an ongoing challenge is to establish the extent to which such techniques used in conjunction with appropriate ground-truthing methodology and optical methods can be used to map both the distribution and status of benthic communities in relation to natural and man-made influences. Such techniques include:

- i) acoustic methods such as sidescan sonar, multibeam bathymetry and acoustic ground discrimination systems (eg QTC<sup>TM</sup> and RoxAnn<sup>TM</sup>);
- ii) underwater remote sensing techniques (eg video and digital stills photography) and;
- iii) Sediment Profile Imaging (SPI).

A limited number of studies further serve to illustrate the combined use of acoustic, optical and traditional methods for seafloor mapping and impact assessment (Kostylev *et al.*, 2005; Kostylev, *et al.*, 2001; Zajac, *et al.*, 2003,1998; DAMOS 2004). The aims of the present study were to assess the advantages of combining conventional grab sampling methods, acoustic techniques, and optical imaging devices to determine seafloor conditions resulting from the disposal of dredged material at a licensed site off NE England. This information has the potential to contribute to the development of improved monitoring approaches within UK waters.

#### 17.2 Methods

#### 17.2.1 Study site and data collection

The Tyne dredged material disposal site (TY070 thereafter, Figure 17.2) was surveyed over the period 2001 to 2004 with a combination of equipment (see Table 17.1 for specific details). During 2001, a pilot survey was conducted covering only three stations (duplicate), two located inside the disposal

**Table 17.1.** Summary of survey techniques applied at TY070 DMDS. SSS=sidescan sonar survey contains the number of swaths used to cover the area. Camera=video stills used for this work. Grab=number of grabs collected during each survey.

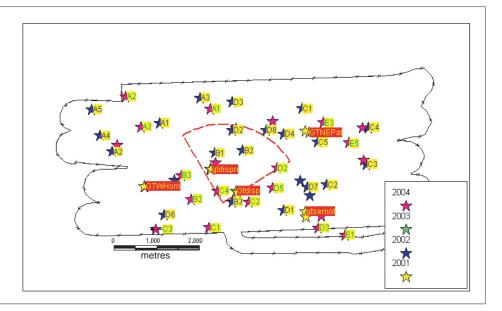
Year	SSS	Multibeam	Grab	Camera	SPI
2001	7		6		
2002	9		21	3	
2003	11	1	21	5	15 (triplicate)
2004	11		21	5	

site) and one to the northeast. There were sampled in order to assess the heterogeneity of the area. The results from the pilot survey conducted in 2001 are limited in comparison to those remaining surveys (ie 2002-2004). Sampling was undertaken using RV Cirolana and RV Corystes (2001 and 2002, respectively) and RV Cefas Endeavour (2003-2004). The full area was approximately 2.7 km by 1.9 km in size. Firstly, the acoustic survey was undertaken with sidescan sonar covering the full area and providing a 100% mosaic of the site. Secondly, ground-truthing was conducting using a 0.1 m<sup>2</sup> Hamon grab for collection of macrobenthos and sediment samples. Thirdly, a camera sledge was also used to provide a visual characterisation of the sediments in the area. Finally, in 2003, a Sediment Profile Imaging (SPI) camera was available to the programme and brought the opportunity to complement previous techniques used for characterisation of the area.

#### 17.2.2 Acoustic survey

Annually, TY070 was surveyed using a Datasonics SIS 1500 digital chirps system with a Triton Isis data logger. Delphmap post-processing software was used to mosaic the imagery and classify texturally different areas of the seabed. The system was operated on a 400 m swathe

Figure 17.3. Indication of the ground-truth (GT) points sampled over 2001-2004 at Dredged Material Disposal Site (TY070).



range, and survey lines were spaced at 400 m intervals in a north/south orientation to ensonify approximately 100% of the survey area. Vessel positioning was provided by Differential Global Positioning System (DGPS) and towed sensor position calculated by vessel heading, cable layback and towfish depth, all of which were logged in real time by the Isis system.

A camera sledge was deployed at several stations across the area surveyed to provide a characterisation of the sediment and habitats present in the area and also to aid interpretation of the sidescan sonar data. In addition during 2002 an acoustic ground discrimination system QTC<sup>TM</sup> (Quester Tangent Corporation, Sydney, BC) was run simultaneously with the sidescan sonar survey using a Furuno single beam hull-mounted echo sounder at frequencies of 200 kHz and 50 kHz (Boyd *et al.*, 2005). Additionally, in 2003, a multibeam survey was also conducted in the area, details of both surveys (QTC and multibeam) are presented in Chapter 5 (Boyd *et al.*, 2006).

#### 17.2.3 Benthic survey

The survey was designed to encompass the total area ensonified by the sidescan sonar survey. The main sampling tool was a 0.1 m<sup>2</sup> Hamon grab fitted with a video camera that provided an image of the undisturbed surface of the sediment adjacent top the grab bucket at each sampling station (Boyd, 2002). This device was employed since it has been shown to be particularly effective on coarse substrata (Kenny and Rees, 1996, 1994; Seiderer and Newell, 1999) and it was therefore considered suitable for sampling the heterogeneous sediments and deposited materials (eg colliery, fly ash and dredged material) known to be present in the area. A sub-sample was also taken from the grab for sediment analysis (particle size analysis-PSA thereafter). The PSA was conducted according to the methodology stated in the guidelines for the conduct of benthic studies at aggregate dredging sites (Boyd, 2002).

A total of 3 (duplicate samples) were collected during the pilot survey in 2001. During the remaining time of the programme a more extensive number of samples (22 samples) collected each year from 2002-2004 along TY070 area were used to provide a detailed characterisation of the site (Figure 17.3).

The grab sampling survey was conducted with a random sampling design within each of the acoustic regions based on the outputs from the sidescan sonar survey. The numbers of stations within each region was allocated in proportion to the size of the area. Once the sample had been collected, the contents of the grab were photographed on the top of the sieving table before the sample was processed. This photographic image was also employed to complement additional visual information (ie video and SPI images) for the interpretation of the acoustic survey. Sieving, sorting, preservation and laboratory processing of samples were conducted following the methodology described in Boyd (2002).

#### 17.2.4 Sediment Profile Imaging

A SPI camera was used to survey TY070 and surrounding areas in 2003. In July 2003, 12 stations located in the inside and in the immediate vicinity of the dredged material disposal site were surveyed with a remotely operated SPI camera that provided undisturbed, in situ, profile images of the upper ~20 cm of the sediment column. However, only a limited number of these stations are reported in this section. The camera used was a Nikon F-801s, and the software used to process the images was Image Analyst for Macintosh version 9.0.3. Three replicate images were taken at each sampling station. From each image, sediment type, sediment boundary roughness (SBR), apparent redox potential discontinuity (aRPD) depth, the Organism-Sediment Index (OSI, Rhoads and Germano, 1986) and the Benthic Habitat Quality index (BHQ, Nilsson and Rosenberg, 1997) were calculated.

#### 17.2.5 Data analysis

Univariate analysis based on total number of individuals (excluding colonial species) and the total number of species were calculated from the Hamon grab samples to provide a quantitative assessment of benthic assemblages within each acoustic region over time.

Additionally, multivariate analyses of data were conducted with PRIMER (Plymouth Routines in Multivariate Ecological Research software), version 6 for Windows. Following square root transformation of the abundance data (excluding colonial taxa), non-metric multi-dimensional scaling ordination (MDS) of Bray-Curtis similarity measures was used to assess changes in species composition (Clarke and Warwick, 1994). Pairwise ANOSIM (Analysis of Similarities, Clarke, 1993) was also conducted to test for significant differences in macrobenthic assemblage composition over time and among acoustically distinct areas.

The SIMPER routine (similarities percentages) within PRIMER was used to determine the contribution of individual species towards the dissimilarity between years and stations, while the BIO-ENV (Biota-Environment) analysis was used to find the best match between the multivariate sample patterns of an assemblage and environmental variables associated with those samples (Clarke and Gorley, 2006) over time at study sites. The environmental variables tested were % gravel, % sand and % silt/clay and annual quantities of licensed dredged material disposed of to the area.

Particle size distributions were analysed using Principal Component Analysis (PCA) to assess spatial and temporal changes at the stations .The variables used were the sorting coefficient, % gravel, % sand and % silt/clay of individual sediment samples.

#### **17.3. Results**

#### 17.3.1 Acoustic survey interpretation

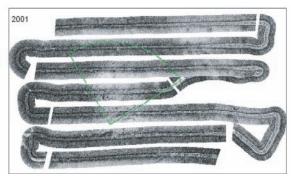
The sidescan sonar mosaic from each of the acoustic surveys is shown in Figure 17.4. Five acoustically distinct areas were delineated over the entire study site (Figure 17.5). The bathymetry in the area sloped gently from around 30 m depth in the west to 50 m in the east (bathymetric survey reported in Boyd, et al. 2006). The seafloor to the west of the disposal site had a hummocky topography (Figure 17.4 section D), whereas the seafloor topography to the east was much smoother (Figure 17.4 section A). Temporal comparison of the sidescan sonar records shows that there was little change in the broad distribution of sediments and features within the survey area between 2001 and 2004.

The surficial sediments in the offshore area east of the disposal site were soft in nature and dominated by relatively featureless, homogeneous sandy muds with small amounts of shell material at the surface (Figures 17.4-A and 17.6-A). The video stills showed that burrowing and tube-forming fauna inhabited these sediments (Turritella, sp. and Antalis sp.). Occasionally, harder linear features were also observed over this substratum. These linear features might be attributable to other impacts (eg demersal fishing activity). At the inshore area, the sediments were observed to be coarser and more heterogeneous, with a band of shellier muddy sands and sandy muds, intermixed with coal and clay, dominating the central part of the survey area (Figure 17.6-B). In the western part, a mixture of sand, mud, coal and other rocks are frequently encountered and can be linked with coal measures known to be present in this area.

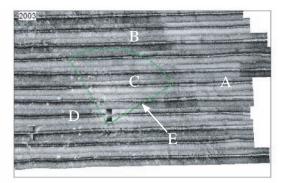
At the disposal site itself sediments were very mixed. The centre of the site is characterised by a lower acoustic backscatter and sediments mainly consist of sandy muds and muddy sands. The southern and central part of the site was generally muddy in nature, with some coarse sand present. The sidescan sonar imagery portrays this as a patch of predominantly finer sediment with areas of coarser, mixed material at the periphery, which appears to represent the acoustic footprint of the deposited sediments. The remainder of the site is mainly shelly, muddy sand with varying quantities of colliery waste and the presence of Alcyonium digitatum, the brittlestars Amphiura sp., and Ophiura sp., hydroids and bryozoans (Figure 17.6-C). Underwater photography showed an area of mixed sediment extending away from the southwest boundary of the licensed area with the presence of Alcyonium digitatum (Figure 17.6-D). It was apparent that the coarser component of this substratum consisted of gravel and cobble-sized coal particles within a muddy sand matrix. Patches of finer sediment (muddy sand/ sandy mud) also appear within this area. From the visual assessment provided by the video and SPI this appeared to be a combination of both disposal operations and natural coal deposits which were exposed at the seabed.

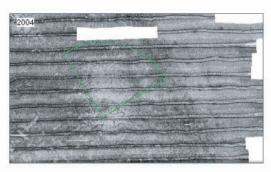
#### 17.3.2 Sediment

In the first year of study (2001) the sediment composition was observed to be an admixture of sand, silt/clay and gravel at all the stations. In the centre of the dredged material disposal site the sediments were mainly a combination of sandy mud and muddy sands, whereas east of the licensed area, sediments were predominantly composed of muddy sand.



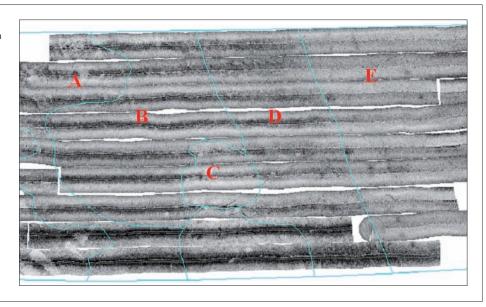






**Figure 17.4.** Temporal change [2001-2004] of the sidescan sonar mosaic at TY070 DMDS. Letters are used to label areas: A= homogenous sand, B=heterogeneous sediments, C= centre of the DMDS, D= hummocky area and E=licensed dredged material disposal site.

Figure 17.5. Sidescan sonar image illustrating delineation of acoustically distinct areas (eg A-E) at TY070.



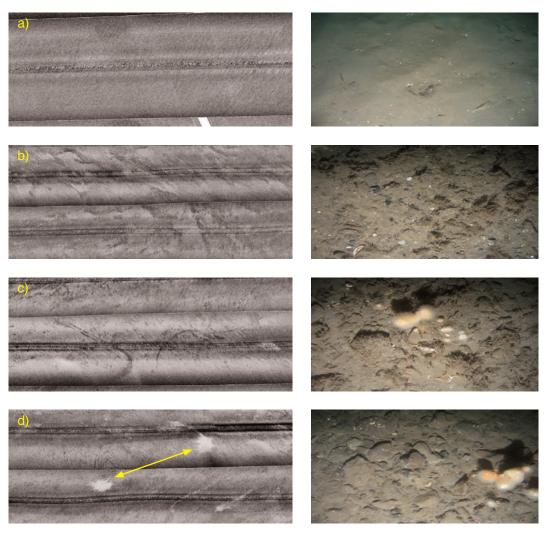


Figure 17.6. Close-up sidescan sonar records for locations A-D in Figure 17.4, along with video images showing seabed types: A) homogeneous sandy mud in the offshore part of the survey area (area shown is approx 200m x 400m); B) heterogeneous sediments composed of shelly muddy sand, sandy mud, coal and clay (area shown is approx 800m x 1600m); C) shelly muddy sand with colliery waste, present inside the disposal site. Also evident are linear trawling marks and possibly anchor drag scars (area shown is approx 300m x 600m) and D) discrete patches of soft material (arrowed) to the west of the disposal site (area shown is approx 600m x 1200m).

The centre of the licensed area is characterised by the presence of sandy muds and muddy sands. The area to the east of the disposal site (area E) is characterised by muddy sands. Sediments to the west of the survey area (area B) were, in contrast, predominantly composed of sandy gravel over time. Area A was observed to be composed of patchy sandy gravels with muddy sands. Principal Components Analysis (PCA) of the sorting coefficient, % gravel, % sand and % silt/clay showed the W-E changes in samples over time (Figure 17.7–A). Samples are spread in the ordination with some tendency of clustering (left side of the ordination) by samples from 2002, 2003 and 2004 (Figure 17.7–A). The distribution of the samples along the PC1 axis accounted for 63% of the variability and could be explained by the inverse variation in % sand. PC2 represented 31% of the

variability, which was mainly accounted for the % silt/clay content. The spatial representation of acoustically distinct areas can also be seen in Figure 17.7-B. There is a tendency for clustering on the left side of the plot of the majority of samples from the acoustic areas E, C and D.

#### 17.3.3 Biological composition

A total of 172 taxa were identified over the four years of the study. In 2001, the pilot survey of the area was designed to assess differences between the centre of the licensed dredged material disposal site and adjacent areas. Hamon grab samples were collected to target and ground-truth the acoustically distinct areas and also to provide an indication of the status of macrobenthic assemblages. Figure 17.8 shows the values of total abundance of individuals and

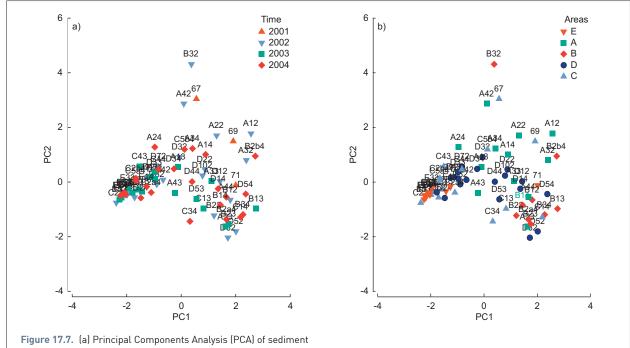
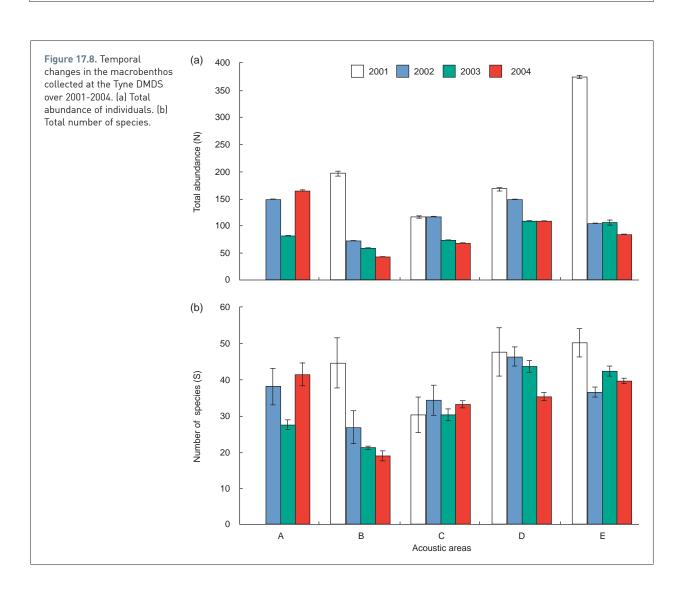
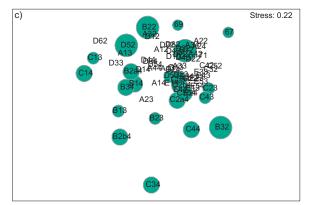


Figure 17.7. (a) Principal Components Analysis (PCA) of sediment characteristics (sorting, % gravel, % sand and % silt/clay) for ground-truth samples at TY070 over 2001-2004. (b) PCA ordination based on acoustically distinct areas delineated at TY070.









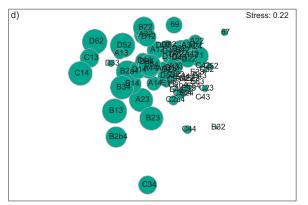


Figure 17.9. (a) Non-metric multidimensional scaling ordination using Bray Curtis similarities measure computed for double square-root transformed species abundances for 2003, (b) 2004, (c) MDS for all years overlain with dredged material quantities (extracted from Figure 17.1) and (d) MDS for all years overlain with % gravel.

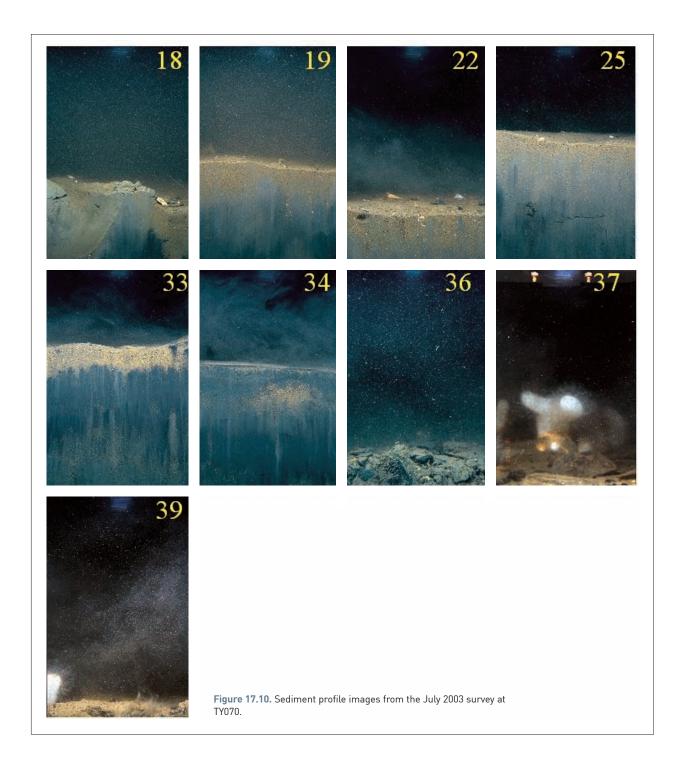
total number of species for the different acoustic areas sampled over the 4 years of the study. In 2001, stations were only collected from within 4 acoustic areas (ie areas B, C, D and E). Over these 5 distinct acoustic areas the values of total abundance of individuals ranged from 45-374 per 0.1 m² and the total number of species ranged from 20-50 per 0.1 m². Lower values of both univariate metrics were observed at area B (west of the dredged material disposal site) in comparison with areas C, D and E over 2002-2004.

For simplicity, the results of multidimensional scaling using Bray-Curtis similarity coefficients were separated over years to assess the pattern of variation. There is an E-W orientation in both ordinations for 2003 and 2004 (Figures 17.9(a) and (b)). An aggregation of samples from the disposal site and adjacent areas is observed to the right side of the ordination, while other samples from the disposal site were more widely dispersed in both years (Figures 17.9(a) and (b)).

Results showed that community composition was best represented by a combination of deposited material, % gravel, and % silt/clay (BIO-ENV analysis; r=0.483). Figures 17.9(c) and (d) show the faunal data overlain with these environmental variables (eg dredged material quantities and % gravel). The species distribution surrounding the disposal site appears to be influenced by the disposal

operation. This is indicated by the separation of samples located in the disposal site and immediate vicinity (Figure 17.9(c)). Additionally, elevated % gravel (Figure 17.9(d)) was noted at areas within the disposal site itself and also at areas A and D. The result of the two-way nested ANOSIM provided evidence of significant differences (r-statistic= 0.26; p<0.002) over time and over acoustically distinct areas (r=0.31; p< 0.001) during the study period.

SIMPER results (see Boyd et al., 2006) showed that the highest values for overall dissimilarity were found for the comparison between 2001 and 2002 (72.11%) and 2001 versus 2004 (73.72%). It is clear that the differences observed over time are attributable to the changes in abundance of a number of taxa over time, resulting from the disposal activities at the site. Examples of species showing appreciable changes include the polychaete Lagis koreni, which has a high average abundance during the first year of the study and lower abundances subsequently. The dissimilarity between the acoustically distinct areas was also analysed. The highest overall dissimilarity was encountered in comparison between areas C and B (73.62%) and E versus B (72.80%). It is clear that decreased abundance at area B can be attributed to the type of substratum encountered (ie sandy gravel) and the high abundance of the carnivorous polychaete Lumbrineris gracilis.



#### 17.3.4 Sediment Profile Imaging

SPI images from July 2003 are shown in Figure 17.10 for stations located in the centre of TY070 and surrounding areas. The sediment distribution appears to be predominantly composed of fine sand in the surface layers of the sediment. Some medium sand and fine muddy material is seen throughout most of the stations sampled. A varying shelly fraction is also visible at the sediment surface at many of the stations surveyed. This material can be seen as the highly reflective fragments scattered across the sediment/water interface in the surface photographs. There was a certain amount of re-suspension of fine material at many of the stations sampled (an artefact of SPI technology: R. Valente pers comm.). An obvious example

of this was observed in the surface and profile images taken at station 19 (Figures 17.10 and 17.11). Coarser sediments were recorded at stations 22 and 25, while boulder field/bedrock outcropping were also recorded at station 36, 37 and 39, to the west of the disposal area.

Surface boundary roughness indicates unevenness of the sediment surface as a result of physical activity and bioturbation processes (Rhoads and Germano, 1986). Values ranged from a low of 0.33 (the very flat sediment surface present at station 33 in the disposal area) to a high value of 1.58 (a biogenically-roughened sediment-water interface at station 30, located to the east of the disposal area). Where high roughness values are recorded in the present survey, it is clear that these are largely attributable

Table 17.2. Results from the analysis of SPI images, including sediment type, penetration, Sediment Boundary Roughness (SBR), apparent redox discontinuity depth (ARPD), Stage (calculated from the Benthic Quality Index) and Organism sediment index (OSI)

Stations	Sediment type	Penetration (cm)	SBR	ARPD depth	Stage	OSI
22	>3-2 phi	5.80	1.00	4.93	II-III	10
25	>4-3 phi	13.43	0.69	5.62	II	9
33	> 4phi	15.39	1.63	2.58	1	5
34	> 4phi	12.96	1.00	1.96	Azoic	2
36	> 4phi				1-11	2
37	>4-3 phi				1-11	2
39	>3-2 phi				1-11	2

to biogenic features. For example, in photographs taken at station 18 (Figure 17.10 and 17.11), this is mainly due to the burrowing activities of decapods whose burrows are evident in several SPI and surface photographs (Stations 18, 30, and 32) or to tube formation and bioturbating activity of infaunal animals eg polychaetes and holothurians.

The apparent redox potential discontinuity (aRPD) depths (the visible line between oxygenated and reduced sediment) recorded from the SPI images is presented in Table 17.2. It is evident that aRPD depth is greatly dependent on the presence/absence of bioturbating fauna. The aRPD presence can be gauged using the combined information from both the profile and the surface images. Where such animals are present (stations 18, 19, 21, 30 and 32) the aRPD layer is relatively deep and uneven, with re-working of sediment observed in the sediment profile and oxygenated voids generally present at depth within the sediment.

At stations where the excavating, irrigation, burrowing and feeding activities of such animals are absent (stations 28, 29 and 33) a shallower aRPD layer occurs, reflecting the presence of reduced sediment recently placed during a dredged material disposal operation. Station 29 reveals a very limited amount of oxygenated sediment present, with an associated low level of biological activities (Figure 17.10). These stations are all located inside the disposal site boundary. It is also relevant that, due to the presence of coarse/rocky substrata, it was not possible to gauge the depth of the aRPD layer at stations 36, 37 and 39 (Figure 17.10). It is important to highlight the variation in different aRPD depths within the disposal site itself, with both relatively high and relatively low values recorded. This may be attributable to the degree of historical disposal activity at the site.

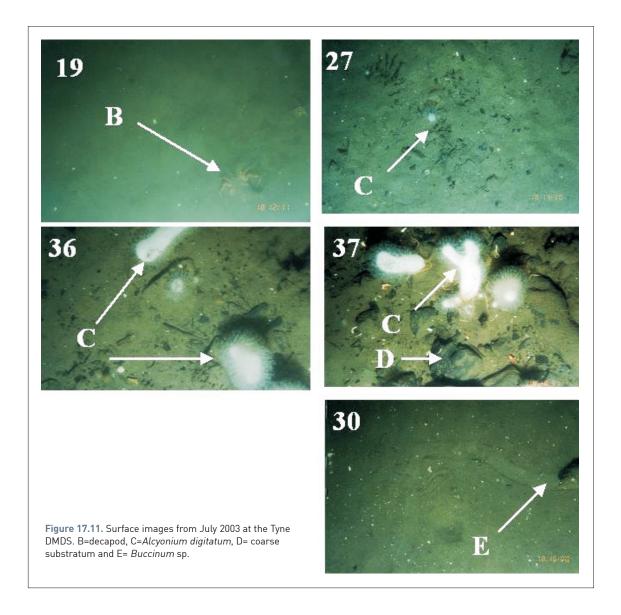
The stations located outside the disposal site returned consistently high aRPD values (Table 17.2). The presence of burrows (attributable to the excavating activities of several infaunal species) can be clearly seen in many of the profile images (eg station 34 with faunal activity at depth); in some cases substantial sections of these burrows are

visible. A large decapod (Figure 17.11 image 19 d) and a pelican foot shell (*Aporrhais pespelecani*) are also visible on the sediment profile image for station 26 (Figure 17.10). Colonies of the anthozoan *Alcyonium digitatum* (soft coral) are visible in the surface images at station 27, 36 and 37 (Figure 17.11). These indicate the presence of suitable substrata for attachment ie gravel, shelly material or coal, although this station is located to the south-west of the licensed area. In the surface image taken at station 30, surface tracks produced by the whelk *Buccinum* sp. are evident (Figure 17.11 image 30 E).

The benthic Habitat Quality Index was calculated for the stations surveyed following the methodology proposed by Nilsson and Rosenberg (1997) (Table 17.2). Successional stages varied from stage I environments (stations 26, 29 and 33, located within the disposal-site were characterised by shallow aRPDs and the absence of discernible biogenic features) to stage III (stations 18, 30 and 32, located outside the disposal area) with their characteristically deep aRPDs, well-developed faunal communities and prominent biogenic features such as burrows and feeding mounds.

#### 17.4 Discussion

Marine benthic habitats are vulnerable to the influence of a wide range of anthropogenic activities (eg dredged material disposal, aggregate extraction, windfarm developments, oil and gas exploitation and fishing impacts). Recent developments in seabed mapping techniques driven by continuous improvements in acoustic systems offer the potential to radically improve single-point sampling approaches to monitor the impacts of such activities. These opportunities provide benthic ecologists with new avenues for studying the structure and dynamics of benthic communities at multiple spatial scales (Zajac et al., 2003). The Tyne dredged material disposal site is used as an exemplar to establish the extent to which such a combination of techniques could be used in the future routine environmental monitoring programmes undertaken in UK waters.



Acoustic techniques have been routinely employed for many years as a tool in disposal site monitoring but the resolution, affordability and accessibility of the technology has greatly increased in recent years (Kenny *et al.*, 2003; Van Lancker *et al.*, 2003). In this study the utility of acoustic techniques was demonstrated by:

- i) the facility for rapid coverage of large areas of seabed,
- ii) facilitating the delineation of sedimentlogically and biologically distinct areas and
- iii) providing information on the footprint of disposal activity.

Examination of the data from grab samples confirmed a distinct area of the seabed within the disposal site, characterised by sediments which were largely muddy in nature, but also included patches of coarse sand. Other substrata present over the survey area also demonstrated agreement between the particle size distribution and SPI images. The particle size distribution data provided the quantitative information to ground-truth the sediments of the area. The use of SPI complemented the PSA information with an *in situ* representation of sediment structure and quality at each station.

On a smaller spatial scale, a video camera attached to the Hamon grab provided instantaneous information on the undisturbed image of the surface sediments, including any associated epifauna. This information only gave a localised and general indication of the footprint of the disposal activity. However, the images at certain stations in the vicinity of the disposal site reveal apparently undisturbed conditions at the sediment surface. Evidence of a legacy of the disposal activity was provided by the vertical SPI images, and the combination of the two sources of information was helpful in evaluating overall seabed status.

The Hamon grab (covering 0.1 m²) provided point-sample information on fauna and sediment composition. These data allowed a quantitative analysis over the different areas and, to a degree, identified changes occurring within and in the near vicinity of the disposal site between 2002-2004. A decline in number of individuals and species over time was observed for area B (to the west of the disposal site), when compared to areas C, D and E. Multivariate analysis indicated a certain degree of similarity in terms of faunal distribution over time including some of the stations located

at the centre of the disposal site (Figure 17.9). However, stations located within the western and southern parts of the disposal site were relatively dissimilar; this was also observed in the sidescan images. As expected, the BIO-ENV identified an influence on the assemblage associated with the disposal of dredge material and sediment type (eg especially variability in % gravel and silt/clay) of species composition with sediment type. The disposal site was directly influenced by a combination of coarse and soft sediments. The areas outside and in the immediate vicinity of the disposal site were mainly composed of soft material (ie muds) and medium sands.

SPI has found wide application worldwide (eg U.S.A. a clear example is the Disposal Area Monitoring System program (DAMOS), Fredette and French, 2004, Solan, et al., 2003; Keegan et al., 2001 O'Connor et al., 1989; Rumohr, 1995) but has only been used to a limited extent in the UK. The present pilot study off the Tyne confirmed that SPI can provide valuable information to assist in interpreting impacts occurring both within and in the near vicinity of the disposal site which could not be discerned from the sidescan sonar or grab sample data alone. The attributes of SPI include:

- assisting in mapping thin layers of deposited dredged material which are not clearly detected by acoustic technology,
- ii) providing complementary information on behaviour and disruption of benthic organisms and sediment quality status,

iii) providing near real-time data return and iv) fast and clear return of data. Therefore, by incorporating the use of SPI in future surveys we can envisage a more informed and efficient monitoring exercise.

We conclude that the combination of approaches employed in this study has the potential for wider application around the UK coast. With the exception of SPI, the techniques employed in this study have been widely used at other disposal sites around the UK coast, although not always in synchrony. Procedures for integrating these approaches, exemplified by the present study, appear to offer significant benefits. Clearly, the scope for all combination surveys will be limited by local circumstances (eg the presence of bedrock provides an obvious constraint on SPI use) and the objectives of the investigation. However, the capability to link point sampling with a wider spatial perspective should, in general, be highly advantageous.

In the particular case of the Tyne, the study provided complementary evidence of the localisation of impacts arising from disposal, but also provided additional insights into the heterogeneous character of the environment in the wider region and the interaction between natural and anthropogenic influences, including those attributable to earlier disposal activities.

# 18. Acoustic monitoring of the Inner Gabbard dredged material disposal site

#### 18.1 Introduction

The Inner Gabbard dredged material disposal site (TH052) currently serves the marine disposal requirements for maintenance dredged material from the Harwich Haven, SE English coast.

The Harwich Haven is formed at the confluence of the estuaries of the River Stour and the River Orwell and encompasses the Port of Felixstowe, the largest container port in the UK, and Harwich International Port. There have been a number of port expansions and consequent capital dredging projects undertaken in the area in recent years. Between 1998 and 2000, in excess of 30 million wet tonnes of predominantly capital dredged material was removed during works associated with the deepening of the port approach channel. The majority of this material was placed at the Roughs Tower disposal site. This site had been used routinely for many years for the disposal of maintenance and capital material arising from Harwich, Felixstowe and other east coast ports. However, ongoing monitoring at Roughs Tower demonstrated that, as a consequence of the capital disposal programme, this site had reached its full capacity and that a new site was required to receive material arising from future maintenance dredging operations within Harwich Haven. Therefore a new site was characterised and Defra (then MAFF) granted a FEPA licence to dispose of maintenance dredged material at the new Inner Gabbard site where disposal commenced in 1998.

The Inner Gabbard disposal site is located off the east coast of England, 30 km east of Harwich/Felixtowe and

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8 km south-west of the Inner Gabbard sandbank (Figure 18.1) and forms a polygon shown in Figure 18.2. The site is located in water depths of 30 – 40 m below chart datum and lies on the western flank of a deep water channel that runs in a roughly north-east/south-west direction.

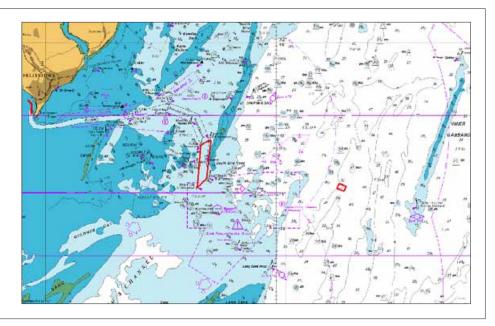
Tidal currents in the vicinity reach up to 1 ms<sup>-1</sup> and as a result it was decided that finer material disposed of at the Inner Gabbard site would not accumulate over time, but would be dispersed over the wider area by natural hydrodynamic processes. Therefore, material placed at the site is, as far as possible, fine in nature and largely composed of maintenance dredgings.

The disposal licence contains certain conditions to ensure that:

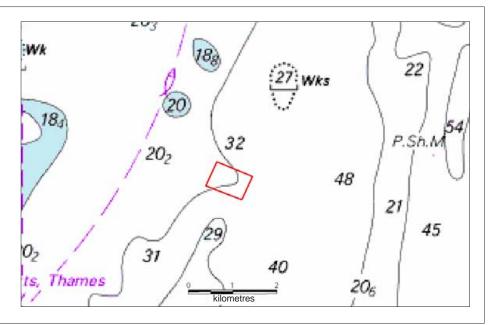
- (i) deposition of material at the site is undertaken in a manner such that material is placed evenly over the area of the site;
- (ii) the rate of deposit is not to exceed 2000 m³ per minute whilst the disposal vessel is underway at a speed of not less than 4 knots through the water and:
- (iii) the licence holder undertakes a programme of monitoring to demonstrate that the use of the deposit area is not having a long-term adverse effect on the biological resources or the legitimate activities of others in the area.

The licence conditions also allow for the disposal of capital material to the site on the condition that it is dissaggregated prior to disposal, thereby ensuring that it will behave in a similar way to maintenance material.

Figure 18.1. Location of the Inner Gabbard site.



**Figure 18.2.** Local bathymetry surrounding the Inner Gabbard site.



The dredged material disposed of at the site by the Harwich Haven Authority arises as a result of 4 or 5 annual dredging campaigns from Harwich and Felixstowe Harbours, the navigation channel and Harwich International Port. Between 1998 and 2003, over 13 million tonnes of material was deposited at the site (Table 18.1).

#### 18.2 Site monitoring

Cefas has undertaken monitoring surveys at the Inner Gabbard disposal area since it became active in 1998, with the most recent survey being completed in 2005. These surveys are intended to provide check monitoring for comparison against more comprehensive monitoring programmes carried out by Harwich Haven Authority as part of their licence conditions. Cefas surveys have incorporated a number of methodologies, including grab sampling for infauna and contaminant analysis, underwater photography and a range of acoustic techniques. This report describes the results of the sidescan sonar surveys conducted at the site between 2001 and 2004.

#### 18.3 Methods

Sidescan sonar surveys provide information about the texture of the seabed within a survey area. From this textural information, it is possible to predict the particulate nature of the sediments and consequently assign sediment descriptions to regions of the seabed. In general, soft sediments provide a low reflection strength which is represented on a sidescan sonar image as a lighter seabed than coarser sediments that might surround them. A very hard seabed, comprising coarse gravel and cobble, will show up as a dark, textured reflector and boulders and bedrock can often be observed as individual hard, dark targets or linear features. These predictions are generally confirmed using conventional sampling techniques, such as grabs

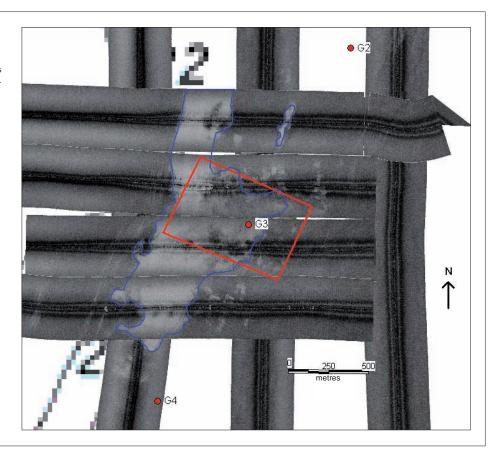
**Table 18.1**. Total amount of maintenance dredgings disposed of to the Inner Gabbard site between 1998 and 2003.

Year of disposal	Disposal quantity (tonnes)
1998	657,064
1999	2,745,679
2000	1,244,423
2001	2,674,549
2002	2,903,108
2003	3,468,655
Total	13,693,478

and cameras. Surveys of this type can distinguish between areas of sand, mud, gravel and rock, and repeat surveys of the same area have the potential to monitor temporal and spatial changes in the distribution of sediments. Sidescan sonar can also discern topographic features such as sand waves and ripples and scour marks, and this information can be used to predict sediment transport pathways. Anthropogenic features such as trawl marks and aggregate dredging tracks can also be identified and mapped using this technology (Limpenny *et al.*, 2002).

Sidescan sonar data were collected using the Benthos™ SIS 1500 digital CHIRP (190-210 kHz) sidescan sonar system in conjunction with the Triton Isis™ data acquisition software. Data were processed, georeferenced and mosaiced using the Triton Isis™ software package to produce continuous acoustic maps of the area surveyed. The vessel position was provided by a DGPS system and the position of the sidescan towfish was calculated by using vessel heading, vessel offsets, towcable layback and towfish depth. Survey line spacing was designed to produce 100% coverage of the areas of interest, using a 400 m acoustic swathe width.

Figure 18.3. Sidescan sonar mosaic of the Inner Gabbard site and the surrounding seabed in 2001. The red dots show the location of ground-truth grab samples.



Ground-truthing of the acoustic mosaic was achieved using samples collected with either a  $0.1~\mathrm{m}^2$  Hamon grab or a Shipek grab. Photographs were taken of the grab samples and these provided useful retrospective information for the subsequent interpretation of the sidescan sonar mosaics. Further information on methodology is given in Boyd, 2002.

#### 18.4 Results

Figures 18.3 to 18.5 and also Figure 18.8 show the sidescan sonar mosaics created from the surveys conducted between 2001 and 2004. The samples which were used for ground-truthing are overlain onto each mosaic. These samples, along with the sidescan sonar images themselves, were used to define the area of the seabed that was covered to a large extent by dredged material for each of the survey years. This area has been delineated by a blue polygon in each image. The exception to this is the image produced in 2002 where the coverage of dredged material is less pronounced. The results from each year are discussed below.

#### 18.4.1 Survey results - 2001

Figure 18.3 shows the nature of the sediments at the seabed some 3 years after disposal began at the site. The sidescan image shows an area of soft sediments (polygon = 0.7 km²) centred on the disposal site, which extends over 500 m to the north and south of the site itself. Only a limited number of ground-truth samples were available in 2001, but sediment descriptions for G3 indicate the presence of fluid mud and hard clay within the disposal site and sediments at G2 and G4 are described as gravelly sand over mud (Table 18.2).

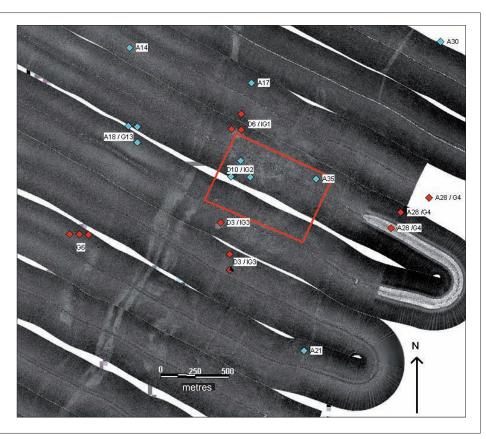
#### 18.4.2 Survey results - 2002

In 2002, the presence of soft material over the wider area was less evident than in 2001. Linear features to the west of the site appear to be composed of rippled sandy sediments indicating that they are dynamic features. The sidescan sonar image does not suggest that large quantities of soft material are present at the seabed within or surrounding the disposal site, and ground-truth data

Table 18.2. Sediment descriptions from grab samples collected in 2001.

Cruise	Station	Sediment description
CIR 5a/01	G1	No description
CIR 5a/01	G2	Gravelly sand over mud
CIR 5a/01	G3	Hard clay with a small amount of gravel. Fluid mud present
CIR 5a/01	G4	Gravelly sand over mud
CIR 5a/01	G5	Gravelly sand over mud

Figure 18.4. Sidescan sonar mosaic of the Inner Gabbard site and the surrounding seabed in 2002. The red and blue diamonds show the location of ground-truth grab samples.



from IG1, IG2 and A35 confirm the absence of fluid mud gravels, with some consolidated clay material present in this area. The sediments over the wider area are largely composed of muddy gravelly sands and muddy sandy

within the samples (Table 18.3).

Table 18.3. Sediment descriptions from grab samples collected in 2002.

Cruise	Station	Sediment description
CIR 3a/02	A14	Muddy shelly gravel
CIR 3a/02	A16	Compacted grey clay lumps with mud and shell
CIR 3a/02	A17	Sandy gravel with clay lumps
CIR 3a/02	A18	No description
CIR 3a/02	A21	Mud and grey clay with some shell and gravel
CIR 3a/02	A27	Muddy shelly sand
CIR 3a/02	A28	Muddy gravel
CIR 3a/02	A30	Muddy sandy gravel and clay lumps
CIR 3a/02	A34	Muddy sandy gravel
CIR 3a/02	A35	Slightly muddy shelly sand with clay lumps
CIR 3a/02	A36	Muddy shelly gravel
CIR 3a/02	A5	Muddy sandy gravel
CIR 3a/02	G6	Muddy sandy gravel
CIR 3a/02	G7	Muddy shelly gravelly sand
CIR 3a/02	IG1	Muddy gravelly sand
CIR 3a/02	IG2	Anoxic muddy gravel
CIR 3a/02	IG3	No description
CIR 3a/02	IG4	Slightly muddy sandy gravel
CIR 3a/02	IG5	Muddy sandy gravel

Figure 18.5. Sidescan sonar mosaic of the Inner Gabbard site and the surrounding seabed in 2003. The red dots show the location of ground-truth grab samples.

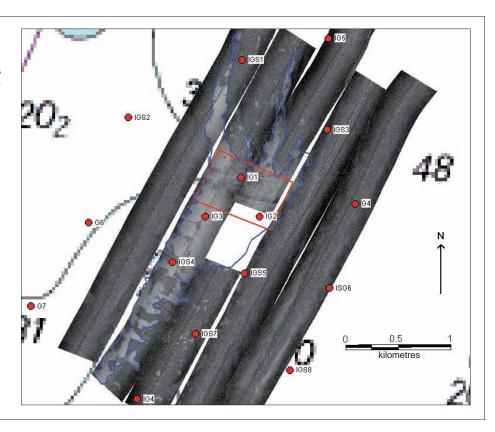




Figure 18.6. Example of the soft, fluid surficial sediments collected from IG3 in 2003.

#### 18.4.3 Survey results - 2003

The sidescan sonar image from 2003 (Figure 18.5) suggests that, once again, soft muddy sediments are present at the seabed both within and surrounding the disposal site. The area of the seabed covered by these soft sediments (represented by the blue polygon) is  $1.4 \, \mathrm{km^2}$ , almost double that present in 2001. These sediments are distributed up to  $1.4 \, \mathrm{km}$  to the north, and  $2.3 \, \mathrm{km}$  to the south of the centre of the disposal site and are aligned roughly parallel to the tidal vector. Sediments collected from IG1, IG2, IG3 and IG4 were described as predominantly mud with small components of other material (Table 18.4), which confirmed sidescan sonar interpretations. The surface sediments covering the wider area are, as in 2001 and 2002, generally composed of muddy sandy gravel and muddy gravelly sand with some consolidated clay material.

At the southern extent of the soft sediments, pairs of tracks made by demersal trawlers can be seen on the sidescan mosaic (Figure 18.7). Each pair of tracks is approximately 20 m apart, 4 m wide, and suggests that the heavy ground-gear of a beam trawl has punctured the soft muddy layer, revealing the coarser substrata beneath.

#### 18.4.4 Survey results - 2004

In 2004, fine sediments were again widely distributed within, and in the vicinity of, the disposal site (Figure 18.8). The area of coverage by fine sediments, as defined by the blue polygon, is approximately 1.7 km², which represents a comparable coverage to the previous year. Ground-truth data again confirm the finer nature of these sediments, particularly at G6 and G10 (Table 18.5). Samples collected at G7 have identified the presence of the 'reef' form of the tube dwelling worm, Sabellaria spinulosa (Figure 18.9).

Table 18.4. Sediment descriptions from grab samples collected in 2003.

Cruise	Station	Sediment description	
END 4a/03	G4	Muddy gravelly sand	
END 4a/03	G7	Gravelly mud with clay lumps	
END 4a/03	IG1	Thin layer of sand. Overlying thin layer of mud (dark grey).	
END 4a/03	IG2	Slightly gravelly mud	
END 4a/03	IG3	Mud over muddy gravelly sand	
END 4a/03	IG4	Sandy gravelly mud with clay lumps	
END 4a/03	IG5	Muddy gravel with lumps of light brown clay	
END 4a/03	IG6	Soft dark grey mud with streaks of black mud. Terrestrial clay present	
END 4a/03	IGS1	Muddy gravelly sand	
END 4a/03	IGS2	Muddy, gravelly sand	
END 4a/03	IGS3	Gravelly, very muddy sand	
END 4a/03	IGS4	soft mud, anoxic at depth	
END 4a/03	IGS5	soft mud over gravelly mud	
END 4a/03	IGS7	muddy sandy gravel with clay lumps	
END 4a/03	IGS7	muddy sandy gravel with clay lumps	
END 4a/03	IGS8	muddy sandy gravel over anoxic sandy mud	
END 4a/03	ISG6	muddy gravelly sand	
END 4a/03	ISG6	gravelly muddy sand with lumps of hard clay	

Figure 18.7. Sidescan sonar image from 2003 showing the tracks on the seabed made by beam trawlers.

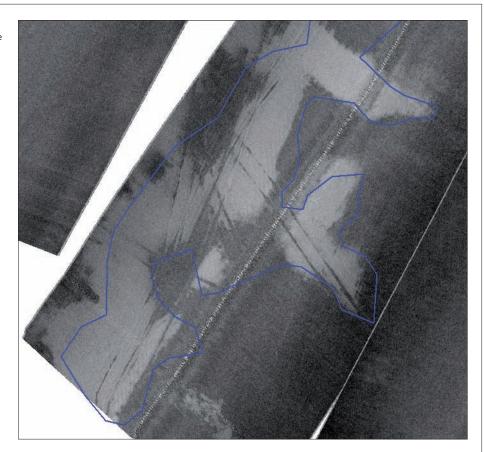


Table 18.5. Sediment descriptions from grab samples collected in 2004.

Cruise	Station	Sediment description
END 6/04	—— G1	Very gravelly muddy sand
END 6/04	G2	Muddy gravelly sand
END 6/04	G3	Gravelly muddy sand
END 6/04	G4	Muddy gravelly sand with Sabellaria
END 6/04	G5	Clean gravelly sand
END 6/04	G6	Soft mud and clay
END 6/04	G7	Muddy sand over consolidated clay, Sabellaria reef
END 6/04	G8	Gravelly muddy shelly sand
END 6/04	G9	Gravelly muddy sand with Sabellaria
END 6/04	G10	Sandy slightly shelly mud
END 6/04	G11	Slightly muddy slightly gravelly sand
END 6/04	G12	Muddy gravelly sand

Figure 18.8. Sidescan sonar mosaic of the Inner Gabbard site and the surrounding seabed in 2004. The red dots show the location of ground-truth grab samples.

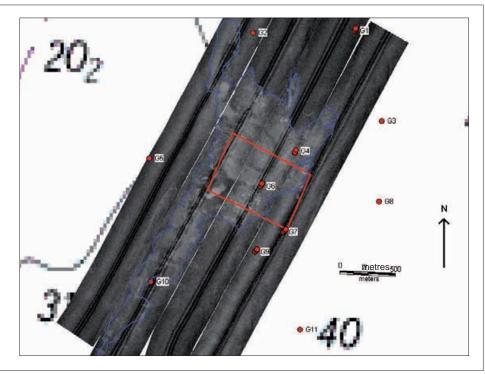




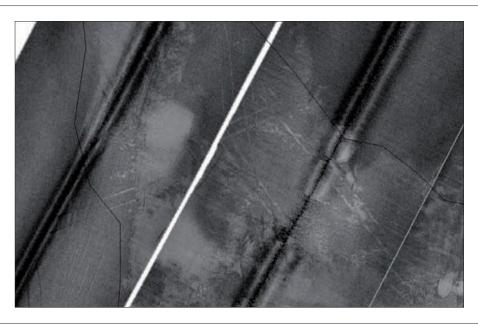
Figure 18.9. Sabellaria reef collected from G7 in 2004, using a Shipek grab.

Beam trawl tracks can be seen on the seabed to the north and south of the disposal site. However, whilst some of the tracks appear to have penetrated the soft muddy sediments at the surface, others tracks which were formed on coarser substrates seem to be in-filled with soft sediments (Figure 18.10).

#### 18.5 Discussion

Ground-truthed sidescan sonar data have shown that fine sediments are present in a discrete area within and surrounding the Inner Gabbard site which appears to be largely attributable to deposited dredged material. The fine material was less in evidence in 2002, and this may be due to the periodic transport of the material away from the site by tides and waves. However, fine sediments

Figure 18.10. Beam trawl tracks on the seabed to the north of the disposal site in 2004. The lighter tracks appear to be infilled with soft sediments.



were again widespread in 2003 and 2004 suggesting that the site accumulates this material, at least over relatively short timescales. Trawl tracks have penetrated the fine material in a number of places suggesting that the coverage is relatively thin in these locations. The Inner Gabbard disposal site was chosen to be dispersive in nature and the results of this work suggest that this is largely the case.

Multibeam bathymetry surveys conducted over the site may shed further light on the longer term potential for accumulation of material in the area. These data will be reported subsequently. Furthermore, sampling for the benthos and chemical contaminant analysis has been conducted by Cefas approximately annually at the site since 1998 and these data are also being worked up. This information will also be reported subsequently and should help to further validate the acoustic data. It will also provide a fuller picture of the biological and physiochemical status of the site.

# 19. Maintenance dredged material for habitat restoration: furthering our understanding of invertebrate recolonisation processes

#### 19.1 Introduction

The disposal of maintenance dredged material constitutes one of the most important problems in coastal zone management (OSPAR, 1998; Bolam et al., in press). Furthermore, since ocean disposal of industrial waste and sewage sludge has been phased out, there is greater focus on behalf of concerned citizens, the media and legislative bodies on dredged material disposal (Vogt and Walls, 1991). This has resulted in a greater emphasis on the relocation of fine-grained maintenance dredged material in such a way as to derive environmental benefits (Murray, 1994; Bolam et al., 2003). As a result, a number of 'beneficial use' options have developed whereby the material is regarded as a potential resource and used to recharge or recreate intertidal habitats. In the USA, dredged material has been used successfully to create new mudflats (Ray, 2000) and saltmarshes (LaSalle et al., 1991; Streever, 2000) which ultimately function like natural systems. In the UK, concerns over the eventual fate of the material and the ecological consequences of placing fine-grained material onto intertidal habitats have limited this practice to smallscale field trials. Currently, less than 1% of the 40 - 50 million m<sup>3</sup> of dredged material produced annually in the UK is used beneficially (Bolam et al., 2003).

Over recent years, the large number of studies investigating invertebrate recovery following a number of intertidal disturbances has resulted in a good understanding of the potential invertebrate recovery rates and mechanisms (Evans et al., 1998; Beukema et al., 1999; Bolam and Fernandes, 2002; Bolam et al., 2002, 2004; Schratzberger et al., 2004a). In general, recolonisation of some species can be rapid, although this depends on the spatial scale and timing of the disturbance, together with the life history characteristics of the recolonising fauna. However, apart from a small number of monitoring studies of variable quality, there have been very few studies investigating the macrofaunal recovery of fine-grained beneficial use schemes in the UK (Atkinson et al., 2001; Bolam and Whomersley, 2003, 2005). Furthermore, there have, as yet, been no published studies regarding meiofauna in this respect. This, together with concerns over the eventual fate of the material, presents a major barrier to the large-scale use of dredged material for habitat creation/ improvement for the foreseeable future.

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When dredged material is placed onto an intertidal mudflat, the resident invertebrates are smothered and recovery occurs via a combination of adult/juvenile settlement and lateral and/or vertical migration (Bolam, 2003; Bolam et al., 2004; Schratzberger et al., 2004b). Which mechanism predominates in any instance depends upon the timing, rate, depth and spatial scale of the recharge, together with changes in the properties of the sediment itself. Here we present the initial findings of a research projectwhich investigated the physical and biological recovery processes of a large-scale intertidal placement of maintenance dredged material. This offered a unique opportunity to further our understanding of the factors contributing to a successful outcome as the placement involved much larger quantities of material than previously conducted in the UK, and the material was placed in different recharge areas, each differing in physical characteristics (primarily tidal height and wave exposure). The early results from ecological modelling of macrofaunal changes with physical and physico-chemical variables are also presented.

#### 19.2 Material and methods

#### 19.2.1 Study area

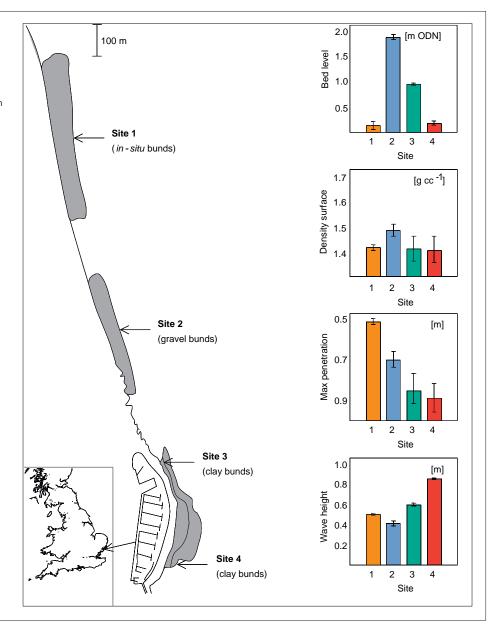
The study area was located on the Orwell Estuary, close to its confluence with the Stour Estuary, on the southeast coast of England (Figure 19.1). The recharge was carried out in phases and in distinct areas. All study sites were recharged in September 2003 with uncontaminated finegrained material from maintenance dredging at Harwich harbour. Each area of placement had a retaining bund or bunds, constructed to help retain the pumped muddy material. The bunds consisted of either *in-situ* bed material (site 1), existing gravelly material (site 2) or clay material arising from a capital dredging operation (sites 3 and 4).

#### 19.2.2. Data and sample collection

The collection of biological samples started one week after the dredged material recharge was completed and was repeated at month 1 (October 2003), month 3 (December 2003), month 6 (March 2004) and month 12 (September 2004) post recharge.

On every sampling occasion, five macrofaunal (0.01 m<sup>2</sup>, 15 cm depth) and three meiofaunal (0.027 m<sup>2</sup>, 5 cm depth) replicate samples were collected at each of the four

Figure 19.1. Location of the study site on the east coast of the United Kingdom. Schematic diagram of the position of the four recharge areas and spatial differences in selected mean  $(\pm SE, n = 3)$  environmental variables averaged over the 12-months study period. These are related to the elevation (bed level), density (density surface), consolidation (max. penetration) and exposure (wave height) of recharged material.



recharge areas. The samples were then fixed using buffered formalin solution (10%) with a 0.01% Rose Bengal stain and stored. In the laboratory, the macrofaunal samples were washed over a 500 µm mesh sieve, the invertebrates were sorted under a dissecting microscope, identified to the lowest possible taxonomic resolution and counted. The meiofaunal samples were first decanted five times onto a 63 µm sieve, then the fauna was extracted from the sediment with Ludox<sup>TM</sup> 40 following the method described in Somerfield and Warwick (1994). The extraction was repeated three times. Sub-samples of 5% of the extracted material were evaporated slowly in anhydrous glycerol and mounted evenly spread on slides for identification and counting. Nematodes, comprising >90% of total meiofauna abundance, were identified to genus or species. One percent of all nematodes in the samples collected at the four recharge sites were randomly selected for the determination of individual length (except long, filiform tails, where present) and width using Image Analysis (Image Pro Plus v 4.5).

For each sampling point, a number of non-biological variables were calculated either from direct *in-situ* measurements or derived from hydrodynamic models. These include tidal flow, bed shear stress, bed density, sediment particle size distribution and redox potential, topography and elevation, water depth and wave characteristics.

#### 19.2.3 Invertebrate infauna

Non-metric multi-dimensional scaling (MDS) using the Bray-Curtis similarity measure based on untransformed (meiofauna) or root-transformed (macrofauna) abundance data was conducted and 2-dimensional ordination plots produced. In such plots, points (samples) close to each other have more similar biological communities than those further away. Two-way crossed analysis of similarities (ANOSIM) tests were carried out (meiofauna only) to test the significance of spatial (ie four sampling sites averaged over all sampling occasions) and temporal (ie five sampling

occasions averaged over all sampling sites) differences in faunal communities.

The nature of the community groupings identified in the MDS ordination was explored further by applying the similarity percentages program (SIMPER) to determine the contribution of individual species to the average dissimilarity between samples. All multivariate analyses were carried out using PRIMER version  $6\beta$  (Clarke and Warwick, 1994).

#### 19.2.4 Modelling

The design of the study is such that it maximizes the amount of variation in the observed biotic and abiotic attributes, ie, a number of recharge sites have been sampled covering a broad range of physical conditions, the repeated sampling giving good temporal resolution. The purpose of the ecological modelling is to explore the relationships between the measured biotic and abiotic parameters so as to define possible gradients in community response both in space and time, and to explore specific species responses to a range of environmental attributes.

Defining quantitative relationships between the numerous variables and cases requires a multivariate approach. Our initial analysis relies on Canonical Ordination to bring together outputs based on regular ordination and multiple regressions. Canonical ordination techniques (such as Canonical Correspondence Analysis (CCA)) are designed to detect the patterns of variation in the species data that can be explained best by the observed environmental variables. The resulting ordination diagrams not only express a pattern of variation in species but also the main relations between the species and each of the environmental variables. The modelling analyses were conducted using the software package CANOCO.

#### 19.3 Results

#### 19.3.1 Meiofauna

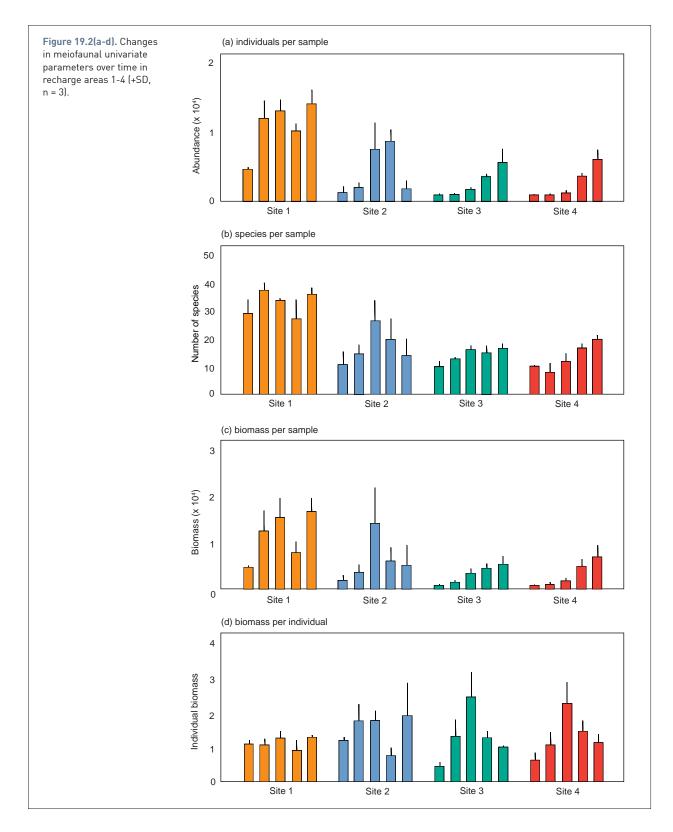
Total nematode (meiofauna) density, number of species and total nematode biomass were all significantly higher at recharge area 1 (Figure 19.2(a - d)). Both density and total biomass increased significantly over time. Nematode density at area 1 tripled within the first month post-recharge whereas at areas 3 and 4, most marked increases were observed in the second half of the study period (ie in the 6 – 12 months post-recharge samples). Total nematode abundance at area 2 peaked 3 - 6 months post-recharge, being significantly reduced after 12 months. Whilst spatial differences in average individual nematode biomass were not significant at the

95% probability level (Figure 19.2(d)), the number of larger specimens was higher in December 2003 (month 3) than at any other sampling occasion. Table 19.1 indicates that these univariate parameters changed significantly over time (except number of species) and spatially between recharge areas (except individual biomass).

The actual composition of nematode assemblages mirrored the spatial and temporal changes in univariate community attributes. The MDS plot reveals that the meiofaunal communities of recharge areas 3 and 4 were structurally similar at each sampling period. The meiofaunal community found at area 1, on the left of the ordination, had the smallest temporal variation but is clearly separated from the others. The community of recharge area 2 exhibited great temporal variability, however, after 12 months, its structure was similar to that after 1 month post-recharge. Results from the two-way ANOSIM test revealed that significant differences in community structure existed both between areas (R = 0.55, p < 0.01) and months (R = 0.43, p < 0.01). Nematode assemblages differed significantly between all sites, except areas 3 and 4, and all sampling occasions except within the first month of the study.

Results from multivariate analyses revealed a statistically significant impact of factors related to the elevation (ie bed level: rs = 0.155, p = 0.003; average water depth: rs = 0.108, p = 0.020), density (ie bed density in 0 - 10 cm depth: rs = 0.230, p = 0.001), consolidation (ie maximum penetration depth: rs = 0.239, p = 0.001) and exposure (ie significant wave height: rs = 0.152, p = 0.002) of bed material on the spatial distribution patterns of nematode species.

Daptonema exhibited a species-specific response to the elevation of the bed material. The relative abundance of Daptonema hirsutum decreased with increasing elevation and decreasing average water depth. Conversely, high proportions of Daptonema oxycerca were characteristic of high bed levels at site 2. In contrast to Daptonema oxycerca, the dominance of Terschellingia longicaudata increased in stable, consolidated sediments. Spatial differences in the trophic structure of nematode assemblages were primarily exerted via changes in the dominance patterns of selective deposit feeders versus non-selective deposit and epigrowth feeders. The decrease of trophic diversity with increased wave height was primarily related to the decline of selective deposit feeders (eg Terschellingia longicaudata) and predators. Non-selective deposit feeders (eq D. hirsutum) and epigrowth feeders (eq Chromadora macrolaima) were abundant at wave-dominated locations such as site 3 and 4.



**Table 19.1.** Results of the Kruskal-Wallis one-way analysis of variance by ranks for spatial and temporal differences of univariate community attributes.

	Recharge area		Time		
	T	р	T	р	
Total abundance [sample <sup>-1</sup> ]	25.65	< 0.01	13.03	0.01	
Number of species [sample-1]	25.65	< 0.01	4.70	0.32	
Total biomass [µg ww sample-1]	19.37	< 0.01	12.31	0.01	
Individual biomass [µg ww individual-1]	0.90	0.83	15.27	< 0.01	

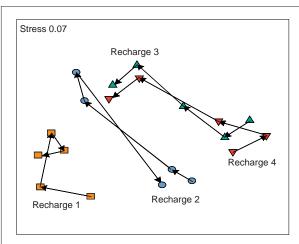


Figure 19.3. Non-metric multidimensional scaling (MDS) plot of untransformed nematode data for areas 1 – 4 from 1 week to 12 months post-recharge (replicates averaged).

#### 19.3.2. Macrofauna

The total numbers of macrofaunal individuals, species and diversity at each area for the first 12 months are shown in Figure 19.4(a - c). These results indicate that there were clear differences in the numbers of macrofauna recolonising these areas, even after 1 week: high abundances were sampled at area 1, moderate numbers at area 2, while very few individuals were found at areas 3 and 4. Between 1 week and 6 months, slow increases were observed in areas 3 and 4 while abundances remained relatively stable at area 2. After 12 months, there were dramatic increases in abundances in the macrofauna in areas 3 and 4, and to a lesser extent at area 1. This was not apparent at area 2 where numbers remained approximately 40 – 50 per core.

The mean number of species per core was consistently higher at area 1 until 12 months post-recharge when the large increases in abundance at areas 3 and 4 also resulted in large increases in species numbers (mean = 12 and 16 for areas 3 and 4, respectively). Numbers of species per core remained continually low at recharge area 2 (3 – 4 per core). There were less obvious differences in the diversity values between the recharge areas over the first 12 month: although area 1 had the greatest diversity, areas 3 and 4 attained comparable levels 12 months post-recharge, while diversity decreased at this time.

Figure 19.5 shows the ordination plot produced from all 4 recharge areas over time (replicates averaged). The plot indicates that areas 1 and 2 are more similar to each other than they are to 3 and 4 (at least in the early stages). Areas 1 and 2 have shown a relatively small degree of

temporal variability, while that of 3 and 4 is large, moving from the left (very different community structures from 1 and 2) to the right after 12 months, resulting in them having communities rather similar to area 1. Noticeably, areas 3 and 4 have undergone very similar temporal shifts and have continually had similar communities as was seen for the meiofauna

Table 19.2 reveals the most influential taxa in characterising each of the 4 recharge areas based on the 12 months post-recharge communities. A large spatial (replicate) variability at recharge area 2 is evident, with an average within-group similarity of 7.2% compared to the within-group similarities > 50% for each of the other 3 areas. Another important feature is that for each of the areas (less so for area 2), a significant proportion of the area's identity can be explained by a single species, and > 70% can be explained by 2 species (except for area 2, 57% is explained by 2 species). The tube-building, surface deposit-feeding worm Streblospio shrubsolii is an important discriminating species for areas 1, 3 and 4, being found at consistently high abundances throughout the replicates in these areas. Species which are typical of high intertidal areas discriminate area 2, notably Diptera and Chironomidae, both insect larvae.

# 19.3.3 Ecological modelling – initial results (recharge area 1)

Figure 19.6 reveals that following Principal Components Analysis (PCA), the 1st principal axis maximizing the community variation between samples can largely be explained by the number of hours exposed during the tidal cycle. This is obviously related to elevation (mODN) in the tidal frame. Variation along the second order axis is more difficult to explain, but significant wave height (Hs) accounts for some of the biological variation observed.

Taking this observation further and investigating the specific relationship between hours of tidal exposure and selected species responses, we see a good fitting response model emerge. For example, in Figure 19.7 the density of *Hydrobia* sp., a gastropod that grazes on unicellular algae, is shown to vary along the first ordination axis (hours of tidal exposure) and that this can be explicitly defined as a quadratic response curve for a range of taxa as shown in Figure 19.8.

Figure 19.8 indicates that *Hediste* sp. and *Hydrobia* sp. occur in greatest densities in areas of the shore that are exposed for between 6 and 10 hours (covered for less than 6 hours) whereas *Cossura* sp. occurs in greatest densities when exposed for less than 4 hours (or conversely covered between 6 – 10 hrs). Cirratulid worms (*Aphelocheata* sp.

Figure 19.4(a-c). Mean macrofaunal individuals, species and Shannon-Wiener diversity per core (+/-SE, n = 5) for recharge areas 1-4 from 1 week to 12 months post-recharge.

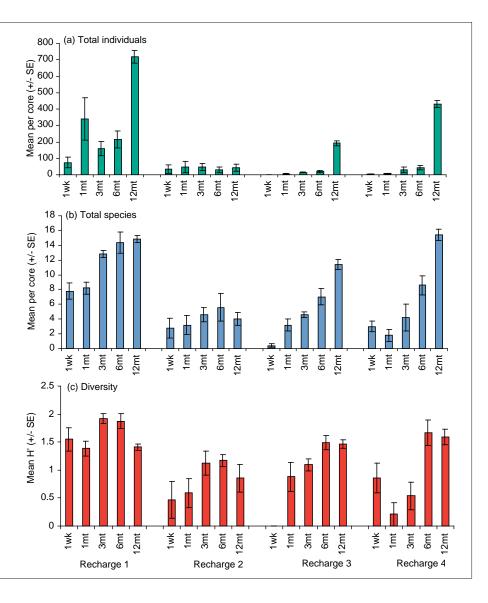
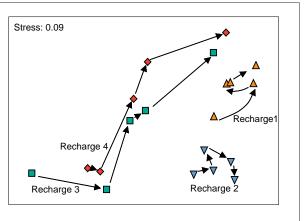
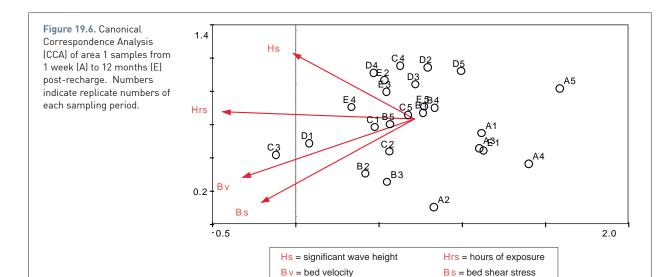


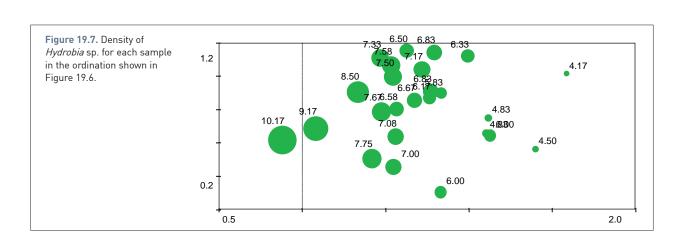
Figure 19.5. Non-metric Multi-Dimensional Scaling of macrofaunal data based on a Bray-Curtis similarity matrix with root-transformed data. Arrows indicate temporal changes from 1 week to 12 months post-recharge.



**Table 19.2.** The most influential taxa (and their % contribution) characterising each recharge area 12 months post-recharge. The average similarity of samples within each area is also given.

Recharge 1 (71.7%)		Recharge 2 (7.	2%)	Recharge 3 (54.9%)		Recharge 4 (69.9%)	
Streblospio shrubsolii	53.3%	Hydrobia ulvae	33.5%	Streblospio shrubsolii	55.2%	Streblospio shrubsolii	49.1%
Tubificoides benedii	33.6%	DIPTERA	24.1%	Pygospio elegans	27.9%	Pygospio elegans	21.9%
Pygospio elegans	4.5%	Chironomidae	23.1%	Hydrobia ulvae	5.6%	Paranais litoralis	13.4%





and *Tharyx* sp.), as well as *Nephtys* sp., show unimodal responses demonstrating increased densities at specific positions in the tidal frame.

#### 19.4 Discussion

The sampling of macro- and meiofaunal communities from four distinct recharge areas (yet recharged concurrently with the same material) within a mudflat system over a 12-month period, accompanied by parallel determination of a wide range of physical and sedimentological variables, has enabled us to evaluate temporal changes in colonist communities and their potential causes. Our study demonstrated that species-rich and diverse assemblages can be established on mudflats which had been enhanced using dredged material. Following the recharge of dredged material, the original invertebrate populations would have been smothered and recolonisation proceeded from the sediment surface, either by planktonic recruitment (macrofauna), settlement of resuspended adults or

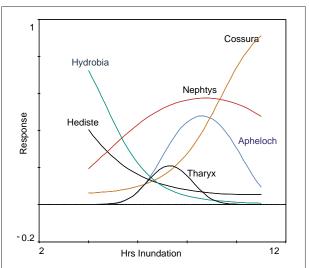


Figure 19.8. Selected species response curves showing the relationship between species density and tidal inundation (exposure) for recharge area 1 throughout the study. Response curves are fitted quadratic functions.

juveniles from the watercolumn (meiofauna), or active adult immigration (macro- and meiofauna). The rapid recolonisation of all replenished sites can be attributed partly to the resilience of mudflat communities, the relatively small scale of each recharge scheme and the rapid de-watering afforded by the bunds used to retain the dredged material (Bolam and Whomersley, 2003, 2005).

The modelling of the physical variables with biological characteristics has enabled a further understanding of the factors affecting biological recolonisation. The results thus far, for recharge area 1, have indicated that the most important factor (in the first 12 months) is tidal immersion. For the other recharge areas, we have found larger temporal variability (eg Figure 19.5), this indicates that factors other than tidal immersion (which is relatively temporally constant) are likely to be more important influential factors for the biology. In this way, different models are likely to be appropriate for the different recharge areas (although the two wave-exposed sites, 3 and 4, are quite likely

to be responding to similar factors). Consequently, we hope to gain an improved understanding of the most important factors affecting biological recolonisation under different circumstances. The response models derived from temporal data at area 1 can be used to predict responses of the same species at the other sites, both in space and time. The response models can therefore be tested, improved and validated, making them suitable estimates of biological response for future recharge events where these species occur. Further analysis will involve using CCA on updated environmental and macrofaunal data, as it becomes necessary, on each recharge area over time (as for area 1 presented here), across all recharge areas at each time, and across all areas and sampling times. We also aim to treat the meiofaunal data with the same rigorous analysis as for the macrofauna. In this way, we aim to establish meaningful relationships between biotic and abiotic variables which can be applied to other beneficial use schemes.

# 20. Monitoring polycyclic aromatic hydrocarbons in sediments from the Rame Head dredged material disposal site

# Authors: Carole Kelly, Kerry Baker and Stephanie Rowland

#### 20.1 Introduction

Rame Head has been used for many years as a disposal site for dredged material from the River Tamar, Plymouth Sound, docks and areas associated with the Port of Plymouth. Local concerns and consequent media interest (particularly from the Western Morning News of Friday 22 October 2004) have implicated Rame Head as a possible source of contamination in nearby Whitsand Bay. Cefas has conducted surveys in this area since 2001, with the results of benthic, acoustic and camera surveys reported previously (Cefas, 2005). Analysis of chemical contaminants in sediments from the disposal ground and surrounding area were also carried out and this report deals with the results from the polycyclic aromatic hydrocarbon (PAH) survey.

PAH enter the environment from both oil and combustion sources. Combustion sources include the burning of fossil fuels, road run-off and the discharge of industrial and sewage effluents. Inputs from oil sources derive from shipping activities, operational and accidental discharges during the use, transport and disposal of oil and its products. PAH are known to have both acute and chronic toxicity to organisms and many of the larger PAH can form carcinogenic metabolites.

#### 20.2 Methods

The sediment samples were collected using a Day grab or, for those sites close to the shore, a hand held van Veen grab. Samples, collected in glass jars, were frozen immediately after collection and not defrosted until required for analysis.

Each homogenised wet sediment sample was extracted using alkaline saponification followed by liquid/liquid extraction. The sample extract was then passed through an alumina chromatography column in order to remove polar compounds, concentrated to 1 ml and sealed in a vial. A suite of alkylated and parent PAH were then determined using coupled gas chromatography-mass spectrometry (GC/MS). Quantification was by means of deuterated internal standards added prior to digestion, with analytical quality control samples being run within each sample batch. Full details can be found in Kelly *et al.* (2000).

#### 20.3 Results

Comparisons of the PAH contaminant concentrations and profiles were made between samples from the disposal site, the point of dredging and the surrounding area. The main sources of PAH in these samples were investigated by apportionment of the PAH compounds to oil or combustion categories in relation to their major sources (Law *et al.*, 1999).

Figure 20.1 shows the sum of PAH at the Rame Head site along with the Cefas station codes.

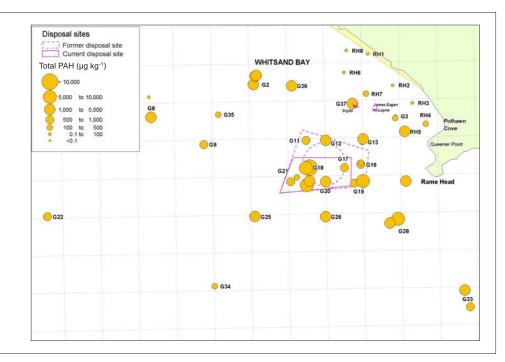
Total (summed) PAH concentrations in the dredged sediments were, in the range of 5,000-30,000  $\mu g~kg^{-1}$  dry weight. These PAH were found to be predominately combustion derived.

The levels of PAH found at the disposal site itself were lower, in the range 400-6,400  $\mu g \ kg^{-1}$  dry weight and, although oil has occasionally been observed in samples taken at this site (station G18 in 2001), again these PAH were predominately combustion derived. The only other site in this survey where oily samples have been collected is at station G37, which is located close to the Scylla reef and so the presence of oil may be as a result of dive or fishing boat activity in this area.

PAH concentrations outside the disposal site were generally lower although with occasional high values. Station G28, for example, showed an elevated total PAH concentration of  $5,500 \mu g kg^{-1} dry weight$ .

The total PAH concentrations at stations close to the beach were much lower, ranging from 10-154 µg kg<sup>-1</sup> dry weight. However, the concentration at station RH4 was higher than would usually be expected for sandy sediments (Woodhead *et al.*, 1999). The same is true for samples taken slightly to the west, with station RH5 (also a sandy sediment) having a total PAH concentration of 2,218 µg kg<sup>-1</sup>. Higher concentrations in sandy sediments could be attributed to enrichment of the organic carbon content, for example as a result of discharges of untreated or primary treated sewage. The Environment Agency discharge locations database shows that in 2004 there were 2 untreated sewage discharges and 2 emergency storm water sewage overflows within Whitsand Bay.

Figure 20.1. ∑PAH at the Rame Head site.



#### 20.4 Discussion

The results show that the PAH profile in samples within the area studied was predominately combustion-derived, which might suggest a more generic ie, urban and industrial source of contamination rather than one related to shipping activities alone.

The source of the PAH contamination in the area cannot yet be discriminated. To further investigate

these preliminary findings additional sediment samples are being collected during 2005. Our intention is to better define the extent of the PAH contamination in this area, and 'track' the contamination and the sources of PAH. We aim to achieve this by comparing the PAH profiles in sediments from Hamoaze (in the lower River Tamar off Devonport – an area which is dredged to maintain navigation) to Looe, Plymouth Sound, the Rame Head disposal ground and Whitsand Bay.

# 21. Seabed indicators derived from acoustic outputs: review and forward look

This review and forward look provides an evaluation of the hope for further derivation of indicators measures from various acoustic systems currently available. Particular attention is paid to potential applications at dredged material disposal and aggregate extraction sites. Summaries of the main categories of acoustic techniques are, in each case, accompanied by an assessment of their indicator potential, by evaluations against agreed criteria governing indicator utility (Sneddon *et al.*, Chapter 14 in this volume; Suzanne Ware, personal communication).

#### 21.1 Introduction

Acoustic surveying offers the advantage of collecting information on the seabed remotely and covering large areas in a relatively short time. Acoustic survey techniques have been in use since early in the 20th Century. After World War I the first single beam echosounders were developed and during World War II sidescan sonar systems were used for military purposes. In the early 1970s, multibeam echosounders were introduced to provide bathymetric data from a swath along the seabed (NOAA, 2005a)

Three main groups of acoustic systems exist to collect data from the seabed surface. These are acoustic ground discrimination systems (AGDS), sidescan sonars and multibeam echosounders. A fourth group of acoustic systems discussed in this report are sub-bottom profilers. These latter only provide information on the structure of the subsurface of the sediment. A review of these acoustic survey systems is presented in the document: an introduction to the operating principle is given for each technique, followed by a discussion of their relevance and future potential in the derivation of quantitative environmental acoustic indicators. None of these methods was specifically used or tested in field experiments for indicator purposes. Further research into the relevance and potential of acoustic indicators is ongoing.

Acoustic methods are primarily suited to study the physical impacts on the seabed and their effects on seabed nature, seabed morphology and seabed bathymetry. Although these factors can currently not be directly related to the status of the seabed environment (polluted or unpolluted) they could potentially identify areas of change and most significant change, level of disturbance, dissimilarity from the surrounding environment, etc. Unlike biological indices this is a fairly new field of research and will require further development to derive the most suitable acoustic indicators.

#### Author: Koen Vanstaen

# 21.1.2 Single beam Acoustic Ground Discrimination Systems (AGDS)

#### Principle

Since the development of single beam echosounders in 1919, they have been the primary instrument to produce and update bathymetric maps for safe navigation of rivers and seas. The scientific community recognised the value to their work early on and hence echosounders played a significant role in scientific discoveries (eg of the Mid-Atlantic Ridge Rift Valley in 1959) and applications. Initially they provided only depth measurements along the track of the ship, but technological advances led to the development of Acoustic Ground Discrimination Systems (AGDS) providing information on the nature of the seabed.

Single beam echosounders emit an acoustic beam to measure the distance between the transducer and the seabed. This acoustic beam has a conical shape and is downward orientated. Signal processing and analysis of the acoustic return allows a study of the nature of the seabed. The strength of acoustic echo returned by the seabed and the way in which the acoustic signal decays with time depends on the nature of the seabed (Foster-Smith, 2005). Therefore, analysis of the acoustic return can be used to distinguish between different seabed types. Hard surfaces will return a strong acoustic echo, whereas a softer seabed will return a weak acoustic signal (Bates et al., 2001).

RoxAnn™, QTC VIEW™ and Echo Plus are the three major commercially available AGDS systems. The theory behind these systems can be found in (Chivers et al., 1990, Burns et al., 1989, Collins, 1996). These systems have proven their success in various applications such as benthic habitats, fish habitats, shellfish habitats and seabed type mapping (Foster-Smith et al., 2004, Kloser et al., 2001, Morrison et al., 2001, Davies et al., 1997, Preston et al., 1999).

Interpretation of the AGDS data requires ground-truthing to characterise the nature of the acoustic regions. Without the collection of seabed samples it is unrealistic to achieve reliable seabed maps using AGDS.

#### Relevance as an acoustic indicator

The numerical values recorded by an AGDS and related to the seabed nature are expected to be the most suitable output from the AGDS for use as an acoustic indicator. The acoustic parameters are influenced by changes in seabed hardness and roughness and AGDS can therefore be an indicator for the impact of those human activities changing the physical properties of the seabed. For example, marine

aggregate extraction leaves an impacted seabed with increased roughness, whereas dredged material disposal may result in softer sediments being present at the seabed surface.

Although the acoustic parameters recorded by an AGDS may be suitable acoustic indicators, some limitations are inherent to the systems tempering the suitability of these parameters. A major disadvantage of the system is the output of uncalibrated values. This prevents the comparison of the actual acoustic values recorded by the system, but still allows the study of relative changes in acoustic parameters. Furthermore, the acoustic parameters are known to vary with the environmental conditions prevailing during the acoustic survey, eg tidal current, concentration of suspended sediment, survey speed. Calibration procedures could be adopted but it is not known how well they perform when comparing results from different surveys and instruments.

The interpolated and interpreted maps which are often the result of AGDS surveys may be more suitable to monitor or map the area impacted by human activities, but these often require a significant input of subjectivity and therefore no longer represent an ideal acoustic indicator.

## Relatively easy to understand by non-scientists and other users

The numerical values recorded by Acoustic Ground Discrimination Systems (AGDS) cannot be interpreted without ground-truth samples. Once calibrated for a specific survey area, it is possible to map the distribution of the seabed sediments. Therefore the raw outputs from these systems are hard to understand for both non-scientists and other users.

# Sensitive and relevant to a manageable human activity

Human activities are likely to impact the physical nature of the seabed. Especially for those activities changing the hardness and roughness of the seabed, AGDS could provide an acoustic indicator related to the change or disturbance at a site.

# Tightly linked to the human activity but not to other causes of change

AGDS are expected to be sensitive to the impacts resulting from human activities. However, as discussed above, changes in the AGDS output are sensitive to prevailing conditions during survey. This will have an impact on the strength of the returned echo defining the acoustic

parameters. As a result it will not be possible to study changes in actual acoustic parameters over time.

#### Easily and accurately measured, with a low error rate

Acquiring AGDS data is easy, does not require expensive equipment and can be easily deployed from small vessels of opportunity. There is no extensive calibration required, reducing the mobilisation time and complexity of the operation.

Although the accuracy of the depth measurements can be assessed easily, it is more difficult to assess the accuracy of the acoustic parameters. This is possible by comparing the data values from different surveys for the same area or looking at the consistency of the acoustic parameters within a classified area. However, this may be influenced by changes in survey conditions. Therefore Foster-Smith and Sotheran (2003) concluded that poor AGDS accuracies should not necessarily imply that AGDS techniques have failed.

Although AGDS are probably the easiest way to acquire acoustic survey data, the low accuracy and the lack of a method to assess the accuracy of the data are a major disadvantage to the use of AGDS systems as a means to derive reliable environmental indicators.

# Affordable and feasible in terms of data collection and manipulation

The basic nature of the system makes it one of the most affordable seabed surveying systems around. Little more than a vessel with DGPS, an echosounder, a processing unit and computer are required to carry out an AGDS survey. The data produced by the systems can be analysed by commonly available software (eg Microsoft Excel™) and more advanced software such as ERDAS Imagine™ and IDRISI™, as well as GIS systems. Therefore this makes AGDS systems a very affordable and feasible tool with which to derive acoustic indicators.

#### Provide early warning

AGDS systems can provide warning when physical changes detectable by the system specifications have occurred in an area. Assuming that small changes will impact the acoustic parameters provided by AGDS, comparing these values from successive surveys could provide early warning of temporal changes at a site. However, as indicated above, the variability of the acoustic parameters with survey conditions, obscuring the cause of the change, may limit the effectiveness of the acoustic parameters as indicators

#### 21.2 Sidescan sonar

#### Principle

A sidescan sonar is a survey instrument that provides an image of the seabed using acoustic waves. A sidescan sonar fish is towed behind a vessel, close to the seabed (Figure 21.1). The low grazing angles allow the detection of small features on the seabed. The sidescan towfish will send out an acoustic pulse at either side of the towfish. These acoustic beams are narrow in a horizontal plane and wide in the vertical plane. This allows the system to insonify and gather information of a narrow stretch of the seabed in the horizontal plane but over a wide distance from the towfish. This provides a wide swathe of seabed information, the extent of which is related to the frequency of the acoustic pulse.

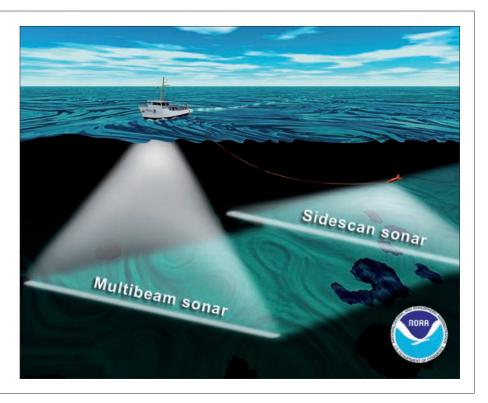
Sidescan sonar systems are available at a variety of acoustic frequencies. For shallow water operations they generally vary between 100 and 500 kHz. Low frequency systems will be able to achieve longer ranges and thus cover larger areas of the seabed. However, to achieve longer ranges the pulse length will have to be longer and therefore the resolution will decrease (Table 21.1).

The interaction at the seabed between the acoustic pulse and the seabed surface will influence the strength and character of the returned acoustic pulse detected by the sidescan receivers. Hard reflectors such as rock outcrops can be differentiated easily from soft muddy seabeds which will only return a weak acoustic return. In addition to the intensity of the returned signal, the texture of the backscatter image can provide additional information to aid the interpretation of the seabed nature.

**Table 21.1.** Relation between frequency, wavelength (related to resolution) and range of common sidescan sonar systems (Fish and Carr, 1990).

Frequency	Wavelength	Range
10 kHz	15 cm	10,000 m
100 kHz	1.5 cm	600 m
500 kHz	0.3 cm	150 m
1,000 kHz	0.15 cm	50 m

Figure 21.1. Acoustic surveying using a towed sidescan sonar (NOAA, 2005b). http://oceanexplorer. noaa.gov/explorations/ 04fire/background/hirez/ multi\_sonar\_hires.jpg



Some care needs to be taken when interpreting sidescan sonar imagery. Although high backscatter intensities are generally related to the seabed roughness, higher backscatter values can also be the result of a seabed slope facing towards the sidescan fish (Figure 21.2). In addition, since the sidescan sonar is towed close to the seabed, acoustic shadows can be created behind obstacles. Acoustic shadows are represented on the sonar record as a weak acoustic return and can therefore be confused with a soft, muddy seabed. For this reason it is advisable for sidescan sonar records to be interpreted by experienced users.

#### Relevance as indicator

A sidescan sonar will measure the strength of the backscatter returned by a swath along the seabed. The backscatter strength is affected by (in decreasing order of importance) (Blondel and Murton, 1997):

- Geometry of sensor-target system (angle of incidence, local slope, etc),
- Physical characteristics of the surface (micro-scale roughness, etc), and
- Intrinsic nature of the surface (composition, density, etc).

Impacts as a result of human activities will influence the above properties and therefore sidescan sonar could be a potential indicator.

The backscatter strength recorded by the system is expressed as an amplitude value. The amplitude is uncalibrated and therefore the backscatter strength may be different during different surveys. Furthermore, system settings such as a different base gain may alter the amplitude values. When using sidescan sonar for comparative purposes this would require calibration of the system for each survey by comparing backscatter amplitudes over the same reference area.

Another characteristic of sidescan sonar, tempering its suitability as an indicator, is the conversion of the backscatter amplitudes to a 256 point grey scale for display purposes. Therefore a sidescan mosaic does not give backscatter amplitude values but only greyscale levels (0 for black, 255 for white). During this process the data range is often reduced from 16-bit to 8-bit (256 data values), losing part of the resolution of the original data, and often the dynamic range is stretched to improve the contrast of the image (histogram equalisation, Figure 21.3). As a result the backscatter values may differ between surveys in the same area as a result of the post-processing corrections.

Figure 21.2. On a homogeneous seabed, shadow zones will be characterised by a weak acoustic backscatter, whereas a strong reflector will produce a high backscatter intensity (Fish and Carr, 1990).

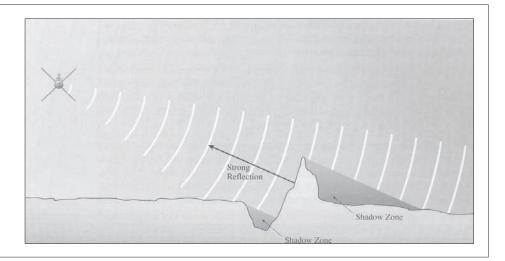
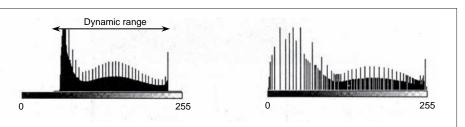


Figure 21.3. Dynamic range of a sidescan sonar backscatter mosaic (left) and histogram after equalisation to improve contrast of the backscatter mosaic (right) (adapted from Blondel and Murton, 1997).



Therefore care should be taken during processing of the data and it may require the production of different backscatter mosaics. For example, one with maximum contrast post-processing settings for mapping purposes and one without corrections specifically for indicator studies. This latter may allow a relative comparison of the backscatter grey scale values.

In addition to comparing the absolute grey scale levels, a potential indicator can be derived from the relative grey scale levels. For example, the heterogeneity of an area can be assessed by textural analysis of the backscatter mosaic.

## Relatively easy to understand by non-scientists and other users

After a sidescan sonar survey, a backscatter mosaic will be produced which provides an image of the seabed. This visual image allows distinctions to be made between different areas by non-scientists and other users. Experienced users can derive information on seabed nature from the backscatter mosaic, although ground-truth samples are required to validate the interpretation.

# Sensitive and relevant to a manageable human activity

Human activities are likely to impact the factors influencing the backscatter amplitude values. Therefore the backscatter of sidescan sonar images will be sensitive to the changes caused by manageable human activities such as aggregate extraction and dredging disposal.

# Tightly linked to the human activity but not to other causes of change

Changes in backscatter amplitude can occur a result of human activities. However, as discussed above, other factors may affect the backscatter amplitude during the survey (seabed slope, gain settings, etc) or during post-processing of the backscatter mosaic. Therefore care should be taken during acquisition and post-processing of the sidescan data.

#### Easily and accurately measured, with a low error rate

Sidescan sonars have been used as survey systems for a long time and are one of the most popular seabed surveying tools. One of the reasons for this is the ease in operating and acquiring the data, not requiring expensive set-ups or complicated calibration procedures. Although easy to measure, it is more difficult to assess the accuracy of the backscatter amplitudes, due to the uncalibrated backscatter values. This might be a disadvantage for sidescan sonars as an indicator tool.

# Affordable and feasible in terms of data collection and manipulation

Sidescan sonars are relatively inexpensive survey instruments and also widely available amongst commercial and non-commercial organisations. The data acquired can be easily processed into mosaics of the seabed surface and can be analysed by experts or using software packages. For use in indicator work, software packages that can analyse the backscatter grey levels will be required. Generally available remote sensing software or GIS are such tools, and therefore make it an affordable and feasible indicator tool.

#### Provide early warning

The smaller the change that the sidescan can detect, the earlier the acoustic indicator can provide warning of changes in the marine environment. However, because of the limitations of the system discussed above, these changes are not necessarily a result of human activities. Therefore absolute greyscale levels may not be a suitable indicator providing early warning of impacts on the marine environment. However, indicators derived from the relative greyscale levels may have future potential a suitable indicator, and will be the subject of future investigation by Cefas.

#### 21.3 Multibeam echosounder

#### Principle

Multibeam echosounders can simultaneously provide seabed bathymetry and sidescan sonar-like backscatter information. This characteristic is unique to multibeam and explains why it has become increasingly the tool of choice for seabed mapping and imaging purposes. The system will send out multiple sonar beams (eg 254 for Cefas' EM3000D multibeam echosounder) in a fan-shaped pattern that is oriented perpendicular to the ship's track (Figure 21.1). The system will detect the bottom echo from the intersection of the 'transmit' and the 'receive' beam footprint. The echo arrival time and angle of the receive beam will provide information on the bathymetry, whereas the backscatter strength is used to construct a seabed image. Combining consecutive pings will allow the creation of a digital terrain model and backscatter mosaic of the surveyed area.

Digital terrain models allow the assessment of changes in seabed topography and morphology, whereas the backscatter mosaic can allow an assessment of changes in seabed nature.

Multibeam echosounders offer a great amount of detail on the bathymetry of a wide area of the seabed, which cannot be achieved using single beam echosounders, nor sidescan sonar. This capability, together with its ability to produce a co-located backscatter image, make multibeam echosounders the tool of choice for many research applications.

Shallow water multibeam systems make use of high frequencies to achieve maximum accuracy. Systems such as Cefas' Kongsberg EM3000D multibeam operate around 300 kHz. This will make the backscatter primarily dependent on the surface scattering, as the signal will only penetrate a small distance into the sediment. The small amount of volume scattering may make it more difficult to distinguish between sediment types. However, this can also been seen as an advantage, as only the top layer will contribute to the volume scattering. Therefore the system may be more sensitive to superficial sediment changes, most relevant to the changes which might occur as a result of human activities.

#### Relevance as indicator

When discussing the relevance of multibeam echosounders as acoustic indicators, two aspects of the system should be discussed: the bathymetry and backscatter component. Both these types of data could provide suitable acoustic indicators on their own, but combined they may have even more potential.

The bathymetry can provide indications of changes in the seabed topography and morphology, whereas the backscatter can be an indicator of changes in the physical characteristics (roughness and nature) of the seabed. Uniquely, multibeam echosounders therefore cover all aspects of physical impacts caused by human activities.

Although the backscatter component of the multibeam system is similar to sidescan sonar, the multibeam backscatter image is often regarded as being of inferior quality. In contrast to the towed sidescan sonar, multibeam echosounders are often hull-mounted (Figure 21.1). This allows sidescan to achieve lower grazing angles and thus better detection of features on the seabed.

However, unlike sidescan sonar, the backscatter strength values from a multibeam echosounder are calibrated during construction of the instrument. Therefore absolute backscatter values are comparable over time and between areas. Calibrated values also allow automated classification methods to perform better. Furthermore, it is possible to use average backscatter strength values per beam for analysis and indicator use, rather than rescaled grey levels. Full resolution backscatter images will also be rescaled to a 256 step grey scale as for sidescan sonar.

The multibeam bathymetry data can be visualised and analysed in various software packages (Fledermaus, Surfer, Idrisi, GIS, etc). This allows the undertaking of advanced tasks such as slope modelling and data re-classification which might be useful to derive acoustic indicators from the data.

## Relatively easy to understand by non-scientists and other users

The bathymetry data from a multibeam system allows the creation of a digital terrain model that can be easily visualised and understood by non-scientists and other users. Advances in technology allow the derivation and visualisation of other parameters such as seabed slope or difference plots from the multibeam data.

In addition to measuring seabed topography, multibeam echosounders record the backscatter strength, which is a proxy of the seabed sediment type. As with sidescan sonar, this allows non-scientists and other users to visualise impacted areas easily.

# Sensitive and relevant to a manageable human activity

Human activities in the marine environment will have an impact on the seabed topography (eg removal of sand and gravel by aggregate extraction; disposal of dredged material) and/or changes in sediment type (eg disposal of fine dredged material). These impacts are within the capabilities to be detected by multibeam echosounders. The outputs from a multibeam system could therefore provide suitable acoustic indicators.

# Tightly linked to the human activity but not to other causes of change

As discussed above, multibeam echosounders are sensitive to the impacts as a result of human activities. The calibrated backscatter values, unique to multibeam systems, remove the uncertainty related to the changes in absolute backscatter levels as a result of human impacts or natural variability. The backscatter values are also corrected (to some extent) for the local seabed slope, reducing the impact on the backscatter strength. This results in data that are closely linked to the human impacts only.

### Easily and accurately measured, with a low error rate

Multibeam echosounders are technologically the most advanced acoustic survey instruments that are able to map the seabed easily and accurately. As an example, the EM3000 system onboard the RV *Cefas Endeavour* can measure depth with a resolution of 1cm and an accuracy

of 5 cm RMS. The backscatter strength measurements have an accuracy of 1 dB. This makes acoustic indicators derived from multibeam echosounders very accurate.

# Affordable and feasible in terms of data collection and manipulation

Multibeam echosounding is becoming a widely available tool for various applications. The experience exists both in research and commercial organisations to undertake multibeam surveying programmes. No major obstructions exist in the collection and manipulation of the acoustic data, allowing their routine collection as a basis for deriving environmental indicators. Compared with other acoustic survey methods, multibeam echosounders will be the most expensive in terms of hardware, but also due to the time needed for installation and calibration of the system.

#### Provide early warning

Multibeam echosounders will be able to detect minor changes in the marine environment due to their accuracy and calibrated backscatter values. These changes will be highlighted when successive surveys are compared with each other using appropriate software packages. Further research is needed to establish the severity of the impact before the acoustic method will identify change.

#### 21.4 Sub-bottom profiler

Sub-bottom profilers provide a 2D slice of the structure of the seabed subsurface along the track of the ship. The frequencies used in sub-bottom profilers are much lower than for echosounders or sidescan sonars and therefore can penetrate the seabed (the lower the frequency, the greater the penetration). The resolution of sub-bottom systems is generally over 10 cm, much lower than some of the impacts as a result of human activities. Different sediments will have different acoustic characteristics, and when the acoustic wave encounters an abrupt change in elastic properties, part of the energy will be reflected and show up on the sub-bottom record.

Recent developments in sub-bottom profiling include non-linear or parametric sub-bottom profilers. These systems simultaneously emit two high frequencies and advanced signal processing is undertaken on the returned signal. This allows calculations of acoustic impedances for the different sediment layers, which are a proxy for sediment type (Table 21.2). An example from a parametric system is given in Figure 21.5.

**Table 21.2.** Acoustic impedance of sediment layers from sub-bottom profiling (U.S. Army Corps of Engineers, 2002).

Material medium	Acoustic impedance (g cm² per sec * 10 <sup>-2</sup> )		
Water	1450		
Silty clay	2016-2460		
Clayey silt	2460-2864		
Silty sand	2864-3052		
Very fine sand	3052-3219		
Fine sand	3219-3281		
Medium sand	3281-3492		
Coarse sand	3492-3647		
Gravelly sand	3647-3880		
Sandy gravel	3880-3927		

All values are corrected for temperature and salinity.

Figure 21.4. Sub-bottom profile showing the structure below the seabed surface (image from http://www.soc.soton.ac.uk/soes/research/groups/geophysics/HRMSG/)

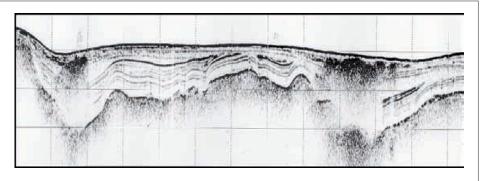
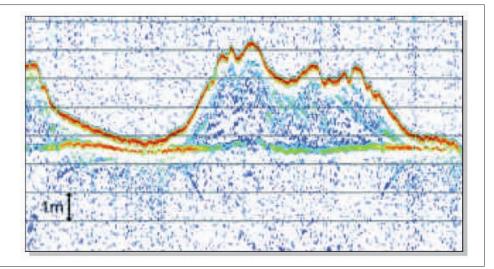


Figure 21.5. Sub-bottom profile from a parametric sub-bottom profiler showing dumped sand on top of hard seabed (Lowag and van den Heuvel, 2002).



Sub-bottom profiles are generally printed on paper for expert interpretation. However, the data can be recorded in a digital format and interpreted digitally in advanced software packages. It is recognised that the outputs from sub-bottom profiling systems are less suited for indicator applications since the main aim is to identify and differentiate between acoustically distinct layers, rather than to characterise them.

Presently, Cefas does not possess in-house capabilities to undertake such work. Further improvements in the precision and accuracy of these systems, along with methods to quantitatively analyse the outputs, may enhance the scope for deriving quantitative indicators. At present we do not consider that a more detailed evaluation is appropriate.

#### 21.5 Case studies

## 21.5.1 Use of calibrated multibeam backscatter strength as a sediment proxy

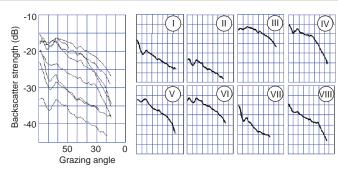
An example of the backscatter capabilities of multibeam echosounders is given by Hughes-Clarke *et al.* (1997). Using a Kongsberg EM1000 (95kHz), angular response curves from the multibeam backscatter were used to distinguish between seabed sediments in the study area (Figure 21.6, Table 21.3).

Table 21.3 lists the sediment types according to grain size. This shows a decrease in backscatter strength with increasing grain size. The example illustrates that multibeam backscatter can be used to distinguish between sediment types. However, no standard deviations were provided and therefore it was unclear whether the sediment classes could be differentiated from each other in a statistically robust way.

**Table 21.3.** Backscatter strength derived from the angular response curves above at an incidence angle of 50 degrees (Derived from Hughes-Clarke *et al.*, 1997).

Sediment facies	Backscatter strength @ 50 degrees grazing angle			
Mud	-38 dB			
Silty mud	-32.5 dB			
Sandy mud	-33 dB			
Silty sand	-27 dB			
Fine sand	-21 dB			
Rippled medium sand, over gravel	-22.5 dB			
Cobble pavement	-19 dB			
Boulder fields	-20 dB			

Figure 21.6. Backscatter strength versus angle of incidence for eight sediment types (Hughes-Clarke *et al.*, 1997).



- I Silty mud
- Mud
- III Cobble pavement
- V Fine sand
- V Rippled medium sand, over gravel
- VI Boulder fields
- VII Sandy mud
- VII Sandy mud

Hughes-Clarke *et al.* (2002) used the multibeam backscatter strengths to monitor the impact of finfish aquaculture cages in Canada. It was concluded that backscatter signatures could indicate changes at the seabed surface as a result of organic enrichment. This illustrates the potential of acoustic indicators in the monitoring of these, and potentially other, human activities.

## 21.5.2 Using calibrated multibeam backscatter strength to monitor changes in sediment type at an aggregate extraction site (Roche *et al.*, 2005)

The Kwintebank is a sandbank on the Belgian Continental Shelf subject to intensive extraction of sand for the construction industry. Multibeam surveys using a Konsgberg EM1002 demonstrated the formation of a depression on the Kwintebank as a result of extraction activities. Since February 2003 an area around this depression was closed for exploitation and has been intensively monitored since. Part of the monitoring is undertaken by collecting multibeam data several times a year. Bathymetry results showed a stabilisation of the depression over time. Monitoring of the multibeam backscatter data demonstrated that no significant changes in seabed nature occurred in the area, which was confirmed by sediment samples.

The success of this method proves the suitability of multibeam backscatter as an acoustic indicator. Although this method was successful in demonstrating the absence of environmental change, it still needs to be demonstrated that it can successfully provide early warning of significant sediment changes.

### 21.5.3 Examples of acoustic indicators derived from Cefas data: preliminary results

An example of an acoustic indicator is given in the context of the physical disturbance of the seabed. A multibeam survey was carried out by Cefas at the Nab Tower dredged material disposal site (East of Isle of Wight) in June 2005. Figure 21.7(a) shows the bathymetry results demonstrating the impact at the seabed surface as a result of the human activities impacting the seabed. The doughnut shaped features on the seabed are a known characteristic of disposal activities. The seabed slope is derived from the seabed bathymetry and is shown in Figure 21.7(b) and allows easy differentiation between areas that are heavily impacted and areas that are less impacted by the disposal activities.

Based on the statistical analysis of the seabed slope surface, unimpacted, moderately and highly impacted areas demonstrated different characteristics. This is illustrated in Figure 21.8 showing the frequency distribution of three 125 x 125 m boxes, showing a very different distribution for each level of impact.

This is also confirmed by the mean seabed slope within each box and the associated standard deviation. The mean seabed slope increases with impact, and similarly the standard deviation will increase (Figure 21.9).

This suggests that an acoustic indicator such as seabed slope could provide an indication of the physical disturbance at a previously undisturbed site.

This is just one example of the potential indicators derived from multibeam data. More acoustic indicators will be tested and evaluated in the next few years.

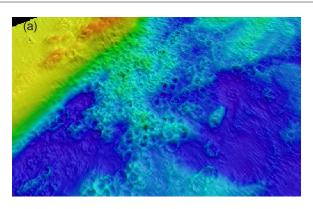


Figure 21.7. (a) Multibeam bathymetry of the NAB Tower dredged material disposal site showing and impacted area; (b) seabed slope derived from the multibeam bathymetry.

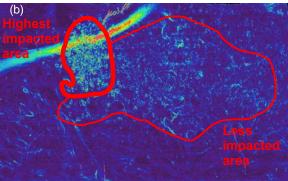


Figure 21.8. Seabed slope frequency distribution for three identically sized areas but with different impact levels

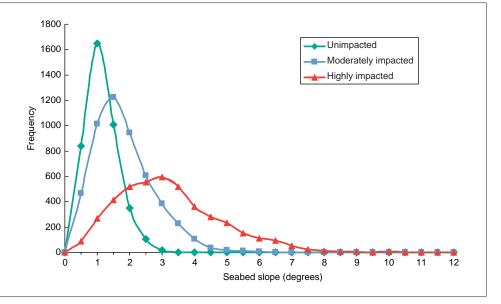
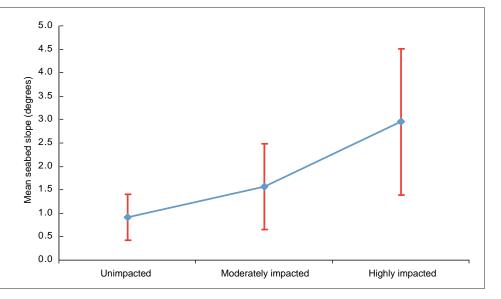


Figure 21.9. Degree of impact versus mean seabed slope showing a positive correlation and increasing standard deviation.



#### 21.6 Conclusions

AGDS was identified as a potentially useful technique to indicate temporal changes in the nature of the seabed, as well as an indicator of spatial differences in seabed nature. However, the potential is tempered by the low repeatability of the raw data values combined with their dependence on several environmental conditions which cannot be controlled or corrected for (eg tidal currents, sediment in suspension).

The use of sidescan sonar as an acoustic indicator was identified as being suitable for the assessment of both spatial and temporal changes in the seabed nature and seabed morphology. To date, sidescan sonar is the preferred technique to map the seabed nature and morphology. However, the non-calibrated backscatter amplitude is a major limitation to its use as an acoustic indicator, combined with the need to reduce its output to a 256 point grey scale raster image.

Acoustic indicators derived from the bathymetry and backscatter strength of multibeam echosounders were identified as suitable means to provide spatial and temporal information on the change in seabed nature, seabed morphology and seabed topography. The ability to provide indicators on seabed nature and seabed topography in a single survey event is seen as a major benefit but will come at a cost, as these systems are the most expensive in purchase, operation, processing and analysis.

A survey of the outcome of qualitative evaluation against agreed criteria governing indicator utility is given in Table 21.4. Each criterion is scored on a scale from 1 (poor) to 5 (very good). We propose to employ the outcome of this initial assessment as a basis for further exploration for deriving useful quantitative environmental indicators from acoustic outputs.

Table 21.4. Assessment against indicator criteria for AGDS, sidescan sonar and multibeam echosounders.

Criterion		AGDS	Sidescan son	ar Multibeam	
Relatively easy to unders	other users	2	4	5	
Sensitive and relevant to a manageable human activity			3	3	4
Tightly linked to the human activity but not to other causes of change			2	3	4
Easily and accurately measured, with a low error rate			1	2	4
Affordable and feasible in terms of data collection and manipulation			5	4	3
Provide early warning			2	3	4
poor	good			very good	
1	2	3		4	5

## 22. Inner Tees Bay: a disposal ground survey 2003

## Authors: Matthew Curtis, Hubert Rees and Sylvia Blake

#### 22.1 Introduction

The Tees estuary is a heavily built-up area with many different industries on its banks including, historically, the manufacture of a number of brominated flame retardants. The Port of Tees authority operates two trailing suction hopper dredges and a small grab dredger, with maintenance dredging being undertaken on a daily basis and with regular placements of small amounts of material at the disposal ground. Approximately 1.5 million tonnes of maintenance material is dredged per year and disposed of at the Inner Tees Bay A disposal ground (Table 22.1). Recently, flame retardant contamination was detected in samples from the Tees estuary (de Boer *et al.*, 2001b) and it became a matter of concern to see if this contamination was being transferred to the dredged material disposal ground.

The Tees Bay A disposal ground was surveyed on 1 June 2003 using the RV *Cefas Endeavour* (Figure 22.1), with the aim of collecting sediment samples for trace contaminant analysis, especially the brominated diphenyl ether (BDE) flame retardants. The opportunity was also taken to sample the benthic macrofauna to investigate any localised effect of the dredged material disposal activity.

#### 22.2 Methods

#### 22.2.1 Survey design

The survey was designed to take into account the most likely route of any dispersing material away from the

Table 22.1. Recent dredging disposal history of Tees Inner disposal ground.

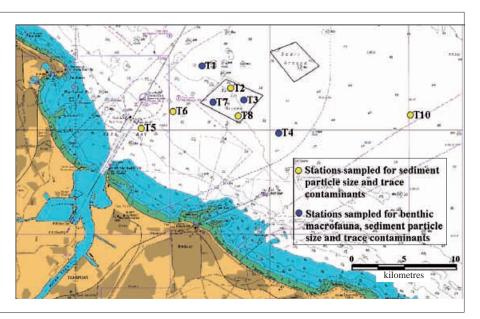
Year	Total tonnage disposed
2000	1,226,699
2001	1,508,831
2002	2,003,029
2003	1,548,345

disposal ground, due principally to tidal action, as well as of suspended material coming from the estuary. Stations were positioned along the main axis of tidal flow, which runs parallel to the coast with a maximum tidal velocity of 0.5 ms<sup>-1</sup> (derived from Admiralty data), and also toward the mouth of the estuary. A total of nine stations were sampled for sediment particle size distribution and trace contaminant analysis, including BDEs, tributyl-tin (TBT), polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs). Additionally, a sub-set of four stations were sampled to provide replicate benthic macrofauna samples.

#### 22.2.2 Field sampling procedures

Replicate samples of sediments for the later analysis of benthic macrofauna, and samples for sediment particle size distribution and trace contaminants were collected using a  $0.1\ m^2$  Day grab. Following the estimation of sample volume, a visual sediment description was

Figure 22.1. Tees survey 2003. Reproduced from Admiralty Chart 152 by permission of the Controller of Her Majesty's Stationery Office and the UK Hydrographic Office (www.ukho.gov.uk). Not to be used for navigation.



recorded and sub-samples were removed for particle size distribution and chemical analysis. The whole sample was then washed over 5 mm and 1 mm sieve meshes to remove fine sediment. The two fractions were combined and back-washed into an appropriate container and fixed in 4-6% buffered formaldehyde solution.

#### 22.2.3 Laboratory procedures

Samples for BDE, PCB and OCP analysis were air-dried in a controlled environment and then ground with anhydrous sodium sulphate prior to Soxhlet extraction with *n*-hexane/acetone. Sulphur residues were removed at this stage with copper. An aliquot of the sample extract was cleaned-up and fractionated by column chromatography using partially deactivated alumina and silica. After addition of internal standards, residues were determined by GC-ECD (electron capture detection) and GC-MS. Full details are given in Allchin *et al.*, (1989) and de Boer *et al.*, (2001a). Samples were not analysed for BDE209 (decabromodiphenylether – the major component of the deca-mix PBDE formulation).

Samples for TBT analysis were extracted wet, and a separate total solid determination was performed to express the result on a dry weight basis. Samples were extracted by shaking with 0.1% sodium hydroxide and methanol. The organotin compounds were then back-extracted into n-hexane, converted to their respective hydrides with sodium borohydride, and analyzed using GC-FPD (flame photometric detection), optimised for tin at 610 nm. All results obtained were calculated as dry weight. Full details of the method are given in Waldock et al., (1989).

In the laboratory, macrofauna samples from each Day grab were first washed with freshwater over a 1mm sieve in a fume cupboard to remove excess formaldehyde solution and then placed on plastic trays and examined under an illuminated magnifier. Specimens were picked from the trays and placed in labelled Petri dishes containing a preservative mixture of 70% IMS for identification and enumeration.

Identified specimens were removed from their containers and rinsed in fresh water before being placed on a dry sheet of high absorbency paper and blotted dry. Once dry, specimens were transferred immediately to a tared container on a balance and the weight of the specimen recorded to 0.0001 g. Specimens were then removed from the balance and placed back in their container.

Analysis of sediment particle size, heavy metals and polycyclic aromatic hydrocarbons (PAHs) has still to be completed.

#### 22.3 Results

#### Visual sediment descriptions

The sediment descriptions from the survey can be seen in Table 22.2. These showed the area to have a homogeneous substratum with most sites consisting of muddy sand. Stations T3 and T7 sampled within the disposal area showed evidence of past dredge disposal activity containing small lumps of black mud and black flecks, which were probably coal.

Table 22.2. Sediment descriptions taken from Day grab samples.

### Station Sediment type

T1	Muddy sand
T2	Muddy sand
T3	Slightly muddy sand with some black flecks - coal? And small lumps of black mud - spoil?
T4	Muddy sand
T5	Muddy sand
T6	Muddy sand
T7	Muddy sand with evidence of dredge spoil - black mud.
T8	Slightly muddy sand
T10	Muddy shelly sand

#### 22.3.1 Trace contaminants

Low levels of BDEs were seen at all stations (Table 22.3) with most samples having concentrations similar to the lowest levels detected in European marine sediments (Law *et al.*, 2004). Stations T2 and T8, inside the disposal ground, and station T1, to the north-west of the disposal ground, all showed slightly elevated levels of BDEs with the highest summed concentration being 1.52 µg kg<sup>-1</sup> dry weight.

Slightly elevated TBT concentrations, 0.013 mg kg<sup>-1</sup> dry weight, were seen at station T7 within the disposal ground (Table 22.3). Stations T1 and T5 showed levels just above the TBT detection level (0.001 mg kg<sup>-1</sup>); at all other stations TBT was not detected. Low levels of PCBs were detected at stations T7 and T8, within the disposal ground, and at stations T4 and T10, to the east of the disposal ground (Table 22.3); these were classified as slightly contaminated (Wells *et al.*, 1989). All stations showed slightly elevated levels of

Table 22.3. BDE, organochlorine pesticides, PCBs and TBT results.

Station	Sum of 10 BDEs (µg kg <sup>-1</sup> )	HCB (µg kg <sup>-1</sup> )	Total HCH (µg kg <sup>-1</sup> )	Dieldrin (µg kg <sup>-1</sup> )	Total DDT (µg kg <sup>-1</sup> )	Sum of 25 PCBs (µg kg <sup>-1</sup> )	TBT (mg kg <sup>-1</sup> )
T1	1.45	0.55	0.57	0.68	0.63	<0.2	0.005
T2	1.34	0.42	0.48	0.64	0.5	<0.2	< 0.001
T3	0.6	0.58	0.94	0.7	0.21	<0.2	< 0.001
T4	0.81	0.38	0.33	0.6	0.45	4.17	< 0.001
T5	0.47	1.2	0.77	0.62	0.21	<0.2	0.005
T6	0.52	0.45	0.66	0.59	<0.2	<0.2	< 0.001
T7	0.46	0.7	0.57	0.98	3.55	5.08	0.013
T8	1.52	0.37	0.35	0.69	0.25	0.53	< 0.001
T10	0.56	0.69	0.31	0.71	<0.2	0.32	<0.001

the OCPs hexachlorobenzene (HCB) hexachlorocyclohexane (HCH) dieldrin and dichlorodiphenyltrichloroethane (DDT) except stations T6 and T10 where no DDT was detected (Table 22.3).

#### 22.3.2 Benthic macrofauna

A total of 116 taxa were identified from stations T1, T3, T4 and T7, of which 36 occurred only once. Bivalves and echinoderms were the most dominant species, both in number and biomass, of which Nucula nitidosa, Mysella bidentata, Abra alba, Chamelea gallina, Dosinia lupinus, and Amphiura filiformis were most abundant. Echinocardium cordatum contributed a high proportion of the biomass at stations T1, T3 and T4, and Leptopentacta elongata contributed a high proportion of the biomass at stations T1 and T4. Phoronis spp. was present in high numbers at station T4 with 1376 individuals encountered.

Stations T3 and T7, inside the disposal ground had a reduced average numbers of species, individuals and biomass compared with stations T1 and T4, outside the disposal ground (Figures 22.2, 22.3 and 22.4). One-way ANOVA of the data showed these differences to be significant (p < 0.05).

Multi-dimensional scaling analysis was performed on the species abundance using PRIMER v.5 (Clarke and Gorley, 2001) (Figure 22.5). This shows that the replicates are closely grouped indicating a high degree of similarity within each station. Stations T1 and T4, outside the disposal ground, were also both closely grouped together, showing the stations to be similar.

The similarity percentages program (SIMPER) was used to indicate which taxa contributed the most towards similarity between replicates from within each station (Table 22.4). Stations T1 and T4 were seen to have similar macrofauna present with the only major difference between them being the presence of the Horseshoe worm, Phoronis spp., in high numbers at station T4. All four stations were generally seen to be dominated by bivalves and echinoderms, which are typical fauna in muddy sands.

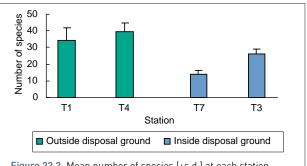


Figure 22.2. Mean number of species (+s.d.) at each station.

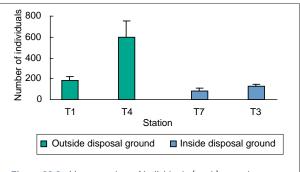


Figure 22.3. Mean number of individuals (+s.d.) at each

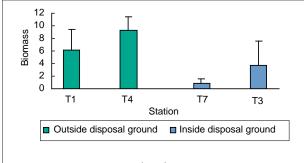
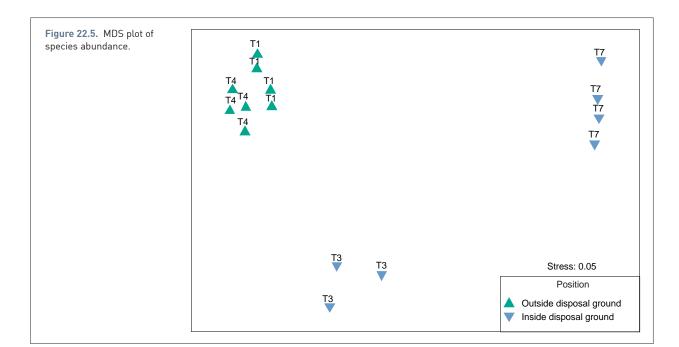


Figure 22.4. Mean biomass (+s.d.) at each station.



 $\begin{tabular}{ll} \textbf{Table 22.4.} & Results of SIMPER analysis of the species abundance data showing average abundance, average similarity, and \% contribution, both individually and accumulatively. \\ \end{tabular}$ 

Station	Taxonomic group	Average abundance	Average similarity	Contribution %	Cumulative %
T1	Amphiura filiformis	7.08	9.86	18.04	18.04
	Mysella bidentata	4.82	6.52	11.93	29.97
	Dosinia Iupinus	3.25	4.43	8.12	38.09
	Diastylis lucifera	2.68	3.87	7.09	45.18
	Chamelea gallina	3.23	3.76	6.89	52.06
T3	Nucula nitidosa	4.76	9.11	14.68	14.68
	Chamelea gallina	4.48	7.7	12.41	27.08
	Amphiura (juv.)	3.09	5.73	9.23	36.31
	Dosinia Iupinus	3.13	5.5	8.86	45.17
	Chaetozone christiei	2.31	4.45	7.17	52.34
T4	Phoronis spp.	18.34	16.67	24.25	24.25
	Amphiura filiformis	7.46	6.75	9.81	34.06
	Chamelea gallina	5.12	4.62	6.73	40.79
	Dosinia Iupinus	3.86	3.72	5.41	46.2
	Mysella bidentata	4.3	3.7	5.38	51.58
T7	Abra alba	4.9	13.92	23.9	23.9
	Bivalvia sp. indet.	4.73	10.93	18.76	42.66
	Nephtys hombergii	2.48	8.71	14.95	57.61
	Chone sp.	2.42	8.23	14.13	71.74
	Nephtys assimilis	1.21	4.09	7.02	78.75

#### 22.4 Discussion

Low levels of BDEs were detected within the survey area, with slightly elevated concentrations within the disposal ground. These levels were similar to the lowest levels detected in other European marine sediments (Law *et al.*, 2004). This suggests that, at present, the disposal ground is not being significantly contaminated with BDEs deriving from dredged material taken from the Tees estuary.

Evidence of past dredge disposal was observed in two stations within the disposal ground; stations T3 and T7 contained small lumps of black mud and black flecks, which were probably coal. Four stations were seen to be slightly contaminated with PCBs, two of these outside the disposal ground; this contamination could be due to the dispersal of contaminated material away from the site. OCPs and TBT were present within the survey area, but were only detected at low concentrations. Sediment particle size, heavy metal and PAH results will be reported at a later date.

The fauna seen in this survey are broadly what would be expected for this area, both within and outside the disposal

site. The results show that the number of species, individuals and biomass were lower within the disposal ground, which is an expected and well-documented consequence of dredge disposal activity. No effects of disposal activity were seen at the stations outside the disposal ground indicating the absence of any far reaching consequences of the dispersal of disposed material by tidal influence on the benthic macrofauna.

#### 22.5 Conclusion

The results to date have identified localised impacts on the benthic macrofauna and sediments arising from dredged material disposal. There was little evidence of adverse effects arising from the tidally induced dispersal of finer particulates away from the disposal site with only slight PCB contamination seen at stations to the east of the disposal ground. The Inner Tees Bay A disposal ground was also surveyed in 2004 and 2005 for trace contaminant analysis and these results will also be reported at a later date.

# 23. Advice on fishery implications of pipeline discharges

#### 23.1 Overview

This section gives a brief summary of activities carried out during 2004 in connection with the provision of advice on fishery implications of pipeline discharges.

Cefas appraisal of applications for pipeline discharges involves consideration of resources in the area, toxicity of the effluent, local hydrographic conditions and any standards set out in national policy or European Union (EU) Directives. This includes the impact of discharges on marine fauna, including fish nurseries and shellfish populations, and specific interactions of chemicals and the marine environment. One important issue in relation to sewage discharges is the microbiological contamination of bivalve mollusc shellfisheries and the associated human health concerns. The reduction of sewage contamination at source is the most effective way of reducing the health risk. It also reduces the burdens on the industry and increases acceptability of product to supermarkets.

## 23.2 Summary of pipeline discharge applications

During 2004 Cefas assessed applications for a total of 469 individual discharges, this represents an increase of over 37% on the previous year (341 in 2003). A total of 264 were assessed for their potential to impact on shellfish waters and/or production areas, Shellfish hygiene issues therefore continued to be the most common concerns addressed. Table 23.1 shows the types of discharge application commented on. The majority of applications were for discharge of domestic or combined domestic and industrial sewage, including storm and emergency sewage overflows.

**Table 23.1.** Numbers of applications of various types commented on within 2004.

Type of application	Number received
Sewage	440
Trade Effluent	29
Total applications received	469
(Shellfish related)	(264)

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. Applications for 196 storm overflow discharges were received in 2004 of which 129 were identified as impacting on shellfisheries. Where discharge improvements were identified to benefit shellfish waters, there is a now an Environment Agency policy requirement to restrict overflow operation to a maximum of ten spills per annum in aggregation with other impacting storm discharges (on average over a ten year period). Previously this requirement had to be negotiated by Cefas on a case by case basis.

Cefas requested annual spill reporting for most of these and where necessary asked for clarification that discharges had been considered in aggregation with others impacting on the same fishery.

Emergency overflows only come into operation when there is a major failure at the sewage treatment works or pumping station. If this should occur, it may cause severe contamination of fisheries in the area. Applications for 129 emergency discharge applications were received, of these 63 had the potential to affect shellfisheries. In advising on such applications Cefas therefore requested urgent notification of emergency events to the local food authority so that appropriate action could be taken to protect public health.

A total of 73 applications were received for continuous discharges of secondary (biologically treated) effluent; these included upgrading of existing works and new treatment works and package plants. In addition 23 applications were received for tertiary treatment, eighty seven percent of which were for year-round UV disinfection aimed at reduction of microbiological contamination. All of these benefited shellfish areas.

## 23.3 Drivers for current pipeline discharge improvements

Most of the applications for discharge improvements were in order to meet the requirements of the EC Directive 91/271 concerning urban waste water treatment (European Communities, 1991a), EC Directive 79/923 on the quality required of shellfish waters (European Communities, 1979) and EC Directive 76/160 concerning the quality of bathing water (European Communities, 1976).

In 1999, ninety five additional shellfish waters were designated bringing the total to 119 in England in Wales, and the Government set a target for all commercially harvested shellfish beds to achieve a microbiological classification of at least Class B as categorised under EC Directive 91/492 (European Communities, 1991b), see Table 23.2.

**Table 23.2.** Classification categories under the Shellfish Hygiene Directive

Class	Criteria —	Requirements		
А	<300 faecal coliforms or 230 E. coli per 100g	Can be collected for direct human consumption		
В	90% compliance with 6,000 faecal coliforms or 4,600 E coli per 100g	Must be purified or relayed to meet class A; may also be heat treated by an approved method		
С	<60,000 faecal coliforms or <46,000 E. coli per 100g	Must be relayed for at least 2 months to meet class A or B; may also be heat treated by an approved method.		
Prohibited	>60,000 faecal coliforms or >46,000 E. coli per 100g	Commercial harvesting prohibited		

Following this and for the first time, Shellfish Waters were included as a driver for investment in water company asset management programmes for 2000-2005 (AMP3). As a result, in 2001, a policy was developed by the Environment Agency for the consenting discharges to achieve the requirements of the shellfish waters directive and to ensure that schemes contributed to meeting Government targets. This policy drew significantly upon scientific advice and recommendations made by Cefas during the preceding years.

## 23.4 Impact of discharge improvements on bivalve molluscan shellfisheries

Human illness arising from the consumption of bivalve molluscan shellfish is a recognized problem. In the UK, the micro-organisms causing such illnesses are almost always the result of sewage contamination of the harvesting areas. The processing techniques, eg purification in clean seawater, will not necessarily remove all of the illness-causing organisms. The processing requirements also have a significant practical impact on the business operation and costs for the producers.

Between annual September classifications in 2000 to 2004 the percentage of Class A areas has decreased from 5.5% to 3.7% and the proportion of Class B areas increased from 66.4% to 84.5%. The proportion of Class C areas decreased from 24.7% to 10.5% and the number of prohibited areas decreased from 3.4% to 1.3% in the same period. The increase in Class B areas and decrease in Class C and prohibited areas in this period, reflect water company investment targeting discharges to tidal waters largely delivered since 1999 and in particular those improvements identified as benefiting shellfish waters in AMP3 to date. The decrease in the proportion of Class A areas is of concern however as Class A confers benefits of access to markets and premium market prices for the fishing industry. Despite significant improvements in water quality as a result of investment in discharge improvements in the last few years fewer than 4% of shellfish production areas in England and Wales achieve the 'Class A' standard which reflects water quality clean

enough to allow marketing of shellfish direct for human consumption without further processing. To achieve class A status for many fisheries it is likely that a combined programme of additional discharge improvements and/or measures to tackle diffuse pollution issues will be required in the future

## 23.5 Drivers for future pipeline discharge improvements

Following Ministers principal guidance in March the number of schemes specifically targeting shellfish waters for inclusion in the next water companies investment programme, AMP4 (2005-2010) were significantly reduced. It is of particular concern that the proposal to invest in the monitoring of storm overflow discharges will not be taken forward. Such information would provide valuable evidence on which to base future investment decisions. Consultation on discharge consent applications and modifications will however be necessary for schemes impacting on tidal waters programmed with other drivers eg Urban Waste Water Treatment and Habitats Directive. At the time of consultation on these schemes, protection of the wider marine environment including shellfish waters concerns will be considered and highlighted to the Environment Agency.

During 2004 a further review of Shellfish Water designations was undertaken and Cefas assisted Defra in the development of a new designation policy with the result that in November 2004 a further five new Shellfish Waters plus five extensions to existing waters were designated bringing the total to 124 waters in England and Wales. Designations confer protection to the shellfish production areas within them under the Shellfish Waters Directive mandatory and guideline receiving water standards and associated design standards incorporated into Environment Agency discharge consenting policy.

#### 23.6 General Advice

In addition to applications for modifications to existing consents and new consents for sewage discharges a

number of applications for temporary relaxation of existing consent conditions were received and responded during the year. A number of requests associated with controversial planning and pollution issues have also been dealt with in the last year.

Other advice provided included that on the pipeline disposal of material contaminated with radioactivity to sea in support of Defra contingency planning, comments to Defra in relation to considerations of tighter controls on direct discharges to groundwater as part of the Groundwater Directive Consultation and advice on the use of groundwater in aquaculture was provided for purposes of the Partial Regulatory Impact Assessment (PRIA). General support was also provided for water

quality liaison with the Environment Agency regions. This has proved effective in championing the consideration of the marine environment and fisheries interests prior to any formal discharge consent application.

#### 23.7 Database maintenance

All applications, consents and authorisations continue to be entered onto a database that contains details of all known discharges to saline waters in England and Wales. The database is being continually developed, provides unique intelligence and is a strategic tool used alongside other Cefas tools to underpin impact assessments and policy decisions in the marine environment.

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