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Bioaccumulation surveillance in Milford Haven Waterway 2007-2008

W J Langston*, S O'Hara, M Imamura & N D Pope
Marine Biological Association
Citadel Hill
Plymouth PL1 2PB

A study undertaken on behalf of

Milford Haven Waterway Environmental Surveillance Group

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*Contact: wjl@mba.ac.uk

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It should be noted that the opinions expressed in this report are largely those of the authors and do not necessarily reflect the views of MHWESG.

Preferred citation

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Executive Summary

Biomonitoring of contaminants (metals, organotins, PAHs, PCBs) has been carried out at sites along the Milford Haven Waterway and at a reference site in the Tywi Estuary during 2007-2008. The species used as bioindicators encompass a variety of uptake routes; i.e. *Fucus vesiculosus* (dissolved contaminants); *Littorina littorea* (grazer); *Mytilus edulis* and *Cerastoderma edule* (suspension feeders that accumulate from both dissolved phase and suspended particulates); and *Nereis diversicolor* (omnivore which often reflects bioavailable contaminants in sediment). Differences in feeding strategy and habitat preference can have subtle implications for bioaccumulation trends though, with few exceptions, contaminant body burdens in Milford Haven (MH) were higher than those at the Tywi reference site.

Substantially elevated metal concentrations were observed at individual MH sites for Mn (molluscs, seaweed), Co (mussels, seaweed), Sn (bivalves), Ni (cockles) and Fe (ragworm), whilst As and Se (molluscs and seaweed) were consistently at the higher end of the UK range for much of the MH Waterway. However, for the majority of metals, distributions in MH biota were not exceptional by UK standards. Several metal-species combinations indicated increases in bioavailability at upstream sites, which may reflect the influence of geogenic or other land-based sources - enhanced in some cases by lower salinity (greater proportions of more bioavailable forms).

TBT levels in mussels were below thresholds considered by OSPAR to be acutely toxic, though based on these guidelines, sub-lethal effects cannot be ruled out at MH sites. TBT (and other BT) levels in the Tywi were close to zero. Phenyltins were not accumulated appreciably in *Mytilus*, whereas some *Nereis* populations in MH may have been subjected to localized (historical) sources retained in sediments.

PAHs in *Nereis* tended to be evenly distributed across most sites, but with somewhat higher values at Dale for acenaphthene, fluoranthene, pyrene, benzo(a)anthracene and chrysene, whilst naphthalenes tended to be enriched further upstream in the mid-upper Haven (a pattern which is seen in mussels for most PAHs). Whilst concentrations in *Mytilus* were above OSPAR backgrounds, there was little indication that generalized exotoxicological guidelines for PAHs would be exceeded (although there has been no ground-truthing of these assumptions). PAH body burdens in Milford Haven biota were generally (but not always) higher than those in the Tywi Estuary.

Lipophilic PCBs in mussels were between upper and lower OSPAR guidelines and were unusual in their distribution in that highest levels occurred at the mouth of MH. This may be a function of better condition and nutritional status (lipids) here, rather than contamination.

Overall, condition indices of bivalves (cockles and mussels) were highest at the Tywi reference site, and at the mouth of Milford Haven, but decreased upstream in the Waterway. There were a number of significant (negative) relationships between CI and body burdens and it is possible that a combination of contaminants could have an influence on this pattern in the CI (and other markers of organism 'health'). Cause and effect needs to be tested more rigorously as there a number of (natural) factors which may be influential. Contextual physicochemical information and published data on sources, pathways and toxicology of contaminants has been included as part of the discussion of bioaccumulation results.

The strategy for biomonitoring undertaken in this project builds on established sampling protocols and is proposed as a basis for a rolling program against which future change could be measured. Complementary, harmonised monitoring in which biological condition and environmental parameters are measured and interpreted alongside body burdens - using multivariate techniques to help assess the status of the site more comprehensively – are also recommended for the future.

Contents

1 Introduction

1.1 Milford Haven Waterway and the requirement for biomonitoring

The Milford Haven Waterway is the only example of a ria-type estuary in Wales and is a component of the Pembrokeshire Marine Special Area of Conservation, encompassing a number of designated conservation features (Burton, 2006). The Haven proper is fully marine for some 12km from the mouth (almost to the mouth of the Pennar river) and consists of a shoreline of >100km. The Daucleddau -the common Estuary of the Cleddau Rivers- is also considered marine (mesohaline) for much of its length because of the small FW inflow relative to the tidal incursion (Nelson-Smith, 1965).

Despite its important conservation status, the Waterway is subjected, potentially, to contaminants from several sources including atmospheric deposition, rivers (dominated by E and W Cleddau), industry (e.g. oil refineries), domestic discharges (WWtW and CSO), diffuse inputs associated with tip leachate, urban development (Milford Haven, Haverfordwest, Pembroke, and Pembroke Dock) and agricultural run-off. Maritime operations, pollution incidents (hydrocarbons and antifouling), dredging and spoil disposal add to this inventory (Atkins, 2002). The importance of Milford Haven as a port is likely to increase in coming years, which could see a rise in some of these pressures. Contamination by biologically-deleterious substances is considered one of the more detrimental aspects arising from human actions, with implications for favourable condition status of the site (highlighted by the '*Sea Empress*' oil spill in 1996); hence the value of establishing a meaningful monitoring programme - to ensure unacceptable deterioration is not occurring (and does not occur in future) as a result of anthropogenic events. Bioaccumulation surveillance, and its interpretation, will help MHPA, CCW, EA Wales, and other members of MHWESG, in their responsibilities to apply appropriate assessments to safeguard against the likelihood of effects.

There are various tools available to environmental managers to predict the likely adverse effects of contamination on marine ecosystems. These include water quality analysis, toxicity testing and ecological survey procedures together with measures designed for the incoming Water Framework Directive. Biomonitoring is valuable because it provides a direct measure of the bioavailability of contaminants. Bioaccumulation is not only an important component of environmental quality assessment but also, for commercial species, can have implications for human health. Shellfish gathering in the Waterway is currently mainly small-scale for mussels, winkles, cockles, clams, oysters and razor fish, although commercial collection of winkles and mussels for seed stock has occurred in the past within the Haven. Limited seaweed harvesting occurs, primarily for the making of laver bread.

It may seem most relevant to base the choice of biomonitoring organism on one or more of the species consumed by humans. However, there are other considerations to be made; these stem from the fact that different contaminants have their own characteristics and that organisms accumulate them from a variety of sources, often at different rates, adopting diverse accumulation strategies. Consequently, there is no single universal indicator organism and the most useful monitoring programmes are likely to include analyses of several species, preferably of differing ecological types (primary producer, detritivor, herbivor, filter feeder). Selection of indicators should be appropriate to the chemistry and form (dissolved, particulate, dietary) of the contaminants of concern. Hence, by integrating results for several different species it should be possible to obtain a broad appraisal of impact to the environment. The selection of species in the current project represents an appropriate blend i.e. *Fucus vesiculosus* (dissolved contaminants); *Littorina littorea* (grazer); *Mytilus edulis* and *Cerastoderma edule* (suspension feeders that accumulate from both dissolved phase and suspended particulates); and *Nereis diversicolor* (an omnivore which often strongly reflects bioavailable contaminants in sediment e.g. Bryan *et al*, 1980, 1985; Langston and Spence, 1995).

By combining the information gained from these species it is likely that a reasonable picture of the significance of biologically-available contaminants in Milford Haven Waterway will be achieved. A similar rationale was adopted for earlier NRA bioaccumulation surveys in Wales (Davies and Ellery, 1995), which, apart from a few MBA data for metals determined almost 30y ago, represent the only long-term bioaccumulation data for the Haven (see review by Bent, 2000). Continuation of this strategy, based on similar species and sites, therefore provides an opportunity to see whether there has been improvement or deterioration in contamination levels, as well as providing a modern baseline against which future change can be gauged.

It is important to stress that biotic factors can sometimes modify responses of organisms to contaminants (e.g. Bryan *et al*., 1980, 1985; Langston and Spence, 1995); in particular seasonal and reproductive variations can cause body burdens to fluctuate (apparently) in the absence of any real change in contamination levels (Langston and Spence, 1995). In the context of detecting environmental change, we have made efforts to identify and, where possible, minimize, the effects of biotic factors such as seasonality and size during sampling, and to pay strict attention to the quality of data.

Finally, as pointed out by Bent (2000), although data on contaminants exists for Milford Haven, interpretation is often lacking. Contextual information on sources, pathways and the toxicological significance of contaminants has therefore been included as part of the discussion of bioaccumulation results. For metals, MBA has data for the same species, for most estuaries in Wales and England, enabling nationwide comparisons, as well as temporal insights, for some contaminants.

1.2 Objectives

The key requirements of the current project were:

- To re-establish bioaccumulation surveillance at stations previously used by Environment Agency Wales.
- To establish a bioaccumulation programme that offers wide coverage within Milford Haven Waterway.
- To undertake bioaccumulation surveillance on a range of metals and organic contaminants of interest to Group members.
- To acquire data on an annual basis to enable regular comparisons with benchmark data.

2 Methods

2.1 Sampling

MBA staff undertook field-survey work in Milford Haven on two occasions. The first phase was between 11th and 14th September 2007: the second phase was between 9th and 11th March 2008. Reference samples for each species were also collected in the Tywi Estuary at these times. Locations of the primary sites are shown in Figure 1; grid references and species occurrences are summarised in Table 1.

Phase 1: The objective was primarily to collect *Nereis diversicolor* and *Littorina littorea* during the specified autumn sampling window for these species. Exploratory sampling and observations of other species were made for future reference. Six locations were sampled for each species within the Milford Haven Waterway, and a further control sample from the Tywi Estuary. Because of different habitat preferences, sites for *Nereis* (infaunal sediment dweller) and *Littorina* (grazer; mainly on rock and seaweed) were not always identical but were as close as practical. Further details of sampling sites and numbers collected can be found in Appendix 1.

Phase 2 of the field-survey was undertaken between 9th and 11th March 2008: The objective, on this occasion, was to collect *Mytilus edulis* (mussels), *Cerastoderma edule* (cockles) and *Fucus vesiculosus* (bladderwrack) from the control site (Tywi Estuary) and suitable locations within the Milford Haven Waterway. This included sites sampled for other species in phase 1 (as close as practical given habitat preferences), plus two further sites. Figure 1 shows the locations of the sites and Table 1 summarises grid references and occurrence of individual species. Further details of sampling sites and numbers collected can be found in Appendix 1.

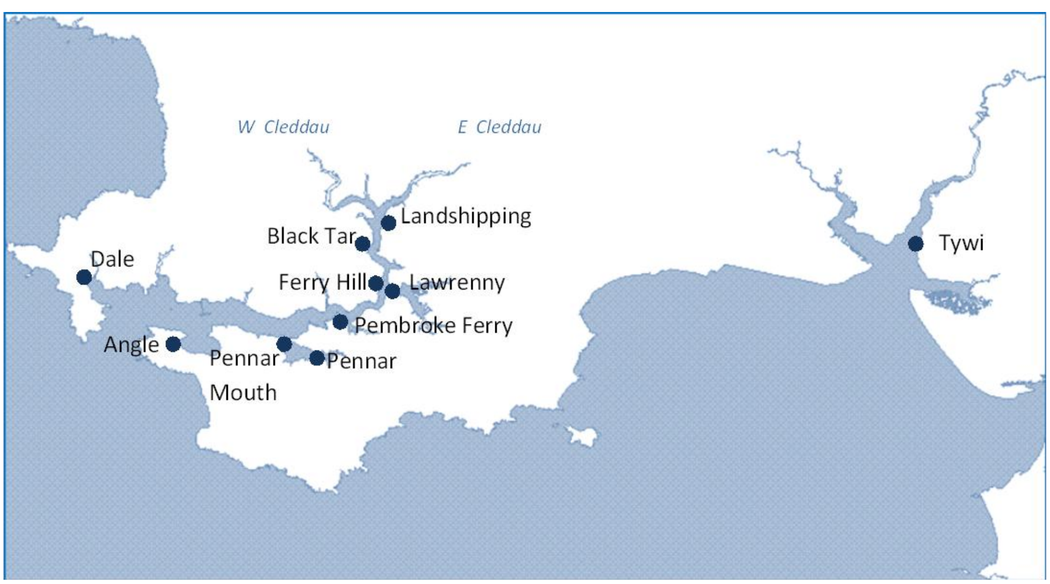


Figure 1. Location of sampling sites for biota, Milford Haven and Tywi Estuary (see Appendix 1 for details).

Table 1. Summary of sampling sites and species distributions

Site	Map ref (sites sampled)	Fuc	Ner	Lit	Myt	Cer
MILFORD HAVEN WATERWAY						
Landshipping	SN011118	(+)	++	(+)	(+)	(+)
Landshipping Quay	SN008108	(++)		++	(+)	(+)
Black Tar	SM999093	++		(+)	++	++
Lawrenny (Cresswell/Carew Mouth)	SN017063	+	++			
Lawrenny (Jenkin's Point)	SN009062	++		++	++	++
Ferry Hill	SN003061	++		(+)	++	++
Pembroke Ferry (Waterloo)	SM982040	++	++	++		
Pembroke Ferry (Ferry Inn)	SM974047	++		(+)	++	++
Pembroke River (Pennar)	SM959020	++	++	++	++	++
Pembroke River (Pennar Mouth)	SM943028	++		(+)	++	++
Angle Bay[b]	SM870027	++		++	++	++
Angle Bay[a]	SM868028		++			
Dale[b]	SM809065	++		++	++	++
Dale[a]	SM815075		++			
TYWI REFERENCE SAMPLES						
Tywi (1.2km u/s of Ferryside)	SN370117		++			(+)
Tywi (St. Ishmael)	SN361082	++		++	++	+

Key: **Fuc**, *Fucus vesiculosus*; **Ner**, *Nereis diversicolor*; **Lit**, *Littorina littorea*; **Myt,** *Mytilus edulis*; **Cer**, *Cerastoderma edule*. + species present and sampled. ++ species numerous and sampled. (+) species present, but not sampled (in some cases, specimens too small or sparse).

All biota were returned live to the MBA and immediately submitted to clean-up procedures in preparation for analysis, as described below.

2.2 Sample clean-up and preparation

On return to the laboratory *L. littorea* were cleaned in filtered, low-contaminant (Eddystone) aerated seawater for 2-3 days (Bryan *et al.,* 1985). Shells were removed in a vice, opercula removed and soft tissues pooled for freeze-drying and analysis as described below. *N. diversicolor* were sieved gently from the site sediments and transferred into fine acid-washed sand covered with filtered 50% (Eddystone) seawater for 6 days before transferring to clean water for a further day (Bryan *et al.,* 1985). Worms from each location were pooled for freeze-drying and analysis. Individual *F. vesiculosus* fronds were washed in filtered 50% Eddystone seawater and adhering particles removed as far as possible with a pastry brush. Thalli were cut up into small pieces, avoiding vesicles and growing tips (Bryan *et al.,* 1985). These were blotted dry of excess water and samples from ~20 plants pooled for freeze-drying and analysis as described below.

Bivalves *M. edulis* and *C. edule* were cleaned in filtered Eddystone seawater for 2 and 3-4 days, respectively (Bryan *et al.,* 1985). Mussels and cockles from each site were measured for shell lengths and total body weight. Soft tissues were dissected from the shells, pooled and weighed in batches of 20 (mussels) or 30 (cockles), and frozen prior to freeze drying.

2.3 Biometric data and Condition Indices.

Whole organism size, weight and tissue wet and dry weight data were recorded for all species collected (see Appendix 2).

Condition indices (CI) for bivalves generally describe the relationship between soft tissue dry weight (meat content) and the organism total size (volume). High CI values are often considered to represent an integrated signal of better 'health' status but may also be a function of greater availability and assimilation of food. The condition index used in this study was CI 4 as defined in Lundebye *et al*., 1997:

$$CI = (\text{Soft tissue dry weight (g)} \times 1000)/(\text{shell length (cm)})^3$$

Biometric data, tissue weights and results from the contaminant analyses described below were input to Microsoft Excel spreadsheets. Statistical analyses were performed using the Statistica package (Statsoft Corp.). Metals data were interlinked to another, larger database holding comparable MBA data for most estuaries in England and Wales.

2.4 Freeze-drying of biological material

Dissected and frozen biological samples for contaminant analyses were freeze-dried to constant weight at -80°C and 10^{-3} torr and were then homogenised by grinding to a fine powder in a ceramic mortar and pestle. Homogenised powders were stored, desiccated, in re-sealable polythene bags. Aliquots of the freeze dried materials were processed and analysed for the following groups of determinands, according to the methods outlined. Detection limits are included.

Metals were analysed in all species; organics were analysed in *Mytilus edulis* and *Nereis diversicolor*. *Nereis diversicolor* and *Littorina littorea* were sampled in autumn 2007; *Mytilus edulis, Cerastoderma edule* and *Fucus vesiculosus* in spring 2008.

2.5 Metals and organotin analyses

Metals

The suite of metals analysed included: Ag, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Sn, and Zn.

Sub-samples (0.5g) of freeze-dried homogenate were digested with 5ml HNO_3 (Fisons *Primar* grade) and 1 ml H_2O_2 in a Milestone (1200 Mega) microwave digestion system. For analysis of Ag, a more traditional hotplate digestion of a separate subsample of freeze-dried homogenate was employed (Langston *et al.,* 1994), since Ag may be lost from nitric acid solution by the dormation of insoluble silver oxide. The clear homogeneous digests were analysed by Flame Atomic Absorption, or, where concentrations were low, by Graphite Furnace AA. Hg and Se were analysed by cold vapour and hydride generation systems, respectively. To prepare samples for arsenic and total tin analysis, 5 ml ashing slurry (6% magnesium nitrate, 10% magnesium oxide) were added to sub- samples of the freeze-dried homogenate; these were ashed in a muffle furnace and dissolved in 10ml HCl prior to analysis by hydride generation AA. Quality assurance included the use of the Certified Reference Materials DORM-2 (National Research Council), LUTS-1 and IAEA-140 (seaweed), which were run as an analytical control with each batch of samples, ensuring that determinations fell within the confidence intervals of the assigned values.

Table 2. Detection limits for metals (µg g^{-1} dw)

	Ag	As	Cd	Co	Cr	Cu	Hg	Ni	Pb	Se	Sn	Zn
LOD	0.02	0.02	0.03	0.06	0.04	0.1	0.017	0.075	0.1	0.02	0.01	0.1

Organotin analysis

Organotin compounds monitored included: monobutyl-, dibutyl- and tributyltin and monophenyl-, diphenyl- and triphenyltin.

The method used for the determination of TBT and other organotins was based on that of Harino *et al*. (2005) developed at the MBA. Tissue samples, including aliquots spiked with standards, were extracted with HCl and acetone, extracted with tropolone-benzene solution, propylated and cleaned on florisil, prior to analysis by GC-FPD. The detection limits were ~0.004µg g^{-1} dry wt. Quality assurance was established using certified reference material, PACS-1, CRM 462,477.

2.6 Polyaromatic hydrocarbon (PAH) analysis

Polyaromatic hydrocarbons assayed and their limit of detection in mussels and ragworm in the current study are shown in Table 3. Their ring composition, molecular weight and most likely primary origin (Law *et al*., 1999) are also indicated.

Table 3. Properties of PAHs analysed in *Mytilus edulis* and *Nereis diversicolor*

PAH	No. of rings	Molecular weight	Petrogenic (Pe) Pyrogenic (Py)	Limit of Detection ($\mu g\ kg^{-1}$)
Naphthalene	2	128	Pe	0.3
1-Methyl-naphthalene	2	142	Pe	0.3
Phenanthrene	3	178	Pe	0.2
Acenaphthene	3	154	Py	0.2
Fluorene	3	166		0.1
Anthracene	3	178	Py	0.1
Fluoranthene	4	202	Py	0.6
Pyrene	4	202	Py	0.7
Benzo[a]anthracene	4	228	Py	0.3
Chrysene	4	228	Py	0.2
Benzo[b]fluoranthene	5	252	Py	0.2
Perylene	5	252	Py	0.2
Benzo[k]fluoranthene	5	252	Py	0.2
Benzo[a]pyrene	5	252	Py	0.2
Dibenzo[ah]anthracene	5	278	Py	0.6
Benzo[ghi]perylene	6	276	Py	0.5
Indeno[1,2,3-cd]pyrene	6	276	Py	0.4

For PAHs, powdered freeze-dried tissue samples were extracted with acetonitrile and tetrahydrofuran (THF) aided by sonication. Clarified, filtered extracts were analysed by HPLC (gradient programming) equipped with scanning fluorescence detector (see Vane *et al*., 2007 for details). The limits of detection for each individual PAH are shown in the above table.

Quality control was achieved by subjecting a well-characterised, low-level PAH proficiency-testing marine sediment (Quasimeme – QPH048MS) to the above procedure. A total of three QCs, three procedural blanks and duplicate sample determinations were conducted at intervals throughout the analysis of the samples.

2.7 PCB analysis

The ICES 7 PCB congeners, 28, 52, 101, 118, 138, 153 and 180 were analysed by gas chromatography - mass spectrometry.

Dried samples were extracted with hexane/acetone in an ASE 200 (Dionex) system. Extracts were cleaned on Florisil prior to gas chromatography-mass spectrometry (GC-MS) on a Fisons 8000 GC directly coupled to Fisons MD-800 single-quadrupole mass spectrometer. The limit of detection (LOD) was between 0.1-0.20 μg/kg. Quality control was achieved by subjecting a PCB certified reference material (LGC 6113) to the above procedure (see Vane *et al*., 2007 for details).

2.8 Contextual information on body burdens

In order to place current Milford Haven biomonitoring data into context, we have used the following criteria.

Metals : *comparisons with other estuaries in MBA database*

In order to make direct comparisons with other UK estuaries, metal concentrations in MH samples was compared to equivalent data for the same species in other UK estuaries, contained in our own database. MH data are ranked in comparison to the rest of the UK and expressed as percentiles of the values present in the database. If current MH data are below the lower quartile value (lowest 25% of values) they are plotted as green bars, red if above the upper quartile (highest 25%). Values in the mid range (25-75th percentile) are represented as grey bars.

PCBs, TBT and PAHs: comparison with OSPAR Guideline values

Extensive data are not available for other organic contaminants. OSPAR Environmental Assessment Criteria (EAC) for TBT and PCBs in mussels have been used to put Milford Haven Data into context.
The OSPAR scheme identifies two types of EAC:

a. "EACs (lower)" – concentrations below which it is reasonable to expect that there will be an acceptable level of protection from chronic effects (presented as green bars in the maps for TBT and PCBs shown in the text).
b. "EACs (higher)" – concentrations above which it is reasonable to expect acute toxic effects on marine species (plotted as red bars). The concentrations in between these upper and lower values indicate sub-lethal effects (such as biomarker responses) cannot be ruled out (plotted in grey).

Black bars are used where reference values have not been set for a particular contaminant.

The lower and higher OSPAR environmental assessment criteria (EAC) for ∑ICES7 chloro-biphenyls (CBs) in mussel are approximately 0.75 and 7.5 µg kg^{-1} wet weight, respectively (OSPAR, 2000; NMMP 2004). These have been converted to dry weight value of 5 and 50 µg kg^{-1} dw by multiplying by the average wet:dry weight ratio of 6.66.

The lower and higher OSPAR environmental assessment criteria (EAC) for TBT in mussel are 0.012 and 0.175 mg kg^{-1} wet weight, respectively (OSPAR, 2004).

For a number of PAHs we have compared values in relation to OSPAR 'Background Concentrations' and 'Background Assessment Criteria' for mussels

a) BC–concentrations expected at undeveloped sites around the North Atlantic

b) 'Background Assessment Criteria' (above BAC concentrations, values can be considered 'above background'). These are summarised below (from OSPAR, 2007).

Concentrations at or below the BC values are plotted in green, those above the BAC in red. Concentrations between these values are plotted in grey. Black bars are used for those PAHs where reference values have not been set.

It should be noted that all such classifications are for guideline purposes only and are based on generic data.

Table 4. Background Concentrations (BC) and Background Assessment Criteria (BAC) for PAHs in mussels (2004/5 data; OSPAR, 2007)

	(µg kg^{-1} dry weight)	
	BC	**BAC**
Naphthalene	1	81.2
Phenanthrene	4.5	12.6
Anthracene	1	2.7
Fluoranthene	7	11.2
Pyrene	5.5	10.1
Benz[*a*]anthracene	1.5	3.6
Chrysene	6.5	21.8
Benzo[*a*]pyrene	1	2.1
Benzo[*ghi*]perylene	2.5	7.2
Indeno[123-*cd*]pyrene	2	5.5

3 Results and Discussion

3.1 Metals

Maps showing the distribution of metals in *Mytilus edulis, Cerastoderma edule, Nereis diversicolor, Littorina littorea* and *Fucus vesiculosus* are shown in Figures 2, 3, 4, 5 and 6, respectively. The raw data are presented in Appendix 3.

There were anticipated species differences in body burdens and spatial trends due to physiological and ecological attributes of individual bioindicators and the chemical properties of different metals. However it is possible to make some general observations regarding bioavailability:

- Milford Haven body burdens were generally either equivalent to, or higher than, those at the site in the Tywi Estuary: the latter appeared to be a suitable regional estuarine reference site for most contaminants. One of the few exceptions was the slightly higher Mn burden in *Nereis* at the upper Tywi sediment site (Figure 4): this may reflect local sediment pore water conditions, particularly lower salinity (i.e. this apparent anomaly may be due to natural factors rather than pollution).

- For the majority of metals and species, concentrations in Milford Haven biota were at the lower-middle part of the UK range (green and grey bars, respectively, in Figures 2-6; see legend for explanation).

- Concentrations of a few elements in certain taxa of MH Waterway were consistently at the higher end of the UK range (within the upper 25% of values, as represented by red bars in Figures 2-6). These included As and Se in molluscs and seaweed. Also, elevated levels were observed for individual sites/species: namely, Mn (molluscs, seaweed), Co (mussels, seaweed), Sn (bivalves), Ni (cockles) and Fe (ragworm).

- Increases in bioavailability at upstream sites were evident in several metal-species combinations, which may reflect the influence of geogenic or other land-based sources. This pattern may be enhanced further by lower salinities upstream (greater proportions of more bioavailable forms and less competition from chloride complexation). The strongest of these gradients were seen for Cd (bivalves), Co (molluscs, seaweed), Mn(bivalves, seaweed), Ag, Ni (bivalves, ragworm, seaweed) and Sn (cockles, winkles).

- Based on current body burden data, there was little indication of localised impact (as indicated in raised levels of bioaccumulation) from sources in the lower part of Milford Haven.

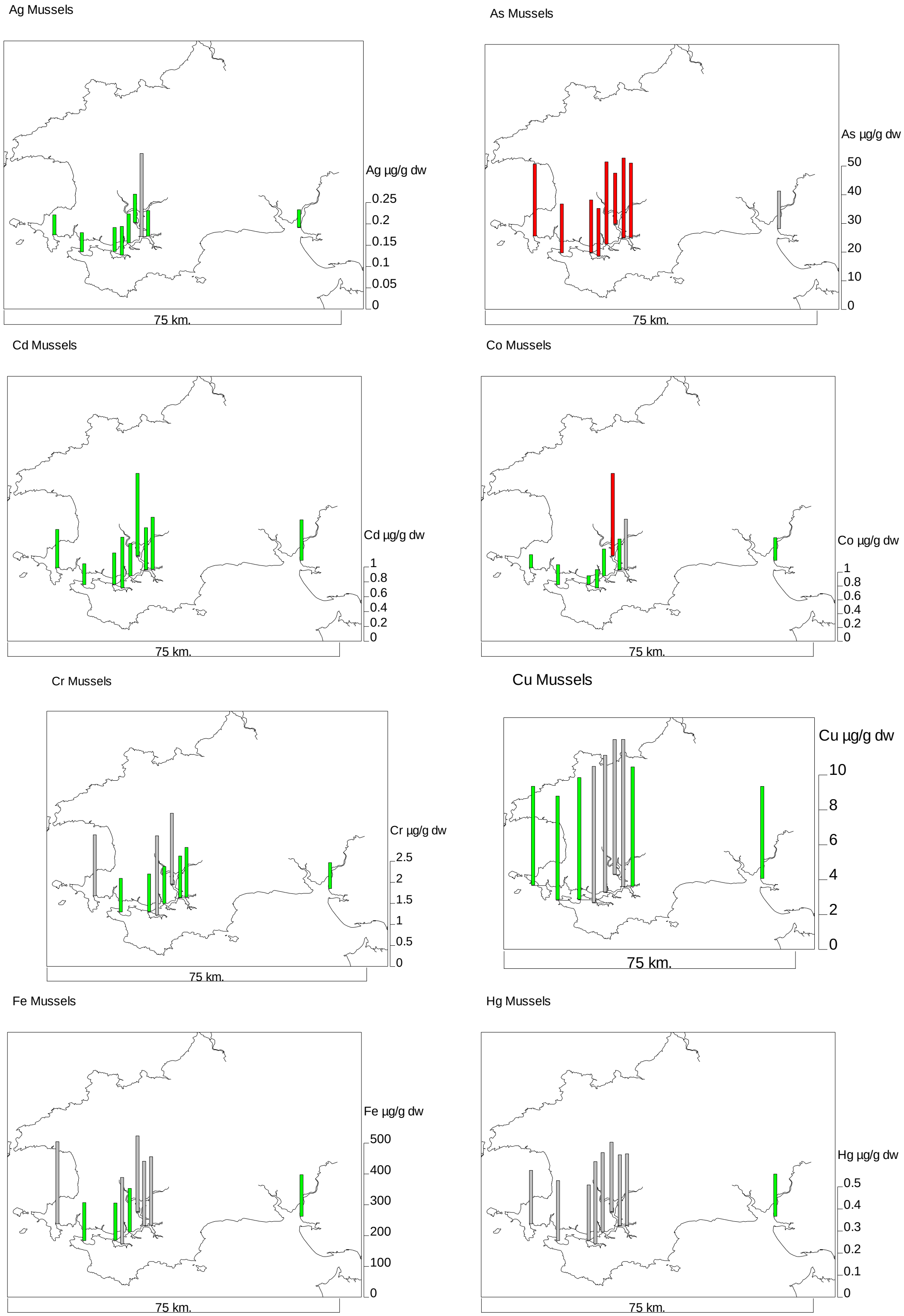
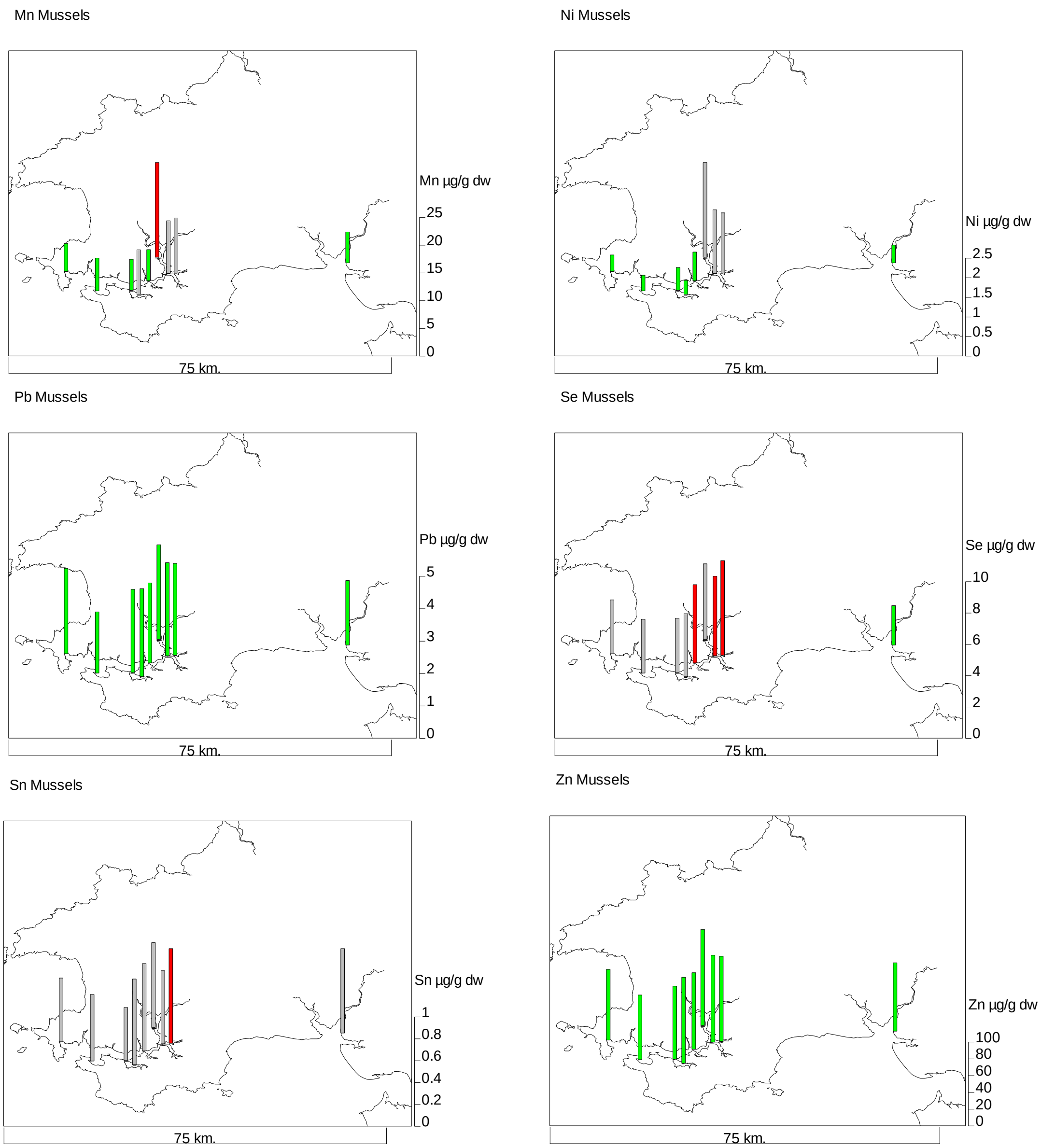


Figure 2. Metals in mussels *Mytilus edulis*, µg g^{-1} dry weight. Values below the lower quartile value (lowest 25%) of values in MBA UK data base are plotted as green bars and red if above the upper quartile (highest 25%). Values in the mid range (25-75th percentile) are represented as grey bars. (cont.)……



...Figure 2 (cont.). Metals in mussels *Mytilus edulis*, µg g^{-1} dw. Values below the lower quartile value (lowest 25%) of values in MBA UK data base are plotted as green bars and red if above the upper quartile (highest 25%). Values in the mid-range (25-75th percentile) are represented as grey bars.

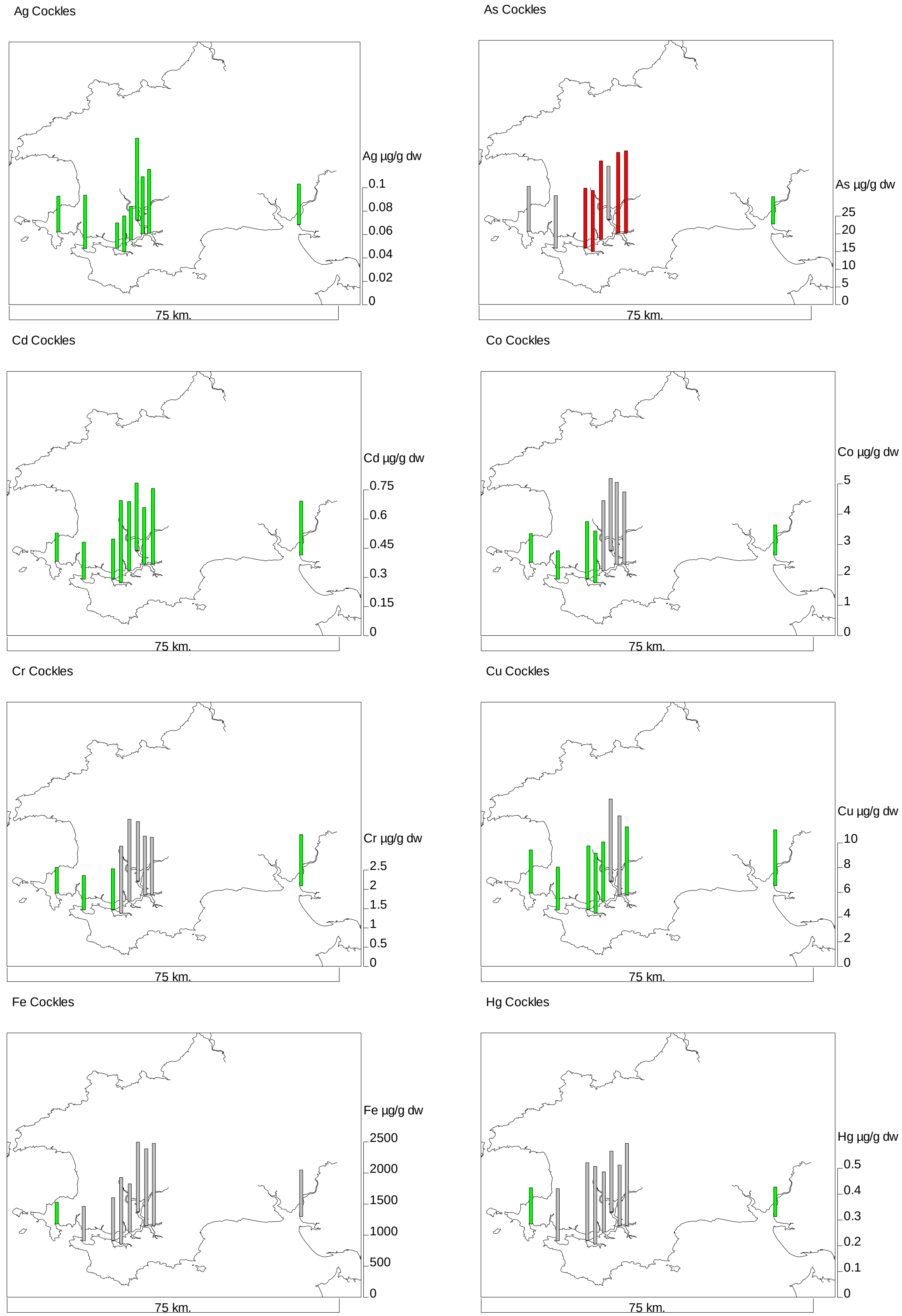
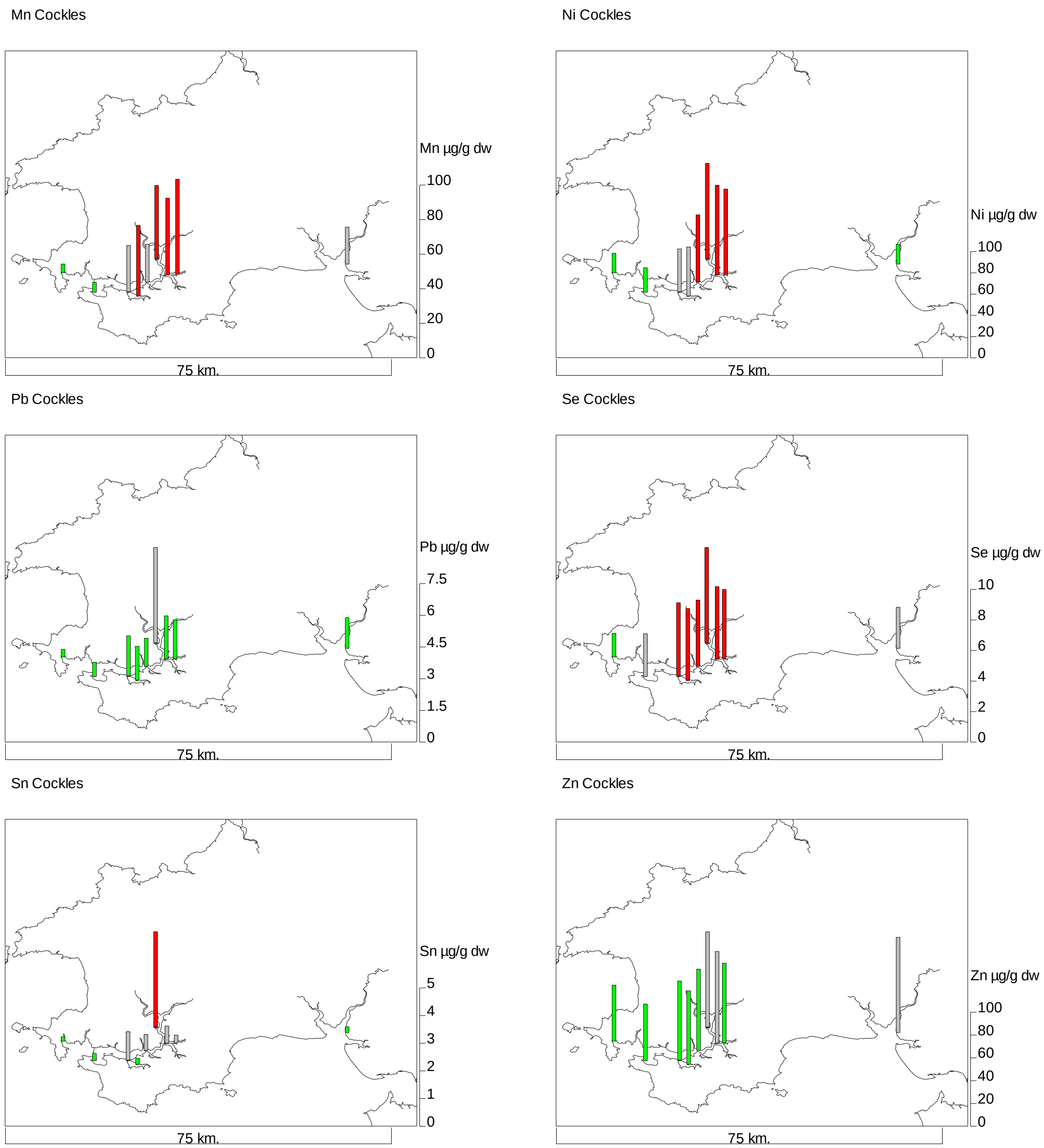


Figure 3. Metals in cockles *Cerastoderma edule*, µg g^{-1} dw. Values below the lower quartile value (lowest 25%) of values in MBA UK data base are plotted as green bars and red if above the upper quartile (highest 25%). Values in the mid-range (25-75th percentile) are represented as grey bars (cont.)....



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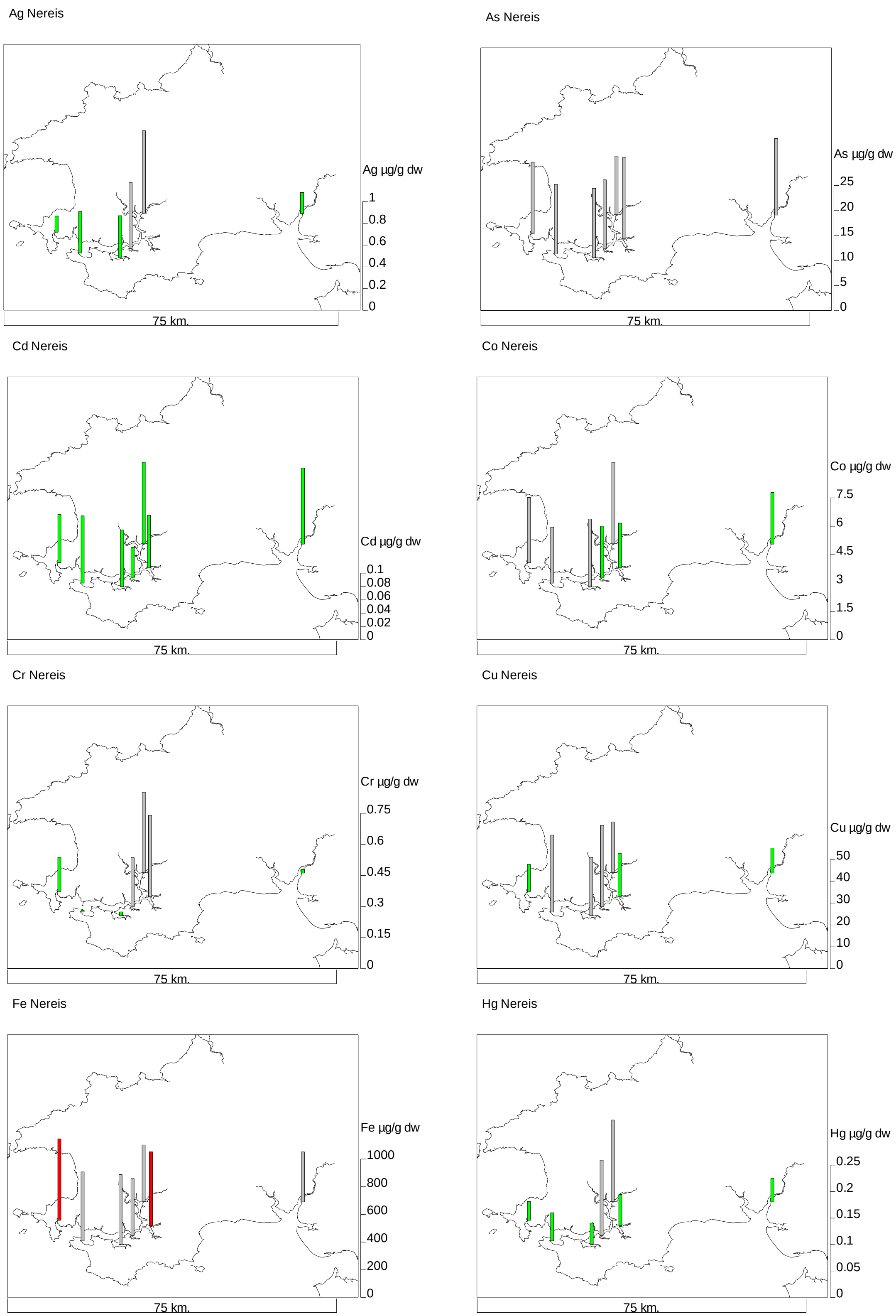
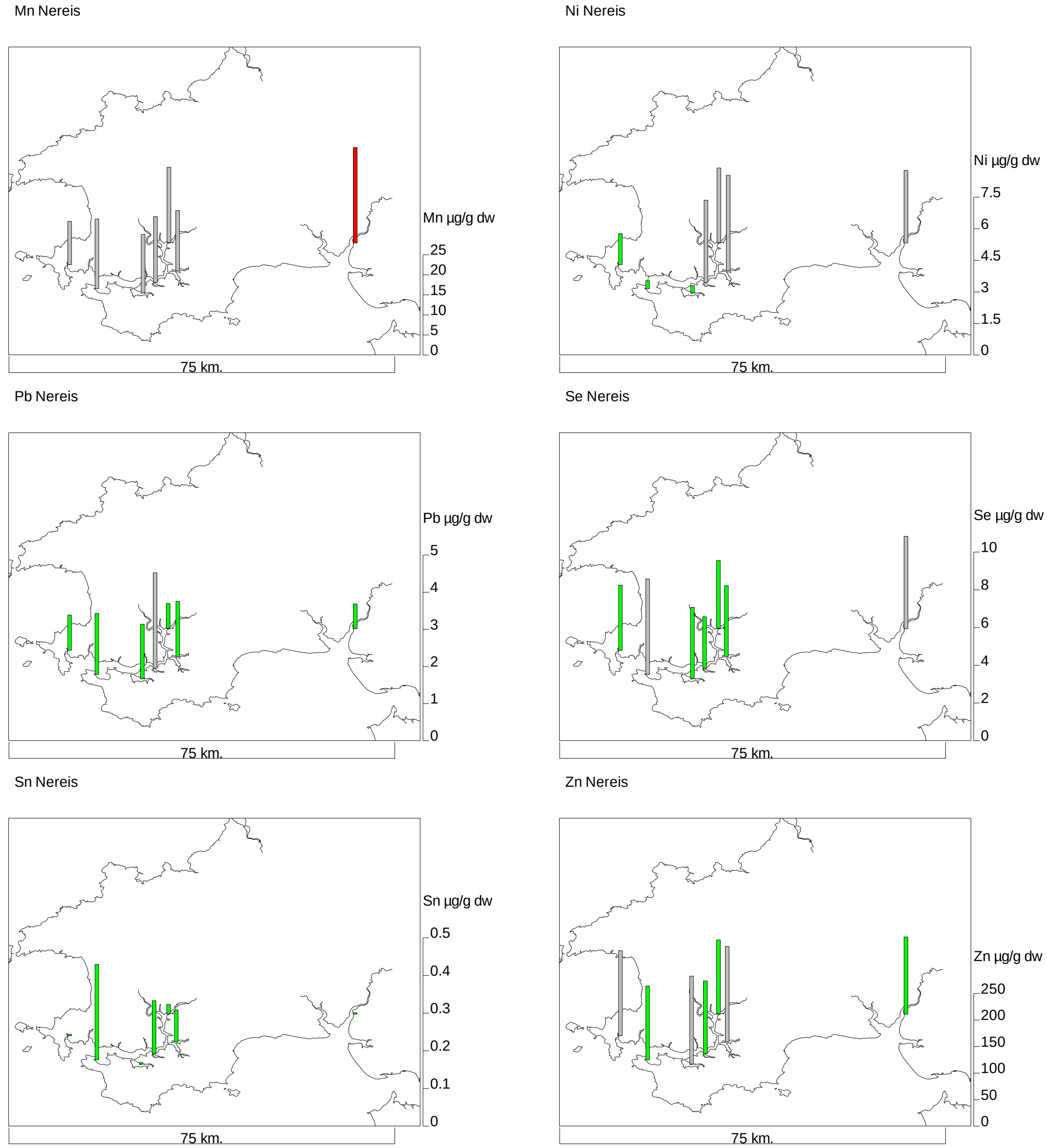


Figure 4. Metals in ragworm *Nereis diversicolor*, µg g^{-1} dw. Values below the lower quartile value (lowest 25%) of values in MBA UK data base are plotted as green bars and red if above the upper quartile (highest 25%). Values in the mid range (25-75th percentile) are represented as grey bars. (cont.)…..



....Figure 4 (cont.). Metals in ragworm *Nereis diversicolor*, µg g^{-1} dw. Values below the lower quartile value (lowest 25%) of values in MBA UK data base are plotted as green bars and red if above the upper quartile (highest 25%). Values in the mid range (25-75th percentile) are represented as grey bars.

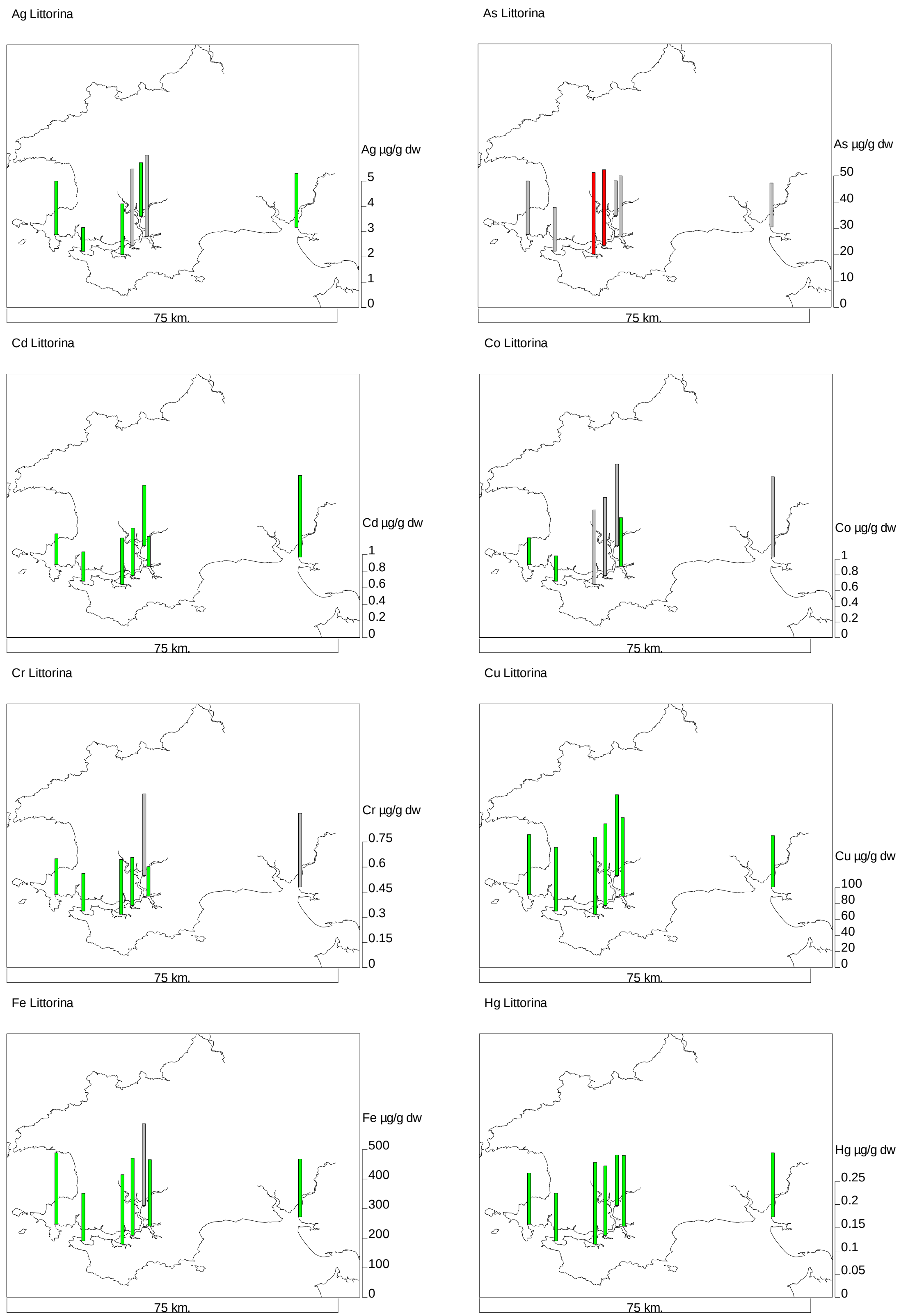
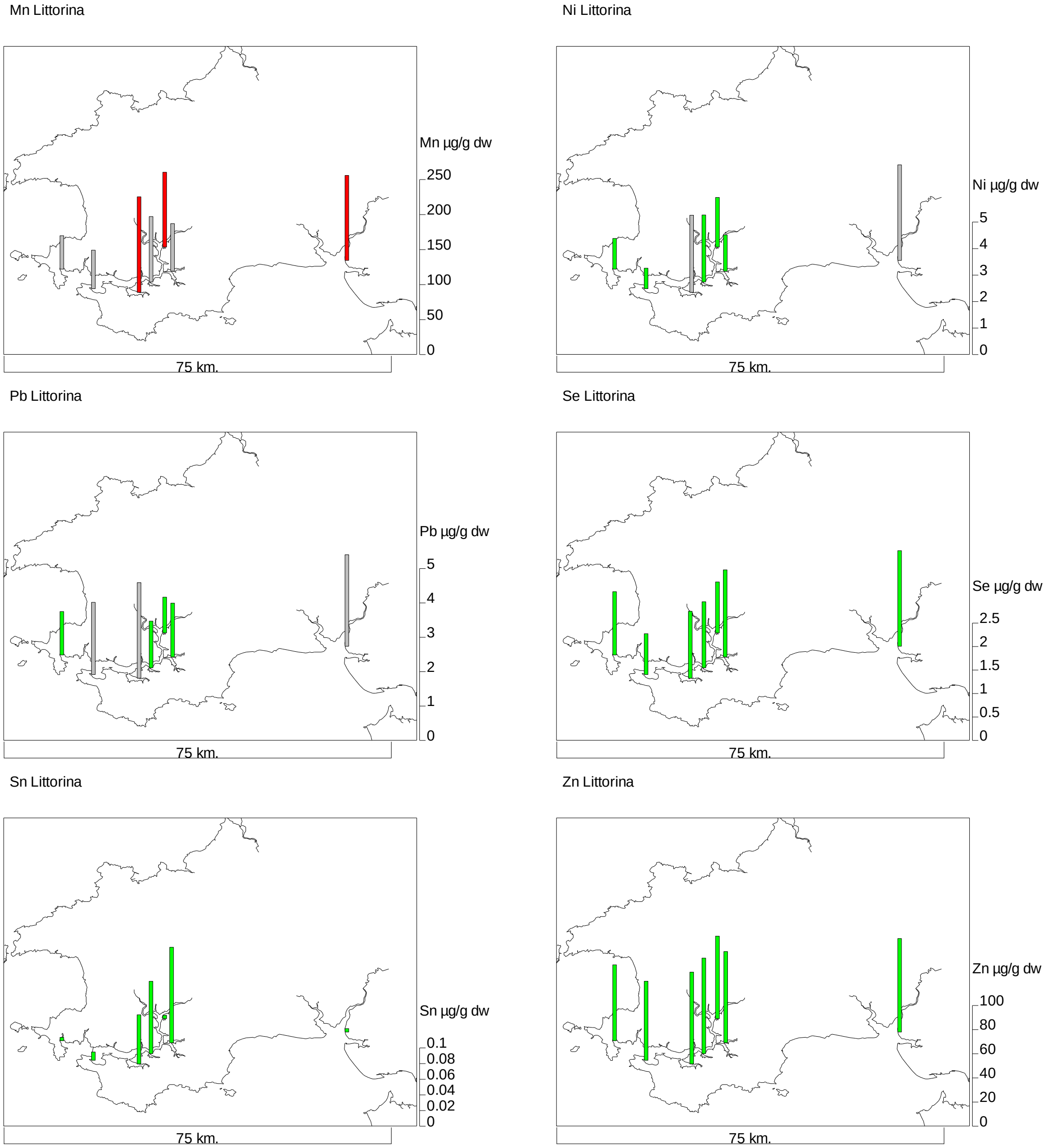


Figure 5. Metals in winkles *Littorina littorea*, µg g^{-1} dw. Values below the lower quartile value (lowest 25%) of values in MBA UK data base are plotted as green bars and red if above the upper quartile (highest 25%). Values in the mid range (25-75th percentile) are represented as grey bars. (cont).....



...Figure 5 (cont.). Metals in winkles *Littorina littorea*, µg g^{-1} dw. Values below the lower quartile value (lowest 25%) of values in MBA UK data base are plotted as green bars and red if above the upper quartile (highest 25%). Values in the mid range (25-75th percentile) are represented as grey bars.

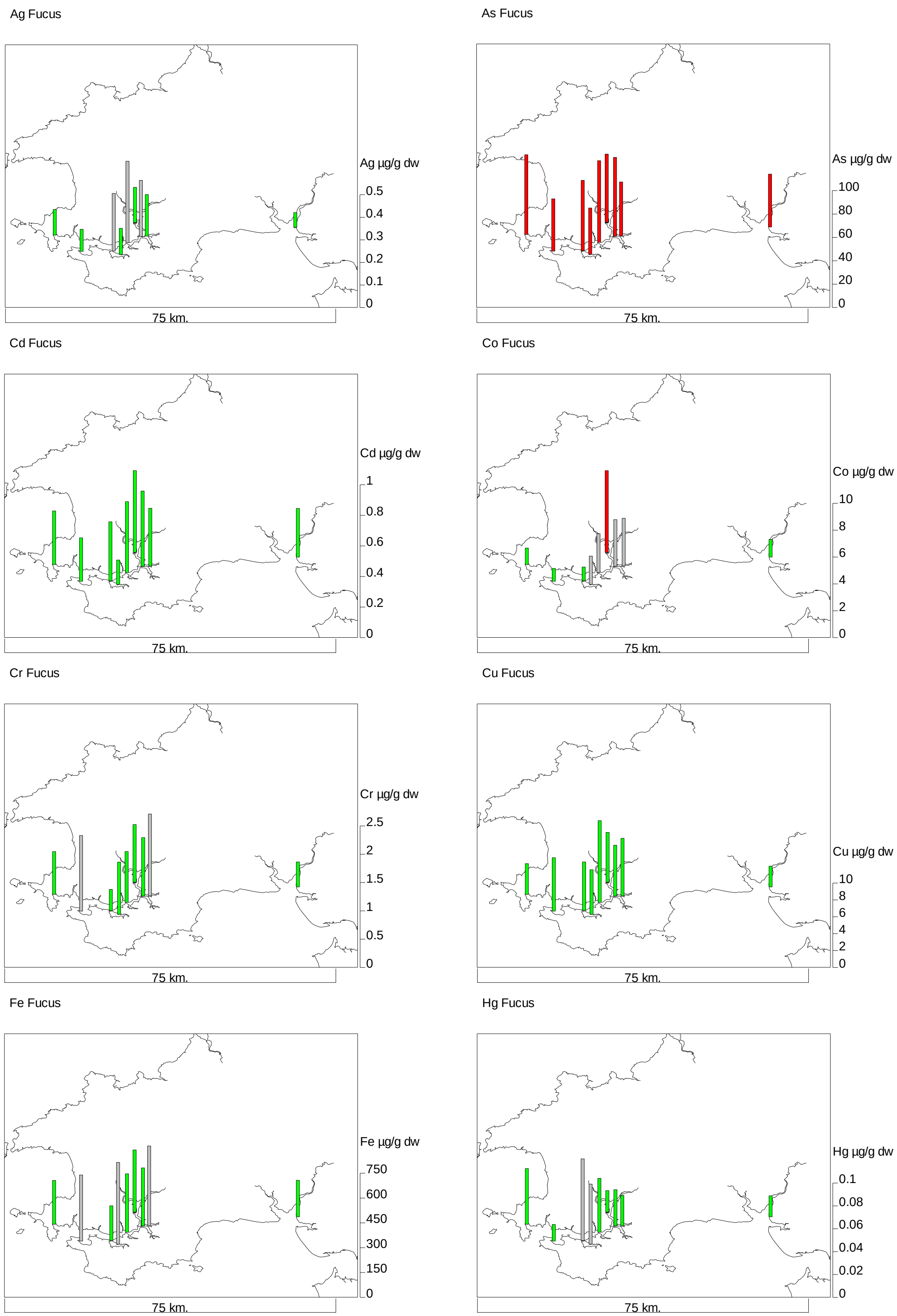
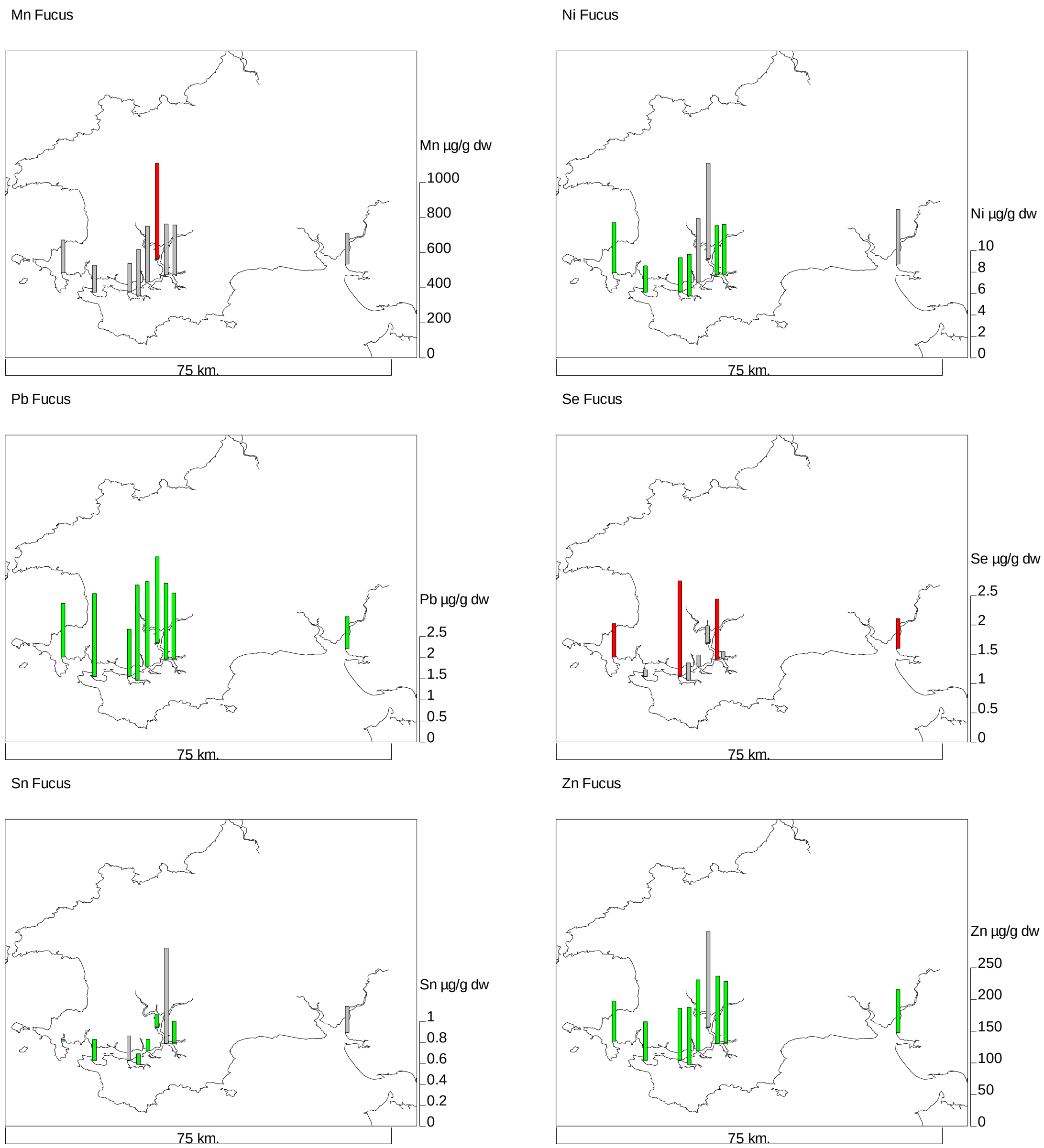


Figure 6. Metals in seaweed *Fucus vesiculosus*, µg g^{-1} dw. Values below the lower quartile value (lowest 25%) of values in MBA UK data base are plotted as green bars and red if above the upper quartile (highest 25%). Values in the mid range (25-75th percentile) are represented as grey bars. (cont.).....



...Figure 6 (cont.). Metals in seaweed *Fucus vesiculosus*, µg g^{-1} dw. Values below the lower quartile value (lowest 25%) of values in MBA UK data base are plotted as green bars and red if above the upper quartile (highest 25%). Values in the mid range (25-75th percentile) are represented as grey bars.

It is, perhaps, simplistic to generalise further over the status of metals in biota from the Haven but, by averaging percentiles across the range of species used, an overview of rankings (for maximum, minimum and mean values in the waterway) can be derived for each metal, in the context of UK ranges (Figure 7). Accepting the limitations, the major features from this data treatment are:

The upper concentrations of As and Mn at MH sites, averaged across all five study species (max percentiles, black bars in Figure 7), suggest that bioaccumulation is consistently high in UK terms, (>80th percentile). Upper values for Co, Fe, Ni, Se and Sn were above the 50th percentile.

Mean levels in MH biota (grey bars, Figure 7) were > 50th UK percentiles for As, Mn and Se confirming widespread degree of contamination throughout much of the estuary. For most other metals, mean levels were close to or below the 25th UK percentile.

Lowest concentrations in MH biota - usually at seaward sites –were within the lowest 10% of UK concentrations in most cases (see white bars Figure 7) and can be considered close to background. Exceptions were As, Mn (Se, Fe) implying some enrichment above background for these elements, even in the least contaminated parts of the Haven.

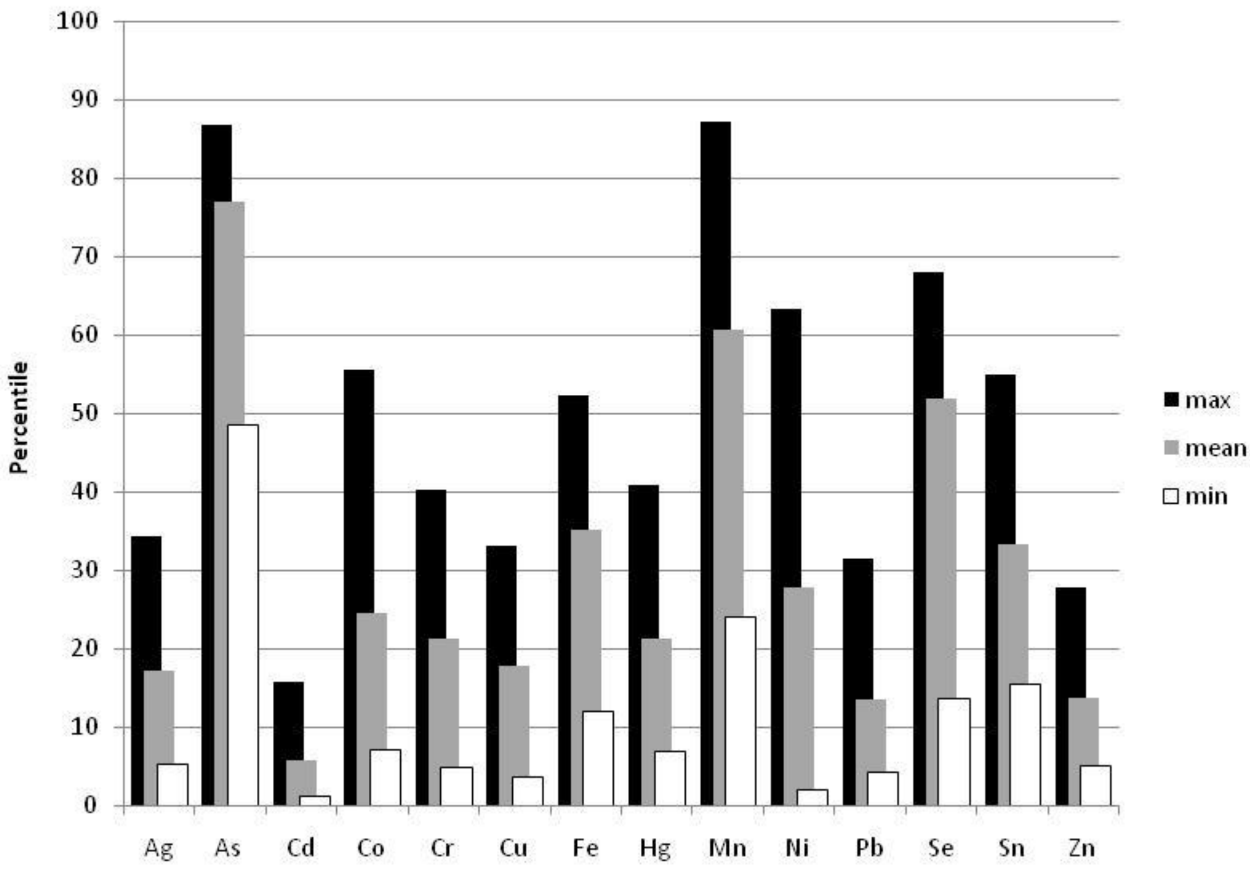


Figure 7. Metals in Milford Haven biota 2007/8. Minimum, maximum and mean values expressed as percentiles of UK ranges (averaged across *Fucus vesiculosus, Littorina littorea, Mytilus edulis, Cerastoderma edule* and *Nereis diversicolor*).

Comparisons with sediments and other sources

There is a *broad* similarity in the spatial patterns of metal bioaccumulation described here with earlier descriptions of sediment-metal distributions, in that highest sediment concentrations tend to be found upstream and minimum values are *generally* those at the mouth of MH Waterway (Smith and Hobbs, 1994). Metal inputs probably occur along much of the waterway from sewage and industrial sources though natural concentrating mechanisms (eg adsorption) can result in net transport of contaminated particles towards the head of the estuary. Here they combine with catchment sources, including a component from weathering of mineralized rocks (Cu, Pb, Zn) and sulphide rich coal measures (Se?). Exceptions to this geochemical trend do occur however: anomalously high sediment concentrations have occasionally been observed near the mouth of the Haven (e.g. Cd and Hg), at the mouth of Cosheston Pill (Hg), and elsewhere, and appear to represent confined 'hotspots'. There is, for example, a landfill site at the industrial estate bordering Cosheston Pill that may be responsible for localized enrichment (Smith and Hobbs, 1994).

It should be stressed that there is a 15y gap between sediment and bioaccumulation surveys and that the current (intertidal) bioaccumulation sites were not the same as (subtidal) sediment sites described by Smith and Hobbs (1994). To improve value, greater harmonization of sampling should be considered in future. On an analytical note, the use of sediment leachates (e.g. 1M HCl) should be considered alongside concentrated acid 'total' digests in future as a better surrogate of biologically-available metal loadings in sediments. This selective extraction technique will also help to distinguish the non-residual (anthropogenic) fraction from the more refractory (less bioavailable) mineral components.

Copper, zinc (and lead) were considered by Smith and Hobbs (1994) to be substantially enriched in most of the MH sediments (compared with world-wide standard shale), partly as a consequence of anthropogenic inputs. In contrast, there were few indications of significant Cu or Zn enrichment in biota during the current study. This is not as contradictory as may appear since many organisms have essential requirements for these elements and may regulate body burdens, thus underestimating environmental contamination – unless at extreme levels. Lead levels in most biota sampled in the present survey were at the lower end of the UK range which may reflect declining levels from anthropogenic sources, notably following removal of alkyl lead from petrol.

The content of most other metals in Milford Haven sediments described by Smith and Hobbs (1994) were, with the exception of Co, similar to typical concentrations in many estuaries in SW Britain (excluding mining-impacted estuaries)- based on comparisons with earlier MBA data (Bryan *et al.,* 1980). Enrichment in Co in sediment from upstream sites in MH waterway was considered indicative of catchment sources, probably natural in origin and was reflected in the pattern of bioaccumulation in seaweed and mussels in the current survey.

Estimates made almost 20y ago indicated that the relative inputs of organic pollution to Milford Haven were divided equally between freshwater, industry and sewage, even though volumes were dominated by freshwater (96%), of which the W and E Cleddau represent some 80% of total FW flow. Inputs of Cu, Pb and Zn from these two rivers were estimated to be in the region of 336, 336, 1000 and 381, 381, 1300 kg y^{-1}, respectively (Hobbs and Morgan, 1992; Bent, 2000). Atmospheric discharges (from Pembroke Power Station, 1993) were estimated to be 4.4 (As), <300 (Cd, Cr, Cu), 6600 (Fe) 13600 (Ni) and 65300 (V) kg y^{-1} whilst <1% of total pollutant load comes from road run-off. Contaminated land/ landfill inputs are potential, if as yet unquantified sources. Likewise, budgets for Cu and Zn leaching from antifouling are unknown. More accurate and updated loadings estimates for contaminants would be a useful item for the future in terms of identifying specific sources of bioaccumulation.

Temporal trends

There are few previous published bioaccumulation data with which to compare current results. Generally, these earlier studies do not indicate exceptional metal bioaccumulation in biota of Milford Haven, in agreement with the current data. Mussel samples from 1996 and 1997 collected by Widdows *et al*. (St Ishmaels, near Dale) are perhaps most reliable and relevant to the 2008 survey (~ 1km across Dale Roads from our Dale samples). Taking the 1996 data as baselines (zero), we have expressed increases and decrease in values as percentage change in Figure 8. Comparison between 1996 baselines and 1997 data of Widdows *et al.* (filled bars) indicates that inter-annual variation is relatively high for several contaminants with As, Hg (and TBT and DBT) increasing over time. In the 2008 survey (unfilled bars, Figure 8), As and Hg remain higher than 1996 baselines, whilst organotins have decreased. There have been consistent declines also for Cd, Ni, Se and, to a small extent, Cu.

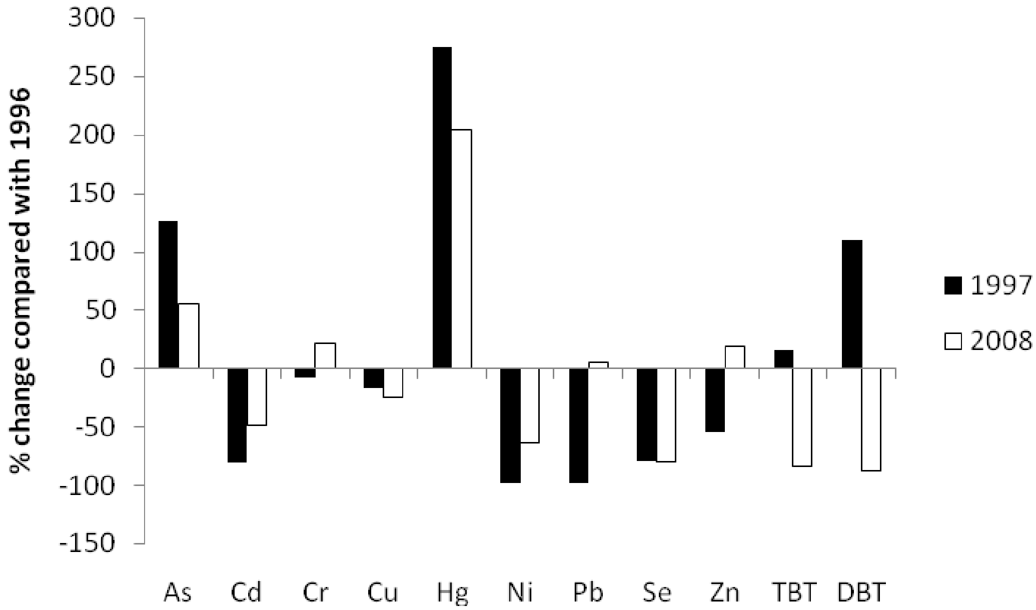


Figure 8. ***Mytilus edulis*** **metal and organotin concentrations –percentage change measured in the current survey (and in 1997) compared with 'baseline' concentrations in 1996 (data from Widdows** ***et al.,*** **2002 and this study).**

Earlier biomonitoring with seaweeds, as part of the NRA Welsh Region Marine Biological Programme, included data for Cd, Cu and Zn at Dale and Lawrenny in Milford Haven, together with Ferryside in the Tywi, between 1993-5 (Davies and Ellery, 1995). These are plotted alongside results for the same sites from the current project (Figure 9). During the intervening period, Cd concentrations have dropped throughout the area by ~50-60% (comparable trends also seen in mussels, above), perhaps in response to the controls on Cd in the wider area (including important regional sources in the Severn Estuary). Copper concentrations have also dropped by ~one-third at MH sites and by more than two-thirds in the Tywi (though the 1995 data appear to have been exceptionally high). In contrast, the changes in Zn concentrations have been relatively small over the last 15 years.

It should be stressed however that the data sets are as yet rather limited to establish temporal trends with any confidence. Further comprehensive surveys of the type described here, using comparable methodology, are needed to confirm long-term change.

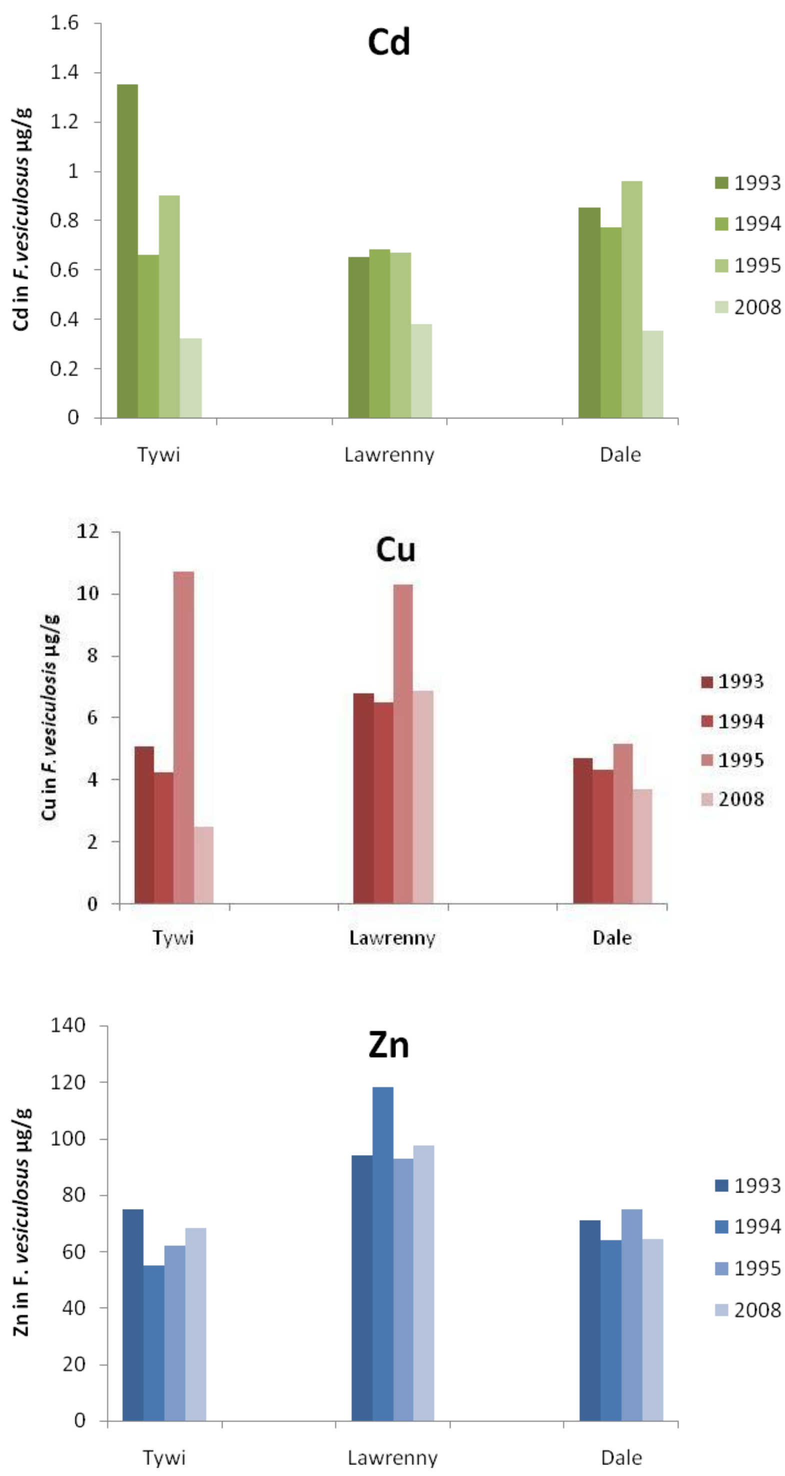


Figure 9. ***Fucus vesiculosus*** **metal concentrations measured in the current survey compared with concentrations in the previous decade (Davies and Ellery, 1995).**

3.2 TBT and other organotins

Data on butyltin body burdens are displayed in Appendix 4. Butyltin (TBT, DBT, MBT) concentrations in mussels are shown in the left-hand series of maps in Figure 10 and for the most part indicate that concentrations increased upstream. The exception was a relatively high value for MBT at Dale. The values for TBT are plotted with respect to Environmental Assessment Criteria (OSPAR) and were above the lower EAC (below which effects are not expected) but below the upper EAC (acute effects expected): the grey bars in the TBT plot signify that sub-lethal effects cannot be ruled out at the Milford Haven sites. In contrast, TBT concentrations (and other BTs) in mussels from the Tywi reference site were close to zero. Black bars are used to represent those organotins where reference values have not been set.

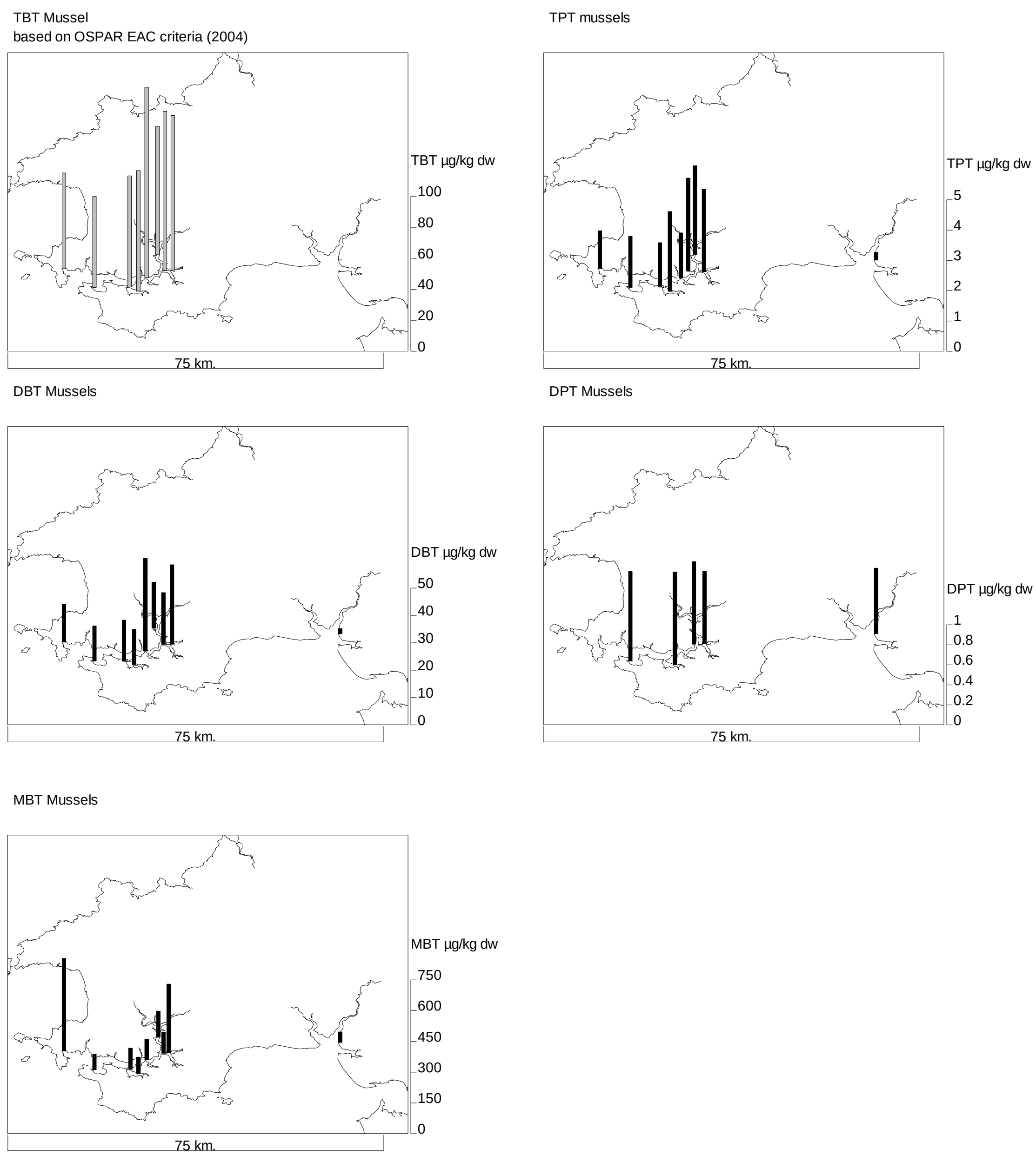


Figure 10. Organotin concentrations (µg kg^{-1} dw) in mussels *Mytilus edulis.*

The proportion of total $\sum$BT (TBT+DBT+MBT) present as the parent compound TBT probably signifies the relative importance of recent sources (a high proportion of TBT indicates fresh inputs). In the current survey, the proportion of TBT varied from 12% at Dale to almost 50% at Pembroke Ferry (mean 31%). In contrast mussels from the reference site in the Tywi estuary contained <1% TBT with most of the BT burden present as the breakdown product MBT. This implies that the influence of recently introduced TBT was greatest in the vicinity of Pembroke Ferry.

The concentrations of phenyltins were much lower than butyltins (TPT and DPT are shown on the right in Figure 10, MPT was below detection). Presumably this reflects the low usage of TPT as an antifouling agent on ships entering the Haven, compared with TBT. Nevertheless the pattern of TPT distribution in mussels closely resembles that of TBT. The proportion of total PT (TPT+DPT+MPT) present as the parent compound, TPT, varied from 66-100% in MH mussel samples (mean 81%), compared with 29% at the reference site. Again this infers more recent input to the MH waterway and that phenyl tins, though present at lower concentrations than butyltins, may persist for longer before degradation.

In a study of mussel condition around the Irish Sea in 1996/7 the concentrations of organotins (OT) were generally low at coastal sites but elevated near harbours, with ΣBT concentrations ranging from 'not detected' to 0.66 μg g^{-1} dry wt (Widdows *et al*., 2002). The highest value on the UK mainland occurred in mussels in Milford Haven, from St. Ishmaels in the vicinity of the oil terminal (Widdows *et al*., 2002). The reported concentrations of TBT (0.44 μg g^{-1}) were above the threshold of 0.2 μg g^{-1} dry wt at which uncoupling of oxidative phosphorylation, the primary mechanism of toxicity, commences. Whilst not threatening the survival of mussels, body burdens of this order would represent a challenge to the reproductive success of TBT-sensitive neogastropods such as dogwhelks *Nucella lapillus*. Previous surveys on OT effects in dogwhelks in western coastal waters have indicated that TBT body burdens and imposex in *N. lapillus* were generally highest near the central and outer part of Milford Haven (where tankers moor), decreasing (with anomalies) with distance seaward, notably outside the Haven (Harding *et al*., 1998). Neyland Marina has also previously been identified as a TBT 'hotspot', whilst levels in the Cleddau were considered typical of UK estuaries (Kitts, 1999; Bent, 2000).

There are few suitable data to establish temporal trends in organotins with accuracy, though the values measured a decade ago in Milford Haven mussels near the oil terminal, described by Widdows *et al*. (2002), were higher than any of the current values (maximum 0.12 and 0.53 μg g^{-1} dry wt for TBT and $\sum$BT, respectively). Theoretically, the global ban on TBT in 2008 should start to produce a further decline in TBT contamination. The current data set is therefore a timely and suitable platform to test this hypothesis and to establish the rate of recovery.

Organotin concentrations in the ragworm *Nereis diversicolor* are shown in Figure 11 Spatial distributions appear somewhat different to those in mussels for a number of possible reasons, including the fact that they live and feed in benthic sediments. Bioavailability of TBT in this phase may influence body burdens to a greater extent than filter feeding mussels whose main source of TBT is probably the overlying water (including suspended organic matter). Secondly, the sites at which *Nereis* were collected were slightly different to mussel sites (and two fewer in number). *Nereis* may also be better at metabolizing TBT to DBT and MBT. Thus concentrations of TBT in *Nereis* were on average lower than in mussels by a factor of four.

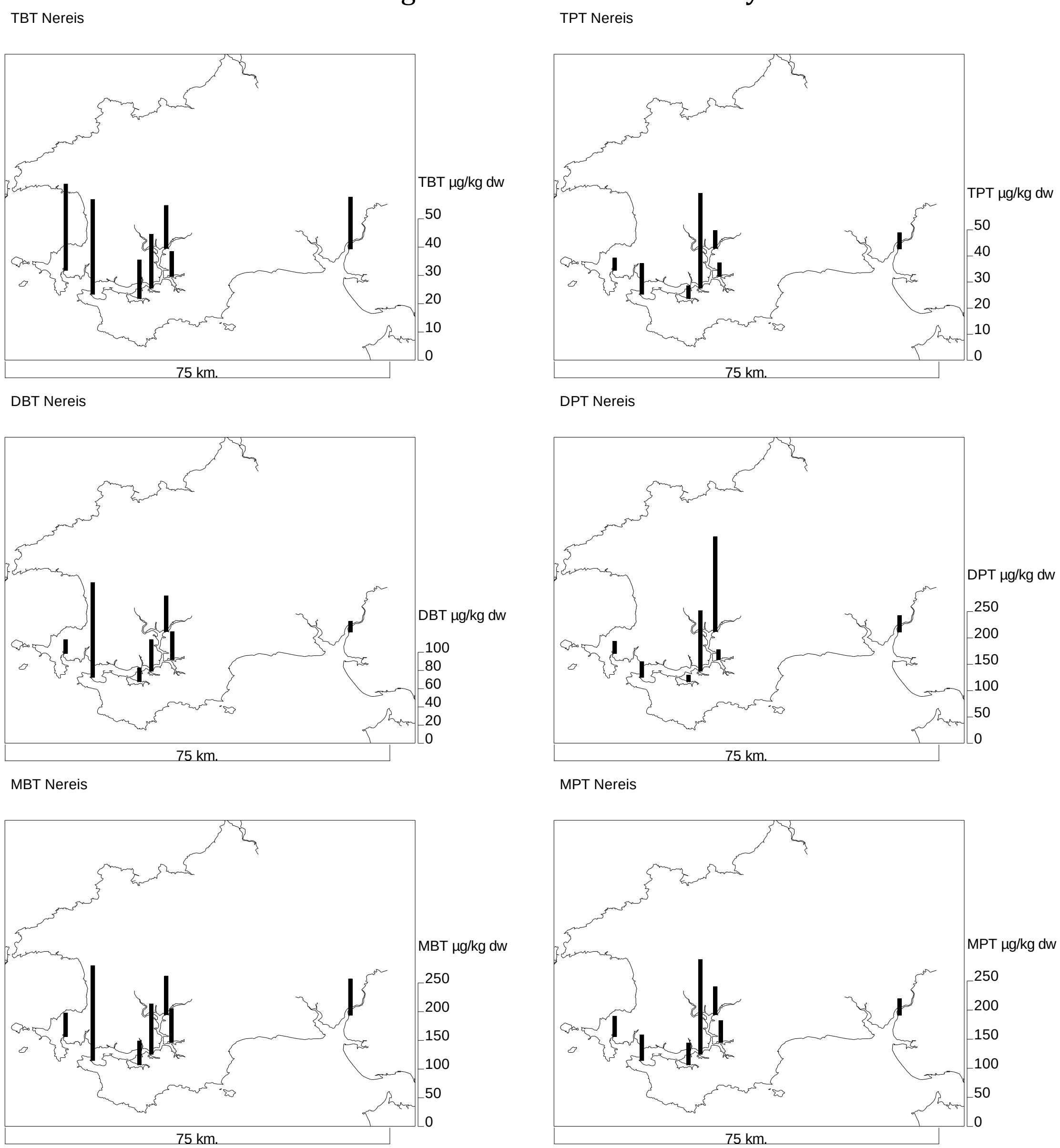


Figure 11. Organotin concentrations (µg kg^{-1} dw) in ragworm *Nereis diversicolor.*

Phenyltin bioaccumulation in *N. diversicolor* is shown in the right-hand column of diagrams in Figure 11. In contrast to mussels, the average triphenyltin (TPT) concentration in worms was five-fold higher than that in mussels (DPT seventy-fold higher), consistent with observations that phenyltins partition more strongly towards sediment and might therefore be more available to sediment-dwelling species. Additionally, the *Nereis* site with highest TPT (and MPT) levels, at Pembroke (Waterloo), was within Cosheston Pill –perhaps subjected to localized sources compared with the mussel site in the main waterway (Pembroke Ferry). The use of TPT in antifouling has been less extensive than TBT in the UK; however, phenyltins have in the past been used, additionally, in agriculture - as pesticides and fungicides.

3.3 PAHs

Data on individual PAHs and total (∑PAH) body burdens are displayed in Appendix 4. Compared with other bivalves such as oysters and cockles, mussels tend to be better accumulators of hydrocarbons and have been used preferentially in monitoring surveys. Results of PAH concentrations in *M. edulis* sampled during the current project are shown in Figure 12, below.

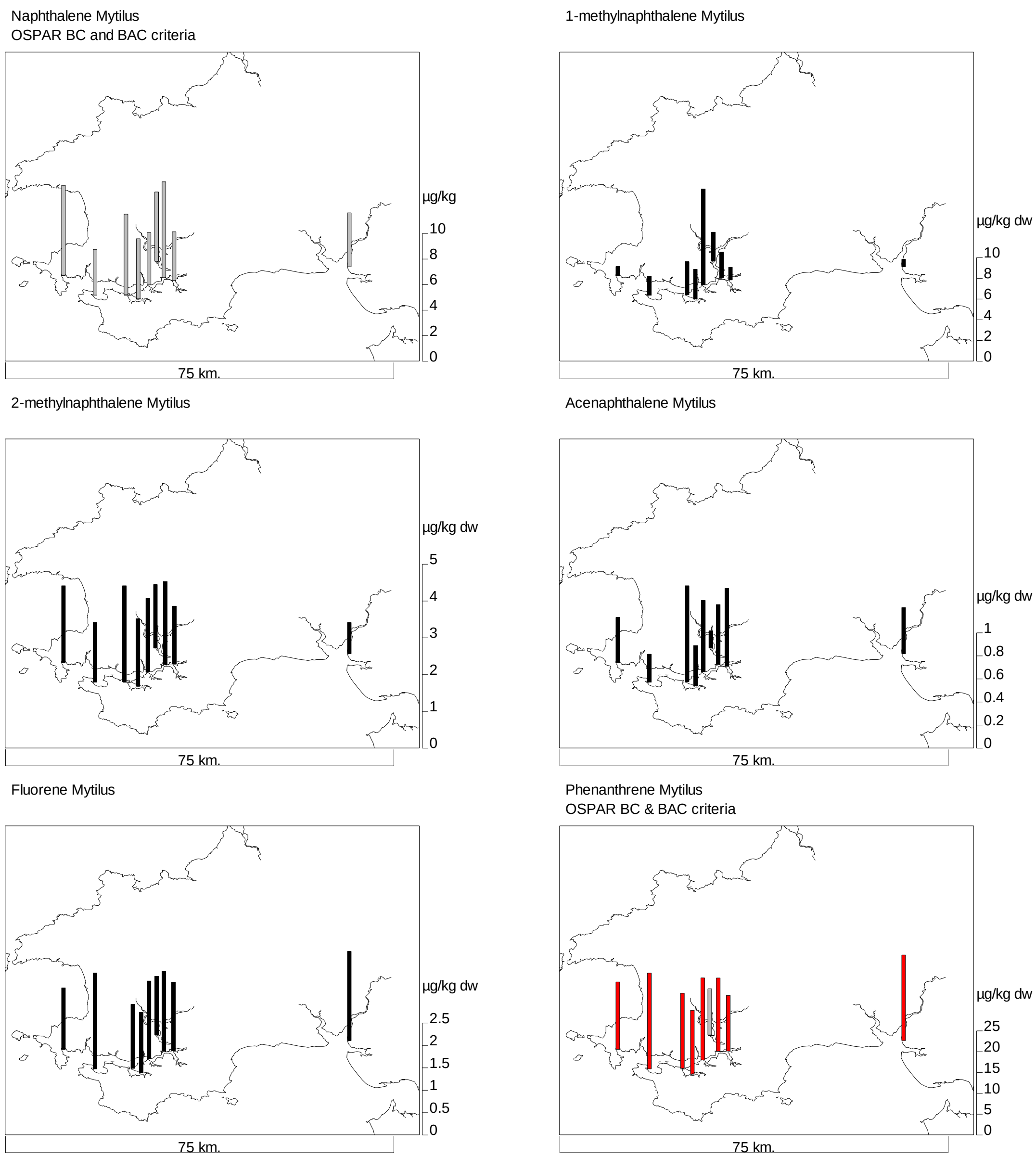


Figure 12. PAHs in *Mytilus edulis* in context with Background Concentration (BC) and Background Assessment Criteria (BAC) for mussels (cont.).....

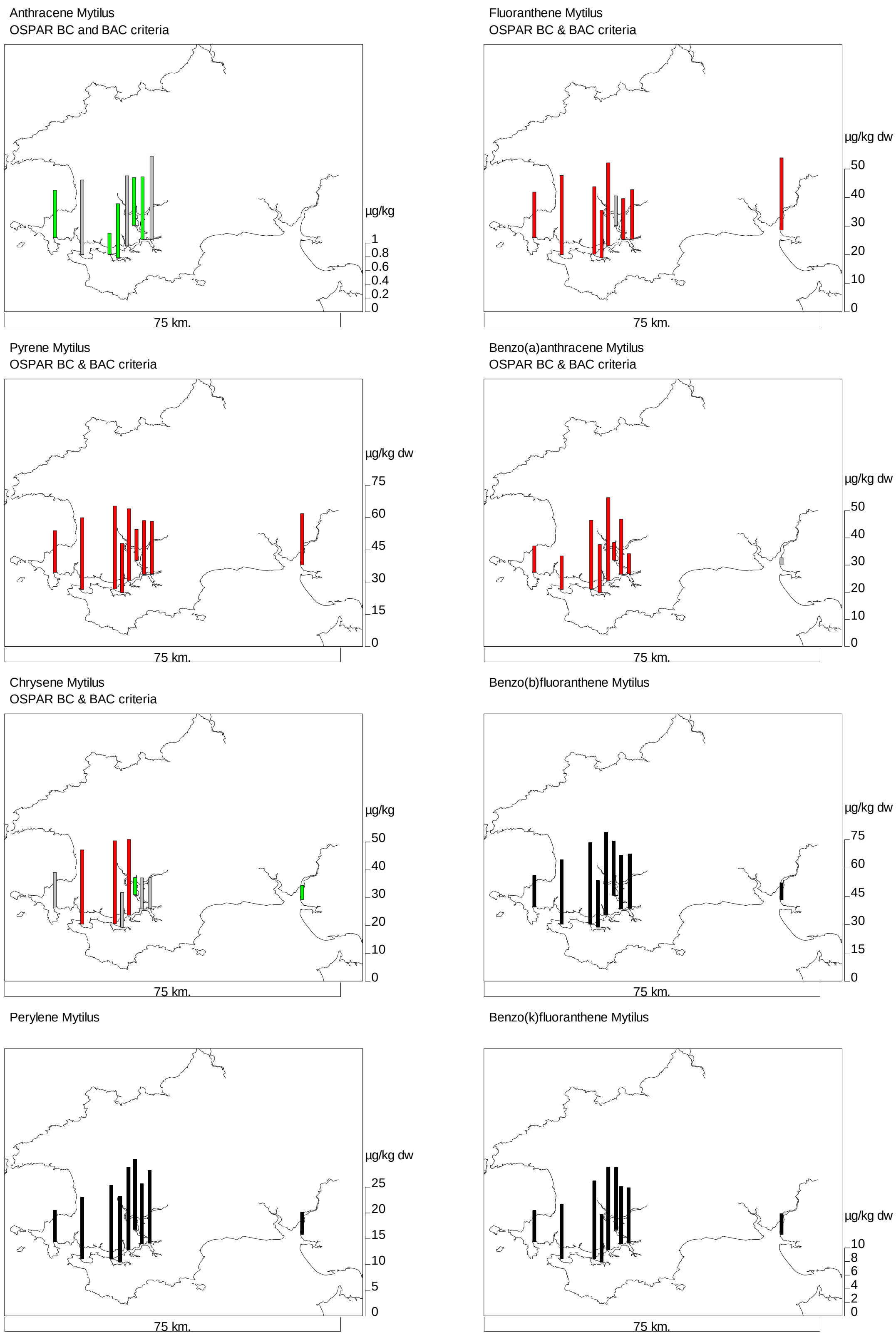


Figure 12 (cont.). PAHs in *Mytilus edulis* in context with Background Concentration (BC) and Background Assessment Criteria (BAC) for mussels (cont.).....

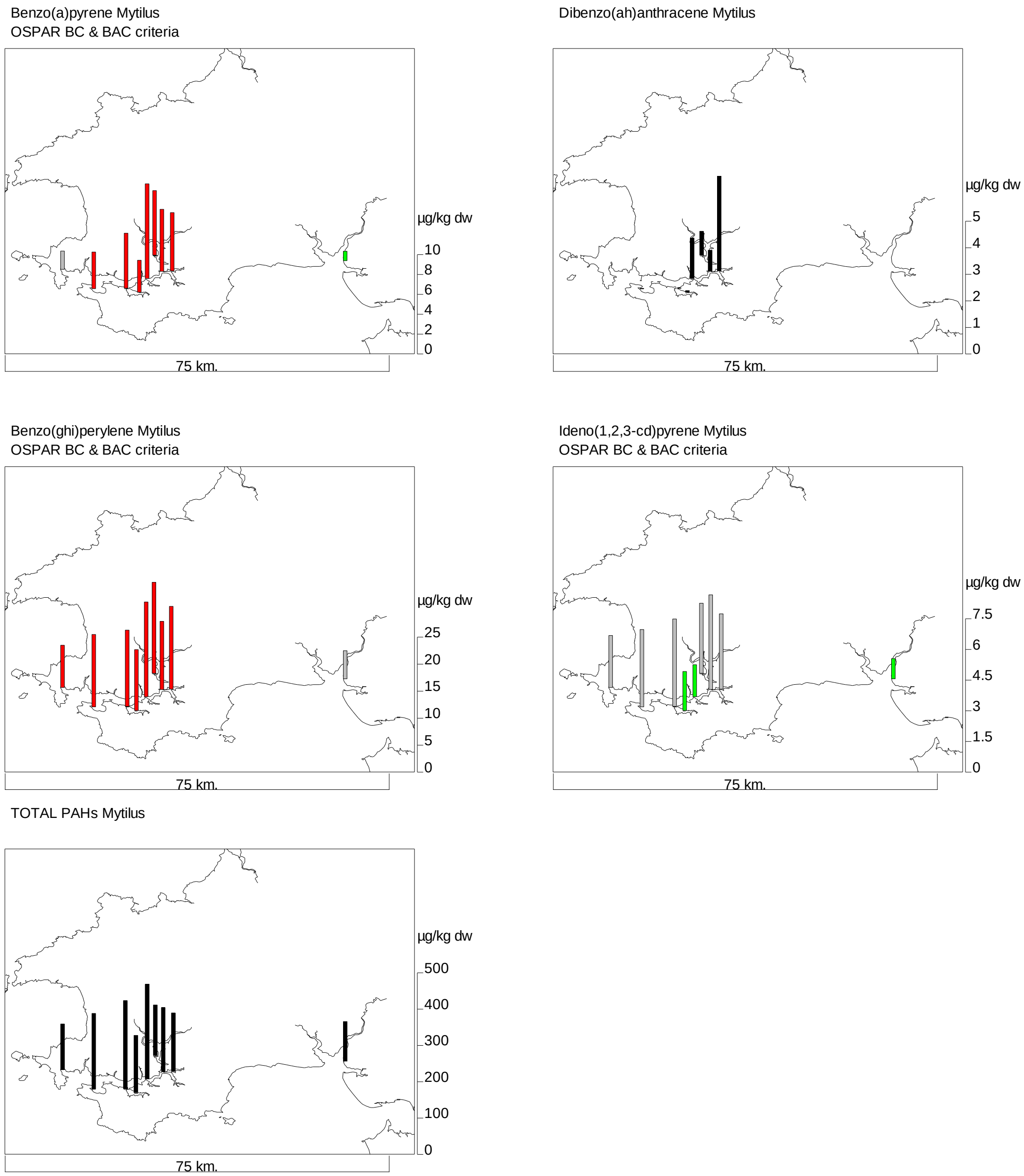


Figure 12 (cont.). PAHs in *Mytilus edulis* in context with Background Concentration (BC) and Background Assessment Criteria (BAC) for mussels.

The main conclusions for PAHs in *Mytilus,* drawn from the data in Figure 12, are:

- Milford Haven body burdens were generally higher than those at the reference site in the Tywi Estuary, although for several PAHs (fluorene, phenanthrene, fluoranthene, and pyrene) concentrations were not substantially different.

- Distributions of individual PAHs within MH waterway tended to fall into three groupings, perhaps reflecting similarity in their origins and chemistry. Thus, some PAHs such as fluorene and phenanthrene varied little across the sites whereas others, including 1-methyl-naphthalene, acenaphthene, benzo(a)anthracene and benzo(a)pyrene, tended to be elevated in the central and upper parts of the system. For the remaining PAHs, most exhibited a gradual upstream gradient. These distributions were subtly different to those in *Nereis diversicolor* (Figure 13, below) and, as discussed in the context of organotins, could be due to in part to localized sampling site differences between the two species and the stronger influence of sediment contamination on *N. diversicolor,* The influence of habitat may explain, for example, why PAH enrichment was not seen at Dale (Pickleridge Beach) in the mussel profiles but was sometimes evident in worms from the nearby muddy creek site. Species differences in the activity of enzymes which metabolise PAHs (e.g. cytochrome P450 system) will also contribute to the variation in bioaccumulation patterns.

- Total ∑PAH profile for mussels shown in Figure 12 presents an even distribution along MH waterway with only slight enrichment upstream; this distribution will obviously be dominated by those PAHs present in highest concentrations.

- There are few published data on PAHs in *Mytilus edulis* with which to compare the current results. The 2008 survey data are plotted in Figure 12 in the context of OSPAR Background Concentration (BC) and Background Assessment Criteria (BAC) for mussels, for those PAHs where reference values are set. Only those concentrations depicted in red would be considered above background (though not necessarily of ecotoxicological importance). This was observed widely for phenanthrene, fluoranthene, pyrene, benzo(a)anthracene, benzo(a)pyrene and benzo(ghi)perylene. In contrast, there were a few PAH concentrations which would be considered as background and representative of undeveloped sites (green bars, Figure 12- see, for example, anthracene). Grey bars lie in between BC and BAC. Black bars are used to represent those PAHs where reference values have not been set.

- Provisional OSPAR ecotoxicological assessment guidelines were proposed for PAHs in mussels in 1995 but do not appear to have been adopted officially. Based on the 2008 body burden data in *Mytilus,* these guidelines would currently not be exceeded in Milford Haven (acute effects not expected), but in reality the extent of sublethal effects is unknown.

Biomonitoring results for PAHs in the sediment-dwelling *Nereis diversicolor* are plotted in Figure 13. Specific 'reference values' for *N. diversicolor* are not available and to provide contextual information, MH samples are compared with reference values for mussels (for those PAHs where baselines have been set), Bars depicted in red in Figure 13 would be considered above background; green bars represent concentrations characteristic of undeveloped sites; grey bars lie in between. Black bars are used to represent those PAHs where reference values have not been set.

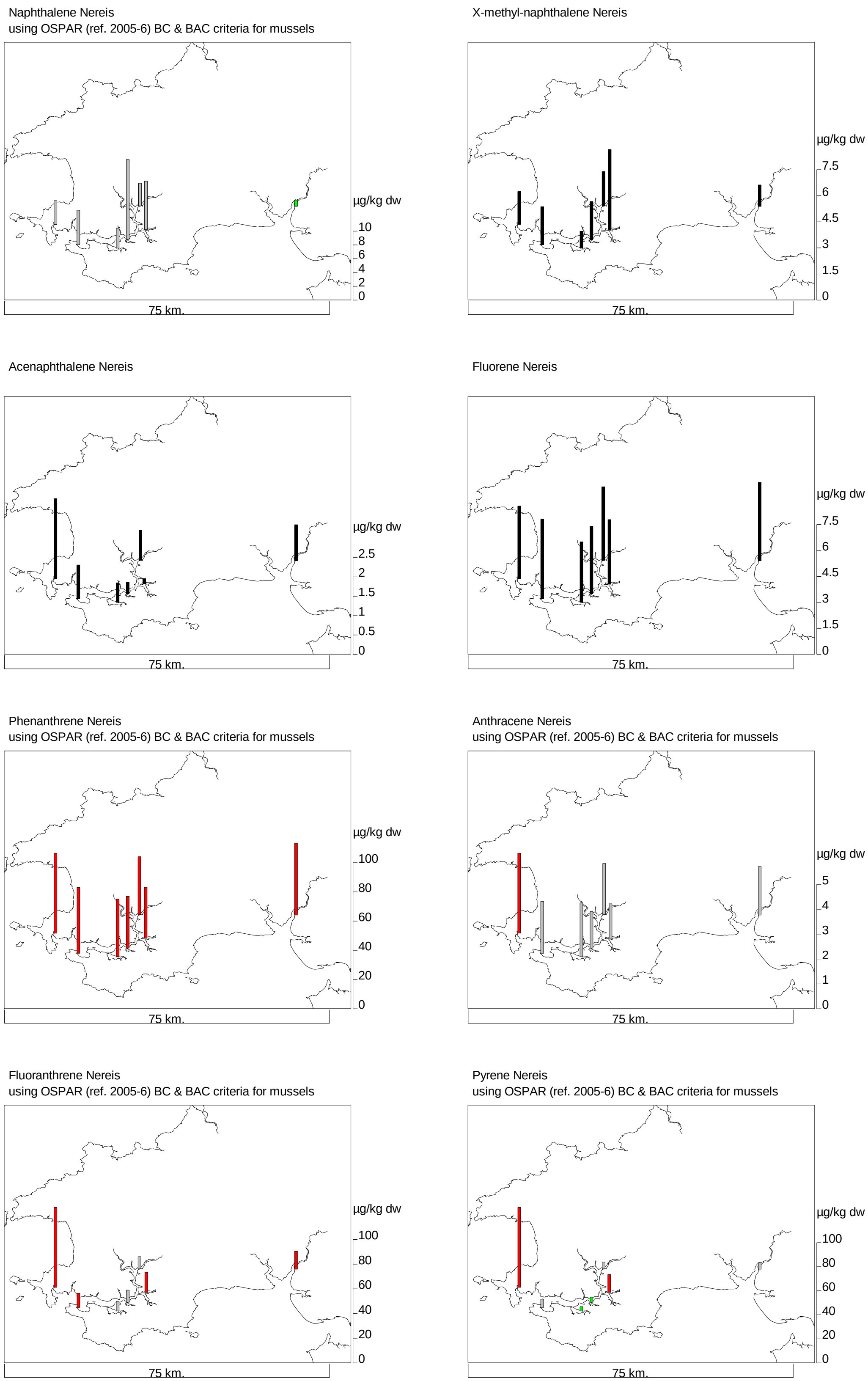


Figure 13. PAHs in *Nereis diversicolor* in context with Background Concentration (BC) and Background Assessment Criteria (BAC) for mussels (cont.).....

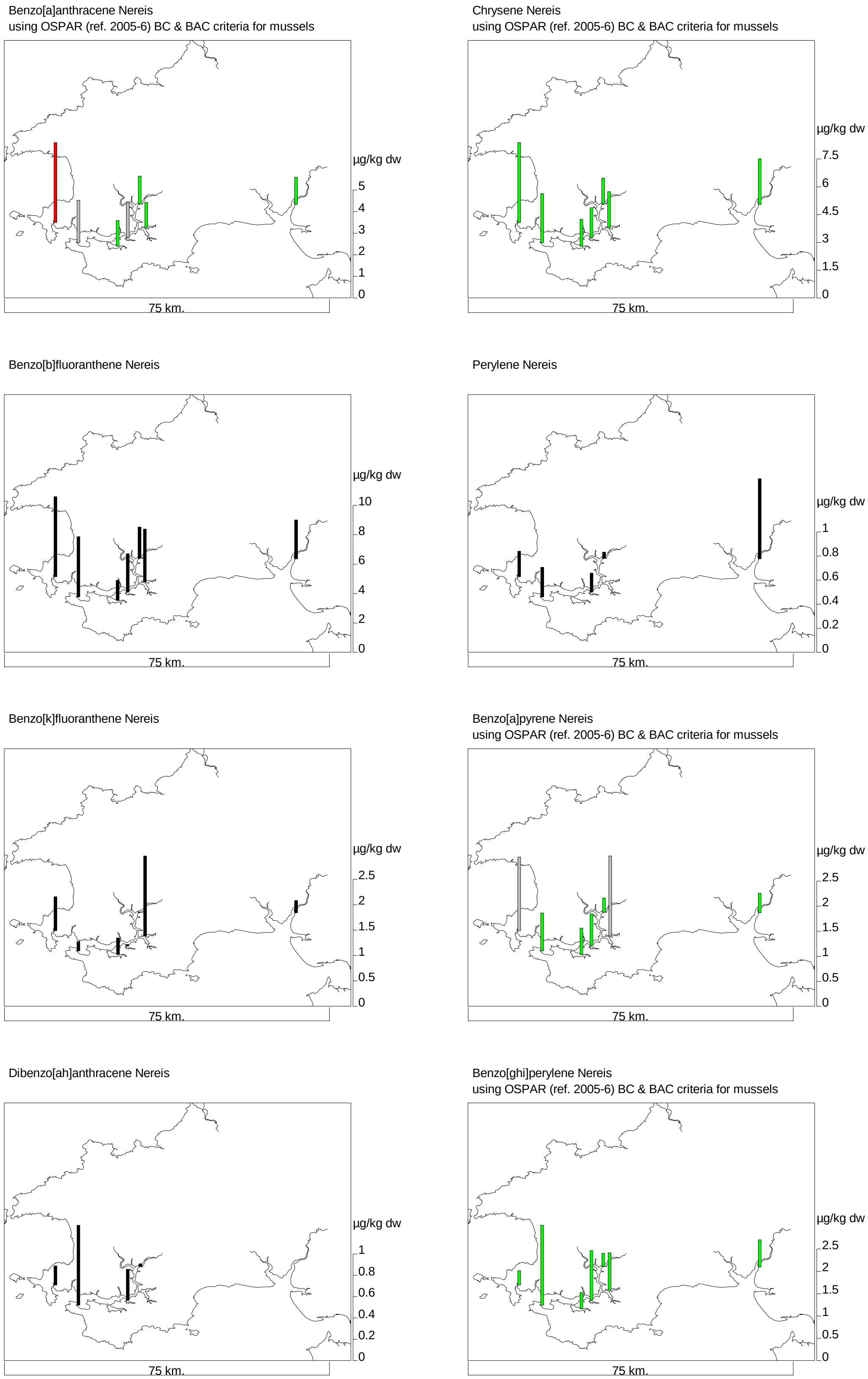
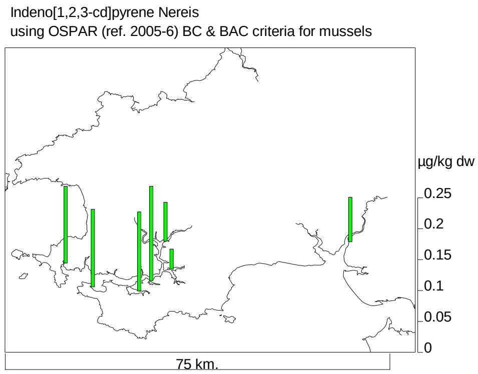


Figure 13 (cont.). PAHs in *Nereis diversicolor* in context with Background Concentration (BC) and Background Assessment Criteria (BAC) for mussels (cont.).....



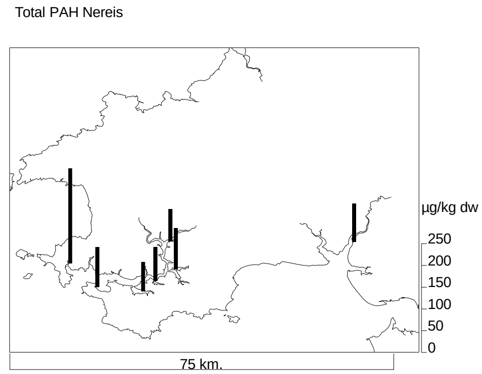


Figure 13 (cont.). PAHs in *Nereis diversicolor* in context with Background Concentration (BC) and Background Assessment Criteria (BAC) for mussels.

The main conclusions for PAHs in *Nereis,* drawn from the data in Figure 13, are:

- Milford Haven body burdens were generally either equivalent to or higher than those at the site in the Tywi estuary, with enrichment most notable for naphthalene.

- As with mussels, however, distributions of individual PAHs tended to follow one of three groupings, presumably reflecting similarity in their origins and chemistry. Group 1 was characterised by naphthalene and 1-methyl-naphthalene, with highest values upstream. Group 2 contained acenaphthene, fluoranthene, pyrene, benzo(a)anthracene and chrysene and was characterized by elevated levels at Dale. Group 3 (fluorene, phenanthrene, anthracene, benzo(b)fluoranthene, indeno (1,2,3-cd) pyrene) was typified be a more even distribution across all sites, including the Tywi reference site.

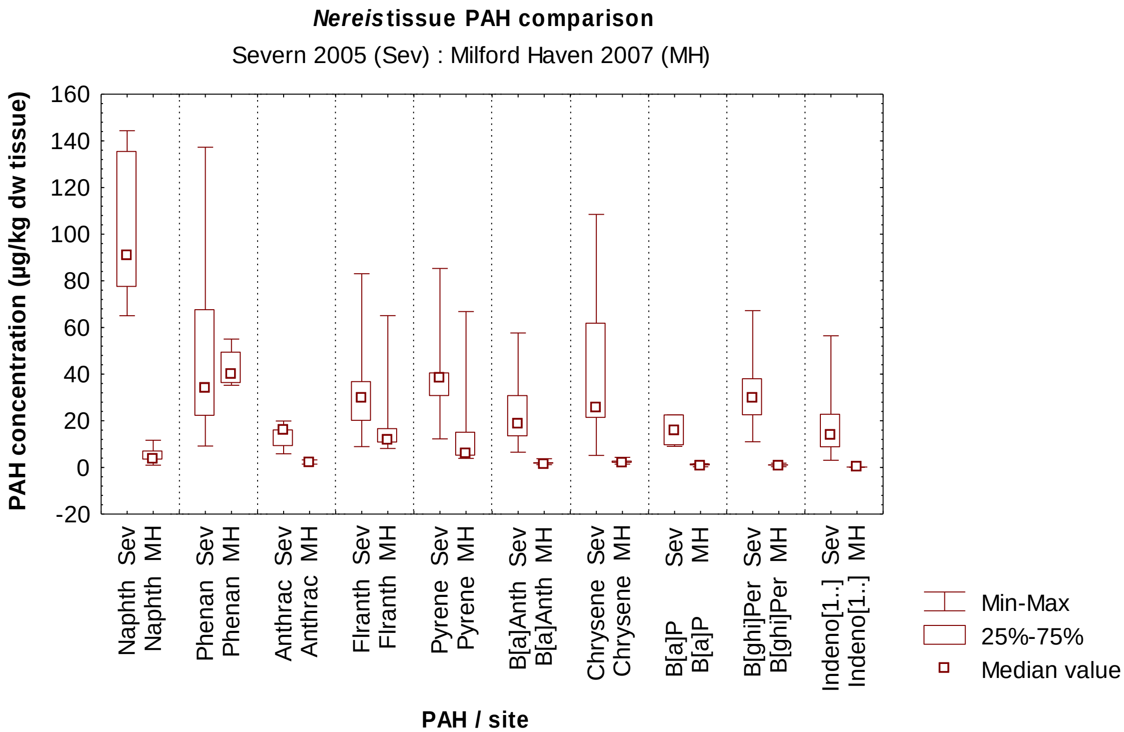
- The bioaccumulation trend for ∑PAH, shown in Figure 13, depicts an even distribution across sites but with somewhat higher values at Dale; this distribution pattern will obviously be dominated by those PAHs present in highest concentrations.

- Though of arguable value, the data are set in context with OSPAR Background Concentration (BC) and Background Assessment Criteria (BAC) for mussels. Concentrations depicted in red in Figure 13 would be considered above background and were observed widely for phenanthrene and, less extensively, for fluoranthene, anthracene, pyrene, and benzo (a) anthracene (Groups 2 and 3 in the above classification mainly). In contrast, green bars - representing concentrations expected at undeveloped sites - are not uncommon for a number of PAHs.

- Based on comparison of body burden data in *Nereis* with provisional OSPAR ecotoxicological assessment criteria (developed for mussels but not adopted) there is little indication that these generic guidelines would currently be exceeded. There is however much uncertainty regarding the overall ecotoxicological importance of PAH burdens and as with mussels the extent of sublethal effects in Milford Haven biota is unknown.

Comparisons with other sources

There are scarcely any published data for PAHs in *N. diversicolor* and to place current results in perspective they are plotted alongside similar data for the Severn Estuary (Axial survey by the EA, 2005) in Figure 14. Although different techniques, sample sizes and timings were used, Figure 14 provides an approximate guide to the relative levels of PAHs in ragworm from the two systems. The ratio between median concentrations are shown in the accompanying table and indicate that Severn worms contained higher levels, mostly, than those in Milford Haven – particularly so for the higher molecular weight (pyrogenic?) PAHs. Only phenanthrene (petrogenic?) was higher in Milford Haven *Nereis*.



Ratios Severn estuary : Milford Haven

Naphthalene	Phenanthrene	Anthracene	Fluoranthene	Pyrene	Benzo[a]-anthracene	Chrysene	Benzo[a]-pyrene	Benzo[ghi]-perylene	Indeno[1,2,3-cd]pyrene
25	0.85	7.7	2.5	6.4	14	13	24	48	110

Figure 14. *Nereis diversicolor.* Comparison of PAHs in Milford Haven samples with those in the Severn Estuary (EA, 2005).

The general pattern in PAH bioaccumulation in mussels along the Welsh coastline of the Bristol Channel/Severn Estuary was demonstrated in a study by Cefas of benzo(a)pyrene (BaP) residues, conducted after the *Sea Empress* grounding in 1996 (CEFAS, 2000; Law *et al*., 1999). A steady increase in bioaccumulation from Freshwater West (at the Mouth of Milford Haven) to Cardiff Flats was tentatively related to the trend in urban development along the coastline (rising most sharply at Cardiff to around 50 µg kg^{-1} ww BaP) and to the delivery, from upstream, of PAHs from other parts of the Severn catchment. This enrichment in mussels from the Severn, relative to that in Milford Haven, mirrors the pattern seen in *Nereis*.

Temporal trends

Hydrocarbons (∑18 PAHs) in mussels collected within the Haven at the time of the *Sea Empress* oil spill rose to extremely high levels within a few days at sites such as Angle and Dale (26189 -100946 µg kg^{-1} ww) though within three months these had dropped back by >90% to concentrations below 500 µg kg^{-1} ww, consistently so for oil-derived PAHs like naphthalene and phenanthrene and their alkylated derivatives (Dyrynda *et al*., 2000). Table 5 shows peak values for some of the alkylated derivatives which predominate in crude oil, 8 days after the grounding of the *Sea Empress* (from Law *et al*., 1999).

Table 5. Oil-derived PAHs in mussels 8days after Sea Empress spill (data from Law *et al*., 1999).

site	date	PAH	µg kg^{-1} ww
Dale	23/2/1996	C_1napthalene	430
		C_2napthalene	5900
		C_3napthalene	22800
		C_1phenanthrene	70000

Pyrogenic PAHs exhibited somewhat different temporal behaviours throughout the period following the grounding of the tanker (Law *et al*., 1999; Dyrynda *et al*., 2000). Bioaccumulation in mussels peaked 23 days after the February spill (up to ~80 µg kg^{-1} ww), before declining close to zero in mid-summer. One year post-spill, further small seasonal (winter) peaks in combustion-derived (high molecular weight) PAHs, such as benzo-a-pyrene (BaP), indeno[1,2,3-*cd*] pyrene and benzo[*ghi*] perylene[1] were observed in mussels from Dale and Angle (upto ~40 µg BaP kg^{-1} ww at the latter site), before returning to baseline levels of a few µg kg^{-1}. It has been speculated that these winter peaks could signify routine cyclical bioaccumulation patterns - coinciding with the deposition of lipids in mussels in autumn/winter, prior to spawning, together with the seasonal increase in oil consumption for

[1] Other combustion derived PAHs include anthracene, fluoranthene, pyrene, benzanthracenes, chrysene, benzofluoranthenes. There was also an indication of a peak in immunosupression effects in these mussels, coinciding with raised PAHs. Dyrynda *et al*., 2000)

heating, and associated increase in atmospheric deposition, sediment resuspension and also higher land run-off. Similar seasonal patterns have been observed for pyrogenic PAHs in N Sea mussels (reaffirming that PAH bioaccumulation is not always related to oil spillage, and may influenced by biotic as well as physicochemical factors). The return to lower PAH levels in spring may be a function of depuration and losses during spawning (gonadal tissues are enriched in PAHs relative to other tissues), subsequent rapid growth (dilution), and perhaps increased photodegradation. Though metabolism of hydrocarbons is considered to be relatively limited in molluscs, this ability will tend to rise with seasonally increasing temperatures (Law *et al*., 1999; Dyrynda *et al*., 2000). The samples taken in our survey of Milford Haven, in March, would coincide with the maxima in pyrogenic PAHs described previously.

Biological consequences of PAHs following the Sea Empress

Milford Haven has been a major oil terminal since the 1960s and has received chronic releases of hydrocarbons from a variety of sources including refineries, power stations, shipping, road run-off and small-scale domestic sources. Overall inputs were estimated to be <250 tonnes pa, predominantly dispersed along the waterway in association with suspended particulates (Little *et al*., 1987; Nikitik and Robinson, 2003). The loss > 70000 tonnes of light crude oil and 480 tonnes of heavy fuel oil from the *Sea Empress* in 1996 represented a significant departure from this trend (though only 15000t are estimated to have reached the shoreline, only part of which entered the Haven, and very little reached the Cleddau Estuaries). Nevertheless, hydrocarbon residues were dispersed along considerable stretches of the MH waterway, with heaviest contamination of sediments reported in the lower reaches (Nikitik and Robinson, 2003). Prior to this (and subsequently) hydrocarbon levels in fine sediments generally displayed a gradient decreasing from the upper estuary, seaward and were more representative of pyrogenic and degraded chronic input than crude oil. The higher values upstream are presumed to reflect the up-estuary transport of fines in this flood-tide dominated system.

To put sediment PAH 'hotspots' in perspective, the concentration of ∑15 PAHs was reported as >100,000 $\mu g\ kg^{-1}$ at Milford Haven (Woodhead *et al*., 1999) – the highest of any recorded value in UK estuarine sediment at the time - above the Threshold for Effects Level (TEL) of 1684 $\mu g\ kg^{-1}$ (dry weight), and also the Probable Effects Level (PEL) for ∑PAHs of 16770 $\mu g\ kg^{-1}$ (dw) *implying* ecotoxicological significance. However up until the time of accident there was little monitoring of biological-effects, particularly sub-lethal 'markers' which may be most relevant in quantifying the 'health' status of populations.

Reports of oil-related biological impacts from the *Sea Empress* imply varying degrees of deleterious effects, though in most cases recovery appears to have been complete within five years. Shellfish including limpets (*Patella* spp), winkles (*Littorina* spp), razor shells (*Ensis ensis*), trough shells (*Mactra corallina*) and cockles *Cerastoderma edule* were subjected to mass mortalities in oiled areas though mussels *Mytilus edulis* were relatively tolerant to oiling and were only acutely affected in areas sprayed with detergent. Cockle *Cerastoderma edule* populations were the only commercial shellfish to suffer major mortalities but recovery appears to have been rapid, albeit fluctuating in scale (Rostron 1998). To guard against tainting in the aftermath of the *Sea Empress*, restrictions on harvesting oysters and some mussel stocks for consumption were in place for 18 months in Milford Haven (up to 7 months for cockles in the Burry Inlet and Three Rivers). Transient levels slightly above background were measured in finfish, lasting only a few weeks (SEEEC 1998). Our own observations in 2007/8 indicate fairly abundant populations of mussels and cockles (and other representative bioindicators) at each of the selected sites in MH waterway, upstream as far as Black Tar. It is possible that distributions may extend even further upstream.

Some burrowing species such as *Echinocardium cordatum* and *Acanthocardia echinata* have been suggested as not fully recovered from oiling at beaches near Dale, however, most communities recovered rapidly and after 5y showed little effect even at previously severely oiled shores. Numbers of seabirds (NB guillemots and razorbills) recovered within similar timescales and total numbers of wetland birds within the Haven appear largely unscathed (avoiding the most contaminated flats in Angle Bay and Pembroke River). Among zooplankton, numbers of barnacle larvae were low post-spill, but apart from minor variations, populations changed little (Batten *et al*., 1998). Biomarkers of genotoxicity revealed responses in some fish species - though not in mussels (Law *et al*., 1998; Harvey *et al*., 1999) - and survival of 0-group bass showed signs of being compromised, the only (tentative) evidence of impact on fish stocks (Lancaster *et al*., 1998). Immunosuppression in mussels was severe immediately following the oil spill, but recovery followed a few months later and the initial effects were not permanent (Dyrynda *et al*., 2000). Reduction of phagocytosis and intra-cellular superoxide production (both defense mechanisms for killing infectious agents) were correlated with mussel body burdens of oil-derived and, in particular, combustion-derived PAHs; in contrast, extra-cellular superoxide production was more strongly related to the oil-derived PAHs.

In a study of physiological stress response in mussels from Irish Sea coastal and outer-estuary sites, reduced scope for growth (SFG) was recorded in samples from Milford Haven in 1996 and 1997, much of which was thought attributable to PAHs and, to a lesser extent, organotins, though the authors suggest that other 'unknown' compounds may have also contributed to effects (Widdows *et al*., 2002). Of all Irish Sea sites sampled, the concentrations of 2- and 3-ring PAHs were highest in mussels from Milford Haven sampled 6 months after the *Sea Empress* oil spill (>20000 $\mu g\ kg^{-1}$ dw). By the following year (1997) the concentrations had declined by ~ 66%, presumably reflecting recovery after the oil-spill, but were still relatively high (4000 to 7800 $\mu g\ kg^{-1}$ dry wt.) – more than an order of magnitude above those measured in the current survey.

Bioassays using sediments from around the waterway indicated sub-lethal responses at some sites (feeding activity in lugworm *Arenicola marina* impaired) though acutely toxic effects were not demonstrated in these assays (Law *et al*., 1998).

At the community level, there were indications of reduced numbers of sensitive amphipods in 1996 following the spill, particularly *Ampelisca spp*., accompanied by relative increases in opportunist polychaetes (e.g. *Capitella*), most immediately in the Middle and Lower Haven and Angle Bay. Diversity appears to have recovered to varying degrees in different parts of the waterway by 2000, even though numbers were still low, generally (Nikitik and Robinson, 2003). Transient effects on saltmarsh vegetation and the diversity of kelp holdfast fauna have been reported (reviewed in SEEEC, 1998; Somerfield and Warwick, 1999; Moore, 2006). Warwick has recently summarized benthic community data for the waterway in a commissioned report to MHWESG.

There have been some valuable lessons on measuring responses to PAHs and other contaminants which have arisen as a result of the *Sea Empress* accident, although many of these were retrospective for obvious reasons, and lacked good background data or co-ordination. There seems a strong case to attempt better co-ordination of chemical and biological monitoring in future - to ensure 'added value' in the development of adequate baselines.

3.4 PCBs

Results summarizing biomonitoring of PCBs in Milford Haven (*Mytilus edulis* and *Nereis diversicolor*) are plotted in Figure 15. These show concentrations of the ∑PCBs based on analysis of the ICES 7 congeners (CBs 28, 52, 101, 118, 138, 153 and 180). Data for individual congeners can be found in the Appendix 5.

The main conclusions for PCBs are:

- Concentrations in mussels showed an unexpected spatial trend in that highest values occured at the mouth of the Haven and decreased in an upstream direction: The lowest values here were comparable to the reference site in the Tywi Estuary.

- Manufacture of PCBs has long since ceased and it seems unlikely that there are recent discharges of any significance. The distribution of PCBs in mussels may reflect historic inputs (PCBs are highly persistent in sediments). However the body burdens in *Nereis* (generally lower than in mussels) showed no clear spatial pattern, and which casts doubt on this explanation.

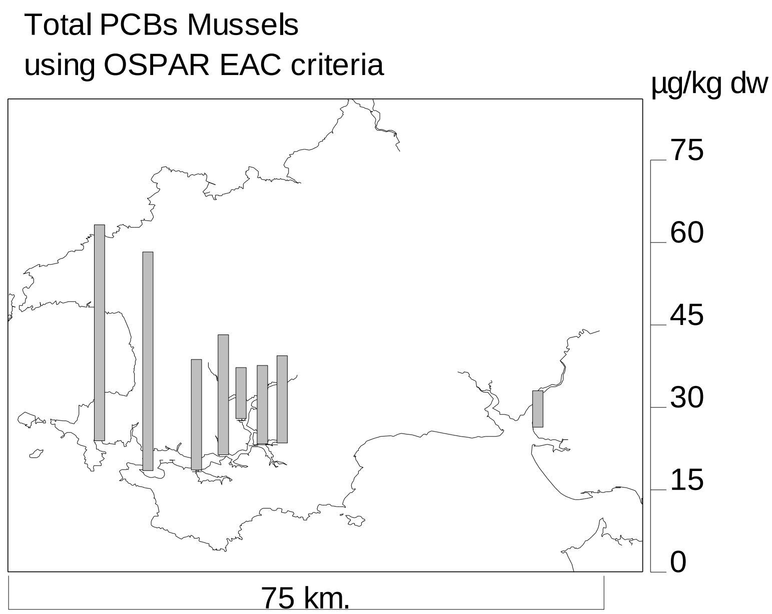
- PCB concentrations in mussels follow a pattern which is similar to the condition index (section 3.5, below). It may be that body burdens of these lipophilic contaminants are a function of lipid reserves which would be anticipated, from condition data, to be highest in those populations at the seaward end of the waterway.

- The lower and higher OSPAR environmental assessment criteria (EAC) for ∑ICES7 PCBs in mussel are 5 and 50 μg kg^{-1} dw and all values for Milford Haven and Tywi samples appear to be below the upper threshold above which effects on marine species might be expected, but above the lower 'no-effects' threshold (hence grey bars in Figure 15). These EAC are only guidelines as to ecotoxicological impact and may be set with overprecautionary 'safety factors': nevertheless the results support the need for supplementary biological effects studies.

- PCB concentrations in *N. diversicolor* are lower than in *M. edulis* and also lie between the upper and lower EAC thresholds (for mussels) indicating that a contribution to sub-lethal effects cannot be ruled out (grey bars in Figure 15). Once again there are caveats regarding the possibility of overprecautionary guidelines and also whether their derivation from mussels and application in worms is appropriate. The rationale is principally to provide context, rather than imply rigorous toxicological significance, and to reinforce the case for relevant effects monitoring.

Further context is gained by comparison of values from MH *N. diversicolor* with EA samples from the Severn Estuary in 2005 (Figure 16). For the lower chlorinated congeners concentrations in both systems are relatively low: for the more highly chlorinated CBs 138, 153 and 180 (hexa- and hepta-chlorobiphenyls), and hence total PCBs, there is considerably more bioaccumulation in the Severn. This is not unexpected since PCBs were previously manufactured at Newport, a known hotspot for contamination.

We are not aware of similar data from earlier MH surveys on which temporal trends could be established. Concentrations of ∑25 PCB congeners (composition not stated) determined in mussels from Milford Haven (St Ishmaels) in 1996/7 were in the range 0.009-0.013 μg g^{-1} (Widdows *et al*., 2002). These were somewhat lower than values determined in the current study. Concentrations in mussels from hotspots in the Irish Sea such as Liverpool Bay and the Mersey Estuary were an order of magnitude higher (Widdows *et al*., 2002).



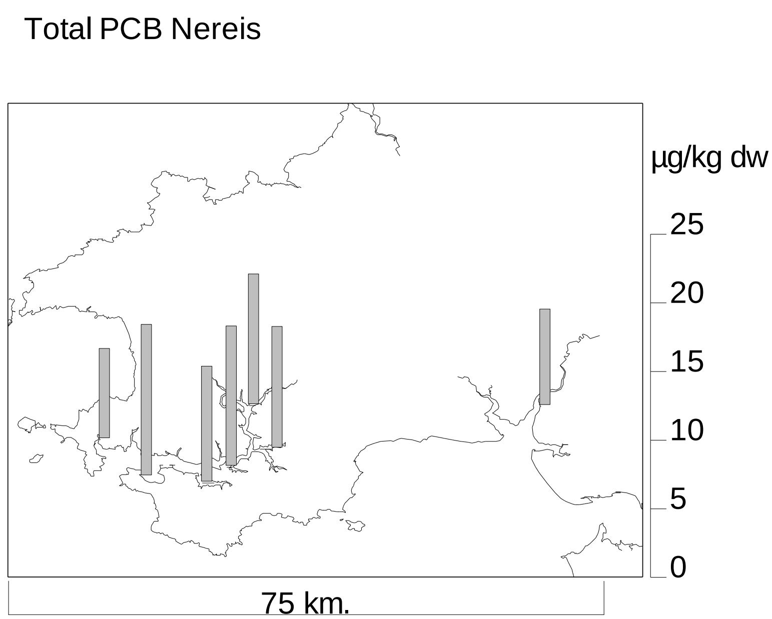


Figure15. PCBs (∑ICES 7 congeners) in mussels *Mytilus edulis* (top) and ragworm *Nereis diversicolor* (bottom). Results have been compared with lower and higher OSPAR environmental assessment criteria (EAC) for mussels (NMMP, 2004; OSPAR 2004). Grey bars signify acute toxicity unlikely; sub-lethal effects cannot be ruled out (see text).

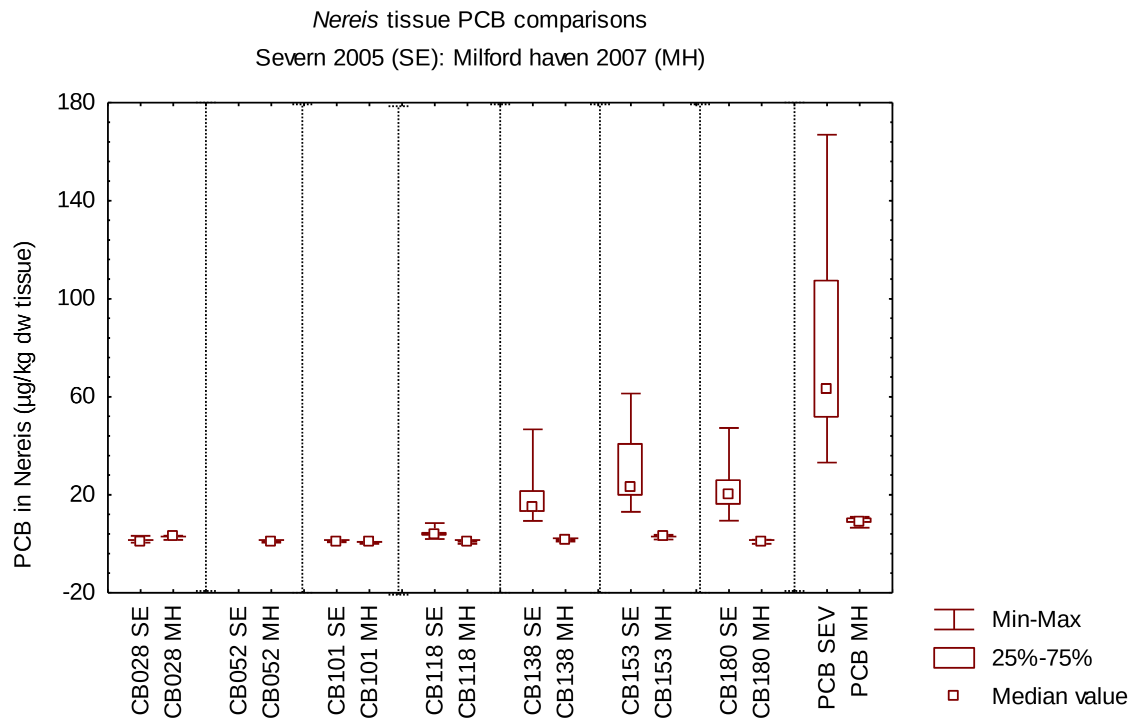


Figure 16. *Nereis diversicolor.* Comparison of PCBs in Milford Haven samples with those in the Severn Estuary (EA, 2005).

3.5 Biometric data

Raw biometric data for *M. edulis* (including Condition Index, CI, based on digestive gland, gill and whole tissue weights) and *C. edule* (CI based on total weight) are given in Appendix 2 along with biometric data for other species.

Mussels *Mytilus edulis*

The condition index (CI) of mussel digestive gland (a measure of weight relative to shell length) is shown in Figure 17A. Highest values were those at St Ishmael (SI -the reference site in the Tywi) and at Angle Bay (A). All other samples were significantly lower than the reference site. Values were generally lowest upstream from Pembroke Ferry (PF). A similar pattern was observed for CI based on whole soft tissues (Figure 17B). In contrast the gill tissue appears less responsive to conditional variation: the only difference relative to the reference value was a slightly higher value at Dale (Appendix 2).

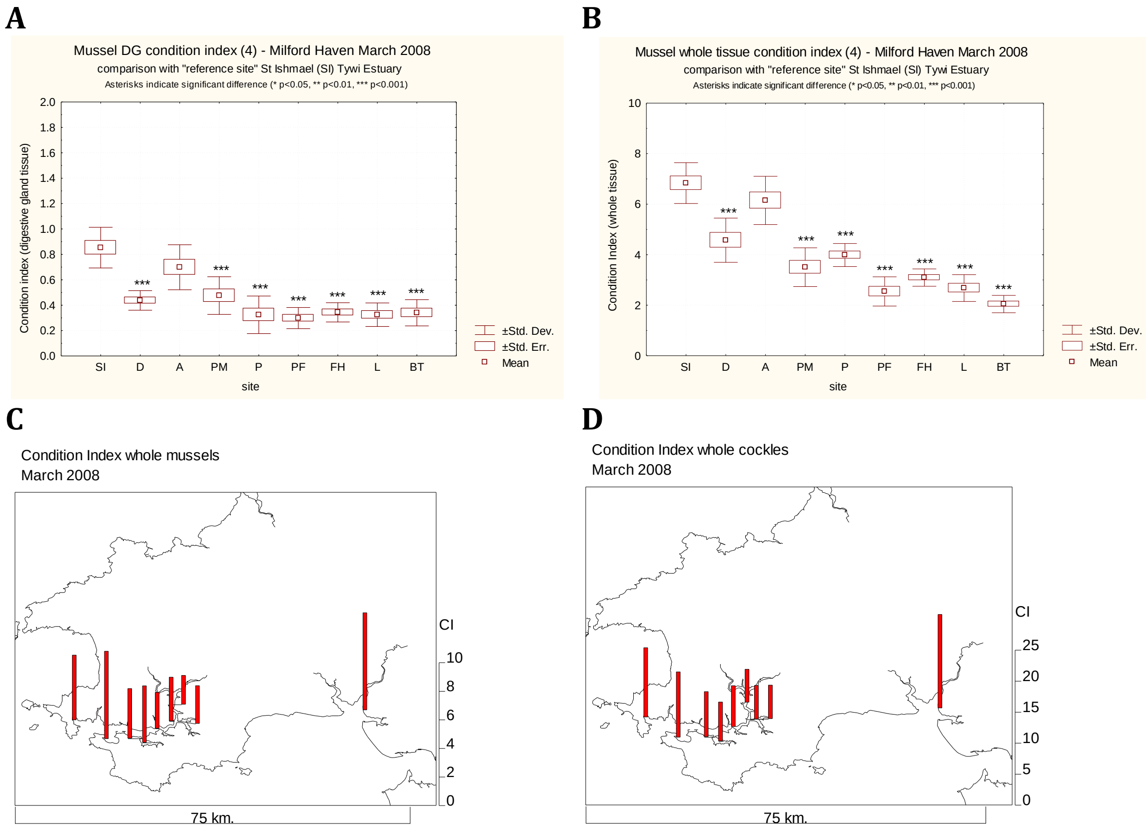


Figure 17. Condition indices in bivalves: *Mytilus edulis* condition indices, based on digestive glands (A) and whole soft tissues* (B) (*Sites significantly different to the Tywi reference site at St Ishmael (SI). Other labels D-Dale; A-Angle; PM- Pennar Mouth; P-Pennar; PF- Pembroke Ferry; FH-Ferry Hill; L-Lawrenny; BT- Black Tar). Maps (C) and (D) show whole body CI for *Mytilus edulis* and *Cerastoderma edule,* respectively.

Cockles *Cerastoderma edule*

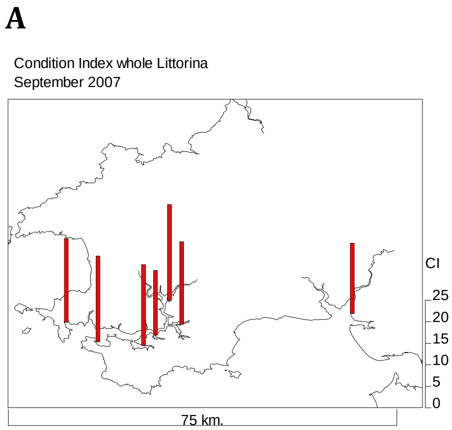
The condition index (CI) of whole cockles (weight relative to shell length) is shown in Figure 17D and resembles strongly the pattern in mussels (Figure 17C). Highest values were those at St Ishmael (the reference site in the Tywi) and at Dale; CI decreased upstream in the Cleddau Estuary.

Winkles *Littorina littorea*

In contrast to cockles and mussels, the condition index of winkles *L. littorea* showed no systematic variation over the study area suggesting less sensitivity to environmental gradients (Figure 18).

Ragworm *Nereis diversicolor*

The average dry weight of ragworm *N. diversicolor* at individual sites were not systematically variable between sites and were highest at Pembroke and St Ishmael and lowest at Dale (the latter may reflect low organic content of sediments towards the mouth of the estuary). However these trends do not suggest marked sensitivity across the environmental gradients sampled (Figure 18).



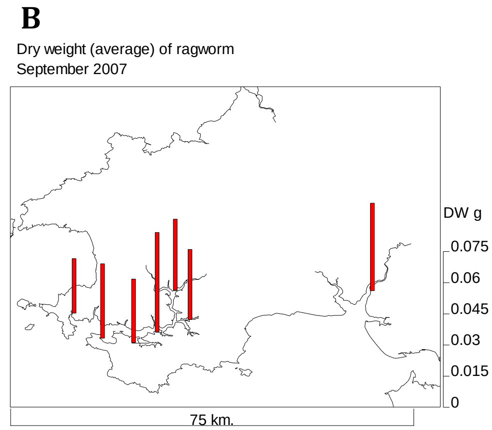


Figure 18. *Littorina littorea*. Whole body Condition Index (A); *Nereis diversicolor* average dry weight (B)

The pattern of condition indices in bivalves was consistent and significant, with a marked reduction in condition upstream. Correlation coefficients between CI and metal burdens were determined for cockles and mussels and highlighted several significant (negative) relationships. Table 6 summarises those with P values <0.05.

Table 6. *Cerastoderma edule* and *Mytilus edulis*. Correlation between Condition Index and metal body burdens (all coefficients shown are significant, P<0.05; ns not significant)

	As	Co	Cu	Fe	Hg	Mn	Ni	Pb	Se	Zn
mussels	ns	ns	-0.8	ns	-0.7	ns	-0.69	-0.86	-0.83	-0.79
cockles	-0.84	-0.88	-0.69	-0.69	-0.84	-0.71	-0.85	ns	-0.82	ns

The condition of mussels decreased in line with increasing body burdens of metals according to the sequence Pb>Se>Cu>Zn>Hg>Ni. The corresponding sequence for metals in cockles was Co>Ni>Hg=As>Se>Mn>Cu=Fe.

For mussels it is possible to extend the range of contaminants in these comparisons to include PAHs, PCBs and organotins. The ranking of contaminants, for those which are significantly (negatively) correlated with Condition Index is shown in Table 7. Some of the more toxic contaminants (e.g. Pb, TBT, Cu, benzo(a)pyrene) appear high on this list and it seems plausible that a combination of contaminants could have an influence on condition index.

Body burdens of a number of contaminants co-vary, both in *Mytilus* and in *Nereis* (Table 8) illustrating possible common sources. However, such covariance contributes to the difficulty in attributing cause effect. There are also other factors which may be influential in the condition of bivalves; notably feeding rate, food availability and salinity.

To establish cause and effect more substantially would require additional measurements of sub-lethal effects and environmental parameters, coupled with multivariate statistical techniques to try and tease out the relative importance of these parameters. This may be a suitable topic for a future R&D in the Milford Haven Waterway, as discussed in subsequent sections (see also Appendix 7).

Table 7. *Mytilus edulis*. Comparison of (negative) Correlation coefficients between Condition Index and metal body burdens (all values are significant, $P<0.05$; ns not significant; nd not determined)

contaminant	Correlation with Condition Index
Pb	-0.86325
TBT	-0.85835
Se	-0.83187
Benzo(a)pyrene	-0.80803
Cu	-0.80061
Zn	-0.78868
Benzo(ghi)perylene	-0.78032
DBT	-0.75958
Dibenzo(ah)anthracene	-0.74688
Perylene	-0.74128
Hg	-0.69862
Benzo(k)fluoranthene	-0.69206
Ni	-0.68709

Table 8. *Mytilus edulis* and *Nereis diversicolor*. Covariance of contaminant burdens (only significant combinations shown)

	Mytilus edulis	*Nereis diversicolor*
Metals	Se vs As, Hg, Ni, Zn Cd vs Co,Cr,Mn,Ni,Zn Co vs Mn, Ni, Zn Fe vs Cr,Pb Cu vs Hg, Pb, Se, Zn Mn vs Ni,Zn Ni vs Se, Zn Pb vs Se, Zn	Cu vs Pb,Sn
Organotins	DBT vs As, Hg, Se TBT vs As, Cu, Hg, Se, DBT	DPT vs Hg MPT, TPT vs Pb MBT,DBT vs Sn MBT vs DBT MPT vs TPT
PCBs		∑PCB vs Cu, Sn, MBT, DBT

4 Conclusions

A substantial baseline study of bioaccumulation has been performed in Milford Haven Waterway based on analysis of metals, organotins, PAHs and PCBs in *Mytilus edulis* and *Nereis diversicolor,* with complementary evidence (for metals) supplied by sampling cockles *Cerastoderma edule*, winkles *Littorina littorea* and seaweed *Fucus vesiculosus*. This multi-species approach to bioaccumulation is appropriate for assessment of water quality threats in the Waterway since it adds confidence that risks from different sources (water, sediment, diet) are addressed and that physiological regulation or detoxification of individual contaminants by a single species do not lead to an underestimation of contamination. Body burdens have the added advantage of integrating water (and sediment) quality over previous months and perhaps longer; they are not subject to the vagaries of transient water sampling or loadings estimates. They have the added advantage of being a direct measure of biologically-relevant fractions.

The availability of these species in reasonable numbers, along much of the waterway, has proved to be a valuable attribute of this Ria. This trait has enabled us to establish a network of monitoring stations for biomonitoring, together with a comparative reference site in the Tywi Estuary for a common suite of bioindicator species. The information presented in the appendices should enable easy resampling and survey in future. All locations are accessible by road, relatively easily, avoiding the need for ship-based sampling. Appendices also list the raw data for contaminants, in separate species, facilitating archiving and future comparisons.

Current status

For the majority of metals, concentrations in MH biota were above the regional Tywi baseline, though were not exceptional by UK standards. However, elevated levels were observed at individual MH sites for Mn (molluscs, seaweed), Co (mussels, seaweed), Sn (bivalves), Ni (cockles) and Fe (ragworm), whilst As and Se (molluscs and seaweed) were consistently at the higher end of the UK range for much of the MH Waterway.

Bioavailability of several metals clearly increases upstream, particularly in the estuary above the Cleddau Bridge. This may reflect overall contamination trends in sediments, and for some elements could be enhanced by decreasing salinity. It would be worth considering investigating bioaccumulation trends further upstream in future, using *Nereis diversicolor* and the clam *Scrobicularia plana* as indicators.

TBT levels in mussels also tended to be highest upstream in MH Waterway and were substantially above (Tywi) reference values - but below thresholds considered by OSPAR to be acutely toxic. Nevertheless, given the levels of bioaccumulation sub-lethal effects cannot be ruled out at MH sites, certainly in more sensitive species such as dogwhelks. This aspect should be investigated further. Phenyltins were not accumulated appreciably in *Mytilus*, however higher levels where recorded in *Nereis* (NB Cosheston Pill), perhaps reflecting localized residual sources in sediments.

∑PAH in *Nereis diversicolor* and *Mytilus edulis* were evenly distributed across most sites but were somewhat elevated in the former species at Dale, and in the latter species at upstream MH sites. ∑PAH values in MH mussels were generally higher than the Tywi reference site. Individual groupings of PAHs exhibited subtle variations in bioaccumulation according to their structure and origins. In *Nereis,* naphthalenes – considered indicative of petrogenic (oil) sources - were enriched upstream in Milford Haven. PAHs such as fluorene and phenanthrene varied little across mussel samples whilst most other PAHs tended to be elevated in the central and upper parts of the system. Variations in bioaccumulation patterns may reflect localized sampling site differences between the two species, the proportionately greater influence of sediment contamination on *N. diversicolor,* and physiological differences. This also applies to other contaminants.

Several PAHs in mussels including phenanthrene, fluoranthene, pyrene, benzo(a)anthracene, benzo(a) pyrene and benzo(ghi) perylene were above OSPAR background (though not necessarily of ecotoxicological importance). Likewise, whilst *Nereis* may be exposed to potential PAH sources in Milford Haven, accumulated burdens were lower than those determined recently in the Severn Estuary.

Lipophilic PCBs in Milford Haven mussels exceeded the lower OSPAR Ecotoxicological guideline value though not the upper threshold, and were unusual in their distribution in that highest levels occurred at the mouth of the waterway. This may be a function of better condition and nutritional status (lipids) of mussels here, rather than contamination. It seems unlikely that bioaccumulation poses an acute threat, though as with the majority of other contaminants measured, site-specific sublethal effects have not been addressed and risk assessment at present is dependent on comparison with generic guideline values.

Identifying trends and gaps

Given the natural variation that occurs in any ecosystem, the only meaningful way to assess such anthropogenic change is to establish long-term time series for a suite of determinands, based on an established set of sites, indicator species and sampling times. Results from current survey represent a core data set against which such future change can be monitored and which will contribute to the overall assessment of status of the Waterway. This may assist the statutory agencies in judging progress to Good Status under the Water Framework Directive and Favourable Condition Status under the Habitats and Birds directive.

There are as yet insufficient data to conclude whether improvement or deterioration has occurred in recent years, though recovery is anticipated to be in progress for organotins (recently banned) and some metals such as Cd and Cu (due perhaps in part to measures to control large scale discharges in the area, particularly the Severn Estuary). In particular, it is a pivotal time in the history of TBT pollution. The global ban on TBT in 2008 should start to produce a decline in TBT contamination and perhaps an improvement in benthic organisms, particularly molluscs. The current data set is therefore an excellent opportunity to test this hypothesis and to establish the rate of recovery.

The current bioaccumulation surveillance is, however, only one part of a long-list of prospective requirements for improved monitoring of the condition of Milford Haven. Given the constraints to funding it is important, for future planning, to identify and prioritise the most (and least) critical parameters.

Bioaccumulation surveys conducted in the early 1990s by the NRA (Welsh Region) established that most persistent organic contaminants analysed were largely undetectable in seaweed and mussel samples from Lawrenny, Dale and Ferryside (Tywi Estuary) (Davies and Ellery, 1995). Similarly, concentrations of organochlorine pesticides (DDT and degradation products, dieldrin, HCB and γ-HCH) in mussels from St Ishmaels (MH) were generally at, or below, detection limits (~0.003 $\mu g\ g^{-1}$ dw) (Widdows *et al.,* 2002). Given these findings there seems little reason to include these determinands in future bioaccumulation studies, as the data would be inadequate for establishing meaningful spatial or temporal trends or assessing toxicological significance. A better use of resources might be to collect and consider evidence of deleterious effects and using these to target extra monitoring for 'unknowns' if justifiable. In this context, there are strong arguments for screening biota using a suite of 'biomarkers' to establish baselines of organism health in Milford Haven Waterway.

There are as yet no statutory environmental quality standards for body burden data – only guideline values which we have used to indicate the relative potential for impact. In order to maximize their value, bioaccumulation trends should be considered alongside biological performance indicators (whether at the population level or at more sensitive, lower levels of biological organization -e.g. biochemical, molecular). Based on current results, the distribution and abundance patterns of the targeted bioindicator species in Milford Haven suggests that any effects, if they occur, are not acute. Again, however this needs to be confirmed by sublethal biological effects measurements (biomarkers and bioassays). In this context it is interesting that the condition indices of bivalves (cockles and mussels) showed significant variations. Values for CI were highest at the reference site in the Tywi Estuary, but decreased upstream in Milford Haven. Given the number of significant (negative) relationships between CI and body burdens it is possible that a combination of contaminants could have an influence on CI of bivalves. However cause and effect needs to be tested more rigorously as there are other factors which may be influential.

Our surveys have indicated that distributions of the chosen suite of bioindicator organisms occur widely, and in reasonable abundance, throughout much of the waterway – provided the appropriate substrate is available and provided there are no severely adverse environmental factors in the vicinity. In the current survey we have avoided sampling near major sources in order to pick out general contamination trends within the waterway. As a result a range of appropriate sites and baseline data for key bioindicator species in Milford Haven is now in place and can be used to assess future estuary-wide change. If in future serious localized contamination is suspected to be an issue at other sites, additional targeted bioaccumulation sampling, using the same species could be used in an investigate capacity (e.g. to identify the influence of selected sources). This might be manifested in anomalous body burdens in some of the key indicator species, accompanied, in extreme cases, by reduced condition or abundance.

A major knowledge-gap for environmental managers concerns the need for better understanding of the significance of accumulated body burdens. In the long term better integration and harmonization of environmental surveys would help to achieve this key requirement. Bioaccumulation studies conducted at a common suite of sites (and times), alongside sediment monitoring, sublethal biological effects measurements and traditional benthic ecology would assist in the interpretation of trends and contribute to the 'weight-of-evidence' approach to impact assessment. Some specific recommendations are discussed further below.

5 Recommendations

Bioaccumulation surveys are an important part of environmental surveillance strategy, provided that appropriate methodology and sampling procedures are employed. These need to control, as far as possible, the influence of those biotic and environmental factors which modify contaminant body burdens (including size, numbers, and seasonality). The survey described here has validated a suitable biomonitoring approach and produced baseline datasets for the Milford Haven Waterway, upon which a future program could be based. The major recommendations for the future are:

- ***Repeat of the bioaccumulation survey in 2009 and in 2011 for metals, TBT, PAHs and PCBs.*** Completion of a series of three comparable surveys in relatively quick succession will address variation in the short-term and help distinguish natural vs anthropogenic trends. Thereafter, a review of the programme should establish requirements for subsequent bioaccumulation sampling intervals, with a view to reducing their frequency to be most cost-effective (e.g. 3-5y intervals). Metals are a continuing presence in the waterway with a variety of historic and contemporary sources. Organotins are still present in most biological samples in significant concentrations and although a global ban has now been implemented, residues and their effects should continue to be monitored until contamination approaches close-to-zero concentrations. In view of the continuing presence of the oil industry and importance of shipping in the waterway, together with associated combustion products, continuation of the database on PAH bioaccumulation is considered essential. The presence of PCBs at ecotoxicologically relevant levels in current samples, coupled with their persistence and biological importance, supports their inclusion in future studies. Pesticide concentrations and other organochlorines might be considered on a 'nice-to-know basis' though the available evidence suggests this is not a major issue in the waterway. Sites and species used in future biomonitoring surveys should build on the current data set. The reference site in the Tywi Estuary is considered to be appropriate for most contaminants; however, consideration should be given to additional reference samples in the 'Three Rivers' and perhaps also further afield.

Bioaccumulation studies on their own cannot satisfy all requirements associated with water quality assessment, since there are no rigorous Environmental Quality Standards for body burdens. This poses the question as to what are the consequences, for the health of the biota, arising from accumulated tissue residues (which may act in combination, alongside other environmental stressors), and how might we measure them? Since acute impacts are no longer anticipated in the post-industrial era, application of novel biological effects indicators (bioassays and biomarkers) might help interpret sub-lethal trends:

- ***Biological-effects surveillance*** (sub-lethal effects monitoring to detect subtle, chronic contamination). A suite of biomarkers and bioassays should be considered to accompany bioaccumulation surveys (and also to compliment traditional ecological surveys of parameters such as abundance and diversity). This strategy should be based on a suite of common sites (see also Bent, 2000). Proposed techniques might include a selection from the following :

Application of biomonitoring techniques	
Technique	**Indicates..**
Immunotoxicity assays	immunocompetence - general well-being of test organism
EROD, PAH metabolites (ethoxyresorufin-O-deethylase)	Exposure to PAHs, PCBs
Metallothionein	Metal exposure (sub-lethal)
Oxyradical scavenging capacity	Combined Oxidative stress: metals and others
Imposex,	TBT
Intersex	endocrine disruption, Impact of STW and other sources Agriculture
DNA damage (micronucleus, comet)	Genotoxicity
Condition indices	integrated picture of the relative health (nutritional) status of different bivalve populations
Toxicity studies on sensitive species (e.g. DTA)	Lethal and Sub-lethal effects To include sediment bioassays
Benthic ecology	Diversity and Abundance

Table 9. Biological monitoring techniques suitable for application to Milford Haven Waterway

Measurement of these sub-lethal measures of 'health' would provide added-value to other forms of surveillance being conducted by MHWESG members, including the biological elements being considered by EA as part of WFD implementation Results would thus help monitor progress towards the requirement to achieve 'Good Status' by 2015. They would also help to prioritise and direct future monitoring towards those areas most at risk. Using methods summarized in Appendix 7, we have successfully trialed two of these biological effects techniques in mussels from Milford Haven, namely TOSC (a measure of oxidative stress) and Metallothionein (MT) induction (a measure of metal exposure). This has been done to demonstrate viability and to test proposed new methodology for integrating data (see below and demonstration results, Appendix 7);

Additional suggestions for future R&D on the use of biological effects tools, and a strategy for their application in Milford Haven Waterway, are given in greater detail on pp xvii of Appendix 7.

- ***Multivariate techniques to investigate links between contaminant bioaccumulation, major environmental variables (salinity, exposure, granulometry etc) and biological effects (biomarkers and abundance/biodiversity of benthic organisms).*** It is important to consider harmonization of chemical and biological sampling as a first step to integrating data sets. Timing should be consistent with that used in the current survey, as far as possible. It would also be beneficial to converge water and sediment quality sampling, bioaccumulation/biomarker studies and intertidal benthic biodiversity studies on a common set of established sites (including those used here), wherever possible. Multivariate statistical packages (e.g Primer-E) can now help to combine large data sets to piece together the linkages between body burdens, environmental variables and measures of biological effects. In turn, this programme can be used to interrogate the data and pick out major associations (an insight into cause and effect), and hence separate sites on the basis of impacts. To illustrate the potential the example in Appendix 7 is based on body burden data, condition, and two biomarkers (MT and TOSC) for mussels collected in the current survey. The output is not optimal as the data is limited and is only intended as a trial; nevertheless distinction between sites, and underlying reasons for their separation is clearly possible. By the addition of more variables and more detailed analysis, this could be refined to provide greater insights into the principal causes of biological and chemical variation throughout Milford Haven.

- '***Contextual' information.*** There are a number of supplementary items which might improve the interpretation of current bioaccumulation data and trends. Mostly this involves a requirement for recent information on contaminant sources, transport and speciation in the waterway. The review of Bent (2000) indicates that raw water (and sediment) quality data may be available as excel spreadsheets held by MHWESG. Scrutiny of these data could provide useful contextual information for the current bioaccumulation results. Production of a readily available synthesis of water quality data (and major physicochemical variables) would be helpful in this respect. Axial surveys of the distribution of dissolved and particulate metals (and TBT, PAHs, PCBs) in relation to salinity and other gradients (e.g. suspended solids) would be beneficial in identifying features that modify bioaccumulation along the waterway. For similar reasons it would be useful to have fixed-station depth-profiles over a tidal cycle, at least at one site in the upper estuary and one in the lower part of the waterway, during different conditions of tide and fresh-water flow. Recent loadings estimates for major discharges and freshwater inputs would also be useful in the interpretation of sources of bioaccumulation, if available (Atkins may have produced this information for MHWESG in their inputs budgets, 2002; EA may also have loadings estimates from the review of consents process).

  Sediment contaminants are believed to have been sampled recently and it is recommended that trends are compared with the current bioaccumulation survey, where appropriate. In future, harmonized sampling might be considered in order to provide added-value. If this common sampling takes place, the use of weak sediment extraction protocols should be considered for testing as a measure of bioavailable (anthropogenic) metal - alongside the more routine concentrated acid digests which measure 'total' metals (the latter include refractory forms which are unlikely to be accumulated by organisms). There should also be careful consideration of normalization techniques used in sediment analysis (based on grain size or major geogenic elements) to ensure that comparisons are on a like for like basis – and that differences are in fact due to contamination rather than grain-size characteristics and geochemistry. A further angle on the toxicological significance of sediments is available for some contaminants by comparison with Guidelines - Threshold and Probable Effects Levels (TELs and PELs).

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7 Appendices

Appendix 1. Sampling Sites[a,b].

[a,b] 'Soft substrate' sites may be slightly different from 'rocky shore habitats'

PHASE1 *Tuesday, 11 September, 2007.*

Tywi control site

River Tywi[a] (1.2km u/s of Ferryside). Grid ref: SN370117.
Nereis diversicolor common; 493 collected in 3 man hours. Mainly around islets of *Spartina* spp and adjacent small streams across mud. Evidence of *Scrobicularia plana* (abundant shells – 6 collected).

◄**River Tywi[b]** (St. Ishmael). Grid ref: SN361082.
Open mussel bank on hard/sandy substratum. *Littorina littorea* common, 290 collected. *Mytilus edulis* common lower on shore, 38 collected. *Fucus vesiculosus* present.

Wednesday, 12 September, 2007.

Angle Bay

Angle Bay[a], Grid ref: SM868028.
Stony mud on the flanks and bed of freshwater stream. *Nereis diversicolor* common; 472 collected in 2.5 man hours. Evidence of *Scrobicularia*, 1 collected.

◄**Angle Bay[b]**, Grid ref: SM870027.
Littorina littorea common on stony patches on mid-shore zone, 105 collected. Cockles *Cerastoderma edule* abundant (small number collected). *Fucus* present.

Pennar

Pembroke River (Pennar), Grid ref: SM959020.
Gritty mud. *Nereis diversicolor* common in mounds dug by bait diggers; 334 collected in 3 man hours. *Fucus vesiculosus* present. *Littorina littorea* common, under/on *Fucus*, 161 collected. Evidence of *Scrobicularia*, 4 collected.

Pembroke Ferry

Pembroke Ferry (Waterloo), Grid ref: SM982040.
Nereis diversicolor common, particularly at edge of small FW stream, below c/park, 459 collected in 3.5 man hours. Some *Scrobicularia*, 3 collected. *Fucus* present. *Littorina littorea* present (large), under/on *Fucus*, 52 collected along edge of slipway.

Thursday, 13 September, 2007.

Dale (lower)

Dale[a] (upper), Grid ref: SM815075.
Stony mud. *Nereis diversicolor* common, 532 collected in 3 man hours. Some *Scrobicularia*, 4 collected.

◄**Dale**[b] (lower), Grid ref: SM809065.
Fucus present. *Littorina littorea* common, under/on *Fucus*, mid-shore, 133 collected. *Mytilus* present lower on shore. Cockles present.

Lawrenny (Cresswell river)

Lawrenny[a] (Jenkin's Point), Grid ref: SN009062.
Stony, rocky beach. *Littorina littorea* common, under/on *Fucus*, mid-shore, 161 collected. *Mytilus* present.

◄**Lawrenny**[b] (mouth of Cresswell River). Grid ref: SN017063.
Gritty mud. *Nereis diversicolor* common, 269 collected, 2 man hours. *Fucus* present.

Landshipping Quay

Landshipping, Grid ref: SN011118.
Gritty mud with freshwater streams. *Nereis diversicolor* common, 389 collected in 3man hours. Some *Scrobicularia*, 1 collected. *Fucus* present. Few v. small *Littorina* spp. on dock wall (not collected).

Friday, 14 September.
◄**Landshipping Quay**. Grid ref: SN008108.
Gritty mud. *Littorina littorea* common on mud and stones, 150 collected in 20 min. *Fucus* present.

PHASE 2

Sunday, 9 March, 2008.

Tywi control site

River Tywi[b] (St. Ishmael). Grid ref: SN361082.
Open mussel bank on hard/sandy substratum. *Mytilus* common on lower shore; 170 collected. Cockle shells were common, but thorough search on both sides of the sand scar failed to show any specimens. Some small cockles, 48, were collected by raking stream edges approximately 400m south of scar. *F. vesiculosus* collected.

Monday, 10 March, 2008.

Angle Bay

Angle Bay[b], Grid ref: SM870027.
Mytilus (smallish) collected from the end of derelict stone quay (120) and from muddy beach, (60), 300m further east. On the beach *Mytilus* were found half buried in muddy, coarse sand. Cockles abundant in sandy mud, approximately 170 collected. *F. vesiculosus* also collected.

Pembroke Ferry

Pembroke Ferry, (Ferry Inn), Grid ref: SM974047.
No evidence of bivalves to the east of the bridge, stony site. *Mytilus* (large) and cockles (in sandy patches) most abundant directly beneath the bridge span. Approximately 100 specimens of each collected. *F. vesiculosus* also collected.

Pennar

Pembroke River (Pennar), Grid ref: SM959020.
Mytilus difficult to find, and were generally small (115 collected), mostly under or attached to small/medium sized stones on the sandy scar. Cockles were commonest towards the lower shore, downstream of the scar, approximately 180 collected. *F. vesiculosus* also collected.

Pennar Mouth

Pennar Mouth, Grid ref: SM943028.
Access was via new road, within housing development site, to old concrete slipway. The shore was stony (and some large rocks) with sandy mud substrate. *Mytilus* (large, approximately 200), cockles (large, approximately 100), and *F. vesiculosus* collected. Some large *Ostrea edulis* were noted, but not collected.

Tuesday, 11 March, 2008.

Dale (lower)

Dale[b], Grid ref: SM809065.
Mytilus abundant, (190 collected). Cockles abundant, (130 collected).
F. vesiculosus also collected.

Black Tar

Black Tar, Grid ref: SM999093.
Mytilus in muddy gravel/rocks either side of slipway (approximately 80 collected). Cockles were found mainly downstream of slipway (approximately 180 collected). *F. vesiculosus* collected.

Ferry Hill

Ferry Hill, Grid ref: SN003061.
Downstream of slipway; sandy-mud substrate with rocks strewn over all. *Mytilus* close to and on rocks, some half buried in substrate (approximately 100 collected). Cockles found in the sandy mud lower on shore (approximately 100 collected). *F. vesiculosus* also collected.

Lawrenny (mussel bed)

Lawrenny[a] (Jenkin's point), Grid ref: SN009062.
Rocky beach with sandy mud substrate. The lower tide state, during this visit, revealed a large mussel bed on the lower shore. *Mytilus* abundant (approximately 140 collected). Cockles were less common (approximately 100 collected). *F. vesiculosus* collected.

Appendix 2. Biometric data.

Nereis diversicolor and *Littorina littorea*, September 2007.

Site	Map Ref.	Dist. from Mouth (km)	Date	No. (specimens)	Size (mm)*	Wet Weight Mean (g)	Dry Weight Mean (g)	Condition index (4)†	W/D ratio
				Nereis diversicolor					
Tywi[a]	SN370117	-	11/09/2007	455	-	0.2654	0.0424	-	6.259
Dale[a]	SM815075	6.5	13/09/2007	590	-	0.1600	0.0265	-	6.038
Angle[a]	SM868028	7.4	12/09/2007	447	-	0.2256	0.0361	-	6.249
Pennar	SM959020	16.8	12/09/2007	453	-	0.1914	0.0308	-	6.214
Pembroke Ferry (Waterloo)	SM982040	19.0	12/09/2007	486	-	0.3128	0.0481	-	6.503
Lawrenny[b]	SN017063	21.8	13/09/2007	252	-	0.2003	0.0340	-	5.891
Landshipping	SN011118	28.0	13/09/2007	389	-	0.2005	0.0345	-	5.812
				Littorina littorea					
Tywi[b]	SN361082	-	11/09/2007	100	17.3	0.1987	0.0851	16.432	2.335
Dale[b]	SM809065	5.5	13/09/2007	35	24.9	1.2569	0.3012	19.512	4.173
Angle[b]	SM870027	7.3	12/09/2007	45	22.7	0.9132	0.2345	20.051	3.894
Pennar	SM959020	16.8	12/09/2007	80	22.4	0.9362	0.2124	18.898	4.408
Pembroke Ferry (Waterloo)	SM982040	19.0	12/09/2007	30	27.3	1.4291	0.3086	15.169	4.631
Lawrenny[a]	SN009062	21.0	13/09/2007	43	21.4	0.8561	0.1892	19.301	4.525
Landshipping Quay	SN008108	26.8	14/09/2007	45	21.0	0.9034	0.2069	22.343	4.366

* Shell length (width) measured with dial calipers.

[a,b] 'Soft substrate' sites may be slightly different from 'rocky shore habitats' : see Appendix 1 for site details

†Condition Index(4) refers to method of calculation – see text for details

Cont…..

Appendix 2. (Cont.)..... Biometric data.

Cerastoderma edule, *Mytilus edulis* and *Fucus vesiculosus*, March 2008.

Site	Map Ref.	Dist. from Mouth (km)	Date	No. (specimens)	Size (mm)*	Wet Weight (g)	Dry Weight (g)	Whole soft tissue Condition index (4)	Gill Condition index (4)†	Digestive gland Condition index (4)	W/D ratio
Cerastoderma edule											
Tywi[b]	SN361082	-	09/03/2008	47	22.25	1.37	0.17	15.16	-	-	8.21
Dale[b]	SM809065	5.5	11/03/2008	60	32.11	3.57	0.37	11.26	-	-	9.57
Angle[b]	SM870027	7.3	10/03/2008	60	28.52	2.62	0.25	10.60	-	-	10.66
Pennar Mouth	SM943028	13.8	10/03/2008	55	30.94	2.62	0.22	7.33	-	-	12.06
Pennar	SM959020	16.8	10/03/2008	60	27.51	1.87	0.13	6.36	-	-	14.15
Pembroke Ferry (Ferry Inn)	SM974047	17.0	10/03/2008	60	31.45	3.04	0.21	6.67	-	-	14.61
Ferry Hill	SN003061	21.0	11/03/2008	60	30.47	2.27	0.16	5.51	-	-	14.60
Lawrenny[a]	SN009062	21.0	11/03/2008	60	28.75	1.88	0.13	5.48	-	-	14.43
Black Tar	SM999093	24.8	11/03/2008	60	29.24	1.87	0.14	5.39	-	-	13.65
Mytilus edulis											
Tywi[b]	SN361082	-	09/03/2008	60	47.36	4.50	0.78	6.83	0.29	0.85	5.81
Dale[b]	SM809065	5.5	11/03/2008	60	46.71	3.40	0.48	4.57	0.37	0.44	7.05
Angle[b]	SM870027	7.3	10/03/2008	60	42.00	2.62	0.38	6.15	0.35	0.70	6.93
Pennar Mouth	SM943028	13.8	10/03/2008	60	51.41	4.45	0.59	3.50	0.29	0.48	7.55
Pennar	SM959020	16.8	10/03/2008	60	35.29	2.05	0.28	3.99	0.37	0.32	7.38
Pembroke Ferry (Ferry Inn)	SM974047	17.0	10/03/2008	60	49.41	3.58	0.49	2.54	0.26	0.30	7.37
Ferry Hill	SN003061	21.0	11/03/2008	60	43.60	2.13	0.23	3.09	0.30	0.34	9.40
Lawrenny[a]	SN009062	21.0	11/03/2008	60	48.74	3.19	0.36	2.68	0.32	0.32	8.92
Black Tar	SM999093	24.8	11/03/2008	60	47.74	2.78	0.28	2.05	0.30	0.34	9.93
Fucus vesiculosus											
Tywi[b]	SN361082	-	09/03/2008	-	-	25.08	5.57	-	-	-	4.58
Dale[b]	SM809065	5.5	11/03/2008	-	-	26.25	4.01	-	-	-	6.51
Angle[b]	SM870027	7.3	10/03/2008	-	-	28.95	5.19	-	-	-	5.60
Pennar Mouth	SM943028	13.8	10/03/2008	-	-	32.03	5.79	-	-	-	5.58
Pennar	SM959020	16.8	10/03/2008	-	-	26.59	5.72	-	-	-	4.65
Pembroke Ferry (Ferry Inn)	SM974047	17.0	10/03/2008	-	-	19.89	3.83	-	-	-	5.17
Ferry Hill	SN003061	21.0	11/03/2008	-	-	33.82	5.23	-	-	-	6.48
Lawrenny[a]	SN009062	21.0	11/03/2008	-	-	13.93	4.05	-	-	-	3.44
Black Tar	SM999093	24.8	11/03/2008	-	-	25.84	4.00	-	-	-	6.45

* Shell length (width) measured with dial calipers.
[a,b] 'Soft substrate' sites may be slightly different from 'rocky shore habitats' : see Appendix 1 for site details
†Condition Index(4) refers to method of calculation – see text for details

Appendix 3. Metals ($\mu g\ g^{-1}$) in *Nereis diversicolor* and *Littorina littorea*, September 2007.

Site	Map Ref.	Dist (Km) from Mouth	Date	Metal $\mu g\ g^{-1}$ (dry weight) Ag	As	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Se	Sn	Zn
Nereis diversicolor																	
Tywi[a]	SN370117	-	11/09/2007	0.198	15.366	0.115	2.759	< 0.04	11.627	364.530	0.045	23.909	3.476	0.680	4.918	<0.01	146.401
Dale[a]	SM815075	6.5	13/09/2007	0.153	14.344	0.073	3.464	0.167	12.563	591.659	0.036	10.891	1.494	0.963	3.478	<0.01	161.240
Angle[a]	SM868028	7.4	12/09/2007	0.386	13.999	0.102	2.984	0.011	35.383	501.412	0.054	17.456	0.402	1.655	5.089	0.255	139.939
Pennar	SM959020	16.8	12/09/2007	0.387	13.920	0.086	3.597	< 0.04	26.845	507.664	0.041	14.680	0.365	1.476	3.801	<0.01	166.423
Pembroke Ferry (Waterloo)	SM982040	19.0	12/09/2007	0.613	13.861	0.047	2.758	0.239	37.454	415.055	0.143	16.517	3.938	2.586	2.756	0.142	137.986
Lawrenny[b]	SN017063	21.8	13/09/2007	na	16.364	0.080	2.391	0.395	20.071	535.924	0.059	15.100	4.564	1.495	3.778	0.086	180.863
Landshipping	SN011118	28.0	13/09/2007	0.762	11.781	0.123	4.332	0.390	23.383	410.654	0.155	18.847	3.565	0.679	3.617	0.026	139.873
Littorina littorea																	
Tywi[b]	SN361082	-	11/09/2007	2.147	16.869	0.988	1.030	0.443	64.902	196.643	0.139	122.087	3.625	2.678	2.047	<0.01	77.845
Dale[b]	SM809065	5.5	13/09/2007	2.139	20.443	0.376	0.350	0.216	75.532	243.180	0.112	48.544	1.178	1.279	1.359	<0.01	63.395
Angle[b]	SM870027	7.3	12/09/2007	0.960	16.798	0.359	0.331	0.228	80.331	162.592	0.104	55.719	0.791	2.114	0.880	0.011	65.933
Pennar	SM959020	16.8	12/09/2007	2.034	31.193	0.563	0.957	0.330	97.330	236.133	0.177	137.283	2.929	2.796	1.435	0.063	76.513
Pembroke Ferry (Waterloo)	SM982040	19.0	12/09/2007	3.056	28.945	0.576	1.002	0.289	102.918	262.093	0.151	94.249	2.547	1.373	1.417	0.093	79.623
Lawrenny[a]	SN009062	21.0	13/09/2007	3.241	23.120	0.365	0.628	0.179	99.059	226.095	0.153	68.731	1.390	1.583	1.869	0.123	76.032
Landshipping Quay	SN008108	26.8	14/09/2007	2.127	13.388	0.732	1.049	0.491	101.750	277.900	0.110	107.660	1.893	1.055	1.096	<0.01	68.824

< in tables denote values below Limit of Detection. na-sample not quantified due to small sample size

[a,b] 'Soft substrate' sites may be slightly different from 'rocky shore habitats' : see Appendix 1 for site details

(cont.)….

Appendix 3. (cont….). Metals (µg g^{-1}) dw in *Cerastoderma edule*, *Mytilus edulis* and *Fucus vesiculosus*, March 2008.

Site	Map Ref.	Dist (Km) from Mouth	Date	Metal µg g^{-1} (dry weight) Ag	As	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Se	Sn	Zn
									Cerastoderma edule								
Tywi[b]	SN361082	-	09/03/2008	0.035	7.83	0.279	1.004	1.338	4.556	755.4	0.116	21.703	19.124	1.474	2.719	0.228	83.91
Dale[b]	SM809065	5.5	11/03/2008	0.031	12.85	0.147	0.965	0.682	3.543	359.1	0.142	5.390	18.849	0.384	1.585	0.183	49.60
Angle[b]	SM870027	7.3	10/03/2008	0.046	14.95	0.193	0.952	0.905	3.508	560.3	0.204	6.077	23.558	0.695	2.830	0.274	50.05
Pennar Mouth	SM943028	13.8	10/03/2008	0.022	16.94	0.207	1.898	1.071	5.191	693.1	0.303	27.021	40.728	1.922	4.829	1.049	69.82
Pennar	SM959020	16.8	10/03/2008	0.031	17.30	0.424	1.708	1.748	4.889	1076.7	0.302	40.998	46.452	1.625	4.724	0.235	64.88
Pembroke Ferry (Ferry Inn)	SM974047	17.0	10/03/2008	0.029	22.29	0.356	2.312	2.136	4.827	781.8	0.235	22.179	63.669	1.350	4.366	0.595	71.69
Ferry Hill	SN003061	21.0	11/03/2008	0.049	22.92	0.294	2.713	1.538	6.443	1245.0	0.237	44.645	84.664	2.069	4.775	0.638	80.87
Lawrenny[a]	SN009062	21.0	11/03/2008	0.055	23.27	0.389	2.384	1.495	5.524	1325.7	0.320	55.362	80.635	1.859	4.561	0.295	70.19
Black Tar	SM999093	24.8	11/03/2008	0.070	15.07	0.347	2.376	1.550	6.670	1125.2	0.237	42.585	89.979	4.529	6.268	3.458	83.82
									Mytilus edulis								
Tywi[b]	SN361082	-	09/03/2008	0.042	13.22	0.551	0.334	0.623	5.303	136.4	0.193	5.647	0.465	2.004	2.548	0.777	82.20
Dale[b]	SM809065	5.5	11/03/2008	0.048	25.19	0.523	0.198	1.458	5.685	268.0	0.244	5.160	0.436	2.651	3.464	0.587	84.79
Angle[b]	SM870027	7.3	10/03/2008	0.046	17.18	0.287	0.295	0.808	5.970	124.8	0.274	6.004	0.413	1.902	3.463	0.613	77.75
Pennar Mouth	SM943028	13.8	10/03/2008	0.057	18.44	0.427	0.129	0.902	7.006	121.9	0.252	5.689	0.597	2.581	3.499	0.490	87.66
Pennar	SM959020	16.8	10/03/2008	0.068	16.72	0.686	0.269	1.891	7.828	216.5	0.374	8.124	0.372	2.730	4.051	0.788	103.21
Pembroke Ferry (Ferry Inn)	SM974047	17.0	10/03/2008	0.069	28.81	0.440	0.395	0.887	7.868	142.1	0.361	5.692	0.743	2.481	5.024	0.803	92.07
Ferry Hill	SN003061	21.0	11/03/2008	0.196	27.98	0.570	0.450	0.995	8.464	210.1	0.322	9.588	1.637	2.886	5.110	0.672	104.21
Lawrenny[a]	SN009062	21.0	11/03/2008	0.061	26.07	0.705	0.730	1.189	6.870	223.4	0.325	10.016	1.550	2.849	6.076	0.870	102.33
Black Tar	SM999093	24.8	11/03/2008	0.067	17.78	1.111	1.195	1.687	7.746	246.5	0.316	17.158	2.429	2.938	4.864	0.780	115.28
									Fucus vesiculosus								
Tywi[b]	SN361082	-	09/03/2008	0.067	45.61	0.321	1.351	0.449	2.510	223.3	0.019	176.607	5.142	0.763	0.517	0.254	68.32
Dale[b]	SM809065	5.5	11/03/2008	0.116	68.59	0.354	1.266	0.765	3.695	267.4	0.049	190.058	4.723	1.282	0.580	0.021	64.17
Angle[b]	SM870027	7.3	10/03/2008	0.100	45.16	0.287	0.995	1.342	6.380	401.5	0.015	157.047	2.519	1.973	0.121	0.208	62.24
Pennar Mouth	SM943028	13.8	10/03/2008	0.257	60.58	0.389	1.062	0.383	5.826	212.8	0.072	163.970	3.236	1.119	1.635	0.236	82.57
Pennar	SM959020	16.8	10/03/2008	0.118	40.00	0.162	2.142	0.927	5.329	496.4	0.053	268.653	3.924	2.263	0.303	0.105	90.70
Pembroke Ferry (Ferry Inn)	SM974047	17.0	10/03/2008	0.363	70.32	0.466	2.923	0.907	9.731	355.6	0.048	321.573	5.972	2.016	0.201	0.112	112.27
Ferry Hill	SN003061	21.0	11/03/2008	0.251	67.85	0.495	3.511	1.042	6.082	354.0	0.032	292.809	4.647	1.804	1.035	0.912	106.82
Lawrenny[a]	SN009062	21.0	11/03/2008	0.186	46.44	0.380	3.582	1.451	6.860	483.8	0.027	284.454	4.705	1.562	0.126	0.210	97.48
Black Tar	SM999093	24.8	11/03/2008	0.158	58.65	0.537	6.114	1.025	5.944	377.9	0.019	545.549	8.926	2.044	0.297	0.122	151.04

[a,b] 'Soft substrate' sites may be slightly different from 'rocky shore habitats' : see Appendix 1 for site details

Appendix 4. Organotins (μg kg^{-1}) in *Nereis diversicolor*, September 2007 and *Mytilus edulis*, March 2008.

Organotins μg kg^{-1} (dry weight)									
Site	**Map Ref.**	**Mouth**	**Date**	**MBT**	**DBT**	**TBT**	**MPT**	**DPT**	**TPT**
			Nereis diversicolor						
Tywi[a]	SN370117	-	11/09/2007	64.03	12.22	18.48	28.72	31.53	6.47
Dale[a]	SM815075	6.5	13/09/2007	41.76	15.84	30.72	35.81	24.02	5.02
Angle[a]	SM868028	7.4	12/09/2007	166.26	104.77	33.72	44.58	30.93	12.04
Pennar	SM959020	16.8	12/09/2007	42.20	15.73	13.85	37.86	13.38	5.13
Pembroke Ferry (Waterloo)	SM982040	19.0	12/09/2007	88.91	35.41	19.24	163.65	115.60	36.58
Lawrenny[b]	SN017063	21.8	13/09/2007	60.15	31.28	9.04	38.54	19.51	5.51
Landshipping	SN011118	28.0	13/09/2007	68.12	39.85	15.41	48.73	180.69	7.14
			Mytilus edulis						
Tywi[b]	SN361082	-	09/03/2008	54	2	<1	<1	0.66	0.27
Dale[b]	SM809065	5.5	11/03/2008	454	14	62	<1	<1	1.26
Angle[b]	SM870027	7.3	10/03/2008	79	13	59	<1	0.9	1.71
Pennar Mouth	SM943028	13.8	10/03/2008	106	15	72	<1	<1	1.48
Pennar	SM959020	16.8	10/03/2008	82	13	78	<1	0.93	2.64
Pembroke Ferry (Ferry Inn)	SM974047	17.0	10/03/2008	104	34	123	<1	<1	1.49
Ferry Hill	SN003061	21.0	11/03/2008	104	19	103	<1	0.83	3.07
Lawrenny[a]	SN009062	21.0	11/03/2008	336	29	100	<1	0.73	2.73
Black Tar	SM999093	24.8	11/03/2008	129	17	83	<1	<1	2.95

< in tables denote values below Limit of Detection.

[a,b] 'Soft substrate' sites may be slightly different from 'rocky shore habitats' : see Appendix 1 for site details

Appendix 5. PAHs (µg kg^{-1}) in *Nereis diversicolor*, September 2007 and *Mytilus edulis*, March 2008.

Site	Naph.	1-Me-naph.	2-Me naph	Ace.	Fluor.	Phen.	Anth.	Fanth.	Pyr.	B(a) A	Chrys.	B(b)F	Pery.	B(k)F	B(a)P	DB(ah)A	B(ghi)P	Ind(123cd)P	Total PAHs
***Nereis diversicolor* (September 2007)**																			
Tywi[a]	0.97	1.26	NA	0.94	4.54	49.47	1.96	14.91	5.86	1.27	2.48	2.65	0.67	0.24	0.40	< 0.06	0.61	< 0.07	88.3
Dale[a]	3.51	1.91	NA	2.07	4.22	55.02	3.23	65.09	66.82	3.72	4.33	5.47	< 0.22	0.67	1.48	< 0.18	0.33	< 0.12	218.4
Angle[a]	5.04	2.19	NA	0.88	4.64	45.39	2.10	11.72	7.20	1.97	2.66	4.12	< 0.25	0.19	0.76	< 0.75	1.80	< 0.13	91.8
Pennar	2.95	< 0.99	NA	0.51	3.51	39.79	2.18	8.12	3.89	1.20	1.46	1.39	< 0.05	< 0.33	0.53	< 0.06	0.37	< 0.13	67.4
Pembroke Ferry (Waterloo)	11.65	2.21	NA	< 0.30	3.92	35.71	1.48	10.20	4.58	1.62	1.62	2.61	< 0.16	< 0.03	0.65	< 0.29	1.12	< 0.15	78.3
Lawrenny[b]	7.09	4.62	NA	< 0.14	3.74	35.21	1.40	16.65	15.08	1.19	1.96	3.62	< 0.05	1.58	1.60	< 0.06	0.85	< 0.03	94.6
Landshipping	3.35	2.00	NA	0.78	4.26	39.94	2.07	10.16	5.97	1.31	1.41	2.15	< 0.05	< 0.01	0.29	< 0.06	0.30	< 0.06	74.2
***Mytilus edulis* (March 2008)**																			
Tywi[b]	4.26	<0.77	0.86	<0.40	2.00	20.6	<0.00	25.4	24.1	2.80	5.23	9.13	4.39	3.08	1.00	<0.00	5.29	1.01	110.4
Dale[b]	7.10	0.92	2.10	<0.40	1.39	16.3	0.70	16.1	19.6	9.92	12.6	17.1	6.26	4.70	1.89	<0.00	7.92	2.57	127.7
Angle[b]	3.62	1.86	1.63	<0.25	2.15	23.1	1.09	27.9	33.6	12.5	26.8	34.2	12.0	8.08	3.74	<0.03	13.5	3.80	209.8
Pennar Mouth	6.34	3.22	2.62	0.84	1.44	18.1	0.31	23.8	38.8	25.5	29.9	43.3	14.3	11.4	5.59	<0.02	14.2	4.30	244.0
Pennar	4.74	2.89	1.83	<0.35	1.35	15.5	0.79	16.9	23.1	17.8	12.6	25.0	12.8	7.01	3.28	<0.08	11.4	1.93	159.3
Pembroke Ferry (Ferry Inn)	4.13	9.29	2.01	0.62	1.75	19.8	1.02	29.2	33.5	30.7	27.4	44.2	16.2	12.2	9.58	1.55	17.6	1.59	262.3
Ferry Hill	7.54	2.52	2.27	0.52	1.80	17.7	0.92	14.5	25.2	20.4	11.3	28.8	11.7	8.46	6.30	<0.82	12.7	4.66	178.1
Lawrenny[a]	3.84	1.28	1.59	0.68	1.54	13.4	1.21	17.5	24.6	7.55	11.2	29.2	14.2	8.22	5.92	1.25	15.4	3.70	162.3
Black Tar	5.46	2.85	1.74	<0.15	1.32	11.2	0.70	10.4	14.5	6.62	6.32	28.7	13.6	9.15	6.54	<0.92	16.9	3.45	140.7

< in tables denote values below Limit of Detection. NA not analysed

[a,b] 'Soft substrate' sites may be slightly different from 'rocky shore habitats' : see Appendix 1 for site details

Appendix 6. PCBs (µg kg^{-1}) in *Nereis diversicolor*, September 2007 and *Mytilus edulis*, March 2008.

Site	Map Ref.	Mouth	Date	PCB 028	PCB 052	PCB 101	PCB 118	PCB 153	PCB 138	PCB 180	Total of 7 PCBs
					Nereis diversicolor						
Tywi[a]	SN370117	-	11/09/2007	1.044	1.181	0.000	0.000	3.111	1.708	0.000	7.044
Dale[a]	SM815075	6.5	13/09/2007	2.022	0.608	0.456	0.000	2.266	1.234	0.000	6.586
Angle[a]	SM868028	7.4	12/09/2007	2.930	0.578	0.364	0.726	3.211	1.551	1.686	11.046
Pennar	SM959020	16.8	12/09/2007	3.336	0.660	0.434	0.623	1.751	0.943	0.705	8.452
Pembroke Ferry (Waterloo)	SM982040	19.0	12/09/2007	2.900	0.493	0.000	0.915	3.279	1.686	0.966	10.239
Lawrenny[b]	SN017063	21.8	13/09/2007	2.648	0.568	0.491	0.776	2.167	1.255	0.974	8.878
Landshipping	SN011118	28.0	13/09/2007	1.579	0.816	0.000	0.625	3.662	1.601	1.253	9.535
					Mytilus edulis						
Tywi[b]	SN361082	-	09/03/2008	0.272	0.558	1.250	1.330	7.406	2.841	0.030	13.688
Dale[b]	SM809065	5.5	11/03/2008	0.633	0.525	11.452	16.019	7.608	2.873	0.382	39.492
Angle[b]	SM870027	7.3	10/03/2008	3.013	0.551	2.677	2.867	23.188	7.364	0.322	39.983
Pennar Mouth	SM943028	13.8	10/03/2008	0.000	0.000	2.496	2.620	10.836	4.039	0.312	20.302
Pennar	SM959020	16.8	10/03/2008	-	-	-	-	-	-	-	-
Pembroke Ferry (Ferry Inn)	SM974047	17.0	10/03/2008	0.498	0.735	2.376	2.584	11.557	3.747	0.561	22.057
Ferry Hill	SN003061	21.0	11/03/2008	0.247	0.261	1.628	1.699	7.781	2.395	0.449	14.459
Lawrenny[a]	SN009062	21.0	11/03/2008	0.504	0.493	1.441	2.192	8.872	2.523	0.052	16.077
Black Tar	SM999093	24.8	11/03/2008	0.545	0.332	1.018	1.604	4.346	1.631	0.002	9.479

[a,b] ‘Soft substrate’ sites may be slightly different from ‘rocky shore habitats’ : see Appendix 1 for site details

Appendix 7. Future R&D: Integrating bioaccumulation, environmental data and biological response using multivariate methods

The purpose of this 'add-on' is to demonstrate a possible way forward for future assessment of Milford Haven in terms of better integration of environmental data with biological consequences (including suggestions for a biomarker style investigation on page xvii). We have trialled two biological response 'biomarkers' namely metallothionein (a measure of metal exposure) and TOSC (total oxyradical scavenging capacity- a measure of oxidative stress)[2] in *Mytilus edulis* samples from the current Milford Haven project. The data have been input to the statistical package Primer-E alongside corresponding data for condition indices, metals, organotins, ∑PAHs and ∑PCBs to examine the principle components responsible for site distinctiveness.

Methods -Biomarkers

A sub-sample of 8 *M. edulis* specimens was taken from each site for biomarker determination –metallothionein (MT) and total oxyradical scavenging capacity (TOSC). These individuals were dissected in to gills and digestive gland tissues. Each tissue sample was weighed into a separate, labeled, Eppendorf tube, "flash"-frozen with liquid nitrogen and stored at -70°C until processed. The remaining soft tissue from each individual was weighed, pooled for each site, and frozen. Individual empty shell weights (blotted of excess water) were also recorded.

Metallothionein and TOSC (total oxyradical scavenging capacity) determinations

Frozen tissues (*M. edulis* gill and digestive gland tissue - stored at -70°C) were processed as described by Chesman *et al*. (2007). Protein content of the final, heat-treated, 28000g supernatants were determined and separate aliquots stored at -70°C for metallothionein and oxyradical scavenging assays. Metallothionein was determined by differential pulse polarography (DPP) using a PARC model 174A analyser, and a PARC/EG&G model 303 static mercury drop electrode, according to methods described previously (Bebianno and Langston, 1989; Langston *et al.*, 2002). Oxyradical scavenging capacity was determined in the same heat-treated samples[3] using the TOSC method, measuring the suppression of ethylene formation by a system containing the peroxy-radical generator 2,2'-azobis-amidinopropane dihydrochloride and a labile substrate α-keto-γ-methiolbutyric acid, as described in Chesman *et al*., 2007.

[2] *Metallothionein is a metal binding protein induced by, and signifying exposure to, metal contamination. TOSC is a measure of a tissue's potential to suppress oxyradical activity and considered an indicator of oxidative stress caused by a range of organic and inorganic chemicals (including PAHs, metals and other factors which may cause an increase in damaging, intracellular, reactive oxygen species).*

[3] *Preliminary studies indicated that oxyradical scavenging capacity of the preparations for peroxy-radicals was not affected by the heat treatment used in the metallothionein procedure, relative to non-heated preparations, whereas for peroxynitrite- and hydroxyl-radicals scavenging capacity was significantly reduced by the heating process.*

Multivariate analysis of Milford Haven data

Data collected in this survey comprise a wide range of variables including biometric data, bioaccumulated metal, organometal, hydrocarbon and PCB burdens and examples of two biomarker responses. It is useful to be able to compare the different sampling sites on the basis of all these combined parameters, for individual species. This enables reduction of complex multivariate data to a much simpler representation of 'similarity' i.e. which sites are most similar to, or different from, each other.

This process can be achieved by using Principal Component Analysis (PCA) of these environmental variables within the PRIMER software package.

Since the data comprise a wide range of units and measurement scales it is essential that pre-treatment of the data is performed so that each variable has equal weighting in the final PCA ordination (otherwise the analysis could be dominated by the numerically large data values). Initially, data were transformed ($\log_{10}$) where necessary to give approximate normality in distributions (typically metal burdens were transformed). Then, ALL data were normalised (subtracting the mean and dividing by the standard deviation, for each variable) prior to PCA ordination so that all variables are presented on a common scale. The mechanics of PCA need not be described here, but essentially the process reduces the location of each sample (in this case sampling site) from a position in multidimensional space (29-D here) to a best-fitting 2-D or 3-D solution which captures as much of the original variability as possible. Each of the PCA ordination axes are described in terms of ratios of the original variables (eigenvalues) and these contributions can be (optionally) represented on the PCA plot as eigenvectors.

As an example we show the results of a 2-D PCA plot for mussel data from the present survey (Table 9 & Figure 19). Data for individual hydrocarbons and PCBs were reduced to their respective totals prior to PCA ordination.

Examination of Table 9 shows that 2-D PCA represents 58.5% of the original 29-D variation, while 3-D ordination captures 72.7%. Further investigation of the individual eigenvectors that contribute to each of these axes shows that there is approximately equal (positive) contribution to PC1 from each of the heavy metals and organotins, together with (negative) contributions from some of the biometric data (weight and condition index). PC2 is dominated by eigenvectors representing total hydrocarbons (positive) and digestive gland TOSC (negative). The sign (positive or negative) refer only to the direction of the eigenvector on the axis, the numerical value represents its magnitude and relative contribution to the axis. We have only shown the 2-D PCA ordination here (Figure 19), but the 3-D ordination adds further refinement by spatially separating variables already described within the 2-d analysis (e.g. size, weight, gill TOSC).

Figure 19 appears cluttered due to superimposition of the eigenvectors for each variable (shown in blue), but more importantly shows how the different sampling sites (black text) relate to each other within this dimensionally-reduced multivariate space. Clearly the 'control' site (St Ishmael, Tywi) is on the far bottom left of the plot, distant from most of the other sites. By considering the eigenvectors, this appears largely to be due to a combination of low metals, low hydrocarbons and PCBs, high levels of digestive gland TOSC (high capacity to scavenge oxyradicals) and high levels of biometrics (size, weight and condition index). Collectively this shows that mussels from St Ishmael were generally larger, in good condition, with low levels of accumulated contaminants and high capacity to cope with pollutant-induced stress.

Outer sites in Milford Haven - Dale and Angle - also separated from inner estuary sites in Figure 19. Dale is at a similar level to St Ishmael on PC2 (hydrocarbons, PCBs and DG TOSC) but further to the right on PC1 indicating higher metal burdens and lower biometrics. In contrast, at Angle a few km southeast across the entrance, there appears to be slightly less influence of metals, but much higher effect of hydrocarbons and PCBs together with a reduction in DG TOSC.

Pennar Mouth, the entrance to the Pembroke River, sits between Dale and Angle in Figure 19, although it shows greatest similarity with Angle, probably reflecting the influence of hydrocarbons and PCBs at this site.

The remaining inner estuary sites fall on the right-hand side of the PCA ordination, with an overall tendency for the most upstream sites to lie towards the bottom of the plot. The location of these sites on the right would appear to result principally from the combined accumulation of metals and organotins together with metallotheionein induction in mussels, while the vertical 'zonation' results from the components in PC2 (principally total hydrocarbons, PCBs and DG TOSC, although the eigenvectors show that several metals also exert influence in this direction as well).

It is tempting to 'over-analyse' PCA plots in determining the factors that best describe the similarities or difference seen between sites, but it must be remembered that PCA is a simplification of the true multivariate data and ALL factors will exert an influence on the final 2-D plot and that inevitably some of the multivariate variability is lost and is not apparent anyway.

Overall, and as a broad summary including the caveat above, it may be regarded that in Figure 19 there is an increasing level of contaminant impact and biological response moving from bottom-left to top right across the PCA plot. Sites closest to each other (in the PCA plot, but not necessarily geographically) are the most similar, while, conversely, those furthest apart are most different in terms of the parameters measured in mussels during this survey.

One of the valuable features of the PRIMER software package to consider for the future is that it also enables linking of biological community data with environmental variable data – using the BIOENV procedure. To do so will of course require that quantitative biological, environmental and contaminant sampling are conducted alongside each other. This linked data analysis then becomes a powerful tool for understandinging the environmental and chemical parameters which most influence the nature of biological communities at different sites. For example, it may show that environmental factors such as salinity, sediment grainsize or exposure exert greater influence on the community than the impact of contaminants. It may be useful in the future to generate such compatible datasets so that we may better understand the principal drivers acting on the biota in Milford Haven.

Table 9. Results of PCA ordination of Milford Haven Mussel data

Eigenvalues

PC	Eigenvalues	%Variation	Cum.%Variation
1	11.8	42.1	42.1
2	4.58	16.3	58.5
3	3.98	14.2	72.7

Eigenvectors

(Coefficients in the linear combinations of variables making up PC's)

Variable	PC1	PC2	PC3
Mean Size	-0.028	-0.095	0.469
Mean Wet Wt	-0.180	-0.099	0.328
Mean Dry Wt	-0.237	-0.088	0.201
Log(0.1+Ag)	0.171	0.102	-0.008
Log(0.1+As)	0.163	0.129	0.261
Log(0.1+Cd)	0.196	-0.277	-0.070
Log(0.1+Co)	0.196	-0.151	-0.012
Log(0.1+Cr)	0.189	-0.101	-0.196
Log(0.1+Cu)	0.238	0.212	-0.011
Log(0.1+Fe)	0.196	-0.247	-0.098
Log(0.1+Hg)	0.234	0.240	-0.095
Log(0.1+Mn)	0.232	-0.151	-0.107
Log(0.1+Ni)	0.228	-0.121	0.145
Log(0.1+Pb)	0.253	-0.086	0.080
Log(0.1+Se)	0.267	0.090	0.110
Log(0.1+Sn)	0.135	-0.066	-0.094
Log(0.1+Zn)	0.271	-0.078	-0.059
Log(0.1+MBT)	0.103	-0.184	0.195
DBT	0.182	0.207	0.255
TBT	0.220	0.227	0.185
TPT	0.265	0.032	-0.129
Total hydrocarbons	0.002	0.410	0.200
Total of 7 PCBs	-0.106	0.146	-0.208
Gill MT (dw)	0.103	-0.007	0.173
Gill MT (prot)	0.098	-0.276	-0.114
Gill TOSC	0.004	0.250	-0.320
DG TOSC	-0.008	-0.385	0.152
whole ST CI (4)	-0.256	-0.062	-0.180

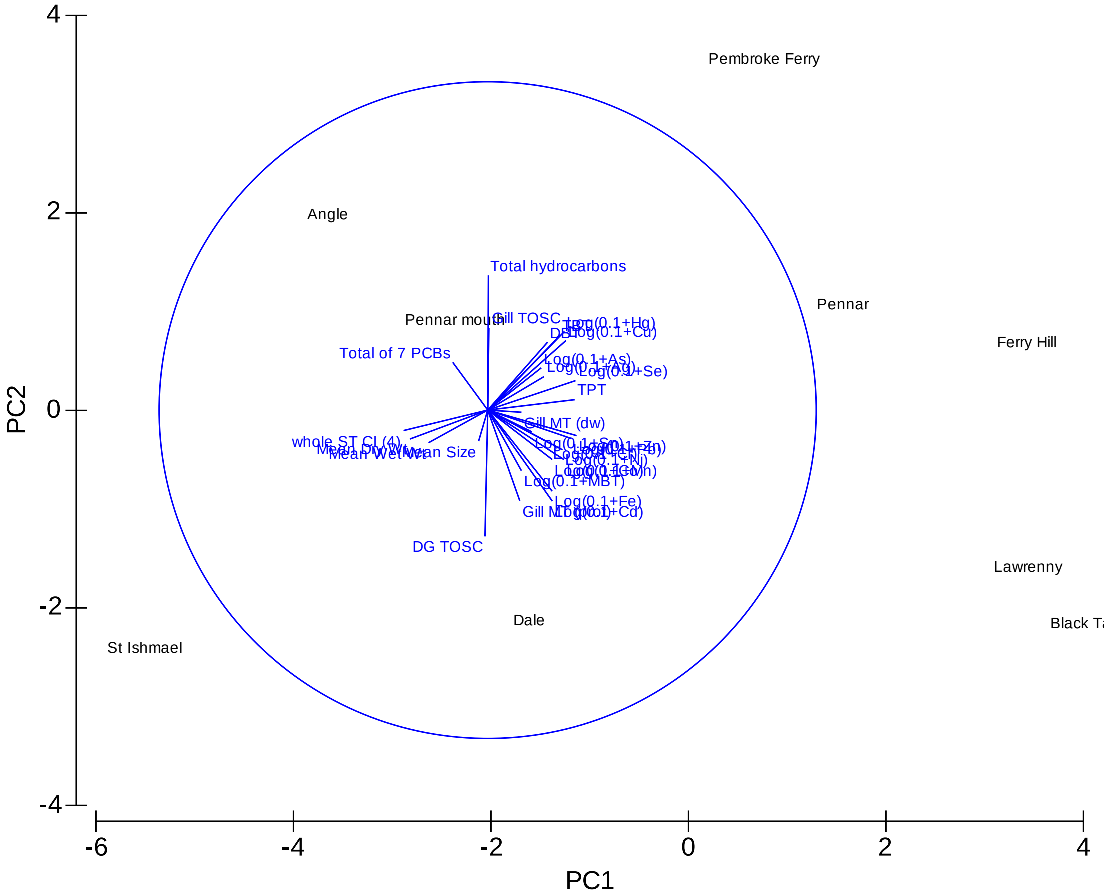

Figure 19. 2-dimensional PCA ordination of Milford Haven mussel data. Eigenvectors for individual variables (contaminants, biomarkers, biometrics) are indicated in blue. Locations of sampling sites (black text) in the diagram show how they relate to each other within this dimensionally-reduced multivariate space (see text).

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APPLICATION OF NOVEL BIOLOGICAL EFFECTS TOOLS TO ASSESS THE CONDITION OF THE MILFORD HAVEN WATERWAY

Evidence has accumulated, worldwide, advocating the application of 'biomarkers of exposure and effect' in environmental monitoring and management. Such schemes have now been developed at Plymouth and applied, recently, to Marine SACs in the south west - to gather information on their sensitivity and potential for informing environmental risk or condition assessments [1]. There is now ample scope for deployment of these 'tools' along the Milford Haven Waterway, to help environmental managers arrive at the most appropriate assessment.

These sensitive, sub-lethal assays are intended to be used alongside more conventional chemical and ecological techniques, to provide a *weight-of-evidence* approach to environmental assessment. To provide this higher level of environmental protection it is suggested that the following suite of techniques, or a subset thereof, may be appropriate to cover the most relevant risks to the Milford Haven Waterway:

'**Exposure biomarkers'**: (these measure response to specific classes of chemicals)

- *Metallothionein* - a protein specifically induced in response to metal exposure
- *Organophosphate and carbamate pesticides* and other neurotoxic compounds. Effects are assessed by measuring inhibition of esterase enzymes
- *Comet Assay* is used to indicate DNA damage and exposure to genotoxic agents.

'**Effects biomarkers'** (these signify general effects on 'health')

- *Immunocompetence* –immune function in invertebrates is determined by measuring the ability of blood cells to isolate foreign particles.
- *Reactive oxygen species* (ROS) including oxygen ions, free radicals, and peroxides, are highly reactive and at times of environmental stress can increase dramatically, resulting in significant damage to cell structures.
- *Total Oxyradical Scavenging Capacity* (TOSC) is a general biochemical marker of oxidative stress induced by, for example, pollutants and fluctuating DO levels associated with eutrophication.
- *Intersex and imposex* provides an assessment of exposure to endocrine disrupting chemicals and reproductive consequences.
- *Sediment bioassays* include burrowing responses and survival in infaunal species and are a measure of sediment quality

A suggested approach in Milford Haven Waterway would involve:

- Implementation of a biological effects measurement, based on the above techniques (or a subset thereof), at a minimum of six sites along the Milford Haven Waterway and at least one reference site outside. Sites used should be based on those for which we have established contaminant data in the bioaccumulation study. We suggest this program focuses on the mussel *Mytilus edulis* which is present in suitable numbers from the mouth of the Haven to well upstream near the confluence of the Eastern and Western Cleddau. Some assays for reproductive effects would also involve clams *Scrobicularia plana,* and dogwhelks *Nucella lapillus.*

Synthesis of results on biological effects alongside other contextual data e.g. bioaccumulation, sediment contaminants (and, ultimately, community data). This integration of data sets is important to better characterise the status and trends of biota in the Milford Haven Waterway and to help separate anthropogenic from natural forcing features. Ideally, body burdens should be determined at the same time (in the same samples) as biological effects measurements; a less satisfactory option would be to use the data in the present study as representative of conditions at the time of future sampling.

The diagnostic value of individual biomarkers, and inter-relationships between different biological responses and environmental data, should be analysed to assess both specific threats and to compare the overall 'health' of animals between sites (based on integrated response data analysed by multivariate methods described above). Baseline information on the status and trends of biomarkers in the Waterway could be used to generate a simple classification scheme for Milford Haven and estuaries, and as a platform for future assessments. Where possible the results should be compared with similar datasets for other estuaries and coastal sites in the UK.

[1]Galloway, T., Langston, W., Hagger, J., Jones, M. (2007) The application of biological-effects tools to inform the condition of European Marine Sites. Final Report, EN contract FST20-18-02 90pp+Appendices