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Endocrine disrupters and European Marine Sites in England

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Endocrine disrupters and European Marine Sites in England

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Preface

When CEFAS conducted this study in 2001 the preliminary risk assessments included in Section 7 were focused on the designated and proposed European Marine Sites, and their interest features, at the time of writing. Subsequent to this study, amendments to the list of interest features for the sites may have either been made, or at least proposed. However, in the majority of cases, this is unlikely to affect the validity of the preliminary risk assessments undertaken. It is also important to note that the sites referred to as Special Areas of Conservation (SACs) in this report are currently candidate SACs (cSACs) unless stated otherwise.[1]

The data included in these assessments were largely limited to those sampling points located within the boundaries of the designated site. It is highly likely that relevant data exist for sub-tidal regions bordering the European Marine Sites, and also in reaches above the tidal limit, but inclusion of these data was outside the scope of this preliminary study. Recently, additional reviews of water quality information, including those data held by Environment Agency Regions, have been conducted for several sites (particularly those located in the South West) to support the Agency's Review of Consents Project.

In Chapter 4, English Nature have highlighted, by way of footnotes, some particularly key 2002 papers, which were produced subsequent to the 2001 review conducted by CEFAS.

The risk of endocrine disruption on SACs and SPAs is an ongoing discussion theme in the work plan of the Environment Agency-English Nature Water Quality Technical Advisory Group.

Dr Mark Taylor
English Nature

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[1] Candidate SACs are sites which have been proposed to the European Commission by the UK government as Sites of Community Importance but have yet to be listed as such by the Commission following agreement with the UK. Following an amendment to the Conservation (Natural Habitats, &c.) Regulations 1994, made in 2000, candidate SACs fall within the definition of European site for the purposes of those Regulations. As such, the relevant provisions of those Regulations apply to those sites as a matter of law. As a matter of government policy, the same level of protection is to be afforded to candidate SACs in other parts of the UK

Contents

1. Executive summary

A large number of anthropogenic substances in the marine environment are suspected of being endocrine disrupters (EDs) i.e. they are known or thought to interfere with the endocrine system in such a way that normal endocrine function is affected and adverse health effects ensue. However, the quality of the evidence for this suspicion is uneven, and much derives solely from *in vitro* assays rather than *in vivo* tests with whole organisms. This means that, for most EDs, it is as yet impossible to conduct reliable environmental risk assessments. This objective is made even harder to achieve by the fact that EDs may occur as complex mixtures, containing some that stimulate, some that block and some that may act partially on common receptors. The toxicity/ED activity of a complex mixture is therefore difficult to predict unless a lot is known about the component chemicals.

Reliable information on the effects of endocrine disrupters in aquatic wildlife is patchy, with the most complete data being available on fish exposed to oestrogens and their mimics, but relatively poor information being available on other marine vertebrates such as birds and mammals. Knowledge of endocrine disruption in invertebrates is even more sparse because their endocrine systems are poorly understood and have been less well investigated, although there is one example (the effects of tributyltin in molluscs) which is well documented.

Within the limitations referred to above, this report lists the known and suspected endocrine disrupters and briefly describes their potential or actual effects in marine wildlife.

This report then collates and evaluates CEFAS and Environment Agency data for the 5-year period 1995-1999 on concentrations of contaminants (mainly suspect endocrine disrupters) in waters, sediments, invertebrates, fish and mammals in certain English Special Areas of Conservation (SACs) and Special Protected Areas (SPAs) based on the site list current in 1999. These data are generally sparse and do not permit a comprehensive risk assessment of endocrine disrupters in any SAC or SPA. However, their possible implications for wildlife are considered in conjunction with data on domestic and industrial discharge volumes in or near the areas of interest. Any known cases of endocrine disruption in the areas of interest are also reported.

Although the environmental risks posed by endocrine disrupters in SACs and SPAs are only dealt with in a preliminary fashion, it is clear that endocrine disruption is occurring, or is likely to be occurring, in some of these areas. Those areas identified as high priorities for future research on this subject (based on presence of large volumes of discharges, elevated concentrations of contaminants, and/or known endocrine disruption) are Teesmouth and Cleveland Coast SPA, Thames Estuary and Marshes SPA, Solent SAC, Poole Harbour SPA, Plymouth Sound and Estuaries SAC, Mersey Estuary SPA and Morecambe Bay SAC. Other areas are given a medium or low priority rating for future research based on this analysis, although other factors such as presence of rare and potentially highly sensitive species (such as shad spp.) may provide a separate reason for requiring local investigations. One such example is the Severn estuary.

Finally, some approaches to the design of investigations of endocrine disruption in SACs and SPAs are outlined. These investigations are required to permit the conduct of reliable environmental risk assessments, although present knowledge on the subject is still too weak to permit fully comprehensive evaluations.

2. General Introduction

English Nature has a statutory responsibility to advise relevant authorities as to the conservation objectives of European Marine Sites in England, and to advise these authorities about any operations which may cause deterioration of natural habitats, or any disturbance of species for which the site has been designated.

European Marine Sites are defined in the Conservation (Natural Habitats, &c) Regulations 1994 as "any part of a European site covered (continuously or intermittently) by tidal waters or any part of the sea in or adjacent to Great Britain up to the seaward limit of territorial waters". European sites include Special Areas of Conservation (SACs) under the Habitats Directive (Council Directive 92/43/EEC), which support certain natural habitats and species of European importance, and Special Protection Areas (SPAs) under the Birds Directive (Council Directive 79/409/EEC), which support significant numbers of internationally important wild birds. Some of these sites partially overlap.

It has been estimated that there are probably more than 60,000 organic pollutants present in the marine environment (Maugh, 1987). These originate from domestic and industrial effluents, leachates from solid waste disposal sites, deliberate application of agrochemicals, agricultural and urban run-off, contaminated land, anti foulants, incineration, atmospheric fall-out, marine traffic, and accidental discharge.

In a recent report for English Nature, one of the key water quality issues identified as likely to affect the interest features of European Marine Sites in the UK was hormone (endocrine) disruption (Cole *et al.,* 1999). The term 'endocrine disrupter' has been defined by the European Commission as **'an exogenous substance that causes adverse health effects in an intact organism, or its progeny, consequent to changes in endocrine function'** (EC, 1997). Reviews carried out by IEH (1995, 1999), CSL (2000) and the Environment Agency (EA, 1998a) have produced lists of known endocrine disrupters (EDs) and potential endocrine disrupters, based on existing evidence from laboratory and field studies. Also, the European Commission has drafted a more comprehensive document (EC, 1999) detailing over 500 known and potential endocrine disrupters, grouped into 38 classes. However, for many of the chemicals listed, there is no available evidence to confirm endocrine disrupting effects.

The objectives of this English Nature project were to:

1. Carry out a preliminary assessment of the potential risks of endocrine disrupters to marine habitats, species and conservation features of European Marine Sites in England.

2. To recommend those sites and features (species) where monitoring and assessment of potential endocrine disruption effects is likely to be most urgently required.

The aims of the literature review (section 3) were to: 1) summarise the existing evidence for the role as potential endocrine disrupters of those chemicals likely to enter the marine environment, 2) to provide an assesment of the sensitivity of different classes of marine organisms to different groups of potential endocrine disrupters and 3) to enable a prediction of the likely or possible effects of EDs on populations of sensitive species

Table 1 lists the substances for which evidence for endocrine disruption is strongest, based on information provided from the reviews cited above and other literature. *In vitro* tests used to evaluate endocrine disruption include cell-free systems, tissue or primary cell cultures of human or animal origin, established cell lines from human or animal tissue and yeast-based assays. They measure a range of biological activities, such as cell proliferation, substance expression and competitive binding of hormones. They are useful as an initial screening tool, being response specific (e.g. oestrogen, androgen etc.), rapid and inexpensive, but lack the ability to accurately model pharmacodynamic/pharmacokinetic, metabolic, bioaccumulation and bioconcentration processes, which are important in causing effects *in vivo*. *In vivo* tests, whilst being generally longer-term and more expensive to run, have the advantage that they are highly integrative, can address mixed mechanisms of action and numerous endpoints and they integrate the potential for metabolism of an inactive compound to active products. *In vitro* tests cannot, therefore, accurately predict endocrine activity *in vivo*, (although reasonable agreement between the two is emerging) (Shelby *et al* 1996; Tremblay & Van Der Kraak 1998). At the present time, the former must be supported by the latter in order to evaluate the environmental relevance of the identified contaminants and reduce the risk of false positive and false negative responses. It was agreed at the European Commission workshop (EC, 1996), that endocrine disrupting activity could only be adequately defined in terms of effects in intact animals (i.e. *in vivo* effects). Chemicals for which evidence for endocrine disrupting activity has been obtained solely by the use of *in vitro* models should be considered only as 'potential' EDs. This report follows that format: those chemicals which have been tested and are positive *in vivo* are described as **known** EDs, while those which have only been tested using an *in vitro* screen are classed as **potential** EDs. Obviously some chemicals can be found in both lists, as they have been found to produce effects both *in vitro* and *in vivo*.

Section 3 of this report reviews the available information on the potential risks to wildlife, which the substances in Table 1 may pose. Section 4 briefly describes the volumes and locations of sewage and industrial waste discharged into the various English SACs and SPAs. Although other sources outside these areas will also contribute to their contamination with EDs, this is considered a reasonable approach for prioritising sites for future investigation. Section 5 summarises the available CEFAS and EA monitoring data for a period of 5 years (1995-99) from the various sites relating to metals and organic substances in waters, sediments and biota. Section 6 then attempts a preliminary evaluation of the risks which these EDs may be causing to wildlife in each area, although the general lack of data (both from monitoring programmes, and from dose-response experiments with EDs) limits this evaluation to a purely qualitative exercise. Finally, the report reaches some brief conclusions, and makes recommendations for future research.

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Table 1. Summary of potential and confirmed endocrine disrupting substances (excluding metals) for which strongest evidence exists

Substance	tested		*in vitro* mode of action on effect	*in vitro* test used	*in vivo* mode of action on effect	*In vivo* test used	references
	in vitro	*in vivo*					
Plant products							
Equol	✓	✓	oestrogen	Sheep uterine receptor	oestrogen	sturgeon	Shutt & Cox (1972); Pelissero *et al* (1991)
b-sitosterol	✓	✓	oestrogen	Human HeLa	impaired steroidogenesis	mosquitofish	Miksicek (1994); Malini & Vanithakumari (1993)
coumestrol	✓	✓	oestrogen	Human MCF-7	oestrogen	sturgeon	Martin *et al* (1978); Pelissero *et al* (1991)
biochanin A	✓	✓	oestrogen	Human HeLa	oestrogen	sturgeon	Miksicek (1995); Pelissero *et al* (1991)
4-vinylguaicol		✓			oestrogen	Vole reproduction inhibition	Berger *et al* (1977)
4-vinylphenol		✓			oestrogen	vole	Berger *et al* (1977)
p-coumaric acid		✓			oestrogen	vole	Berger *et al* (1977)
ferulic acid		✓			oestrogen	vole	Berger *et al* (1977)
formononetin	✓	✓	oestrogen	Human HeLa	oestrogen	sturgeon	Miksicek (1995); Pelissero *et al* (1991)
daidzein	✓	✓	oestrogen	Human HeLa	oestrogen	sturgeon	Miksicek (1995); Pelissero *et al* (1991)
genistein	✓	✓	oestrogen	Human HeLa	oestrogen	sturgeon	Miksicek (1995); Pelissero *et al* (1991)
chalcone derivatives	✓		oestrogen	Human HeLa			Miksicek (1995)
flavones	✓		oestrogen	Human HeLa			Miksicek (1995)
flavanones	✓		oestrogen	Human HeLa			Miksicek (1995)
Mycotoxins							
zearalenone	✓		oestrogen	Human MCF-7			Martin *et al* (1978)
zearalenol	✓		oestrogen	Human MCF-7			Martin *et al* (1978)
Organochlorine pesticides							
o,p'-DDD	✓		oestrogen	Human MCF-7			Soto *et al* (1992)
p,p'-DDT	✓		oestrogen, anti-androgen	Human MCF-7, rat androgen receptor			Soto *et al* (1995); Kelce *et al* (1995)
o,p'-DDT	✓	✓	oestrogen	Human YES	oestrogen	mosquito fish VTG	Arnold *et al* (1996); Denison *et al* (1981)

Substance	tested		*in vitro* mode of action on effect	*in vitro* test used	*in vivo* mode of action on effect	*In vivo* test used	references
p,p,'-DDE	✓	✓	anti androgen	Rat androgen receptor	oestrogen	Rats developmental toxicity	Kelce *et al* (1995); Kelce *et al* (1994)
chlordecone	✓		oestrogen	Human MCF-7			Soto *et al* (1995)
endosulfan	✓		oestrogen	Human MCF-7			Soto *et al* (1994)
aldrin/dieldrin	✓	✓	oestrogen	Human MCF-7	pituitary disfunction	teleost fish	Soto *et al* (1994); Kime (1995)
lindane		✓			oestrogen	Guppies histopathology	Wester (1991)
methoxychlor	✓	✓	oestrogen	Human MCF-7	oestrogen	Mouse reproductive tract stimulation	Soto *et al* (1995); Walters *et al* (1993)
1-hydroxychlordene	✓		oestrogen	Human MCF-7			Soto *et al* (1995)
toxaphene	✓		oestrogen	Human MCF-7			Soto *et al* (1994)
Other pesticides							
Vinclozolin (dichloroanilide fungicide)		✓			anti-androgen	Rats sex differentiation	Gray *et al* (1994)
pyrethrins	✓		anti androgen	Human fibroblast + hormone binding			Eil & Nisula (1990) +
bioallethrin	✓		anti androgen	Human fibroblast + hormone binding			Eil & Nisula (1990) +
fenvalerate	✓		anti androgen	Human fibroblast + hormone binding			Eil & Nisula (1990) +
fenothrin	✓		anti androgen	Human fibroblast + hormone binding			Eil & Nisula (1990) +
fluvalinate	✓		anti androgen	Human fibroblast + hormone binding			Eil & Nisula (1990) +
permethrin	✓		anti androgen	Human fibroblast + hormone binding			Eil & Nisula (1990) +
resmethrin	✓		anti androgen	Human fibroblast + hormone binding			Eil & Nisula (1990) +
chlorvenvinphos		✓			enzyme inhibitor/impaired vitellogenesis	teleost fish	Kime (1995)

Substance	tested		*in vitro* mode of action on effect	*in vitro* test used	*in vivo* mode of action on effect	*In vivo* test used	references
diazinon		✓			pheromone antagonist	salmon	Moore & Waring (1996)
carbamates		✓			pheromone antagonist	salmon	Moore & Waring (1996)
atrazine		✓			impaired GtH release	rat	Cooper *et al* (1996)
trifluralin		✓			pituitary hormone antagonist	minnow	Couch (1984)
3-tri fluoromethyl-4-nitrophenol		✓			oestrogen	Trout VTG	Hewitt *et al* (1998)
Alkylphenols							
4-tert-butylphenol	✓		oestrogen	Human MCF-7			Soto *et al* (1995)
4-tert-octylphenol	✓	✓	oestrogen	Human MCF-7	oestrogen	trout VTG	Soto *et al* (1995); Jobling *et al* (1996)
4-sec-butylphenol	✓		oestrogen	Human MCF-7			Soto *et al* (1995)
4-tert-pentylphenol	✓		oestrogen	Human MCF-7	oestrogen	carp VTG	Soto (1995), Gimeno *et al* (1998)
4-iso-pentylphenol	✓		oestrogen	Human MCF-7			Soto *et al* (1995)
4,4odihydroxybiphenyl	✓		oestrogen	Human MCF-7			Soto *et al* (1995)
4-nonylphenol	✓	✓	oestrogen	Human MCF-7	oestrogen	trout VTG, flounder VTG	Soto **et al** (1995); Jobling *et al* (1996); Christensen *et al* (1999)
4-nonylphenol-diethoxylate	✓	✓	oestrogen	Human MCF-7	oestrogen	trout VTG	White *et al* (1994); Jobling *et al* (1996)
4-nonylphenoxycarboxylic acid	✓	✓	oestrogen	Human MCF-7	oestrogen	trout VTG	White *et al* (1994); Jobling *et al* (1996)
p-sec-amylphenol	✓		oestrogen	Rat oestrogen receptor			Meuller & Kim (1978)
1-naphthol	✓		oestrogen	Rat oestrogen receptor			Meuller & Kim (1978)
2-napthol	✓		oestrogen	Rat oestrogen receptor			Meuller & Kim (1978)
tetrahydronapthol-2	✓		oestrogen	Rat oestrogen receptor			Meuller & Kim (1978)
Phthalates							
di-n-butylphthalate	✓		oestrogen	Human MCF-7			Jobling *et al* (1995)
butylbenzylphthalate	✓		oestrogen	Human MCF-7			Jobling *et al* (1995)

Substance	tested		*in vitro* mode of action on effect	*in vitro* test used	*in vivo* mode of action on effect	*In vivo* test used	references
Natural and synthetic hormones							
ethynylestradiol	✓	✓	oestrogen	Human MCF-7	oestrogen	trout & flounder VTG	Soto *et al* (1995); Sheahan *et al* (1994) Allen *et al* (1999a)
Diethylstilbestrol	✓	✓	oestrogen	Human YES	oestrogen	Rat uterotrophic assay	Arnold *et al* (1996)
hydroxyflutamide	✓		anti androgen	Rat androgen receptor			Kelce *et al* (1995)
17B-oestradiol	✓	✓	oestrogen	Human YES	oestrogen	fathead minnow VTG	Routledge *et al* (1996); Panter *et al* (1999)
oestrone	✓	✓	oestrogen	Trout hepatocyte	oestrogen	fathead minnow VTG	Jobling *et al* (199X); Panter *et al* (1998)
Resin components							
bisphenol A	✓	✓	oestrogen	Rat MCF-7	oestrogen	trout VTG	Krishnan *et al* (1993); Lindholst *et al* (2000)
t-butylhydroxyanisole	✓		oestrogen	Human MCF-7			Soto *et al* (1995)
Industrial chemicals							
Some PAHs	✓	✓	oestrogen and antioestrogen	Trout hepatocyte	oestrogen and antioestrogen	Trout VTG	Anderson *et al* (1996a); Anderson *et al* (1996b)
PCBs	✓		anti-oestrogen	Human MCF-7			Krishnan & Safe (1993)
TCDD	✓		anti-oestrogen	Human MCF-7			Safe (1994)
PCDD	✓		anti-oestrogen				Safe (1994)
PCDF	✓		anti-oestrogen				Safe (1994)
benzo(a)pyrene	✓		anti-oestrogen				Safe (1994)
3-methylcholanthrene	✓		anti-oestrogen				Safe (1994)
indole[3,2,-b]carbazole	✓		anti-oestrogen				Safe (1994)
methyl mercury		✓			impaired steroidogenesis	guppies	Wester (1991)
TBT		✓			androgen (impaired synthesis of oestradiol)	gastropods	Bryan *et al* (1987)

+ See also Tyler *et al* 2000 for data on pyrethroid insecticides and their metabolites.

3. Review of endocrine disrupting chemicals

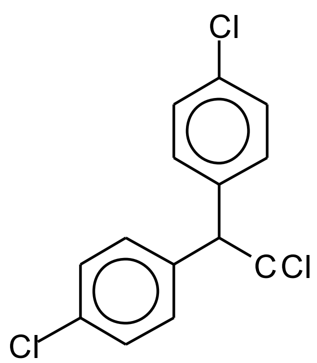
3.1 Introduction

Several major reviews have been written detailing current knowledge of endocrine disruption. IEH (1999) provides an assessment of the significance of ED chemicals in the environment for reproductive health at different levels in the ecological hierarchy, and identifies priorities for further ecological research. SETAC (1999) recently published the proceedings of a workshop, which reviewed the status of our understanding of invertebrate endocrinology, and assessed the applicability of invertebrates for ED testing and environmental monitoring. The Environment Agency (1998a) produced a review of the scientific evidence for endocrine disruption and outlined its strategic approach to protect the environment from endocrine disrupting substances. The Central Science Laboratory (CSL, 2000) has reviewed the research carried out on endocrine disrupters in top predators. Currently underway is an extensive review for WHO/UNEP/ILO International Programme on Chemical Safety, which will provide a global assessment of the State-of-the-Science of endocrine disrupters

The following section, based extensively on existing reviews, summarises the existing evidence for the role as endocrine disrupters of the major categories of chemicals likely to enter the marine environment, and provides examples of chemical structures for each group. In general, persistent chemicals may present a high risk for causing effects, due to the long-term exposure which they produce and the fact that they are more likely to be found in relatively remote parts of the marine environment. In the case of endocrine disrupters, even quickly degradable substances may be considered as potentially hazardous at points close to discharges and other inputs, because short-term exposure in very sensitive life stages may result in severe long term effects.

Endocrine disrupting chemicals can be classed according to their mode(s) of action. Much of the initial research on endocrine disruption focussed on the vertebrate sex steroid hormone systems. Substances that mimic or block the natural female sex hormones in animals are termed oestrogens and anti-oestrogens respectively; similarly, those that mimic or block the natural male sex hormones are classed as androgens and anti-androgens respectively. Endocrine disrupters can, in addition, interfere with sex steroid synthesis and metabolism, and it is now widely recognised that other endocrine systems, such as the pituitary, thyroid and thymic can also be affected.

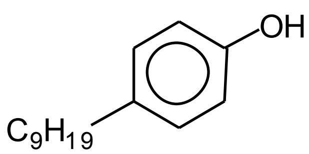
3.2 Pesticides



p,p'-DDT

Insecticides, such as DDT, dieldrin, aldrin, lindane, dichlorvos, endosulfan, permethrin, diazinon, chlorfenvinphos, herbicides such as trifluralin, atrazine, simazine and linuron, and fungicides such as vinclozolin have been shown *i* vitro or *in vivo* to exert effects on the endocrine system in a variety of ways. Some organochlorine pesticides are capable of interacting with the oestrogen receptor (e.g. *o,p'*-DDT (Donohoe and Curtis, 1996)), and others act as anti-androgens (e.g. p,p'-DDE (Kelce *et al.,*1995)) i.e. they block the androgen receptor therefore diminishing the effects of testosterone. They may also interfere with normal production of gonadotrophic hormone (GtH). The triazine herbicides are thought to have an effect on the pituitary, while some anticholinesterase pesticides are known to interfere with sensitivity to pheromones in fish at concentrations as low as 1 µg/l (Waring and Moore, 1997). Possible oestrogenic and anti-androgenic effects of pyrethroid insecticides have been reported. Fenvalerate, permethrin and resmethrin have been reported to act as anti-androgens in human *in vitro* assays (see Eil and Nisula, 1990 in Environment Agency, 1998a). Permethrin is a substance that acts as an oestrogen and disrupts androgen synthesis (reviewed in Lyons, 1996). Many of these compounds have shown adverse effects *in vivo*, therefore are considered to be known EDs. A number of marine surveys for pesticides have been undertaken. The National Monitoring Programme Survey of the Quality of UK Coastal Waters detected elevated atrazine and simazine concentrations in some estuarine waters (e.g. Humber, Thames, Tamar, Mersey and Severn), but these were below the proposed statutory Environmental Quality Standard (EQS) of 2µg/L (MPMMG, 1998). It should be noted here that most standards set for water quality are based on toxicological data which do not as yet take into account ED effects (but there are exceptions such as TBT). It may be that in the future standards will have to be revised if there is increased evidence of deleterious ED effects at lower concentrations than the existing EQS. Significantly more research is required into the ED effects of those chemicals for which EQSs exist (and those that are in the pipeline), in order to obtain evidence supporting a change to the set level. For the organochlorine pesticides, estuarine and coastal water concentrations measured as part of surveys carried out by the Environment Agency and the National Monitoring Programme were generally below the analytical limits of detection (which are well below the respective EQS concentrations) and therefore indicate low background concentrations in UK marine waters (Cole *et al*., 1999; EA, 1998a). They are ubiquitously present in estuarine sediments, but again at background levels (MPMMG, 1998); dieldrin, lindane and metabolites of DDT have been detected in shellfish and fish liver (MPMMG, 1998) and marine mammal tissues (CEFAS, 1998). A discussion concerning tributyltin (TBT) can be found in section 2.8.

3.3 Alkylphenol Polyethoxylates and Alkylphenols

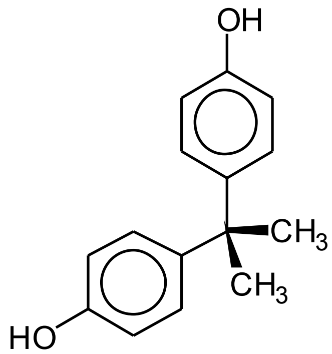


4-nonylphenol

Alkylphenol polyethoxylates are nonionic surfactants used in industrial detergents and some pesticide formulations and cosmetics. They are also used in plastics manufacture. The annual UK production of nonylphenol polyethoxylates is approximately 18 000 tonnes (Department of the Environment, 1993). Their alkylphenol degradation products have been shown to be weakly oestrogenic (see Servos, 1999 for a review) both *in vitro* (e.g. Routledge and Sumpter, 1996) and *in vivo* (Harries *et al.,* 1995). The ethoxylates are broken down in sewage treatment plants and the resulting alkylphenols remain stable in the effluent and sewage sludge. Some alkylphenols and their lower ethoxylates (e.g. nonylphenol (NP), nonylphenol monoethoxylate (NP1EO) and octylphenol (OP)) have been found to be weakly oestrogenic (when compared to the potency of the natural oestrogen 17ß –oestradiol), both *in vivo* and *in vitro* (White *et al.,* 1994; Harries *et al.,* 1997) but are nevertheless in themselves some of the more potent weak oestrogens. *In vivo* effects (VTG synthesis in trout) of nonylphenol and octylphenol were apparent at 20 and 4.8 μg/l respectively (Harries *et al*., 1995). Higher ethoxylates are not oestrogenic (White *et al*, 1994). Alkylphenolic compounds have been measured in effluents discharging to the estuarine and freshwater environments, in surface waters (Blackburn and Waldock, 1995; Blackburn *et al*., 1999; Environment Agency, 1998a) and in fish tissues (CEFAS, 1997; Lye *et al*., 1999). Concentrations of total extractable alkylphenols (which include the alkylphenols and mono- and di-ethoxylates only) in the Mersey and Tees estuaries have been reported to be 11 and 76 μg/L respectively (Blackburn *et al*., 1999), which are close to or exceed the no observed effect concentration (NOEC) for induction of vitellogenin in caged trout (5-20 μg/L total extractable alkylphenols). Concentrations of nonylphenol and nonylphenol monoethoxylate in a major sewage effluent discharging to the Tyne estuary were 3 and 45 μg/L respectively (Lye *et al*, 1999). Concentrations of these compounds in Tees estuary sediments reached 9 μg/g (NP) and 4 μg/g (NP1EO), while concentrations of NP in fish (flounder) tissues from the same area ranged from 30-180 g/g wet weight (Lye *et al*., 1999).

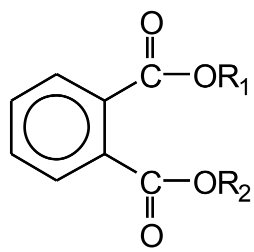
Nonylphenol has been shown to inhibit settlement of barnacle cypris larvae at concentrations above 0.1 μg/l (Billinghurst *et al*., 1998), and a LOEC for survival of 91 μg/l was observed for the midge *Chironomus tentans* (Kahl *et al*., 1997). A single intraperitoneal injection of NP at a dose of 25 mg/kg induced vitellogenin and zona radiata protein synthesis in juvenile Atlantic salmon (Arukwe *et al*., 2000). Similarly, 2 injections (weekly) of 10μg/g NP induced VTG synthesis in male flounder (Christensen *et al*., 1999). Water concentrations of 50 μg/l and above induced intersex in male Japanese medaka (Gray and Metcalf, 1997).

3.4 Bisphenol –A



Bisphenol –A (BPA) is used in the production of polycarbonate epoxy resins lining food cans and water pipes, dental coatings and fillings, flame retardants and PVC polymers. The annual world-wide production of BPA in 1993 reached 640 000 tons, 0.017% of which was estimated to be released into the environment (Benjonathon and Steinmetz, 1998; Staples *et al.*, 1998). It has been shown to be weakly oestrogenic (although, like the alkylphenols, is among the more potent of the weak oestrogens) both *in vitro* and *in vivo* (Krishnan *et al.,* 1993; Kloas *et al.,* 1999; Lutz and Kloas, 1999). In a recent study, Lindholst *et al.* (2000) demonstrated oestrogenic effects *in vivo* (vitellogenin induction in rainbow trout) at water concentrations as low as 40-70 μg/L. Although there are limited data on environmental concentrations, its wide use in many products and high production volume implies that it is liable to enter the marine and estuarine environments (EA, 1998a). Concentrations between 80 to >4000 μg/l in effluents have been reported (see EA, 1998a).

3.5 Phthalates



General formula for phthalates

Phthalates are used in the production of vinyl floor tiles, adhesives and synthetic leather, as plasticisers in food packaging and to impart flexibility into plastics. Because they are produced industrially in large quantities, they are one of the most abundant man-made chemicals in the environment (Murature *et al.,* 1987). Global annual production is estimated to be about 2.7 million tonnes (IEH, 1999). The ubiquity of these compounds in the aquatic environment is well documented; reviews focusing on the presence and risk assessment of phthalates in the aquatic environment are provided by Sheahan *et al.* (2000) and Staples *et al.* (2000).

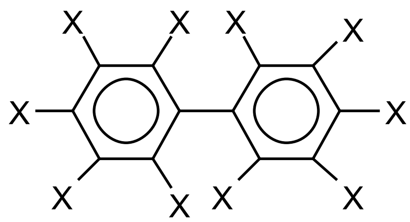
Concentrations have been measured in river, waste and drinking waters as well as in biota and sediments (e.g. Fatoki and Vernon, 1990; Hites and Biemann, 1972 and references in Jobling *et al.,* 1995). Di-2-ethylhexyl-phthalate (DEHP) is the most widely used phthalate ester (Sheahan *et al.*, 2000), and along with di-n-butyl phthalate (DBP), dimethyl phthalate (DMP), diethyl phthalate (DEP) and butylbenzene phthalate (BBP) are some of the most commonly detected phthalates in the aquatic environment (Bedding *et al.,* 1982; Lewis *et al.*, 1998). For marine water samples DEHP was measured at concentrations >0.05μg/l in 37 of 38 samples collected from U.K. estuaries and coastal sites as well as from the River Seine (Law *et al.*, 1991). The highest concentration of DEHP measured in this survey was 8.4 μg/l from a sampling site within Plymouth Sound. In the marine sediments the concentrations of phthalates is generally lower than those found in freshwater sites (Sheahan *et al.*, 2000). In the UK, the highest reported sediment concentrations of DEHP, BBP and DBP were 702, 18 and 698 μg/kg respectively, in the Mersey estuary. The only report of DMP and DEP in marine sediments measured maximum concentrations of 39 and 233 μg/kg respectively in the River Mersey (Preston and Al-Omran, 1986).

The toxicity of a range of phthalate esters to aquatic microorganisms, algae, invertebrates and fish has been reviewed by Staples *et al.* (1997). The low molecular weight phthalates (which includes all those mentioned above) consistently exerted acute and chronic effects at concentrations below the limit of solubility across all test types and species. EC50s ranged from 0.003 mg/l to 537 mg/l, depending on the test species and endpoint. Oestrogenic activity may be found in sediments and attributed to phthalates (EDMAR, in prep.). However, for marine sediment, environmental concentrations for the UK are at least an order of magnitude below the toxicity values derived from laboratory studies, mainly based on water column tests (Sheahan *et al.*, 2000). Although the endpoints measured in toxicity studies were not chosen specifically to determine endocrine disruption, some of the observed chronic effects on growth and reproduction may be due to subtle effects on endocrine systems. Further research on this point is required.

DEHP, DBP and BBP have been shown to be weakly oestrogenic *in vitro* (human breast cancer cell lines and trout oestrogen receptor; Jobling *et al.,* 1995). Similar results have been demonstrated by Knudsen and Pottinger (1999) for three major teleost steroid-binding sites. Tissue levels for DEHP in different fish species from UK waters have been measured in the range 13 to 51 µg/kg wet weight (Waldock, 1983). For molluscs a higher range of 9.2 -214 µg/kg was reported. The oestrogenic *in vitro* effects in fish were only established at concentrations equivalent to several orders of magnitude in excess of tissue concentrations (i.e. between 10^{-6} and 10^{-4} M), and even then the response measured was a weak one. Although restricted in number, such ED studies in aquatic species indicate that such effects are likely to be limited in the aquatic environment. The greatest potential for either toxic or endocrine disrupting effects is likely to arise in molluscs associated with contaminated sediments (e.g. dredge spoil sites) since many of the phthalates concentrate in the sediments and molluscs metabolise phthalates more slowly (Sheahan *et al.*, 2000).

Regardless of these above facts, the potential endocrine disrupting hazard of these compounds to aquatic wildlife remains uncertain, due to the lack of information of *in vivo* effects and discrepancies in calculated bioaccumulation factors (EA, 1998a).

3.6 PCBs and other organochlorines

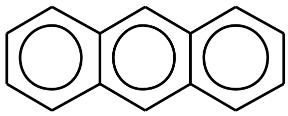


X= H or Cl

Polychlorinated biphenyls (PCBs) were used in various industrial materials and processes such as in cutting oils, hydraulic lubricants, flame retardants and heat transfer fluids; as antioxidants in paint and paper and in dust reducing agents and organic diluents. While no longer manufactured, they are still widely present in some types of equipment and in waste disposal sites – total world production by 1970 was estimated to be hundreds of millions of kilograms (IEH, 1995). They are generally very stable materials which, because of their hydrophobic properties and resistance to degradation, can accumulate and persist in biological systems. The endocrine disrupting properties of PCBs vary depending on the structure; for example, less chlorinated PCB congeners (e.g. PCB 104 and 188) and their hydroxylated derivatives have been shown to exhibit oestrogenic properties in human breast cancer cell lines and trout hepatocyte assays at concentrations of 0.01µM and above (Andersson *et al.,* 1999). The more highly chlorinated congeners have been found to be antioestrogenic, i.e. they bind with the oestrogen receptor and block it (Krishnan and Safe, 1993) or tend to be inactive (EA, 1998a). Some PCBs have also been found to affect steroid (including oestradiol, progesterone and testosterone) metabolism (*in vitro* test only) via induction of the cytochrome P-450 enzyme system (Drenth *et al.,*1994).

Polychlorinated *p*-dibenzodioxins (PCDDs) and polychlorinated *p*-dibenzodifurans (PCDFs) are not used commercially, but are formed as unwanted by-products in a range of processes, such as the manufacture of chlorinated compounds, incineration processes, paper pulp bleaching and emissions from cars and steel manufacture. Only a very small amount is estimated to enter the environment (e.g. only approximately 15kg annually in the USA (IEH, 1995)), and subsequently measured environmental concentrations are in nanogram or picogram quantities. PCDDs and PCDFs are usually found in the aquatic environment as complex mixtures. The action of PCDDs and PCDFs is strongly antioestrogenic, with EC50s for inhibition of an estradiol-induced protein in MCF-7 cells ranging from 8.6 to 1900 nM (Krishnan and Safe, 1993; Safe *et al*., 1991).

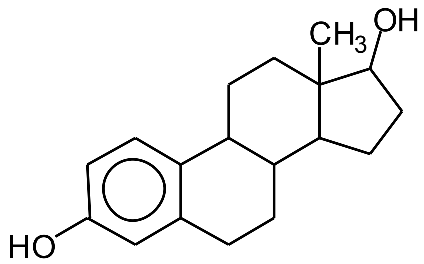
3.7 PAHs



Phenanthrene

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in the marine environment. Although they can be formed naturally, their predominant source is anthropogenic. Their widespread occurrence results largely from formation and release during the incomplete combustion of coal, oil, petrol and wood, but they are also components of petroleum and its products. PAHs enter the marine environment via sewage discharges, surface run-off, industrial discharges, oil spills and deposition from the atmosphere. PAHs have been measured in seawater, sediments and fish tissues around the UK (CEFAS, 1998; MAFF, 1995; Matthiessen *et al.,*1998). Significant concentrations (up to 10.7 µg/L in surface seawater; >10 000 µg/kg dry weight in sediments) have been found in a number of estuaries, particularly those which are heavily industrialised (CEFAS, 1998; Law *et al*., 1997). In a survey of PAHs in seawater carried out between 1993 and 1995, 30% of samples were taken from areas in which bivalve molluscs may be locally contaminated to an unacceptable level (based on the EU Maximum Admissable Concentration for protection of human consumers eating organisms derived from contaminated waters of 200 ng/l total PAH) due to bioaccumulation. The endocrine disrupting effects of PAHs in invertebrates have not been described. Nicolas (1999) reviewed the existing literature describing the effects of PAHs on various phases of vitellogenesis in fish. Although PAHs have a deleterious effect on vitellogenesis, this can vary with species, population and even between individuals, making general conclusions uncertain; possible mechanisms of action have still to be identified. Other reported effects include reduction in circulating hormones (e.g. Anderson, 1996b), oestrogenic and antioestrogenic effects (e.g. Anderson *et al.,* 1996a; Anderson *et al.,* 1996b), retardation of oocyte maturation (e.g. Thomas and Budiantara, 1995) and reduction of reproductive success (e.g. Kocan *et al.,*1996).

3.8 Natural and synthetic oestrogenic hormones



17ß-oestradiol

Natural oestrogen hormones such as oestrone (E1) and 17ß-oestradiol (E2) and synthetic hormones such as ethynylestradiol (EE2) can enter the aquatic environment via sewage treatment plants. The natural oestrogens are synthesised and excreted by all vertebrates and some invertebrates, while the synthetic oestrogens are excreted by women taking the contraceptive pill and hormone replacement therapy. As expected, they readily interact agonistically with the oestrogen receptors, therefore they are very potent even at very low concentrations (Desbrow *et al.,*1998; Routledge *et al.,*1996). It is unlikely that unconjugated (i.e. biologically active) oestrogens enter the sewage system directly, because a major proportion of these steroids are conjugated before being excreted. However, many studies (Harries *et al.,*1996, 1997; Jobling *et al.,*1998; Lye *et al.,*1997; Purdom *et al.,*1994) have demonstrated that sewage effluents contain substances that are oestrogenic to fish, and these have been subsequently identified as "free" oestrogens, predominantly oestrone, 17ß-oestradiol and ethynylestradiol (Desbrow *et al.,*1998). Measured concentrations of these three substances in seven domestic sewage treatment work effluents ranged from 1.4-76, 2.7-48 and 0.2-7 ng/L respectively. Panter *et al.* (1999) exposed fathead minnows to a metabolite of ethynyloestradiol (oestradiol –3- glucuronide) and to effluent generated from laboratory simulations of sewage treatment processes, also containing the metabolite (dosed at 553 ng/l of oestradiol –3- glucuronide). They found oestrogenic activity (VTG induction) in the latter exposure system, suggesting that microbial activity was capable of degrading the steroid metabolite into a biologically active oestrogen.

There are few data on the presence or effects of other steroid hormones (e.g. androgens) in the aquatic environment, but it is to be expected that some of these are having an influence, at least near sewage discharges. The ED activity of Hydroxy flutamide is discussed in Daughton and Ternes (1999). The EDMAR (Endocrine Disrupters in the Marine Environment) programme (p 60) is investigasting oestrogenic and androgenic activity in surface waters and sediments and attempting to identify causative substances.

3.9 Tributyltin (TBT)

$$(C_4H_9)_3—SnOSn—(C_4H_9)_3$$

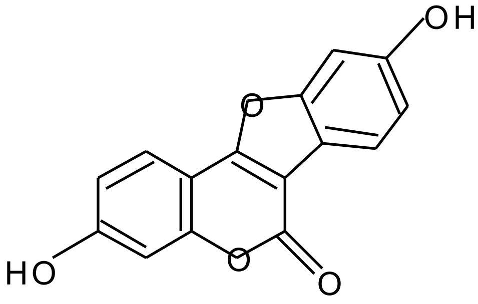
Bis (tri-n-butyltin) oxide

Organotin compounds, in particular tributyltin (TBT) are widely used as biocides in a variety of consumer and industrial products. The main source of organotins in the aquatic environment is through their use in antifouling paints. Concentrations in estuarine waters and sediments in the UK have measured as much as 40 ppb and 28 ppm respectively (Sylvia Blake, pers. comm.; CEFAS, 2000). Deleterious effects on non-target organisms (reviewed by Bryan and Gibbs, 1991), particularly gastropod molluscs (Bryan *et al.,*1986), led to a nationwide ban, in 1987, on the use of TBT on vessels under 25 m length. The main effect of TBT in gastropod molluscs is to cause a condition known as imposex, whereby the females' ability to reproduce is impaired due to masculinisation (i.e. the females grow a penis which blocks the oviduct). It has now been established that this is due to endocrine disruption, caused by elevated testosterone titres. The precise mechanism of action of TBT is still equivocal, but the weight of evidence suggests that inhibition of enzyme activity in the hormonal pathway which converts testosterone to 17ß-oestradiol is probably occurring (see Matthiessen and Gibbs, 1998 for a review of the possible mechanisms).

Although the use of TBT-based antifoulants was restricted to large vessels from the late 1980s, they can still pose a hazard for molluscs and other invertebrates, due to TBT's earlier widespread use and persistence. Impacts from current inputs (especially near dry docks, harbours and sewage treatment works) are also still occurring although further bans are now proposed on larger vessels. The incidence of imposex in dogwhelks (*Nucella lapillus*) has been used extensively as a biomonitor of TBT levels in seawater; at ambient concentrations of 1-2 ng/L the condition is fully developed, and at concentrations above 5 ng/L female dogwhelks are fully sterilised (Matthiessen and Gibbs, 1998). These effect levels have been used to set the UK EQS for TBT in marine waters. In a review of current knowledge of fate and distribution of TBT in the marine environment, Law and Evers (submitted) noted that current concentrations remaining in French waters range from below the limit of detection to 280 ng/L (Michel and Averty, 1999), which is above the toxicity threshold of 1 ng/L. Levels of imposex in populations of dogwhelks around the coast of Wales in 1996 and 1997 indicated that levels of TBT were well above the UK Environmental Quality Standard of 2 ng/L (Morgan *et al.,*1998). Water concentrations in marinas, estuaries and harbours between 1986 and 1989 ranged from <1 to 1960 ng/L (Waite *et al.*, 1991).

It should also be noted that TBT in sediments (generally at concentrations above 0.02 µg/g dry wt) has been demonstrated to have adverse effects on benthic communities, including direct and indirect effects on species other than molluscs, although the extent to which these effects are caused by endocrine disruption has not been established (Rees *et al.*, 1999; Waldock *et al.,* 1999)

3.10 Phytoestrogens



Coumestrol

Phytoestrogens are natural chemicals found in plants, notably whole grains, fibres and soy products. They have been listed by the UK Environment Agency as chemicals with oestrogenic and antioestrogenic potential, but are not recommended as a priority for management action (EA, 1998b). The major concern is over human exposure through consumption, but these chemicals can enter the aquatic environment from food processing plant and paper pulp-mill effluents. The hazard that phytoestrogens pose, and their presence in the UK aquatic environment remains uncertain (EA, 1998a). However, it has been well established in Scandinavia and North America that pulpmill effluents can have a variety of endocrine disrupting effects in exposed fish populations (Bortone *et al.*, 1989; Gagnon *et al.*, 1994; Van der Kraak *et al.*, 1998).

3.11 Metals

A number of metals, namely cadmium, lead and mercury have been implicated as having ED effects. The major use of cadmium is in electroplating, and it is also used as a constituent of easily fusible alloys, as a deoxidiser in nickel plating, in nickel-cadmium storage batteries and in process engraving. It is widespread in the marine environment and has been routinely measured in water, sediments and animal tissues in several surveys of UK coastal and estuarine waters (MPMMG, 1998). Concentrations tend to be higher in estuaries that are heavily industrialised; the Severn estuary, for example, had median water concentrations of 0.4 μg/L and the highest median mussel tissue concentration (9.78 mg/kg). The Tyne and Tees estuaries had the highest levels in sediment (median concentrations of 4000 μg/kg).

Very high cadmium concentrations (1 mg/L) have been shown to induce non-receptor-mediated VTG production in female Atlantic croaker (*Micropogonias undulatus*), the mechanism for which may be through altered gonadotrophin hormone (GtH) secretion by the pituitary (Thomas *et al.,*1989, 1990). The same concentration of cadmium has recently been shown to increase the secretion of gonad-inhibiting hormone (GIH) from the sinus gland in fiddler crabs (*Uca pugilator*), leading to inhibition of ovarian growth (Rodriguez *et al.*, 2000).

Lead, because of its corrosion-resistant properties, is used in containers for corrosive liquids, in ammunition, cable-covering, plumbing and storage batteries. However, due to health and environmental concerns its use in paints, fuel and insecticides has been drastically curtailed or eliminated. It has been measured (MPMMG, 1998) in UK estuarine waters (median concentrations in Tyne, Tees and Thames of 5 μg/L), sediments (median conentration of 450 mg/kg in the Tyne and Tees), mussel tissue (highest median concentrations of 15 mg/kg found in the Severn estuary) and fish liver (highest median concentration of 0.6 mg/kg found offshore of the Tees). At present there are no studies that specifically investigate the endocrine disrupting properties of lead in fish or invertebrates, but mammalian studies (rat *in utero* exposures) have shown that lead acts in two ways – at the level of the hypothalmic pituitary unit and directly at the level of gonadal steroid biosynthesis.

Mercury has a wide range ofuses, including manufacture of thermometers, barometers and many other instruments, the recovery of gold from ores, in advertising signs, batteries and catalysts. As with cadmium and lead, it has been measured in several marine matrices (MPMMG, 1998). Concentrations in marine waters were essentially below the limit of detection, typically tens of ng/L; median concentrations in sediments ranged from limit of detection to 3700μg/kg, with relatively high concentrations observed in the Tees and Thames estuaries. Mercury in mussel tissue was highest offshore of the Lune and Wyre estuaries (median concentration = 0.804 mg/kg) and in fish muscle, highest concentrations (0.26 mg/kg) were found in Liverpool Bay and Morecambe Bay, areas that have been subject to considerable inputs of mercury via discharges from the chlor-alkali industry.

Research studies of the ED effects of mercury are limited, but it is thought to have the same mechanisms of action in mammalian systems as outlined above for lead.

4. Effects of endocrine disrupting chemicals in marine and freshwater wildlife

4.1 Fish

This section provides an overview of the evidence for endocrine disruption in marine and freshwater fish species, a phenomenon which is now well established. It also identifies a number of cases where ED is possibly occurring. The main focus is on effects in wild fish but experimental studies will also be discussed. The distinction between *well-established* and *possible* ED has been based primarily on the quantity and quality of supporting experimental and mechanistic data. In essence, if mode of action is unknown, it is impossible to be sure that endocrine disruption has occurred. It is important to distinguish between ED *sensu strictu*, and more general effects of pollution on, for example, fish reproduction and development (e.g. Kime 1995, Monosson 1997) or reproductive behaviour (e.g. Jones and Reynolds, 1997). The distinction between effects on the fish endocrine system, and direct effects on gamete development and viability is made by Kime (1999).

4.1.1 Mechanisms of endocrine disruption in fish

In fish, there are several possible ways in which ED effects are known to be manifested: a) Vitellogenesis or zonagenesis in juvenile or male fish; b) Premature, delayed or abnormal gonad development; c) Effects on sex steroid titres; d) Abnormal secondary sex characteristics; e) Interference with the pheromonal control of reproduction; f) Altered adrenal physiology. These will each be briefly described below.

Detailed coverage of our current knowledge of ED in fish can be found in several publications: Arukwe and Goksøyr (1998); Börjeson and Norrgren (1997); Giesy and Snyder (1998), Hontela (1997), Hontela *et al.* (1993), Kime, (1998), Leatherland (1993, 1994), Marcquenski and Brown (1997), Matthiessen (1998), Matthiessen and Sumpter (1998), Schwaiger and Negele (1998), Stahlschmidt-Allner *et al.* (1997), Sumpter (1995), Sumpter *et al.* (1996), Tyler *et al.* (1998) and Van Der Kraak *et al.* (1998 a and b).

4.1.1.1 Vitellogenesis and Zonagenesis

Processes

During normal vitellogenesis in fish, the yolk precursor protein vitellogenin (VTG) is produced by the livers of mature females when stimulated by the presence of oestradiol (E2) in the blood, which in turn is produced by the ovaries in response to various external stimuli. Under normal circumstances (i.e., in mature females) the vitellogenin is transported via the blood back to the ovaries and deposited in the developing oocytes. Similarly, zonagenesis involves the production of zona radiata protein (ZRP) by the liver under the direct control of E2, and is also carried to the ovary, where it becomes part of the extracellular oocyte envelope that prevents polyspermy during fertilisation and provides mechanical protection for the embryo. Male fish do not normally have VTG or ZRP in their blood (presumably because they have a minimal amount of endogenous estrogen which is below the threshold for induction) although they do possess the receptors for oestrogens in the liver and therefore have the capacity to produce VTG and ZRP if exposed to exogenous oestrogens (Emmersen *et al.,* 1979; Hyllner *et al.,* 1991; Le Guellec *et al.,* 1988). This process has been widely used as a tool for detecting the presence of natural and synthetic oestrogens and their mimics in natural waters (e.g. Allen *et al.,*1999 a,b; Harries *et al.,*1997).

Oestrogens and oestrogen mimics act by binding with the oestrogen receptor protein molecule in target tissues. A ligand-receptor complex is formed which then interacts with the oestrogen responsive element in the DNA; this in turn switches on the transcription of VTG and/or ZRP genes. Anti-oestrogenic substances, on the other hand, bind with the oestrogen receptor and prevent oestradiol from triggering its normal responses. Liver hypertrophy usually accompanies VTG induction, because the tissue responds to the exogenous oestrogenic signal and starts producing large quantities of protein. Unlike in females, the induced VTG in males has no available target tissue (i.e. the ovaries), so it can remain in the blood for many weeks, with the potential for causing kidney damage and calcium deficiency (Herman and Kincaid, 1988). Furthermore, large scale VTG synthesis represents a considerable investment in energy and food for a male fish which would normally be directed elsewhere. Thus, although VTG induction in male fish should primarily be regarded as a biomarker of exposure to oestrogens, its presence in males at high concentrations may also have deleterious consequences in its own right. Limited data are available (e.g. Läenge *et al.,*1997) which suggest that VTG induction in males can co-occur with interferences in reproductive success (in the form of hatching success of broods): VTG induction could therefore become a biomarker of effect. Vitellogenesis in juveniles and males is an excellent tool for monitoring oestrogenic contamination of both freshwater and marine aquatic environments (Kime *et al.,*1999; Tyler and Routledge, 1998). Detections of VTG or ZRP in male blood plasma have several advantages as survey techniques; for example, they can only be induced by interactions with the E2-receptor, and not through general stress or other mechanisms of toxicity.

Laboratory and field studies

The first report of induction of a VTG-like protein in male laboratory fish after exposure to an oestrogen-mimic (the insecticide □–HCH) was made by Wester *et al.* (1985). Subsequently, there has followed a large number of other experimental studies with natural and manmade substances. There is no doubt that oestrogenic hormones and their mimics all act as receptor agonists, although natural oestrogenic hormones and their analogues are several orders of magnitude more potent than the hormone mimics.

Oestrogenic effects in UK freshwater fish, such as rainbow trout and roach, have been well documented (Harries *et al.,*1996, 1997; Jobling *et al.,*1998b; Purdom *et al.,*1994) and have been attributed to several substances – nonylphenol (Jobling *et al.,*1996) and natural and synthetic oestrogenic hormones (oestradiol, oestrone and ethynylestradiol; Desbrow *et al.,* 1998; Routledge *et al.,*1998). The latter enter sewage as inactive conjugates, but can be de-conjugated by bacterial enzymatic activity (Panter *et al.,* 1999).

Research in freshwaters outside the UK has revealed similar vitellogenic effects in various fish species, notably in male carp (*Cyprinus carpio*) from various parts of the USA (Bevans *et al.,* 1996; Folmar *et al.,* 1996; Goodbred *et al.,* 1997), in caged trout held in German sewage effluent for 6 months (Hansen *et al.,* 1998), in caged fathead minnows held for 3-5 weeks in American sewage effluent (Nichols *et al.,*1999), in caged whitefish held for 4 weeks in treated pulp and paper mill effluent (Mellanen *et al.,* 1999), and in trout held in a Swedish river for 2 weeks (Larsson *et al.,* 1999). In general, the degree of VTG induction was less than that seen in some UK locations, and this could be due to different sewage treatment processes and/or greater dilution. Bisphenol –A was added to the list of probable causative substances (in addition to NP, E1, E2 and EE2) as a result of the research performed in Sweden.

The first to report the presence of VTG in male marine fish was Lye *et al.* (1997, 1998). European flounder (*Platichthys flesus)* caught in the Tyne estuary, UK, near a STW discharge had elevated VTG concentrations, and a number of testicular abnormalities. These effects were partly associated with exposure to, and bioaccumulation of, several oestrogenic alkylphenols (Lye *et al.,* 1999). Subsequent more extensive investigations in Britain (Allen *et al.,*1999 a and b; Matthiessen *et al.,* 1998) measured high levels of VTG (up to 20 mg/ml plasma) in male flounder from heavily industrialised estuaries (Tyne, Tees, Wear and Mersey) and from one coastal area near the Mersey. Lower or undetectable levels of VTG have been measured in flounder from estuaries draining urban areas with relatively little heavy industrial activity, or rural areas. Up to 18% of the male fish in the Tyne and Mersey showed ovotestis (i.e. intersex), but this has not been seen in other estuaries, although a few intersex fish have recently been caught offshore in Liverpool Bay and the Wash. There was no correlation between occurrence of intersex and plasma concentrations of VTG. Male vitellogenesis and ovotestis have now also been seen in another estuarine fish species (the viviparous blenny *Zoarces viviparus*) caught in oestrogen-contaminated areas (Tyne and Clyde estuaries, EDMAR programme, unpublished)[*]. The causative substances are not yet known, but as with freshwaters, are expected to be a mixture of natural and synthetic substances. Unpublished EDMAR programme data show that the causative substances in the Tyne and Tees are a mixture of natural and synthetic hormones as well as industrial substances. The majority of the oestrogenic activity is associated with sediment particulates and remains to be identified.

The implications of oestrogen exposure for marine fish populations are still under investigation. Premature vitellogenesis in female flounder, but none in males, has been seen in mesocosms in which fish were held on contaminated harbour dredgings (Janssen, 1996; Janssen *et al.,*1997), but the causes of this effect are likely to be different from those described above. Elevated VTG titres have been seen in female flatfish caught in contaminated American harbours (Pereira *et al.,* 1992).

As mentioned previously, although VTG has essentially been used as a biomarker of oestrogenic exposure, high levels of VTG induction in juvenile or male fish can be harmful in their own right. Excessive VTG induction is often associated with other adverse endocrine endpoints such as testicular growth retardation, liver hypertrophy, ovotestis, and possibly premature female maturation and retarded smoltification althjough some of these effects may in fact be due to toxicity of the VTG inducing chemical (Janssen *et al.,*1997; Jobling *et al.,*1996; Kramer *et al.,*1998; Madsen *et al.,*1997; Panter *et al.,*1998). Consequently, the VTG field data reported here indicate that adverse effects of oestrogenic endocrine disruption at several levels in fish may be rather widespread in the more contaminated situations, for example near certain discharges.

[*] Subsequent to the production of the main body of this report, Matthiessen *et al* (2002) published a summary of progress in the EDMAR programme, which includes further reference to papers resulting from the research undertaken.

Polycyclic aromatic hydrocarbons (PAHs) have been found, in general, to produce reductions of VTG titres in female fish. Plausible mechanisms of action for these substances include anti-oestrogenic action leading to lowered oestradiol levels (Nicolas, 1999), and/or increased liver mixed function oxidise (MFO) metabolism, which indirectly results in increased metabolism and excretion of natural steroids.

Non-receptor-mediated VTG induction has been observed in female Atlantic croaker (*Micropogonias undulatus*) exposed in the laboratory to a very high cadmium concentration (1 mg/L), which may have been caused by altered gonadotrophin hormone (GtH) secretion by the pituitary (Thomas *et al.,*1989, 1990). However, this a mechanism of action has not been repoted. In field studies It seems likely that some heavy metals (particularly cadmium, lead and mercury) are indeed able to act as endocrine disrupters through their ability to interfere with the cells in the pituitary which synthesise steroid-controlling hormones (Bleau *et al.,*1996; Norris *et al.,*1997, 1999; Thomas and Khan 1997), but the precise mechanisms are not fully understood. It is also worth noting that toxic effects of heavy metals (e.g. mercury and cadmium) on the steroid synthesising organs may also lead to reductions in steroid titres, triggering reduced negative feedback on the pituitary and increased release of pituitary hormones (Bleau *et al.,*1996; Kirubagaran and Joy 1989, 1991).

There are as yet no reported occurrences of ZRP induction from the field, but several laboratory studies have been conducted with oestrogen-injected fish (Arukwe *et al.,*1997b; Arukwe *et al.,*1998; Hyllner *et al.,*1991; Knudsen *et al.,*1998). These indicate that ZRP induction is likely to be as useful a biomarker of oestrogenic exposure as VTG, and it has been shown that juvenile salmon (*Salmo salar*) exhibit zonagenesis when exposed in aquarium water to oil refinery effluent dilutions as low as 10% (Arukwe *et al.,*1997b).

4.1.1.2 Premature, delayed or abnormal gonad development

Fish gonad development, and hence normal reproductive function, may be damaged through both endocrine and non-endocrine mechanisms. However, it is rarely, if ever, possible to distinguish between the two when effects are observed in the field. General pollution-related stress can cause impacts on gonad development without the primary involvement of the endocrine system (see Monosson 1997 for a summary of field observations), or only indirectly via the inhibitory action of cortisol on reproductive hormone secretion (Carragher and Sumpter, 1990). There are several cases of *possible* but uncorroborated ED-related impacts on gonad development which could be explained in terms of a range of possible endocrine mechanisms (including receptor-mediated effects, effects on the pituitary-gonadal pathway, or interference with steroid metabolism), but non-endocrine toxicity, or non-contaminant effects, cannot be ruled out. Some examples include premature maturation in flatfish from the southern North Sea (Rijnsdorp and Vethaak, 1997), decreased egg weight and increased atresia (i.e. egg resorption) in flatfish from contaminated harbours in the eastern USA (Johnson *et al.,*1992) and delayed and reduced ovarian development in flatfish after the *Amoco Cadiz* oil spill in Brittany (Stott *et al.,*1983). The two examples described below rely on weight of evidence to support the case for ED.

Abnormal gonad development has been observed in oestrogen-exposed fish in UK rivers and estuaries; in this case, oestrogenic ED is definitely occurring, as evidenced by VTG induction in males. In rivers, the more extreme cases of oestrogenic contamination have been accompanied by retardation of testicular growth in caged adult trout (Harries *et al.,*1997). Caged fish held in the River Aire, which was contaminated with alkylphenols (concentrations ranged between 82 and 180μg/l total exctractable alkylphenols at sites up to a distance of 5km downstream of a STW effluent input), demonstrated testicular effects similar to those seen in laboratory exposures of trout to environmentally realistic concentrations of alkylphenols (Jobling *et al.,* 1996). Ovotestis in wild male roach (*Rutilus rutilus)* and gudgeon (*Gobio gobio*), presumably caused by oestrogenic exposure during gonadogenesis, have been observed in several UK rivers downstream of sewage treatment works (STWs) (Jobling *et al.,*1998b; Tyler and Routledge, 1998). The severity of these effects ranged from the occasional oocyte in otherwise normal testicular tissue, to large regions of mature ovarian tissue interspersed with rather abnormal testicular material. Their prevalence ranged from a few percent of the male population, to 100% in stretches of rivers like the Nene and Aire. In some of these fish, the vas deferens was also feminised or absent, and milt production was impaired (Jobling *et al.,* 1998a)[**]. Feminisation of the vas deferens has also been described in all-male carp larvae exposed in the laboratory to 4-*tert*-pentylphenol (Gimeno, 1997; Gimeno *et al.,*1996, 1998a), and there is clearly a window of sensitivity during gonadogenesis.

Wild flounder (*Platichthys flesus*) caught in heavily industrialised UK estuaries (Mersey and Tyne) have recently been shown to have similar effects on spermatogenesis (Lye *et al.,* 1998) and ovotestis (Allen *et al.,*1999 a and b; Matthiessen *et al.,*1998), although a smaller proportion of males (up to 18%) were affected compared to the worst cases in roach. In most of the other estuaries surveyed, ovotestis in male flounder was absent. It is hypothesised by Matthiessen *et al.* (1998) that flounder do not show higher prevalences of ovotestis (in estuaries where VTG is nevertheless induced) because their larvae probably undergo gonadogenesis while still at sea, where conditions are relatively uncontaminated. The causes of these effects in UK estuaries are not yet known, but circumstantial evidence indicates that both natural and synthetic oestrogens and their mimics are probably responsible.

It is likely that fish species which inhabit a contaminated estuary for their whole lifecycle and therefore are exposed to ED substances continually are at greater risk of ED effects than other anadromous or catadromous species. It is generally perceived that the larval stages are the most sensitive to ED, and exposure to EDs at this stage could lead to the development of intersex conditions and other reproductive abnormalities. It is impossible to determine at this stage the relative risks to anadromous vs catadromous species, as both migrate through estuaries when they are adults or pre-adults. Obviously, the risks of ED occurring will be greater for those species which take longer to migrate through a contaminated estuary and/or those which feed during their migration.

Exposing fish to sufficiently high concentrations of oestrogens or androgens during gonadogenesis is usual in aquaculture to change the phenotypic sex (Hunter & Donaldson, 1983). Several pollutants (e.g. nonylphenol, PCBs, E2) have been shown to alter the apparent sex ratio of a population in laboratory studies (Gray and Metcalfe, 1997; Hartley *et al.,* 1998; Matta *et al.,*1998; Nimrod and Benson, 1998), but there are very few examples of this from the field which can be linked to contaminants.

One such field example concerns a population of *Coregonus hoyi* in Lake Michigan, USA, which in the 1960s contained up to 97% females, but which saw a shift to sexual equality in the late 1980s (Monosson *et al.,* 1997). The increase in the proportion of males was strongly correlated with a reduction in body residues of *p,p'*-DDE, a known anti-androgen, although

[**] More recently, Jobling *et al* (2002) demonstrated that wild intersex roach have impaired gamete production and quality, resulting in decreased fertilisation success compared to normal males.

the oestrogen mimic *o,p'*-DDT also declined over the same period. If the feminisation effects were indeed pollution-related, it therefore appears that they may have had at least two causes. There are no field examples which have established a stronger relationship than this between skewed sex ratios and pollution. This may be because the doses of hormone mimics required to achieve complete sex reversal, as opposed to intersex induction, are rather high (e.g. 100 µg nonylphenol/L; Gray and Metcalfe, 1997).

4.1.1.3 Effects on sex steroid titres

Sex steroid titres can be affected by a large range of possible pollution-related mechanisms and substances, and several field observations have been made regarding influences on circulating sex steroids. Male carp caught near oestrogenic STW discharges in the USA had reduced titres (Folmar *et al.*, 1996), while oestradiol/ketotestosterone ratios were elevated in a few wild carp that contained higher-than-normal levels of pesticides residues (Goodbred *et al.,*1997). Female fish (*Paralabrax clathratus*) in the South California Bight showed depressed plasma GtH and E2, but elevated ovarian testosterone production, both being correlated with increased body burdens of organochlorines (Spies and Thomas, 1997) and possibly being caused by interference with normal GtH production. Female flounder (*Platichthys flesus)* held for 3 years on harbour sediment contaminated mainly with PCBs and PAHs showed elevated testosterone and oestradiol titres which could have been due to decreased steroid clearance via the cytochrome P450 system (Janssen *et al.,* 1997). Correct interpretation of this type of data should take into consideration the natural cycles of sex steroids in fish, which vary with the seasons.

4.1.1.4 Abnormal secondary sex characteristics

Endocrine disruption has been reported as being responsible for altering the external sexual differentiation of fish. The best examples derive from studies of pulpmill discharges in Canada and Scandinavia. Although the number of these types of discharges to UK marine and freshwaters is extremely limited, it is still worth mentioning these effects, as the causative compounds may well be present in discharges from other industrial sources.

One hundred percent of female mosquitofish (*Gambusia affinis)* in a stream below a paper mill in Florida showed strong masculinisation of the anal fin (Howell *et al.,* 1980). As the development of the anal fin in males is under androgenic control, it was reasonable to conclude that the females had been exposed to an exogenous androgen derived from the paper mill effluent. Denton *et al.* (1985) and Howell and Denton (1989) showed that partially biodegraded mixtures of plant sterols (β-sitosterol, campestrol and stigmastanol) were able to induce non-reversible masculinisation of the anal fin in adult female *G. affinis*, but that undegraded sterols were inactive. Laboratory studies (Bortone and Drysdale 1981; Drysdale and Bortone 1989) also showed that newly-hatched females exposed to bleached kraft mill effluent (BKME) developed elongated anal fins, and those in males became precociously elongated. More recently, a low proportion (2%) of spawning female channel catfish (*Ictalurus punctatus*) has been found to have male secondary sexual characteristics in the Red River of the North (North Dakota), but the cause of this abnormality has not been established (Hegrenes, 1999).

The reverse of these effects has been observed in *Catastomus commersoni* exposed in the field to BKME from pulp mill discharges in Canada (Munkittrick *et al.,*1991). In these, males show an absence of secondary sexual (nuptial) tubercles which may be due to the reduced plasma steroid (11-KT) titres. It is not known whether the apparent paradox of masculinisation caused by BKME in Florida and de-masculinisation by BKME in Canada are due to differential sensitivity of the species in question, or to a different mix of discharged substances.

In Australia, wild male mosquitofish (*Gambusia affinis holbrooki*) have recently been found to have reduced gonopodium length (Batty and Lim, 1999). These partially demasculinised male fish were caught downstream from a sewage treatment plant discharge, and the effects were attributed to either oestrogens or anti-androgens. However, spermatogenesis did not seem to be impaired, so the ecological significance of the observations is not yet known.

Feminised secondary sexual characteristics have been observed in two species of sand goby, *Pomatoschistus minutus* and *P. lozanoi*, from oestrogen-contaminated locations in UK estuaries (EDMAR programme, unpublished)*. Many males from these areas possess urogenital papillae, a structure used for gamete deposition, with morphology intermediate between males and females. The mode of causation and implications of these observations are still being investigated.

4.1.1.5 Interference with the pheromonal control of reproduction

Recent laboratory studies have shown that interference with the pheromonal control of reproduction can occur at environmentally realistic pesticide concentrations. Moore and Waring (1996, 1998) and Waring and Moore (1997) have shown that brief (30 minute to 5 day) exposures to low levels of organophosphate (1μg/l), carbamate (1μg/l) and triazine (2 μg/l) pesticides are able to interfere with the ability of mature male salmon (*Salmo salar*) to make the final maturational steroid hormone changes in response to pheromones released by ovulated females. This ultimately resulted in lowered plasma titres of several sex steroids, and reduced volumes of expressible milt. There are currently no field data on this type of effect.

Other olfactory mediated responses, particularly with regard to imprinting and homing in salmon, should also be considered. During smoltification, which is controlled by the endocrine system, the fish imprint to a particular smell characteristic of their home river. It is therefore possible that pesticides may inhibit the ability of the salmon to imprint and reduce their ability to home when they return from sea. In addition, pesticides may affect the adults' ability to recognise the home river even if they have imprinted as smolts. Sholtz *et al* (in press) have shown that chinook salmon exposed to 0.4 μg/L diazinon for 30 minutes do not home as well as the control group.

4.1.1.6 Altered adrenal physiology

Fish stimulated by acute chemical or physical stress secrete adrenocorticotropic hormone (ACTH) in the pituitary (Hontela, 1997; Hontela *et al.,*1993). This in turn induces the kidney to secrete the steroid hormone cortisol which is mainly responsible for mobilising the energy

* Subsequent to the production of the main body of this report, Matthiessen *et al* (2002) published a summary of progress in the EDMAR programme, which includes further reference to papers resulting from the research undertaken.

required to deal with stress. These are normal adaptive responses, and their triggering by acute stress (including chemical exposure) cannot generally be described as endocrine disruption. However, chronic stress leading to prolonged elevation of cortisol has a variety of undesirable effects including muscle wastage and reduced growth, inhibited gonadal growth and immunosuppression.

A number of field studies have now demonstrated that chronically stressed fish become unable to produce the cortisol response due to 'exhaustion' of the pituitary-interrenal system. For example, in Canada, *Perca flavescens* and *Esox lucius* from sites contaminated with PAHs, PCBs and heavy metals were unable to produce cortisol in response to acute handling stress, and the cells which secrete ACTH were atrophied (Hontela *et al.,* 1992, 1995). The authors speculate that this atrophy is caused by prolonged hyperactivity of these cells, and that it impairs the ability of the fish to regulate their energy metabolism, but it is also possible that cortisol clearance is speeded up by xenobiotic-induction of the cytochrome P450 mixed function oxidase system. In both sexes of *P. flavescens*, gonads were smaller and thyroid hormone (T4) titres lower. Hontela *et al.* (1997) later showed that BKME also produced similar effects to PCBs, PAHs and metals. Norris *et al.* (1997, 1999) studied heavy metal-exposed *Salmo trutta* populations in the USA and found the same inability to make the ACTH and cortisol response to acute stress. Recent work by Girard *et al.* (1998) has shown that *P. flavescens* from contaminated sites show a smaller cortisol response to injected ACTH than do fish from control sites. In other words, the impairment of the cortisol response could not be remediated by ACTH, indicating that damaged interrenal tissue is contributing to the observed effects.

Some effects of the type described above have also been seen in chronic laboratory exposures of fish to mercury (Kirubagaran and Joy, 1991), but more experimental corroboration is needed. Also, there are as yet no field data to indicate what the population-level consequences of these effects may be. The observations nevertheless show that many common pollutants are able to cause long-term damage to the cortisol stress response in fish, and it seems likely that this will cause knock-on problems for energy mobilisation and salinity adaptation.

4.1.2 Cases of possible endocrine disruption in fish

The studies described more briefly in this section involve effects which could conceivably be due to endocrine disruption, but for which little or no supporting evidence yet exists and their role at this stage is speculative.

4.1.2.1 Reduced reproductive success

The effects of certain endocrine disrupters on sex steroids, gonad development, sex ratios and secondary sex characteristics, as described above, are likely to have consequent impacts on reproductive output which potentially affect population viability (although in many fish species there is considerable redundancy built into reproductive output). Various laboratory studies with EDs have shown that such effects are possible: exposure of larval or adult fish to a range of oestrogenic substances have resulted in reductions (up to 100% in some cases) in egg and larval production (e.g. Kramer *et al.,*1998; Läenge *et al.,*1997; Nimrod and Benson 1998; Waring *et al.,*1996). Nagler and Cyr (1997) have shown that male flatfish held on naturally ED-contaminated sediments produce sperm which are less effective in producing viable larvae, although it is not certain that this effect was indeed an example of ED.

However, there are very few field studies which have explicitly investigated these higher level effects as they relate to ED. There are, of course, many reports of reproductive effects in wild fish populations which have been more or less firmly linked to contaminants (e.g. Jones and Reynolds, 1997; Kime, 1995; Monosson, 1997), but these certainly include effects due to non-endocrine mechanisms of action. These include direct toxic damage to reproductive tissues, reduced energy availability and growth, general debility, and some types of altered behaviour.

Puget Sound on the United States west coast, an area contaminated with PAHs and PCBs, provides perhaps the best example of possible ED-linked reproductive impacts in the field (Collier *et al.,* 1998). A range of reproductive effects have been observed in several flatfish (*Pleuronectes bilineatus* (Johnson *et al.,*1998), *Pleuronectes americanus* (Johnson *et al.,* 1992), *Parophrys vetulus* (Casillas *et al.,*1991; Johnson *et al.,*1988, 1995, 1997; Landahl *et al.,*1997) including precocious female maturation, inhibited ovarian development, reduced egg weight, reduced spawning success and reduced larval survival. No evidence for population effects has been presented however. The precise mechanisms behind these reproductive effects are not entirely clear, although they are variously correlated with elevated levels of PAHs, DDT/DDE, and PCBs. The majority of the effects (especially reduced fecundity and spawning success) could possibly be explained either in terms of the anti-oestrogenic effects of some PCBs and PAHs, or the interactions of PAHs or dioxins with the aryl hydrocarbon (Ah) receptor. The Ah receptor is involved with CYP1A1 gene expression and EROD activity, and the induction of these is associated with diminished response of liver cells to 17β-estradiol in terms of synthesis of vitellogenin (Anderson *et al*., 1996). Stein *et al.* (1991) have shown that *P. vetulus* injected with extracts of sediments from Puget Sound experience reduced plasma E2 titres. This effect may also be due to increased steroid clearance by the PAH- or organochlorine-induced P450 system. Precocious female maturation seen in *P. vetulus* from the Hylebos Waterway (Collier *et al.,*1998; Johnson *et al.,*1997) could be due to the oestrogenic effects of various organochlorines. However, non-endocrine modes of action are also possible due to the large number of contaminants present in the Puget Sound system.

4.1.2.2 Fry and juvenile mortality/abnormality syndromes

Fry mortality syndromes in salmonids have sometimes been linked to effects of contaminants and may in some instances be due to endocrine disruption although this has not ben clearly demonstrated. Several species of *Salvelinus*, *Oncorhynchus* and *Salmo* on the Great Lakes, USA, have shown conditions known as Early Mortality Syndrome (Marquenski and Brown, 1997) and Blue-Sac Disease (Symula *et al.,*1990). In the Baltic Sea, *Salmo salar* are suffering a similar syndrome known as M-74 (Bengtsson *et al.,* 1999; Börjeson and Norrgren, 1997). Both Early Mortality Syndrome (EMS) and M-74 are caused by thiamine deficiency, and could therefore be entirely natural, perhaps related to differential thiamine or thiaminase contents in prey species.

Blue-Sac Disease in *Salvelinus namaycush*, on the other hand, involves oedema of the yolk sac, and is most likely caused by a complex mixture of organochlorines (Giesy and Snyder 1998) There are also some data which suggest that contaminants are involved in EMS and M-74. For example, Tillitt *et al.* (1996) showed that thiamine can protect against the deleterious effects of dioxin in fish embryonic development, so natural thiamine deficiency could exacerbate the effects of organochlorine contamination. There also seems to be a correlation between high levels of M-74 and elevated body burdens of polychlorinated dibenzofurans

(PCDFs) and coplanar PCBs (Vuorinen and Keinänen, 1999; Vuorinen *et al.,*1997a). Furthermore, recent experiments with rainbow trout larvae have indicated that substances (e.g. dinitrobenzene) metabolised by electron reduction can cause thiamine deficiency. There is also weak evidence that M74 may be associated with toxicant-induced abnormalities in thyroid hormone and retinol titres (Vuorinen *et al.,*1997b). It must be borne in mind, however, that none of these syndromes has been clearly shown to be caused by endocrine disruption, although at this stage such mechanisms cannot be ruled out.

Studies in Canada have established a somewhat stronger relationship between juvenile mortality and EDs. River basins sprayed with an aminocarb-based insecticide (Matacil 1.8D) formulated with nonylphenol (NP) as a surfactant, had a coincidental significant proportion of the lowest salmon (*Salmo salar*) catches, and heavy salmon smolt mortality; a similar product with no NP (Matacil 1.8F) had no such effects (Fairchild *et al.*, 1999). The researchers estimated that the concentrations of NP in river water that would have resulted from the spraying were sufficient to cause oestrogenic effects in fish. In the laboratory, NP can both inhibit smoltification and impair hypo-osmoregulation in salmon (Madsen *et al.*, 1997). It is known that sex steroids, and the entire process of sexual maturation, have antagonistic effects on smoltification and seawater acclimation, although the precise mechanisms by which they and NP act are unclear.

4.1.2.3 Hypothyroidism

Hypothyroidism (or goitre) is a condition of enlargement of the thyroid gland and occurs widely in vertebrates due to natural iodine deficiency in the diet (Leatherland, 1994). However, with the exception of Pacific salmon (*Oncorhynchus* spp.) introduced into the Great Lakes, outbreaks of thyroid enlargement are rare in fish. In the Great Lakes, although the salmon are not generally deficient in iodine (Leatherland, 1992,1993), they suffer from various degrees of thyroid lesion, suggesting that perhaps environmental goitrogens (antithyroid compounds) are responsible. These may either inhibit iodide metabolism by the thyroid or interfere with iodination. Effects of this general type have been produced in fish in the laboratory through exposure to substances like thiocyanate (Lanno and Dixon 1996), sodium pentachlorophenate (Hickie *et al.,*1989), mercury (Kirubagaran and Joy, 1989), and endosulfan (Bhattacharya, 1995). Furthermore, depressed thyroid hormone titres have indeed been observed in many salmonid stocks in the Great Lakes, particularly Lakes Michigan and Erie (e.g. Leatherland and Sonstegard, 1980, 1981, 1984; Leatherland *et al.,*1989).

It was originally suspected that various organochlorines might be responsible (because these can affect thyroid hormones in mammals, and some Great Lakes salmonids contain high levels of organochlorine residues). However, the evidence for this remains equivocal. Salmon chronically dosed with PCBs exhibited lowered thyroid hormone titres (Leatherland and Sonstegard, 1978), but trials in which rainbow trout and coho salmon were fed with organochlorine-contaminated fish from the Great Lakes failed to produce anti-thyroid effects (Leatherland, 1992, 1993; Leatherland and Sonstegard, 1982). Nevertheless, contaminated fish fed to rodents did produce goitres and depressed serum thyroid hormone (Leatherland, 1992, 1993), indicating the presence of bioaccumulated goitrogens of some type. The causative substances affecting Great Lakes fish are yet to be established, but there is some evidence (Leatherland, 1993, 1994) that compounds produced by microflora associated with eutrophication have anti-thyroid activity. A correlation does exist between the degree of eutrophication in the Great Lakes and the severity of thyroid lesions. In conclusion, it seems feasible that the thyroid abnormalities in Great Lakes salmonids may indeed be a manifestation of endocrine disruption, but possibly caused by both anthropogenic substances and by the anthropogenic activities leading to eutrophication. More research on this phenomenon is clearly required.

4.1.3 Conclusions

Endocrine disruption is indubitably occurring in wild fish populations across Europe and North America, through a variety of mechanisms as described above. Known or suspected causative substances range from natural phytoestrogens and vertebrate-derived oestrogens, through heavy metals, to a variety of synthetic organics. However, it must be stressed that in most cases described in section 4.1.2 the precise modes of action are still not entirely clear, partly due to an incomplete knowledge of fish endocrinology. The species which have so far been studied are limited to the gonochoristic teleosts; circumstantial evidence suggests that as-yet unidentified substances (including those which are not ED substances) are contributing to the effects, and the true severity and geographic extent of the effects is unknown. One of the few cases where the relationship between cause and effects of ED is more clearly understood is that of intersex roach in UK rivers (Jobling *et al.*, 1998). There, a highly significant relationship has been found between the occurrence of intersex and the concentration of STW effluent. For the range of sites studied, the proportion of intersex fish in any sample could perhaps be predicted and, since the regression between the intersex index and average annual effluent concentration was significant, the average intersex index in a population of roach from a particular site could be estimated from the average concentration of sewage effluent.

Many substances may be able to act together via receptor-mediated mechanisms. Mixtures of endocrine disrupting chemicals may interact additively, synergistically or antagonistically, but these interactions have yet to be adequately investigated, and will require substantial research to be fully understood.

The most extensively researched mode of action is via the oestrogen receptor. Other important effects, such as those on pituitary function, steroid synthesis and the corticosteroid system are less well understood, and studies of impacts on the thyroid system have not yet produced conclusive results. Receptor-mediated androgenic, anti-androgenic, anti-oestrogenic, thyroidal and anti-thyroidal effects of contaminants have not yet been definitively identified in field investigations, but it seems possible that impacts caused through at least some of these mechanisms.

There is still very little understanding of how the endocrine disruption already known to be occurring in fish is impairing the ability of populations and communities to survive and thrive. There is as yet no published work which clearly links endocrine disruption in a fish species to adverse effects at the population or higher levels, even though it is in some cases not unreasonable to conclude that such phenomena are occurring[**]. There are naturally many contingent factors which intervene between an ED effect in an individual fish, and a change in population structure, so it should not automatically be assumed that the one will always follow the other. Strict application of the precautionary principle may suggest that if, for example, all the males in a fish population are synthesising vitellogenin, then one does not need to prove that the population is endangered before requiring the situation to be rectified.

[**] More recently, Jobling *et al* (2002) demonstrated that wild intersex roach have impaired gamete production and quality, resulting in decreased fertilisation success compared to normal males.

However, evidence beyond a simple biomarker signal such as VTG induction is likely to be required in many cases before remediation is undertaken. The degree of precaution taken at a particular site will depend upon the confidence that ED is occurring, and the scale of risk at the site. For example, it would be advantageous to be more precautionary in areas where rare fish species (for example, shads) are at risk. Such species usually occur at low population densities, hence interference with reproductive effort may be less likely to be compensated for by density-dependent effects for example. Although no conclusive information is yet available, other lifecycle/behavioural characteristics which may predispose a species to greater risk of ED must be taken into account, such as bottom-dwelling vs pelagic species (sediment dwellers may be at greater risk through exposure via ingested sediment than water column species), and anadromous vs catadromous vs estuary breeding species (discussed previously).

The available data suggest that marine fish are equally as susceptible to ED as freshwater species, and that in the more contaminated marine habitats (especially industrialised estuaries), one can observe ED-related effects which approach the severity of those seen in contaminated freshwaters.

4.2 Invertebrates

The utilisation of hormones to control and co-ordinate biochemical, physiological and behavioural processes is common to all major invertebrate taxa. Neuropeptide signalling mechanisms which utilise the peptide products of specialised neurosecretory cells are the predominant effectors among the endocrine systems so far characterised in invertebrates, but non-peptide endocrine messengers are of importance in many groups. Both of these systems are potentially susceptible to interference by ED's, but, at present, there is little evidence in any animal group of endocrine disruptive effects being exerted via a peptide signalling system (but see Oberdörster and McClellan-Green, 2000). However, if all non-peptide signalling systems are potential targets for ED's then every invertebrate group must be considered to be potentially susceptible. Invertebrate species may be particularly sensitive to ED's during critical developmental periods such as moulting, metamorphosis and during the vigorously growing larval stage.

The presence and function of steroid hormones, and evidence for ED effects, in invertebrates has recently been reviewed by LeBlanc (2000). The evidence is, as yet, restricted, the majority of research having focused on fish and higher vertebrates. The reason for this lack of information is the limited knowledge of invertebrate endocrinology (Tattersfield *et al.,* 1997). As yet there is inadequate understanding of the endocrine mechanisms controlling moulting, growth, reproduction, or sexual determination for most of these following taxa: rotifers, nematodes, polychaetes, oligochaetes, amphipods, *Artemia* spp., cladocerans and copepods (De For *et al*, 1999). A more complete understanding of the endocrine-mediated processes is urgently required before assessment of ED in invertebrates can be considered comprehensive.

4.2.1 Known cases of endocrine disruption in invertebrates

There are very few cases of endocrine disruption in invertebrates that have been associated specifically with an established cause. In this section the evidence for ED effects of certain insecticides and of the antifouling agent tributyltin are fully discussed; these are the only two scenarios where a cause-effect relationship is clear. This is followed by a review of studies, both in the laboratory and the field, where the effects observed may have been caused by endocrine disruption. It must be stressed however, that at present, much of the evidence is circumstantial because many studies were carried out before the concept of 'endocrine disruption' was recognised; the endpoints measured were not chosen *a priori* to determine ED but were directed at measuring general toxic responses.

4.2.1.1 Insect growth regulators

The most well studied invertebrate endocrine system is that of the insects, owing to their economic, ecological, and agricultural importance. The most widely reported effects of endocrine disruption in invertebrates are those of certain insecticides. In this case, chemicals have been purposefully synthesised to disrupt the endocrine system of a number of insects in order to reduce risk of insect-borne disease or insect-mediated destruction of food crops. Identification of the hormones controlling insect growth and development has permitted the synthesis of compounds designed to mimic, block or otherwise interact with normal hormone systems (i.e. ED) of the targeted insect. Ecdysone inhibitors are designed to inhibit insect moulting, and juvenile hormone analogues have been the basis of some insecticides which attempt to mimic the natural hormone and disrupt normal maturation and metamorphosis of the insect (Gorbman and Davey, 1991; Jepson, 1993; Lagadic *et al.,*1994; Nimmo and McEwen, 1994; Slama, 1971).

Because these chemicals have been specifically developed to disrupt arthropod endocrine systems, there is potential for them to pose an environmental ED hazard to non-target organisms (Tattersfield *et al.,* 1997). A number of insect growth regulators have been found to affect several aspects of freshwater, estuarine and marine crustacean growth and development patterns, although at the moment it has not been confirmed that these effects are hormonally mediated in those taxa.

Laboratory studies have shown that exposure to methoprene (a juvenile hormone analogue used, for example, to control mosquitoes), in the low parts per billion concentrations can (1) alter larval development in marine crabs and shrimp (Celestial and McKenney, 1994); (2) affect both development and reproduction in freshwater cladocerans (Chu *et al.,*1997; Templeton and Laufer, 1983); and (3) retard juvenile growth, delay the onset of reproduction, and reduce reproductive capacity in an estuarine mysid, *Mysidopsis bahia* (McKenney and Celestial, 1996). These concentrations are similar to those that are lethal to a number of target insects, including salt marsh mosquitoes.

A series of studies using the mud crab *Rhithropanopeus harrisii* have demonstrated that several juvenile hormone analogues (i.e. insecticides) increase the time required for complete larval development and limit metamorphic success at concentrations similar to those which are effective against developing larvae of various target insects. These effects were observed between 0.1 and 1 ppm, concentrations which are two to three orders of magnitude lower than those lethal for fish and other non-target organisms (Bookhout and Costlow 1974; Celestial and McKenney 1994; Christiansen *et al.,*1977; Costlow 1977). An ecdysone mimic accelerated moulting of zoeae of this crab (Clare *et al.,*1992) and exposure to tributyltin altered growth rates of larvae (Sanders *et al.,*1985). Swimming speeds and phototaxis of larval *R. harrisii* were altered with sublethal exposure to the insect growth regulator hydroprene, while methoprene exposure resulted in no modifications in crab larval behaviour (Forward and Costlow, 1978), it is not clear whether the effects observed were due to endocrine disrupting mechanisms. Methoprene exposure inhibited vitellogenesis in this species by arresting oocytes in a terminal previtellogenic stage (Payen and Costlow, 1977).

The juvenile hormone analogue, hydroprene, (Gomez *et al.,*1973) and a synthetic juvenile hormone (juvenile hormone I; Ramenofsky *et al.,*1974) both caused cyprid larvae of the acorn barnacle, *Balanus galeatus* to metamorphose prematurely. Treatment of cyprids from another barnacle, *Eliminius modestus*, with juvenile hormone analogues resulted in abnormal intermediates with both cyprid and naupliid characteristics (Tighe-Ford, 1977), similar to larval-pupal intermediates reported for insects treated with juvenile hormone analogues (Downer and Laufer, 1983; Sehnal, 1983;). Juvenile hormone analogues produced morphological abnormalities in crab megalopae larvae (Costlow, 1977) and metamorphosed lobster larvae (Charmantier *et al.,*1988). Exposure to precocene caused toxicity to early developmental stages of brine shrimp and barnacles (Landau and Rao, 1980).

Completion of larval development of *Palaemonetes pugio* (the grass shrimp) has also been used to evaluate the impacts of insect growth regulators on non-target crustaceans (McKenney, 1998). As with the mud crab studies, larvae of grass shrimp exhibited reduced rates of metamorphic success at concentrations of methoprene similar to those that are effective against developing larvae of various target insects. Exposed larvae were more sensitive to toxicity during the early and final larval stages rather than during intermediate stages. Methoprene in these exposures retarded growth in early larval stages and postlarvae, but enhanced growth in premetamorphic larvae.

In summary, although insect growth regulators can clearly disrupt crustacean development, there is as yet little evidence for such effects occurring in the field and it is not clear, in many cases, whether the mechanism of action is via endoctrine discruption. The main application of IGRs is agricultural-based; once applied, they tend to bind to soil particles and are not readily mobilised from this substrate. They are rapidly degraded, with half-lives ranging from a few hours to several days. For these reasons, coupled with the fact that there are no direct inputs of IGRs to estuarine and marine waters, it is unlikely that significant concentrations are found in these environments.

4.2.1.2 Tributyltin

The effects of tributyltin (TBT), an ingredient of antifouling paint for boats and ships, provides one of the few clear examples of endocrine disruption in marine invertebrates. It is now well established and understood that imposex and intersex in gastropod molluscs is induced almost uniquely by TBT, which is based on results of laboratory experiments using the dog-whelk, *Nucella lapillus* (Bryan *et al.,* 1987). Comprehensive reviews covering the adverse effects and mechanism of action of TBT on marine organisms can be found in Bryan and Gibbs (1991), Alzieu (1996) and Matthiessen and Gibbs (1998). Briefly, imposex is the phenomenon whereby masculinisation of the females takes place, in the form of development of male sex organs such as a penis and vas deferens, leading to prevention of normal breeding activity and eventual population disappearance. Intersex is a related condition found in littorinid mesogastropods which also leads to inability of the females to lay eggs (Matthiessen and Gibbs, 1998) In this latter condition females develop ross malformations of the oviduct (eg ventral splitting and prostate gland formation, both of which are male characteristics) that prevent copulation and capsule formation, thus inhibiting egg laying.. TBT exposed females have increased levels of testosterone titres, the likely cause of which is the competitive inhibition by TBT of cytochrome P450-mediated aromatase (Spooner *et al.,*1991).

Species susceptible to imposex exhibit in general a higher TBT sensitivity than the intersex-developing snail *Littorina littorea,* where the aquatic threshold concentration for development of intersex characteristics is 5.0 ng TBT-Sn/l compared with <0.5 ng TBT-Sn/l for imposex development in *Nucella lapillus* (Oehlmann, 1998). The most sensitive species known currently seems to be *Ocinebrina aciculata* with a threshold concentration of 0.1 ng TBT-Sn/l.

In contrast to intersex, with *Littorina littorea* being the only known affected species currently, imposex is widespread across species of gastropod mollusc. On the other hand, some prosobranch snails do not develop imposex even when exposed to high aqueous TBT concentrations or sampled in highly contaminated areas. Within these species are the limnic archaeogastropod *Theodoxus fluviatilis*, the mesogastropods *Bithynia tentaculata*, *Hydrobia ventrosa* and *Potamopyrgus antipodarum* as well as the buccinid species *Columbella rustica*. Other marine species like *Capulus ungaricus* and *Turritella communis* are not able to develop imposex because males are aphallic and do not possess a vas deferens (Oehlmann, 1998). Although most cases of imposex reported to date are confined to coastal and shoreline areas, it is worth noting that imposex has been observed in juvenile edible whelks (*Buccinum undatum*) in the open North Sea, in the vicinity of offshore shipping lanes (Hallers-Tjabbes *et al.,*1994).

In addition to gastropods, several species of bivalve molluscs exhibit biological effects in response to exposure to TBT. In the early 1980s, Pacific oysters (*Crassostrea gigas*) from some cultivation areas showed unusual shell morphology. The valves became greatly thickened and developed internal chambers, and in extreme cases, the oysters became ball-shaped, and the meat yield was greatly reduced to the extent that the oysters were no longer commercially acceptable. It was noted that the deformity became less severe with distance from marinas, and subsequent research showed that the effect was caused by exposure to TBT. It was also demonstrated that at high concentrations of TBT, the female reproductive systems were masculinised and spermatogenesis could occur. TBT has been reported to have various effects on a range of bivalve species, including *Ostrea edulis, Mytilus edulis, Scrobicularia plana, Mercenaria mercenaria*. (Lapota *et al.,*1993; Laughlin *et al.,*1988, 1989; Ruiz *et al.,*1995a, b; Thain *et al.,* 1986) and these have been shown to be linked to alterations of the endocrine system. It is not clear whether shell thickening in Pacific oysters is a consequence of some form of endocrine disruption or arises from interaction between TBT and other aspects of the biochemistry of the oysters.

There is relatively little information on the ecosystem-level impacts of TBT, but it is expected that the endocrine disrupting effects of TBT in molluscs will have consequent effects for some marine communities. Experiments conducted on the Isle of Man (Spence *et al.,*1990) in which all *Nucella lapillus* individuals were removed in an attempt to simulate the consequences of severe imposex, showed that there was a subsequent increase in the population density of limpets, and to a lesser extent those of mussels and barnacles (on which dogwhelks prey). The increase of limpets in turn led to a decline in macrophytes due to heavy grazing pressure. More recent field studies have shown that TBT from yachts in the Crouch estuary, Essex, had a major effect on the benthic invertebrate community, including not only molluscs but also crustaceans and ascideans (Rees *et al*, 1999. Crustaceans have been shown to be highly susceptible to TBT (Bushong *et al*., 1990), so it is not inconceivable that their populations in this estuary were directly affected. It is not known if ascidians are sensitive to TBT: their populations in the Crouch estuary may have been indirectly affected by altered interspecific competition and predation. Recovery of the Crouch community after the use of

TBT on small boats was banned in 1987 took approximately a decade (Rees *et al.*, 1998, 1999; Waldock *et al.*, 1999). Concentrations of TBT in water and sediment decreased from 45 to 2 ng/l and 0.16 to 0.01 μg/g dry weight respectively, over the period 1987 to 1992.

Triphenyltin (TPhT), as well as TBT, promoted the development of imposex during injection experiments in the rock shell (*Thais clavigera)*, and its potency was estimated to be approximately the same as that of TBT (Horiguchi *et al.,*1997a). Results of injection experiments using 18 kinds of organotin compounds showed that 4 organotins (tripropyltin (TPrT), TBT, tricyclohexyltin (TCHT) and TPhT) promoted the development of imposex in the rock shell (Horiguchi *et al.,* unpublished data). The estimated order of potency for promoting imposex was ; TPhT = TBT > TCHT > TPrT (Horiguchi *et al.,* unpublished data). In the case of the dog-whelk, TPhT did not promote the development of imposex although TPrT and tetrabutyltin slightly promoted it (Bryan *et al.,* 1988). It is also reported that monophenyltin (MPhT) induced imposex in *Ocenebra erinacea* (Hawkins and Hutchinson, 1990). It is possible that sensitivity to organotin species inducing or promoting the development of imposex differs among gastropod species, although little is known about such interspecific differences.

4.2.2 Other evidence of endocrine disruption in marine invertebrates

Among aquatic invertebrates, one of the best studied endocrine systems is that of crustaceans, particularly the decapods. Although there are some examples of laboratory-based experiments demonstrating endocrine disruption in crustaceans, the conclusions are often equivocal. Field-based data are generally lacking. Crustaceans may serve as a significant indicator species of endocrine disruption due to their economic importance (aquaculture and fisheries), ecological significance (as keystone species in many food webs), and extensive use as model invertebrates in laboratory toxicity evaluations.

The following section summarises the existing information on studies of ED (or potential ED) with a variety of marine organisms, with particular focus on the crustaceans.

Several studies on potential ED compounds, such as PAHs, TBT and methoxychlor, have been conducted with the commercially important blue crab species *Callinectes sapidus.* Measured endpoints included moulting, developmental success (oocyte, embryonic and larval), claw regeneration, lipovitellin production, and steroid metabolism (Bookhout and Costlow, 1975; Bookhout *et al.,*1976; Lee and Noone, 1995; Lee *et al.,*1996; Mothershead and Hale, 1992; Oberdörster *et al.,*1998; Schlenk, *et al.,*1993). P450 isoenzymes and heat-shock proteins were increased at doses of 250-500 μg/kg TBT, and this response was thought to aid metabolism and elimination of the compound. Cadmium and TBT have been found to reduce oocyte growth at concentrations of 20 μg/L and 2 μg/L respectively. In addition, hatching success was reduced by 50% at cadmium and TBT concentrations of 0.25 μg/L and 0.047 μg/L respectively. Newly moulted crabs exposed to PAH in water had higher hepatopancreatic concentrations than inter-moult crabs (9560 ng/g vs 3360 ng/g), and it was postualted that this was due to either increased water uptake and shell permeability at ecdysis or a decrease in metabolism of PAH during moulting.

The effects of insect growth regulators on the grass shrimp *Palaemonetes pugio* have been described in section 4.2.1.1. There are also reports in the literature describing the physiological responses of grass shrimp to other potential EDs. *P. pugio* was adversely affected by the pyrethroid insecticide fenvalerate bound to artificial sediment at a nominal concentration of 100µg/kg. Significant mortality occurred at metamorphosis, when larvae changed from pelagic individuals to benthic organisms i.e. when settling on to and being in direct contact with the contaminated sediment (Weber *et al.,*1996). Fenvalerate-laden sediment altered the growth and metabolism of developing *P. pugio* dependent on fenvalerate concentration, age of shrimp, and whether shrimp were premetamorphic or postmetamorphic in development (McKenney *et al.,*1998). Continuous exposure to 0.03 µg/L of endrin, an organochlorine pesticide, delayed the onset of spawning and reduced the number of viable offspring per female (Tyler-Schroeder, 1979). Several dithiocarbamates inhibited limb regeneration in this shrimp (Conklin and Rao 1982) and resulted in additional physiological responses coupled with histopathological deformities (Rao *et al.,*1982). Tributyltin has been found to inhibit telson regeneration in grass shrimp (Khan *et al.,*1993), as well as claw regeneration in other crustaceans (Weis *et al*., 1987). Similarly, other xenobiotics such as PAHs, DDT, and other pesticides can affect moulting or regeneration in a wide variety of crustaceans (Weis *et al.,*1992).

Exposure of *Procambarus clarkii* (red swamp crayfish, native to USA) to cadmium causes induced hyperglycemia, which is thought to be mediated by crustacean hyperglycemic hormone (Reddy *et al.,*1994). Cadmium (at a concentration of 0.5µg/g body weight) arrested ovarian maturation of *P. clarkii* by inhibiting release of gonad stimulating hormone (Reddy *et al.,*1997) and decreasing fecundity and hatching success (Naqvi and Howell 1993). Reddy *et al.* (1997) also reported arrested ovarian maturation by mercury (at a concentration of 0.5 µg/g body weight) due to inhibition of the stimulatory effects of serotonin. Ovarian atresia was induced by naphthalene which was reported to block release of gonad stimulating hormone by brains and thoracic ganglia (Sarojini *et al.,*1994, 1995).

Billinghurst *et al.* (1998) investigated the effects of the known environmental oestrogens 4-nonylphenol and 17ß-oestradiol on the settlement of the cypris larvae of the barnacle *Balanus amphitrite*. This cyprid stage contains high levels of cyprid major protein (CMP) which is very similar to the oocyte protein vitellin, found in some invertebrates. Vitellin is analagous in its function to vitellogenin, which has been used as a biomarker for oestrogenic exposure in lower vertebrates. The hypothesis was that since CMP is closely related to vitellin, xeno-oestrogens may elevate the levels of CMP which would in turn enhance cypris larvae settlement. The results showed that there was a significant reduction in larval settlement, and the authors concluded that there was no evidence for endocrine disruption. However, potentially harmful toxic effects of 4-nonylpenol at environmentally realistic concentrations (1 µg/l and above) were demonstrated, which in themselves may give cause for concern. Further work is now in progress to investigate the potential endocrine disrupting effects of these endocrine disrupters at different developmental stages of barnacles (Billinghurst *et al.,* 2000). However, it has been shown (EDMAR programme, unpublished)* that vitellin production in shrimps and crabs does not respond to oestrogen exposure. Recent work has now confirmed that crustacean vitellin does not act as a biomarker of vertebrate androgen or oestrogen exposure, and normal vitellogenesis in female crabs is not perturbed by exposure to these hormones or their mimics.

* Subsequent to the production of the main body of this report, Matthiessen *et al* (2002) published a summary of progress in the EDMAR programme, which includes further reference to papers resulting from the research undertaken.

Exposure of oyster (*Crassostrea gigas*) embryos to a range of concentrations of 4-nonylphenol (0.1-10000 µg/L) resulted in a decrease in the number of D-shaped larvae larvae (at 100 µg/L and above) and delayed development to D-shape (0.1 µg/L and above; Nice *et al.*, 2000). It was concluded that further work is required to determine whether these effects were due to endocrine disruption, or some other mode of toxic action, and longer-term studies to assess population effects are necessary.

Amphipods are generally one of the most toxicologically sensitive invertebrate taxa in benthic aquatic communities (Burton *et al.,*1992; Lamberson *et al.,*1992). *Corophium volutator* has recently been used in long term experiments to determine effects of nonylphenol on growth and sexual differentiation (Brown *et al.,*1999). The population density of surviving amphipods was reduced and growth retarded at the lowest concentration tested (10µg/L), and exposed males had significantly longer second antennae than control males. The authors hypothesised that nonylphenol may act via the androgenic gland which controls the development of secondary sexual characteristics in crustaceans.

Field data on endocrine disruption in marine invertebrate species in the natural environment is limited, with the exception of the well documented cases of tributyl tin (TBT) exposure in molluscs (Matthiessen and Gibbs, 1998). High prevalence of intersex in harpacticoid copepods has been reported from the vicinity of a sewage outfall in the Firth of Forth, Scotland (Moore, 1991; Moore and Stevenson, 1994). However, no conclusive causal relationship between the sewage outfall and the levels of intersex was determined. Similarly the lobster, *Homarus americanus*, has been observed with ovotestes in Nova Scotia but it was not concluded whether this was a natural or site-related phenomenon (Sangalang and Jones, 1997).

Some hormonally controlled processes in echinoderms, such as oogenesis and gonadal development, have been observed to be affected by cadmium (effect concentrations = 25µg/l Cd; Besten *et al*., 1989, 1991a and b). Results from field studies are, however, inconclusive. Sea stars (*Asterias rubens*) exposed to 25 µg/l cadmium in laboratory studies or fed with PCB contaminated mussels (26 µg Σ_8-PCBs/g lipid) in the laboratory exhibited significant reductions in the levels of progesterone, testosterone and cytochrome P450 in the pyloric caecae of males and females (*Besten et al*., 1991b). Animals were also collected at different sites in the North Sea, in order to study the quality of their offspring and to perform biomarker measurements. No pollution gradient-related effects were observed on oocyte maturation or on the early development of sea star embryos. However, slight effects were observed on the microsomal cytochrome P450 monooxygenase system in the pyloric caecae of female sea stars collected from polluted sites within the influence of rivers like the Elbe (Germany), the Rhine, the Western Schelde (Netherlands) and the Humber (UK) (Besten *et al.,* 1996; Postma and Valk 1996, 1997). Because rates of P450-dependent steroid metabolism in *Asterias rubens* can be decreased as a result of exposure to contaminants in semi-field studies (Besten *et al.,* 1996; Postma and Valk 1997), measured decreased P450 activities found in the North Sea field study may signal potential adverse effects.

In general, ED-impacted invertebrate populations have faster recovery times than many vertebrates (due to their shorter life cycles), although field data on the effects of TBT (Waldock *et al.,* 1999; Rees *et al.,* 1998, 1999) suggest that once concentrations have been reduced, marine invertebrate community recovery may take at least a decade, particularly if natural immigration is slow and the community comprises long-lived, slowly reproducing species. However, it must be remembered that some ED's are very persistent, so even when their inputs are restricted, residues in sedimentary and other sinks may, if remobilised, continue to exert their effects for many years.

In summary, there are only a few well-supported examples of invertebrate endocrine disruption in the field, and, only a small number of these have clearly demonstrated effects operating through a known causative agent, resulting in endocrine disruption that modifies individuals and results in population and community level changes. The best example is that of TBT in molluscs, as described above. Its widespread distribution has been associated with locally severe effects on marine mollusc populations. While there are many examples of endocrine disruption in terrestrial invertebrates, these studies are associated with pesticide field trials. Studies of wild terrestrial populations where endocrine disruption has been identified as a serious problem are severely limited.

Most field studies do not focus on mechanisms but rather on high level responses. It seems probable, however, that there are many real-world examples where endocrine disruption is affecting invertebrate populations and communities in both the freshwater and marine environments. The conclusion that effects of ED's on invertebrates in the field are likely to be widespread, though undetected, stems from several lines of evidence and reasoning (SETAC, 1999). First, the conservatism in basic mechanisms of endocrine function suggests that if various vertebrate endocrine systems are affected (see Kendall *et al.,*1998), then other animals exposed to the same or similar compounds may be similarly affected although the lack of a relationship between vitellin production in crustacea and exposure to vertebrate oestrogens and androgens suggests that such effects are not always predictable. Second, invertebrates are exposed to a great variety of compounds known to alter hormone function in both vertebrates and invertebrates, although the evidence for effects on invertebrates is mostly from laboratory research. The significance of invertebrate ED has been recognised during more than three decades of research and testing to develop such compounds intentionally for insect control programmes. However, the majority of invertebrate taxa have received far less attention than the vertebrates, and understanding population effects of environmental contaminants is generally less well understood. As a result, effects on invertebrate populations may remain undetected for some time, and it is notable that populations effects have been documented for ED in TBT-exposed snails throughout the world.

4.3 Marine mammals

Population declines and abnormal reproductive function have been attributed to the effects of endocrine disruption in several marine mammals, including seals and whales. Organochlorines, including PCBs and DDT have been inferred as the causative agents for population declines of grey and ringed seals in the Baltic and Wadden Seas (Jensen *et al.,* 1979), otters in the UK, Sweden and Norway (Mason, 1995; Mason and Macdonald, 1993; Smit *et al.,*1996) and beluga whales in the St Lawrence seaway in Canada (De Guise *et al.,*1995). In all cases, elevated tissue concentrations of these chemicals have been measured and, in some instances, there was an association with reproductive abnormalities.

Some links have been made between immune system functioning and chemicals which have endocrine disrupting properties. It is known that the antibody and lymphocyte components of the immune system are regulated by oestrogens and androgens. In addition corticosteroids and thyroid hormones are involved in immune function (Reijnders, 1999). Mass mortalities of harbour seals and striped dolphins in various parts of the world have been attributed to contaminant-induced immuno-suppression (Dietz *et al*., 1989). A high incidence of severe lesions in Beluga whales, caused by opportunistic and mildly pathogenic bacteria, is also thought to be due to contaminant-induced immuno-suppression (Martineau *et al*., 1988).

Known or suspected endocrine disrupting chemicals have been detected in the tissue of other marine mammals. For example, butyltin compounds, which have an ED effect in gastropod molluscs, have been detected in sea lion tissues in Japan (concentrations ranging between 150 to 220 ng/g wet weight) (Kim *et al.,*1996), in bottlenose dolphins (mean concentration in liver: 1400ng/g) along the US Atlantic and Gulf coasts (Kannan *et al.,*1997) and also in the liver tissue of harbour porpoises (22-640 ng/g) and grey seals (0-22 ng/g) from UK coastal waters (Law *et al.,* 1998). The reported effects of butyltins in mammals such as rats include immunosuppression, but further study is needed of the possible toxic effects and the risk of accumulation of these compounds in marine mammals.

Although there are correlations between tissue concentrations of contaminants in sea mammals and reproductive impairment in one form or another, it is very difficult to prove that these are due to endocrine disrupting effects (Environment Agency, 1998). One semi-field study has linked reproductive failure of common seals from the Wadden Sea to PCB pollution (Reijnders, 1986). Two groups of seals were fed either flatfish from the Wadden Sea or herring caught in the northeast Atlantic, for a period of two years. Females fed Wadden Sea fish had a lower pregnancy rate, with altered associated hormone profiles at the time of embryo implantation. These effects were attributed to the higher concentrations of PCBs (mean daily intake in seals 1.5 mg vs 0.22 mg) and *p,p'*-DDE (mean daily intake 0.4 mg vs 0.13 mg) in the flatfish, although a mechanism of action was not proffered. Later analysis of plasma samples from this feeding study showed that a diet of fish highly contaminated by PCBs led to vitamin A and thyroid deficiency (Brouwer *et al*., 1989), and this may have contributed to the reproductive failures observed. It has been suggested that this level of intake poses a risk to UK seal populations, based on measured residues of PCBs and DDE in UK fish (Central Science Laboratory, 2000).

4.4 Birds

The endocrine system of birds, as with all other animals, is intimately involved in the regulation of many physiological functions, and exposure to exogenous chemicals, including natural chemicals and environmental contaminants, can disrupt endocrine function in a variety of ways. Two of the most sensitive functions are sexual differentiation and reproduction. EDs can have a powerful, permanent influence on the sexual characteristics of birds, including adult behaviour, if they exert their effects during critical periods of embryonic development (i.e. in the egg), when both the brain and gonads are being developed. The avian embryo can be exposed to EDs during the critical period through transferral of contaminants from the female to its eggs. Bird eating raptors and some fish eating birds (e.g. cormorants and pelicans) are particularly sensitive to substances such as DDE bioaccumulation effects (Institute for Environmental Heralth, 1999). There are several routes of exposure to EDs for birds of marine and estuarine habitats, whether for all or part of the year. Food will likely be a major uptake route, through the consumption of marine invertebrates or fish. The risk to marine bird species will vary depending on the prey species ability to bioaccumulate ED substances. Birds which prey upon mussels, which are strong bioaccumulators, may be at a greater risk of exposure than those which eat crustaceans or plant material. Those species which feed on mud flats exposed at low tide may also directly ingest ED contaminated sediment.

The most concrete evidence for endocrine disruption in a bird species is that provided by the studies of Fry and Toone (1981), who found that gull embryos exposed to the organochlorine pesticide DDT (at concentrations 2-5 ppm) and its metabolite DDE (20-100 ppm) exhibited several reproductive defects, such as feminisation of male birds, egg shell thinning, suppression of egg formation, embryo mortality and abnormal reproductive behaviour e.g. female-female pairing. Exposure was due partially to primary contact with the pesticide but mainly through secondary uptake via the food chain. DDT is a persistent lipophilic organochlorine which is readily transferred to lipid-rich yolk; fish-eating birds can acquire substantial body burdens of such contaminants from their diet, and then transfer these body burdens to the egg yolk. However, it should be noted that there is still some doubt as to whether the widely reported effect of egg shell thinning by DDT/DDE is caused by endocrine disruption, rather than by direct toxic effects on the shell gland.

Other pesticides, such as dieldrin, aldrin and lindane, and the industrial contaminants PCBs and dioxins, have also been implicated in endocrine disrupting effects in birds (Peakall and Fox, 1987; Fry, 1995). Poor reproductivity of herring gulls in the Great Lakes was due to a high incidence of egg loss (caused by abnormal incubation behaviour in adults) and embryonic death, linked to high levels of residues of several xenobiotics, including dioxins and PCBs (Peakall and Fox, 1987. Fry (1995) reviewed the evidence for reproductive effects (which may or may not be ED mediated) in birds exposed to pesticides and industrial chemicals, including organochlorine and organophosphate pesticides, PAHs and heavy metals. A diverse range of physiological effects on breeding adult birds and developmental effects on embryos are reported. Several herbicides, fungicides, PCBs and organochlorine mixtures have been suggested as environmental oestrogens possibly affecting populations of breeding gulls in southern California, the Great Lakes and Puget Sound.

Laboratory studies of ED in birds have examined both reproductive or developmental effects. The chlorinated insecticide kepone binds to the chicken oestrogen receptor, with a potency of about 5000 times less than the natural oestrogen 17ß –oestradiol (Eroschenko and Palmiter, 1980). This same chemical also stimulated growth and severe morphological changes in the oviduct of immature Japanese quail (Eroschenko and Palmiter, 1980). *O,p'*-DDT and the di-methylated metabolite of methoxychlor also bind to the oestrogen receptor and produce oestrogenic effects *in vivo* (Kupfer and Bulger, 1980). Organochlorine chemicals (including DDE and PCBs) fed to breeding Ring Doves resulted in a reduction and/or delay in behaviourally- related increases in sex hormones and altered reproductive behaviours, such as abnormal female responses to male courtship behaviour and a reduction in the time spent feeding offspring (McArthur *et al.,* 1983). Crude oil administered orally to herring gull and black guillemot nestlings produced elevated plasma corticosterone and thyroxine levels, which seemed to be related to depressed growth of the birds (Peakall *et al.,*1981). Natural phytoestrogens (isoflavones from plant extracts) incorporated into the diet resulted in a delayed onset of reproduction and decreased egg production in Californian quail (Leopold *et al.,*1976).

Field observations of abnormalities and deformities have also been attributed to endocrine disruption. In the North American Great Lakes, the incidence of crossed bill syndrome in cormorants was correlated with concentrations of polychlorinated hydrocarbons (PCH) in eggs (Fox *et al.,* 1991). This syndrome mimics chick oedema disease, which was observed in the offspring of adult hens fed polychlorinated dibenzodioxins and –dibenzofurans. In the 1970's, herring gulls on Lake Ontario in Canada were noted to have a poor hatching success rate, and surviving chicks showed characteristic symptoms of chick edema disease. Later analysis of eggs collected throughout the 70's revealed that they contained high levels of TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin), thought to have been discharged from a herbicide manufacturer (Gilbertson, 1985). Similar biological effects were demonstrated in Forsters terns, double-crested cormorants and Caspian terns in Green Bay (Lake Michigan), but in this case, dioxin-like non-ortho-substituted PCBs (at concentrations greater than 3.5 ppm) were implicated (Tillitt *et al.,*1991). Although the latter compounds are relatively less potent than TCDD, they are much more abundant in the environment and thus may have toxic effects on wildlife species in the field.

Altered levels of thyroxine and vitamin A, which are crucial for the normal development of sexual and immune functions, have been reported to co-occur with embryonic abnormalities in some bird populations exposed to synthetic halogenated hydrocarbons (Gilbertson *et al.,* 1991). PCHs can influence both thyroid hormone, an important regulator of development and metabolism (Zile *et al.,* 1997), and cause changes in the status of vitamin A (which is necessary for normal embryonic development) in plasma or liver, and has been implicated birth defects observed in birds (Spear and Moon, 1985).

MAFF Central Science Laboratory (2000) reviewed the evidence for endocrine disruption in top predators, including birds, and assessed the risks posed by ED chemicals by comparing residues in prey with levels shown to cause detrimental effects in top predators. Although data on critical dietary levels of ED chemicals for fish-eating birds were not available, it was predicted that residues of ED chemicals in prey could exceed critical dietary levels, assuming that these were similar to raptor species, for which data do exist (Peakall *et al*., 1990). Bioaccumulation factors (of DDE for example) for fish to fish eating bird eggs, were found to be as high as 57-fold, indicating that low residue concentrations in prey species can still have adverse effects on birds. It was also found that larger fish had a disproportionately greater level of residues than smaller fish, hence it is predicted that greater effects are likely to be observed in birds whose diet contains a greater proportion of large fish.

There is potential greater risk of exposure to organotin compounds of mollusc-feeding waterfowl (and hence their predators), compared with those species that feed on macroalgae, for example. No data exist on organotin residues in predatory birds or waders for which molluscs may form a significant part of the diet.

4.5 EDMAR programme progress

The EDMAR (Endocrine Disruption in the Marine Environment) programme, jointly funded by MAFF, DETR, SNIFFER (Scotland and Northern Ireland Forum for Environmental Research) and CEFIC (European Chemical Industry Council) was implemented in 1998 and is due to end in 2001[*]. Much progress has been made in the development of biomarker methods for measuring ED in fish and invertebrates, the isolation and identification causative substances and also in monitoring ED in fish in UK estuaries. Major progress within the programme to date is summarised below:

[*] Subsequent to the production of the main body of this report, Matthiessen *et al* (2002) published a summary of progress in the EDMAR programme, which includes further reference to papers resulting from the research undertaken.

Two biomarkers of androgenic exposure in female stickleback (spiggin induction and increase in kidney epithelial cell height - KEH) have now been successfully developed and calibrated. They show similar, and dose-dependent, sensitivity to the model androgen methyl-testosterone, and the effects are antagonised by the anti-androgen flutamide. Caged sticklebacks have been placed in estuaries to identify for androgenic and anti-androgenic effects in the field.

Histochemical methods for locating endocrine biomarkers has progressed to a stage where a workable procedure for localising flounder VTG in thin sections will shortly be available for use in field surveys. A successful method for localising oestrogen receptors in flounder tissue (using rainbow trout receptor antibody) has also been developed, which could be applied to fish from a range of estuaries to examine the possibility that oestrogen exposure can induce an increase in the numbers of receptors. This may have several consequences, including elevated sensitivity to oestrogens.

Invertebrate biomarker assays for vitellin have been developed and tested for in two species of crustaceans (brown shrimp and shore crab). However, it has been confirmed that this protein does not act as a biomarker of vertebrate androgen or oestrogen exposure in these crustacea, and normal vitellogenesis in female crabs is not perturbed by exposure to these hormones or their mimics.

Detailed analytical chemistry and TIE work to isolate the oestrogenic fractions from water and sediments from the Tees and Tyne has identified a number of substances which are contributing to the oestrogenicity present. These include 17β-oestradiol, estra-1,3,5-trien-3-ol, androsterone, *bis*(2-ethylhexyl)-phthalate and nonylphenol. The majority of the activity in the Tees is associated with the solid phase in sediments, and there are a number of substances in this compartment which have still to be identified. However, of those substances identified to date, natural hormones or their degradation products contribute the most activity. A national survey of androgenic activity in estuarine waters has shown little activity in many places, but higher activity in sewage effluent from two locations (Irvine Valley sewer and Dalmuir STW in Scotland). Toxicity Evaluation and Identification (TIE) procedures are now in progress using Irvine Valley effluent in order to identify the androgenic component(s).

Field surveys have confirmed that flounder are still being heavily exposed to oestrogenic substances in some industrialised estuaries, including Clyde, Tyne, Tees and Mersey. Surveys of sand goby, a species which lives and breeds in estuaries, have shown no male intersex on VTG gene induction. However, what appears to be feminisation of a secondary sexual characteristic (the uro-genital papilla, which is thought to be involved in gamete deposition) has been observed in many male fish in the Tees and Mersey. This morphological abnormality is rare or absent in the Alde (the clean control estuary), Crouch and Thames. The biological significance of this is still being investigated, and it will be necessary to investigate whether the effect can be reproduced in laboratory fish exposed to a model oestrogen.

A final round of fieldwork with a variety of fish species will take place up to spring 2001. There will be a focus on viviparous blennies, with the objective of linking oestrogenic exposure with reproductive success, samples of sand goby will also be taken for further investigation of feminised secondary sexual characteristics (urogenital papillae), and flounder will again be sampled in key estuaries to establish whether oestrogenic exposure has declined since 1997.

Research on migratory salmonids has begun, using sea trout adults and smolts in the Tees, with particular focus on VTG induction and on the smoltification process.

4.6 Conclusions

This review has described cases of known and possible endocrine disruption in the four main groups of animals to be found in the UK marine and estuarine environments, namely fish, invertebrates, birds and mammals. These studies have drawn upon both laboratory studies and field observations. A large and ever-increasing number of chemicals are being placed on "priority lists" of endocrine disrupters. The major chemicals/sources which are currently of concern in the marine/estuarine environments, and which are implicated in causing effects across several groups of animal include organochlorine compounds, steroids and alkylphenols in sewage effluent and organotin compounds.

It can be difficult to prove that a particular effect observed in the field is caused by a given mechanism, even when deploying supportive laboratory experimental studies. In the natural environment, multiple mechanisms (some of which are mutually antagonistic, others of which will reinforce each other) may well be operating in areas where large numbers of active substances (natural and anthropogenic) are present in relatively high concentrations. At the moment, we cannot predict whether complex waste discharges are likely to cause endocrine disruption, because there are, as yet, no internationally standardised procedures for testing substances or effluents which can definitively identify ED action (Tattersfield *et al.,*1997). However, based upon a weight-of-evidence approach, we consider that these sources are likely to cause.

The population-level significance of endocrine disruption in individuals within a population remains largely unknown. The only proven case where endocrine disruption by a particular pollutant has led to population-level and community-level effects is that of the decline in mollusc population densities and marine benthic community diversity as a result of exposure to TBT. In the case of fish exposed to oestrogenic effluents, there is little evidence that population declines are in progress except possibly in some highly polluted rivers, where non-endocrine contaminants are probably playing at least as important a role as sewage-derived oestrogens. We do not really understand why endocrine disruptions had major effects in the former case, and rather minor ones in the latter. It must be borne in mind that reproductive interference caused by ED is not as clear cut in fish as it is for gastropods, and the processes that regulate their populations are likely to differ.

Because our current understanding of change at the community and ecosystem level is still rudimentary, future research on endocrine disruption should focus on effects at both the individual and population levels in order to establish firmer predictive links. A serious issue concerning research on endocrine disrupters and wildlife is not so much whether this particular pollution mechanism is operating, but if it is intrinsically possible to predict population- and community-level change from a knowledge of reproductive and other impacts on individuals. There is also a need to understand the possible effects of complex mixtures of endocrine disrupters. These problems are common to all of ecotoxicology, and are certainly not unique to endocrine disrupters.

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5. Volumes of sewage and industrial effluent discharged to SACs and SPAs

The aim of this exercise was to obtain information about the major sources of direct dischargings of potential endocrine disrupting chemicals into European Marine Sites, in order to allow a preliminary evaluation of the risks for a selection of SACs and SPAs and hence prioritisation of sites for further investigation. The available data do not, unfortunately, permit the description or quantification of contaminant inputs to the relevant SACs and SPAs. However, in order to obtain a general picture of the waste inputs to these areas, data on sewage treatment works (STW) discharges (which generally include some trade waste) and separate industrial discharges (excluding power station cooling waters, which are not major sources of contaminants), have been obtained from the CEFAS discharge database. This database has been compiled from Environment Agency data, and most of it was updated during 1999.

The information available on the chemical constituents of these discharges is patchy and very incomplete. For that reason, we have only used data on the discharge location, type and consented volume. Discharges which have been included in Table 2 are those which are either located within the SAC or SPA itself, or in immediately adjacent estuaries. Discharges in rivers above the tidal limit have not been included although other sources within a catchment but outwith the site boundary may be important and may eventually reach and impact on estuaries and coastal waters, the major data retrieval process involved was not within the scope of this project. It was considered that this shortcoming was acceptable given the other constraints in this preliminary prioritidation exercise. The data in Table 2 have also been plotted on maps of the relevant areas in Figures 1-20. The size of the symbols is proportional to the consented volume of each discharge.

Table 2. Total consented volumes of sewage and trade effluent (excluding power station cooling water) discharged either directly to SACs and SPAs, or in immediately adjacent estuaries.

SAC or SPA	Sewage effluent (m^3/day)	Trade effluent (m^3/day)
Berwickshire and North Northumberland Coast SAC	8,711	12
Teesmouth and Cleveland Coast SPA	138,403	258,902
Flamborough Head SAC	16,863	2,500
Humber Flats, Marshes and Coast SPA	255,488	325,720
Wash and North Norfolk Coast SAC	39,402	33,236
Essex Estuaries SAC	41,895	23,423
Thames Estuary and Marshes SPA	3,967,096	611,606
Thanet Coast SAC	74,331	13,702
Solent and Southampton Water SAC	299,047	685,688
Isle of Wight SAC	54,926	2,346
Poole Harbour SPA	134,399	6,167
Chesil and The Fleet SAC	19,602	6,200
Plymouth Sound and Estuaries SAC	95,304	121
Fal and Helford SAC	54,510	20,022
Isles of Scilly SAC	No data	No data
Lundy SAC	0	0
Severn Estuary SPA/pSAC	1,028,773	65,472
Dee Estuary SPA	49,937	62,335
Mersey Estuary SPA	1,173,674	649,117
Ribble Estuary SPA	200,644	12,000
Morecambe Bay SAC	170,751	336,622
Duddon Estuary SPA	187,401	39,897
Solway Firth SAC	6,729	0

6. Monitoring data on potential endocrine disrupters in SACs and SPAs

The purpose of this section was to collate and summarise existing information on the distribution and measured concentrations of known and potential endocrine disrupters in each of the selected SACs and SPAs, in order to carry out a preliminary risk assessment for each site. While this type of approach takes into account both diffuse and point sources of endocrine disrupters, it does have limitations. The number of samples taken in any one area tends to be small, and the suite of determinands used in routine monitoring is very restricted; many of the ED chemicals listed in Table 1 of the literature review are simply not measured.

Chemical monitoring data for five matrices - water, sediment, fish tissue, invertebrates and mammals, for the five year period 1995-1999 have been obtained from the CEFAS BLISS database (held at Burnham-on-Crouch), and the Environment Agency National Centre for Environmental Data and Surveillance's database (held at Bath). Additional data are held by local offices of the EA, but this is not always stored in a standardised format and the level of data processing required was not within the scope of this project. Furthermore, this data will often relate to compliance measurements made close to outfalls, rather than background monitoring. Nevertheless, much of the routine chemical monitoring data for the period in question have been collated.

The data have been summarised by calculating the median and the 25 and 75 percentile values for the entire period for each determinand and matrix (Appendices 1-6). In view of the fact that data were generally rather sparse, the animal tissue matrices were aggregated as follows:-

Invertebrates = all invertebrate tissues (generally whole body, expressed as mg/kg wet wt. And generally sediment-dwelligno r benthic invertebrates).

Fish = all fish tissues (generally muscle or liver, expressed as mg/kg wet wt.). Note that these highly mobile animals may have been exposed to contaminants at points remote from the area of sampling.

Mammals = all marine mammals (generally blubber tissue from stranded pinnipeds and cetaceans, expressed as mg/kg wet wt.). Note that these highly mobile animals may have been exposed to contaminants at points remote from the area of sampling.

In addition, data on **Sediment** (expressed as mg/kg dry wt.) and **Water** (expressed as ng/l or µg/l) are presented where available.

Note: no data on residues in birds were available for use in this study.

The determinands were largely chosen on the basis of the foregoing literature review suspected or known to be endocrine disrupters. However, for the sake of completeness, all available data on metals have been included (in addition to the suspect ED metals cadmium, mercury and lead), as well as all organochlorines and total hydrocarbons (including the suspect PAHs). This assists in giving a broad picture of the chemical contaminant status of the areas in question. Many of the substances listed in Table 1 have not been measured routinely in the relevant areas, and so will not be included in Appendices 1-6. As well as separating the data for SACs and SPAs into different tables (and only showing data for an SPA where there is no overlap with an SAC), the data in the Appendices have also been split into three sets of tables for ease of reading: Metals; PCBs and Pesticides; and Miscellaneous (includes Nonylphenol, Octylphenol, Total Hydrocarbons [THC] including PAHs, and Tributyltin).

For each SAC or SPA, where data exist, the contaminant information has been plotted on histograms which have then been pasted onto a map of the area for ease of reference (see Figures 21-35). The exception to this is the Solent SAC for which no map was available at the time of the analysis. The locations of relevant sample points have also been shown on the maps.

7. Preliminary risk assessments for endocrine disrupters in SACs and SPAs

Using the data collected in sections 4 and 5 above, and information from English Nature on the key features for which specific European Marine Sites have been selected, the aim of this section is to relate the risks of significant exposure to particular groups of EDs to the relative sensitivities predicted for key species within each site. Subsequently, on the basis of this preliminary assessment, recommendations are made for those sites where further investigation and/or action is a priority.

There are a number of reasons why these assessments can, at best, only be regarded as preliminary:

- There are few data on inputs of EDs to SACs or SPAs. Consented discharge volumes are only a very crude indication of point-source inputs, and although it would be possible to estimate consented loads (e.g. from the Pollution Release Inventory) such estimates would not include comprehensively all EDs. In addition, consented discharge volumes used have excluded diffuse and upstream point inputs from rivers and the atmosphere.
- The available monitoring data are generally very sparse, often with small sample sizes, or completely missing matrices i.e. water, sediment, mammals, fish or invertebrates.
- No monitoring data exist at all for many known or suspect EDs. For example, steroids are known to be major causes of endocrine disruption in some UK rivers, but no routine marine monitoring data are available for this group
- Few data are available on the concentrations of EDs which are known to have effects on aquatic life. Where available, such data usually concern concentrations in water, however the monitoring data for this matrix are very few
- Invertebrates are important in all SACs and SPAs, but less is known about their responses to EDs than for marine fish. A similar situation exists for birds and mammals. Even where physiological responses may be predicted, population responses are much more difficult to predict.

Because of this severe information shortage, the assessments which follow are purely qualitative and to an extent subjective. The main recommendation arising from this study can therefore be given now: if the potential environmental risks which EDs pose in SACs and SPAs are to be reliably described, a large amount of new chemical and biological fieldwork is required.

The risk assessment for EDs will be described below for each site in turn, starting with the northeast English coast, and moving round clockwise to the northwest coast. Th approach taken was to concurrently consider effluent inputs, presence and levels of known and potential EDs from the monitoring data, known ED effects occurring in the area, and the key species/habitats of that area, including information on their rarity and sensitivity where available. On the basis of this information a judgement is made about the potential risks to fauna. In the case of the SACs, it will be found helpful to consult Appendix 7 which lists their main features of conservation interest. Similar data have not been presented for the

SPAs, since they have been selected for their importance to birds (mainly ducks, geese and waders), many of which feed on benthic and epibenthic invertebrates. Birds are most likely to be at risk from the biomagnification of EDs via their food and this was assumed in the following appraisals. Comments relate to potential risks associated with water-borne contaminants, or those present in sediments and aquatic biota.

Berwickshire and North Northumberland Coast SAC

This SAC probably receives few polluting inputs because almost all discharges are from small rural STWs and the volume is very small (total consented volume = 8,723 m^3/day). The major source of contaminants is likely to be the Tweed at Berwick, and this is all derived from domestic sewage. Important conservation features include extensive eelgrass beds and a large population of grey seals, as wel as important bird populations.

Not unexpectedly, contaminant concentrations are generally low, so there is not expected to be widespread chronic stress of the type which can exhaust the cortisol response.

Metals data were obtained only available for a few (4-7) invertebrate samples, but those implicated in endocrine disruption (especially lead, mercury, and cadmium, which at high concentrations have been linked to effects on the pituitary-interrenal and pituitary-thyroid axes) do not appear to have been bioaccumulated to significant levels with the exception of cadmium which is above background (median value of 2.4 mg Cd/kg) but probably not in the range of concern.

None of the organic determinands appear to be present in invertebrates or marine mammals (no fish data were obtained) at significant concentrations, although sample sizes are very small (1-5) and several determinands are missing from the dataset. The highest median concentrations are 1.7 mg THC/kg in invertebrates and 1.9 mg ΣDDT/kg in marine mammals. These concentrations represent background levels and are well below those found in animals from contaminated estuaries. On the basis of this, neither of these levels are judged to pose a significant problem. There are no data for organics in water or sediment.

In summary, the dataset used is very fragmentary, but polluting inputs are probably small and the available contaminant data do not give serious cause for concern. The priority for further investigation is therefore low.

Teesmouth and Cleveland Coast SPA

This is a wetland of international importance with a range of habitats (intertidal flats, rocky shores, saltmarsh) used by waterfowl, the significant species of which include terns, knot, redshank, sanderling, ringed plover, shelduck and teal. There are some discharges within the SPA itself, as well as large industrial and domestic discharges close by in the Tees estuary (total consented volume of 397,305 m^3/day, excluding power station cooling waters). As a result, the Tees is probably one of the most contaminated estuaries in the UK (Gray, 1976; Tapp *et al.* 1993; Turki, 1998). Furthermore, male flounder from the Tees are amongst the most highly feminised fish to have been caught around the UK coastline, as a result of oestrogenic substances discharged within the estuary (Allen *et al.* 1999a). Much of the oestrogenic contamination is located in sediments, but although a proportion has been identified as being of steroidal or industrial origin, the majority remains to be identified (EDMAR programme, unpublished data). There are also significant movements of large ships into the Tees which are a source of TBT contamination. Oil refineries in the estuary are a source of hydrocarbons including PAHs, and chemical manufacturing plants discharge a complex mix of anthropogenic substances. Nonylphenol (NP) is manufactured beside the Tees estuary, and total NP concentrations in Tees estuary water in 1993 were 0.09-5.5 µg/l (Blackburn and Waldock, 1995). Total NP concentrations in Tees water in 1998 ranged from 0.22 to 2.6 µg/l, probably reflecting reductions in inputs during the intervening period (CEFAS unpublished data). In 1998, NP concentrations in Tees sediments ranged from 0.2 to 42 mg/kg, showing that historically contaminated sediments may be a significant source of this material (CEFAS unpublished data). However, NP only appears to be a minor contributor to the total oestrogenicity of Tees sediments (EDMAR programme unpublished data). Historical mining operations have resulted in elevated lead levels in sediments within the estuary (Plater *et al.* 1998).

Few monitoring data were obtained for the SPA itself. The only matrix for which metals data were obtained is sediment (n=2-4) in which Cd, Hg and Pb levels are all elevated above background (medians of 6.6, 0.32 and 39.5 mg/kg respectively). An unknown proportion of this will, however, be associated with metalliferous minerals and is therefore expected to be relatively unavailable to biota. A single mammal sample showed a moderately high level of PCBs (54.7 mg/kg), but the few organics measured in sediments (n=2) were not markedly elevated, and no data were obtained for water, invertebrates or fish.

These data form an inadequate basis on which to make a preliminary risk assessment. However, there is evidence for strong oestrogenic activity in the Tees estuary, probably caused by a variety of domestic and industrial sources, and it is to expect that this is causing some adverse effects in the immediately adjacent SPA. The possible effects that may be looked for. include damage to the populations of fish and invertebrates on which birds feed, and conceivably biomagnification-related effects in the birds themselves (although steroids do not bioaccumulate significantly, and nonylphenol is only moderately bioaccumulative). This conservation area is therefore a high priority for further research.

Flamborough Head SAC

This internationally important area is characterised by extensive subtidal chalky reefs and sea caves supporting a range of rocky shore, kelp forest, faunal turf and faunal cushion/crust communities. Important species include fish, molluscs, crustaceans, annelids, sponges and coelenterates. Direct STW and industrial inputs to the area are small (total consented volume = 19,363 m^3/day).

No monitoring data was obtained, so potential endocrine disruption cannot be estimated. However, the lack of significant inputs suggests that contaminant effects are likely to be small, and relative to some other areas the priority here for research is considered to be low.

Humber Flats, Marshes and Coast SPA

This contains extensive areas of reedbed and salt marsh supporting a wide range of birds, including terns, harriers, short-eared owl, pochard, brent geese, shelduck, widgeon, teal, mallard, scaup, oyster catcher, plover, lapwing, knot, dunlin, ruff, godwit, curlew, redshank, turnstone and bearded tits. There are a number of large STW and industrial discharges within the SPA, as well as in adjacent parts of the Humber estuary (total consented volume of 581,208 m^3/day). Despite these inputs, feminisation of male flounder in the area is only moderate, probably because of the large dilution available in the estuary (Allen *et al.* 1999a).

No monitoring data was obtained from the CEFAS and EA databases, probably because the habitats included in the SPA are essentially terrestrial (though intermittently flooded). A preliminary risk assessment cannot therefore be made. However, the presence of several large discharges, coupled with the observation that male fish in the estuary are somewhat feminised, suggests that this SPA is a medium priority candidate for further research.

Wash and North Norfolk Coast SAC

This varied SAC includes barrier beaches, saltmarsh, intertidal flats and subtidal sandbanks. It supports one of Europe’s largest populations of common seal, as well as intertidal and subtidal polychaetes (including the ‘reef-building’ worm *Sabellaria spinulosa*), bivalves, crustaceans and echinoderms. Discharges in the area are generally small, although there are several large discharges in the King’s Lynn area (total consented volume = 72,638 m^3/day). The River Nene (which discharges to the Wash) shows at least partial contamination with oestrogens, with 100% incidence of intersex male roach found at one site around a STW (Jobling *et al.* 1998).

Contaminant levels are generally low, so it is unlikely that non-specific contaminant-related stress is causing exhaustion of the adrenocorticoid system. No metals data for fish, and only one datapoint for water was obtained. However, levels of Hg, Cd and Pb are not seriously elevated in invertebrates (medians of 0.02, 0.44 and 0.36 mg/kg, respectively), mammals (4.85, 0.03 and 0.05 mg/kg respectively), or sediments (0.05, 0.29 and 23 mg/kg respectively).

Concentrations of organics are also fairly low, with median ΣDDT levels of 0.006 mg/kg in invertebrates, and 3.6 mg/kg in mammals. PCBs in mammals are also low (7.8 mg/kg). Relatively low TBT concentrations in sediment (0.016 mg/kg) reflect the fact that shipping activity in the area is minor.

In summary, the priority for research on EDs in this area is generally low, although further research into the possible oestrogenic effects of the Nene discharge in the Wash is warranted.

Essex Estuaries SAC

This area includes the estuaries of the Blackwater, Colne, Crouch and Roach extending to the limit of the seaward mud- and sand-flats. The SAC contains the most southerly breeding ground in the North Sea of the common seal (in the Crouch), and the breeding ground of a herring subspecies (in the Blackwater). The Colne is the last known UK site of a coregonid fish known as the houting (*Coregonus oxyrinchus*). The area has rich intertidal and subtidal invertebrate communities, including sponges, annelids, molluscs, echinoderms, crustaceans and ascideans. The Maplin Sands are host to nationally-important eelgrass beds and high densities of the hydroid *Sertularia* spp. Direct discharges to the area are small to medium in size and mainly from STWs (total consented volume = 65,318 m^3/day), but there are large numbers of small boats and marinas which give rise to elevated levels of copper and zinc from antifoulings and sacrificial anodes (Matthiessen *et al.*, 1999). In earlier years, TBT inputs from these boats were high and caused serious effects on the local oyster industry and on benthic invertebrate communities, but since TBT-use was banned on small vessels in 1987, these communities have recovered (Rees *et al.*, 1999, 2000; Waldock *et al.*, 1999). Low or medium levels of oestrogenic substances probably enter these estuaries via sewage effluent, and some male flounder caught in the lower reaches of the Crouch were moderately feminised, showing slight induction of vitellogenin (Allen *et al.* 1999b).

In general, contaminant concentrations are low, but copper and zinc concentrations in sediments range up to maxima of 22,871 and 4,717 mg/kg, respectively, so non-specific stress leading to interference with the adrenocorticoid system is a possibility. Cd, Hg and Pb concentrations in invertebrates and sediment are not significantly elevated, but no metals data were obtained for water, fish or mammals.

Organochlorine concentrations in invertebrates are all low (e.g. median ΣDDT levels of 0.0029 mg/kg; median ΣPCB levels of 0.0025 mg/kg), but are no data were obtained for other matrices. Furthermore, few data were obained for other organics with the exception of THC in invertebrates which are slightly above background (median = 12.5 mg/kg).

In summary, contaminant data examined were sparse, but they do not indicate a serious problem. However, the fact that sewage discharges in the Crouch are causing moderate feminisation in male flounder (probably through discharge of steroids) suggests that these estuaries are not in optimal condition. No data are available on feminisation of fish in the Blackwater and Colne, but the presence there of rare fish populations suggests that some research on this subject may be justified. The priority for further research is therefore considered to be medium.

Thames Estuary and Marshes SPA

This area mainly covers the marshes and flats on the south side of the outer Thames estuary. It is a wetland of international importance with a complex of intertidal habitats, saltmarsh and lagoons. It is important both as a breeding and wintering area for birds, being used regularly by approximately 75,000 water fowl. These include avocet, plover, dunlin, knot, godwit, redshank, ruff, gadwall, shelduck, teal, pintail, shoveler, tufted duck, pochard, terns, swans, hen harriers, short-eared owl and kingfisher.

There are very large inputs of sewage and industrial effluent discharged to the Thames estuary as a whole, although little is discharged direct to the SPA (total consented volume discharged to the estuary = 4,578,702 m^3/day). It is known that male flounder in the upper estuary are moderately feminised (Allen *et al.*, 1999 a & b), but those caught in the outer estuary near the SPA appear normal. There are major shipping movements in the estuary, so it is to be expected that TBT inputs are still significant. Studies of TBT in the Thames indicate that this has probably causedg adverse effects in molluscs and other invertebrates.

Unfortunately, there are no contaminant data available from samples collected within the SPA, probably because it is largely above mean low water. No data from below the mean low water mark were collated for this review. Consequently, it is not possible to carry out more than a very preliminary assessment of risk. However, a more intensive review of existying data should enable this preliminary assessment to be refined.

In summary, because of the large effluent inputs to the estuary, it is to be expected that contaminants are causing some endocrine disruption in the SPA, although the lack of feminisation in outer estuary flounder is encouraging. The potential exists for birds to accumulate certain EDs through feeding on contaminated invertebrates, and although little is known about endocrine disruption in birds, it has been established that they can become feminised by, for example, high doses of certain organochlorines. The evidence available suggests that the SPA may be a high priority for future research.

Thanet Coast SAC

This area is notable for its internationally important chalk reefs, cliffs and sea caves. There is a rich intertidal algal flora, and a range of important animal groups, including rock-boring bivalves, crabs, polychaetes, bryozoans, hydroids, ascidians and coelenterates. Local discharges are moderate in size and mainly STW effluents (total consented volume discharged to the area = 88,033 m^3/day).

Unfortunately, no contaminant data were obtained for this SAC, so no preliminary risk assessment can be made.

In summary, the small size of the discharges in this area, coupled with the good dilution afforded by the open sea, suggests that endocrine disruption is likely to be an insignificant factor. The priority for research is therefore low at this stage.

Solent SAC

Most of this site is a complex of estuaries and harbours, with six coastal plain estuaries and four bar built estuaries. There is an unusual strong double tidal flow. The intertidal zone is dominated by sediment habitats including mudflats dominated by eelgrass beds and *Enteromorpha* algae. These are often fringed with saltmarsh, and unusual features include rare sponges in the Yar and polychaete 'reefs' in the entrance to Chichester Harbour. On firmer substrates, boring bivalves and faunal turf consisting of hydroids, bryozoans ans sponges can be found, while whelks and hermit crabs characterise the mobile sandy sediments.

Domestic and industrial discharges are fairly large (total consented volume discharged to the area = 984,735 m^3/day), including significant inputs from oil refining and other heavy industries. Furthermore, there is heavy shipping traffic which introduces TBT to the system, and some TBT still resides in sedimentary sinks (CEFAS, 2000). Formerly, TBT caused major adverse effects in areas of pleasure boating such as the Hamble estuary, but such problems are now mainly confined to the vicinity of shipping lanes and dockyards. Allen *et al*. (1999a) found moderate feminisation of male flounder in Southampton Water, probably related to sewage discharges.

No chemical monitoring data were obtained for this review, so a preliminary risk assessment cannot be made. However, the substantial discharges in the area, coupled with the relatively poor dilution available in Southampton Water and, to a lesser extent in the Solent, suggest that endocrine disruption may be occurring, and it has indeed been demonstrated in flounder. This area is therefore a high priority for research.

Isle of Wight SAC

This encompasses the southern shore of the Isle of Wight, which includes a number of extensive sublittoral and littoral chalk and limestone reefs. The rock is bored by bivalves and sponges, and the area supports diverse marine life including some rare seaweeds. Discharges in the area are fairly small and dominated by sewage effluents (total consented discharge = 57,272 m^3/day).

There are almost no contaminant monitoring data from this area. Two sediment samples contained low levels of Cd, Hg and Pb (median values of 0.02, 0.04 and 18.0 mg/kg respectively), but there is no other information in the CEFAS and EA databases.

Although it is not possible to conduct a preliminary risk assessment, it is clear that discharges in the area are small, so it is probable that significant endocrine disruption is not occurring. The priority for research is therefore low.

Poole Harbour SPA

This is a large natural harbour which includes extensive tidal mudflats and saltmarshes with associated reedbeds etc. It is of European importance from an ornithological point of view because it is host to large populations of waders (especially avocet and black-tailed godwit), Mediterranean gull, common tern, and shelduck. Other bird populations of interest or national importance include golden plover, dunlin, ruff, curlew, redshank, greenshank, pochard, black-headed gull, sandwich tern, Bewick’s swan, Brent geese, teal, goldeneye, merganser, various divers, Slavonian grebe, various harriers, short-eared owl, peregrine, kingfisher, and cormorant. Peak mean populations of waterfowl exceed 25,000.

Sewage treatment works discharges to Poole Harbour are moderately large (134,399 m^3/day consented volume) considering that it is a fairly enclosed area. Industrial inputs are small (6,167 m^3/day), although they include discharges from a small oilfield and a chemical manufacturing plant.

Inputs of TBT from pleasure craft used to be substantial (although current inputs from large vessels are small), and this is reflected in the fact that TBT concentrations in sediment are still rather high (median = 0.91 mg/kg; 75%-ile= 4.0 mg/kg – see Appendix 6). Metal and organochlorine levels are apparently fairly low, although data are only available for sediment and invertebrates, and sample numbers are generally small. However, it is known that Poole Harbour contains waters and sediments that are acutely toxic to some bioassay organisms

(CEFAS, 1998), so it is possible that the adrenocorticoid system of some organisms in the area has been affected by non-specific stress.

In summary, monitoring data are rather sparse, but inputs to Poole Harbour are not insignificant and contaminants are known to be causing some non-endocrine impacts on invertebrates. The concentrations of TBT suggest this may also be causing endocrine effects on molluscs. It is possible that toxic effects are occurring to invertebrates, in addition other specific endocrine disruption. It seems unlikely that there are high concentrations present of bioaccumulative endocrine disrupters sufficiently to affect birds directly as a result of biomagnification, but indirect effects on their food organisms appear possible. Priority for research in Poole Harbour is therefore high.

Chesil and the Fleet SAC

This area is notable for the extensive shingle ridge of Chesil Beach, and the enclosed saline lagoon known as the Fleet. This lagoon contains a wide range of habitats, including cobbles, pebbles, bedrock, sand and mud. Marine invertebrates (e.g. sea slugs, rare bivalves and snails, rare crustaceans and annelids, several sea anemones and a rare starfish), plants and algae are very diverse, including sponges, two species of eelgrass, and the tassel grass *Ruppia*. There are several important spawning fish species (gobies, mullet, pipefish, bass) and some rarities.

Discharges in the area are small and dominated by sewage effluent (total consented discharge = 25,802 m^3/day). Unfortunately, contaminant monitoring data available for this review were very sparse, being confined to 1-2 samples of invertebrates, in which contaminants appear to be present at very low concentrations.

Although no preliminary risk assessment is possible, the lack of large discharges in the area suggests that endocrine disrupting effects are likely to be small. The priority for research is therefore low.

Plymouth Sound and Estuaries SAC

This is a complex drowned ria, comprising large shallow inlets and bays, estuaries and shallowly-flooded sublittoral sandbanks and gravel. There is a corresponding high diversity of habitats with an extremely rich fauna and flora, making this site of international conservation importance. Important and rare invertebrate populations from many different phyla are found in these habitats, and the area is also an important nursery and spawning ground for several species of fish. There are substantial eelgrass beds as well as subtidal reefs supporting boring bivalves.

Due to the presence of the city of Plymouth, sewage inputs to the system are moderately large (total consented volume = 95,425 m^3/day), but there is hardly any direct industrial effluent from the shipyards and other industries. However, male flounder in the estuary do not appear to be feminised (Allen *et al.* 1999a), although few fish have been sampled to date. There are probably still some TBT inputs to the system from shipping (see below), but these used to be much larger before TBT use on small vessels was banned in 1987.

Contaminant levels are predominantly moderate (with the exception of TBT which is high), when compared to background levels found in unpolluted estuaries and offshore, so it is possible that non-specific stress is causing exhaustion of the cortisol response in some species. Cd, Hg and Pb levels are not generally elevated in fish, invertebrates, seawater or sediment (no data for mammals), although the Pb level in sediment is slightly above background (median = 69.0 mg/kg), possibly due to historical mining activities. This is supported by zinc and copper concentrations which are elevated in invertebrates and sediment. On the other hand, several organic contaminants appear to be present above background concentrations. TBT concentrations in seawater are high enough to cause adverse effects in molluscs (median = 28 ng/l), although TBT levels in sediment appear low, suggesting that current inputs are still important. PCBs are slightly elevated in fish (median = 0.71 mg ΣPCB /kg), and DDT levels in sediment are low (median = 0.009 mg ΣDDT/kg). Furthermore, concentrations of several other pesticides (including organophosphates, endosulfan, simazine and trifluralin) are elevated in seawater (up to 30 µg/l).

In summary, Plymouth Sound appears to be contaminated at significant concentrations by a range of organic contaminants with known or suspected endocrine disrupting properties. The data a still too sparse to draw firm conclusions, but the priority for further research is high.

Fal and Helford SAC

This SAC comprises two rias and the adjacent Falmouth Bay. There is a large diversity of habitats, including large shallow inlets and bays, intertidal mud- and sand-flats, and subtidal sandbanks. The largely marine fauna and flora are of international conservation significance. The rias and bays contain important sediment communities, including maerl with two species of unattached coralline algae, and eelgrass beds with diverse invertebrate fauna (including bivalves, crabs, amphipods, polychaetes, anemones, and echinoderms). There are also patches of gravel, of rock pools and of sublittoral rock, the latter containing rich sponge and sea-squirt communities. Many nationally rare algae, invertebrates and fish occur in the rias, including Couch's goby (*Gobius couchi*) which is only found at this site in the UK. The algal communities associated with rock pools in the Helford area, and with maerl beds, are particularly diverse, and include many rare seaweeds.

The total volume of sewage and industrial discharges is relatively small (74,532 m^3/day), with sewage predominating. However, there are substantial TBT inputs to the system from shipping, especially in the vicinity of Falmouth Harbour from which dredgings often contain high levels of this substance. This is supported by the data presented in this report, which show that the median level of TBT in sediment is 2.3 mg/kg, although there are no TBT data for other matrices. Although data are sparse, and contaminant levels generally appear fairly low, copper and zinc levels are somewhat elevated in sediments and invertebrates – probably due to shipping and historical mining activities. It is known that these elevated metal levels in sediments are causing effects on benthic communities in parts of the area (especially in Restronguet Creek e.g. Bryan and Gibbs, 1983; Millward and Grant, 2000), so non-specific impacts on the adrenocorticoid system of some organisms may be occurring. Cd levels in sediments and invertebrates are low, although Hg and Pb levels in sediment are slightly elevated (respective median concentrations of 0.62 and 63.5 mg/kg). There are no metals data for seawater, fish or mammals.

Organic contaminant data are only available for a few (n=3) invertebrates, and these suggest that organochlorine levels are generally low.

In summary, although data are sparse, there is evidence that some metals (especially copper and zinc) are present in parts of the area at levels which may be sufficient to cause impacts on the adrenocorticoid system in fish and which are certainly causing community effects in invertebrates. Furthermore, TBT is known to be a significant contaminant in the vicinity of Falmouth Harbour. Thus, although contaminant inputs generally are fairly low, endocrine disruption is probably occurring in localised areas. In view of the conservation importance of both fish and invertebrates in this SAC, and of the relative lack of contaminant data, it is therefore concluded that Fal and Helford is of medium priority for further research.

Scilly Isles SAC

This area is internationally noted for a large diversity of intertidal and subtidal sandbanks, and contains a range of undisturbed marine sediment fauna and flora (including the most extensive eelgrass beds in Britain) which are probably unique in north-west Europe. Noted fish include seahorses (*Hippocampus* spp.), and invertebrate communities include cuttlefish, rare gastropods and bivalves, crabs, polychaetes, anemones and burrowing echinoderms.

No data on discharges were obtained for this review, but these are certainly small, and almost exclusively confined to domestic sewage. Unfortunately, there are no data available on contaminant concentrations in the area, but the remoteness of this SAC from major contaminant inputs suggests that endocrine disrupters are unlikely to be a problem.

Despite the complete lack of information on inputs and contaminants, it is expected that endocrine disruption is not an important factor in the marine environment of the Scilly Isles, so this priority for investigations in this area is low.

Lundy SAC

This is a small granite island in the outer Bristol Channel, which is of international importance for its very diverse marine reef habitats and communities. Over 316 species of algae have been recorded around Lundy, and these are associated with very diverse invertebrate communities. These include many rare and unusual species, some of which (e.g. solitary corals, seafans and sponges) have some very long established colonies due to the stable conditions. There is a high proportion of Mediterranean-Atlantic species of international importance. Lundy is a breeding area for grey seals, and there are important fish species present, including various wrasse species in the kelp forests.

There are no no sewage or industrial inputs to this SAC, and due to its remote location, it is expected that diffuse inputs from further afield are minimal. Furthermore, no contaminant monitoring data were available for this review, but it is likely that contaminant concentrations are low.

In conclusion, despite the lack of contaminant monitoring data, the marine communities around Lundy are expected to be among the most pristine in the UK, and endocrine disrupting effects are unlikely to be occurring. The priority for research on this subject is therefore low, although Lundy could be considered as a reference area for rocky bottom communities with which to compare communities from more contaminated locations.

Severn Estuary SPA/pSAC

The Severn Estuary has an extremely wide tidal range and a substantial number of different habitats, including the estuaries of 8 rivers, and both subtidal and intertidal mud- and sand-flats which contribute significantly to habitat diversity. It is of international importance as a large coastal plain estuary with the second highest tidal range in the world, resulting in strong tidal streams, seabed scouring, and high turbidity. Littoral sediments have high densities of infaunal invertebrates dominated by polychaetes, amphipods and gastropods which provide food for important wintering wildfowl populations. Some sheltered areas support up to 3 species of eelgrass. In subtidal areas, there are extensive reef colonies of polychaete worms (*Sabellaria* spp.) which are the largest in Britain. The fish fauna is very diverse (>110 species), and the estuary is an important location for several rare fish including twaite shad (*Alosa fallax*), allis shad (*Alosa alosa*), river lamprey (*Lampetra fluviatilis*), and sea lamprey (*Petromyzon marinus*), all of which feed at sea but migrate into estuaries and/or rivers to breed.

Consented discharges to the Severn estuary are large (total consented volume = 1,094,245 m^3/day), although the proportion of industrial effluent is relatively small (65,472 m^3/day). There is also substantial shipping traffic, which is likely still to be introducing fresh TBT residues, although there are no data available on TBT concentrations in the Severn. Offsetting these large inputs is the fact that the estuary is large and well-mixed, thus providing substantial dilution capacity.

The only matrices from the Severn for which contaminant data were obtained for this review available are seawater and sediment, and the range of determinands is rather poor. However, organochlorine concentrations are low, as are most metal concentrations. Levels of cadmium and mercury in seawater are well below the relevant environmental quality standards. The only other available information (EDMAR programme, unpublished data) originates from two flounder individuals caught in the Severn (Swash Channel) in 1999, one of which was a male showing mild feminisation. A risk to fish (possibly including the rare species found in this system) from endocrine disrupters may therefore exist, although its degree of severity is impossible to judge on available evidence.

In summary, although the large waste inputs to the Severn are balanced by a massive dilution capacity, there are hints (albeit tentative) that some endocrine disruption may be occurring. Given the rarity of fish species for which this site is important, the large volume of sewage inputs and not insignificant industrial discharges, the priority for further research in this area is judged to be high.

Dee Estuary SPA

The Dee Estuary consists mainly of a large area of intertidal sand- and mud-flats, with extensive saltmarshes on the landward side. The estuary is nationally and internationally important for waders and wildfowl which are supported by rich invertebrate in-fauna. There are internationally important populations (3-6% of total) of oystercatcher, knot, bar-tailed godwit and redshank, and the maximum monthly count of wildfowl exceeds 14,000. Other important bird species include pintail and shelduck. There are also some small sandstone islands with a small winter population of purple sandpiper and large flocks of dunlin. A substantial herd of grey seal (15% of the Welsh population) is present on West Hoyle Bank.

There is considerable industrial development at the head of the estuary, and although the total volume of consented discharges is only of moderate size (112,272 m^3/day), the majority of this (62,335 m^3/day) is industrial effluent. There is also some shipping traffic, and TBT concentrations in seawater (median = 10 ng/l) exceed the concentration that causes endocrine disruption in some molluscs. There are only negligible TBT data available from other matrices.

Organochlorine concentrations in all matrices are generally low, with the highest concentrations of ΣPCBs (23.1 mg/kg) appearing in a single mammalian sample. Metals are generally present at low levels. Male flounder sampled from the estuary between 1997 and 1999 repeatedly fail to show any signs of feminisation, despite the close proximity of the highly oestrogenic Mersey estuary (EDMAR programme unpublished data; Allen *et al.* 1999a).

Overall, contaminant levels in the Dee Estuary are low, with the exception of slightly elevated levels of TBT in water. This suggests that endocrine disruption is probably not a major factor in this area (a prediction supported by the lack of feminisation in flounder), either for the birdlife, or for their invertebrate food. The priority for research is therefore low.

Mersey Estuary SPA

This is a wetland of international importance, which includes large areas of saltmarsh and intertidal sand- and mudflats, interspersed with smaller areas of brackish marsh and rocky shores. The peak mean bird population is over 78,000, including >47,000 waders and >30,000 wildfowl. There are internationally important numbers of migratory wildfowl, including shelduck, teal, pintail, dunlin and redshank. There are also internationally important numbers of ringed plover, and nationally important wigeon, grey plover, black-tailed godwit and curlew.

The Mersey estuary contains one of the largest concentrations of heavy industry in the UK, as well as a large human population in its catchment. Not surprisingly, the total consented volume of discharged effluent is therefore very large: 1,822,791 m^3/day, of which 649,117 m^3/day is directly discharged industrial waste. There are also high numbers of shipping movements in the Mersey, so TBT inputs are probably significant. However, few TBT data are available – there are only 5 sediment samples, containing a median TBT concentration of 0.12 mg/kg. This is relatively low, but the small sample size cannot be considered representative.

Metal concentrations in fish and sediment are generally low, with median Cd, Hg and Pb levels of 0.15, 0.40 and 2.0 mg/kg respectively in fish, and 0.34, 0.59 and 58.0 mg/kg in sediment. The somewhat elevated lead levels are probably due to inputs from organolead manufacturing plant in the area. No metals data were obtained for water, invertebrates or mammals. For organochlorines, significant data only exist for fish (however, the sample size were low), and median concentrations are not high, although ΣPCBs are somewhat elevated (median = 1.4 mg/kg). However, despite these sparse monitoring data, the information that <u>is</u> available shows that the Mersey is one of the most contaminated UK estuaries (Matthiessen *et al.* 1993; NRA, 1995; Fox *et al.* 1999), and it is to be expected that endocrine disruption is occurring. Indeed, Allen *et al* (1999 a & b) have shown that male flounder from the Mersey are among the most strongly feminised in the UK.

In summary, it seems likely that some Mersey wildlife in addition to fish is potentiallyexperiencing endocrine disruption. However, whether birds are at risk from effects on their prey, or through direct biomagnification of residues in the foodchain, is impossible to say based on the recent review. The Mersey is a high priority for further research into the effects of endocrine disrupters.

Ribble and Alt Estuaries SPA

The Ribble and Alt estuaries in summer support nationally important breeding populations of common tern, ruff, black-headed gull and lesser black-backed gull. In addition, they are host to nationally or internationally important wintering populations of Bewick's swan, whooper swan and golden plover. Other important species include pink-footed geese, shelduck, wigeon, teal, pintail, oystercatcher, grey plover, lapwing, knot, sanderling, dunlin, godwit, curlew and redshank. In total, the average peak populations comprise >161,000 waders and >57,000 wildfowl.

These estuaries receive substantial amounts of sewage effluent (consented volume = 200,644 m^3/day), but relatively little industrial waste (12,000 m^3/day), and few vessels of a size that would be antifouled with TBT. Data obtained on contaminants were extremely sparse, with no representative sample sizes of any matrix. Indeed, the only available samples concern a few invertebrate tissues (n = 1-2) which do not appear to be significantly contaminated with metals or organochlorines. Despite this, it is to be expected that the relatively large volume of sewage effluent will be introducing a number of endocrine disrupting contaminants, including steroids. Unfortunately, there are no data on feminisation of Ribble flounder.

In summary, although there are no data on the subject, it is probable that some wildlife in the Ribble and Alt Estuaries is exposed to endocrine disrupters due to the large inputs of sewage effluent. However, the small inputs of industrial waste suggest that persistent and bioaccumulative substances of the type which might biomagnify and affect birds are unlikely to be a serious problem. In view of the uncertainties, the prioity for research is therefore considered to be medium.

Morecambe Bay SAC

This major embayment comprises a large area of internationally important intertidal mud- and sand-flats. Four minor estuaries discharge into the bay. Very large areas of flats are exposed at low tide, and these support a number of in-faunal communities living in sediments ranging from sands to fine muds. There are particularly high numbers of polychaetes, bivalve molluscs and amphipods, and these in turn provide food for a large number of waders and wildfowl. In the Walney Channel, there is an in-faunal community characterised by the sipunculid worm *Goldfingia vulgaris*. In certain places, there are very extensive mussel beds, and small areas of reef with fucoid algae. There is also a rich community of sponges on pebbles and cobbles. The Bay provides an important nursery and spawning ground for several fish species including flounder and plaice, and there are migratory populations of trout and salmon. Finally, small numbers of grey seal can be found in the area.

Morecambe Bay receives very large volumes of effluent (total consented volume = 507,373 m^3/day), of which the majority (336,622 m^3/day) is comprised of waste from a wide range of industries. Although this is discharged into a large area, the average depth of water is small, so dilution may not be sufficient to allow contaminant (including EDs) concentrations to fall below effect threshold levels. However, shipping traffic is generally light and this is reflected in low TBT concentrations in sediments, although the median concentration in water (10 ng/l, n=4) is above the no-effect level for gastropod molluscs. A survey of effects of TBT in Western coastal waters found that ~8% of the female population of dogwhelks at Heysham, which is a busy port with large commercial boats and ferries to the Isle of Man, were sterile due to imposex (Harding *et al.*, 1998). Nonylphenol and octylphenol concentrations in water and sediments are low (n=2-3), as are heavy metal concentrations in water, sediments, invertebrates and fish (e.g. median Cd, Hg and Pb levels in invertebrates = 0.27, 0.05 and 1.0 mg/kg respectively). Finally, organochlorine concentrations in water and sediment are low, and bioaccumulated amounts of these materials in fish and invertebrates are not of concern (e.g. median Σ DDT and Σ PCB in fish = 0.096 mg/kg and 0.277 mg/kg, respectively).

In summary, despite the large volumes of discharges to Morecambe Bay, and relatively small dilution capacity, levels of contaminants in the various matrices (no data for mammals) are generally low. Widescale endocrine disruption is therefore unlikely to be occurring, although there may be localised effects near discharges. The fact that Morecambe Bay is an important breeding ground for a variety of fish species, combined with a lack of data on endocrine disruption in fish from this bay and evidence for ED effects on gastropod molluscs, justifies a recommendation that this area should be given high priority for further investigation.

Duddon Estuary SPA

This relatively small, sandy estuary is of European importance for birds because it hosts large populations of sandwich terns, pintail, knot and redshank. Peak mean populations of waterfowl exceed 31,000.

Considering the small size of the receiving water, the total volume of discharges is moderately large (consented volume = 227,298 m^3/day), although only a small proportion (39,897 m^3/day) consists of directly discharged industrial effluent. Shipping movements are negligible, but there are no readily usable data on TBT contamination. Contaminant data were only obtained for 1-2 samples of invertebrates, and although these do not indicate a problem, they cannot necessarily be considered to be representative.

In summary, there are insufficient data for even a preliminary risk assessment, but the large volumes of effluent suggest that endocrine disruption may be an issue. The priority for research is therefore considered to be medium given this uncertainty.

Solway Firth SAC

This is a large, sandy estuary with both inter-tidal and sub-tidal mud- and sand-banks. The area of estuarine inter-tidal flats is the third largest in Britain. Tidal currents and wave energy are strong, and due to the shallowness of the water, there is considerable seasonal fluctuation in water temperature. There are varied in-faunal communities of invertebrates, including bivalve molluscs, polychaetes, crustaceans and echinoderms. Exposed boulder clay supports a rich epifauna including sponges, seamats, sea-squirts, hydroids, horse mussels, crabs, lobsters, and the reef-building worm *Sabellaria alveolata*. The Solway Firth is a fish spawning and nursery ground, and an important site for a number of rare migratory fish species including sea trout, salmon, allis shad (*Alosa alosa*), twaite shad (*Alosa fallax*), river lamprey (*Lampetra fluviatilis*) and sea lamprey (*Petromyzon marinus*).

The Solway Firth is close to pristine in terms of the very small volumes of effluent which are discharged into it (total consented volume = 6,729 m^3/day, all from STWs). Few people live in its catchment, and there is very little industry. Unfortunately, few samples were available for contaminant analysis, although these do not indicate a contaminant problem. This is supported by the observations of Lye *et al.* (1997) who did not detect significant feminisation in flounder from the Solway.

In summary, the Solway Firth is very unlikely to be significantly contaminated with endocrine disrupters, and despite the lack of comprehensive contaminant data in this review, the priority for research is considered to be low. The Solway would probably constitute a good reference area with which to compare more contaminated sandy or muddy estuaries.

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8. Conclusions and recommendations

As mentioned above, the available to this review data on contaminants and their effects for the period 1995-1999 are simply too sparse to make firm conclusions about the environmental risks posed by endocrine disrupters in the English SPAs and SACs. However, endocrine disruption due to oestrogen exposure is undoubtedly feminising male fish in or near to several of these locations (notably in the Tees, Humber, Essex estuaries, Thames, Solent, Plymouth Sound, Severn and Mersey), and the effects of current and past uses of tributyltin (TBT)-based antifouling paints are probably still causing masculinisation of molluscs in areas of heavy shipping activity (most of the above locations).

It is impossible to gauge the severity or ecological significance of possible endocrine disrupting effects in these areas (except in the case of TBT in the Crouch estuary where both population and community effects have been described), although it should be borne in mind that the present degree of feminisation in flounder does not appear to have been translated into major effects at the population level (with the possible exception of the Tees). Furthermore, the most devastating effects of TBT are probably now in the past, and mollusc populations and benthic communities that were formerly badly affected are now often recovering. Nevertheless, these are not sufficient grounds for complacency, because the linkages between individual-level and population- or community-level changes are poorly understood. In addition, there are large numbers of endocrine disrupters whose presence or absence in SACs and SPAs is unknown, and we understand almost nothing about their possible interactive effects. There are also many organisms (especially invertebrates) whose responses to endocrine disrupters have never been investigated. Finally, one should not forget that there are many other contaminants which are able to cause polluting effects without interfering with the endocrine system, so the absence of endocrine disrupters from an area cannot be considered a clean bill of health. In fact, parts of many English estuarine areas (and certainly all the industrialised ones) contain non-endocrine contaminant concentrations in water and/or sediment which are able to cause adverse biological effects. In addition to assessing the risks of ED in English SACs and SPAs, this study has provided a comprehensive up-to-date chemical information database for these areas, which will significantly contribute to ongoing studies under the Environment Agency's review of consents programme under the Habitats Regulations English Nature to identify those sites at which non-endocrine toxicological effects may be occurring, and which may therefore require more detailed investigation.

The lack of firm data on endocrine disrupters and their effects in SACs and SPAs suggests the inevitable need for more research to support the development of reliable environmental risk assessments. An evaluation of the priority for such research has been made in each of the above preliminary assessments, on a scale of Low, Medium or High. Low priority for research has been assigned to those areas where discharges are small and/or where contaminant levels are also known to be low. High priority is attached to areas where discharges are large, contaminant levels are elevated, and/or endocrine disruption is already known to be occurring, this judgement also takes into account the presence of particularly sensitive species. Medium priority has generally been used to designate areas where discharges are large, but dilution capacity may also be large, and evidence of elevated contaminant levels and endocrine disrupting effects is either sparse or absent. The assigned research priorities are summarised below in Table 3.

Table 3. Priorities for research on the presence and effects of endocrine disrupters in English SACs and SPAs.

Low priority	Medium priority	High priority
Berwickshire and Northumberland Coast	Humber Flats, Marshes and Coast	Teesmouth and Cleveland Coast
Flamborough Head	Essex Estuaries	Thames Estuary and Marshes
Wash and North Norfolk Coast	Fal and Helford	Solent
Thanet Coast	Ribble and Alt Estuaries	Poole Harbour
Isle of Wight	Duddon Estuary	Plymouth Sound and Estuaries
Chesil and the Fleet		Severn Estuary pSAC
Scilly Isles		Mersey Estuary
Lundy		Morecambe Bay
Dee Estuary		
Solway Firth		

The approach to research will vary from site to site. Furthermore, our understanding of endocrine disruption is not yet sufficiently strong to allow a comprehensive programme of investigatory work to be designed. However, it is clear that many unrelated substances (both natural and anthropogenic) are able to interfere with the normal workings of the endocrine system, and many have probably not yet been identified. For that reason, it is recommended that research work should be primarily biology-led. In other words, the most sensible general approach is to use the integrating properties of organisms to detect the holistic effects (if any) of endocrine disrupters. Examples of this approach which are feasible now are the use of fish to detect the presence of oestrogens and androgens (and their respective antagonists). There are also a number of *in vitro* screens based on cell or tissue culture which are able rapidly to detect the presence of many endocrine disrupters in extracts of water, sediment or tissue. Once effects have been established, it is then possible to identify their causes through the use of Toxicity Identification and Evaluation (TIE) techniques. The most difficult part of such an investigation is to assess the ecological significance of any effects found. It is generally not considered sufficient to establish that endocrine disruption is occurring; it should also be shown that there is a significant risk of impacts occurring at the population level. However, a more precautionary approach will be needed for SACs and SPAs that are noted for the presence of populations of very rare and/or vulnerable species.

At present, available knowledge limits such investigations to oestrogenic and androgenic effects in fish (caused by oestrogens, androgens and their mimics), adrenocorticoid effects in fish (caused by multiple stressors), and androgenic effects in molluscs (caused by TBT). There are also possibilities for studying oestrogenic effects in birds, although these have several scientific and practical difficulties. Depending on the situation, such work can either be done with wild organisms or using caged test animals. *In vitro* bioassays are also available which permit the use of TIE to identify oestrogens and androgens (and their antagonists).

Currently, under the EDMAR programme[*], there are various site-related investigations underway, noteably on the Tyne, Tees, Mersey, Thames and Southampton Water. These include surveys of sand goby and viviparous blenny (east coast only) to measure feminisation

[*] Subsequent to the production of the main body of this report, Matthiessen *et al* (2002) published a summary of progress in the EDMAR programme, which includes further reference to papers resulting from the research undertaken.

and reproductive effects respectively, caged stickleback studies to investigate andgrogenic activity, and TIE work to identify and track sources of oestrogenic and androgenic activity. It is noteworthy, however, that EDMAR has not yet been able to adequately addres monitoring approaches for marine invertebrates, while organisms from this group constitute some of the most important in SACs. Future marine ED research will therefore need to pay much more attention to invertebrates. It will also be desirable for future EDMAR-type programmes to focus on some of those areas identified in this study as high priority for further investigation, but which have not been significantly studied to date (e.g. Severn estuary and Morecambe Bay).

Due to the considerable ignorance concerning the prediction of ED effects from a knowledge of chemical concentrations, a more promising approach to the investigation of such effects in SACs and SPAs will be to deploy a range of ED-sensitive bioassays which are able to integrate the effects of many substances. These can be used both to monitor the ED potential of discharges (i.e. Direct Toxicity Assessment), and to study effects in wild sentinel species.

In conclusion, the environmental risks posed by endocrine disrupters in English SACs and SPAs are still unknown. Certain of these areas are considered a high priority for future research, using the responses of wild or test organisms to identify possible effects and their causes. This approach is the most practicable and precautionary until a comprehensive investigatory programme can be designed which can encompass all possible types of endocrine disruption.

9. Appendices

Appendix 1 Summary of metals - all SACs

Units: Tissues mg/kg (wet) ; Sediments mg/kg (dry) ; Seawater µg/L.

SITE	MATRIX		AG	AL	AS	CD	CO	CR	CU	FE	HG	NI	PB	SE	ZN
Berwickshire and North Northumberland Coast	invertebrates	n	5		5	7		5	5	5	5	5	5	5	4
		25	0.04		0.32	0.17		0.41	0.22	0.06	0.04	0.31	0.42	0.45	6.75
		Median	0.26		2.40	2.40		0.53	0.76	40.0	0.05	0.41	0.64	0.48	10.50
		75	9.0		2.50	12.0		162.0	1.20	82.0	0.17	3.60	18.60	26.0	44.0
Chesil and The Fleet	invertebrates	n	1		1	1		1	1	1	1	1	1	1	1
		25	0.41		1.5	0.11		1.5	20.0	70.0	0.02	0.89	0.14	0.50	254.0
		Median	0.41		1.5	0.11		1.5	20.0	70.0	0.02	0.89	0.14	0.50	254.0
		75	0.41		1.5	0.11		1.5	20.0	70.0	0.02	0.89	0.14	0.50	254.0
Essex Estuaries	invertebrates	n	25		25	29		25	25	25	25	25	25	25	25
		25	0.24		1.30	0.07		0.57	1.10	67.0	0.02	0.52	0.15	0.52	10.0
		Median	0.28		1.60	0.12		0.90	1.30	223.0	0.02	5.80	0.41	0.59	13.0
		75	0.41		1.90	0.48		1.10	8.20	380.0	0.03	10.0	0.56	0.73	116.0
	seawater	n					2	2	2			2			2
		25					0.10	0.00	4.01			1.89			7.54
		Median					0.11	0.00	4.30			2.38			7.84
		75					0.11	0.00	4.58			2.86			8.13
	sediment	n	8		6	7		7	7		7	7	7		7
		25	0.00		6.45	0.10		17.40	5.35		0.04	8.70	8.20		31.0
		Median	0.00		8.85	0.11		30.0	10.0		0.05	16.0	15.0		47.0
		75	0.00		9.53	1.97		31.50	22871.0		0.07	34.50	18.0		4717.0
Fal and Helford	invertebrate	n	3		15	15		15	15	15	15	15	15	3	15
		25	0.08		1.55	0.26		0.30	104.50	41.50	0.01	0.11	0.16	0.54	576.50
		Median	0.16		1.70	0.29		0.32	115.0	66.0	0.01	0.13	0.24	0.58	651.0
		75	0.17		1.90	0.32		0.35	142.50	75.0	0.01	0.17	0.28	0.63	681.0
	sediment	n			18	18		18	18		18	18	18		18
		25			35.0	0.27		17.25	160.25		0.28	11.25	52.50		271.25
		Median			39.0	0.37		20.50	168.50		0.62	13.0	63.50		296.0
		75			75.25	0.57		22.00	307.25		0.98	17.0	67.0		353.0

SITE	MATRIX		AG	AL	AS	CD	CO	CR	CU	FE	HG	NI	PB	SE	ZN
Isle of Wight	sediment	n	6		2	2		2	2	2	2	2	2		2
		25	0.00		15.38	0.02		43.50	19.25	2.60	0.04	25.0	17.50		16.0
		Median	0.00		21.25	0.02		44.0	19.50	3.40	0.04	26.0	18.0		18.0
		75	0.00		27.13	0.02		44.50	19.75	4.20	0.04	27.0	18.50		20.0
Morecambe Bay	fish	n	676		30	48			115	676	144		48	676	115
		25	0.00		4.93	0.05			0.30	0.00	0.09		0.14	0.00	5.10
		Median	0.00		6.50	0.11			3.10	0.00	0.14		0.21	0.00	19.0
		75	0.00		9.75	0.20			9.10	0.00	0.20		0.27	0.00	25.50
	invertebrates	n	4		5	5		5	5	4	5	5	5	5	5
		25	0.01		1.90	0.20		0.89	1.10	93.25	0.04	0.59	0.64	0.71	20.0
		Median	0.11		2.20	0.27		1.10	1.30	123.0	0.05	0.67	1.00	0.73	23.0
		75	0.31		2.30	0.31		1.60	1.40	152.0	0.06	0.97	1.20	0.75	24.0
	seawater	n				5	1	9	5		5	5	5		5
		25				0.041	0.04	0.00	1.17		0.02	0.43	0.14		3.70
		Median				0.045	0.04	0.00	1.25		0.02	0.59	0.20		5.75
		75				0.069	0.04	0.20	1.38		0.02	0.76	0.30		6.67
	sediment	n	18	8	41	43		43	43		42	42	42		42
		25	0.00	2112.0	4.00	0.12		9.30	2.0		0.01	5.40	7.28		23.25
		Median	0.00	2444.5	5.80	0.20		18.0	6.36		0.06	9.90	15.10		40.50
		75	0.00	6869.75	7.80	0.26		33.0	17.0		0.31	16.75	23.50		82.0
Plymouth Sound and Estuaries	fish	n	50		3	3			6	50	6		3	50	6
		25	0.00		2.80	0.25			0.31	0.00	0.04		0.20	0.00	6.30
		Median	0.00		2.90	0.30			10.31	0.00	0.05		0.20	0.00	20.8
		75	0.00		3.15	0.30			20.0	0.00	0.05		0.20	0.00	35.75
	invertebrates	n	6		6	7		6	6	6	6	6	6	6	6
		25	0.19		1.20	0.18		0.43	30.25	48.25	0.01	0.40	0.26	0.54	180.0
		Median	0.23		1.35	0.37		0.72	79.50	61.0	0.01	0.47	0.36	0.65	352.0
		75	0.30		1.65	0.45		0.79	167.0	69.25	0.03	0.55	0.42	0.81	482.75
	seawater	n				8		16	8		8	8	8		8
		25				0.00005		0.00	2.48		0.00001	0.78	0.2		10.05
		Median				0.00005		0.25	2.6		0.00001	1.15	0.25		18.6
		75				0.00006		0.5	3.38		0.00001	1.9	0.3		28.18
	sediment	n	7	2	61	77		77	77		77	77	77		77
		25	0.00	22815	22.0	0.17		21.0	66.0		0.23	16.0	57.0		100.0
		Median	0.00	22885	30.0	0.25		25.0	82.		0.30	17.0	69.0		128.0
		75	0.00	22955	42.0	0.39		29.0	97.0		0.47	19.0	122.0		161.0

SITE	MATRIX		AG	AL	AS	CD	CO	CR	CU	FE	HG	NI	PB	SE	ZN
Severn	seawater	n				12		12	12		12	12	12		11
		25				0.26		0.35	3.40		0.029	1.39	0.10		7.45
		Median				0.4		0.37	3.76		0.065	1.42	0.19		11.80
		75				0.64		0.67	4.00		0.13	1.48	0.27		14.95
	sediment	n		2	3	2		2	2		3	2	2		2
		25		8485.5	8.0	0.26		18.78	7.48		0.15	12.77	36.41		10485
		Median		13517	13.90	0.33		27.32	12.07		0.29	17.84	44.94		13007
		75		185485	17.00	0.41		35.86	16.65		0.36	22.92	53.48		15528
Solway Firth	invertebrates	n	1		2	2		2	2	1	2	2	2	2	2
		25	0.07		1.20	0.17		0.45	1.40	0.00	0.02	1.37	0.37	0.70	13.50
		Median	0.07		1.30	0.20		0.59	1.70	0.00	0.02	2.41	0.40	0.80	14.0
		75	0.07		1.40	0.22		0.73	2.00	0.00	0.02	3.46	0.42	0.90	14.50
	seawater	n				3		3	3		3	3	3		3
		25				0.05		1.10	1.28		0.02	0.64	0.26		8.43
		Median				0.05		2.0	1.44		0.02	0.68	0.30		10.6
		75				0.052		2.0	1.83		0.02	0.77	0.69		11.95
	sediment	n		2	3	3		3	3		3	3	3		3
		25		2564.75	5.87	0.04		8.20	2.0		0.02	6.10	5.65		15.15
		Median		2819.50	7.74	0.08		8.57	2.0		0.03	7.20	6.07		17.30
		75		3074.25	10.07	0.29		12.29	3.05		0.04	7.55	7.94		26.65
Wash And North Norfolk	invertebrates	n	6		6	11		6	6	6	6	6	6	6	6
		25	0.04		1.73	0.11		0.35	0.80	59.50	0.01	0.35	0.25	0.45	10.0
		Median	0.17		1.85	0.44		0.45	1.09	1310	0.02	0.49	0.36	0.56	13.0
		75	0.23		1.98	11.50		0.53	32.58	19575	0.02	0.69	0.53	0.71	20725
	mammals	n				4					4		4		
		25				0.02					2.45		0.01		
		Median				0.03					4.85		0.05		
		75				0.09					9.78		0.08		
	seawater	n						1			1				
		25						0.00			2.5				
		Median						0.00			2.5				
		75						0.00			2.5				
	sediment	n	3		8	5		5	5		5	5	5		5
		25	0.00		9.20	0.23		33.0	11.0		0.05	17.0	22.0		59.0
		Median	0.00		9.45	0.29		34.0	11.0		0.05	18.0	23.0		59.0
		75	0.00		12.50	0.34		34.0	12.0		0.06	18.0	24.0		60.0

Appendix 2 Summary of PCBs and pesticides - all SACs

Units: Tissues mg/kg (wet) ; Sediments mg/kg (dry) ; Seawater ng/L.

SITE	MATRIX		TOTAL PCBs	Total OP	Aldrin	Atrazine	DDD	DDE	DDT	Dieldrin	Endrin	Endo-sulfan	HCB	AHCH	BHCH	GHCH	Isodrin	Simazine	Triflur Alin
Berwickshire and North Northumberland Coast	invertebrates	n	5				5	5	5	5			5	4	4	5			
		25	0.003				0.00056	0.001	0.0008	0.0005			0.0003	0.00035	0.00053	0.00035			
		M	0.003				0.00056	0.001	0.001	0.00053			0.0005	0.000425	0.00054	0.0005			
		75	0.005				0.001	0.001	0.0016	0.00053			0.0005	0.0005	0.004155	0.0005			
	mammals	n	3				3	3	3	3			3	3	1	3			
		25	4.7355				0.0515	1.04	0.555	0.205			0.0185	0.0135	0.0007	0.0005			
		M	4.82				0.064	1.2	0.63	0.28			0.027	0.016	0.0007	0.0005			
		75	13.867				1.082	10.1	1.715	0.74			0.1935	0.0165	0.0007	0.00625			
Chesil and The Fleet	invertebrates	n	2				2	2	2	2			2	2	1	2			
		25	0.00025				0.0005	0.0005	0.00063	0.0005			0.0005	0.0005	0.0005	0.0005			
		M	0.0005				0.0005	0.0005	0.00075	0.0005			0.0005	0.0005	0.0005	0.0005			
		75	0.00075				0.0005	0.0005	0.00088	0.0005			0.0005	0.0005	0.0005	0.0005			
Essex Estuaries	invertebrates	n	26				26	26	26	26			26	26	26	26			
		25	0.001				0.00056	0.00045	0.00063	0.00053			0.0003	0.00035	0.0005	0.00035			
		M	0.0025				0.00056	0.00075	0.0016	0.00053			0.0003	0.00035	0.00054	0.00075			
		75	0.00775				0.001	0.002	0.0016	0.002			0.0005	0.0005	0.00054	0.001			
	sediment	n	1																
		25	0.01076																
		M	0.01076																
		75	0.01076																

SITE	MATRIX		TOTAL PCBs	Total OP	Aldrin	Atrazine	DDD	DDE	DDT	Dieldrin	Endrin	Endo-sulfan	HCB	AHCH	BHCH	GHCH	Isodrin	Simazine	Triflur Alin
Fal and Hereford	invertebrates	n	3				3	3	3	3			3	3	3	3			
		25	0.0195				0.00056	0.001	0.0016	0.00053			0.0003	0.00035	0.00054	0.00035			
		M	0.025				0.00056	0.001	0.0016	0.00053			0.0003	0.00035	0.00054	0.00035			
		75	0.0295				0.00128	0.0015	0.0016	0.00227			0.0003	0.00035	0.00054	0.00035			
Morecambe Bay	fish	n	75				75	75	75	75			75	75	45	75			
		25	0.1525				0.0135	0.019	0.0055	0.0045			0.0008	0.0005	0.0005	0.0005			
		M	0.277				0.035	0.036	0.025	0.009			0.003	0.001	0.0007	0.002			
		75	0.523				0.0595	0.059	0.0345	0.0245			0.004	0.007	0.0007	0.006			
	invertebrates	n	8				8	8	8	8			8	8	7	8			
		25	0.00375				0.0017	0.000875	0.0015	0.00053			0.0003	0.00035	0.00052	0.00088			
		M	0.009				0.0035	0.0015	0.0016	0.00059			0.0003	0.000425	0.00054	0.001			
		75	0.01175				0.005	0.002	0.0017	0.00099			0.0005	0.0005	0.00062	0.001			
	seawater	n			4		3	3	7			6	4	4	4	4	4		4
		25			5.00		5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00		10.00
		M			5.00		5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00		10.00
		75			5.00		5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00		10.00
	sediment	n	6		7		11	12	12	11	7		11	4		4			
		25	0.00105		0.00		0.00168	0.00175	0.00175	0.00	0.00		0.0012	0.02		0.0975			
		M	0.00223		0.00		0.0068	0.0046	0.0053	0.001	0.00		0.002	0.05		0.175			
		75	0.00448		0.0005		0.17	0.02	0.02	0.165	0.0005		0.065	0.125		0.255			

SITE	MATRIX		TOTAL PCBs	Total OP	Aldrin	Atrazine	DDD	DDE	DDT	Dieldrin	Endrin	Endo-sulfan	HCB	AHCH	BHCH	GHCH	Isodrin	Simazine	Triflur Alin
Plymouth Sound and Estuaries	fish	n	3				3	3	3	3			3	3	3	3			
		25	0.5965				0.0325	0.0395	0.023	0.0175			0.006	0.0005	0.0005	0.0045			
		M	0.708				0.033	0.048	0.03	0.019			0.007	0.0005	0.0005	0.005			
		75	0.727				0.0385	0.0495	0.037	0.021			0.007	0.0005	0.0005	0.005			
	invertebrates	n	11				11	11	11	11			11	11	9	11			
		25	0.02				0.0009	0.0015	0.001	0.00083			0.0004	0.000425	0.0005	0.0005			
		M	0.027				0.002	0.002	0.001	0.001			0.0005	0.0005	0.0005	0.0005			
		75	0.0355				0.0025	0.002	0.0015	0.002			0.0005	0.0005	0.0007	0.00075			
	seawater	n		64	8	8			8	8	8	16	8	8	8	8	8	8	8
		25		10000	0.5	30.0			2.9	0.6	1.00	600	0.7	0.3	2.1	0.78	1.00	29475	11400
		M		15000	0.5	30.0			2.9	0.6	1.00	1250	0.7	0.3	2.1	1.4	1.00	30000	11400
		75		20000	0.5	30.0			2.9	0.6	1.00	2000	0.7	0.3	2.1	1.73	1.00	30000	11400
	sediment	n	27		2		2	2	2	2	2		2						
		25	0.01648		0.0015		0.0017	0.0017	0.0029	0.0015	0.0012		0.0015						
		M	0.0197		0.0017		0.0022	0.0023	0.0047	0.0017	0.0015		0.0017						
		75	0.02794		0.002		0.0026	0.003	0.0066	0.002	0.0017		0.002						
Severn	seawater	n		85	12	12		1	13	12	12	7	12	12	12	12	12	11	11
		25		5.00	5.00	10.00		0.005	5.00	5.00	5.00	10.00	5.00	5.00	5.00	5.00	5.00	10.00	5.00
		M		5.00	5.00	10.00		0.005	5.00	5.00	10.00	10.00	5.00	5.00	5.00	5.00	5.00	11.00	5.00
		75		8.00	5.25	11.5		0.005	5.00	5.25	10.00	2508	5.25	5.25	5.25	5.25	6.5	15.00	5.5
	sediment	n			2				2	2	2		2						
		25			0.0017				0.0017	0.0017	0.0034		0.0017						
		M			0.0018				0.0018	0.0018	0.0036		0.0018						
		75			0.0019				0.0019	0.0019	0.0038		0.0019						

SITE	MATRIX		TOTAL PCBs	Total OP	Aldrin	Atrazine	DDD	DDE	DDT	Dieldrin	Endrin	Endo-sulfan	HCB	AHCH	BHCH	GHCH	Isodrin	Simazine	Triflur Alin
Solway Firth	invertebrates	n	2				2	2	2	2			2	2		2			
		25	0.00				0.00063	0.0005	0.0005	0.0005			0.0005	0.0005		0.0005			
		M	0.00				0.00075	0.0005	0.0005	0.0005			0.0005	0.0005		0.0005			
		75	0.00				0.00088	0.0005	0.0005	0.0005			0.0005	0.0005		0.0005			
	seawater	n			3		3	3	6	3	3	6	3	3	3	3	3		3
		25			5.00		5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00		10.00
		M			5.00		5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00		10.00
		75			5.00		5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00		10.00
	sediment	n	1		2		2	2	2	2	2		2						
		25	0.0108		0.00		0.001	0.001	0.001	0.00	0.00		0.0011						
		M	0.0108		0.00		0.001	0.001	0.001	0.00	0.00		0.0013						
		75	0.0108		0.00		0.001	0.001	0.001	0.00	0.00		0.0014						
Wash and North Norfolk	invertebrates	n	7				7	7	7	7			7	7	7	7			
		25	0.003				0.0009	0.002	0.00125	0.00083			0.0004	0.000425	0.0005	0.001			
		M	0.004				0.002	0.002	0.0016	0.002			0.0005	0.0005	0.0005	0.001			
		75	0.005				0.002	0.002	0.002	0.002			0.0005	0.0005	0.00052	0.001			
	mammal	n	4				4	4	4	4			4	4	3	4			
		25	6.463				0.33075	1.325	0.251	0.8265			0.1813	0.026375	0.0006	0.16513			
		M	7.851				0.97	2.2	0.445	2			0.435	0.0455	0.0007	0.325			
		75	9.4475				1.775	3.95	0.72	3.6			0.6325	0.05675	0.0007	0.435			

Appendix 3 Summary of miscellaneous - all SACs

Units: Tissues mg/kg (wet) ; Sediments mg/kg (dry) ; Seawater ng/L.

Site	Matrix		Nonylphenol	Octylphenol	Total Hydrocarbons	Tributyltin
Berwickshire and North Northumberland Coast	Invertebrates	n			3	
		25			1.55	
		M			1.70	
		75			6.35	
	Mammals	n				1
		25				0.0015
		M				0.0015
		75				0.0015
Chesil and The Fleet	Invertebrates	n			1	
		25			5.20	
		M			5.20	
		75			5.20	
Essex Estuaries	Invertebrates	n			6	
		25			12.0	
		M			12.5	
		75			13.75	
	Mammals	n				1
		25				0.002
		M				0.002
		75				0.002
	Sediment	n			1	16
		25			11.45	0.003375
		M			11.45	0.0045
		75			11.45	0.02175

Site	Matrix		Nonylphenol	Octylphenol	Total Hydrocarbons	Tributyltin
Fal and Helford	Invertebrates	n			1	
		25			29.00	
		M			29.00	
		75			29.00	
	Sediment	n				20
		25				0.81
		M				2.354
		75				3.21725
Morecambe Bay	Invertebrates	n				1
		25				0.012
		M				0.012
		75				0.012
	Seawater	n	3	3		4
		25	0.075	0.05		8.75
		M	0.1	0.05		10.00
		75	0.1	0.05		10.00
	Sediment	n	2	2		27
		25	0.02375	0.01625		0.0035
		M	0.0325	0.0225		0.0045
		75	0.04125	0.02875		0.0385
Plymouth Sound and Estuaries	Fish	n			2	
		25			41.00	
		M			45.00	
		75			49.00	
	Seawater	n	8	8		8
		25	0.05475	0.03375		28.00
		M	0.059	0.04		28.00
		75	0.1125	0.04675		28.00
	Sediment	n	4	4		49
		25	0.0325	0.0225		0.0056
		M	0.035	0.025		0.036
		75	0.03875	0.03125		0.048

Site	Matrix		Nonylphenol	Octylphenol	Total Hydrocarbons	Tributyltin
Solway Firth	Invertebrates	n				1
		25				0.002
		M				0.002
		75				0.002
	Seawater	n				3
		25				10.00
		M				10.00
		75				10.00
	Sediment	n				3
		25				0.001
		M				0.002
		75				0.006
Wash and North Norfolk	Mammal	n				1
		25				0.016
		M				0.016
		75				0.016
	Sediment	n				5
		25				0.014
		M				0.016
		75				0.02

Appendix 4 Summary of metals - all SPA that do not overlap SACs

Units: Tissues mg/kg (wet) ; Sediments mg/kg (dry) ; Seawater µg/L.

SITE	MATRIX		AG	AL	AS	CD	CO	CR	CU	FE	HG	NI	PB	SE	ZN
Dee	fish	n			5	7			10		12		7		12
		25			4.1	0.053			0.46		0.05675		0.195		8.1
		Median			4.2	0.054			6.27		0.0735		0.22		25.45
		75			4.5	0.125			13.5		0.15175		0.47		30.25
	invertebrates	n				4					4		4		
		25				0.01					0.02		0.65		
		Median				0.02					0.03		0.83		
		75				0.05					0.04		1.03		
	seawater	n				14		14	14		14	14	14		14
		25				0.04		0.35	1.56		0.012	0.83	0.13		11.25
		Median				0.044		0.42	1.88		0.02	1.04	0.22		13.85
		75				0.057		1.69	2.50		0.02	1.13	0.29		15.65
	sediment	n		3	4	4		4	4		4	4	4		4
		25		1782.50	6.82	0.0005		10.14	4.25		0.00003	4.63	6.70		30.58
		Median		1848.00	8.36	0.10		20.07	8.40		0.011	10.16	13.63		77.35
		75		1716550	10.34	0.24		34.79	14.33		0.067	25.98	26.01		14420
Duddon Estuary	invertebrates	n				1					1		1		
		25				0.192					0.037		0.643		
		Median				0.192					0.037		0.643		
		75				0.192					0.037		0.643		
Mersey	fish	n			4	1			5		5		1		5
		25			4.85	0.15			0.59		0.38		2.00		9.50
		Median			5.95	0.15			0.79		0.40		2.00		10.00
		75			6.85	0.15			0.79		0.77		2.00		11.00
	sediment	n			5	5		5	5		5	5	5		5
		25			8.50	0.34		36.00	23.00		0.54	13.00	50.00		115.00
		Median			8.70	0.34		38.00	24.00		0.59	15.00	58.00		127.00
		75			8.80	0.52		39.00	26.00		0.64	16.00	72.00		129.00

SITE	MATRIX		AG	AL	AS	CD	CO	CR	CU	FE	HG	NI	PB	SE	ZN
Poole Harbour	invertebrates	n				2					2		2		
		25				0.04					0.05		0.25		
		Median				0.04					0.05		0.25		
		75				0.04					0.05		0.25		
	sediment	n			22	22		22	22		22	22	22		22
		25			5.85	0.27		21.25	19.25		0.19	11.25	24.75		54.25
		Median			6.30	0.33		24.00	24.00		0.22	12.00	29.00		68.00
		75			6.65	0.43		26.00	71.00		0.34	12.75	54.00		117.75
Ribble Estuary	invertebrates	n				2					2		2		
		25				0.11					0.04		0.54		
		Median				0.16					0.04		0.57		
		75				0.21					0.04		0.61		
Teesmouth and Cleveland Coast	sediment	n			2	4		4	4	2	2	4	4	2	4
		25			15.50	0.14		0.27	35.25	25.50	0.30	0.23	18.75	57.50	83.25
		Median			16.00	6.58		23.15	40.50	26.00	0.32	9.12	39.50	61.00	91.50
		75			16.50	13.00		48.00	45.50	26.50	0.33	18.25	60.75	64.50	97.00

Appendix 5 Summary of PCBs & pesticides - all SPAs that do not overlap SACs

Units: Tissues mg/kg (wet) ; Sediments mg/kg (dry) ; Seawater ng/L.

SITE	MATRIX		TOTAL PCBs	Total OP	Aldrin	Atraz-ine	DDD	DDE	DDT	Dieldrin	Endrin	Endo-sulfan	HCB	AHCH	BHCH	GHCH	Iso-drin	Sima-zine	Triflur-alin
Dee	fish	n	5		2		7	7	7	7	2		7	7		7			
		25	0.383		0.000443		0.04191	0.017405	0.01941	0.007905	0.0009		0.00141	0.00066		0.00141			
		M	0.453		0.000565		0.1	0.041	0.046	0.015	0.0011		0.002	0.002		0.002			
		75	0.489		0.000688		0.135	0.0485	0.053	0.0185	0.0014		0.0055	0.002		0.0045			
	invertebrates	n	4				4	4	4	4			4	4	4	4			
		25	0.0053				0.00056	0.00045	0.00145	0.00053			0.0003	0.00035	0.00053	0.00035			
		M	0.017				0.00328	0.001225	0.0016	0.001265			0.0003	0.00043	0.00054	0.00068			
		75	0.03				0.00675	0.00225	0.0016	0.00225			0.00035	0.00088	0.00054	0.001			
	mammals	n	1				1	1	1	1			1	1		1			
		25	23.133				0.4	1	0.3	0.088			0.009	0.0005		0.005			
		M	23.133				0.4	1	0.3	0.088			0.009	0.0005		0.005			
		75	23.133				0.4	1	0.3	0.088			0.009	0.0005		0.005			
	seawater	n		90	14	8	6	6	20	14	14	16	14	14	14	14	14	8	14
		25		5.00	5.00	10.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	10.00	5.00
		M		5.00	5.00	10.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	10.00	5.00
		75		20.00	5.00	10.00	5.00	5.00	5.00	5.00	8.75	6.25	5.00	5.00	5.00	5.00	5.00	10.00	10.00
	sediment	n			2		2	3	4	2	2		2						
		25			0.0012		0.00125	0.0014	0.001	0.0012	0.0024		0.0012						
		M			0.0014		0.0015	0.0018	0.0014	0.0014	0.0028		0.0014						
		75			0.0016		0.00175	0.0019	0.0019	0.0016	0.0032		0.0016						
Duddon Estuary	invertebrates	n	2				2	2	2	2			2	2	2	2			
		25	0.001				0.0011	0.000625	0.00075	0.000538			0.00035	0.00063	0.00055	0.001			
		M	0.002				0.0014	0.00075	0.001	0.000575			0.0004	0.00075	0.0006	0.001			
		75	0.003				0.0017	0.000875	0.00125	0.000613			0.00045	0.00088	0.00065	0.001			
Mersey	fish	n	2				2	2	2	2			2	2		2			
		25	1.21				0.38	0.165	0.06975	0.042			0.00725	0.002		0.00425			
		M	1.389				0.41	0.2	0.0705	0.043			0.0075	0.002		0.0045			
		75	1.568				0.44	0.235	0.07125	0.044			0.00775	0.002		0.00475			
	sediment	n	4																
		25	0.0526																
		M	0.0607																
		75	0.0826																

SITE	MATRIX		TOTAL PCBs	Total OP	Aldrin	Atraz-ine	DDD	DDE	DDT	Dieldrin	Endrin	Endo-sulfan	HCB	AHCH	BHCH	GHCH	Iso-drin	Sima-zine	Triflur-alin
Poole Harbour	invertebrates	n	2				2	2	2	2			2	2	2	2			
		25	0.001				0.00056	0.00045	0.0016	0.00053			0.0003	0.00035	0.00054	0.00035			
		M	0.001				0.00056	0.00045	0.0016	0.00053			0.0003	0.00035	0.00054	0.00035			
		75	0.001				0.00056	0.00045	0.0016	0.00053			0.0003	0.00035	0.00054	0.00035			
	sediment	n	6																
		25	0.1726																
		M	0.3454																
		75	0.5167																
Ribble Estuary	invertebrates	n	2				2	2	2	2			2	2	2	2			
		25	0.0235				0.00875	0.003	0.00078	0.002			0.00035	0.00063	0.00051	0.00063			
		M	0.025				0.0095	0.003	0.00105	0.002			0.0004	0.00075	0.00052	0.00075			
		75	0.0265				0.01025	0.003	0.00133	0.002			0.00045	0.00088	0.00053	0.00088			
Teesmouth and Cleveland Coast	mammals	n	1																
		25	54.668																
		M	54.668																
		75	54.668																
	sediment	n	3				2	2	2	2			2	2		2			
		25	0.014				1.5	1.125	2.2	2.125			6.55	0.32		0.675			
		M	0.0198				1.6	1.15	2.3	2.85			6.9	0.35		0.69			
		75	0.0212				1.7	1.175	2.4	3.575			7.25	0.38		0.705			

Appendix 6 Summary of miscellaneous - all SPAs that do not overlap SACs

Units: Tissues mg/kg (wet) ; Sediments mg/kg (dry) ; Seawater ng/L.

Site	Matrix		Nonylphenol	Octylphenol	Total Hydrocarbons	Tributyltin
Dee	Invertebrates	n			4	
		25			14.25	
		M			19.00	
		75			23.25	
	mammals	n				1
		25				0.0015
		M				0.0015
		75				0.0015
	seawater	n				6
		25				10.00
		M				10.00
		75				10.00
	sediment	n				2
		25				0.0025
		M				0.005
		75				0.0075
Duddon Estuary	invertebrates	n				1
		25				0.012
		M				0.012
		75				0.012
Mersey	sediment	n				5
		25				0.05
		M				0.12
`		75				0.29

Site	Matrix		Nonylphenol	Octylphenol	Total Hydrocarbons	Tributyltin
Poole Harbour	invertebrates	n			2	
		25			3.80	
		M			3.80	
		75			3.80	
	sediment	n				22
		25				0.371
		M				0.91
		75				3.9625
Ribble Estuary	invertebrates	n			1	1
		25			11.00	0.014
		M			11.00	0.014
		75			11.00	0.014
Teesmouth and Cleveland Coast	mammal	n				1
		25				0.047
		M				0.047
	sediment	n			1	2
		25			2203	0.01675
		M			2203	0.0235
		75			2203	0.03025

Appendix 7 Features of conservation interest in English SACs

SAC	Marine habitats of interest	subfeatures	organisms present of importance to the site						
			mammals	fish	molluscs	crustaceans	annelids	sponges	miscellaneous
Solway Firth	estuaries	rocky scars		*Salmo trutta* (sea trout)	*Mytilus edulis* (common mussel)	*Semibalanus balanoides* (barnacle)	*Sabellaria alveolata*	*Halichondria panicea*	
				Salmo salar (salmon)	*Patella vulgata* (limpet)	*Elminius modestus*	*Sabellaria spinulosa*		
				Alosa alosa (allis shad)	dogwhelks				
				Lampetra fluvialtillis (river lamprey)					
				Petromyzon marinus (sea lamprey)					
				flatfish - flounder, plaice , sole, dab					
				Alosa fallax (twaite shad)	*Littorina littorea* (periwinkle)				
	intertidal sand- and mudflats	muddy sand communities			*Cerastoderma edule* (common cockle)		*Arenicola marina*		
					Macoma balthica (Baltic tellin)				
		sandy mud communities			*Macoma balthica* (Baltic tellin)	*Corophium volutator* (amphipod)	*Hediste diversicolor* (ragworm)		
					Hydrobia ulvae (laver spire shell)				
		gravel and sand communities			*Angulus tenuis*	*Bathyporeia* spp	*Nephtys cirrosa*		
					Donax vittatus		*Nephtys hombergii*		
							Scoloplos armiger		
							Arenicola marina		
	subtidal sandbanks	infralittoral gravel and sand communities		spawning/nursery grounds for various fish spp	*Fabulina fabula*	*Bathyporeia elegans*	*Nephtys cirrosa*		
					Spisula subtruncata		*Magelona mirabilis*		
					Tellina tenuis		*Microphalamus similis*		

SAC	Marine habitats of interest	subfeatures	organisms present of importance to the site						
			mammals	**fish**	**molluscs**	**crustaceans**	**annelids**	**sponges**	**miscellaneous**
					Modiolus modiolus (horse mussel)				
Drigg Coast	estuaries				razor shells	*Corophium volutator* (amphipod)	*Hediste diversicolor* (ragworm)		*Echinocardium cordatum*
					Macoma balthica	*Eurydice pulchra* (isopod)	*Arenicola marina*		
					Mytilus edulis	*Semibalanus balanoides*			
					Littorina littorea	*Elimnius modestus*			
					Littorina saxitalis	*Carcinus maenas*			
Flamborough Head	Reefs	rocky shore communities		*Lipophrys pholis* (shanny)	*Hiatella arctica* (wrinkled rock borer)		*Polydora* spp.		
					Zirfaea crispata (piddock)	*Semibalanus balanoides* (barnacle)			
					Patella spp (limpets)				
		kelp forest communities		*Labrus bergylta* (ballan wrasse)		nursery area for crab and lobster			
				Labrus mixtus (cuckoo wrasse)					*Perophora listeri* (sea squirt)
		subtidal faunal turf communities			*Modiolus modiolus* (horse mussel)		*Sabellaria spinulosa*	sponges	sea-firs
					Mytilus edulis (common mussel)				sea mats
								Polymastia boletiformis (yellow sponge)	sea squirts
									Archidistoma aggretatum
									Thuiaria thuja (hydroid)
									Diphasia alata (hydroid)
									Smittina affinis (bryozoan)

SAC	Marine habitats of interest	subfeatures	organisms present of importance to the site						
			mammals	**fish**	**molluscs**	**crustaceans**	**annelids**	**sponges**	**miscellaneous**
	sea caves	faunal cushion and crust communities			*Mytilus edulis* (common mussel)	*Semibalanus balanoides* (barnacle)	*spirobid polychaetes*	*Halichondria panicea* (breadcrumb sponge)	*Actinia equina* (beadlet anenome)
					Nucella lapillus (dogwhelk)		*Pomatoceros triqueter* (tubeworm)	*Clathrina coriacea*	
								Clionia celata (chalk-boring yellow sponge)	
Morecambe Bay	Large shallow inlets and bays	intertidal boulder and cobble skear communities			*Mytilus edulis*		*Sabellaria alveolata* (honeycomb worm)		
		subtidal boulder and cobble skear communities			*Mytilus edulis*			cushion sponges	hydroids
									sea squirts
		brittlestar beds							*Ophyothrix fragilis* (brittlestar)
		intertidal boulder clay			*Barnea candida* (piddocks)				
	intertidal mudflats and sandflats	sand communities			*Macoma balthica* (Baltic tellin)	*Bathyporeia* spp	*Arenicola marina* (lugworm)		
					Cerastoderma edule (edible cockle)	*Eurydice pulchra* (isopod)			
		mud communities			*Scrobicularia plana* (peppery furrow shell)	*Corophium volutator* (amphipod)	*Hediste diversicolor* (ragworm)		
		eelgrass bed communities		important nursery and spawning grounds for fish and shellfish					

SAC	Marine habitats of interest	subfeatures	organisms present of importance to the site						
			mammals	**fish**	**molluscs**	**crustaceans**	**annelids**	**sponges**	**miscellaneous**
Plymouth Sound and Estuaries	Large shallow inlets and bays	intertidal rock and boulder shore			*Hiatella arctica*		*Myxicola aesthetica*		*Dendrodoa grossularia* (sea squirt)
							Polydora spp		
		subtidal rocky reef			*Okenia elegans* (Sea slug)			sponges	*Aiptasia mutabilis* (anenome)
									soft corals
									Eunicella verrucosa (sea fan)
									hydroids
									bryozoans
									Ophiopsila arenea (brittle star)
									Alcyonum digitatum (dead mans fingers)
									Amphianthus dohrnii (anenome)
		kelp forest communities				important nursery area for crabs and lobsters			
		subtidal mud communities			*Philine aperta*	*Goneplax rhomboides* (angular crab)			*Edwardsia claparedii* (anenome)
						Calianassa subterranea (burrowing shrimp)			*Ophiura* spp (brittle star)
						Upogebia delturna			*Virgulaira mirabilis* (sea pen)
						Upogebia stellata			

SAC	Marine habitats of interest	subfeatures	organisms present of importance to the site						
			mammals	fish	molluscs	crustaceans	annelids	sponges	miscellaneous
	estuaries	subtidal mud communities		feeding grounds for juvenile fish, eg. Solea solea					
		intertidal mixed muddy sediment communities			*Cerastoderma edule* (cockle)				
					Ostrea edulis (native oyster)				
		subtidal mixed muddy sediment communities							*Hartlaubella gelatinosa* (rare hydroid)
		estuarine bedrock, boulder and cobble communities				*Balanus crenatus* (acorn barnacle)	*Sabella pavonia* (peacock worm)	*Hymeniacidon perleve* (orange peel sponge)	*Cordylophora caspia* (hydroid)
						Carcinus maenas (shore crab)			
		saltmarsh and reedbed communities		Nursery areas for juvenile bass and other fish species					
	sandbanks which are slightly covered by seawater all the time	eelgrass bed communities		seahorses	cuttlefish	swimming crabs			anenomes
						hermit crabs			*Echinocardium cordatum*
						shore crabs			brittle stars
		gravel and sand communities			*Spisula elliptica*		*Pisione remota*		*Echinocardium cordatum*
					Dosinia lupinus		*Polygordius lacteus*		
					Gari tellinella				
					Glycera lapidum				

SAC	Marine habitats of interest	subfeatures	organisms present of importance to the site						
			mammals	**fish**	**molluscs**	**crustaceans**	**annelids**	**sponges**	**miscellaneous**
		muddy sand communities			*Abra alba*		*Melinna palmata*		
					Chamelea gallina				
					Thyasira flexuosa				
Lundy	Reefs	rocky shore communities			limpets	barnacles			*Balanophyllia regia* (cup coral)
		kelp forest communities		*Labrus bergylta* (wrasse)		*Cancer pagurus* (edible crab)		sponges	sea squirts
				Labrus mixtus		*Hommarus gammarus* (lobster)			anenomes
		vertical and overhanging circalittoral rock communities						*Axinella* spp (branching sponges)	*Leptopsammia pruvoti* (cup coral)
									Parazoanthus spp (colonial anenome)
									Alcyonium glomeratum (red sea fingers)
		circalittoral bedrock and stable boulder communities						*Axinella* spp (branching sponges)	*Eunicella verrucosa* (pink seafan)
									Amphianthus dohrnii (fan anenome)
									Pentapora foliacea (ross coral)

SAC	Marine habitats of interest	subfeatures	organisms present of importance to the site						
			mammals	**fish**	**molluscs**	**crustaceans**	**annelids**	**sponges**	**miscellaneous**
Isles of Scilly	Sandbanks which are slightly covered by seawater all the time	Eelgrasss bed communities				*Necora puber* (swimming crab)			*Laomedea angulata* (hydroid)
				Hippocampus spp (seahorses)	*Sepiola* spp (cuttlefish)	hermit crabs			*Anemonia viridis* (snake-lock anenomes)
						shore crabs			*Echinocardium cordatum*
									Amphiura spp (brittlestars)
		sand and gravel communities			*Mactra and Spisula* spp (trough shells)	sand hoppers	polychaete worms		*Spatangus purpureus* (sea urchin)
					Gibbula magus (snail)				*Cereus pedunculatus* (daisy anenome)
					Jujubinus striatus (sea snail)				brittlestars
					Bittium simplex (sea snail)				starfish
					Callista chione (bivalve)				sea urchins
									Molgula oculata (sea squirt)
		mixed sediment communities			*Gibbula magus*		*Cirriformia tentaculata*		*Cereus pedunculatus* (daisy anenome)
					Lacuna vincts (chink shell)		*Notomastus latericeus*		
	mudflats and sandflats not covered by seawater at low tide	sand communities			*Angulus tenuis*	*Cestopagurus timidus* (hermit crab)	*Arenicola marina*		*Spatangus purpureus* (purple heart urchin)
					other bivalve molluscs		polychaete worms		*Echinocardium cordatum*

SAC	Marine habitats of interest	subfeatures	organisms present of importance to the site						
			mammals	**fish**	**molluscs**	**crustaceans**	**annelids**	**sponges**	**miscellaneous**
					Dosinia exoleta (rayed artemis)		*Lanice conchilega* (sand mason worm)		
					Ensis siliqua (razor shell)				
					Acanthocardia aculeata (spiny cockle)				
Fal and Helford	Large shallow inlets and bays	rocky shore communities			*Verruca stroemia* (barnacle)	*Procellana platycheles* (broad-clawed porcelin crab)	spirobid worms	rich sponge fauna	bryozoans
							serpulid worms		hydroids
		subtidal rock and boulder communities							*Psammechinus miliaris* (sea urchin)
									Asterina gibbosa (cushion star)
								Halichondria panicea (breadcrumb sponge)	*Pentapora foliacea* (ross coral)
								Hymeniacidon perleve (orange peel sponge)	*Eunicella verrucosa* (seafan)
									Alcyonium digitatum (dead mans fingers)
		subtidal mud communities		important feeding grounds for fish					
	sandbanks which are slightly covered by seawater all the time	Maerl bed communities		*Gobius couchi* (Couch's Goby)	bivalves	crabs	polychaetes		*Cerianthus lloydii* (burrowing anenome)
				other fish					other anenomes

SAC	Marine habitats of interest	subfeatures	organisms present of importance to the site						
			mammals	**fish**	**molluscs**	**crustaceans**	**annelids**	**sponges**	**miscellaneous**
		gravel and sand communities			bivalves	*Pagurus bernhardus* (hermit crab)	*Owenia fusiformis*		*Echinocardium cordatum*
					Ensis spp (razor shells)		*Lanice conchilega* (sand mason worm)		*Asterias rubens* (starfish)
							terebellid worms		
	mud and sandflats not covered by seawater at low tide	intertidal sand and gravel communities			bivalves	amphipods	polychaetes		*Leptopentacta elongata* (sea cucumber)
		intertidal muddy sand communities					*Myxicola infundibulum*		*Ophiura ophiura* (brittlestar)
							Branchiomma bombyx		*Amphiura brachiata* (brittlestar)
									Echinocardium cordatum
							Lanice conchilega (sand mason worm)		
							Arenicola marina		
		intertidal mud communities			*Scrobicularia plana*		*Hediste diversicolor* (ragworm)		
					Abra alba				
Chesil and The Fleet	Lagoons			important nursery ground for several fish species eg sand and common gobies, grey mullet, pipefish	*Tenellia adspera* (sea slug)	*Gammarus insensibilis* (lagoon shrimp)	*Armandia cirrhosa* (lagoon sandworm)	Suberites massa	*Nematostella vectensis* (starlet sea anenome)
				designated bass nursery	*Akera bullata*			*Halichondria bowerbankii*	*Anemonia viridis* (snake-locks anenome)
					Cerastoderma glaucum (lagoon cockle)				*Asterina gibbosa* (starfish)
					Caecum armoricum (DeFolin's lagoon snail)				

SAC	Marine habitats of interest	subfeatures	organisms present of importance to the site						
			mammals	fish	molluscs	crustaceans	annelids	sponges	miscellaneous
North East Kent (Thanet Coast)	Reefs	intertidal red algaal turfs communities			several spp of chalk-boring piddock		Polydora spp.		
		kelp dominated communities on animal bored rock			*Venerupis saxatilis* (bivalve)	crabs	polychaete worms		anenomes
									Sagartia troglodytes (anenome)
		subtidal animal bored chalk communities			piddocks *Barnea* spp, *Pholas dactylus, Hiatella actica, Perticola pholadiformis*				*Alcyonidium digitatum* (bryozoan)
									Nemertesia spp, *Sertularia* spp, *Halecium* spp (hydroids)
									Molgula manhattensis (ascidian)
Berwickshire and North Northumberland Coast	Reefs		*Halichoerus gryptus* (grey seal)						
	Mudflats and sandflats not covered by seawater at low tide								
	submerged or partly submerged sea caves								

SAC	Marine habitats of interest	subfeatures	organisms present of importance to the site						
			mammals	**fish**	**molluscs**	**crustaceans**	**annelids**	**sponges**	**miscellaneous**
Solent and Isle of Wight	Reefs				piddocks	hermit crabs	polychaetes	v. rare sponges	hydroids
	Estuaries				whelks		*Sabellaria spinulosa*		bryozoans
Essex Estuaries	Estuaries		breeding ground for common seal	small spawning ground for a distinct subspecies of herring	commercial cockle beds		*Sabellaria spinulosa*	rich sponge communities	brittlesatrs
	Mudflats and sandflats not covered by seawater at low tide						*Arenicola marina*		ascidians
									Sertularia spp (hydroid)
The Wash and North Norfolk Coast	Large shallow inlets and bays		important area for breeding and moulting of *Phoca vitulina* (common seal)		large nos of bivalves	large nos of crustaceans	large nos of polychaetes		brittlestars
	mudflats and sandflats not covered by seawater at low tide				tellin bivalves		*Sabellaria spinulosa* (reef building worm); *Arenicola marina* (lugworm)		
	sandbanks which are slightly covered by seawater at low tide				*Scrobicularia plana* (peppery furrow shell)		*Lanice conchilega* (sand mason worm)		cumaceans

SAC	Marine habitats of interest	subfeatures	organisms present of importance to the site						
			mammals	**fish**	**molluscs**	**crustaceans**	**annelids**	**sponges**	**miscellaneous**
Severn Estuary	estuary			v. diverse - >110 spp.	gastropods	amphipods	polychaetes		internationally important wintering wildfowl
	mudflats and sandflats not covered by seawater at low tide				gastropods	amphipods	polychaetes		
	sandbanks which are slightly covered by seawater at low tide			river lamprey	*Spisula,* Tellina		*Sabellaria alveolata*		Beds of *Zostera angustifolia*, *Z. noltii* and Z. *marina*
				twaite shad			*Sabellaria spinulosa*		
				sea lamprey					
				allis shad					

10. Figures

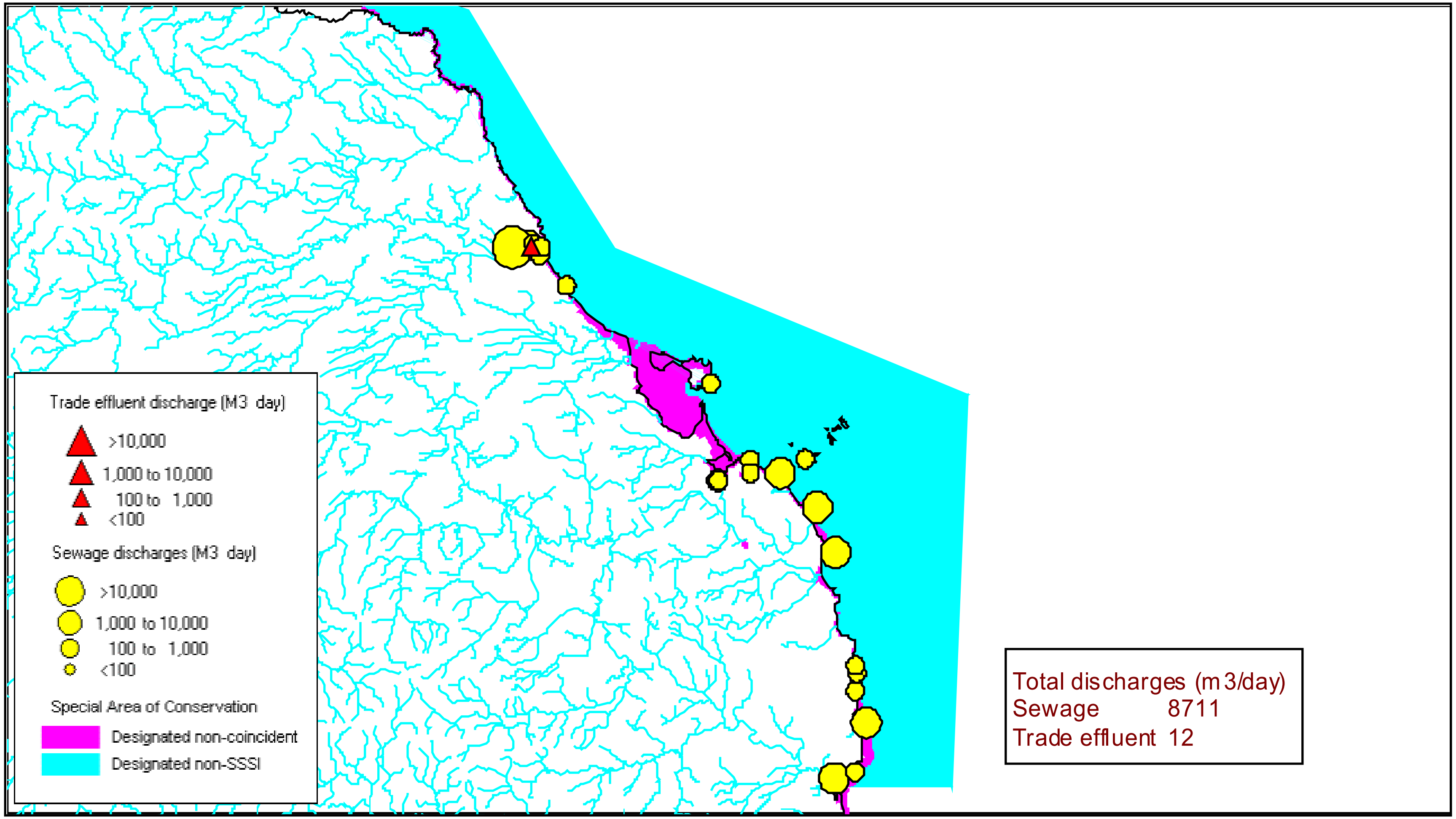

Figure 1. Berwick and North Northumberland Coast discharges

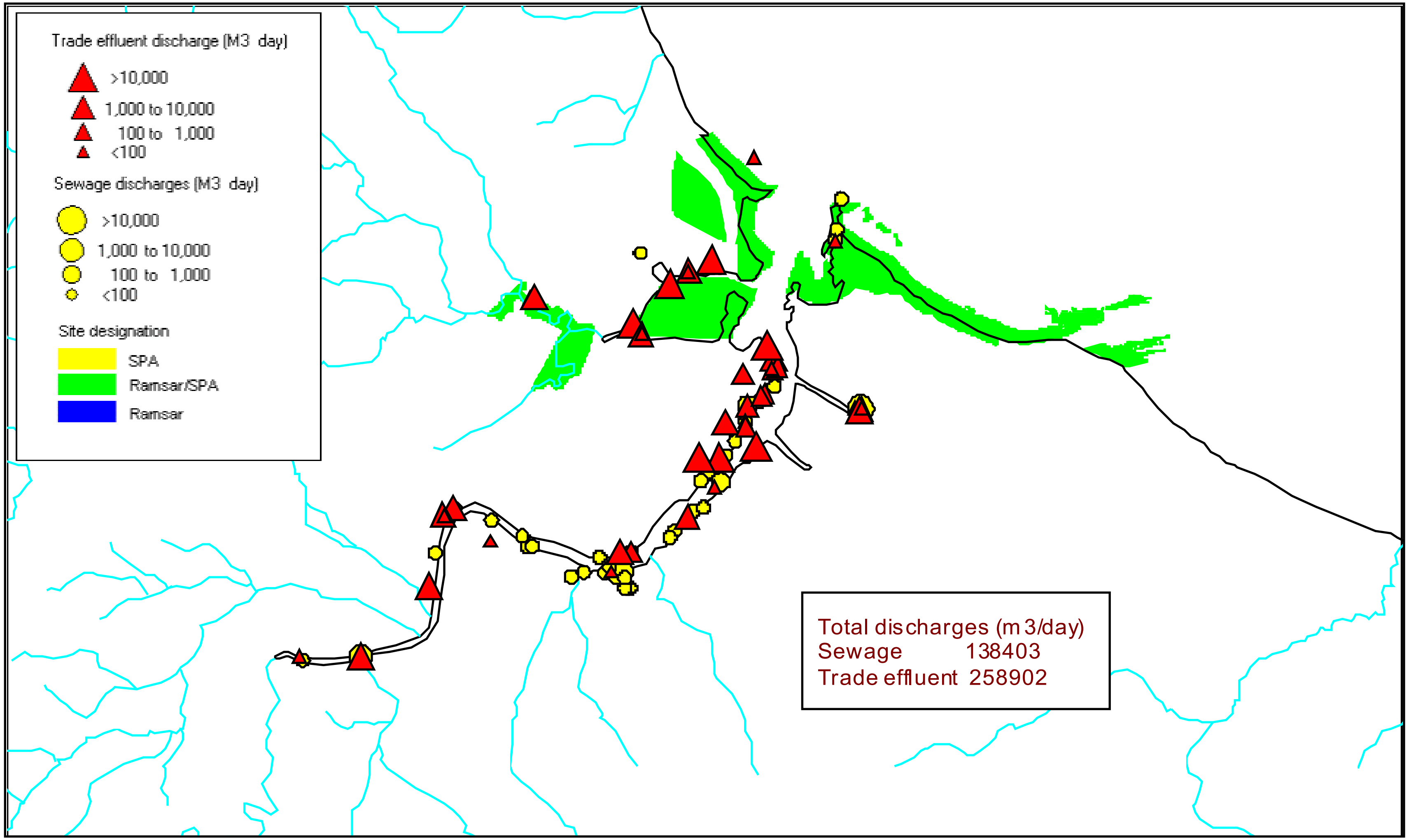

Figure 2. Teesmouth and Cleveland Coast discharges

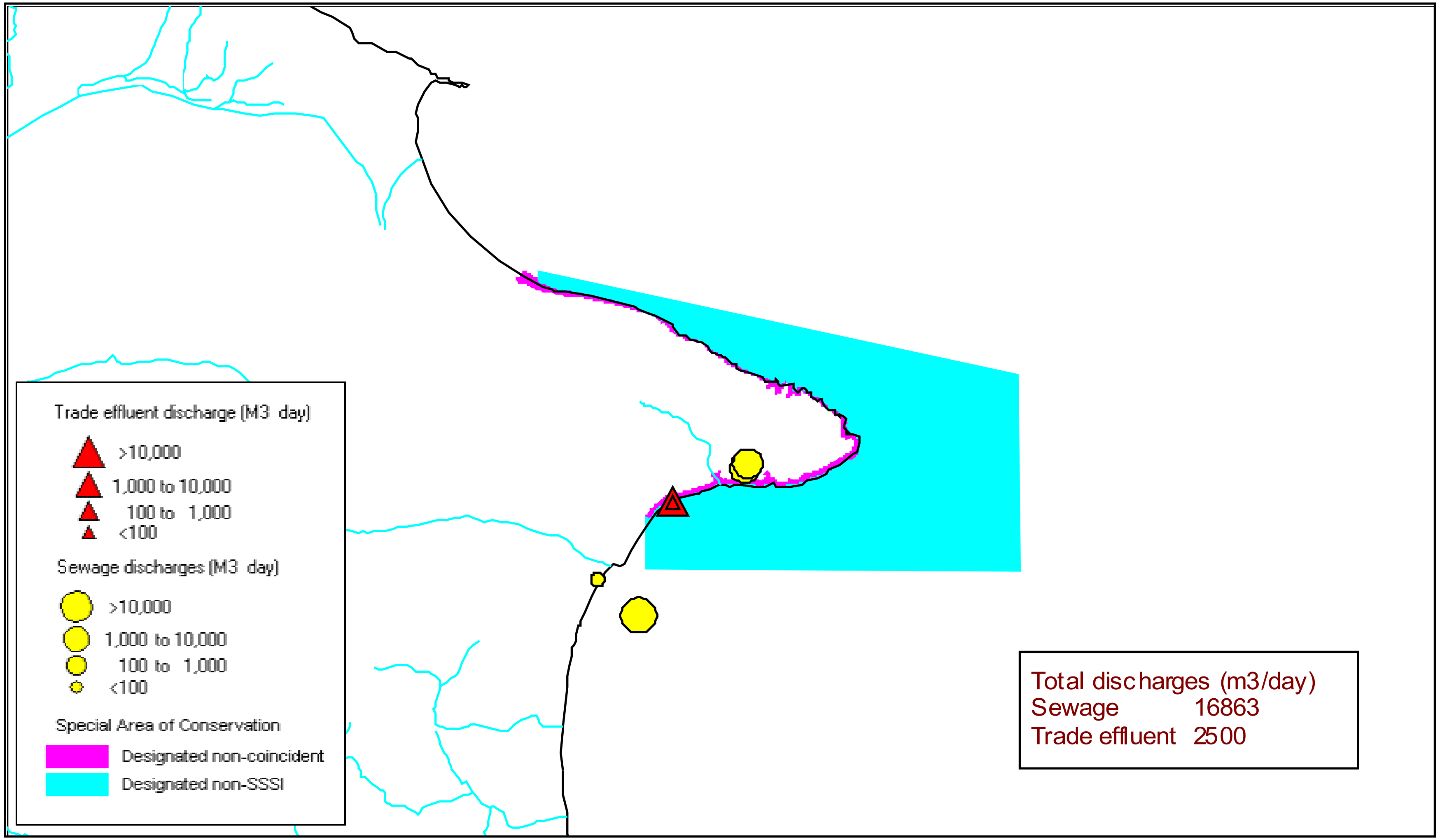

Figure 3. Flamborough Head discharges

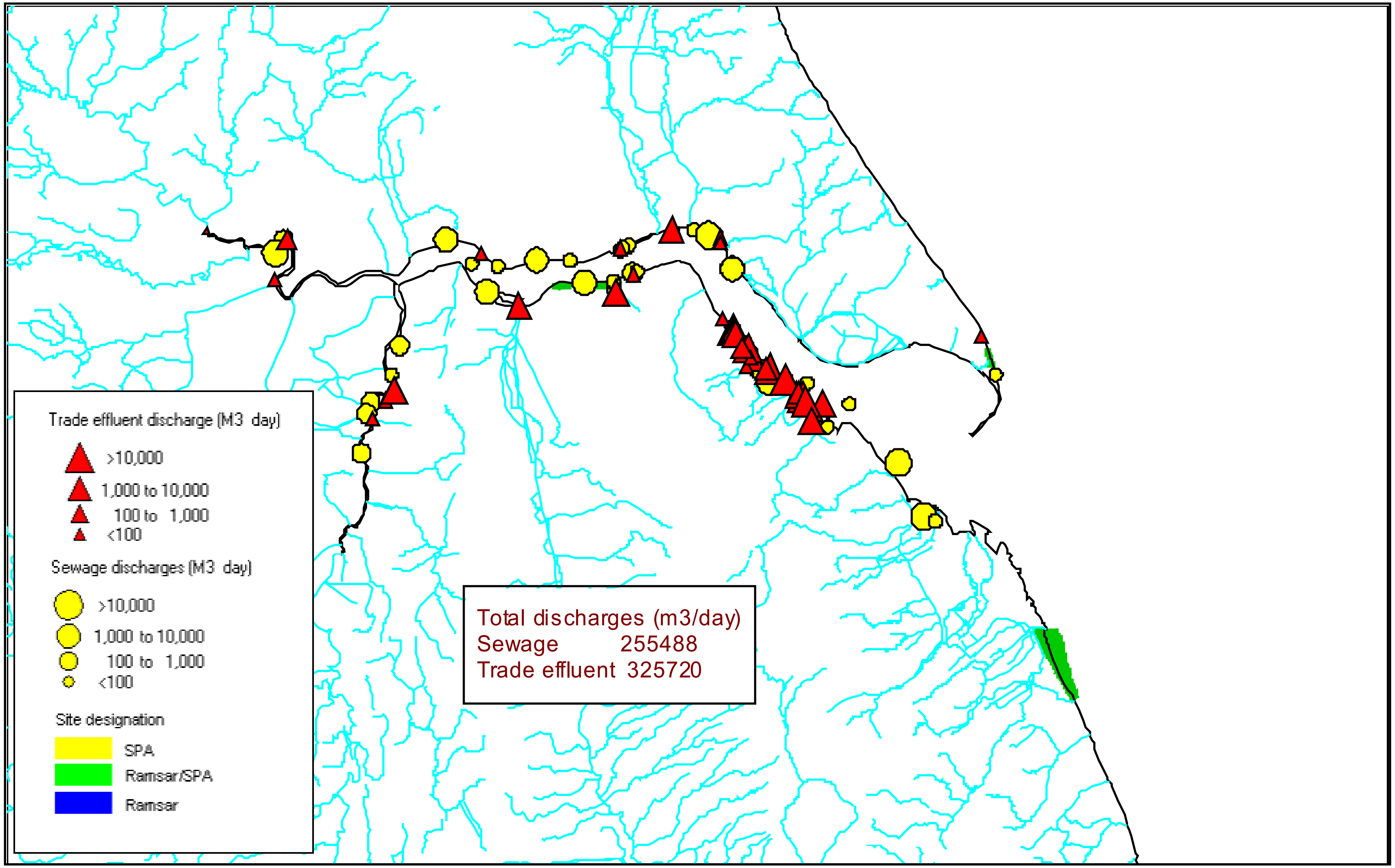

Figure 4. Humber Flats, Marshes and Coast discharges

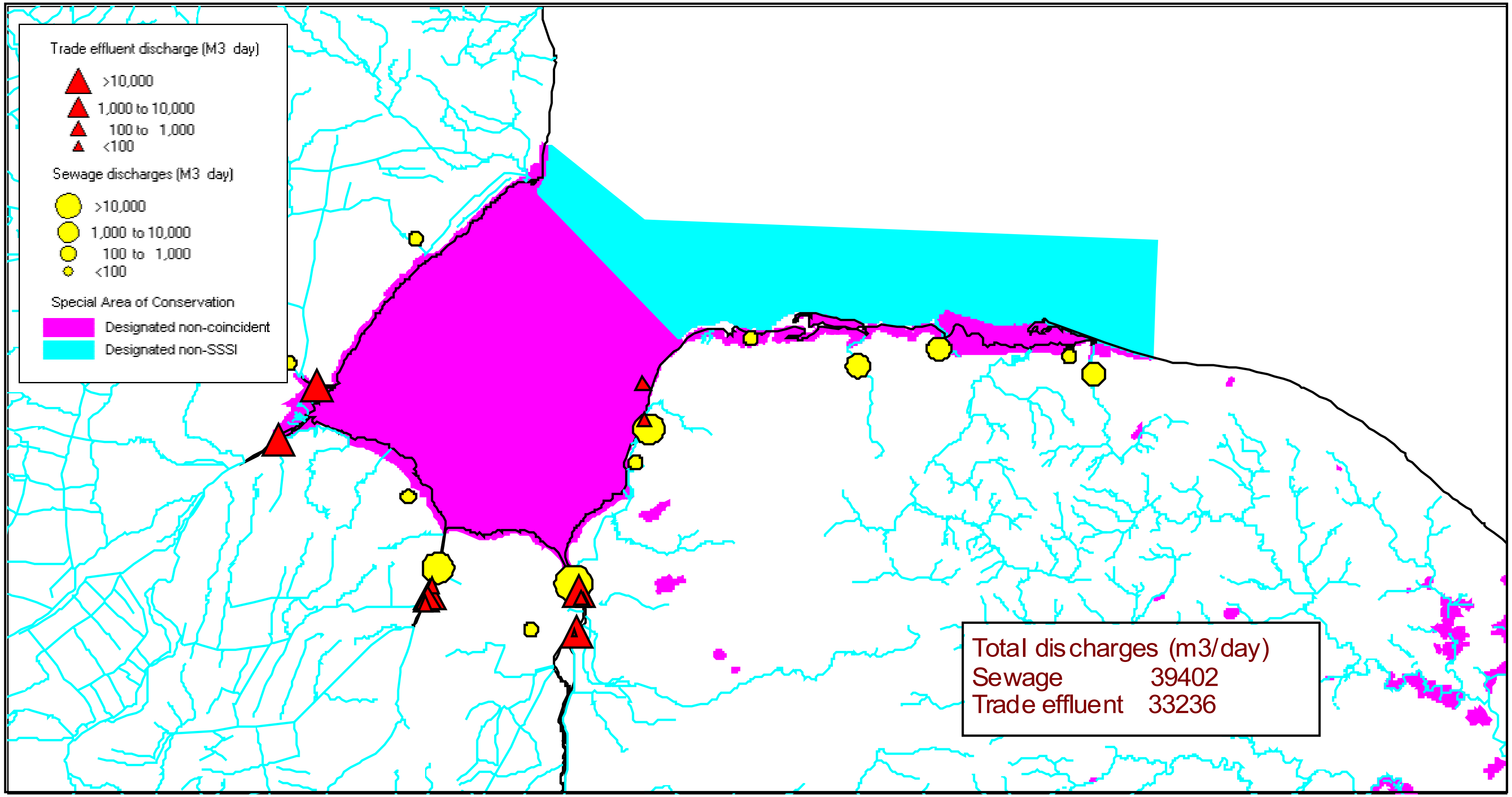

Figure 5. Wash and North Norfolk Coast discharges

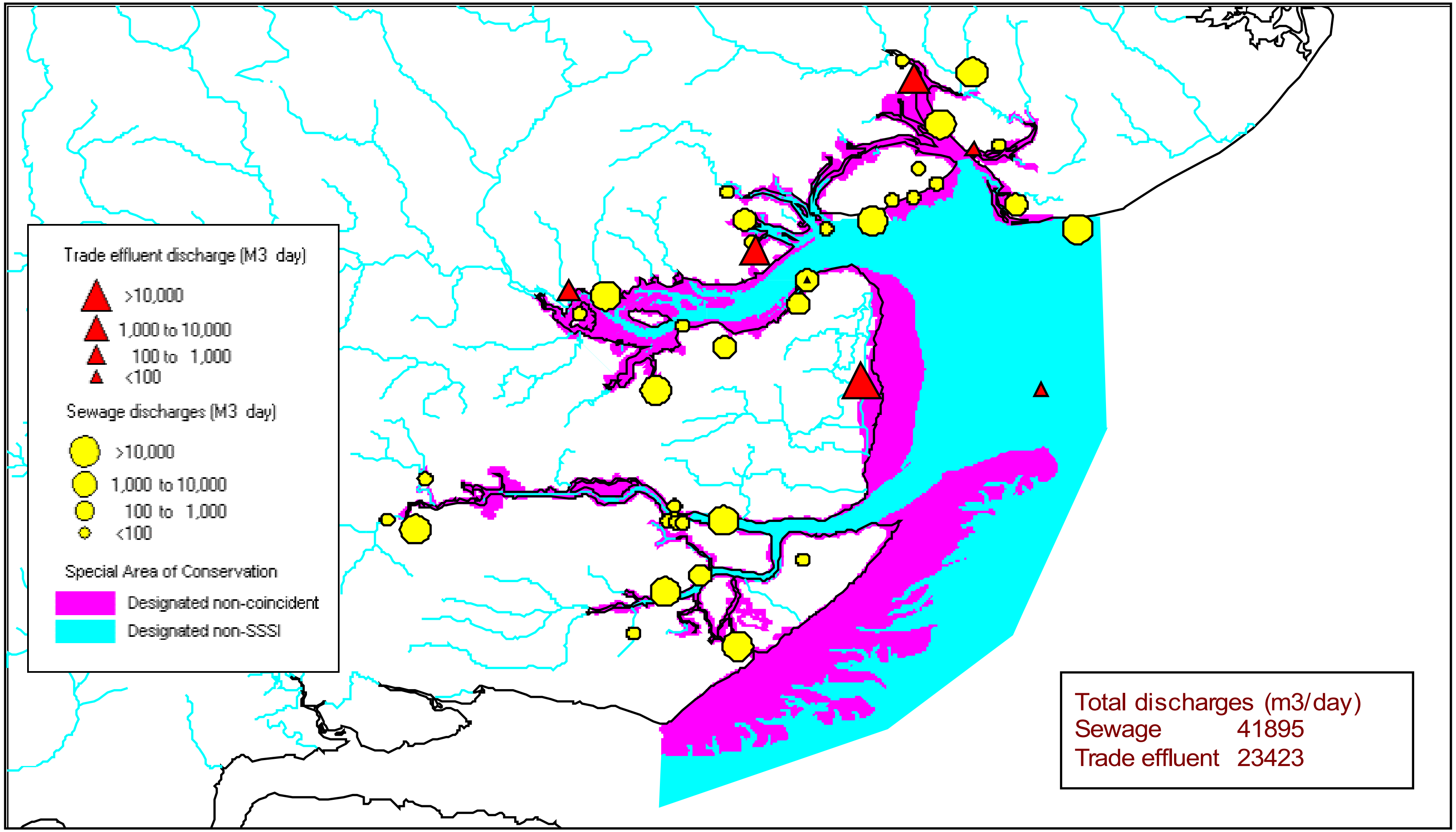

Figure 6. Essex Estuaries discharges

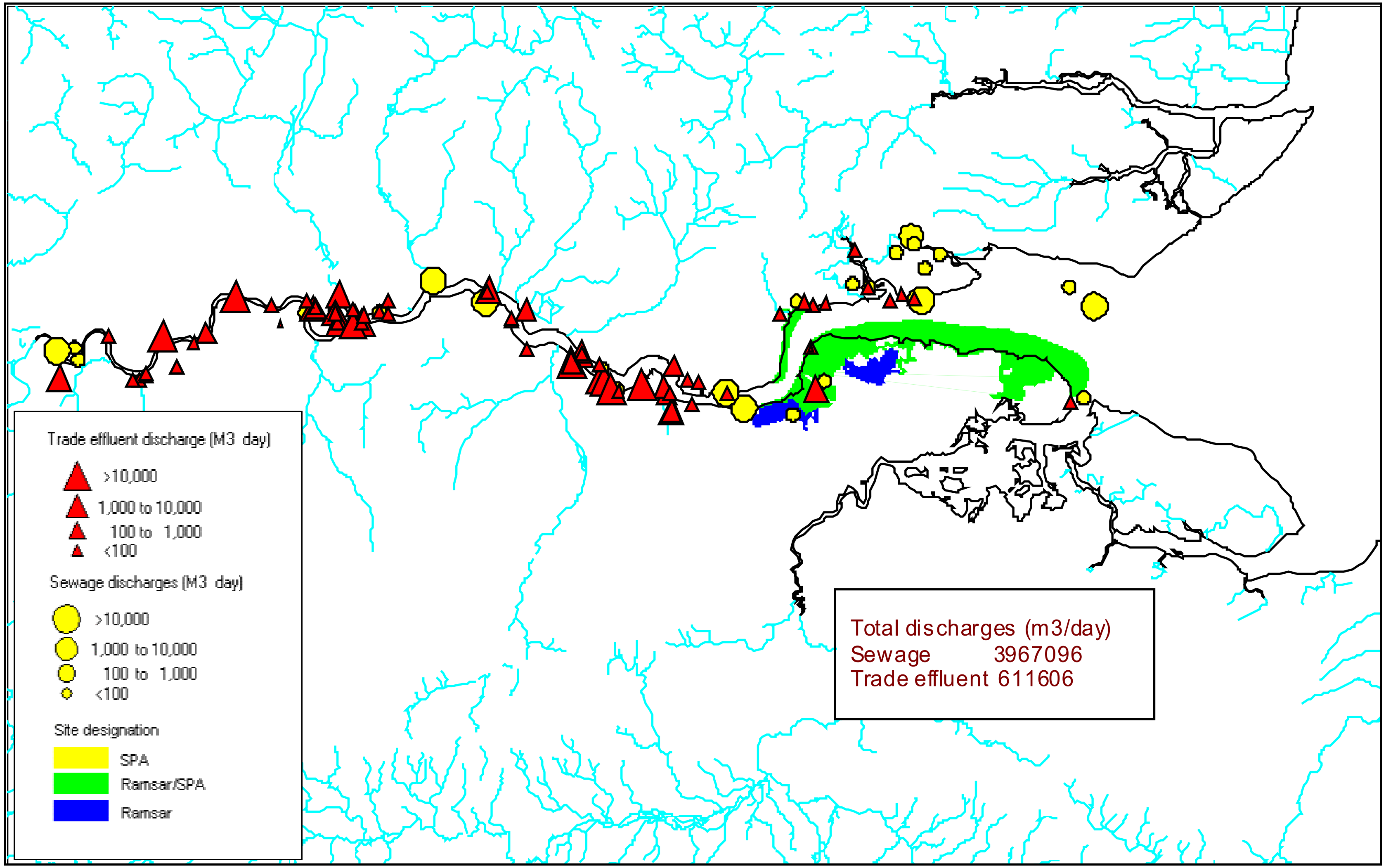

Figure 7. Thames Estuary and Marshes discharges

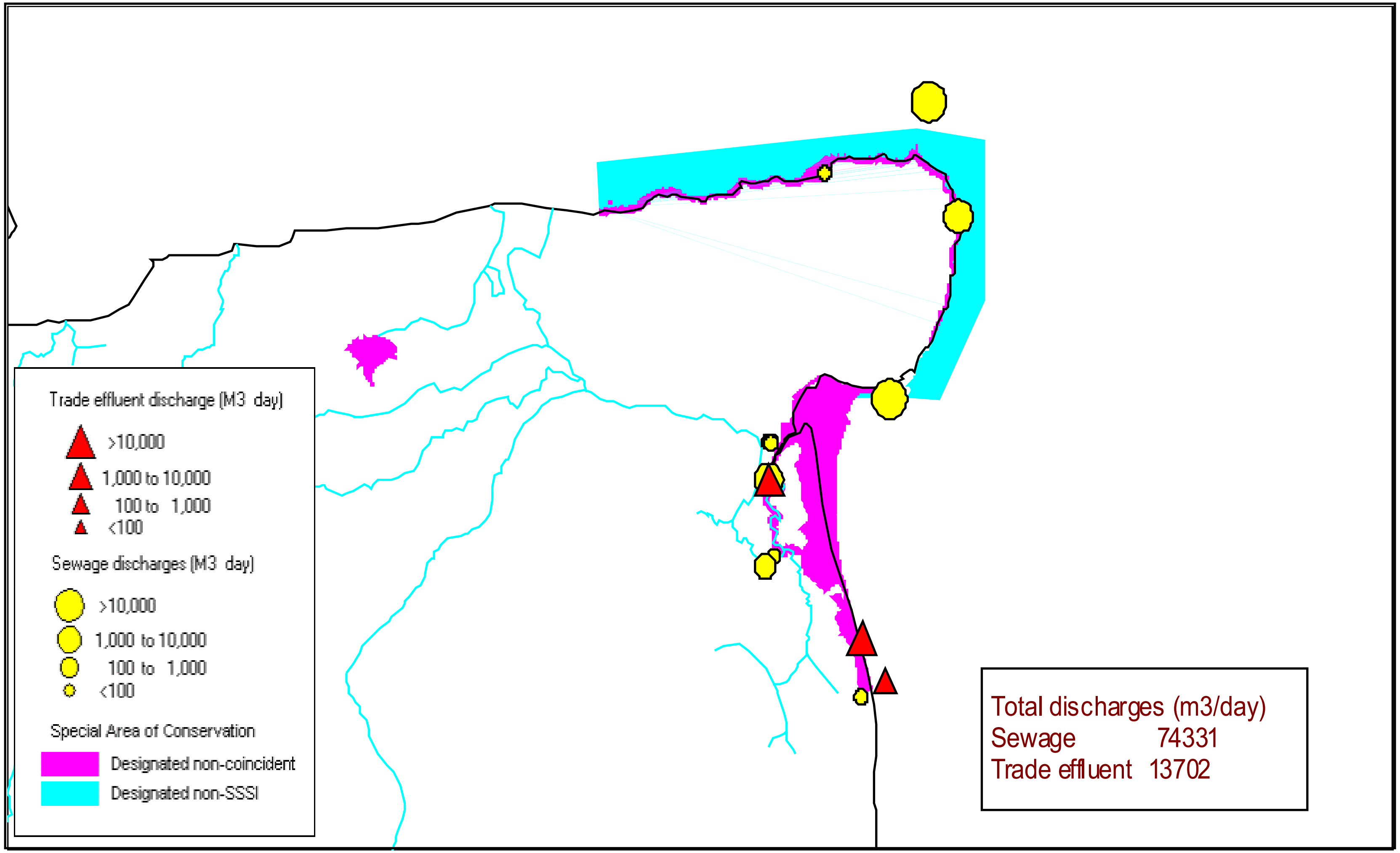

Figure 8. Thanet Coast discharges

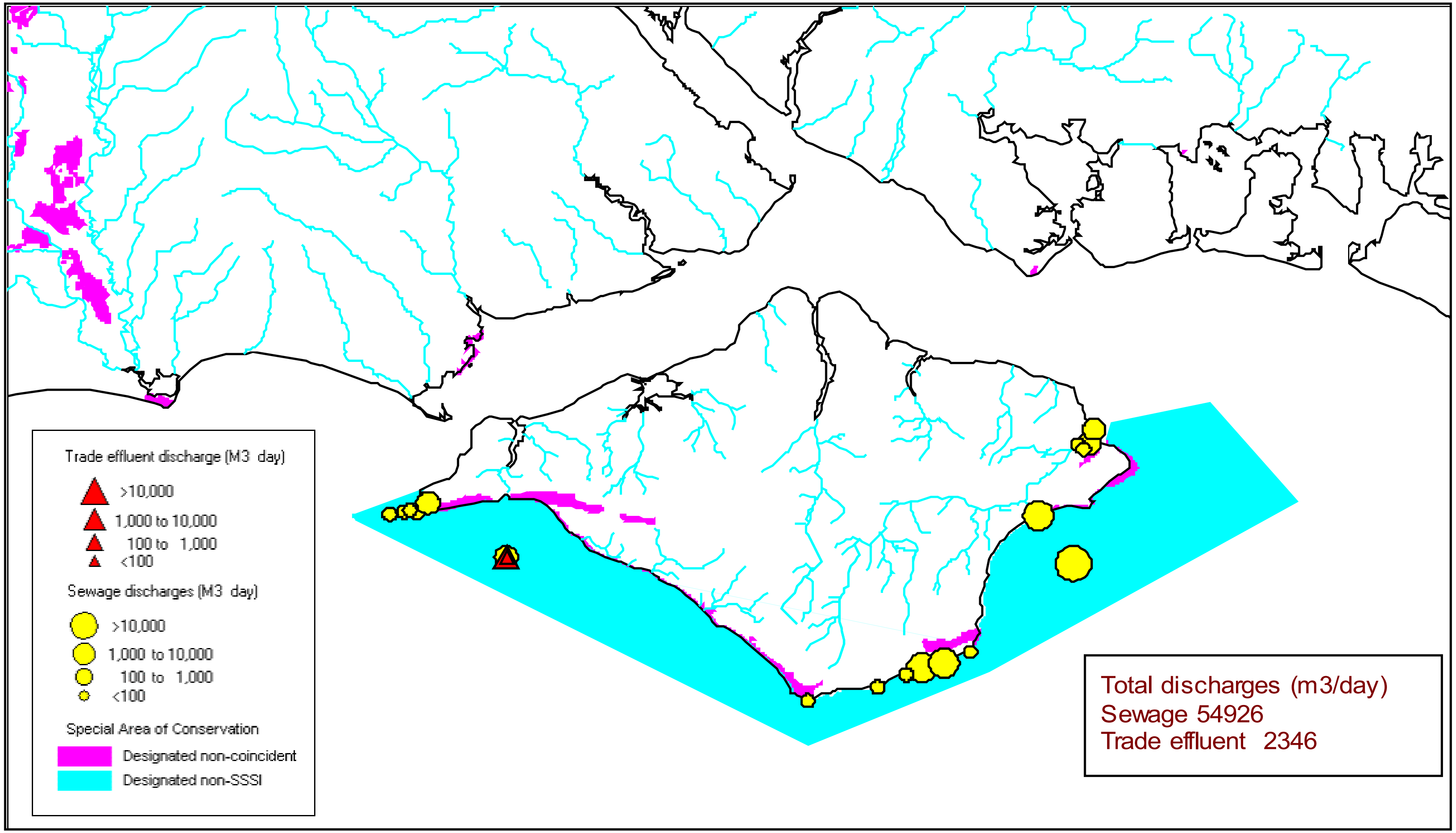

Figure 9. Isle of Wight Maritime discharges

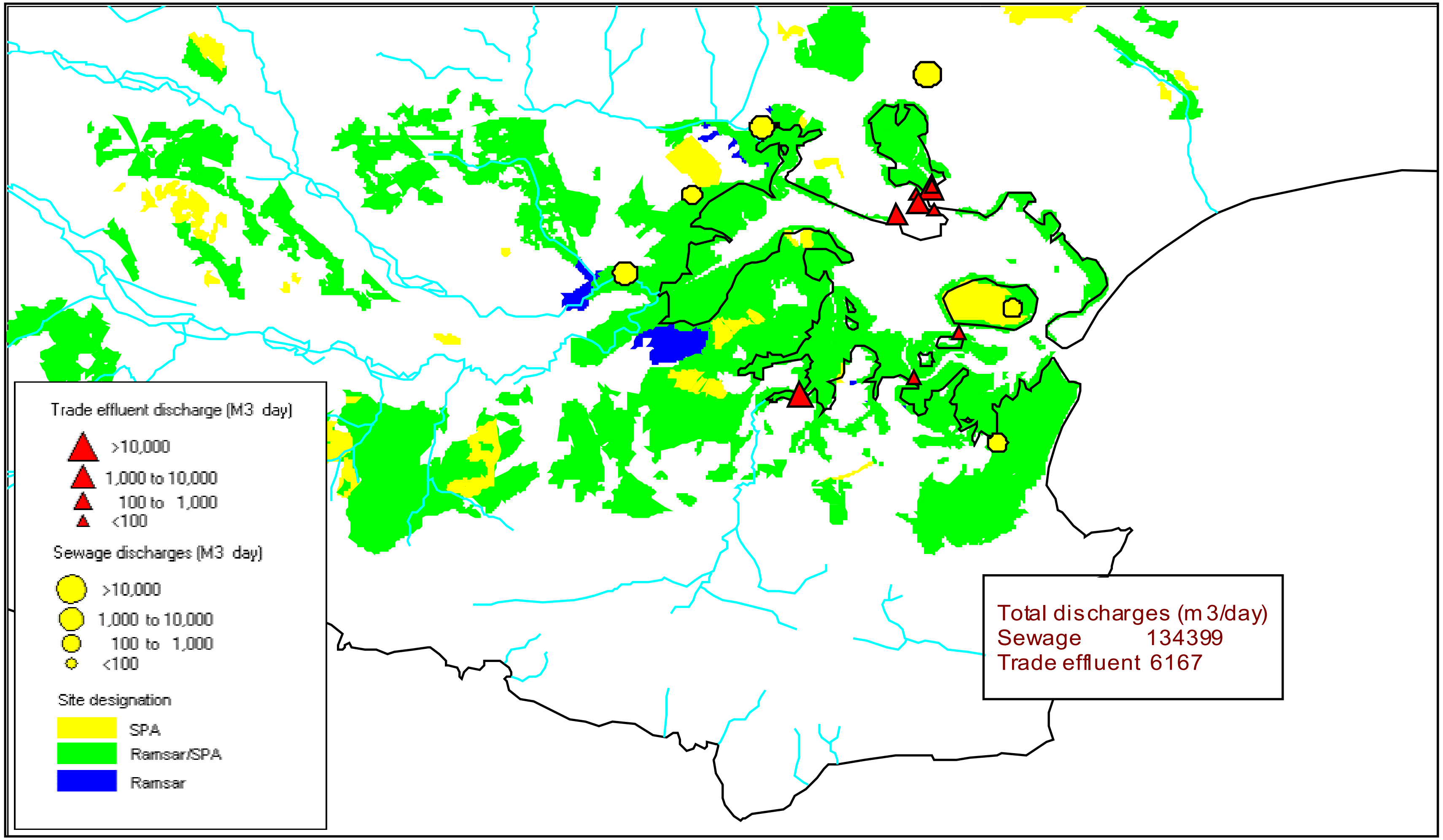

Figure 10. Poole Harbour discharges

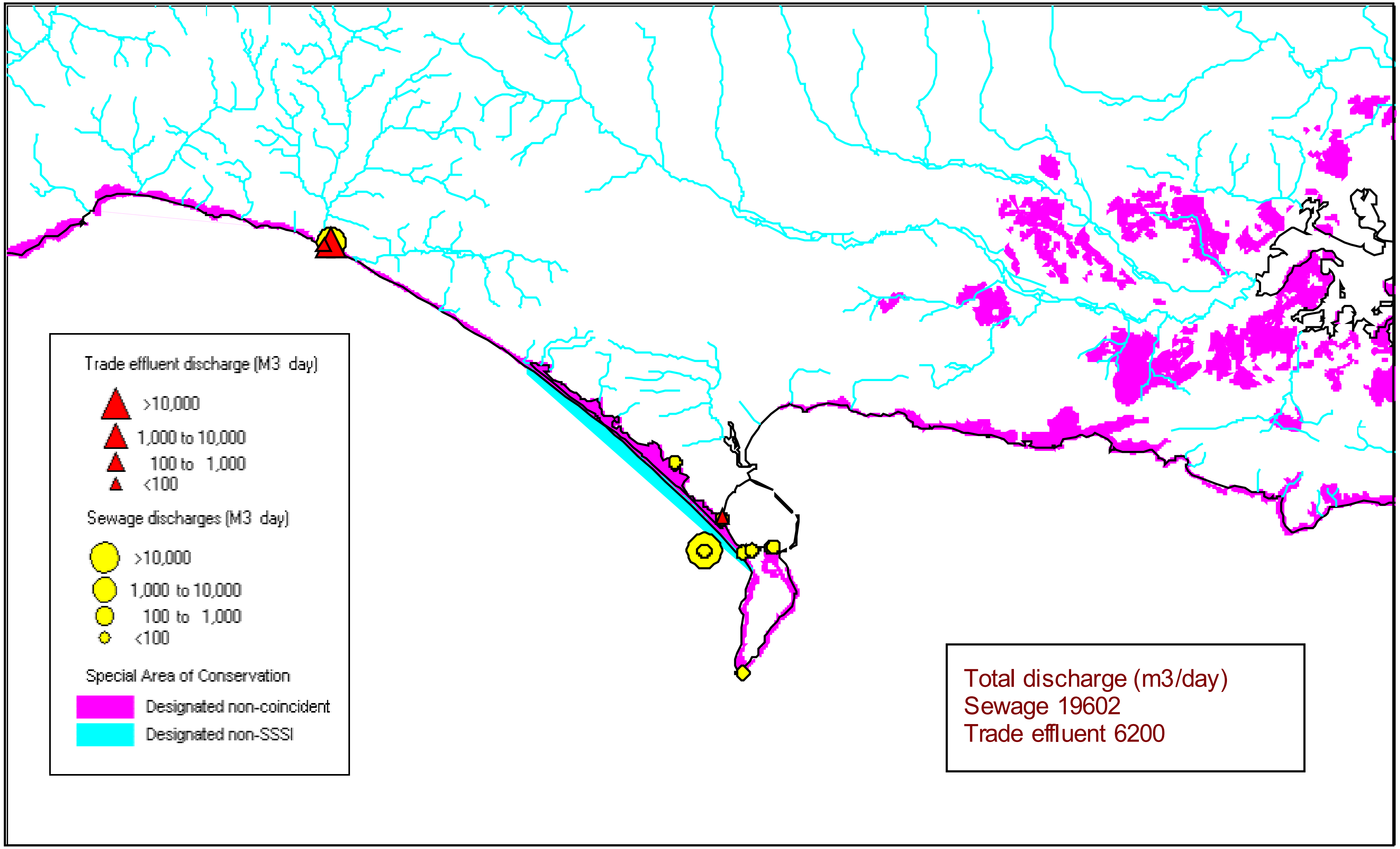

Figure 11. Chesil and the Fleet discharges

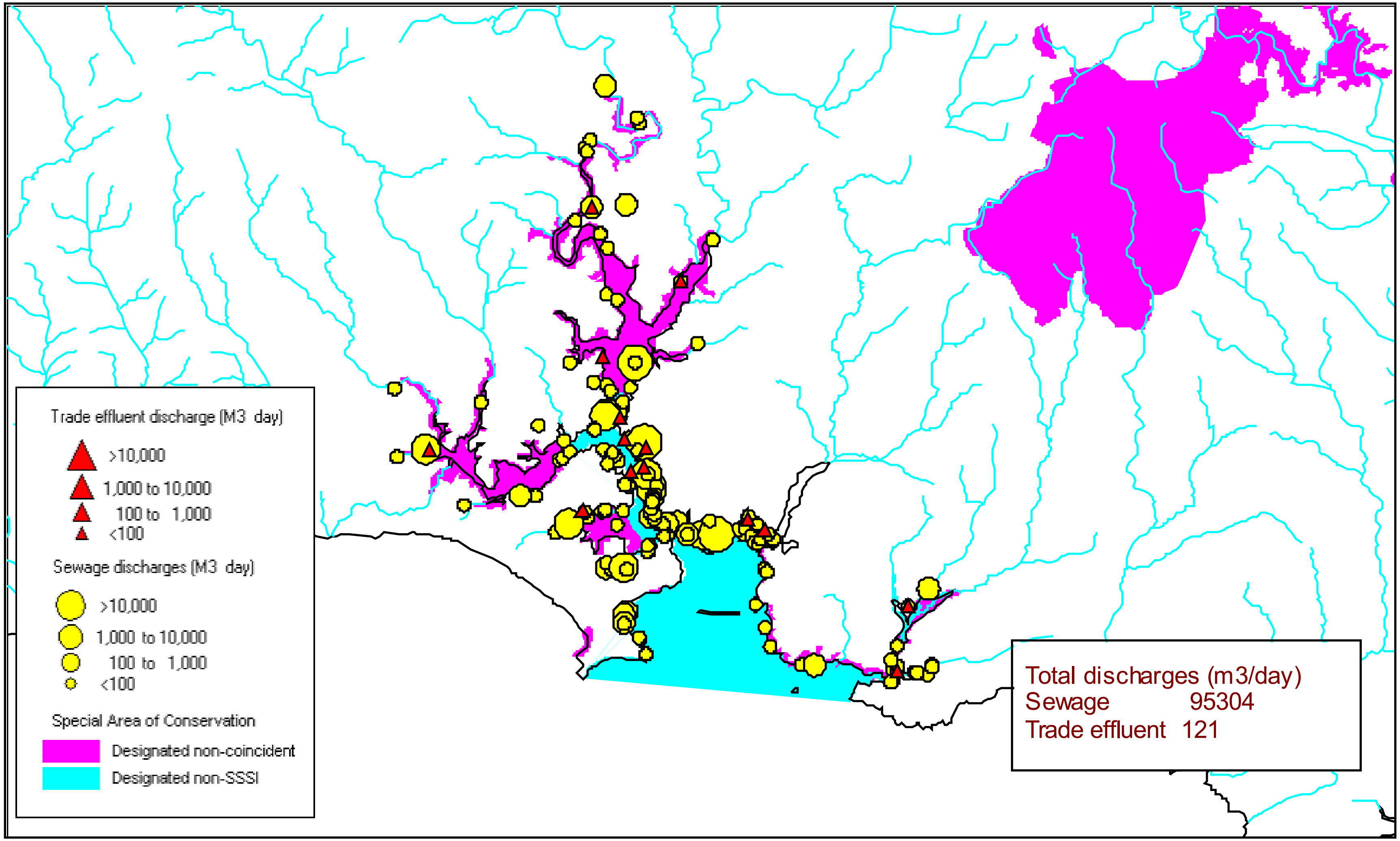

Figure 12. Plymouth Sound and Estuaries discharges

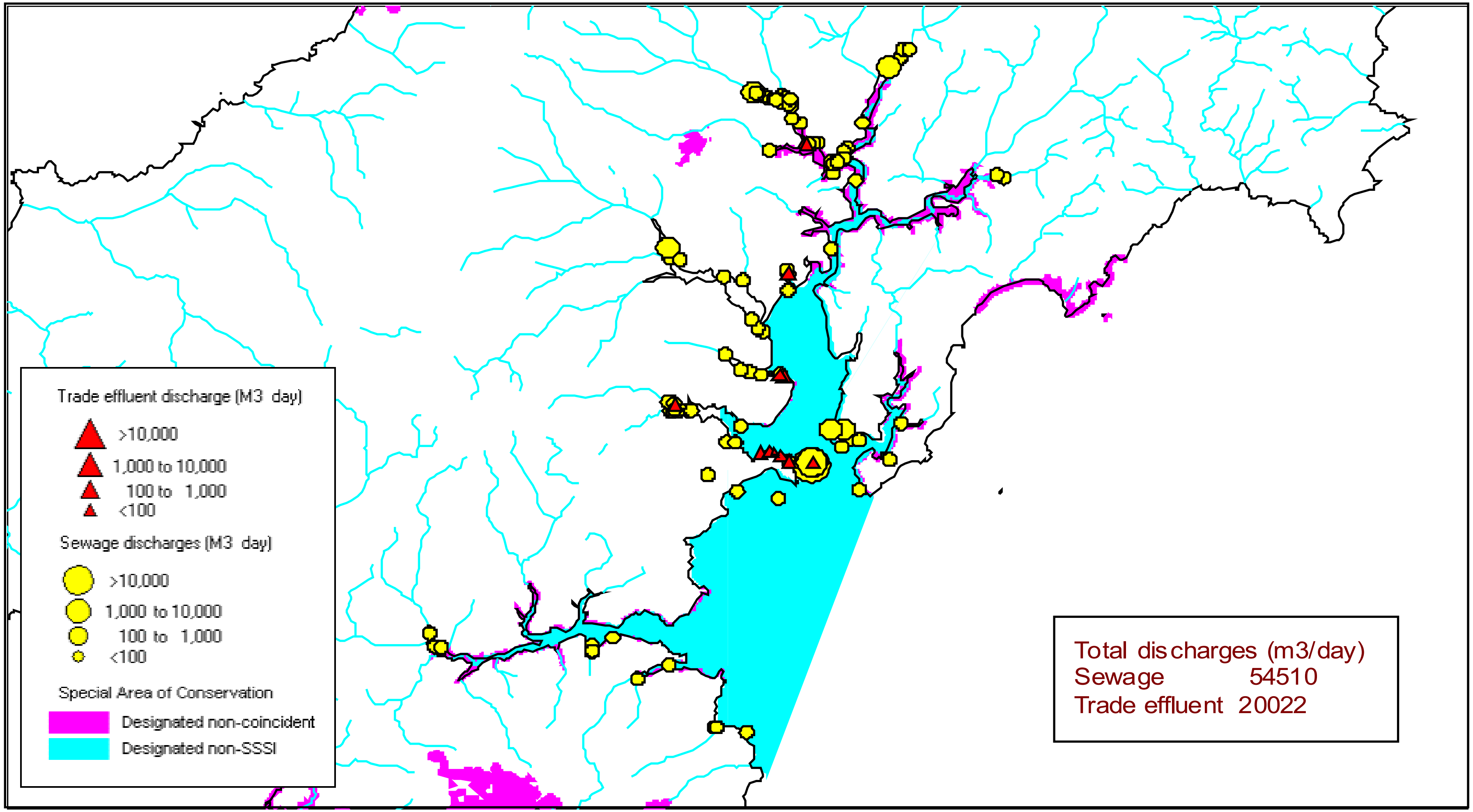

Figure 13. Fal and Helford discharges

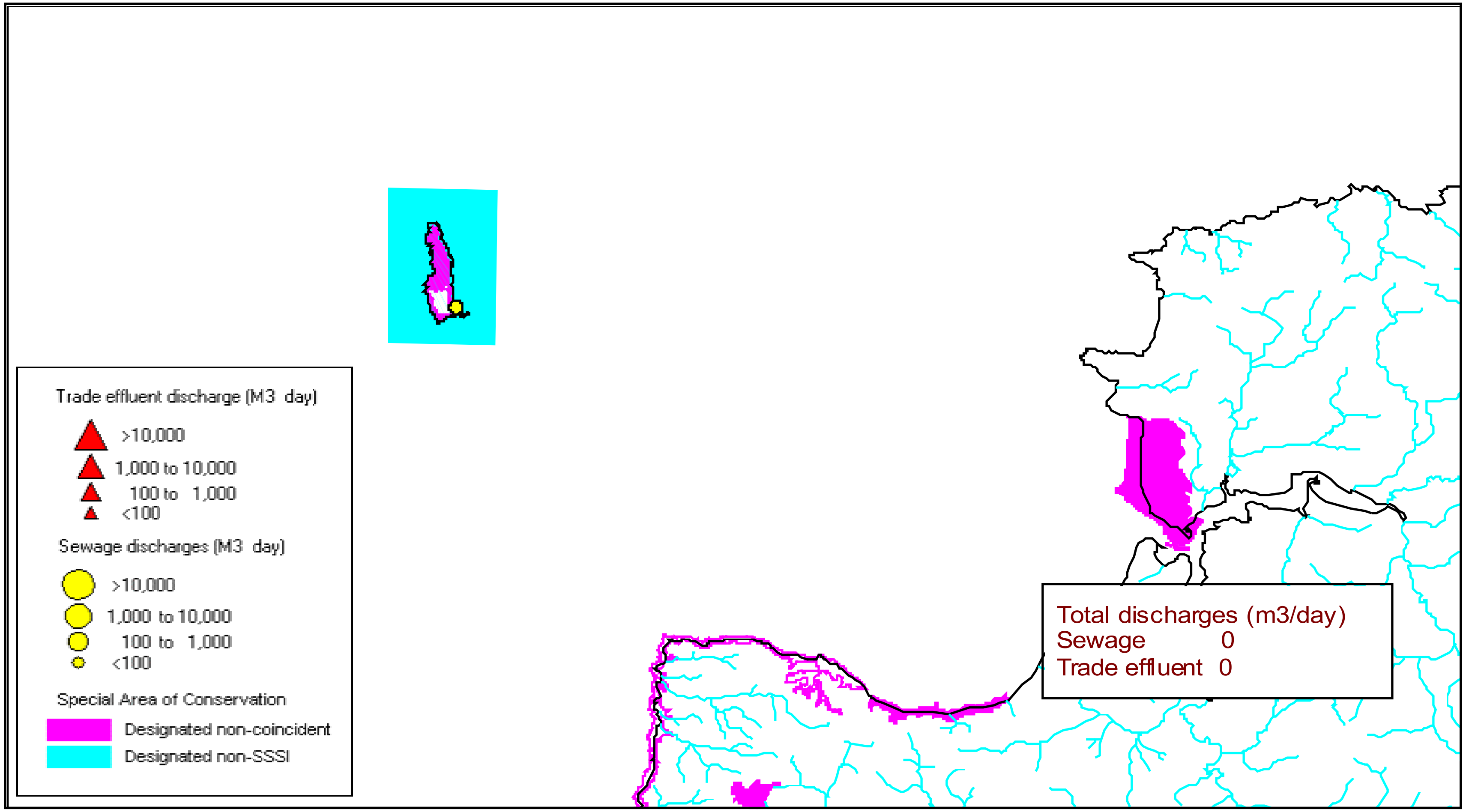

Figure 14. Lundy discharges

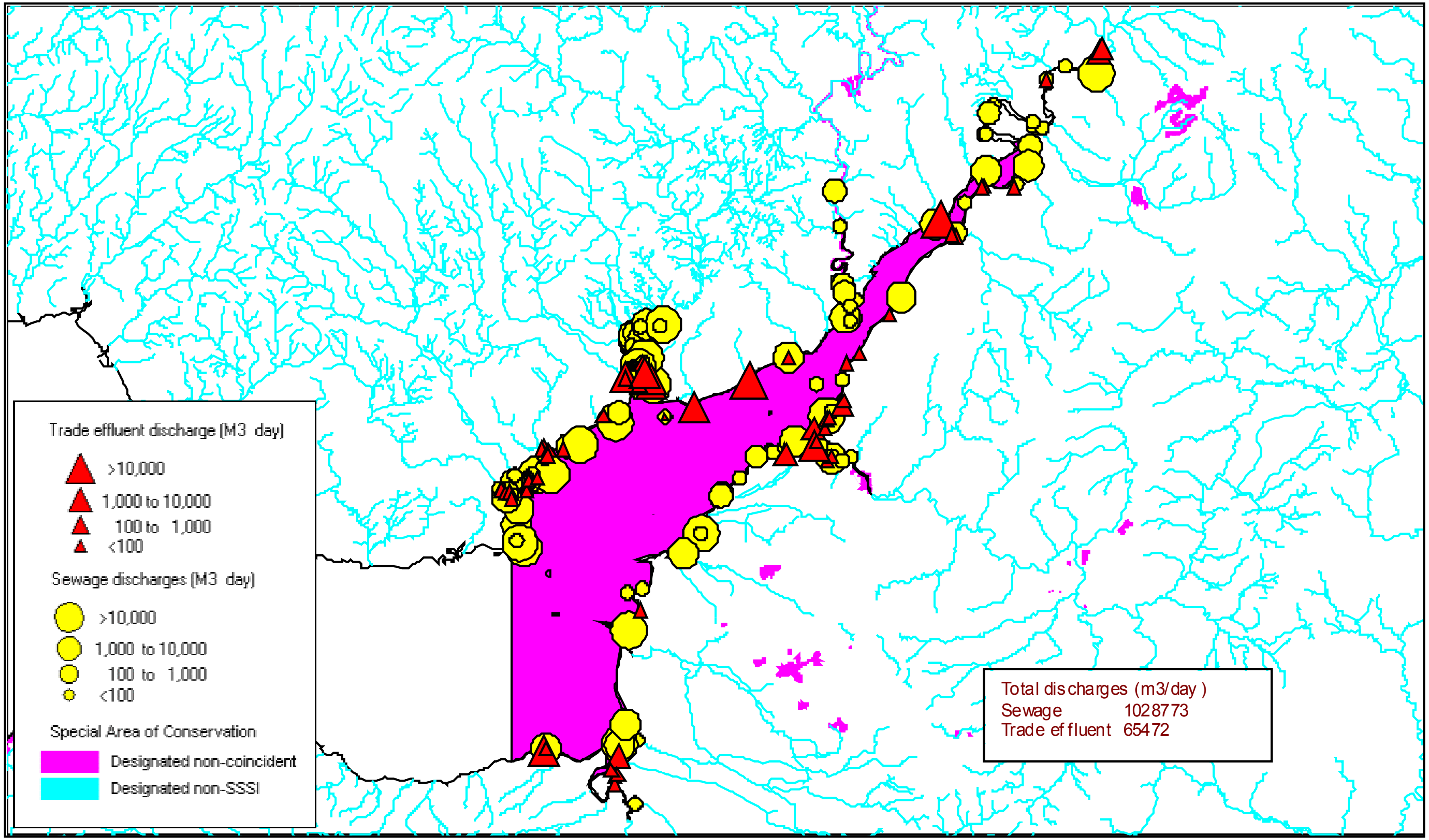

Figure 15. Severn Estuary discharges

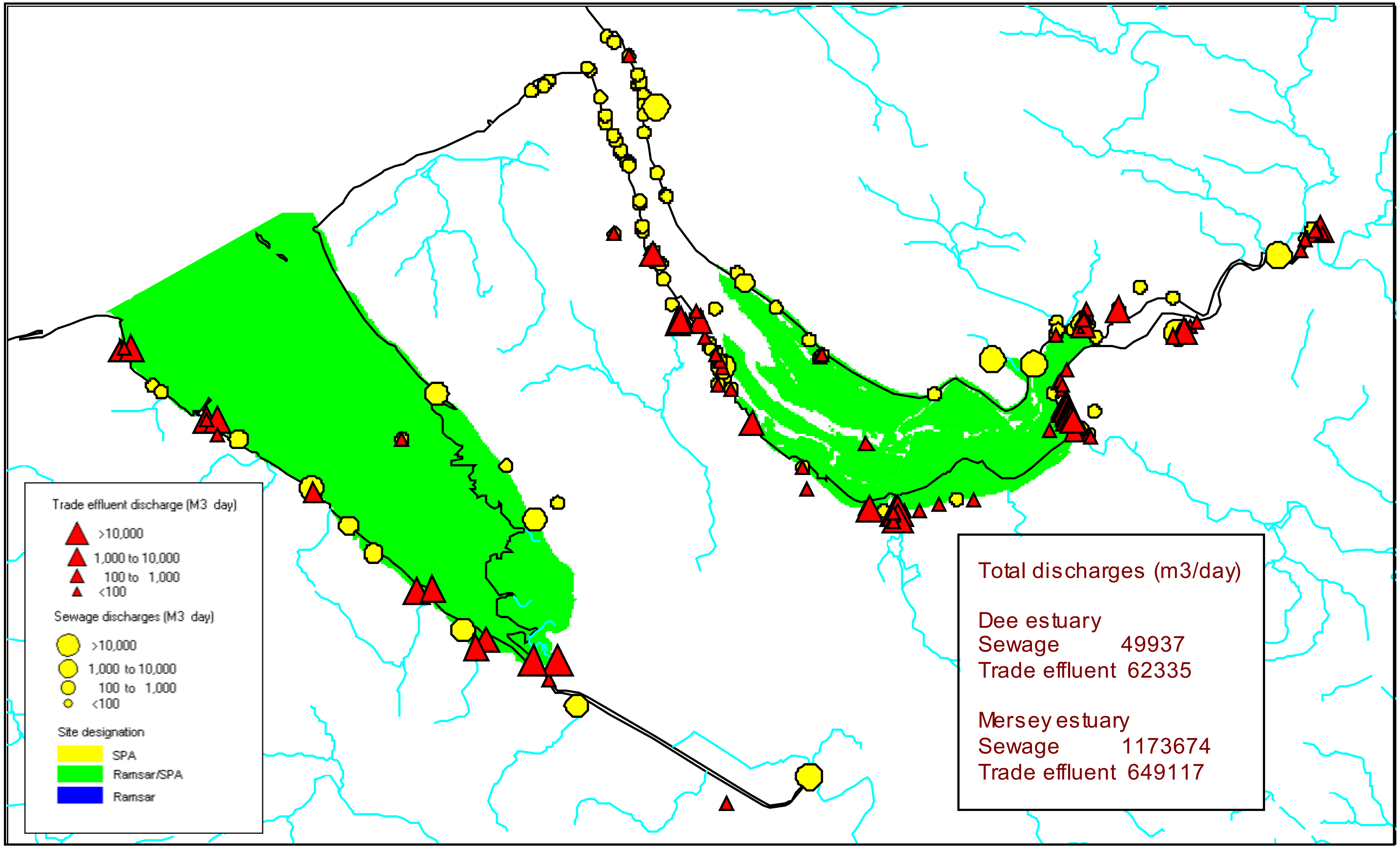

Figure 16. Dee Estuary and Mersey Estuary discharges

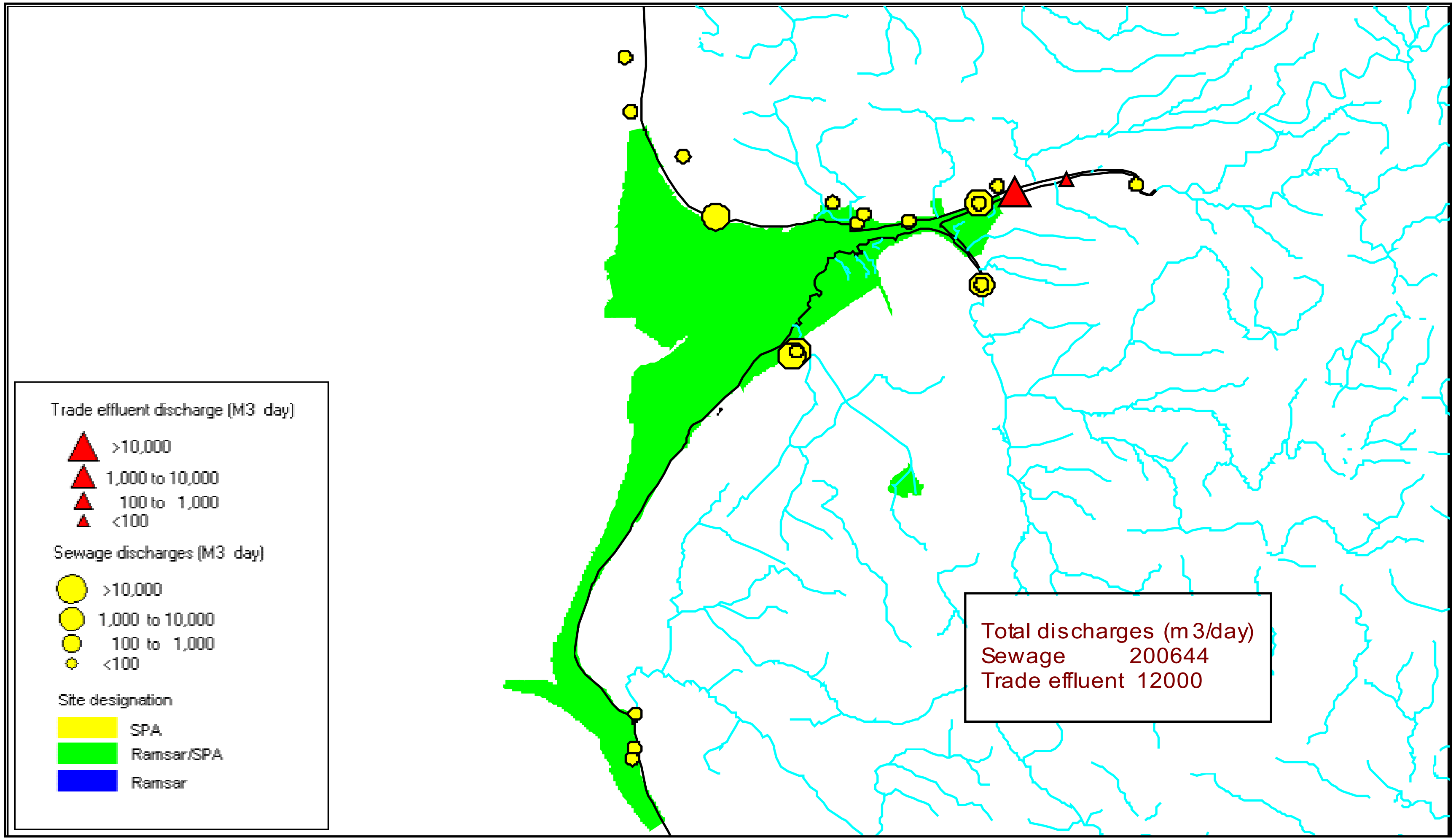

Figure 17. Ribble and Alt Estuary discharges

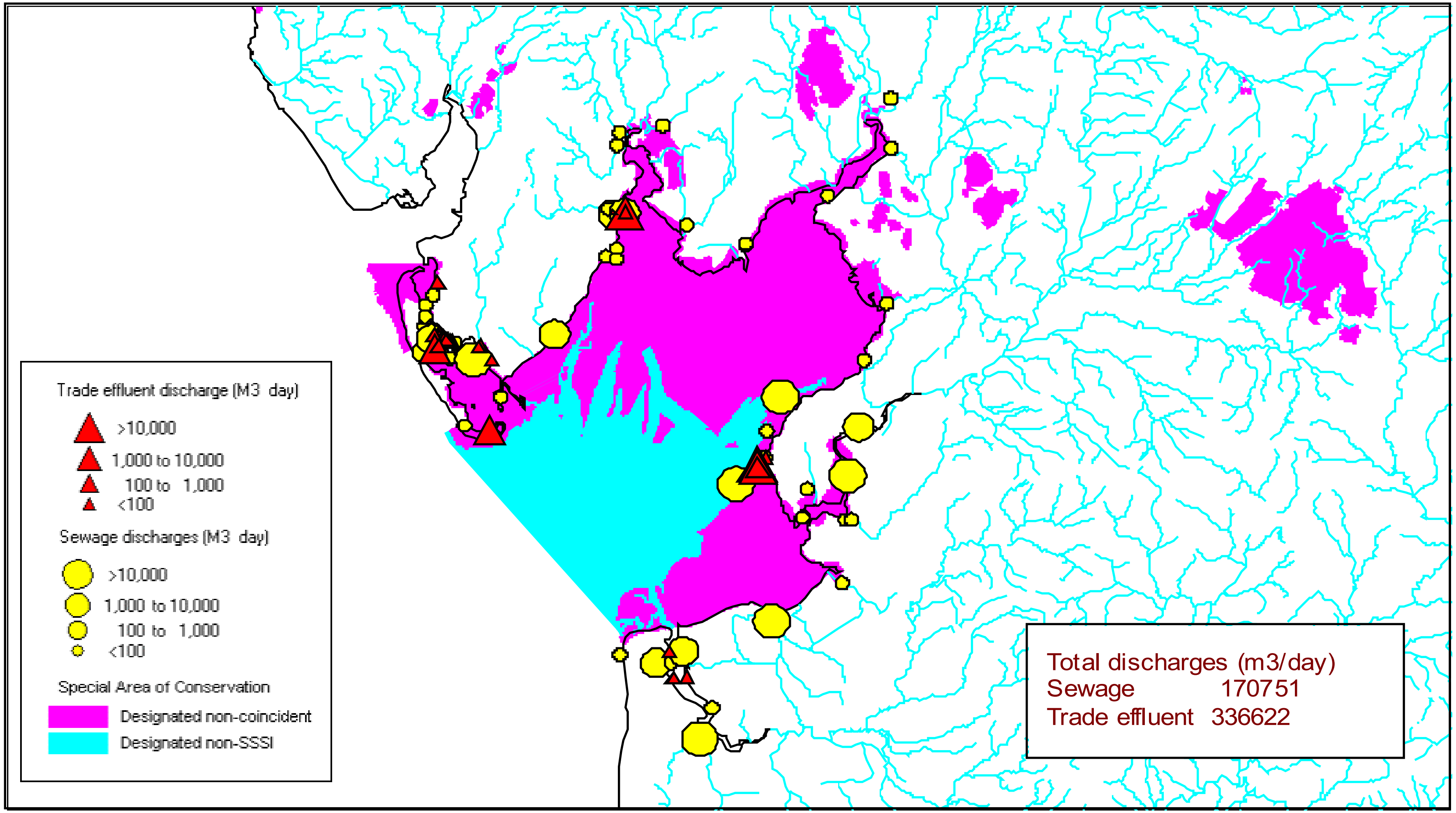

Figure 18. Morecambe Bay discharges

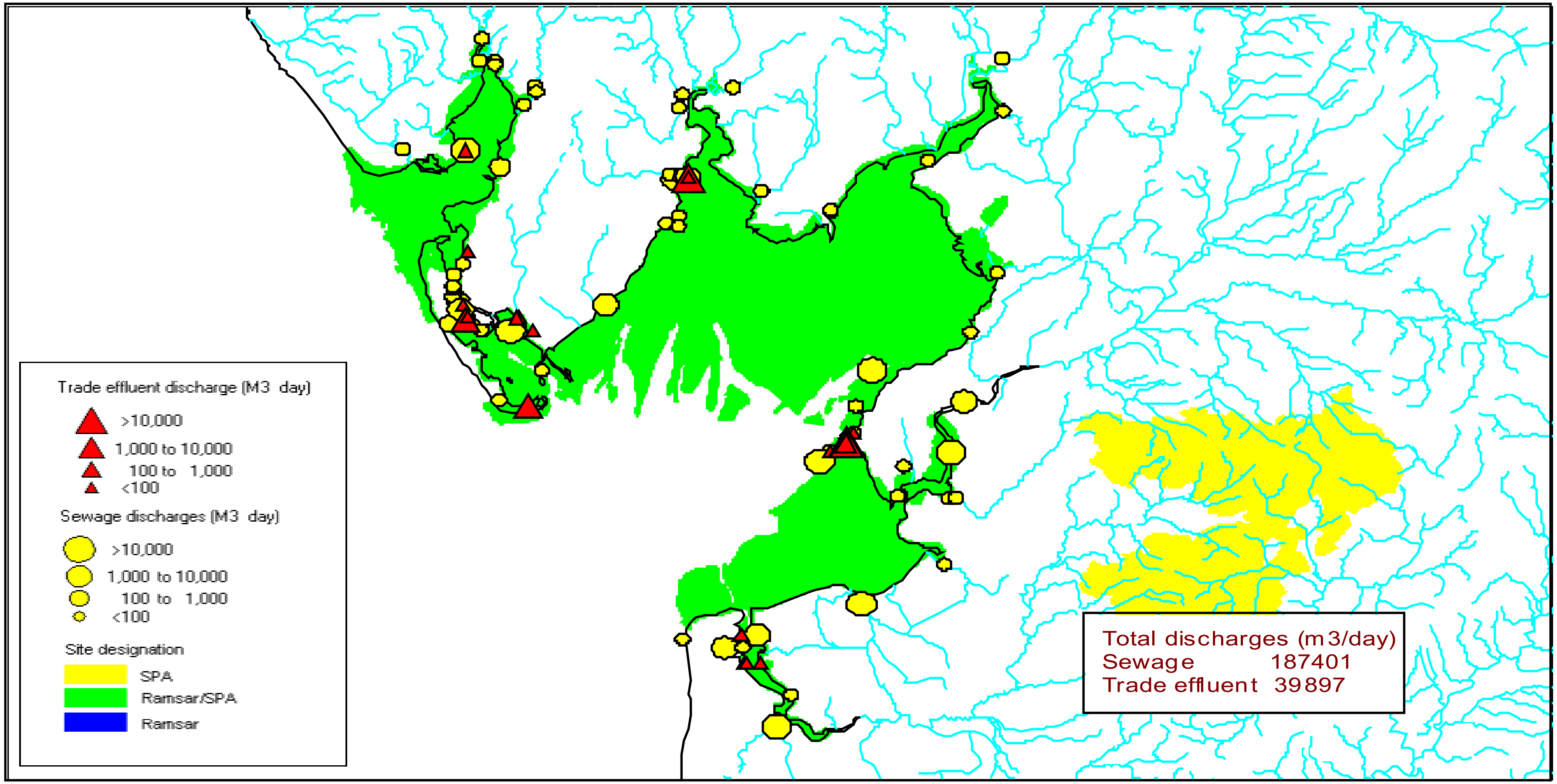

Figure 19. Duddon Estuary discharges

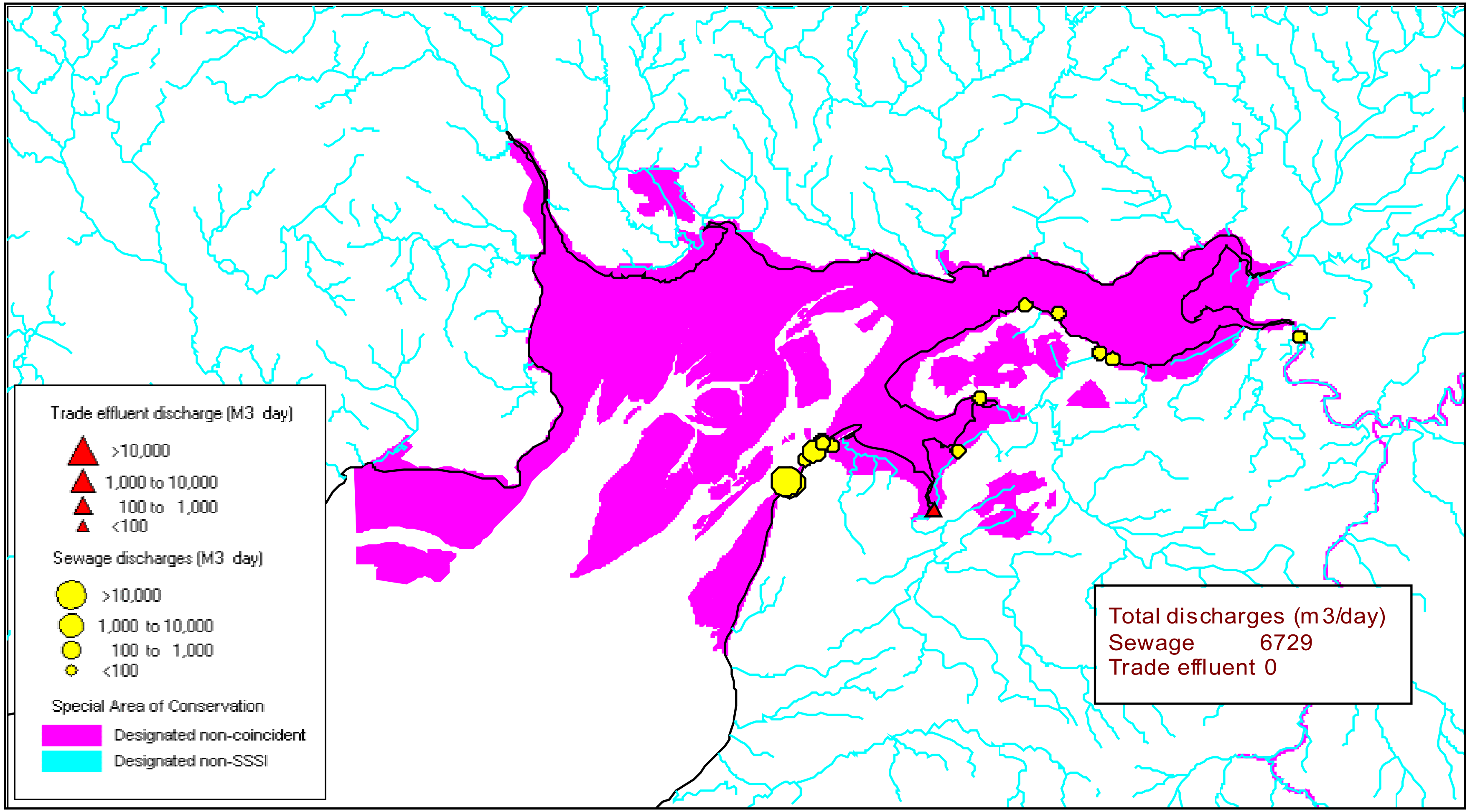

Figure 20. Solway Firth discharges

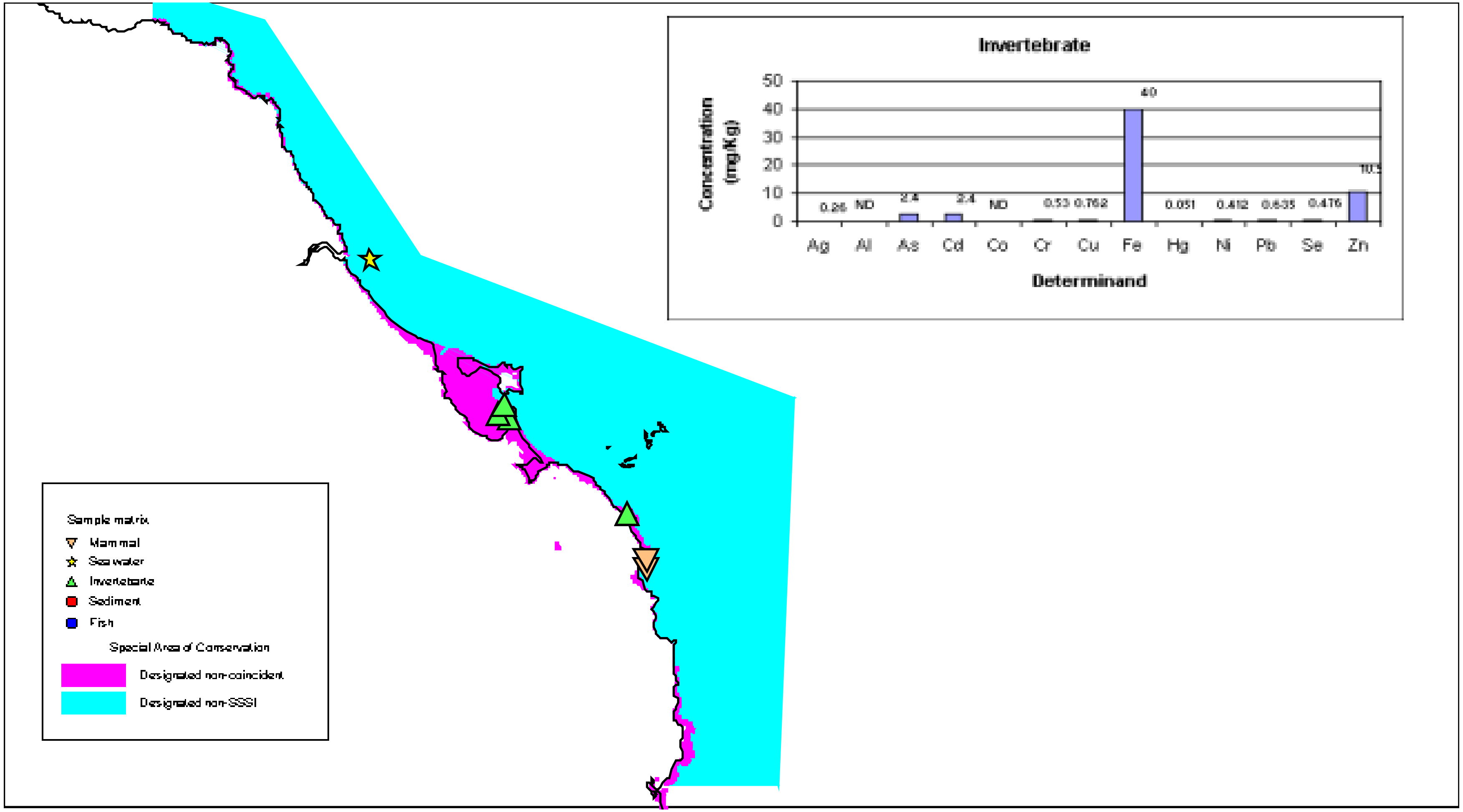

Figure 21a. Metals: Berwick and North Northumberland Coast

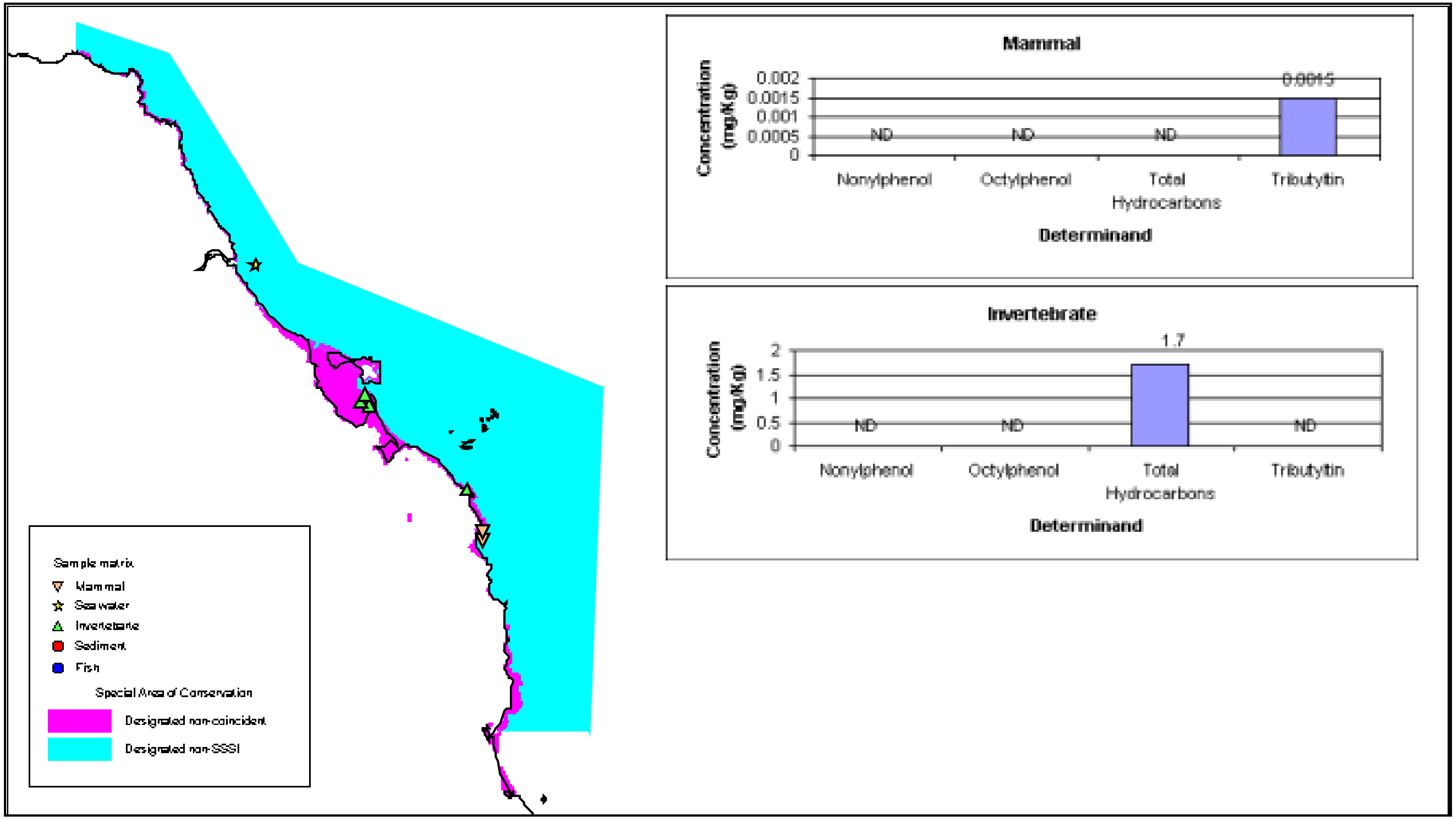

Figure 21b. Miscellaneous contaminants: Berwick and North Northumberland Coast

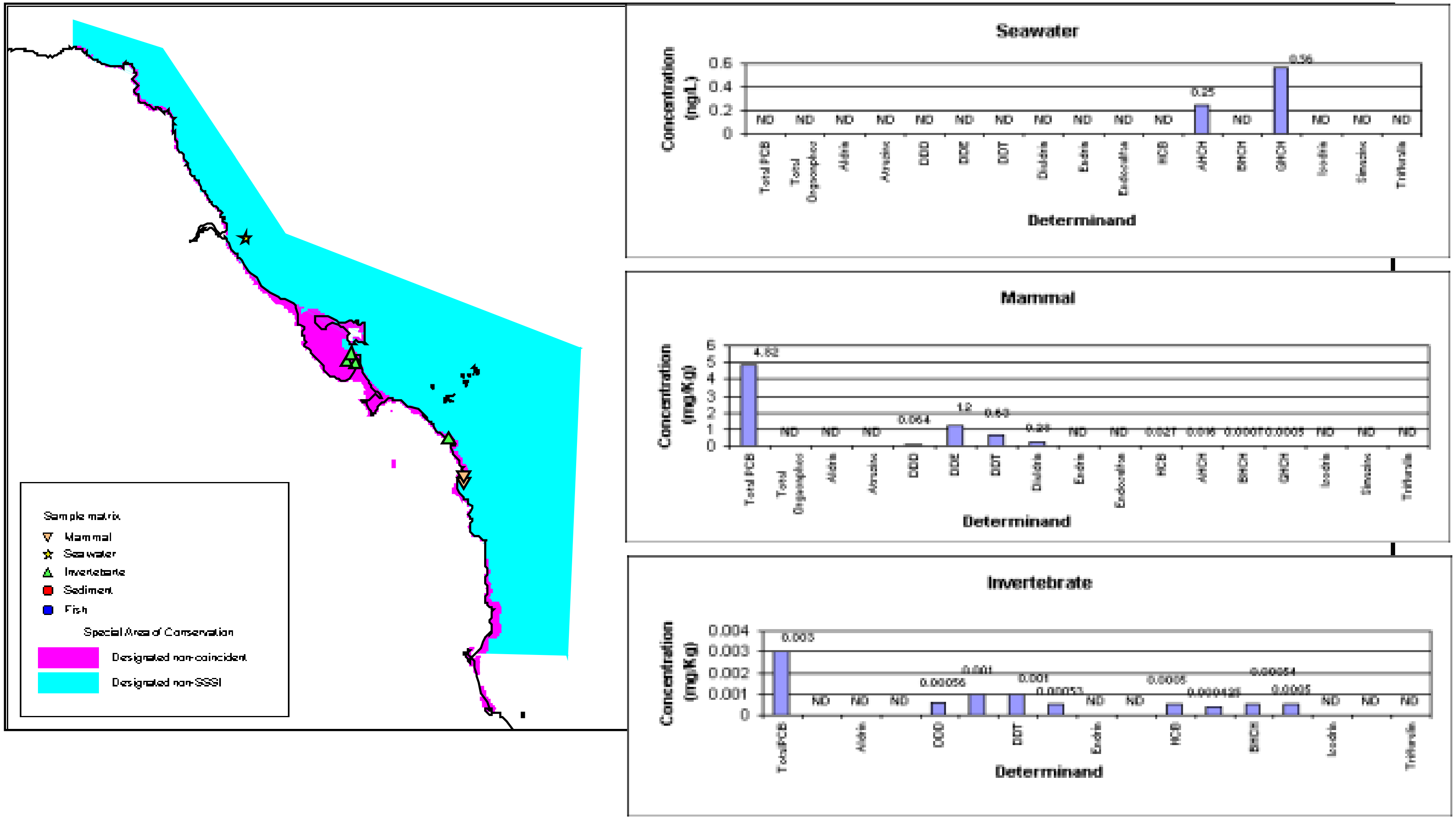

Figure 21c. PCB and pesticides: Berwick and North Northumberland Coast

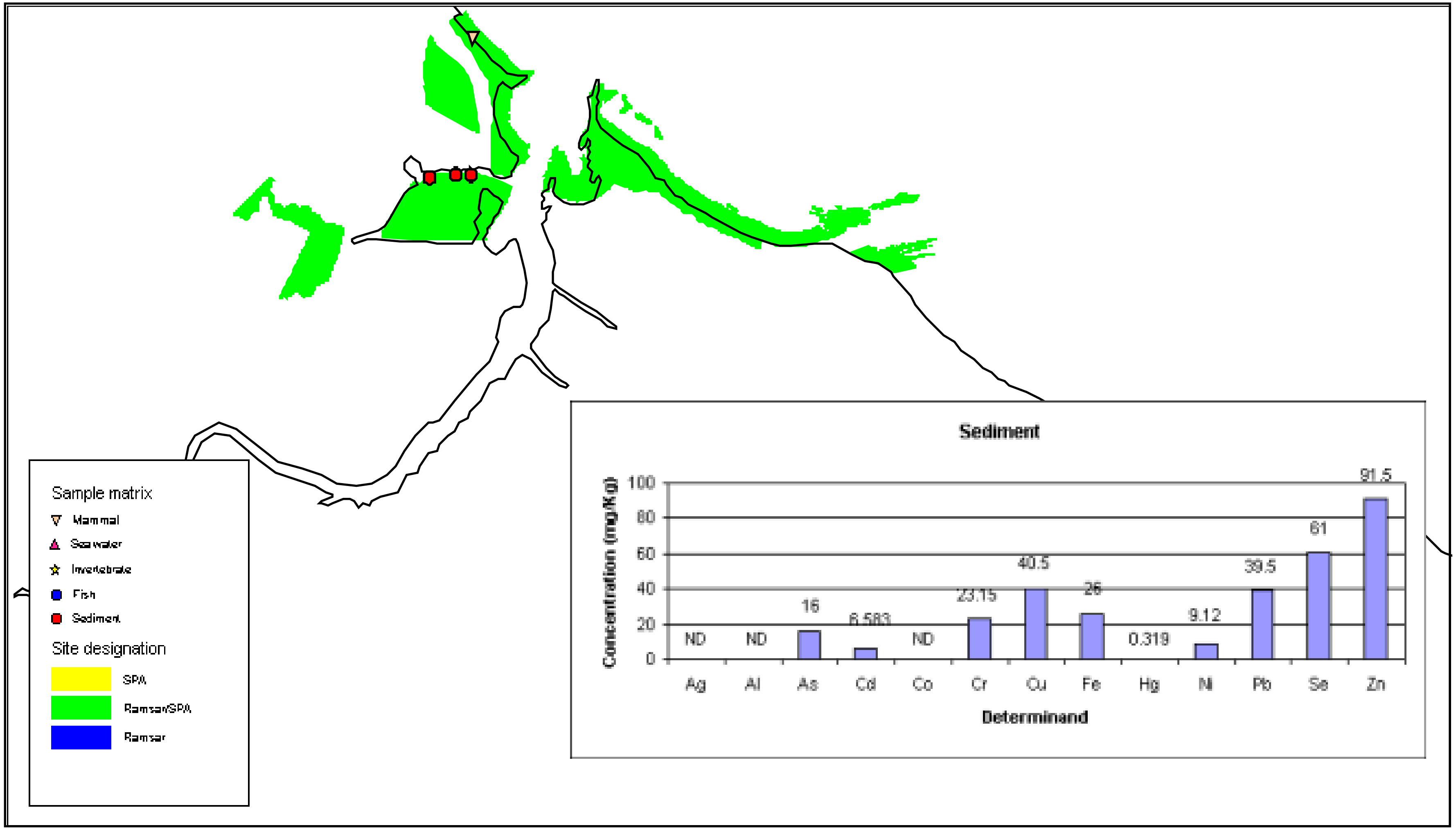

Figure 22a. Metals: Teesmouth and Cleveland Coast

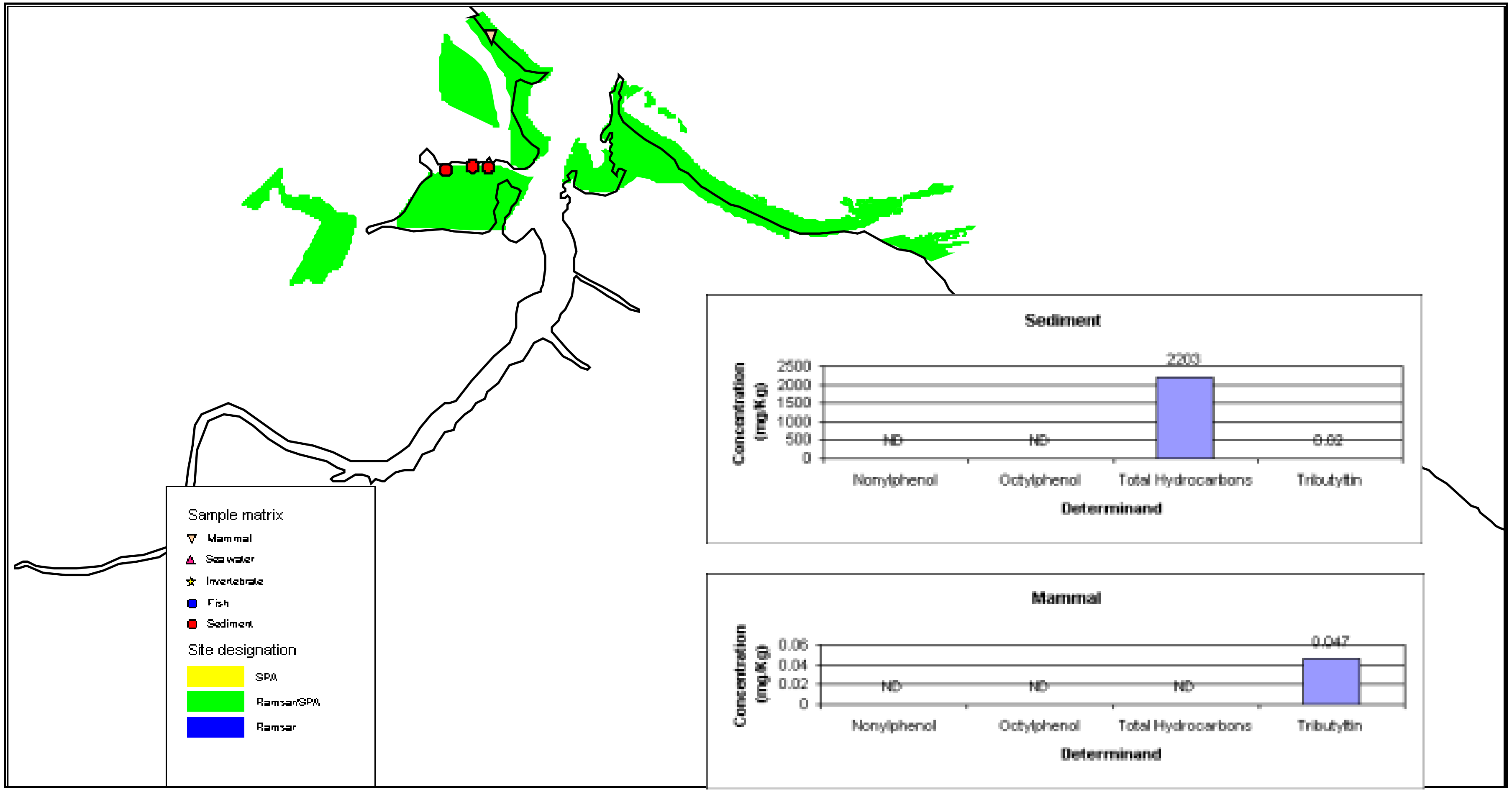

Figure 22b. Miscellaneous contaminants: Teesmouth and Cleveland Coast

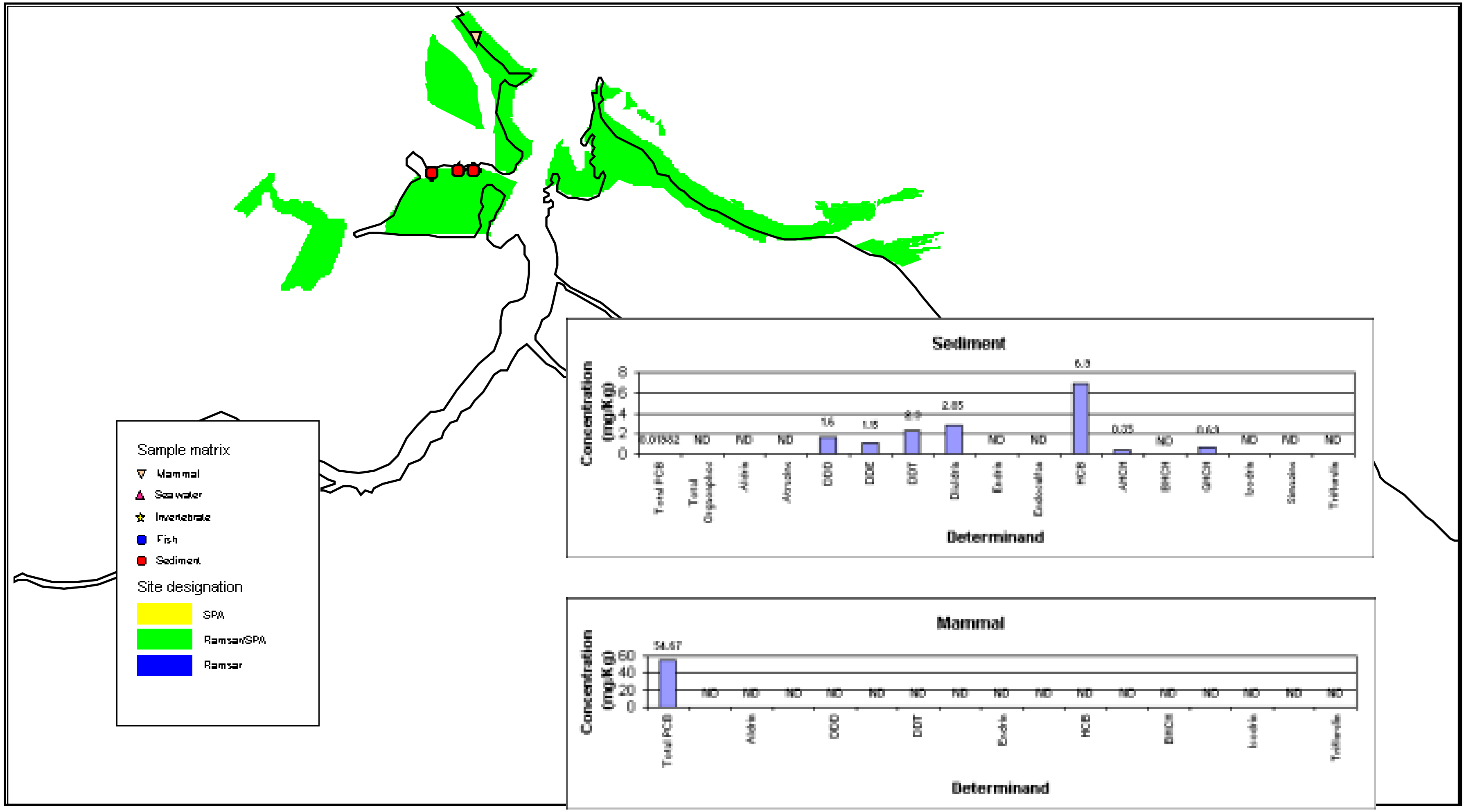

Figure 22c. PCB and pesticides: Teesmouth and Cleveland Coast

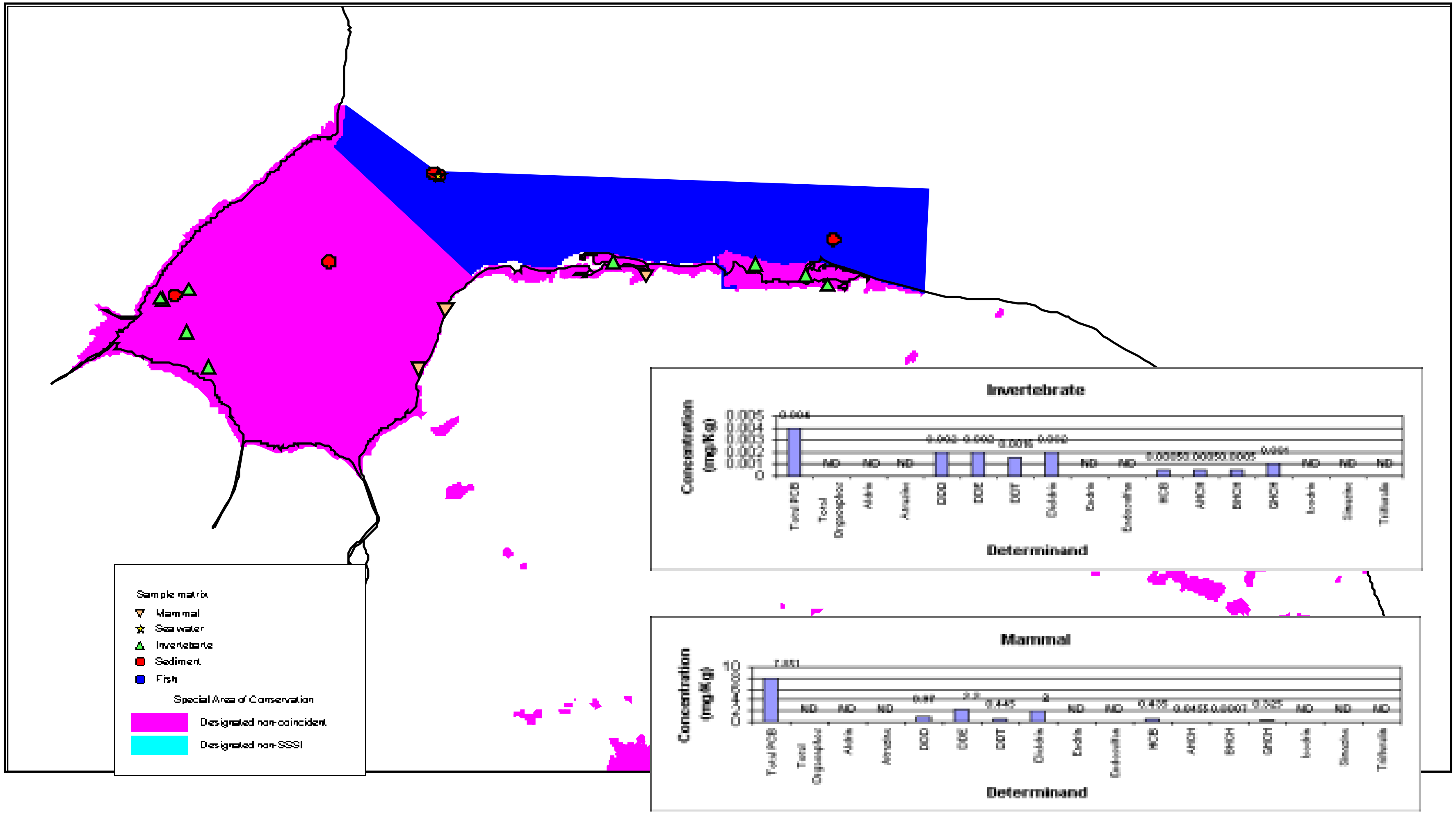

Figure 23c. PCB and pesticides: Wash and North Norfolk Coast

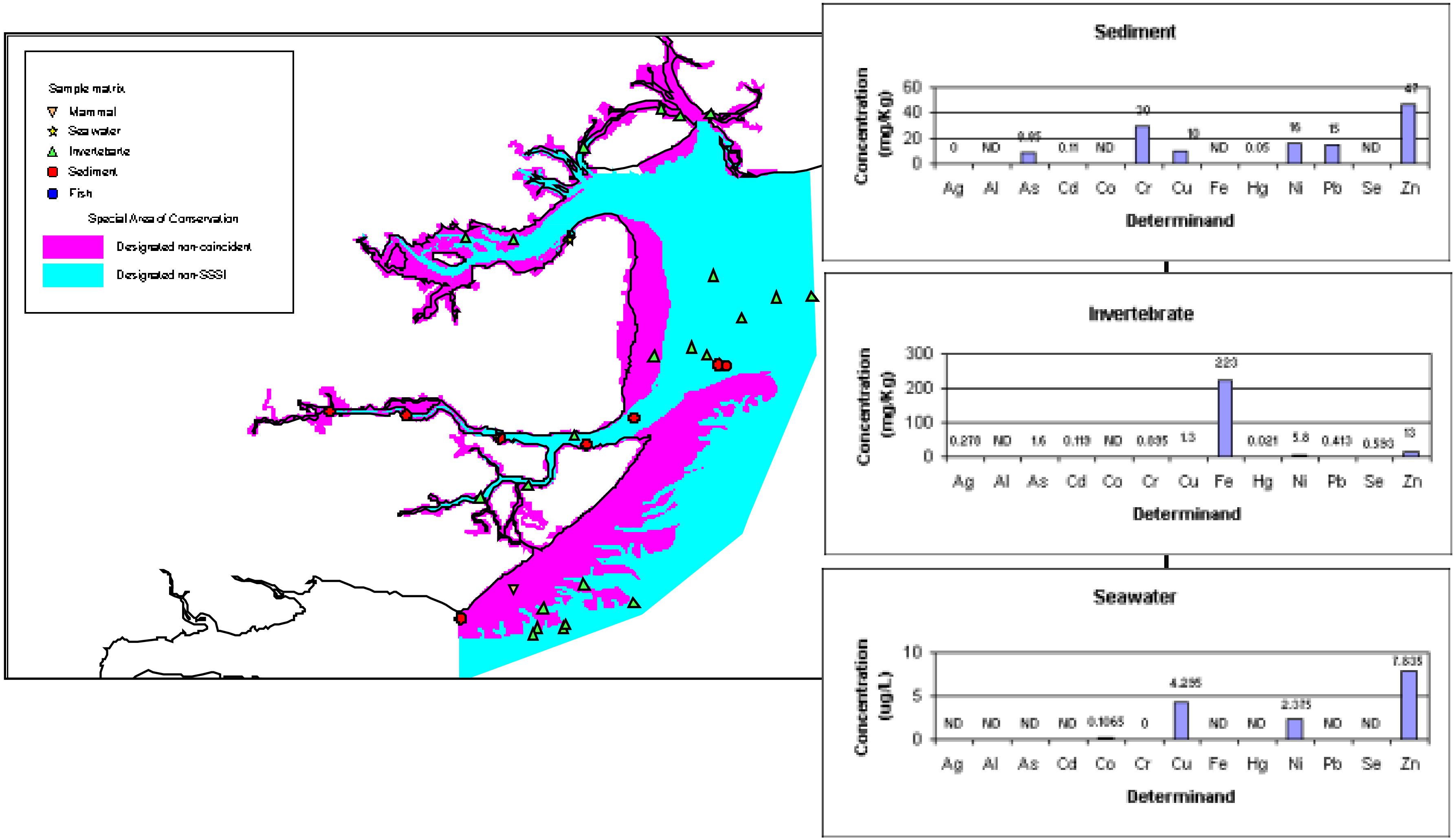

Figure 24a. Metals: Essex estuaries

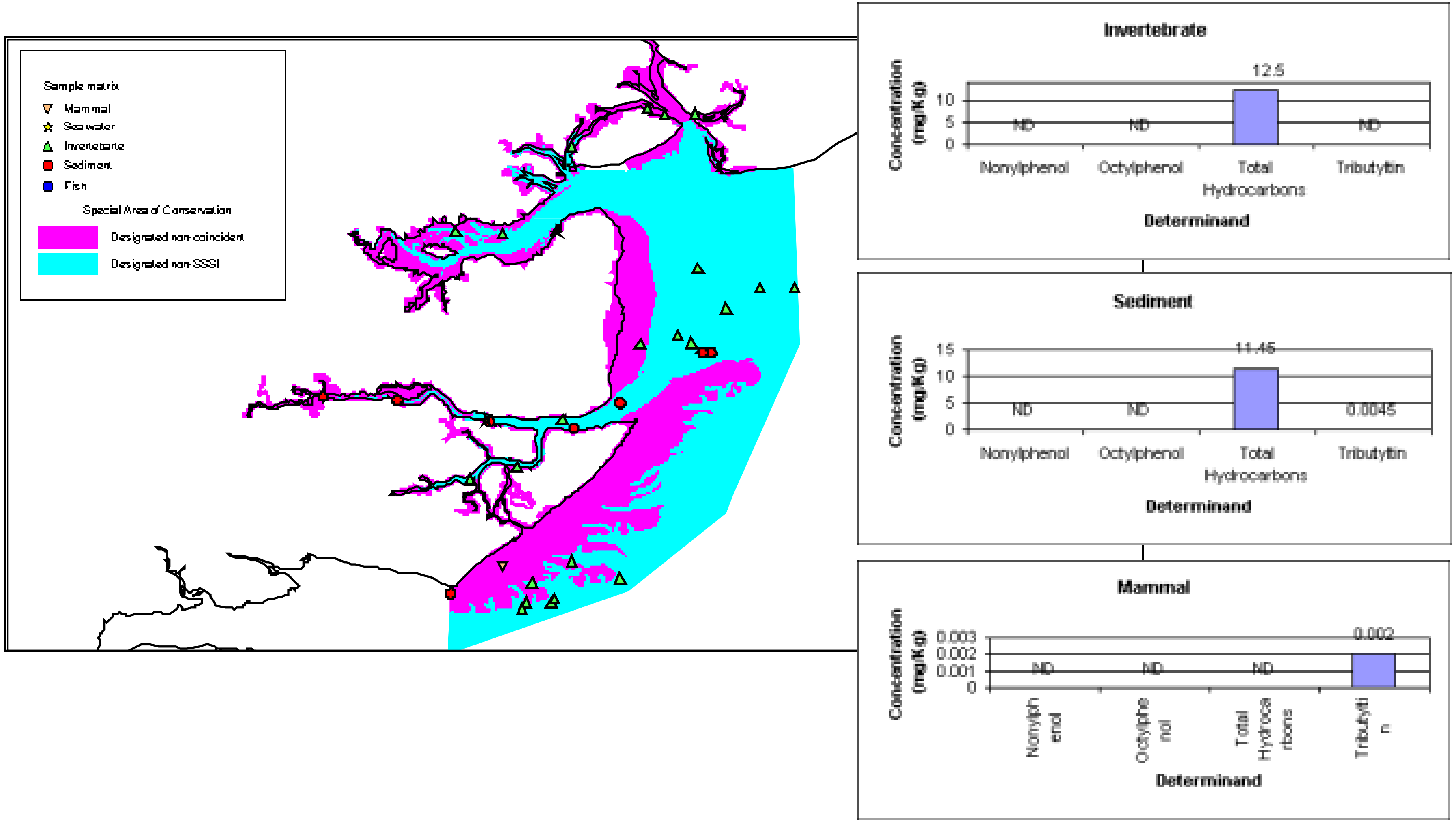

Figure 24b. Miscellaneous contaminants: Essex Estuaries

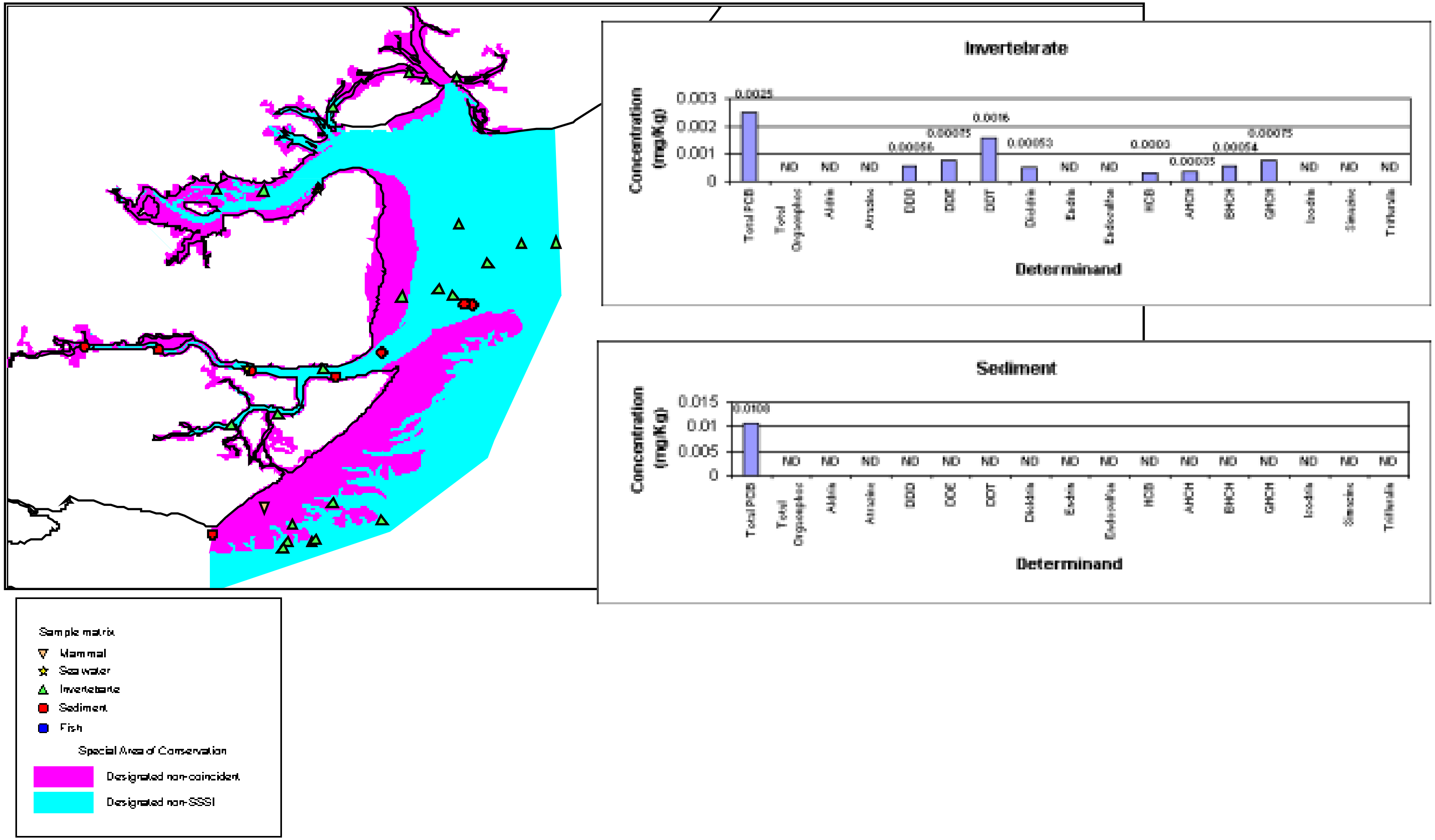

Figure 24c. PCB and pesticides: Essex Estuaries

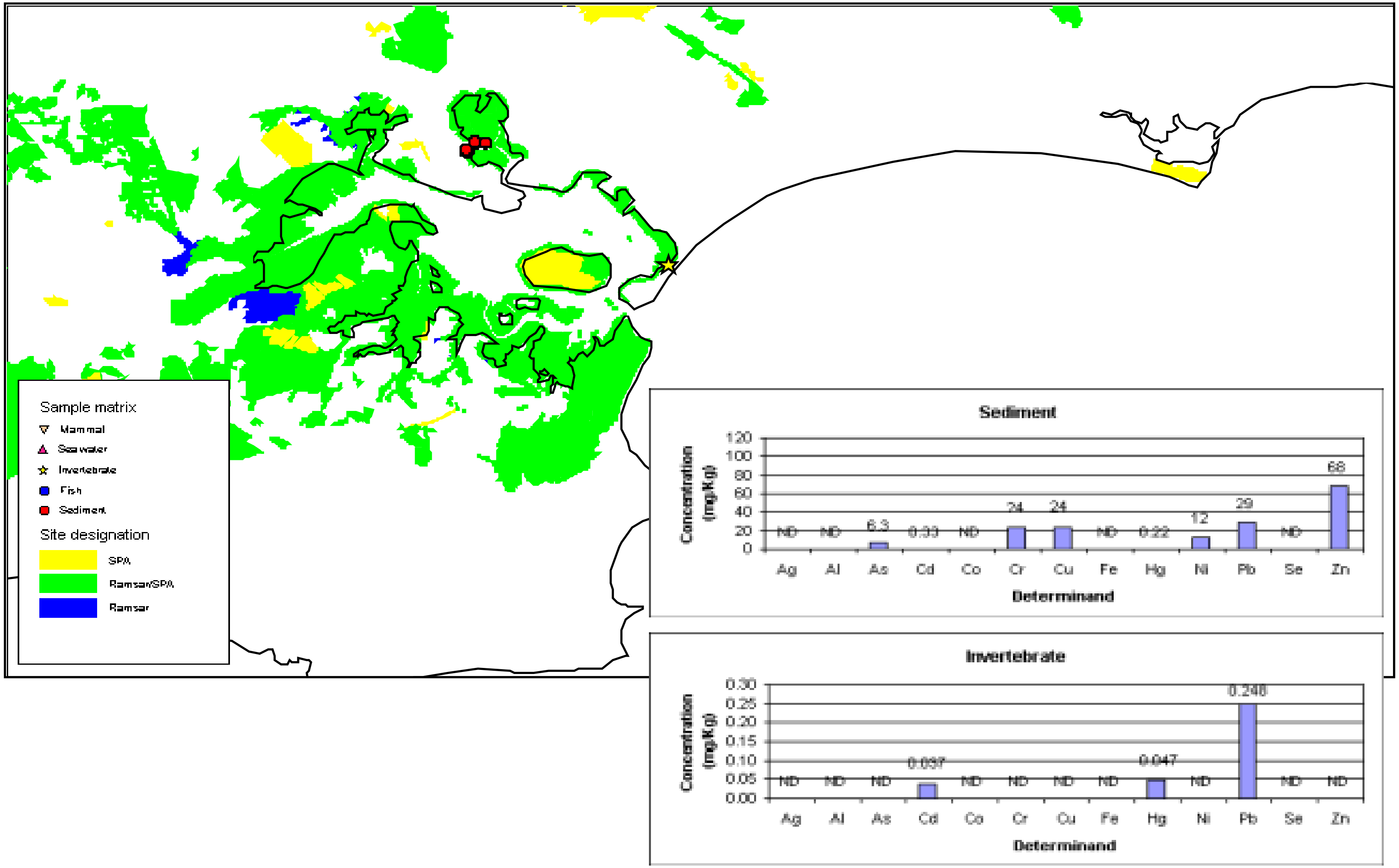

Figure 25a. Metals: Poole Harbour

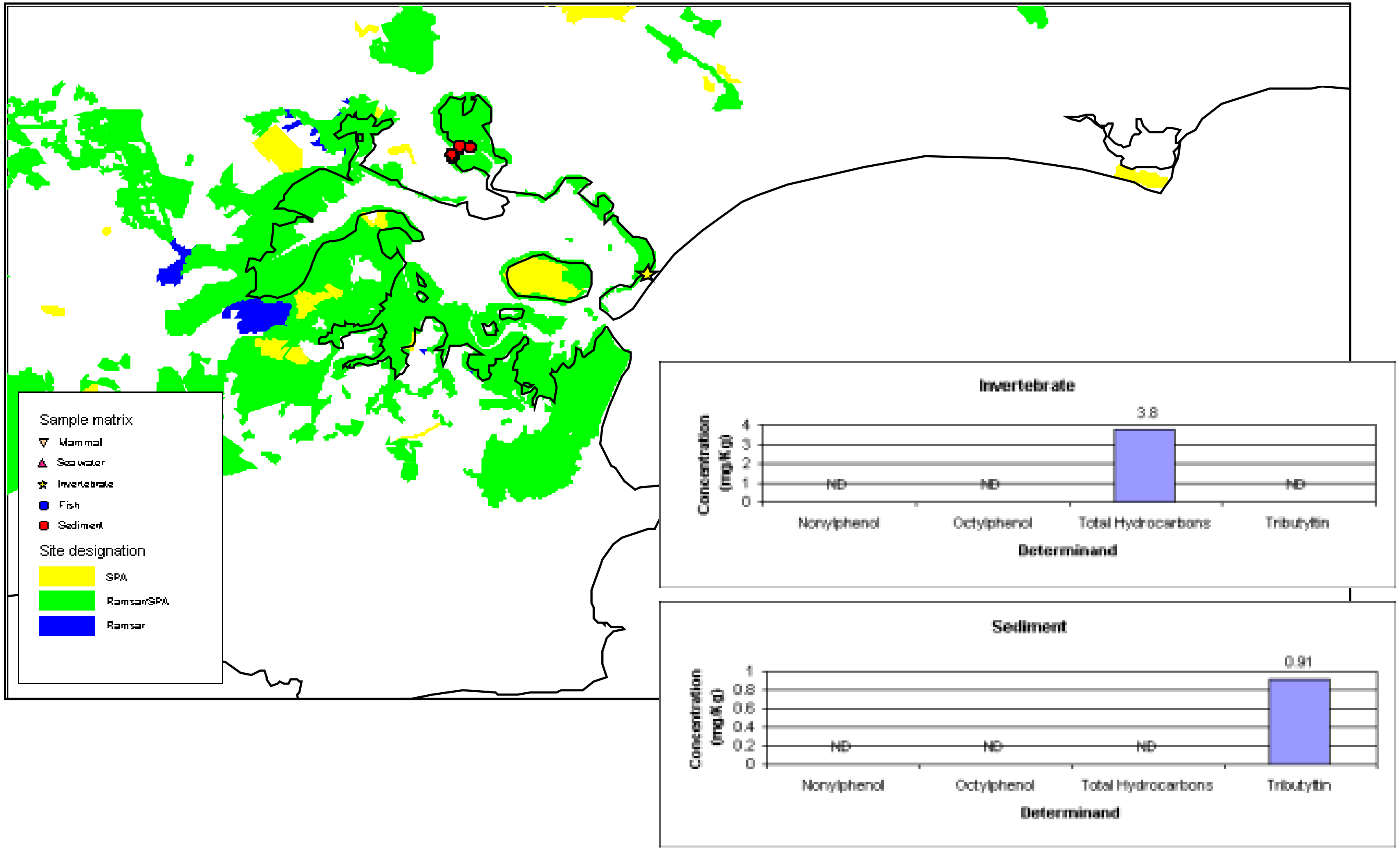

Figure 25b. Miscellaneous contaminants: Poole Harbour

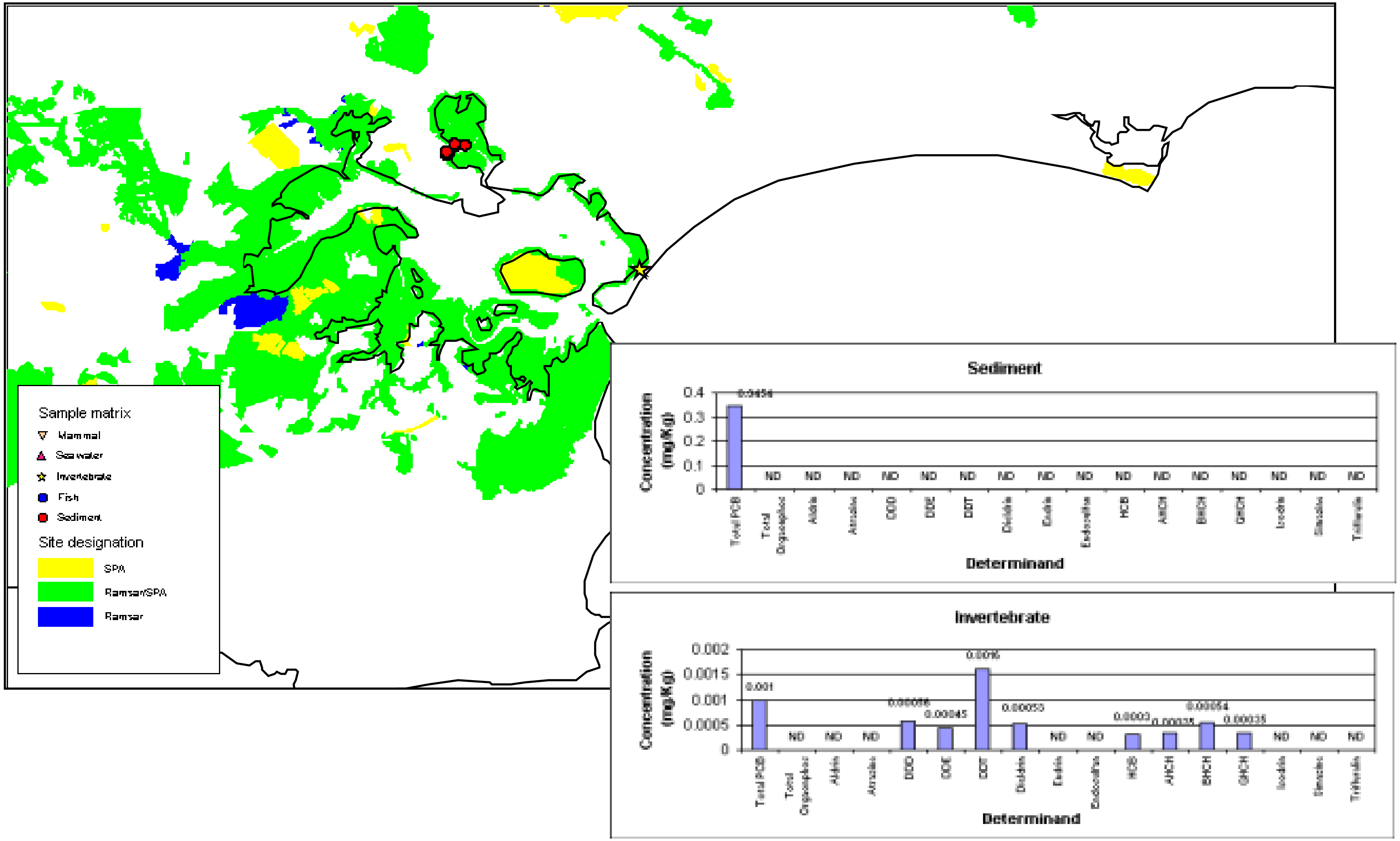

Figure 25c. PCB and pesticides: Poole Harbour

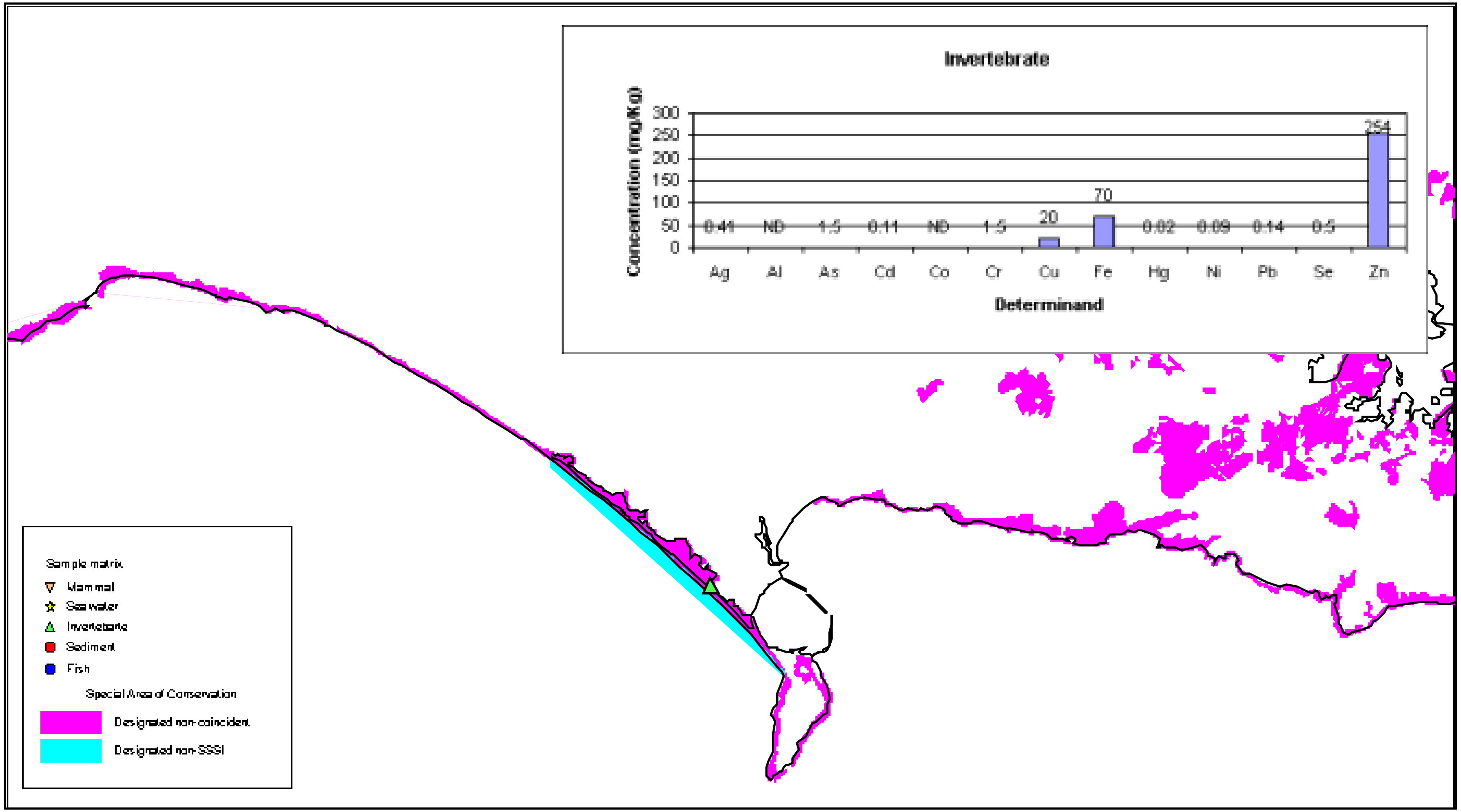

Figure 26a. Metals: Chesil and the Fleet

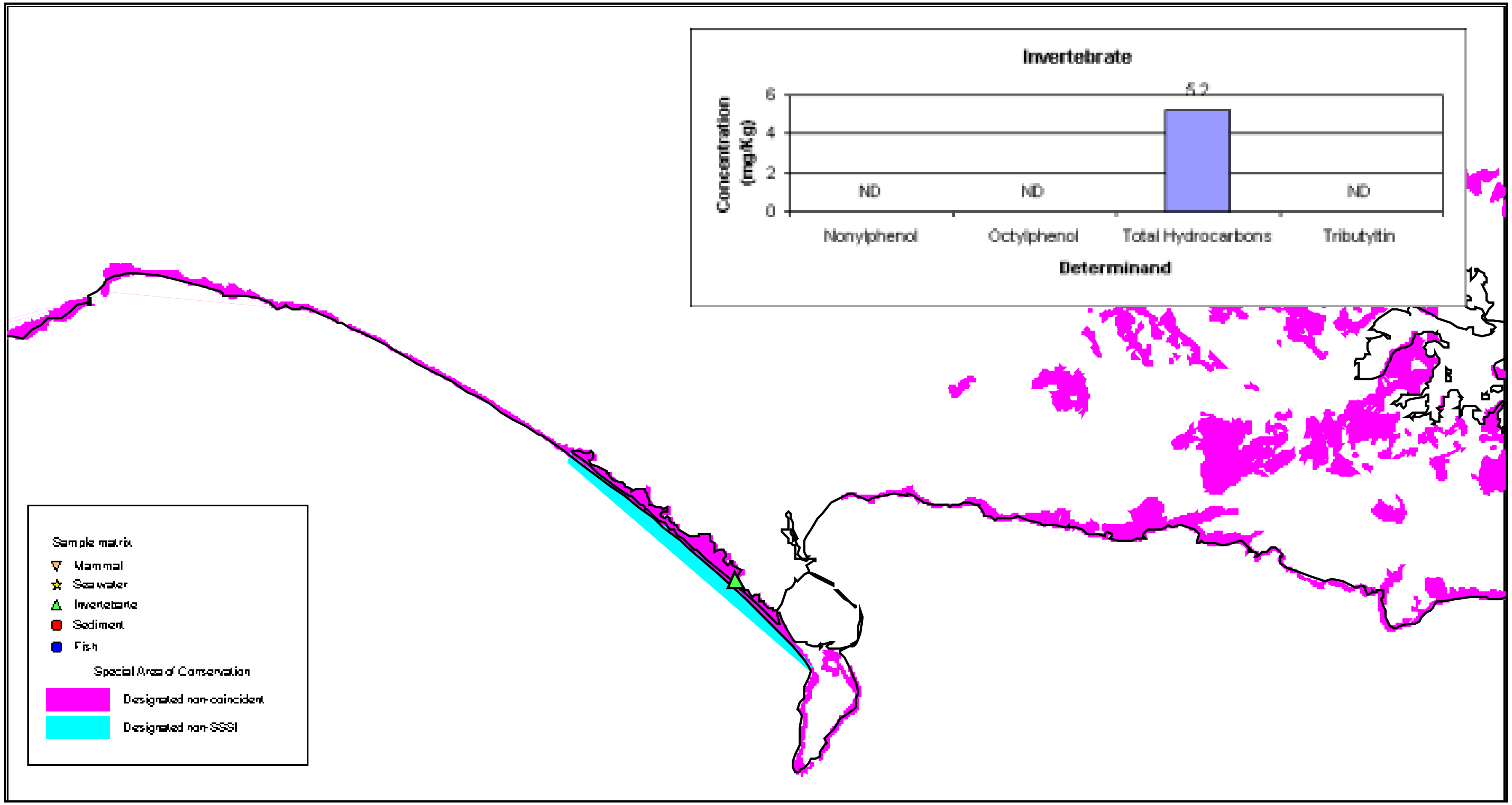

Figure 26b. Miscellaneous contaminants: Chesil and the Fleet

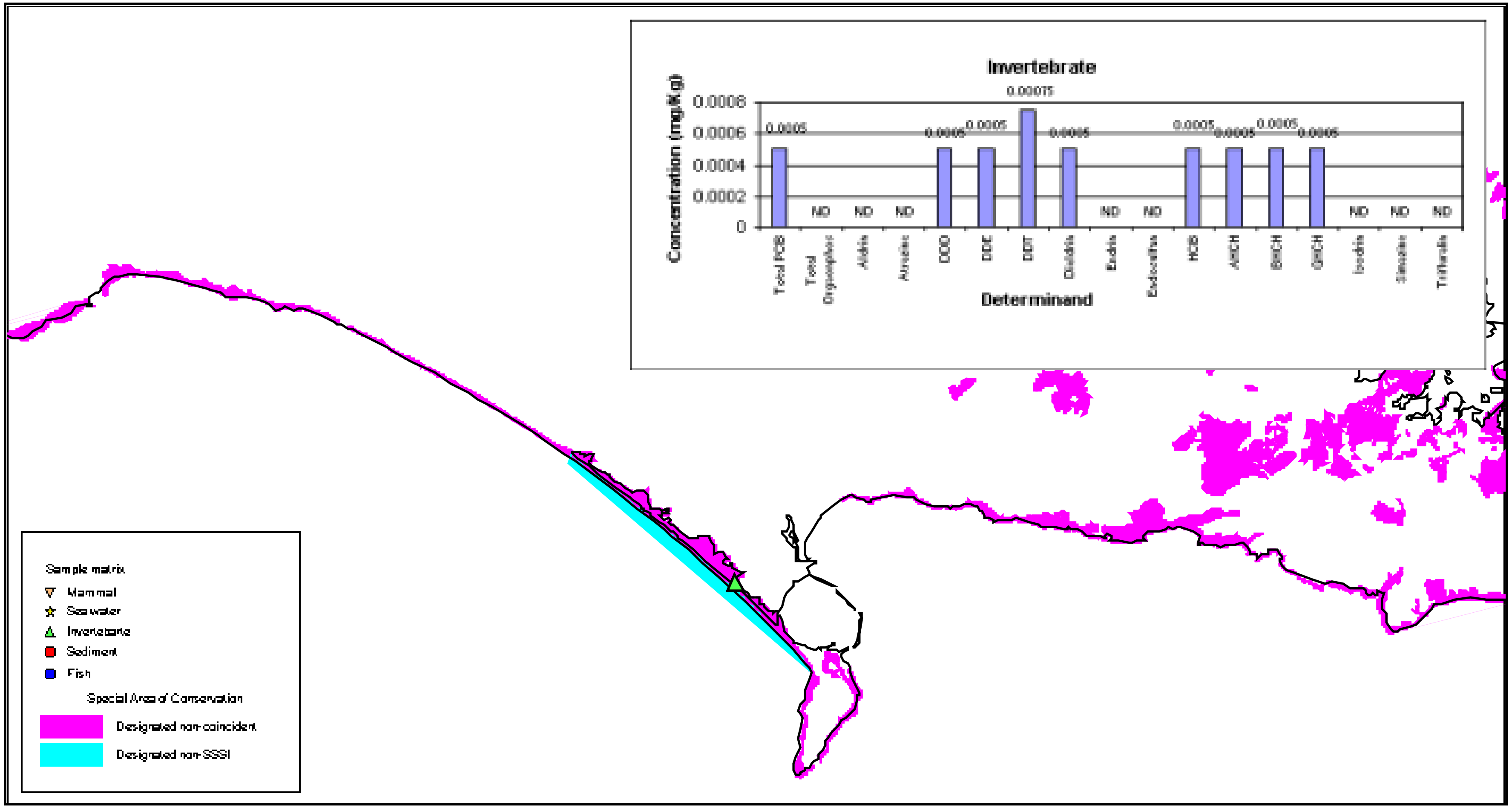

Figure 26c. PCB and pesticides: Chesil and the Fleet

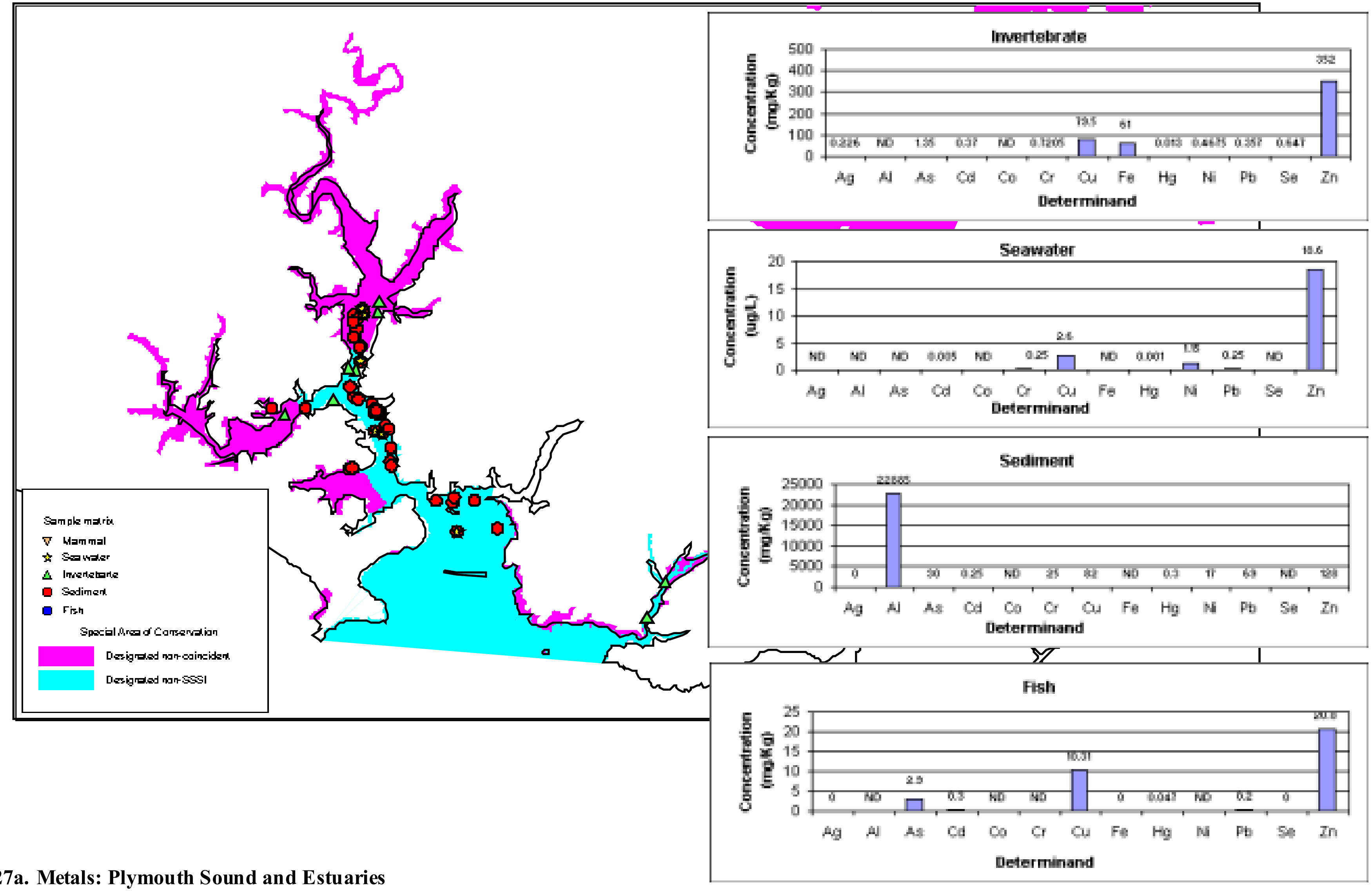

Figure 27a. Metals: Plymouth Sound and Estuaries

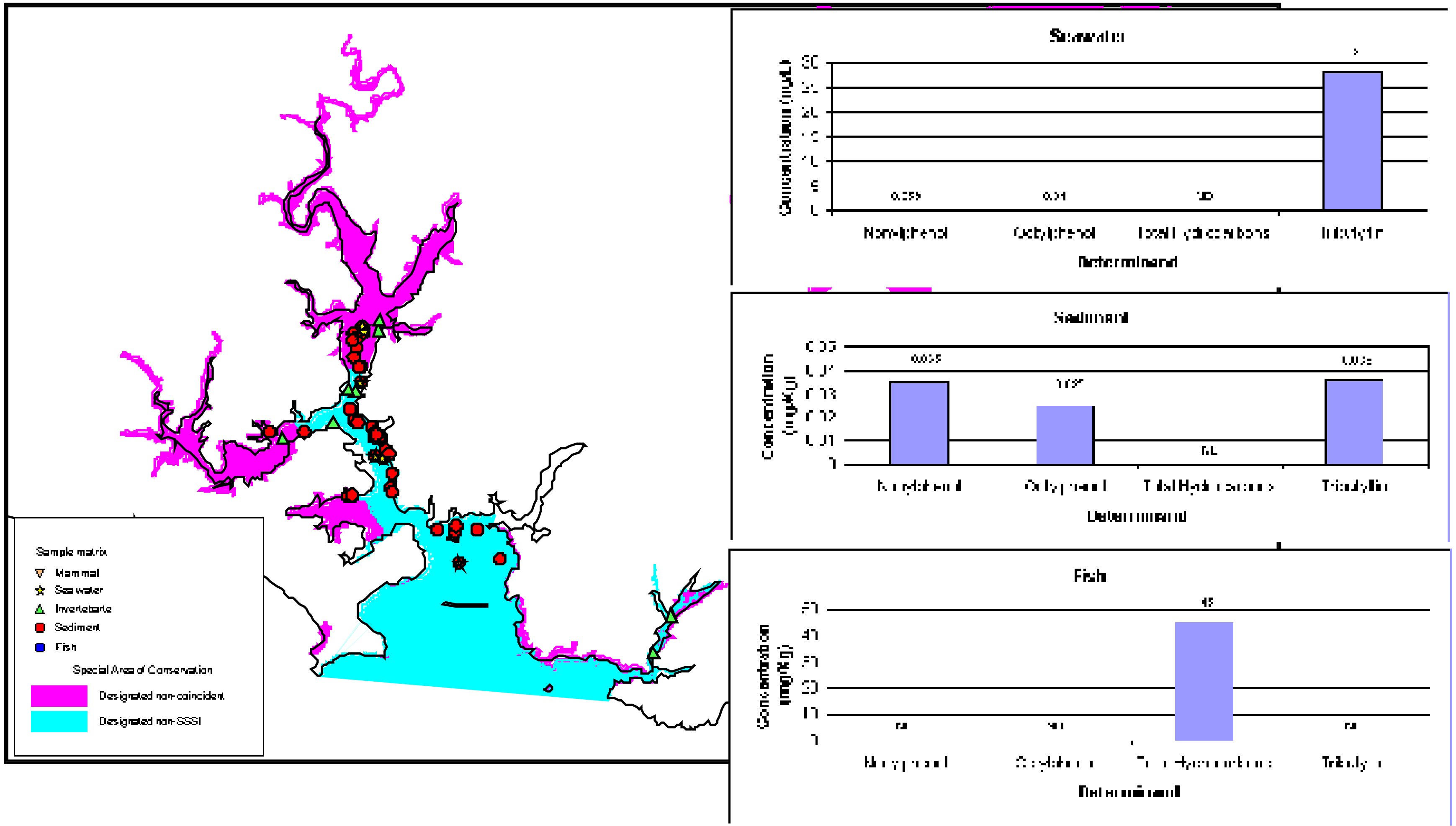

Figure 27b. Miscellaneous contaminants: Plymouth Sound and Estuaries

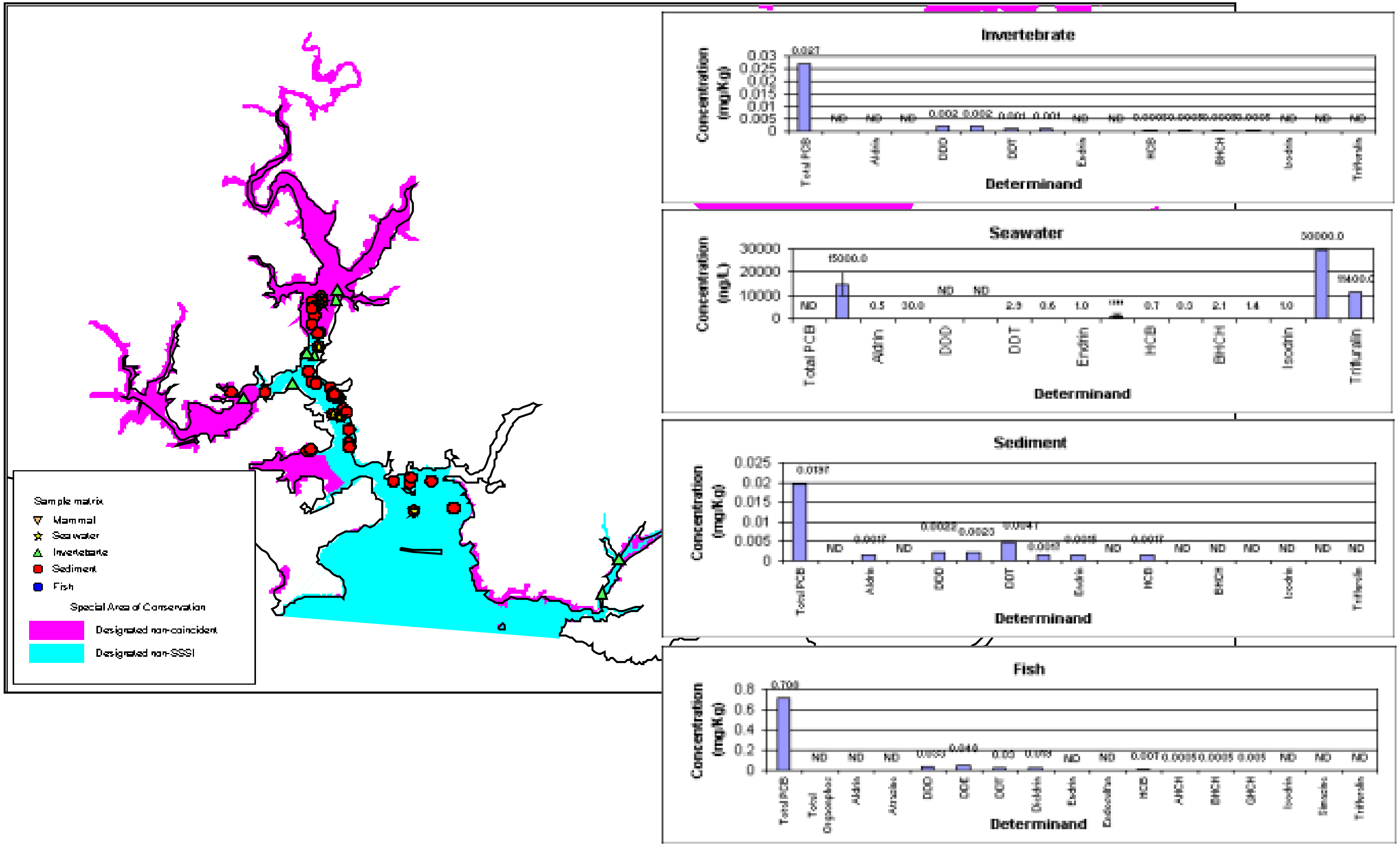

Figure 27c. PCB and pesticides: Plymouth Sound and Estuaries

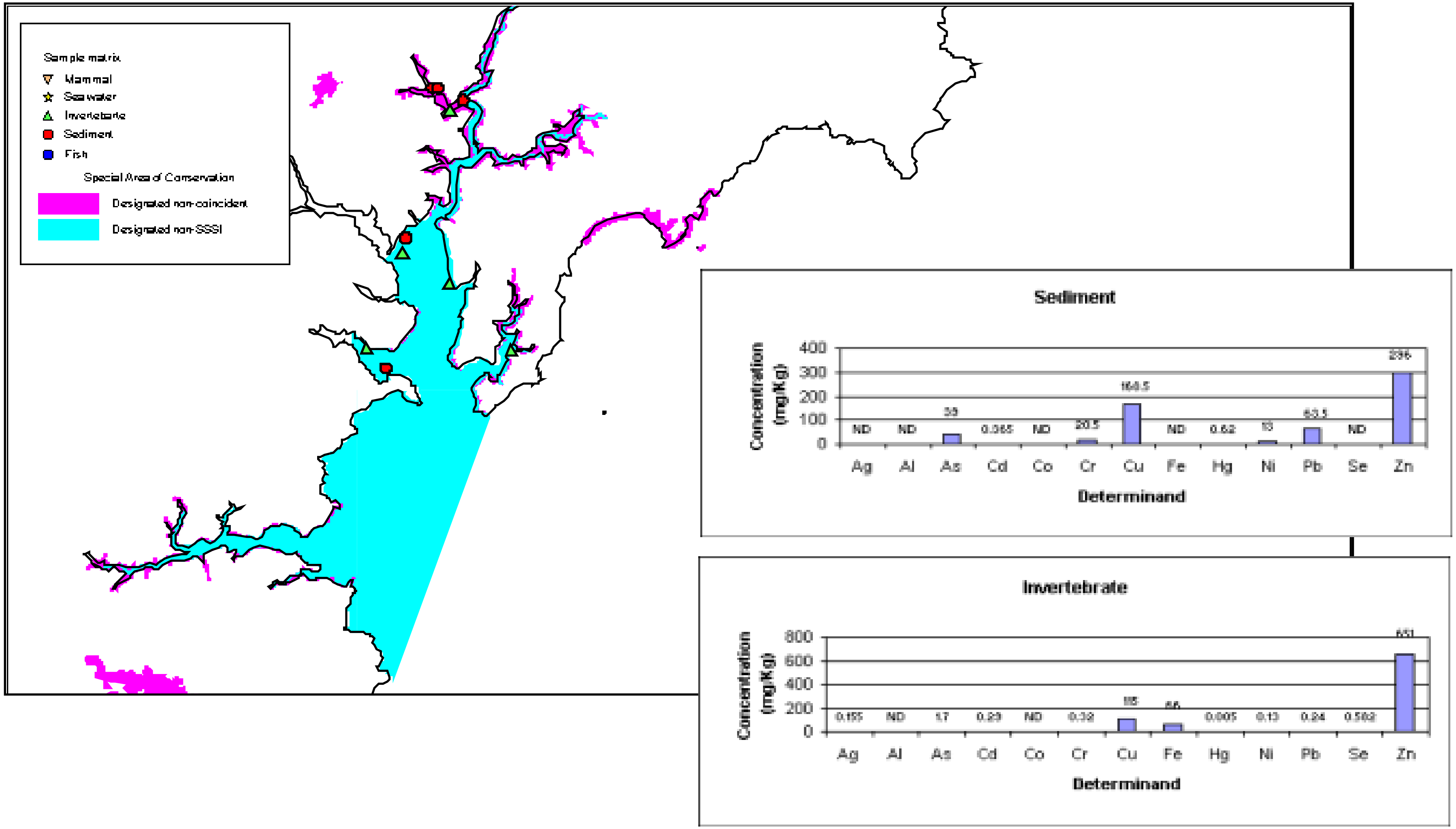

Figure 28a. Metals: Fal and Helford

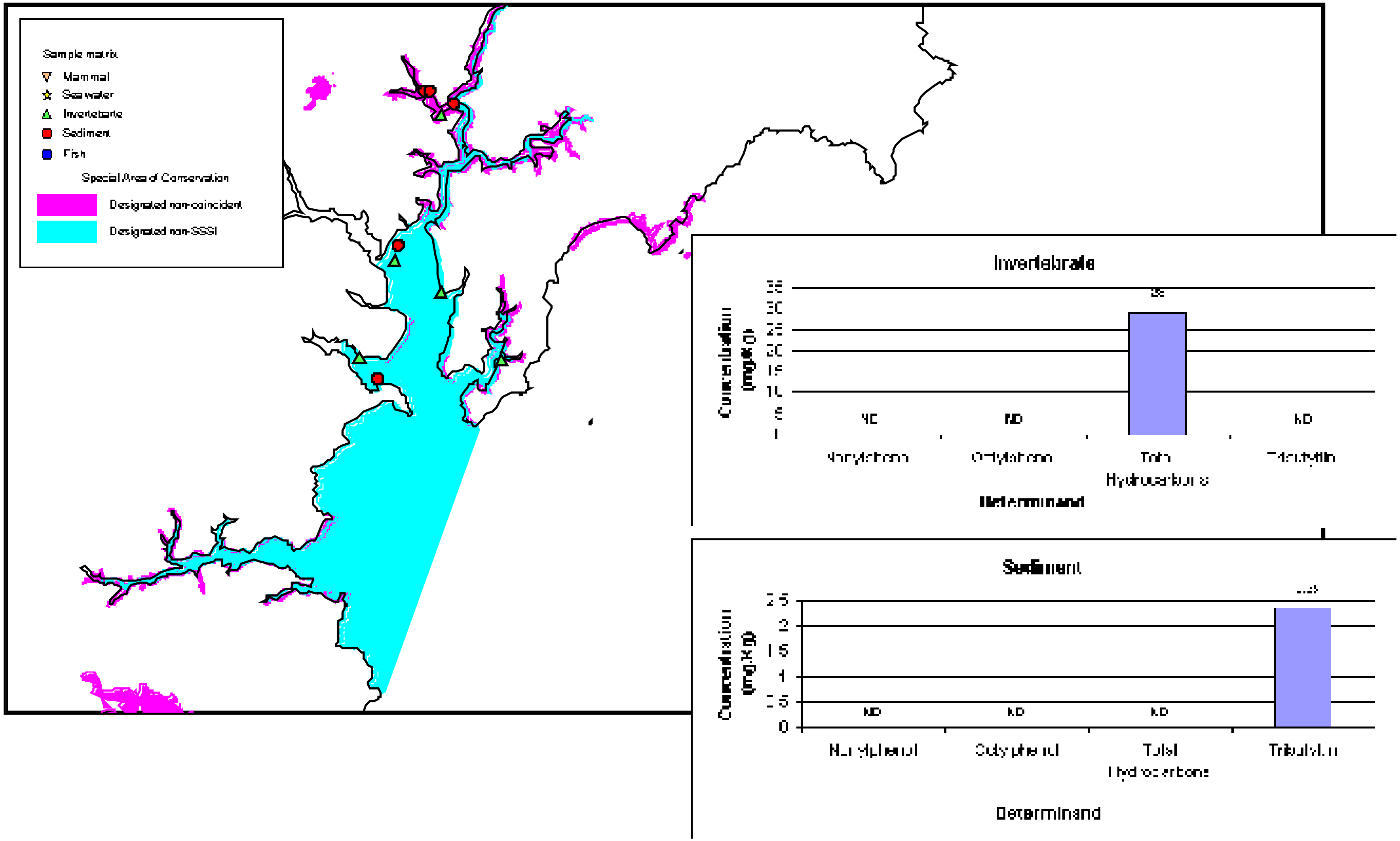

Figure 28b. Miscellaneous contaminants: Fal and Helford

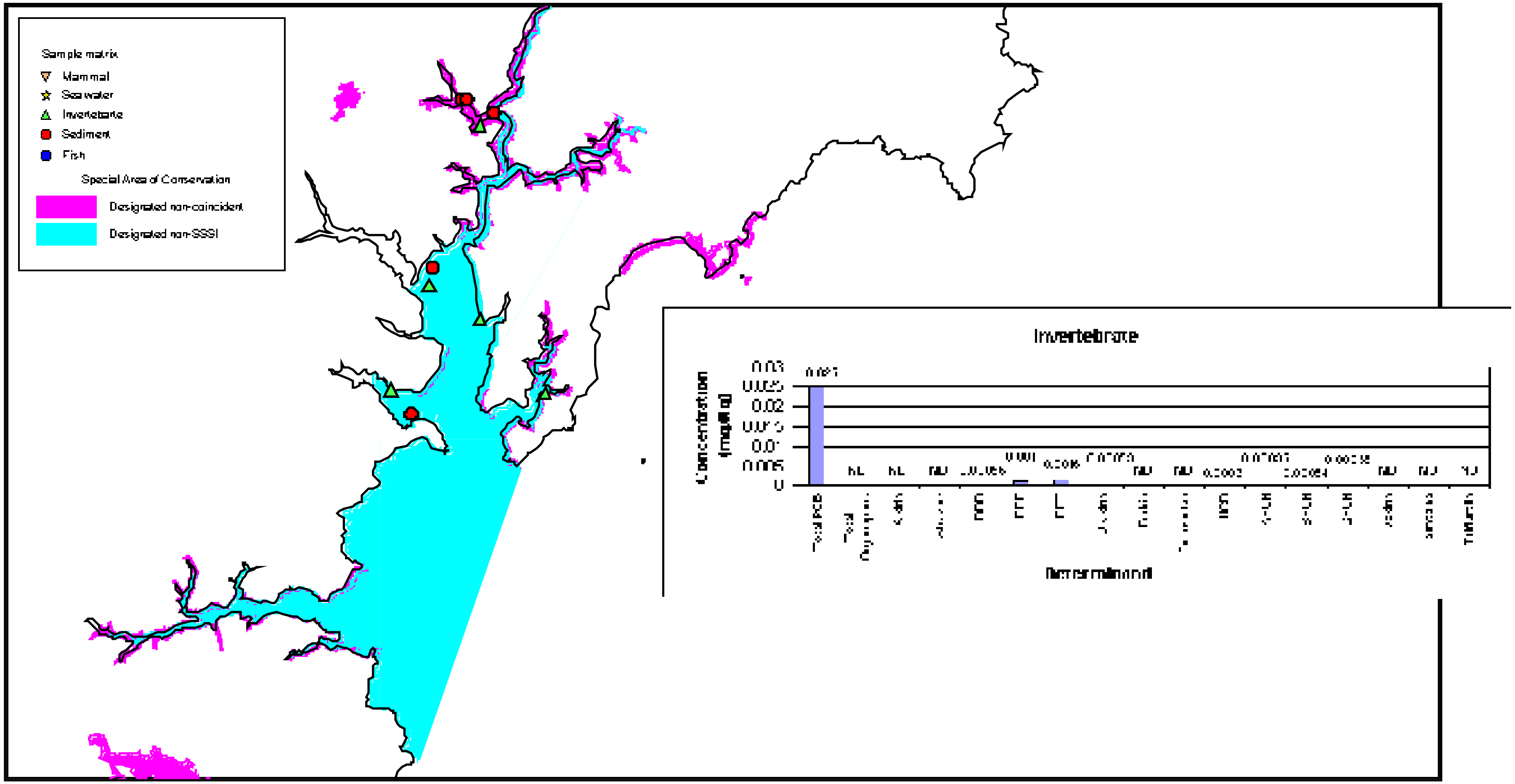

Figure 28c. PCB and pesticides: Fal and Helford

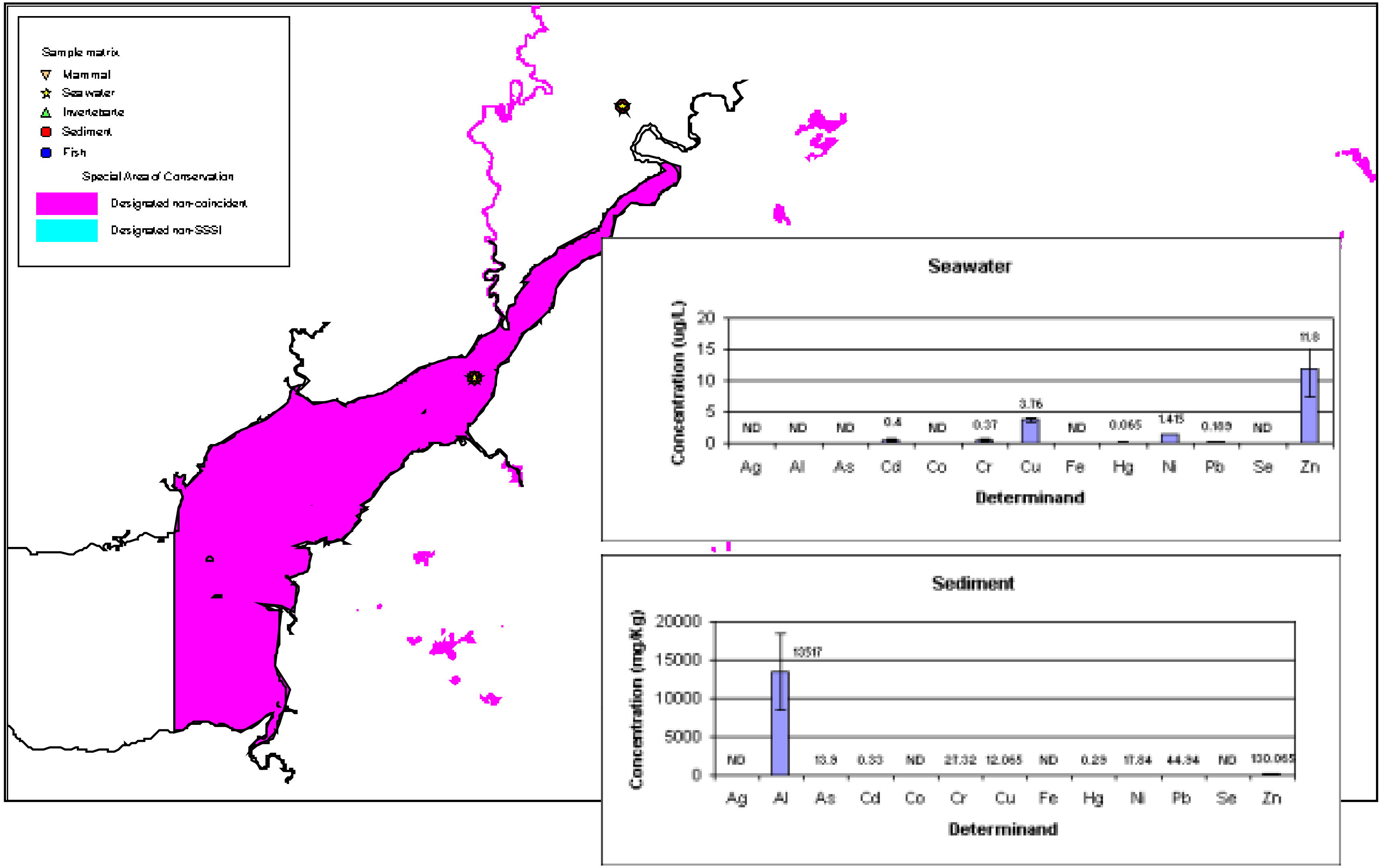

Figure 29a. Metals: Severn Estuary

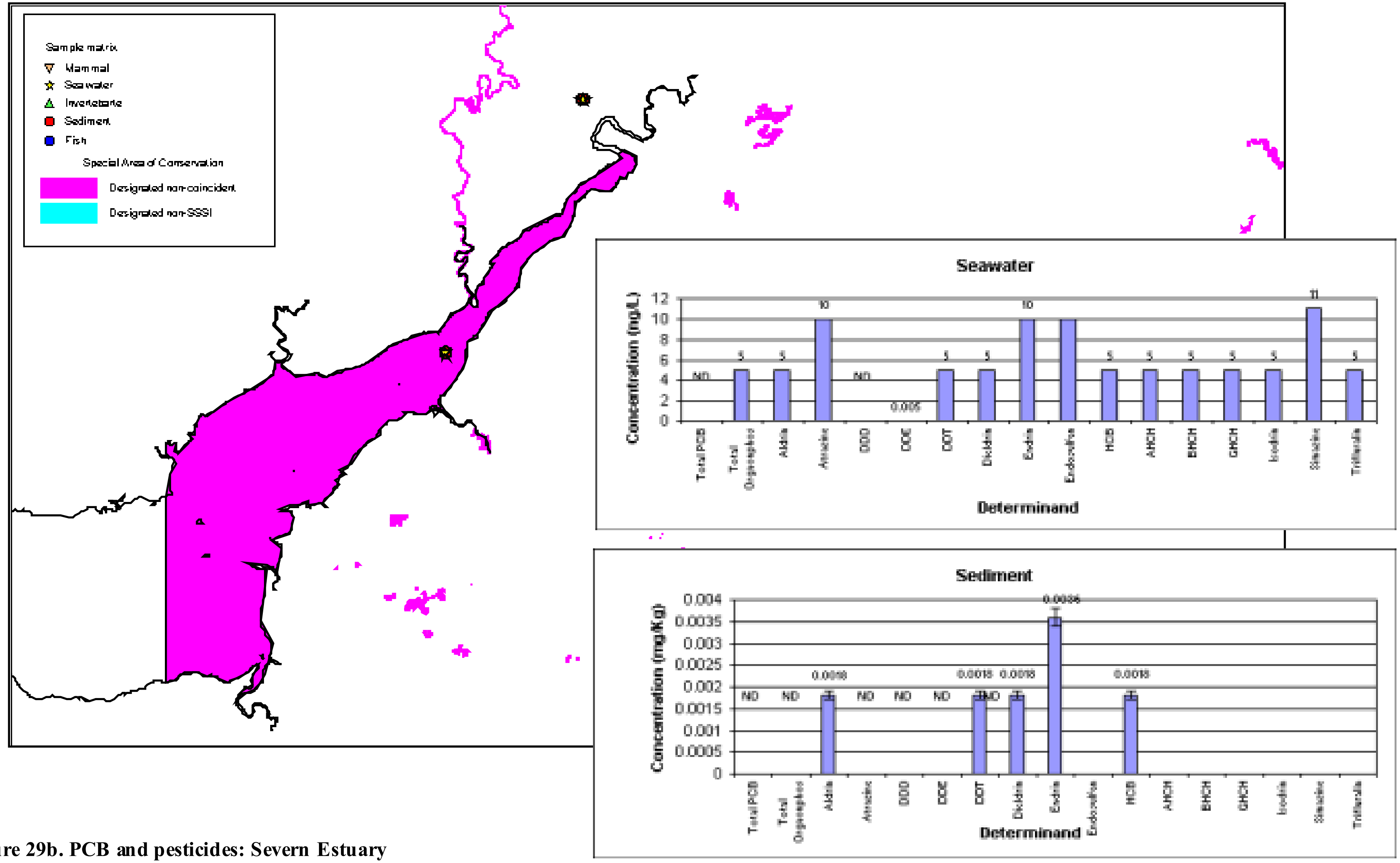

Figure 29b. PCB and pesticides: Severn Estuary

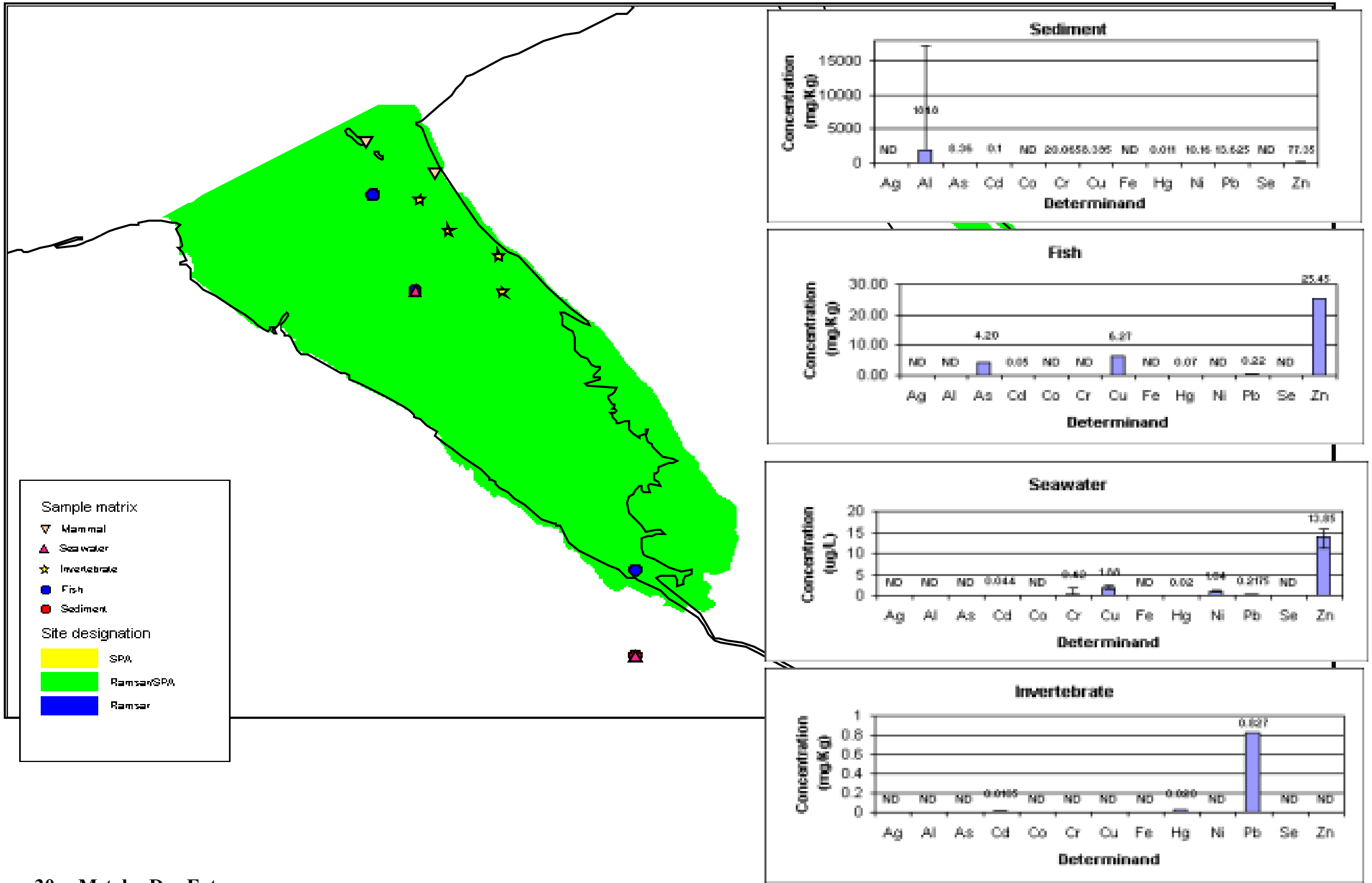

Figure 30a. Metals: Dee Estuary

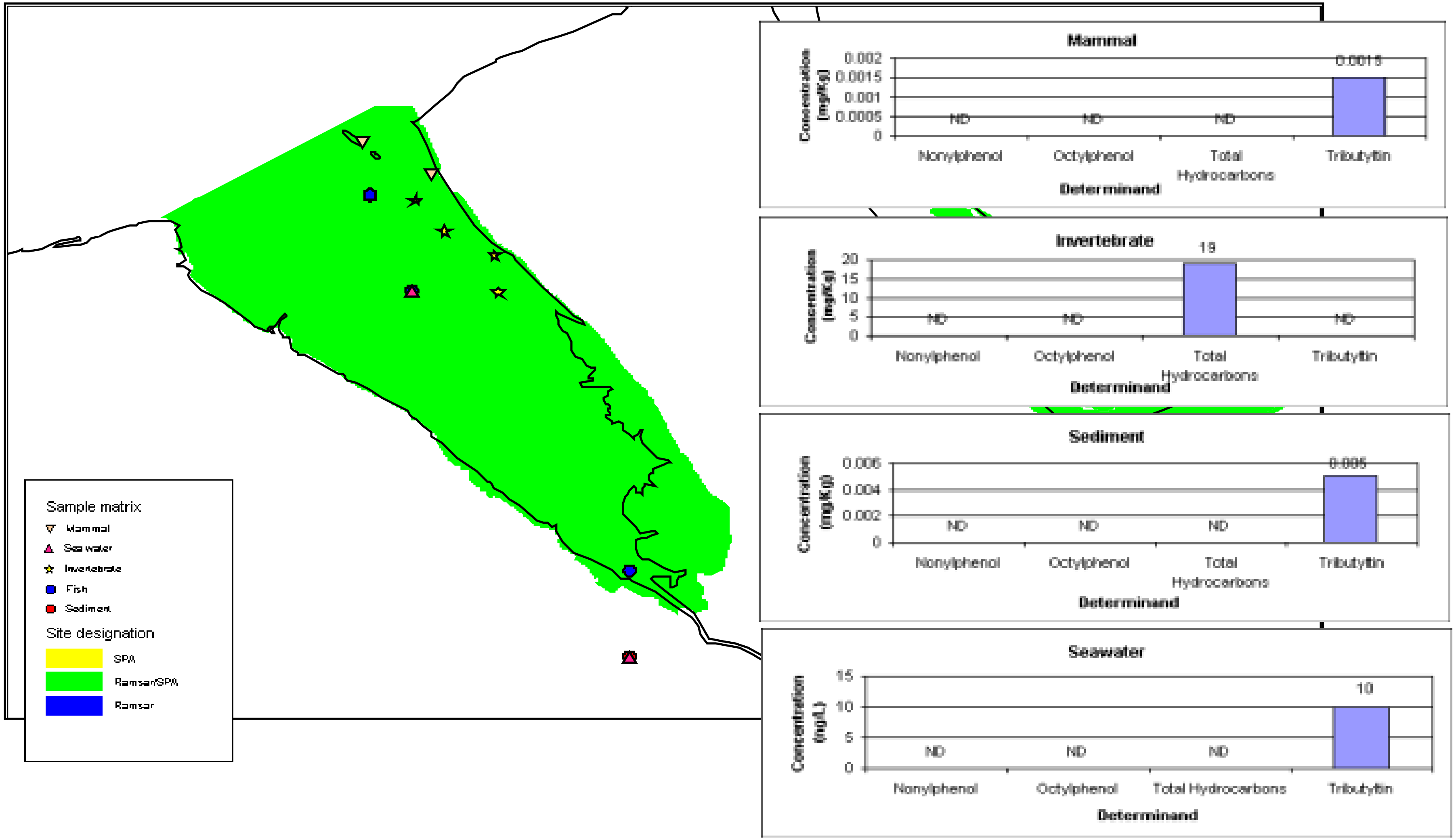

Figure 30b. Miscellaneous contaminants: Dee Estuary

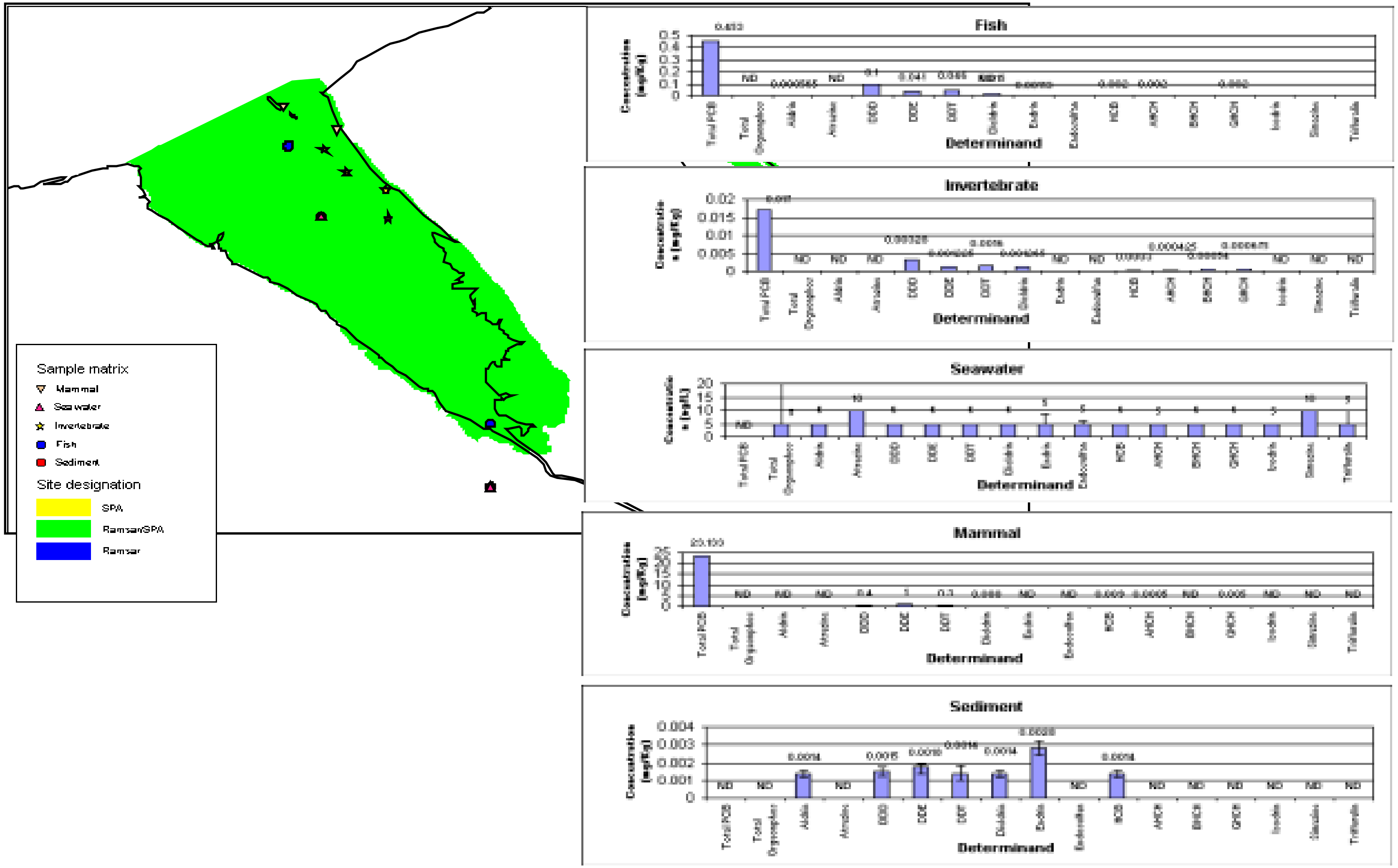

Figure 30c. PCB and pesticides: Dee Estuary

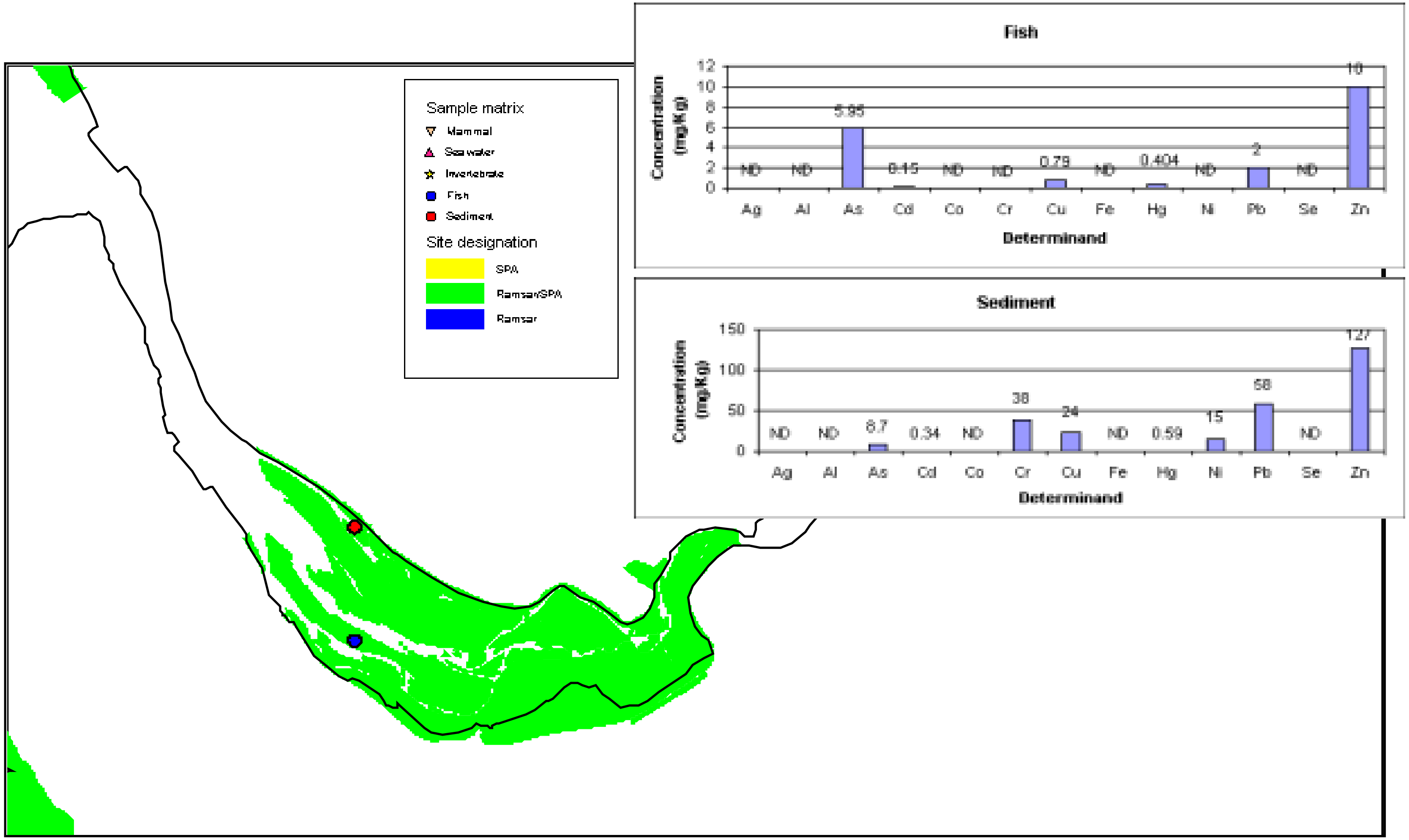

Figure 31a. Metals: Mersey Estuary

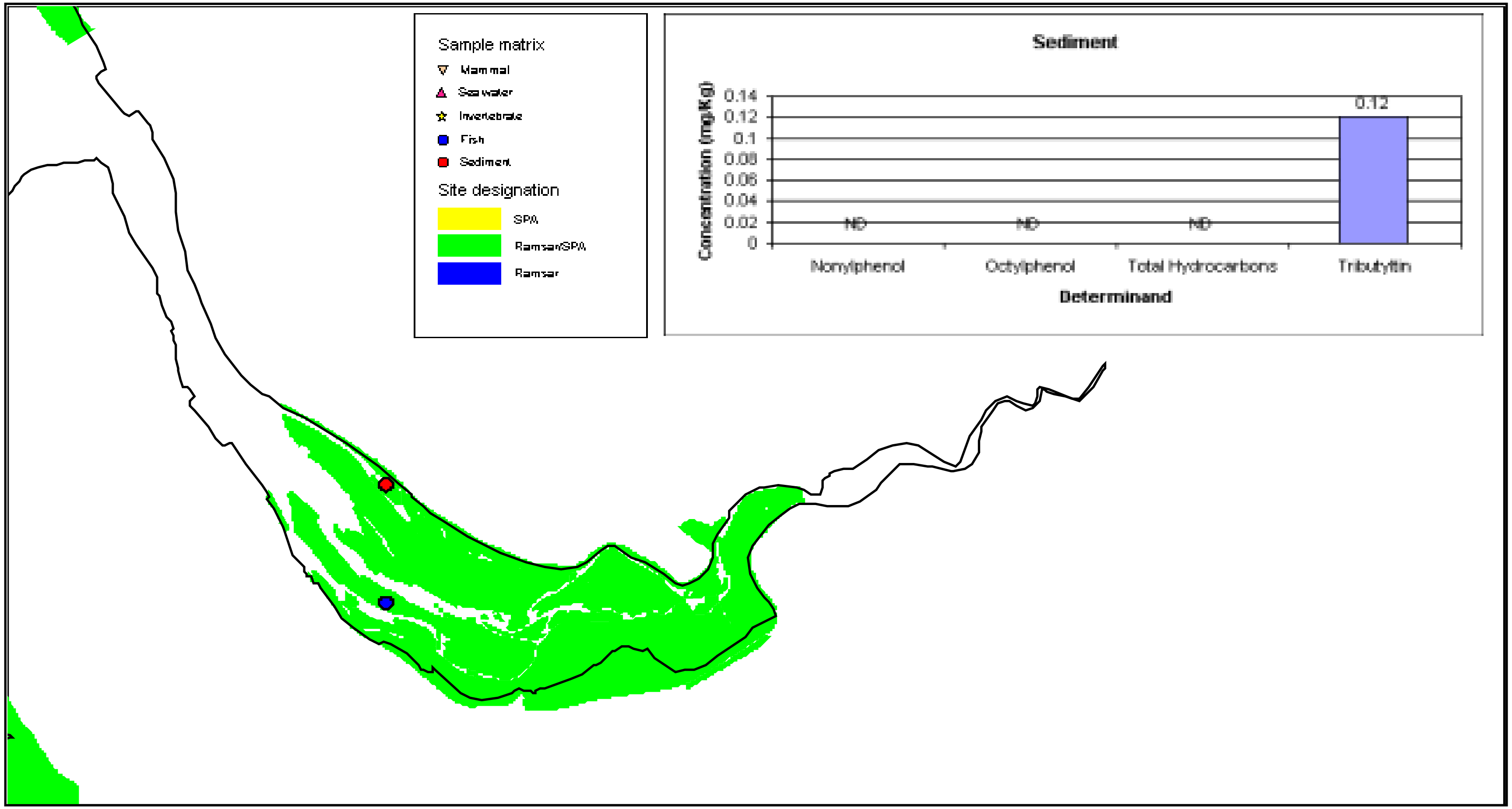

Figure 31b. Miscellaneous contaminants: Mersey estuary

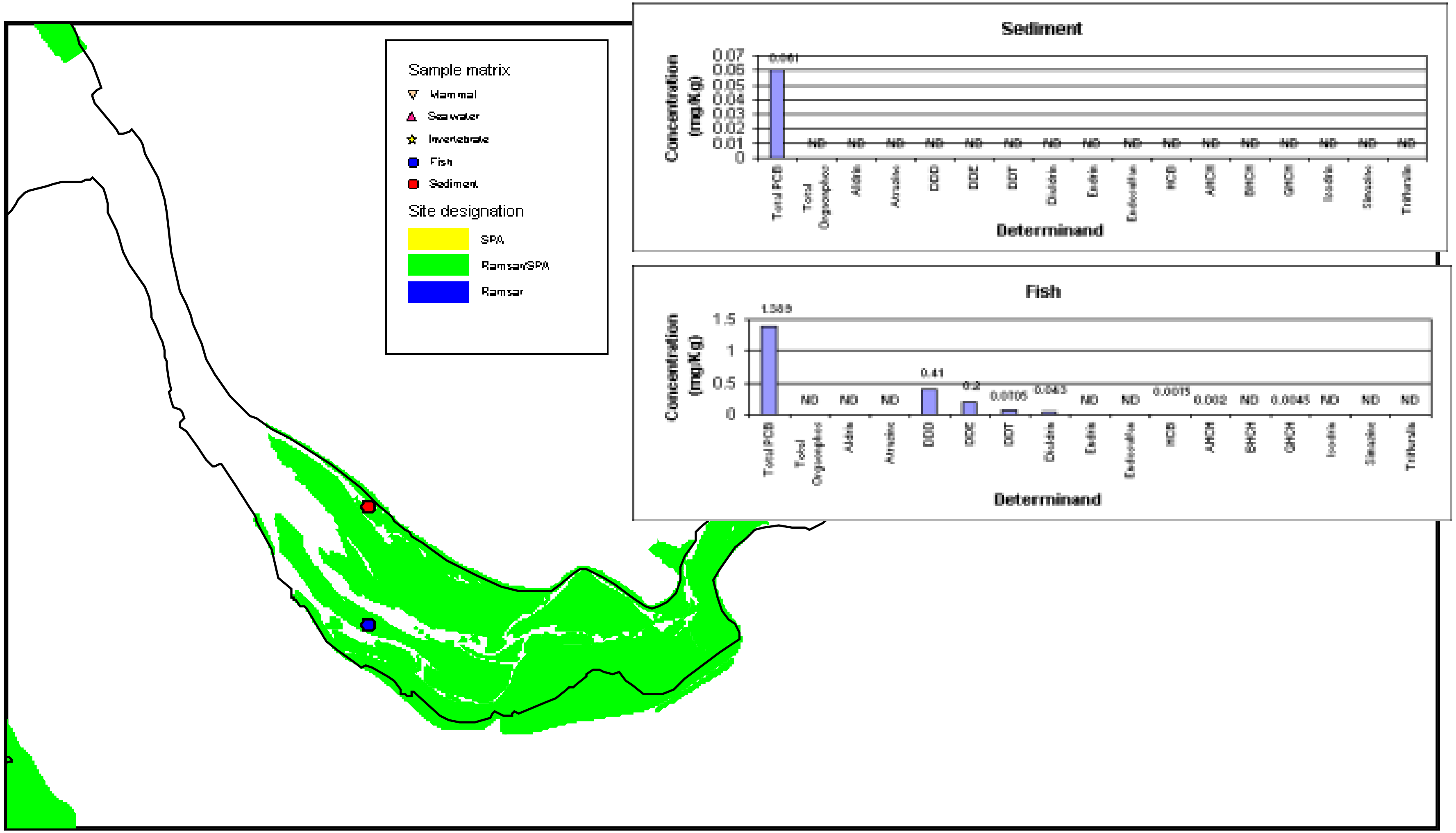

Figure 31c. PCB and pesticides: Mersey Estuary

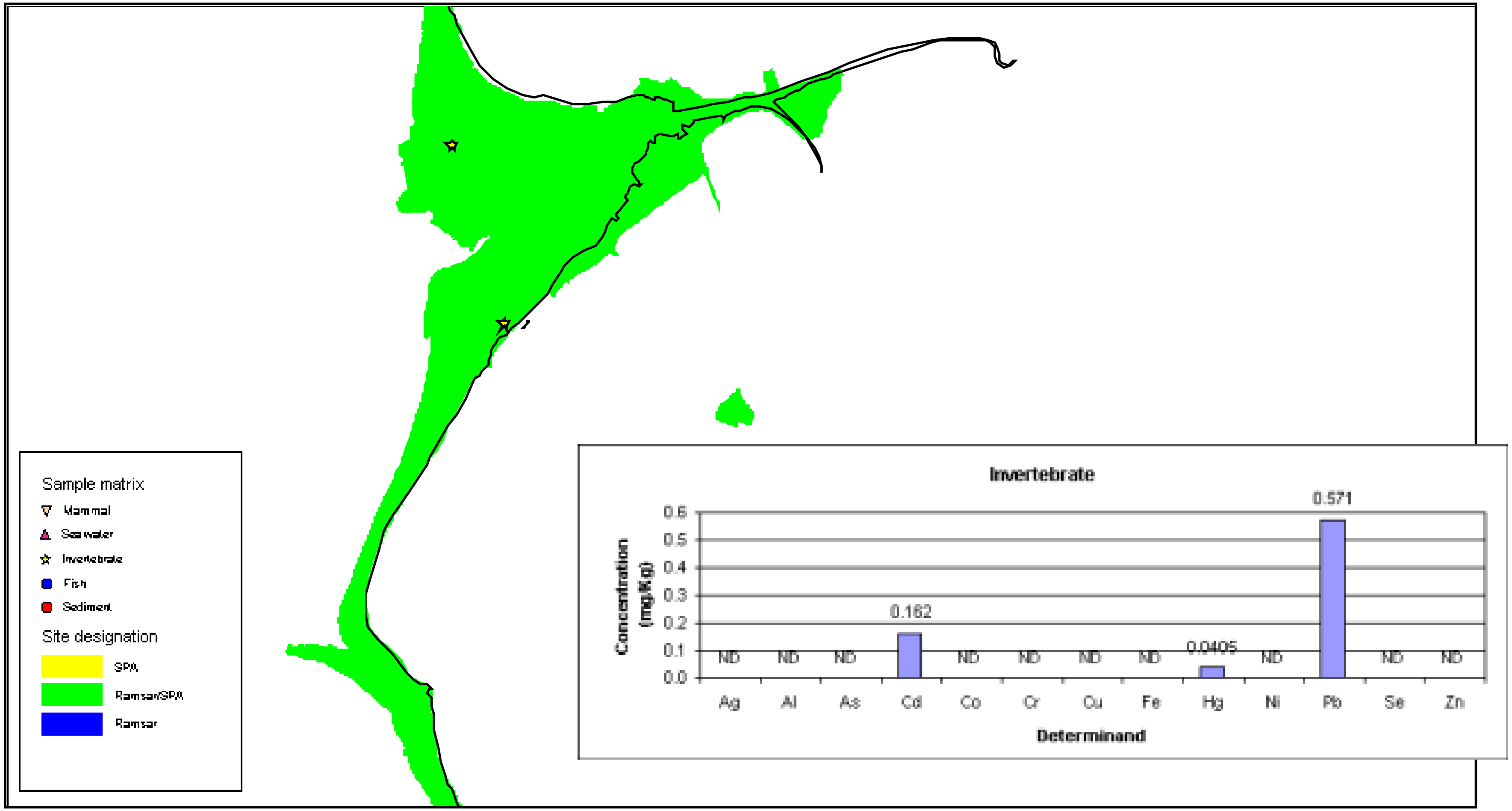

Figure 32a. Metals: Ribble Estuary

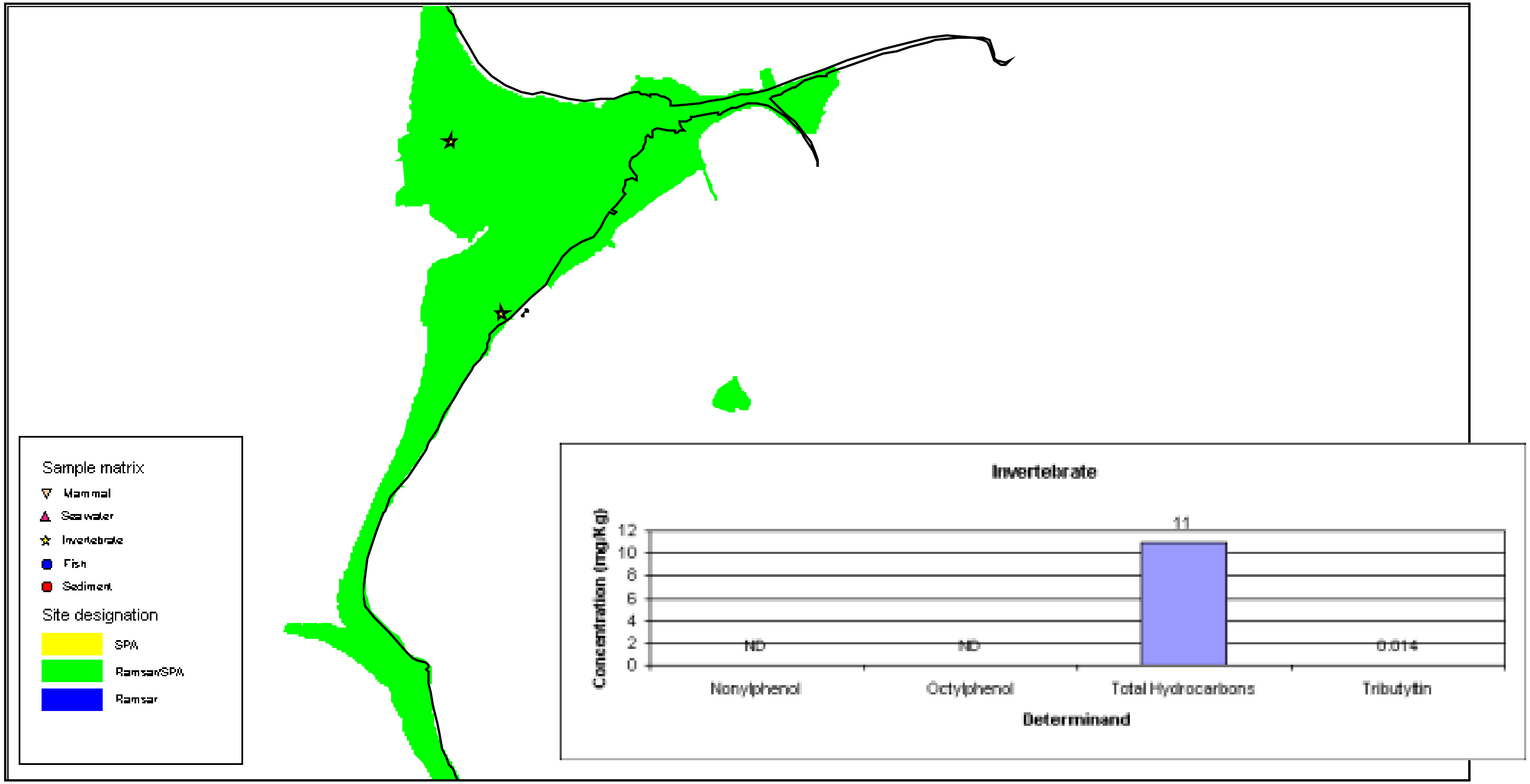

Figure 32b. Miscellaneous contaminants: Ribble Estuary

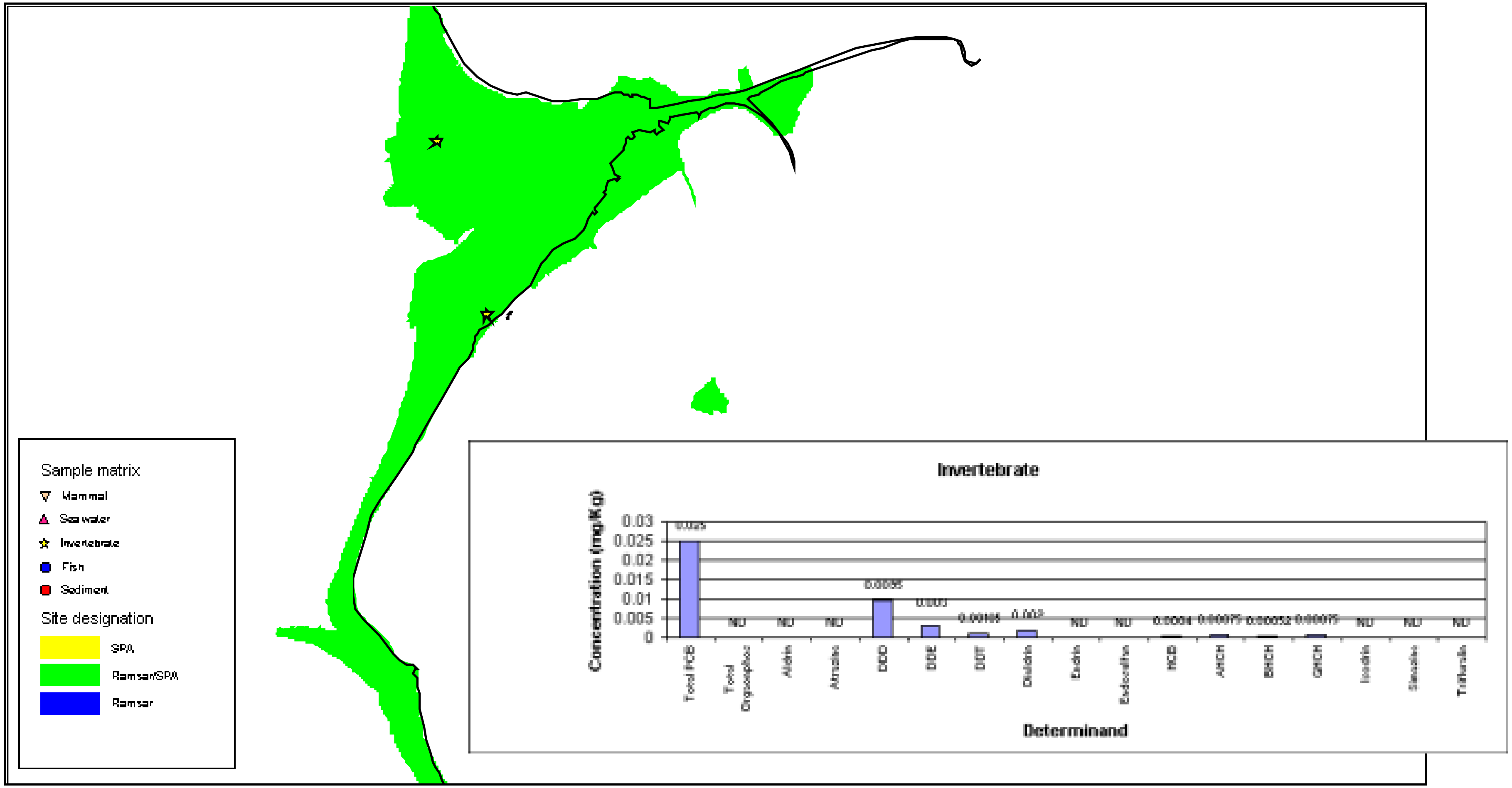

Figure 32c. PCB and pesticides: Ribble Estuary

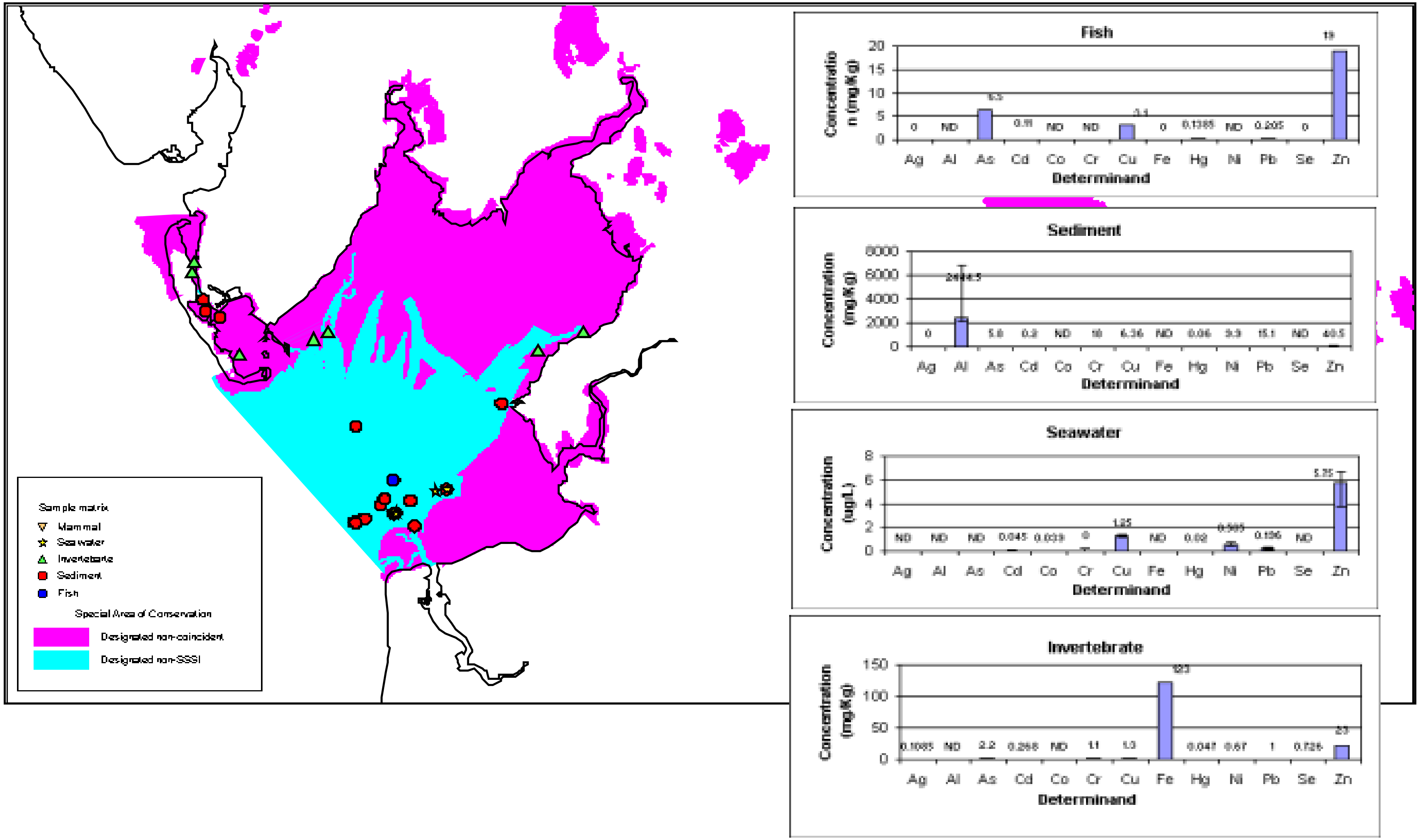

Figure 33a. Metals: Morecambe Bay

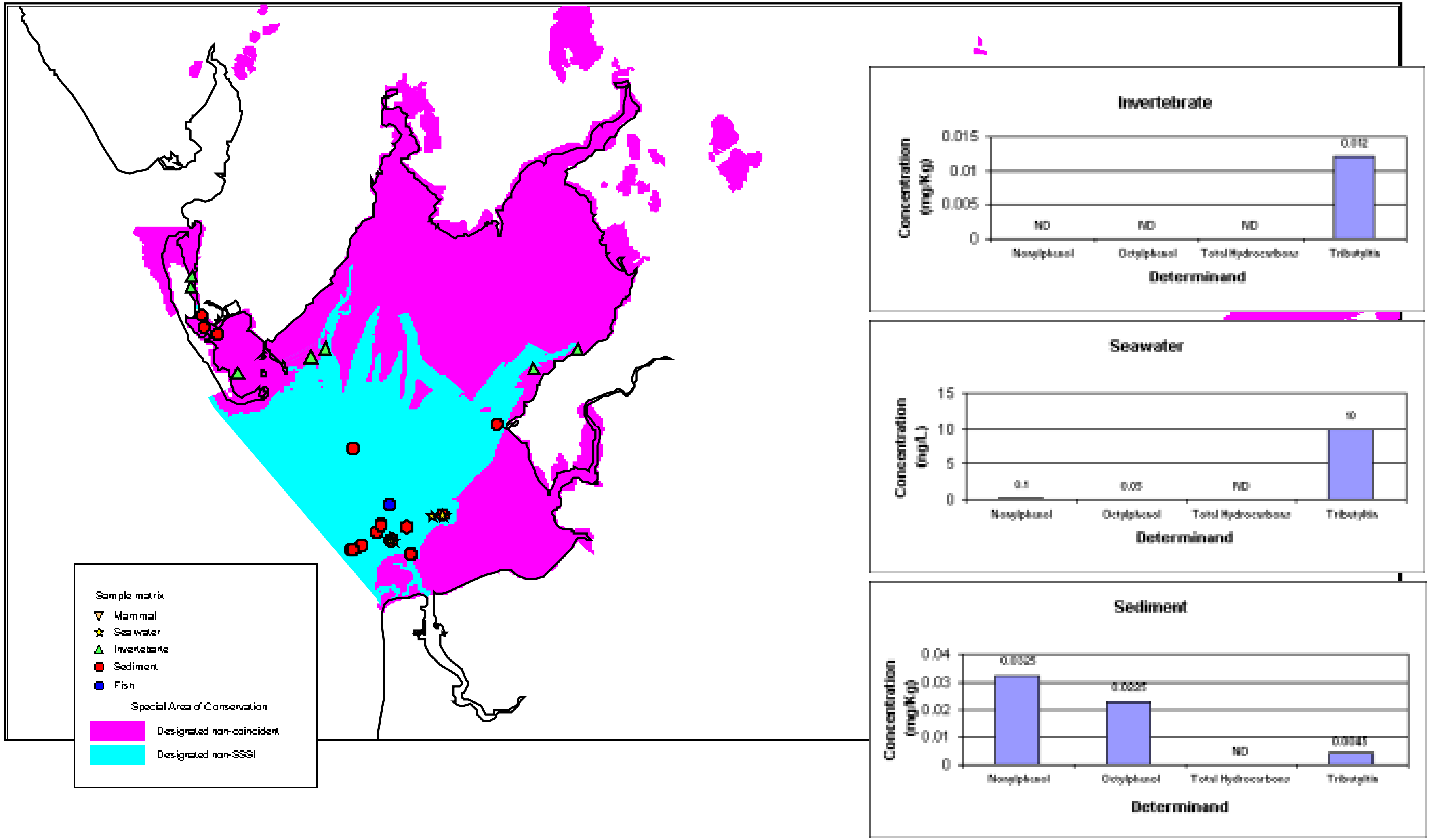

Figure 33b. Miscellaneous contaminants: Morecambe Bay

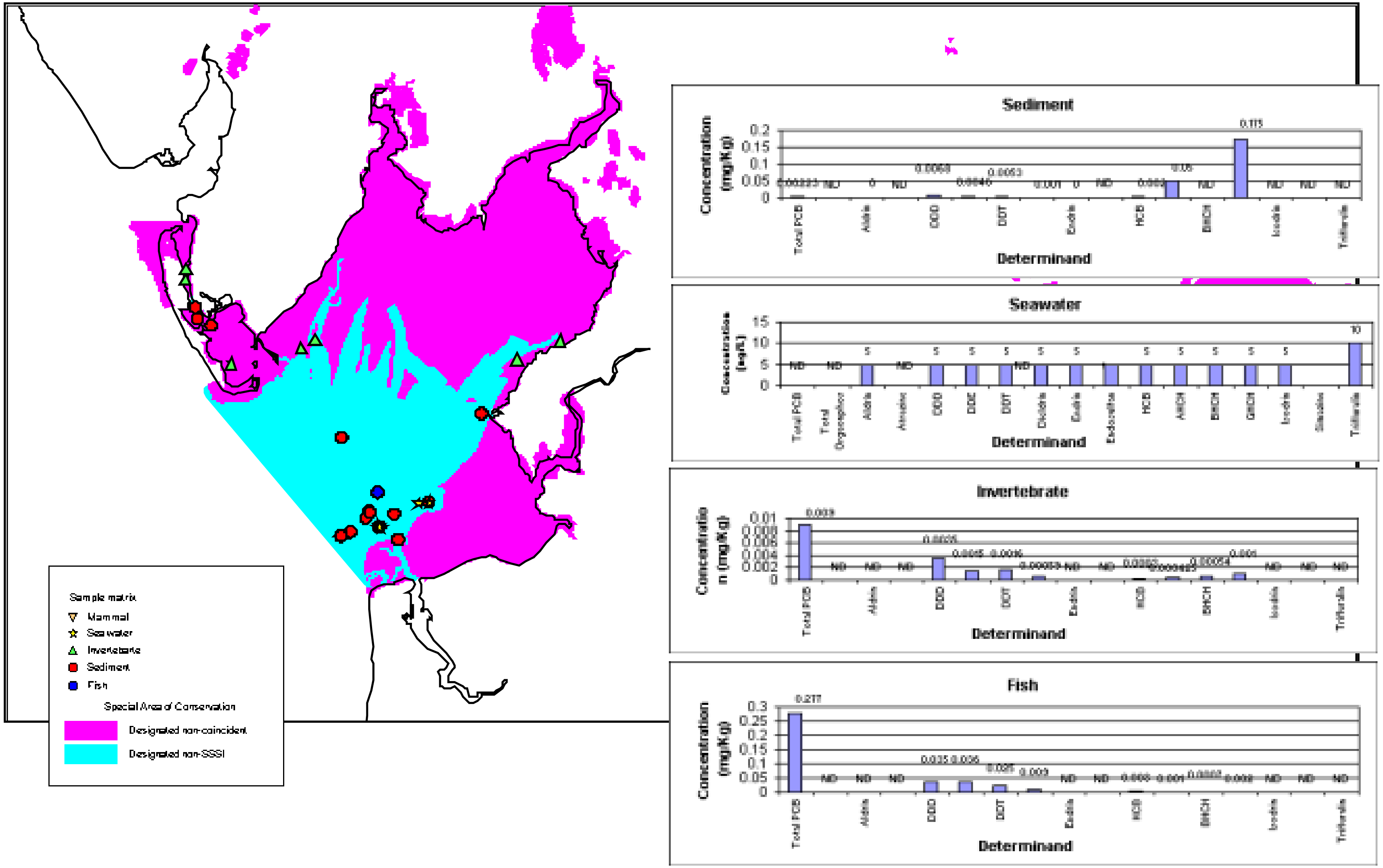

Figure 33c. PCB and pesticides: Morecambe Bay

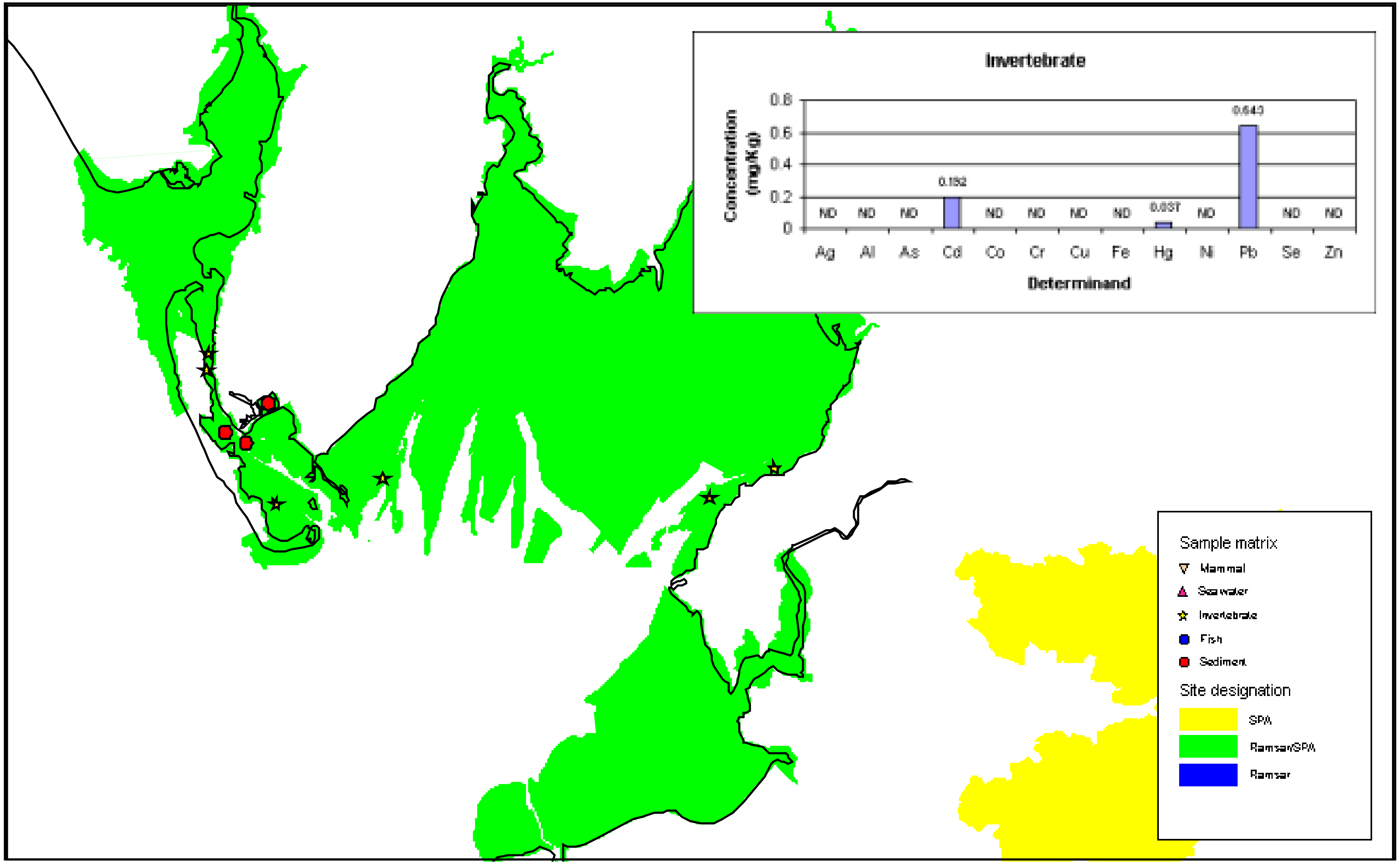

Figure 34a. Metals: Duddon Estuary

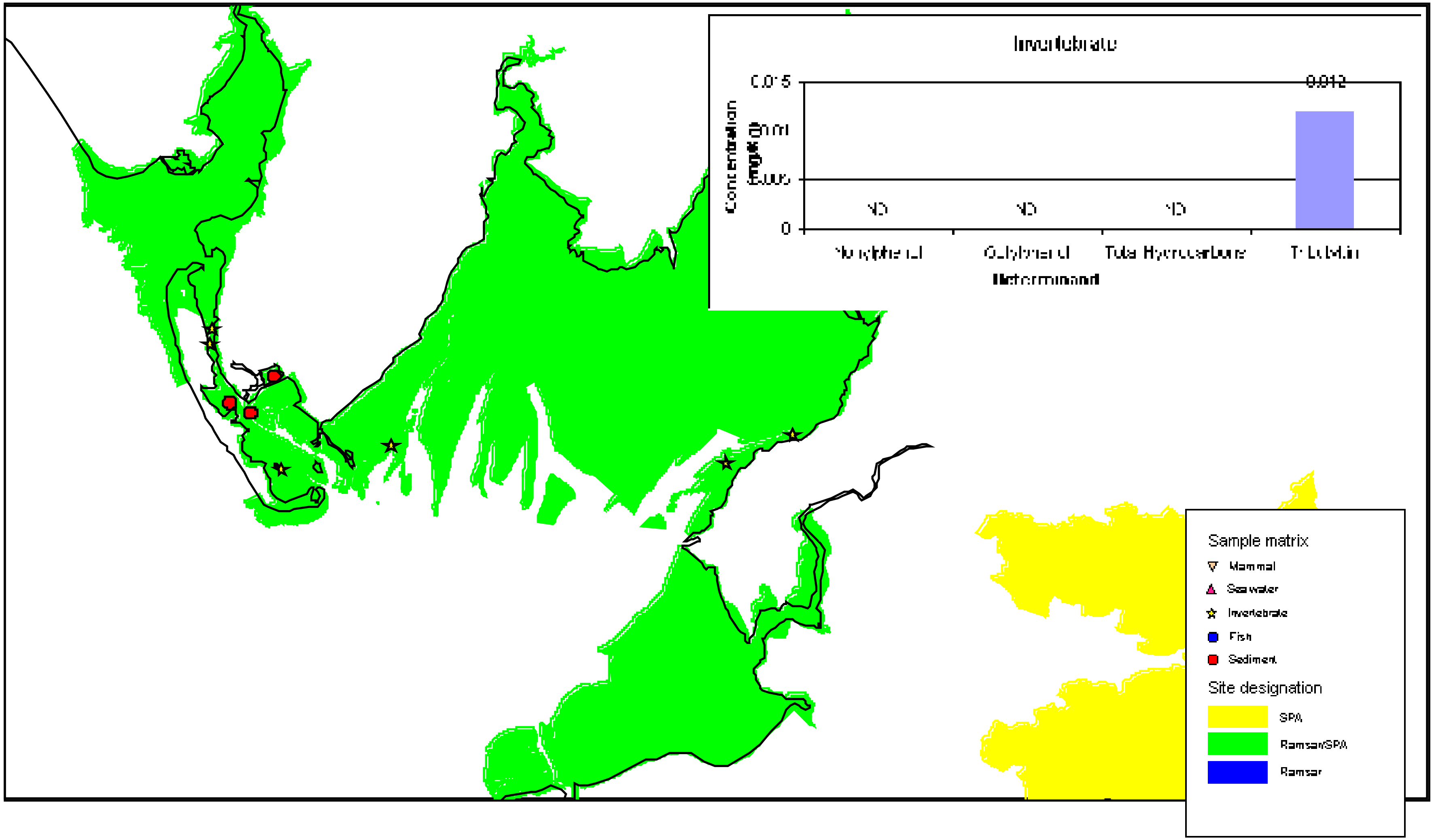

Figure 34b. Miscellaneous contaminants: Duddon Estuary

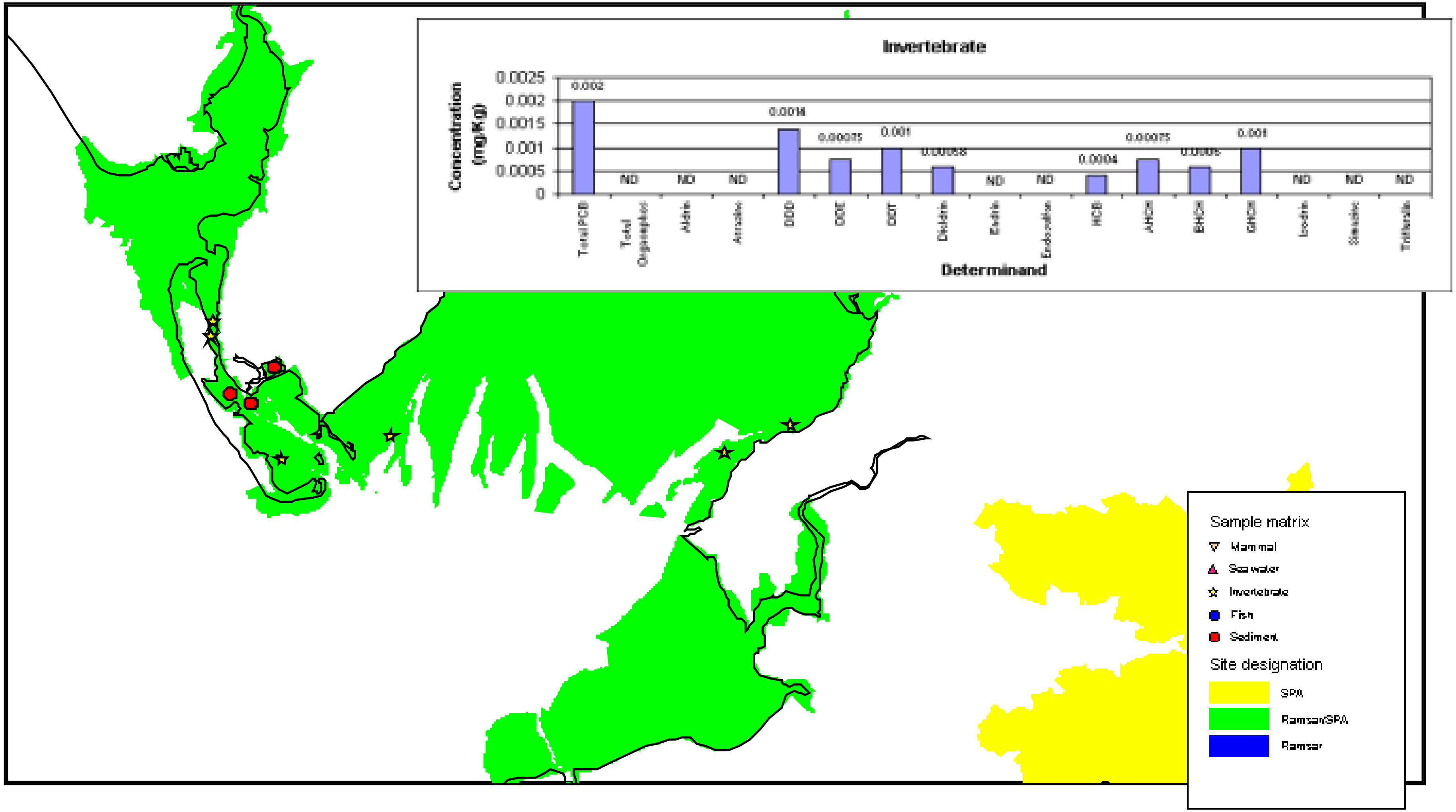

Figure 34c. PCB and pesticides: Duddon Estuary

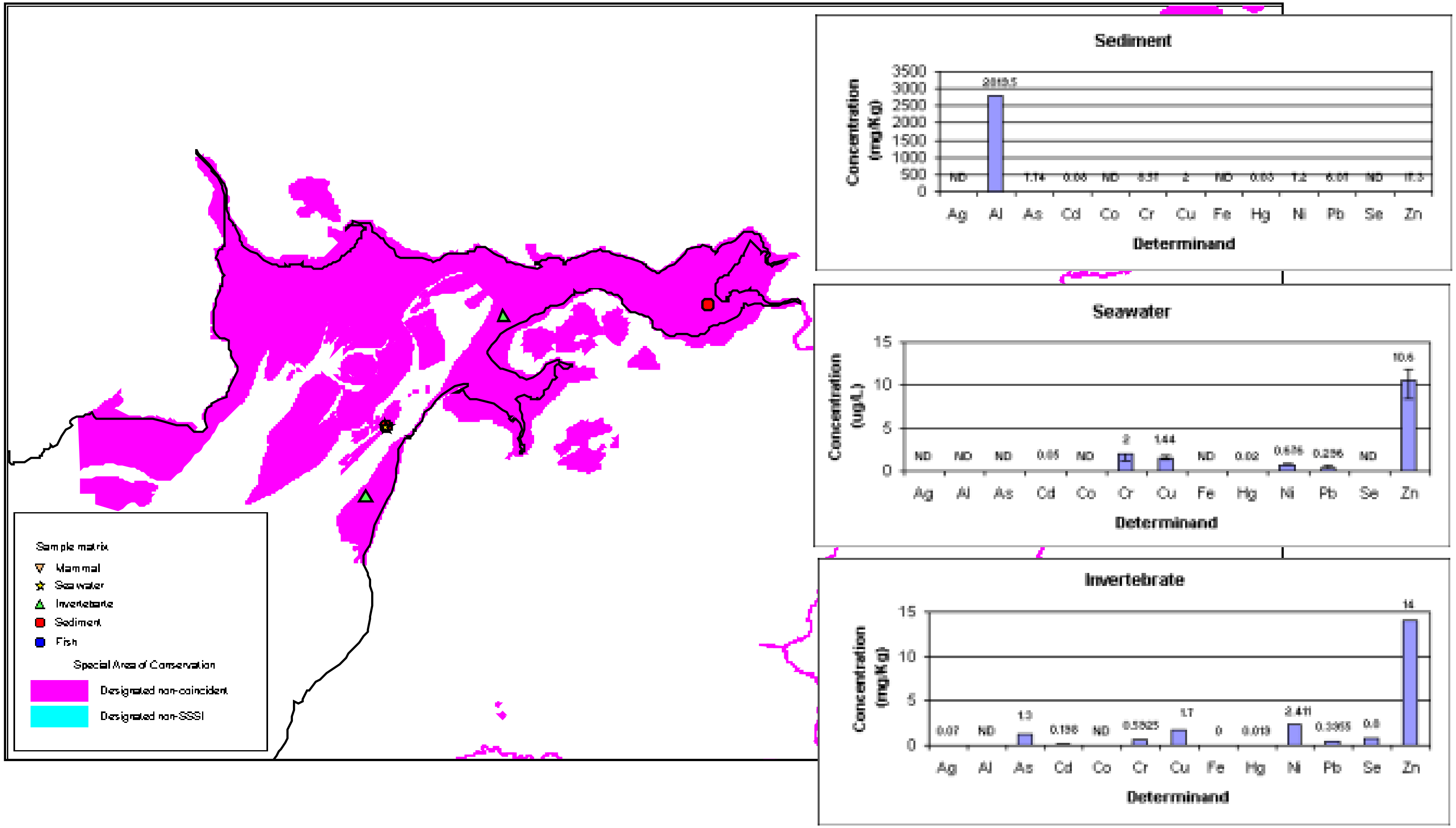

Figure 35a. Metals: Solway Firth

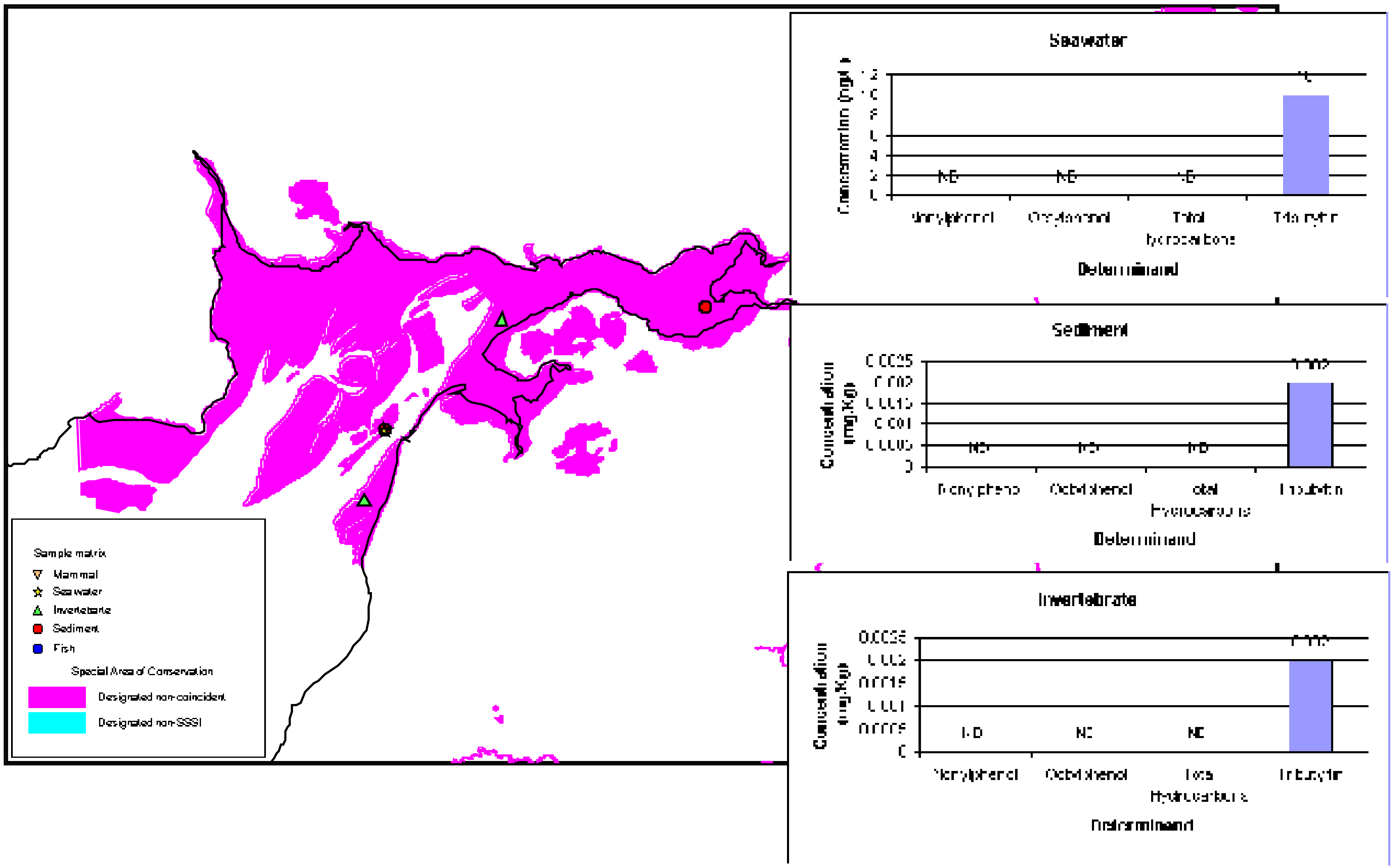

Figure 35b. Miscellaneous contaminants: Solway Firth

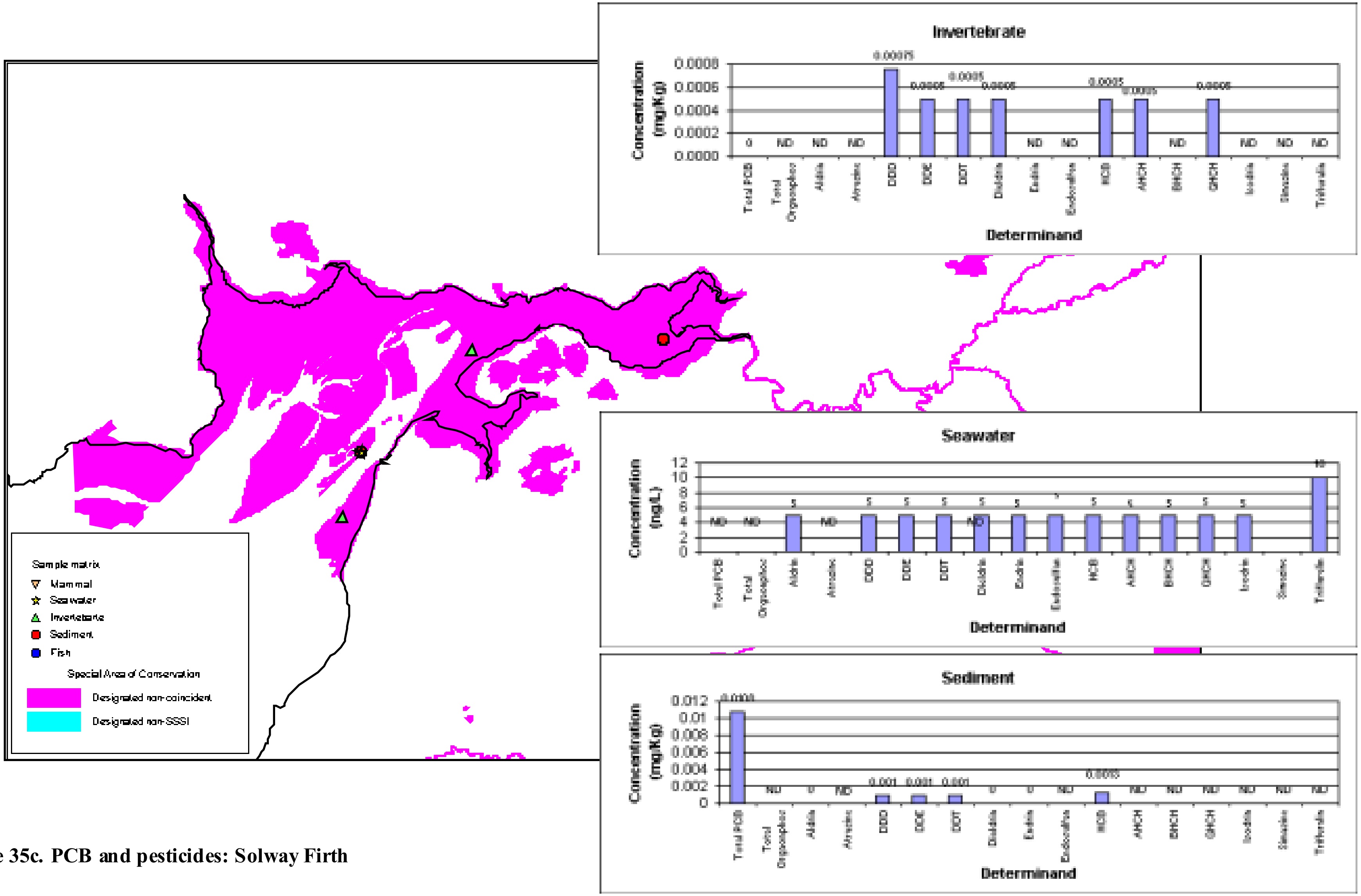

Figure 35c. PCB and pesticides: Solway Firth

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Middle left: English Nature bat warden with a whiskered bat near Holme, Devon.
Paul Glendell / English Nature 24,795
Bottom left: Radio tracking a hare on Pawlett Hams, Somerset.
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