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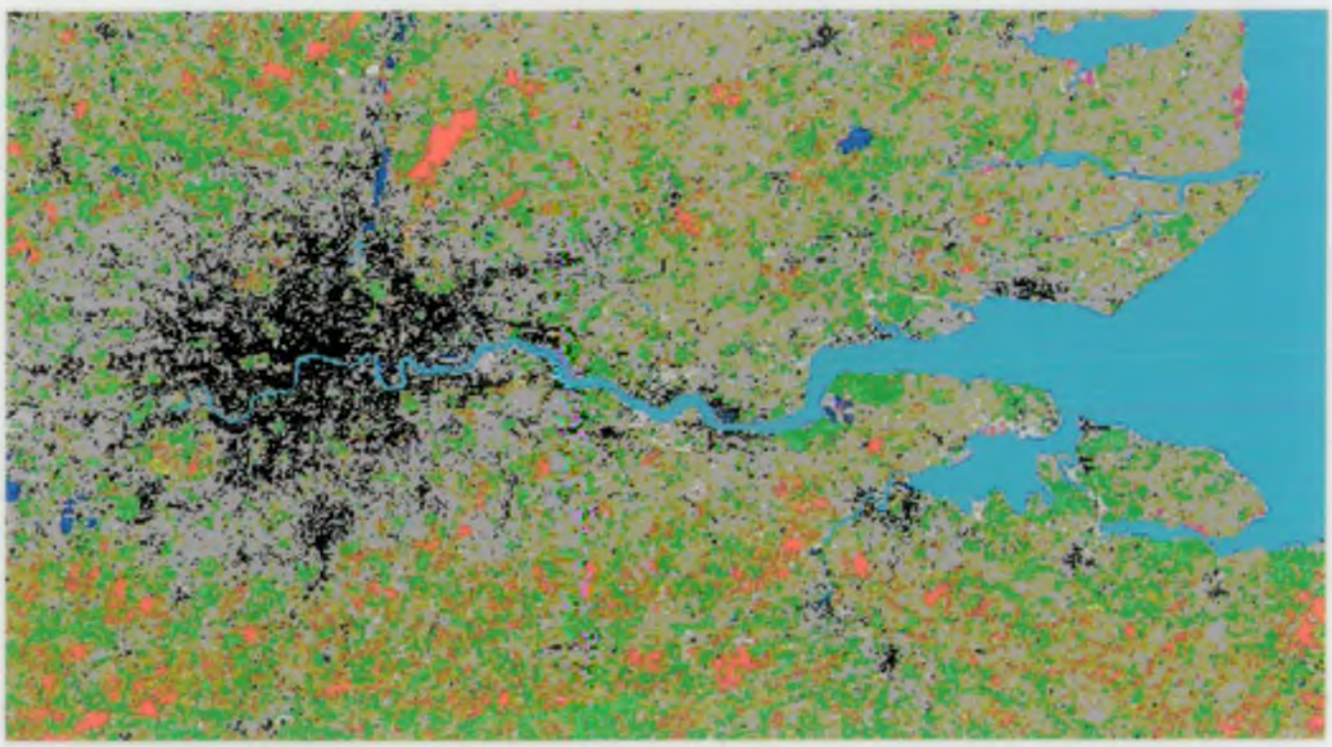

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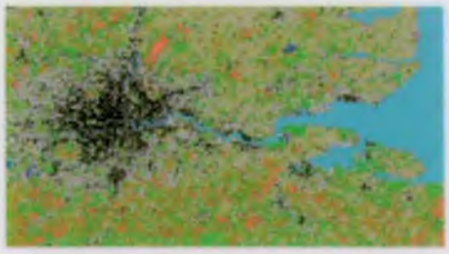

Front cover illustration - The Thames Estuary, from 'The Land Cover Map of Great Britain 1990'.
Acknowledgements: Institute of Terrestial Ecology, Monks Wood.

EA - THAMES WATER QUALITY

Metallothionein in eels from the Thames Estuary: A biochemical indicator of environmental quality

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Plymouth Marine Laboratory
NATURAL ENVIRONMENT RESEARCH COUNCIL

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A collaborative study between Plymouth Marine Laboratory and the Environment Agency (Thames Region).

Other Titles in 'Thames Estuary Environmental Quality Series':

Tributyltin (TBT) Impact in the Thames Estuary
Thames Estuary Environmental Quality Series, **1**, 67pp (March 2000).

Bioaccumulation of Metals in the Thames Estuary - 1997.
Thames Estuary Environmental Quality Series, **2**, 116pp (October 2000).

SUMMARY

Metallothioneins (MTs) are cysteine-rich, low molecular weight, metal-binding proteins whose functions include detoxification, storage and metabolism of metals such as cadmium (Cd), copper (Cu), mercury (Hg), silver (Ag) and zinc (Zn). Enhanced synthesis of MT signifies an effort to reduce the toxicity of excessive amounts of metal ions in cells, and as such, MT is potentially important as a specific biochemical indicator of metal pollution. The purpose of this study was to assess the suitability of the MT 'bioassay' in environmental monitoring, using livers of eels *Anguilla anguilla*, and hence, to establish the bioavailability and toxicological significance of accumulated metal burdens in eels from the Thames Estuary.

Hepatic MT levels and metal concentrations were determined in *A. anguilla* collected on three occasions during 1998 at sites along the Thames, from Richmond Lock seawards to Blythe Sands, and also from a reference site in SW England. MT was present, inherently, at basal levels of about 2 mg g^{-1} (dry weight) in livers of 'control' eels, predominantly in association with Cu and Zn - presumably to regulate requirements for these essential metals. MT concentrations were found to be variable within each eel 'population' but were generally highest (up to 11 mg g^{-1} in individual eels) at the more contaminated upper- and mid-estuarine sites in the Thames. There were significant differences in mean hepatic MT levels between eel populations from both Brentford and Kew (inner estuarine sites) and Blythe Sands at the mouth of the estuary.

Season, sex, reproductive status and salinity may have influenced MT levels to some extent, although metals were the most significant factor. Hepatic MT levels in all eels sampled were highly correlated with their metal burden (Zn, Cu, Ag and Cd), indicating that MT synthesis is induced in direct response to metal bioavailability - notably to Cu and Ag enrichment in the upper- and mid-estuary.

Chromatographic profiles of hepatic extracts (cytosol) showed that, with the exception of Zn, the majority of metals were primarily associated with MT. Furthermore, proportions of MT-bound Cu, Ag and Cd increased as total amounts of these metals in the cytosol (and in whole livers) increased. There was no indication of threshold levels being reached. Thus, despite causing induction of MT, excess bioavailable Cu, Ag and Cd appear to be successfully detoxified in eels over the range of environmental contamination encountered along the Thames Estuary. Superficially, it would seem that eel populations have become adapted to metal contamination. However, raised levels of MT at upstream sites infer an attempt to respond to contamination which, in itself, is a signal that the fish are affected. The cost to the animal has yet to be determined.

The methodology developed in this study has considerable potential for monitoring the effects of metal exposure on a broader basis. *A. anguilla* is a common inhabitant of estuarine and fresh waters throughout Europe. By observing the suggested sampling criteria, further studies with Thames and other eel populations would help to build on the valuable baseline data for hepatic MT, and would provide a better understanding of responses to metal contamination. Sampling of additional reference sites is recommended for comparative purposes and to improve interpretation of metal binding patterns. It would be useful to determine, for example, whether the amounts of Zn associated with high molecular weight proteins (sometimes elevated in Thames eels) merely reflect the role of this essential metal in metalloenzymes, or whether they are of toxicological significance. Nevertheless, results from the current project have demonstrated, that measurements of hepatic MT in eels may serve as an effective biochemical indicator of estuarine 'health' and environmental quality.

CONTENTS

1. Introduction

1.1 Sub-lethal bioindicators of metal pollution

Estuaries are a major recipient of anthropogenic discharges of pollutants, which, over time, can have serious consequences for estuarine biota in terms of, for example, elevated levels of infection and disease, and decreased growth and reproduction rates. Such deleterious effects may not become apparent until changes occur at the ecosystem level, a point at which it may be too late to take effective countermeasures. A concept of ecotoxicology is that these changes are preceded by sublethal effects to individual organisms at the molecular and cellular level. Detection of such subtle sublethal effects in estuarine organisms can serve as an early indication of contaminant stress and provide valuable information regarding the extent, and possible consequences of prolonged or continued exposure. Induction of the protein metallothionein has been proposed as one such sublethal biological indicator of metal pollution.

1.2 Metallothionein

Metallothionein (MT) was first identified as a result of studies into elevated cadmium concentrations in the tissues of man and other mammals. These led to investigations to identify the protein carrier of cadmium. Margoshes & Vallee (1957) described a low molecular weight cadmium-binding protein in equine renal cortex which was later purified, characterised and designated as metallothionein (Kagi & Vallee, 1960, 1961).

Since its discovery, the structure and metal binding properties of MT have been extensively studied and the protein has been reported to occur throughout the animal kingdom, in vertebrates (fish, amphibians, reptiles, birds, mammals) and invertebrates (echinoderms, annelids, molluscs, crustaceans, insects) as well as in plants, several eukaryotic micro-organisms, and in some prokaryotes (Engel & Brouwer, 1989). The structure and metal regulation of MTs are highly conserved throughout most phyla (George & Olsson, 1994).

MT is a soluble, heat stable protein with a molecular weight of 6800 Daltons, based on amino acid analysis (60-62 amino acid residues). Approximately 30% of these residues are cysteine and serve as ligands for metal chelation, binding Class B metal cations including silver (Ag), cadmium (Cd), copper (Cu), mercury (Hg) and zinc (Zn) (Langston *et al.*, 1998). Vertebrate MT binds 6-7 gram atoms of Cd or Zn by tetrahedral co-ordination with the cysteine residues and up to 11-12 gram atoms of Cu and Hg. Metal ions are bound in two metal thiolate clusters giving the MT molecule a prolate ellipsoid shape which on size exclusion chromatography results in an apparent (overestimated) molecular weight of 10-15kDa. MT appears to be present in dimerised form in extracts from certain species -particularly invertebrates - with an apparent molecular weight in the region of 20kDa (George & Langston, 1994).

Whilst the binding affinity for metals to cysteine residues in MT is generally considered to be highest for Hg, followed in decreasing order by Cu, Cd and Zn, this is not reflected universally in the ability of these metals to induce MT synthesis. In most of the vertebrate species studied Zn is the most potent inducer, Cd and Hg being less effective, whilst Cu is

often a poor inducer. Consequently, Zn usually saturates the binding sites of MT although it can be displaced by increasing amounts of Cu, Cd and other metals in high concentrations (Palmiter, 1994). In aquatic invertebrates this may not be the case; in mussels, for example, Cd is the most potent inducer of MT and Zn the least (Langston *et al.*, 1998).

Production of MT is regulated by genes and the protein is thought to be involved in various intracellular processes associated with metal metabolism. It occurs primarily in the cytoplasm, although it has also been detected in the nucleus and lysosomes. Synthesis of MTmRNA can occur in most tissues in response to metals, although translation seems most effective in livers of teleosts (bony fish) and consequently concentrations of the protein tend to be highest in this tissue (George *et al.*, 1996). MT acts as a storage and supply site for essential metals such as Zn and Cu which are utilised in protein synthesis, nucleic acid metabolism and other metabolic processes (Roesijadi, 1994). In addition to this regulatory function, MT may also play a role in metal detoxification. An excess of intracellular free metal ions, whether essential or non-essential, can have damaging effects, impairing the conformation and activity of enzymes and metalloproteins. Under conditions of elevated metal concentration, MT synthesis is induced, providing more binding sites for metal ions and limiting latent damage. The induction of MT is, therefore, a potentially powerful biochemical indicator of response to metal contamination.

Another potential 'signal' of sub-lethal impact from excess metal is the appearance of atypically high metal concentrations in non-thionein (non-MT) pools. Such disturbances in the normal partitioning of metals among different cellular components (cytosolic pools) - the so-called 'spillover' effect - signifies that the homeostatic capacity of detoxifying proteins (i.e. MT) are reaching saturation and that deleterious effects may follow (Brown *et al.*,1987).

To date, metallothionein has been identified as playing a homeostatic and regulatory role in several marine and freshwater fish species, including salmoniformes (rainbow trout), pleuronectiformes (flounder and plaice), cypriniformes (stone loach and goldfish) and gadiformes (cod), with most MT activity occurring in the liver (Olsson, 1996). These laboratory experiments confirm, generally, that teleost MTs are inducible by Cd, Cu, Zn and Hg, that MT levels increase in proportion to the dose of administered metals, and that the metals are bound to MT (reviewed by George & Olsson, 1994). There are a variety of other conditions that could also result in MT induction: basal levels of hepatic MT in some fish species have been shown to vary with time of year, reproductive state, water temperature and developmental state (Overnell *et al.*, 1988, Olsson *et al.*, 1987), though, overall, MT induction is far greater in response to metal contamination.

Few studies have focused on metallothionein levels in the order Apodes (eels) despite the apparent suitability of the common eel *Anguilla anguilla* to act as an indicator organism (see below). One early report suggested that MT is a normal constituent of eel liver, and that levels are increased by exposure to Cd, Zn and Cu (Noël-Lambot *et al.*, 1978), but a review of the literature has revealed no further work on MT in the species. In fact, field studies on MT in any teleost are remarkably few in number, particularly along estuarine gradients.

1.3 Objectives and benefits of present research

The primary objective of this study is to assess the toxicological significance of accumulated metal burdens in populations of the native eel *Anguilla anguilla* from different sites along the length of the Thames Estuary. The measurement of MT levels in livers of these organisms, coupled with determination of the relative proportions of metals in various subcellular components, comprise a new approach to monitoring environmental quality which, it is hoped, will provide an indication of biological impact, as well as an assessment of contamination. The basic hypothesis under test is that induction of the protein MT, above basal levels, constitutes a sublethal response to metal contamination, in that the organism is attempting to adapt to elevated levels of bioavailable metals. If successful, this investigation of MT concentrations and associated metal-binding properties in the common eel will not only provide valuable baseline information on the quality of the Thames Estuary, but will justify a similar approach on a wider basis (including freshwater habitats). *Anguilla* is one of the few species of any taxonomic group whose distribution covers the entire salinity range from river to the sea.

The current study is closely linked to other joint PML-EA projects on the Thames Estuary (see, for example, published reports on TBT and metals in the *Thames Estuary Environmental Quality Series*, 1 and 2). Results of these studies, and others on stress indices, (reports in preparation), will contribute towards a greater understanding of the status of contamination and impact along this major tidal waterway.

2. Materials and methods

2.1 *Anguilla anguilla* - the common eel

The common eel *Anguilla anguilla* (Plate 1) has been an important part of the Thames estuarine biota for many years, with eel fisheries in the Thames recorded as long ago as the 11th century in the Domesday book (Anon, 1086). *A. anguilla* breeds in the Sargasso sea and migrates as the larval stage (leptocephalus) to Europe, where metamorphosis changes the larvae into more recognisable elvers or glass eels. Juveniles, referred to as 'yellow' eels due to the colour of the underside, are common in the waters of rivers and estuaries, penetrating the Thames freshwater catchment as far as the head waters above Oxford (Naismith & Knights, 1993). Juveniles are known to remain in European waters for 7-20 years until changes in pigmentation (silvering) and morphology herald the start of the journey back to their Sargasso breeding grounds. Mature silver eels leave the inner Thames in late autumn. Yellow eels are territorial and maintain local home-ranges of up to 450 feet in diameter (Slayter, 1981). They are generally nocturnal, and hide in mud, weed beds or shady pools during the day (Naismith & Knights 1990). Eels feed on benthic invertebrates but will consume almost any aquatic fauna occurring within the area. Feeding is seasonal - restricted to the months between April and September; in colder months eels burrow into the mud, entwine together and pass the winter in a torpid state (Slayter, 1981). The gender of young eels is difficult to distinguish even under the microscope, as ribbon-like testes can easily be mistaken for ovaries (Tesch, 1977). Females can attain lengths of 90-150 cm whilst male eels rarely exceed 50 cm (Deelder, 1984).

Habitat and feeding preferences make the eel vulnerable to the type of sediment-associated contamination which occurs in estuaries, and, as a relatively stationary, territorial organism *A. anguilla* fulfils the criteria for selection as an indicator species. It's role as a possible bioindicator of sediment bound metals is a particularly important area for investigation.

2.2 Sample sites and collection of samples

Routes for metal uptake in fish vary but are primarily *via* absorption across the gills from water, or gastrointestinal absorption from food. Sediments represent a potentially important, but, as yet, largely unquantified, assimilation pathway into benthic species such as eels. Irrespective of uptake route, following their absorption, metals are distributed by the blood to different tissues - notably to the liver and kidneys - where they may be sequestered by MT or induce MT synthesis (Olsson *et al.*, 1998). The present survey was therefore designed to investigate levels of MT and associated metals in liver samples from different populations of *Anguilla anguilla,* in relation to suspected trends in environmental contamination (sediment and water) along an axial transect of the Thames Estuary.

During 1998 sampling of eels took place at various sites from Richmond Lock seawards to Blythe Sands as indicated in Figure 1. Known and accessible eel habitats were chosen as sampling sites, coinciding, where possible, with sediment- and water-collection sites surveyed for metals in the previous year.

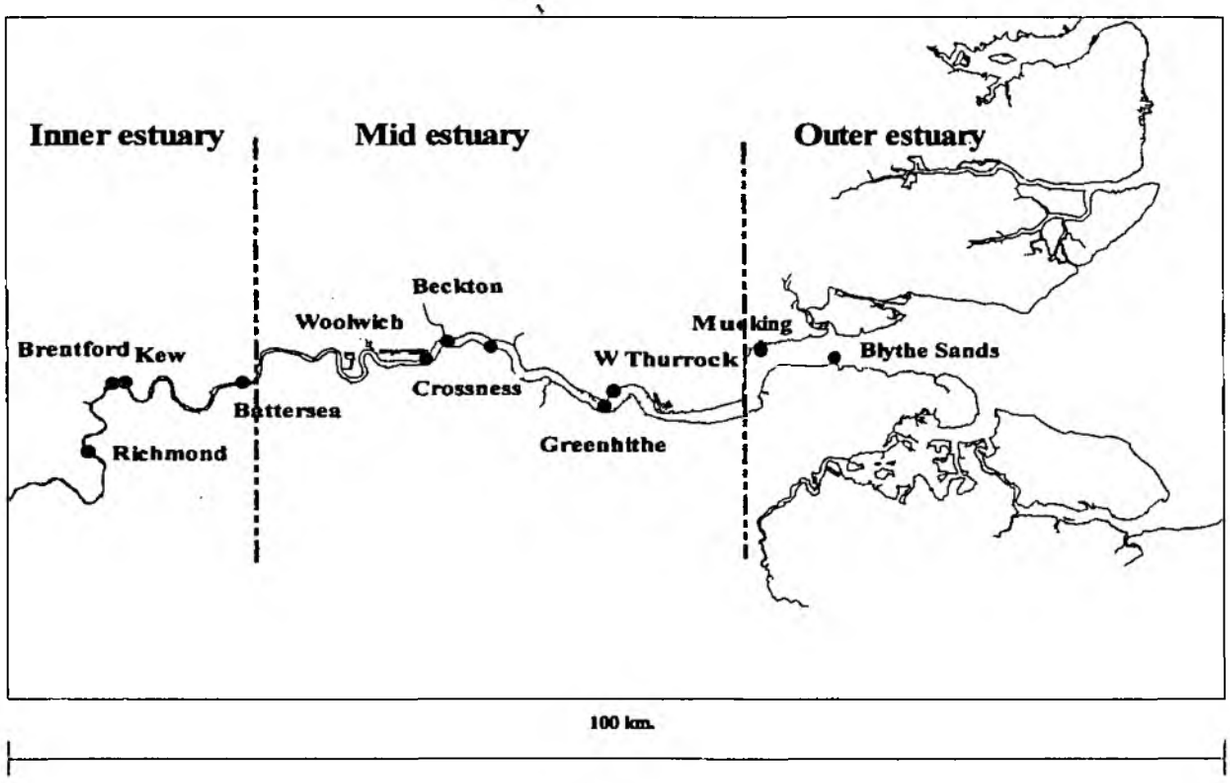

Figure 1. Location of eel sampling sites along the Thames Estuary (see also Table 1 for map references)

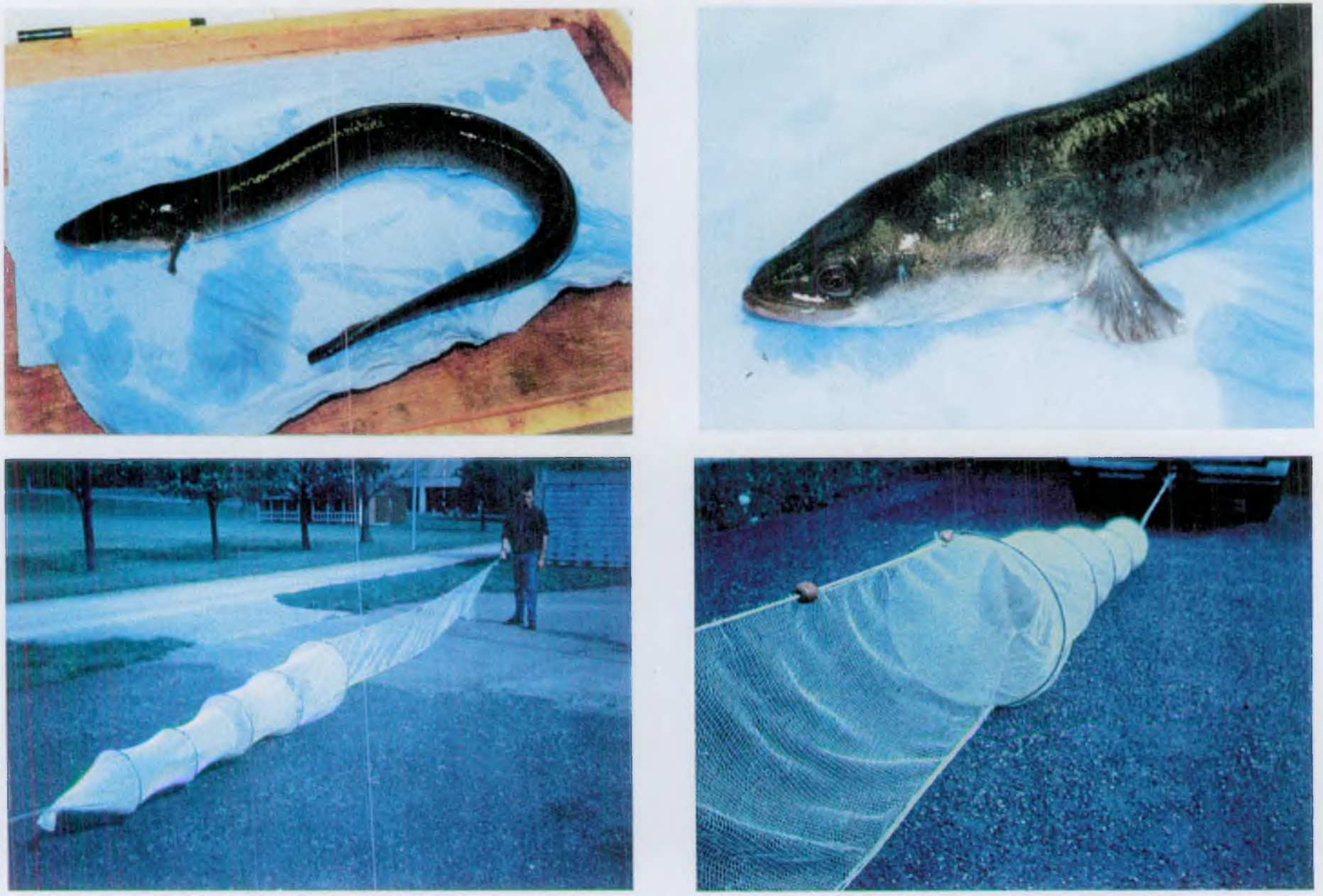

Plate 1. The common eel, *Anguilla anguilla*, and fyke-nets used in Thames Estuary surveys.

Table 1. Sample sites, dates and numbers of eels sampled during 1998 (see Fig.1 for locations)

Location	Map Reference	Dist from Teddington Lock (km)	Date	No. of eels in sample	Section of estuary**
Richmond	TQ 180 740	5	May	8	inner
	TQ 164 731		Aug	7	
	TQ 180 740		Nov	8	
Brentford	TQ 187 777	8	May	8	
	TQ 187 777		Aug	8	
	TQ 187 777		Nov	1	
Kew	TQ 192 778	9	Aug	8	
Battersea	TQ 265 768	25	May	8	
Woolwich	TQ430 795	43	May*	7	mid
Beckton	TQ 454 816	47	Aug	8	
Crossness	TQ 472 815	51	May	8	
	TQ 472 815		Nov	8	
Greenhithe	TQ 580 755	61	May*	8	
West Thurrock	TQ 616 762	62	May*	8	
	TQ 616 762		Aug	8	
	TQ 616 771		Nov	8	
Mucking	TQ 712 811	80	Nov	2	outer
Blythe Sands	TQ 770 800	100	May*	1	
	TQ 770 800		Aug	6	

* Denotes samples which were trawled (others sites sampled using fyke nets)

** see Figure 1

Eels were collected in May, August and November 1998. Sampling was carried out in conjunction with Agency staff and local eel fishermen, who contributed valuable background knowledge of the estuary, eel fishing methods and sites. During the May sampling period some sites were trawled for eels, but this proved to be inefficient. Subsequently, all samples were collected in fyke-nets (Plate 1) set parallel to the shore in pairs and left for at least one night, over one tide. Nets were then taken up and the catch transported live to the EA laboratory at Crossness in ambient water. Sampling in November was problematic; unusually high spring tides and flood waters hampered the setting out and collection of nets, restricting sampling to five sites. In addition, catches at some sites were low, probably reflecting the semi-dormant state of eels during colder

winter months. Nonetheless data for November has been included in the report, partly for reference purposes, but primarily because results provide interesting analogies with studies of MT in other fish species.

The gender of individual fish was not determined due to well-acknowledged difficulties in differentiation, but the length of the eel serves as a rough guide, as males rarely exceed 50cm. After recording lengths and weights, the fish were killed by a blow to the head and the spinal cord severed. Livers were excised without rupturing the gall bladder and frozen in liquid nitrogen, before being transported to Plymouth Marine Laboratory where they were stored at -80°C prior to analysis.

For reference purposes a sample of eels was also taken from Neal Point on the River Tamar (Map Ref. SX 436 613) in the South West of England in August 1998.

2.3 Treatment and analysis of samples

The number of livers sampled in eel populations at each site varied between one and eight, depending on fish availability (Table 1). For the MT assays, 0.5-1g portions from individual livers were allowed to thaw slowly on ice. The samples were homogenised in five volumes of 0.02M Tris-HCl buffer in an ice bath. An aliquot of each homogenate (3-5ml) was centrifuged (MSE HI-Spin 21) at 28000g for 40 min at 4°C. After this first centrifugation step an aliquot of the supernatant (one sample from each site) was subjected to gel chromatography as described below (section 2.5).

To help partially purify MT, the remainder of the above supernatant (cytosol) was decanted and heated in a water bath at 80°C for 10 mins in order to denature high molecular weight proteins which might interfere with MT analysis. Heat-treated supernatant was then re-centrifuged at 28000g for a further 40 mins at 4°C and the MT-containing cytosol separated from precipitated proteins.

A further, measured aliquot of liver homogenate, for determination of total hepatic metal concentration (and wet:dry weight ratios), was dried at 80°C for 24hrs, re-weighed, and digested with concentrated HNO_3 on a hotplate. Digested samples were evaporated to near dryness before dissolution in 1M HCl. Cu and Zn analysis of the digests was carried out by flame atomic absorption spectrophotometry using an air-acetylene flame (Varian® AA20). Concentrations of Ag and Cd were determined by graphite furnace atomic absorption spectrophotometry (Varian® 300 Zeeman) using standard addition methods. All metal concentrations are expressed on a dry weight basis. The certified reference material DORM-2 (National Research Council, Canada), and internal standards, were run with samples as a check on analytical performance.

2.4 Metallothionein determination

Metallothionein was measured directly in the partially-purified (heat-treated) supernatant, using differential pulse polarography (DPP) according to the method described by Bebianno & Langston (1989). This method takes advantage of two features of MT: firstly, the stability of MT at high temperatures is relatively unique and enables other, potentially interfering, proteins to be removed by heat denaturation; secondly, the high cysteine content typical of MT can be used as a basis for determining concentrations of the metal-binding protein directly, using DPP. This quantitative sulphydryl-specific procedure is

based on the linear relationship between the thiol (-SH) concentration in proteins and the height of the corresponding polarographic wave, formed as samples - complexed with excess Co - are reduced during a potential scan between -1.4 and -1.6V. The assay was carried out using a PARC model 174A analyser and a PARC/EG&G model 303 static mercury drop electrode (SMDE). An aliquot of heat-treated cytosol (1-2µl), together with 300µl Triton-X (0.025%, v/v), were added to 10ml hexamminecobalt chloride buffer (the electrolyte) and the polarographic response of the eel MT plotted on an X-Y flat-bed recorder. Comparisons of measured peak heights with those from standard additions of purified rabbit MT (Sigma) enabled quantification of MT in eels, expressed on a dry weight basis (Bebianno & Langston, 1989). Wet weight conversions have also been performed (see appendix) to allow comparisons with published data on other species.

2.5 Gel chromatography and preliminary validation of the assay

To establish cytosolic metal-binding characteristics, and the scale of involvement of MT in eels, the supernatant (before heat treatment) from one individual liver per site was fractionated by size-exclusion gel chromatography. Approximately 0.5ml of supernatant (prepared as described above) was applied to a 1.5 x 60cm Sephadex G-75 column equilibrated at 4°C with 0.02M Tris-HCl buffer, pH 8.6. Samples were eluted at 0.5ml min^{-1} using the same buffer and collected as 3ml fractions for metal and -SH analysis. Optical absorbance in the eluate was measured at 254nm on a Varian® Cary-1 UV visible spectrophotometer as a general marker for proteins and amino acids.

For validation purposes, an aliquot of heat treated supernatant was fractionated in the same way in order to confirm the precipitation of the majority of high molecular weight proteins and the heat stability of MT. The chromatography column was calibrated using bovine serum albumen, hen egg albumen, carbonic anhydrase and cytochrome C as standard molecular weight markers.

Typical UV-absorbance profiles (254nm) from both heat-treated and non heat-treated hepatic cytosol are plotted in Figure 2A. The first major peak to elute, between fractions 15-20, represents high molecular weight (HMW) proteins, including enzymes, and, as expected, is dominant in non heat-treated cytosolic extracts. Very low molecular weight (VLMW) compounds such as amino acids are responsible for the second peak in absorbance between fractions 40-55. Comparison of the two absorbance profiles in Figure 2A indicates that heat treatment removes >95% of the HMW proteins from eel cytosol samples. Heat denaturation, followed by centrifugation should, therefore, minimise interference from most other thiolic proteins during metallothionein determination.

In order to confirm the effectiveness of this 'purification' step, chromatographic fractions from eel samples (heat-treated and untreated) were analysed for thiolic proteins by DPP (Figure 2B). With an apparent molecular weight in the region of 10-20 kDa and high cysteine content, MT was anticipated to elute, and be detectable, between fractions 24-40. In untreated cytosol samples, a high thiolic (cysteine) content was measurable both in the HMW protein pool (fractions 15-20) and in the MT-containing fractions (24-40) as indicated in Figure 2B. After heat-treatment of cytosol samples, there was little polarographic response associated with HMW proteins (mostly precipitated, together with any associated metal) whilst that attributable to MT remained unchanged (Fig 2B). There was virtually no contribution from very low molecular weight, cysteine-containing, fractions (which would include the free amino acid). Therefore, it is concluded that heat

treatment and high-speed centrifugation separates MT from most potentially interfering compounds in eel cytosol samples, allowing direct quantification of the protein by polarography. Other than the expected precipitation of metals associated with HMW proteins, heat treatment had no effect on distribution of metals in other fractions - MT or VLMW (data not shown).

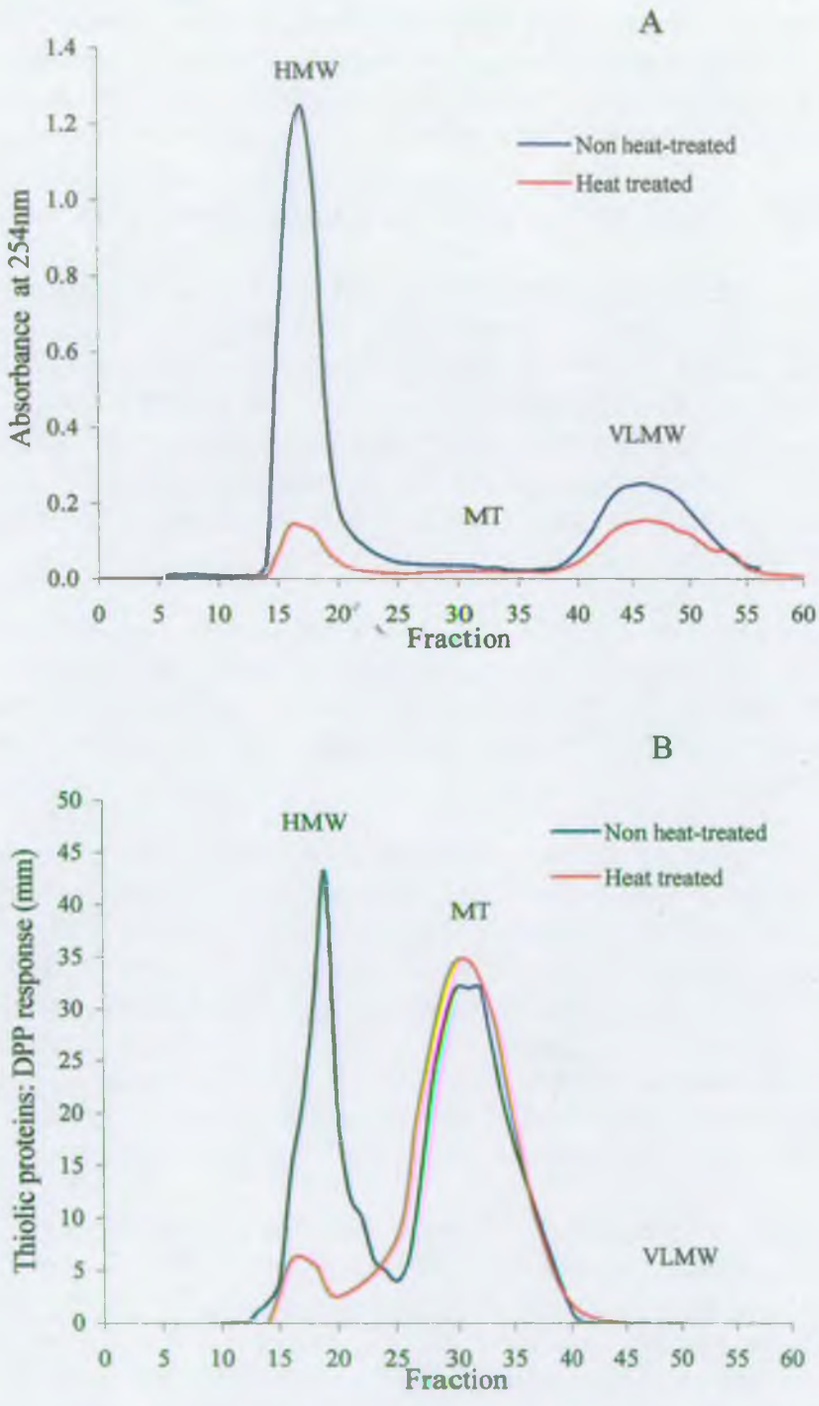

Fig. 2. *Anguilla anguilla* (Battersea, May 1998). Profiles of absorbance at 254 nm (A) and polarographic response (B) obtained from gel filtration (Sephadex G-75) of hepatic cytosol, before (blue line) and after (red line) heat-treatment. This comparison shows effective removal of HMW proteins. Thiolic (cysteine) residues remaining after heat denaturation are predominantly associated with MT.

Metal-partitioning behaviour in eel cytosol (non-heat-denatured) was investigated by measuring Ag, Cd, Cu and Zn in eluted fractions of chromatographed samples, using flame or graphite furnace atomic absorption (described in section **2.3**). Examples of typical distributions in the cytosol of Thames eels (Kew) are illustrated in Figure 3, confirming the importance of the association of these metals with the metallothionein fraction. The sizeable association of Zn, and, to a lesser extent, Cu, with the HMW fraction is consistent, partly, with the role of these two essential metals in metalloenzymes. Minor quantities of Zn and Cu were associated with the VLMW pool (amino acids and 'free' metal).

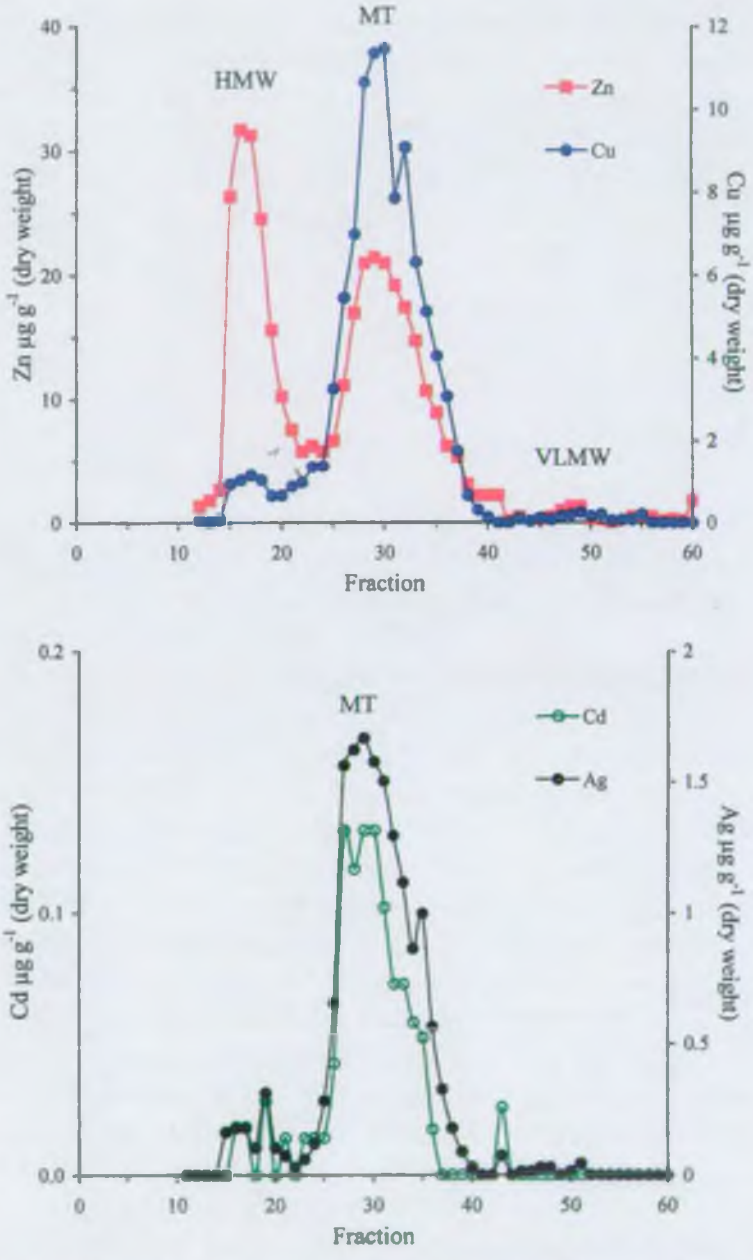

Fig. 3. *Anguilla anguilla* (Kew, August 1998). Typical distribution of metals in (non heat-treated) cytosolic fractions of Thames eel liver, following size exclusion chromatography on Sephadex G-75. Upper figure, Cu and Zn; lower figure, Ag and Cd. Note different scales. All metals were bound in significant proportions to MT; Zn was also associated to a large extent with HMW proteins.

2.6 Data processing and analysis

Data on MT and metal concentrations in eel livers (including various fractions, described above), together with data for metal concentrations in sediments and water - collected in a separate survey (July 1997)[1] - were recorded in *Microsoft Excel* spreadsheets. Mean MT concentrations were calculated for each eel population and plotted to show the hepatic MT distributions along the estuary on the three sampling occasions. Correlation and regression analyses of these data were performed to reveal spatial and temporal trends. Relationships between MT concentrations and individual metals (total, cytosolic or MT-bound) were also examined.

For the MT data, homogeneity of variances was determined by Levenes test. A $\log_{10}$ transformation was used to transform data where the variances from raw data were not homogenous. The data set was imported into *Statistica* and subjected to one-way Anova (Analysis of Variance) and Tukeys HSD (Honest Significant Difference) tests, to reveal any significant differences in MT concentration between sites.

3. Results

Data showing the length, weight, hepatic MT and metal concentrations in the livers of individual eels analysed in this study are included in the appendix at the end of this report. The following sections present our interpretation of the results.

3.1 Hepatic metallothionein concentrations

Levels of hepatic metallothionein in *Anguilla anguilla* from the Thames Estuary varied between 1.19 - 11.15 mg g^{-1} (dry weight) overall. MT levels were variable within each population but, generally, concentrations were highest in populations from inner and mid-estuarine sites. One-way Anova, and Tukey's HSD tests on the logarithmically transformed Thames data (all dates) confirmed that MT levels in eels from both Brentford and Kew (mean MT - 4.8 and 4.9 mg g^{-1}, respectively) in the inner estuary were significantly higher (by a factor of 2) than in eels from Blythe Sands (2.1 mg g^{-1}) at the mouth of the estuary ($p<0.05$). These trends are illustrated in Figure 4, which shows mean MT concentrations decreasing with distance downstream from Teddington Lock, on each of the sampling dates. Linear regression analyses of the data, though not necessarily the best model, serve to confirm that gradients in MT concentrations decreased consistently toward outer estuarine sites. The slope of the regression lines differed, slightly, depending on the time of sampling. This may be due partly to the fact that different populations were sampled in each of the three surveys, though seasonal factors cannot be ruled out. The steepest of gradients, and most significant R^2 ($P<0.05$) value, was that of the August sample (Fig. 4).

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[1] Bioaccumulation of Metals in the Thames Estuary - 1997. *Thames Estuary Environmental Quality Series*, **2**, 116pp (October 2000).

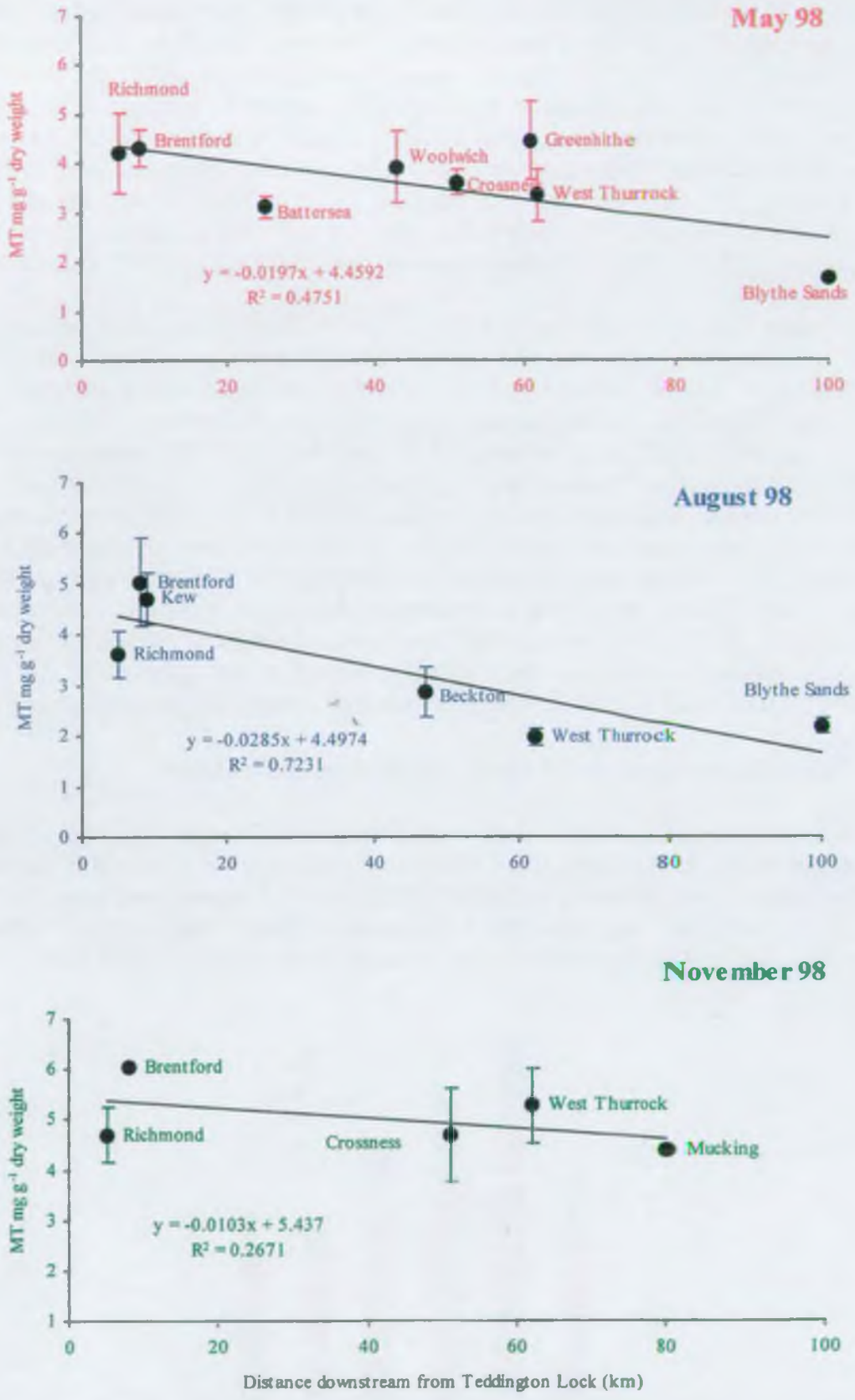

Fig 4. *Anguilla anguilla*. Hepatic MT levels (mg g^{-1} dw) in eel populations of the Thames Estuary, plotted as a function of distance downstream from Teddington Lock. (Error bars represent standard error of the mean where n>2; linear regression equations shown).

The mean MT concentration in eels from the Tamar Estuary, south west England, was 2.99 mg g^{-1} - comparable to levels in eels from West Thurrock and Blythe Sands in the outer Thames Estuary (1.95 and 2.22 mg g^{-1} respectively), collected during the same month (August 1998). Until more reference sites have been examined, there can be no guarantee that these values represent background levels. Historically, sections of the Tamar have been subjected to metal contamination from mining; similarly there is a long tradition of waste dumping at the mouth of the Thames Estuary. Nevertheless, the mean MT concentrations of 5-6 mg g^{-1} for some eel populations in the inner Thames are clearly high in comparison to these existing 'reference' values, and signify elevated MT induction.

It would seem from the literature that MT in certain fish species might sometimes be induced, to a limited extent, by endogenous factors other than metals – notably size. Unfortunately, it was not always possible to sample eels of a consistent length at the Thames sites. Therefore, in order to rule out the influence of allometric parameters on MT levels, weight and length were investigated as variables. Regression analyses revealed a slight positive correlation between MT levels and the weight ($r=2.85$; $P=0.001$) and length ($r=0.24$; $P=0.006$) of individual eels, but as there was an even weight distribution among sites, size does not explain the observed MT gradients. If anything, eels sampled from the outer estuary were marginally (though not significantly) the longest on average, whereas MT levels were lowest here. This is contrary to the expected trend if size, rather than metals, was the dominant factor. It was therefore concluded that the size of eels used in this survey was not influencing observed MT gradients to any great extent, although in future work endeavours should be made to use eels of a more consistent weight and length.

3.2 Metallothionein and hepatic metal concentrations

Preliminary results indicated that there were four principal metals (Zn, Cu, Ag and Cd) associated with MT in the livers of eels from the Thames Estuary. Concentrations of these metals in whole livers decreased in the order Zn>Cu>>Ag>Cd and were generally highest in eels from inner- and mid- estuarine sites, on all sampling dates. Figure 5 shows the average hepatic metal concentrations of eel populations sampled in August 1998.

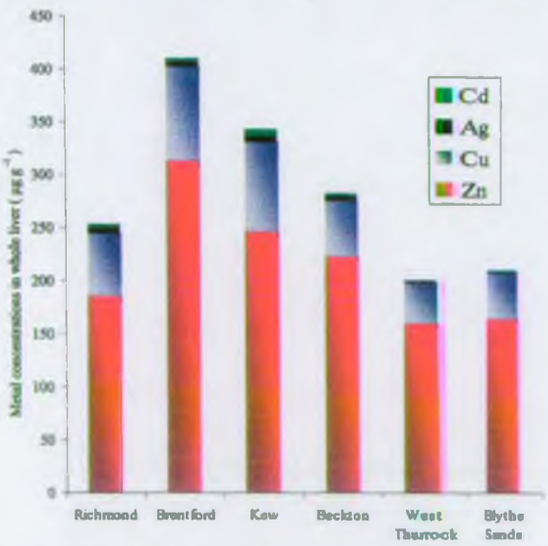

Figure 5. *Anguilla anguilla.* Mean concentrations of metals in livers of eel populations from Thames Estuary sites (August, 1998).

Table 2 summarises data describing the relationships between MT levels and molar concentrations of metals (individually and combined) in whole livers. Considering the entire data set as a whole, total hepatic metals ($\sum$ Zn+Cu+Ag+Cd) and MT levels were significantly correlated ($r=0.57$, $p=2.95 \times 10^{-12}$)

Correlations for individual sampling dates are illustrated in Figure 6, which shows the profiles of MT and total hepatic metals ($\sum$ Zn+Cu+Ag+Cd) in eel populations along the estuary in May, August and September (1998) surveys.

Concentrations of individual metals were also highly correlated with MT levels, most significantly for Cu ($r=0.78$, $P=3.35 \times 10^{-27}$, using combined data for the three surveys). With the exception of Cd in May and November, significant correlations between individual hepatic metals and MT levels were found on all sampling dates (Table 2).

Table 2. ***Anguilla anguilla.*** **Relationships between MT levels and metal concentrations (molar) in livers. Summary of results from statistical tests for individual surveys and for the entire data set. r = correlation coefficient, p-value = significance level obtained by regression analysis.**

Date	Zn		Cu		Ag		Cd		Zn+Cu+Ag+Cd	
1998	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value
May	0.3	0.027	0.76	8.3×10^{-12}	0.39	3×10^{-3}	0.22	0.10	0.48	2.21×10^{-4}
Aug	0.88	2.2×10^{-15}	0.93	1.8×10^{-20}	0.56	6.3×10^{-5}	0.56	5.6×10^{-5}	0.92	2.08×10^{-19}
Nov	0.65	2.7×10^{-4}	0.77	2.1×10^{-6}	0.64	2.99×10^{-4}	0.31	0.12	0.72	1.9×10^{-5}
All	0.49	5.5×10^{-9}	0.78	3.3×10^{-27}	0.53	9×10^{-11}	0.29	8.1×10^{-4}	0.57	2.95×10^{-12}

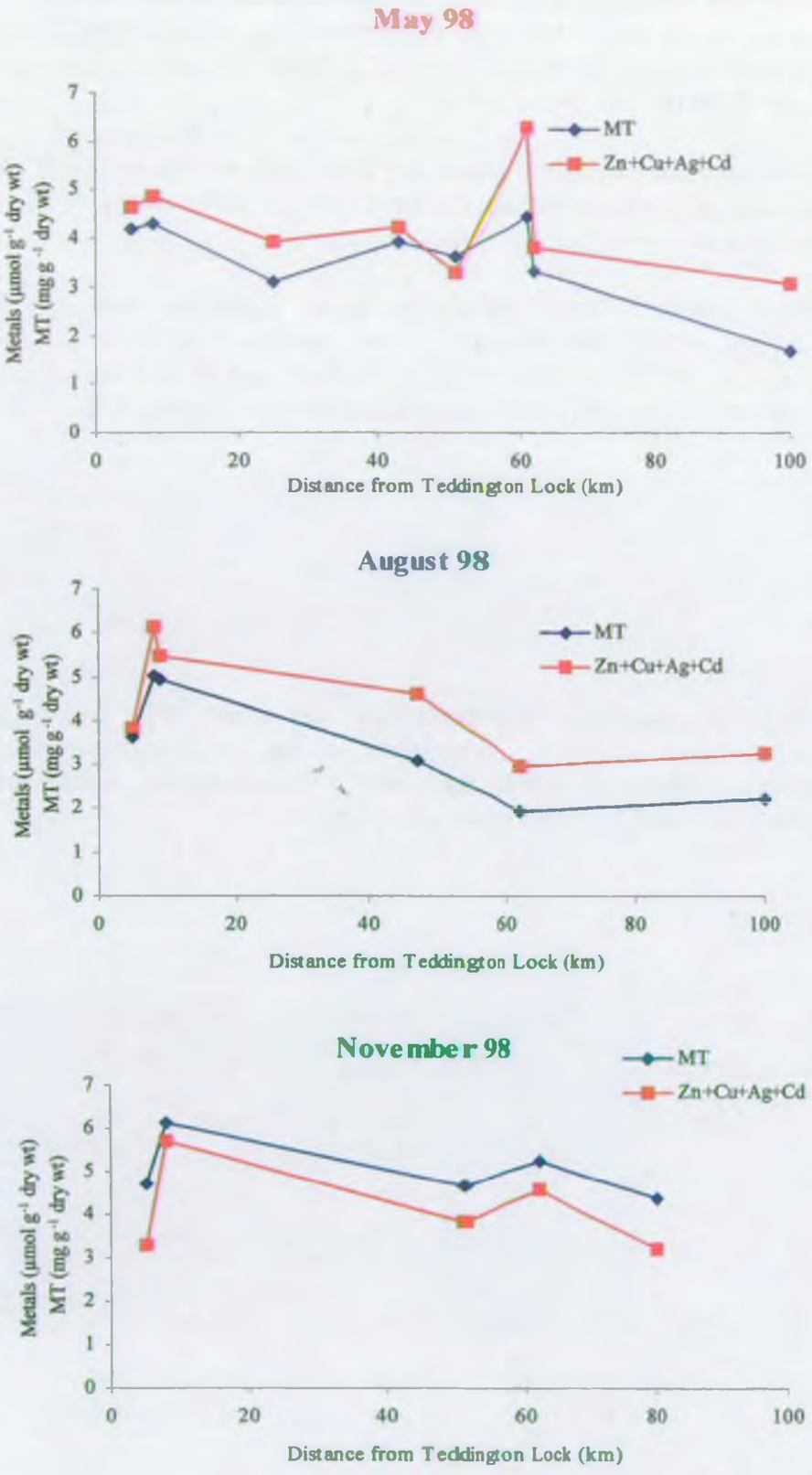

Figure 6. *Anguilla anguilla*. Profiles of total hepatic metal concentrations (∑Zn+Cu+Ag+Cd, expressed as µmol g^{-1} dry wt) and MT levels in eel populations along the Thames Estuary (May, August and November, 1998).

Summation of the molar quantities of metal (∑Zn+Cu+Ag+Cd) bound to MT allows the approximate concentration of the protein to be calculated stoichiometrically (assuming that one MT molecule binds 7 g-atoms of metal), and hence compared with direct measurements by polarography (as further assurance of the validity of data). MT concentrations calculated by these two different methods way were, in fact, in good agreement (Figure 7, r =0.7798; P<0.0001) - considering the assumptions made in stoichiometric estimations. To achieve such good agreement ∑Zn+Cu+Ag+Cd must account for most of the metal-binding capacity of MT with only a small proportion available for other metals. This is further evidence that MT induction in the livers of Thames eels is a direct function of bioaccumulated metal burdens.

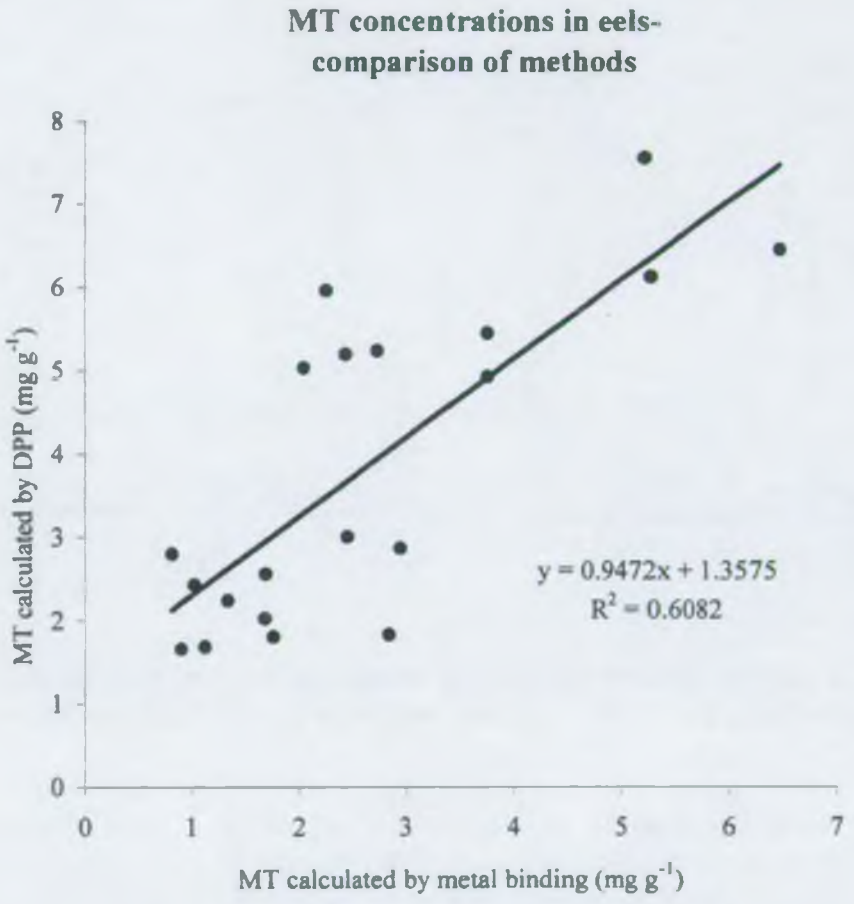

Figure 7. Comparison of MT determinations in eel livers by different methods: direct measurement by Differential Pulse Polarography (DPP) and estimation from metal binding (assuming 1 mole of MT binds 7 g-atoms metal).

3.3 Subcellular distribution of metals in hepatic cytosol

Chromatographic separation of eel liver cytosol extracts showed that each of the metals measured (Zn, Cu, Ag and Cd) was associated with the MT fraction, often predominantly so. Typical profiles for livers of eels from Richmond (inner estuary) and West Thurrock (mid/outer estuary) are illustrated in Figures 8 (see also Kew, inner estuary; Fig 3). The ratios of MT-bound metals, calculated in moles, varied, systematically, depending on the region of the estuary where eels were sampled. For inner estuarine sites the sequence of metals bound to MT, in terms of decreasing molar quantities, was generally Cu ≥ Zn>Ag>Cd but in eels from the outer estuary the sequence Zn>Cu>Ag>Cd was more

usual. These results might imply that Cu was the metal most responsible for the observed MT induction (in the inner estuary), though it should be noted that concentrations of all metals bound to MT increased in an upstream direction.

Figure 8A shows the chromatographic profiles of metals in hepatic cytosol of an eel caught at Richmond in August 1998. The MT concentration in the liver of this eel was 5.2 mg g^{-1}. Cu and Zn were the principal metals bound to MT; Ag and Cd concentrations were considerably lower (note different scales in Fig 8A). The profile for Zn (and, to a lesser extent, Cu) also shows the expected association with HMW proteins. However, most of the cytosolic Cu (Ag and Cd) was bound to MT. Only minor quantities of each metal were associated with the VLMW pool.

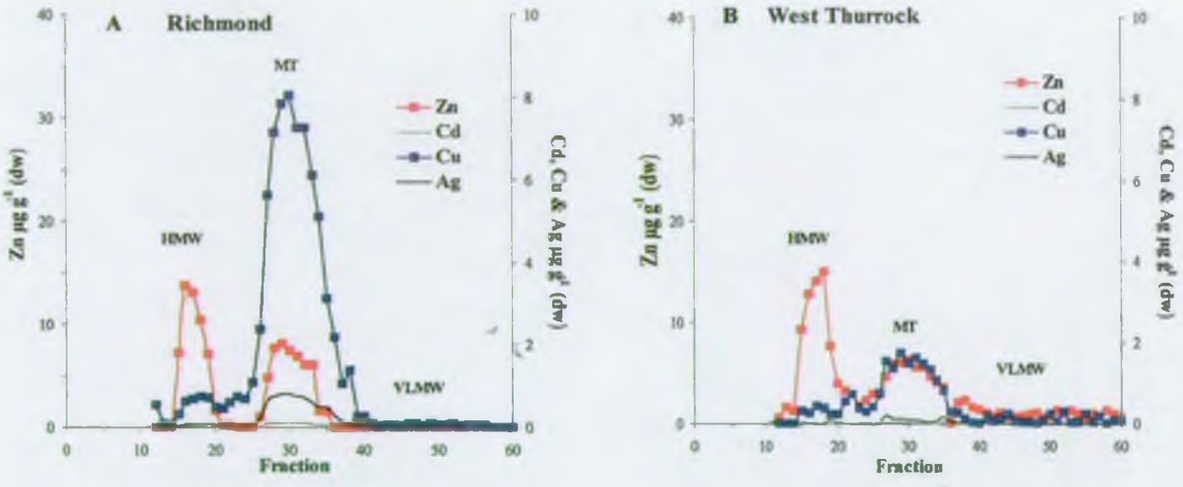

Fig. 8. *Anguilla anguilla*. Elution profiles of metals in hepatic cytosol (non heat - treated) from eels collected at Richmond (A) and West Thurrock (B) in August 1998.

For comparison, metal distributions in the hepatic cytosol of an eel caught at West Thurrock in the mid/outer estuary, in August 1998, are shown in figure 8B. The MT concentration in this eel was 1.81 mg g^{-1} and, with the exception of Zn concentrations (which were similar), metal levels were lower than those at the inner estuarine site, Richmond (Fig 8A). Considerably less Cu, Ag and Cd was bound to the MT fraction in the W. Thurrock sample, although it still represented the major metal-binding pool for these metals. The resulting ratio of Cu:Zn bound to MT was characteristically higher at the upstream site (1) than at W. Thurrock (0.25).

Statistical analysis reveals the strength of correlations between MT levels and associated metals (in moles), as summarised for August and November surveys in Table 3. This information serves as a further guide as to which metals are sequestered preferentially - and induce MT. However it must be remembered that these relationships were derived from one eel per site only, and, unfortunately, the number of samples can affect the apparent statistical significance of results. Consequently, although r-values for the relationships between MT levels *vs* MT-bound metals (Table 3) were generally higher than for MT vs total hepatic metals (Table 2), p-values were also higher (less significant,

statistically) because of the small sample size. Thus, in November, whilst $Zn_{[MT]}$ and $Ag_{[MT]}$ were correlated significantly ($p<0.05$) with MT levels, as were the combined metals ($\sum$Zn+Cu+Ag+Cd) in the MT fraction, $Cu_{[MT]}$ and $Cd_{[MT]}$ were not ($p>0.05$), despite relatively high values for the regression coefficient (Table 3). In the August survey (slightly larger sample range), all MT-bound metals except Zn were significantly correlated with MT levels ($p<0.005$ for Cu; $p<0.05$ for Ag and Cd). Profiles of MT levels and MT-associated metals, for August samples, are plotted on a spatial scale in Figure 9 and illustrate the close similarity in distributions.

Larger, more uniform sample sizes would be preferable in future surveys, to eliminate possible bias in the strength of relationships between MT and metals caused by variable sample numbers and locations.

Table 3. ***Anguilla anguilla.*** **Statistical parameters describing relationships between concentrations of MT and MT-bound metals. (r = correlation coefficient; p-value = significance level of the linear regression analysis).**

Date 1998	Zn		Cu		Ag		Cd		Zn+Cu+Ag+Cd	
	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value
Aug	0.58	0.17	0.96	5.7×10^{-4}	0.93	0.02	0.82	0.02	0.78	0.04
Nov	0.96	0.01	0.73	0.16	0.99	7×10^{-4}	0.86	0.06	0.95	0.01

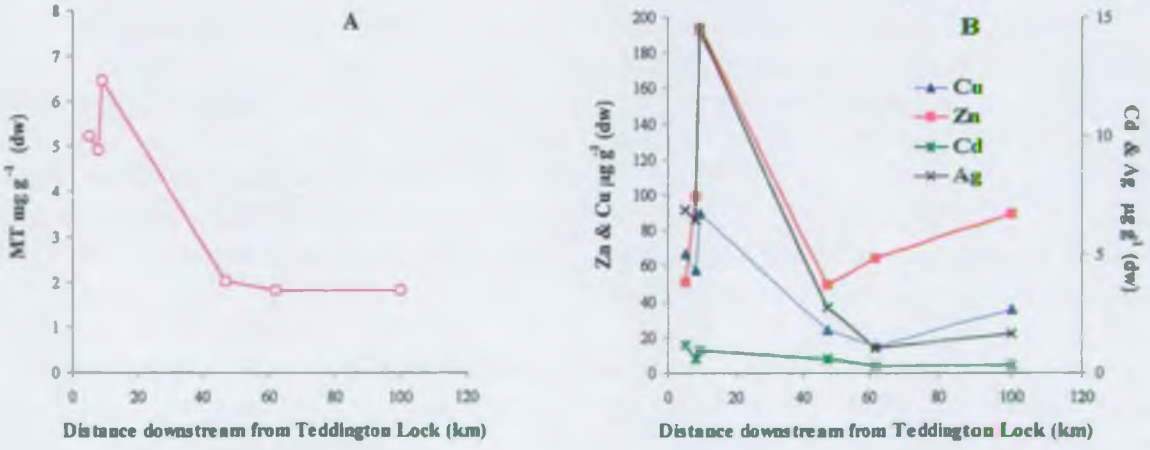

Fig. 9. ***Anguilla anguilla.*** **Profiles of MT (A) and MT-bound metals (B) in livers of eels collected along the Thames Estuary, August 1998.**

The high r values for relationships between MT and both total hepatic- and MT-associated metals are a further indication that induction of MT in eels is a direct result of elevated metal burdens. Without experimental evidence, it is not possible to conclude which metal is most effective in provoking MT production, since substitution reactions between metals are possible once the protein has been synthesised. Thus, the metals bound to MT reflect their binding affinity, as well as abundance, and need not necessarily be directly responsible for induction. Nevertheless, on current evidence, Cu was most significantly correlated with MT and may, therefore, have the greatest influence on MT levels.

Metal concentrations in the cytosol of Thames eels, together with data on metal partitioning, are summarised in Table 4. The results shown are for eels collected in August 1998 and are representative of other surveys (May and November). Similar data for the reference population (Tamar, August,1998) are included in Table 4, for comparison.

The data set in Table 4 confirms that, with exception of Zn, the majority of cytosolic metals were dominated by the MT fraction. The highest concentration of Cu in cytosolic form (100 $\mu g\ g^{-1}$) - of which 89% was associated with MT - was found in an eel from Kew (inner estuary). Cu bound to HMW and VLMW ligands constituted only minor proportions of cytosolic Cu at this site (9 and 2% respectively). A similar distribution was observed at other upstream sites, Richmond and Brentford. In eels from the mid- and outer estuary (Beckton, West Thurrock and Blythe Sands) cytosolic Cu concentrations were lower (20 - 58$\mu g\ g^{-1}$) and, on average, 73% of this was bound to MT, 19% was associated with the HMW pool and 9% with the VLMW cytosolic pool. The proportion of cytosolic Cu associated with MT in Thames eels was higher than in the Tamar reference population. In the latter, total cytosolic Cu was only 6.4$\mu g\ g^{-1}$, with 61% of this associated with the MT pool. This difference in concentration and partitioning behaviour is further confirmation that eels from the upper Thames are subjected to excess Cu burdens.

Cytosolic distributions of Ag were similar in proportion to those of Cu (Table 4). In eels from sites at the fresh water end of the estuary, Ag associated with the MT pool represented 83-92% of cytosolic Ag, with relatively minor amounts in HMW and VLMW pools (6-15% and 1.5-2%, respectively). Total cytosolic Ag was highest at Kew (15.9 $\mu g\ g^{-1}$). For mid- and outer estuary sites, proportions of MT-associated Ag were slightly lower (76-83%) with 11-13% and 5-9% present in HMW and VLMW pools, respectively. Total cytosolic Ag at these more marine sites ranged from 1.4-4.7$\mu g\ g^{-1}$. Again, the distribution of Ag in the cytosol of the Tamar and Thames eels differed: a much smaller fraction (49%) was associated with MT in the Tamar reference population, whilst 30 and 21% (respectively) were found in HMW and VLMW pools. Total cytosolic Ag in the Tamar eels (0.7$\mu g\ g^{-1}$) was also significantly lower (by up to twenty-fold) than in samples from the inner Thames. Clearly, enhanced bioavailability of Ag could be a contributory factor towards MT induction in the latter populations.

Partitioning of Cd between cytosolic pools in Thames eels followed the same pattern as that of Cu and Ag (Table 4), although the overall concentrations were much lower (Cd concentrations in cytosol ranged from 0.46-1.42$\mu g\ g^{-1}$). Most of this soluble Cd (73-89%) was associated with MT in eels from inner estuary sites. In fish from the middle and outer estuary, slightly lower proportions (57-72%) of the total cytosolic Cd were bound to MT. At the Tamar reference site, less than half (48%) of total cytosolic Cd was associated with MT. Relatively higher proportions of Cd than other metals were found in VLMW pools, both in Thames (25%) and Tamar (33%) eels.

Table 4. ***Anguilla anguilla.*** **Partitioning of cytosolic Zn, Cu, Ag and Cd in livers of eels from the Thames and Tamar estuaries, August 1998.**

Site	Total metal in cytosol ($\mu g\ g^{-1}$)	HMW ($\mu g\ g^{-1}$)	(%)	MT ($\mu g\ g^{-1}$)	(%)	VLMW ($\mu g\ g^{-1}$)	(%)
Zinc							
Richmond	105.0	53.7	51	51.2	49	0.0	0.0
Brentford	243.1	124.5	51	99.2	41	19.4	8.0
Kew	396.6	165.3	42	194.3	49	37.1	9.4
Beckton	181.4	121.3	67	49.9	28	10.2	5.6
West Thurrock	154.1	75.1	49	61.7	40	17.3	11.0
Blythe Sands	313.9	157.0	50	112.6	36	44.3	14.0
Tamar (control)	122.4	51.7	42	56.8	46	13.9	11.0
Copper							
Richmond	74.0	6.1	8	66.9	90	1.0	1.4
Brentford	65.0	6.9	11	57.8	89	0.4	0.6
Kew	100.2	8.9	9	89.0	89	2.2	2.0
Beckton	32.9	7.2	22	24.1	73	1.6	4.8
West Thurrock	20.1	3.6	18	14.6	73	2.0	9.9
Blythe Sands	58.1	9.2	16	42.4	73	6.5	11.0
Tamar (control)	6.4	2.0	31	3.9	61	0.5	8.0
Silver							
Richmond	7.43	0.44	6	6.87	92	0.12	1.6
Brentford	7.76	1.18	15	6.42	83	0.16	2.0
Kew	15.89	1.21	8	14.46	91	0.22	1.5
Beckton	3.38	0.44	13	2.77	82	0.16	4.9
West Thurrock	1.36	0.20	14	1.04	76	0.12	9.0
Blythe Sands	4.74	0.53	11	3.95	83	0.25	5.0
Tamar (control)	0.70	0.21	30	0.35	49	0.15	21.0
Cadmium							
Richmond	1.42	0.20	14	1.17	82	0.05	3.7
Brentford	0.84	0.08	10	0.61	73	0.15	17.0
Kew	1.08	0.09	9	1.00	89	0.03	2.5
Beckton	0.97	0.14	15	0.58	60	0.25	25.0
West Thurrock	0.46	0.04	9	0.33	72	0.09	19.0
Blythe Sands	0.56	0.20	35	0.32	57	0.05	8.0
Tamar (control)	0.90	0.17	19	0.43	48	0.30	33.0

The distribution of cytosolic Zn differed from that of Cu, Ag and Cd, because of the more significant proportion associated with the HMW protein pool (Zn_{HMW}). Inter-site variation in Zn partitioning was less pronounced than for other metals, as indicated for the August survey in Table 4. The highest concentration of cytosolic Zn was 397 $\mu g\ g^{-1}$, in the Kew eel sample, 49% of which was associated with the MT pool (Zn_{MT}) and 42% present as Zn_{HMW}. At other inner-estuarine sites (Richmond and Brentford), Zn_{HMW} accounted for 51% of the total cytosolic Zn. For the mid-estuarine sites of Beckton and West Thurrock the proportions of Zn_{HMW} were 67 and 49%, with 28 and 40 % present as (Zn_{MT}). Cytosolic Zn concentrations in eels from Blythe Sands, at the mouth of the estuary, were, perhaps, somewhat higher than expected (314 $\mu g\ g^{-1}$) - second only to Kew, nevertheless, partitioning of Zn in the Blythe sample was virtually identical to that at West Thurrock, upstream. In the Tamar population, the soluble Zn concentration (122$\mu g\ g^{-1}$) was at the lower end of the range determined in Thames eels though, again, the distribution pattern was similar (46 and 42% associated with MT and HMW pools, respectively).

Such consistency in Zn-binding behaviour in eels from different sites suggests that this essential metal may be regulated efficiently in the liver. MT is almost certainly involved in the regulation and storage of Zn, though the precise mechanism has yet to be demonstrated. In contrast, MT probably plays a more definitive, detoxifying role in sequestering excess Cu (and Ag) in Thames eels, as reflected in the estuarine gradients for MT and accumulated metals, established above.

Thus, from data in Table 4, there appears to be a trend towards a higher concentration of Cu, Ag and Cd (but not Zn) bound to MT in livers of eels collected at inner Thames Estuary sites. This suggests increased bioavailability of metals at upstream sites, coinciding with raised induction of the metal-binding protein in the liver. Again it must be stressed, however, that chromatographic profiles of metal distributions are derived from only one eel at each site. In Figure 10 the averaged data for inner, mid and outer Thames Estuary sites (see Fig. 1 and Table 1 for divisions) have been grouped to illustrate more definitively the trends in cytosolic metal-binding behaviour along the estuary, and to compare partitioning with the Tamar reference site.

To summarise, the majority of cytosolic Cu, Ag and Cd in eel livers was associated with MT, even at the reference site. However, there was also a tendency for a higher proportion of these metals to be bound by MT as contamination (and MT levels) increased upstream, towards the inner Thames sites. In the less heavily-contaminated outer estuary, the sub-cellular distribution of Cu, Ag and Cd more closely resembled that of Tamar 'controls'. In contrast to other metals, proportions of Zn_{MT} varied little between sites and there was a much more significant proportion of Zn associated with HMW proteins. This is consistent with an essential, regulated rôle for Zn in metalloenzymes, though the implications of higher Zn_{HMW} concentrations in some of the more contaminated Thames eels are not known.

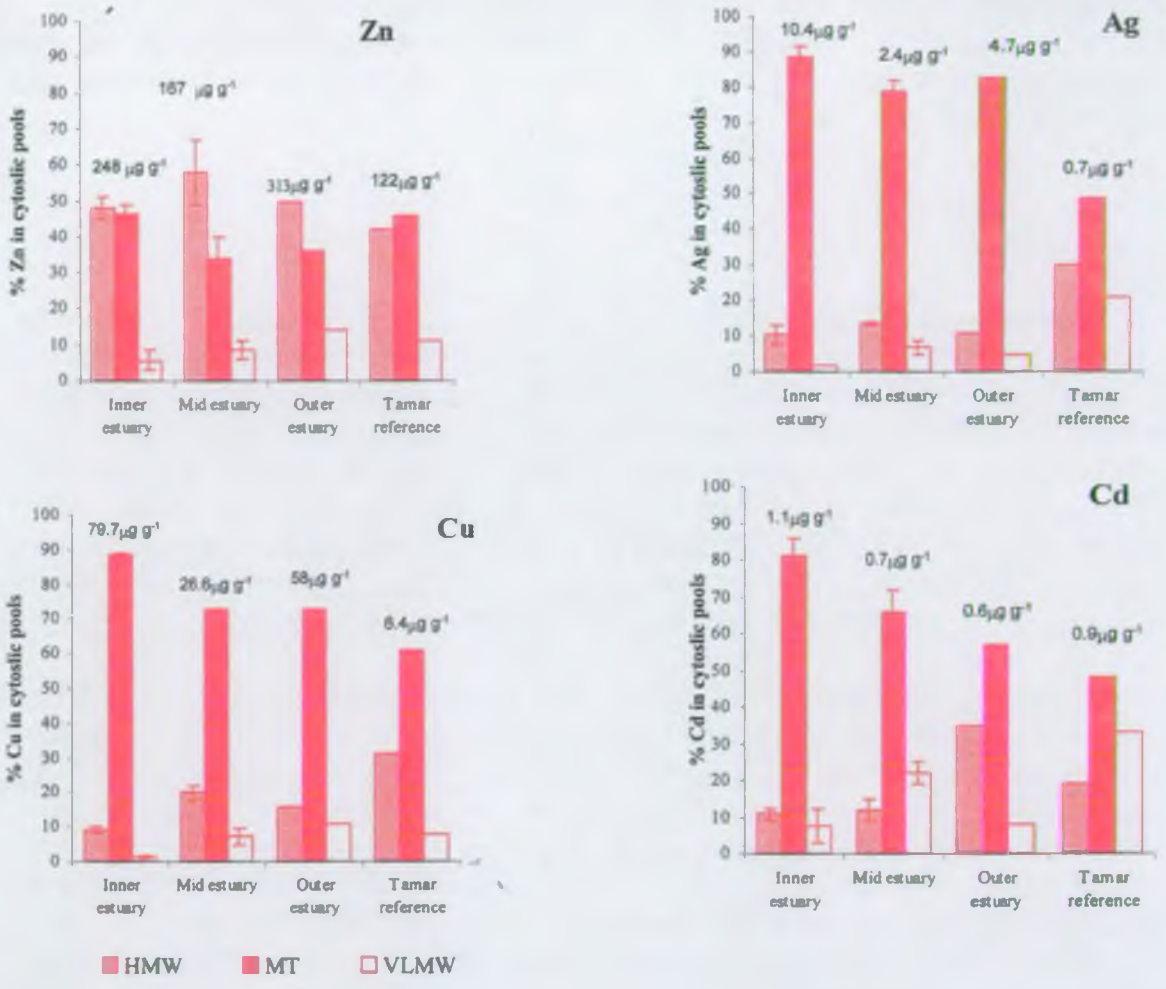

Figure 10. ***Anguilla anguilla.*** **Partitioning (%) of metals between cytosolic pools (high molecular weight proteins - HMW; metallothionein - MT; and very low molecular weight ligands - VLMW) in eels from the Thames and Tamar Estuaries, August 1998. (Means ± SD, where n>2). Figures above histograms are concentrations of total cytosolic metals. For sites included in inner/mid/outer Thames Estuary groupings, see Fig. 1 and Table 1.**

3.4 Metallothionein levels and metals in sediment and water

The previous section has established close links between metallothionein levels and accumulated (bioavailable) metals in eel livers. In order to try and extend the diagnostic capabilities of this approach further, data on metal levels in Thames sediments and waters (see *Thames Estuary Environmental Quality Series*, No **2**,) were used to test relationships between patterns of contamination in the environment and MT induction in eel populations along the estuary. For fish collected in May 1998, MT levels were strongly correlated with all metals in the sediment ($p<0.005$ for Zn, Cu and Ag; $p<0.05$ for Cd) whilst in August only Ag and Cd ($p<0.05$) showed any relationship. The more significant correlations with particulate metals in the spring samples may reflect the fact that eels overwinter in the sediment. Conversely, for dissolved metals, MT levels were more highly correlated with water in August (Zn, Ag and Cd; $p<0.05$) than in spring (Cd; $p<0.05$). In the November survey, relationships between MT and either sediment- or dissolved-metals were not

statistically significant: however, the database for this survey was particularly limited. It would be beneficial, in future, to try to establish predictive relationships between environmental contamination and MT induction in eels on a more extensive, and contemporary, range of samples.

4. Discussion

One of the outstanding difficulties encountered in environmental impact assessments is how to determine the biological significance of contaminants, including metals. Although many investigations have demonstrated toxicological effects and endpoints in the laboratory, the transfer of techniques to the field, particularly in estuaries, remains an urgent priority. The development of sub-lethal indices of impact has expanded encouragingly in recent years, but, in terms of adequate validation under natural conditions, most are still at an early stage of evolution. To add to the uncertainties over whether these techniques will be adequate for the environment under investigation, all too often there may be doubts as to the selection of appropriate organisms.

The measurement of metallothionein levels, and disturbances in distributions of metals among various subcellular components, comprise one possible approach to monitoring environmental quality which, if adequately tested, may provide an indication of sub-lethal response, as well as an assessment of metal contamination (George and Langston, 1994). Metallothionein synthesis in several fish species has been shown, experimentally, to be induced in direct proportion to dosages of administered metals, including Zn, Cu, Cd and Hg (Roch and McCarter, 1984a&b; Olsson and Hogstrand 1987; Overnell *et al.*, 1987; George and Langston, 1994). Furthermore, there are indications from initial field studies that elevated levels of hepatic MT in fish are linked to metal contamination in lakes, rivers and estuaries. For example, in a survey of flounder (*Platyichthys flesus*) along the River Forth Estuary, Scotland, variation in hepatic MT induction was shown to be location-dependent, with the highest levels occurring in an area where heavy metals from chemical industries had accumulated in the sediments (Sulaiman *et al.*, 1991; George and Langston, 1994). Similarly, a strong correlation between hepatic MT levels and metal contamination was found in rainbow trout *Salmo gairdneri* from a series lakes along the Campbell River system in Vancouver which were subjected to different degrees of metal (mining) pollution, (Roch & McCarter, 1984b).

In order to progress the application of this type of approach, the current study was designed to examine the relationship between hepatic MT and accumulated metal burdens in populations of the common eel *Anguilla anguilla* in the Thames Estuary, and to determine the suitability of the eel for environmental monitoring. For many reasons the species would seem an ideal choice; notably because of its tolerance to the full range of salinity from fresh- to sea-water, territorial behaviour, benthic habitat, suitable size and widespread availability. The outstanding questions are, 'Can induction of MT in eels, be used as a measure of response?' and thus, 'Are eel populations in the Thames attempting to adapt to elevated levels of bioavailable metals?'.

At least four metals (Zn, Cu, Ag and Cd) were found to be associated with hepatic MT in eels from the Thames, though this itself does not necessarily signify impact. Even in the absence of contamination, MT, by virtue of it's high metal binding capacity, is thought to act as an intracellular metal reservoir - capable of modulating homeostasis and transferring

essential elements such as Zn and Cu to and from metalloenzymes (Roesijadi, 1994). Low levels of MT are therefore to be expected in the livers of most species - even at pristine sites. However, elevated intra-cellular (free) metal concentrations can have pathological effects, and a further function of MT is to bind excess metal ions (essential and non-essential), rendering them unavailable to exert damage (Langston *et al.* 1998). It is the induction of MT synthesis (above baseline levels), in response to an increased influx of metals, which is one of the important diagnostic features being tested here.

In the absence of contamination, the constitutive MT in eel livers appears to be a Cu and Zn regulating protein. However, along the Thames Estuary, MT levels in eels are highly correlated with their associated metal burden, and MT synthesis is induced as a result of elevated metal bioavailability at a number of sites. Analysis of livers suggests this response may be attributable, primarily, to Cu and Ag contamination. It is possible that Zn and Cd are also involved in induction, but are displaced by the more strongly-binding metals - as also indicated for trout by Roch and McCarter (1984a.). To date *in vitro* experiments with *A. anguilla*, have established that Cd can replace MT-bound Zn, but not Cu (Noël-Lambot *et al.*,1978). Based on combined evidence, it would seem that, in eels, the affinity of metals for hepatic MT follows the sequence Cu=Ag>Cd>Zn.

Of the sites sampled in the current survey, the response to excess intracellular metals was most pronounced in eels from the upper (riverine) section of the tidal Thames. This reflected the overall trend in environmental contamination: depending on the time of year, MT levels were a direct function of metal concentrations in surrounding water or sediment. It is concluded, therefore, that MT determinations in eel livers are useful indicators of environmental quality and sublethal response to metals.

In the survey of hepatic MT in flounders *Platichthys flesus* from the Forth Estuary, described above, induction was most evident in an area where metal concentrations were high in benthic muds, implying possible uptake of metals from this sedimentary source (Sulaiman *et al.*, 1991; George and Langston, 1994). Metal uptake routes are not well documented for *A. anguilla* - other than an early study by Noël-Lambot & Bouquegneau (1977) which suggests that uptake of Cd and Hg from water is more important than from food. There appears to be no published evaluation of uptake from sediments, but it may be significant that, in the current study, relationships between MT and particulate metals were particularly strong in May - after the eels had emerged from over-wintering in benthic muds. Since eels in 'hibernation' do not feed, dietary assimilation of metals will be negligible during this period. It seems likely, therefore, that eels assimilate metals across the integument, either from interstitial water or by direct contact with sediment, throughout the winter months. At other times they may ingest particulate metals during feeding. The potential for biomonitoring contaminated sediments, using eels, should be examined further in future surveys.

By producing MT in proportion to accumulated metal concentrations, eels from some of the inner Thames sites are, in effect, attempting to adapt to stress. The question is, 'how successfully?' The characterisation of cytosolic metal-partitioning behaviour was employed here to help address this issue, the rationale being, that, when the balance of intracellular metals is disturbed by an excess influx, normal homeostatic ligands such as MT become saturated. This latter process will be manifested by an increase in the proportion of metals present in either the HMW enzyme-containing pool, or as 'free' (VLMW) metal. It has been proposed that this so-called 'spill-over' effect coincides with the onset of toxicological effects in marine organisms and that MT normally helps to prevent such dis-

equilibria (Pruell & Englehardt, 1980; Roesijadi, 1982; Brown *et al.*, 1987). George (1989), for example, has shown that in plaice (*P. platessa*) the relationship between MT and Cd deviates from linearity at higher doses, and that when this overload occurs hepatotoxicity becomes apparent. Measuring disturbances in the sub-cellular distribution of metals therefore represents a further possible 'diagnostic test' with which to asses the significance of accumulated tissue burdens, complementing measurements of MT induction. In the livers of Thames eels, Cu and the non-essential metals Ag and Cd were predominantly associated with MT and displayed linear relationships with the thiolic protein, even at the most polluted sites. There was little sign of saturation of MT, or spill-over. If anything, MT tended to account for an increasing proportion of cytosolic Cu, Ag and Cd, as concentrations increased. This implies that the induction of hepatic MT in eels was effectively acting as a detoxifying system for these metals, by preventing non-specific metal-binding to enzymes and other sensitive molecules.

The partitioning of Cu, Ag and Cd among different sub-cellular fractions (HMW, MT, VLMW) of Thames samples, expressed as a function of total cytosolic metal, is summarised in Figure 11. This illustrates how, if eels are subjected to contamination, the rate of Cu, Ag, and Cd-binding to MT increases in comparison with other fractions (steeper slopes). Only Zn differs from this pattern; $Zn_{[HMW]}$ increases with increasing total cytosolic Zn at a similar rate to that of $Zn_{[MT]}$ (Figure 11D). These data for Zn are in accord with it's behaviour in trout *S. gairdneri* (Roch *et al.*, 1982) where there appears to be little detoxification by MT synthesis, against Zn, when in the presence of other, more strongly-bound metals, especially Cu (Ag and Cd). Brown *et al.* (1987) also consistently found a similar pattern for Zn in California tonguefish *Symphurus atricauda* (one of three fish species investigated), but suggested that increases in $Zn_{[HMW]}$ were only manifested at lower degrees of contamination - up to the point where the HMW pool becomes saturated: this is thought to correspond to the point at which the Zn-requirements of the enzymes have been met. Thereafter, with increasing concentrations of Zn, equilibrium-dependant exchange between the subcellular pools probably occurs (Brown *et al.*, 1987). Our observations suggest that this explanation may be applicable to Zn partitioning in Thames eels, although further work is needed to verify the precise status of Zn, and to confirm whether or not the increase in $Zn_{[HMW]}$ is indicative of toxicity (as a result of saturation of MT) at the more contaminated sites.

Thus, the concept of 'spill-over', despite its general appeal as a mechanism for toxicity, has still to be widely confirmed. It has been demonstrated in certain species, following acute laboratory exposure experiments (Roesijadi, 1982; Pruell & Englehardt, 1980; George, 1989), however, major disruption in partitioning may be less evident in organisms chronically exposed to metals, and which are able to adapt (Brown *et al.*, 1987). This is consistent, partly, with the observation that the MT system of Thames eels is not saturated by Cu, Ag and Cd: unlike Zn, there is little evidence that these metals show enhanced association with the HMW enzyme pool, at any of the sites, despite elevated concentrations (Fig. 11). Again, however, further work it is needed to clarify responses.

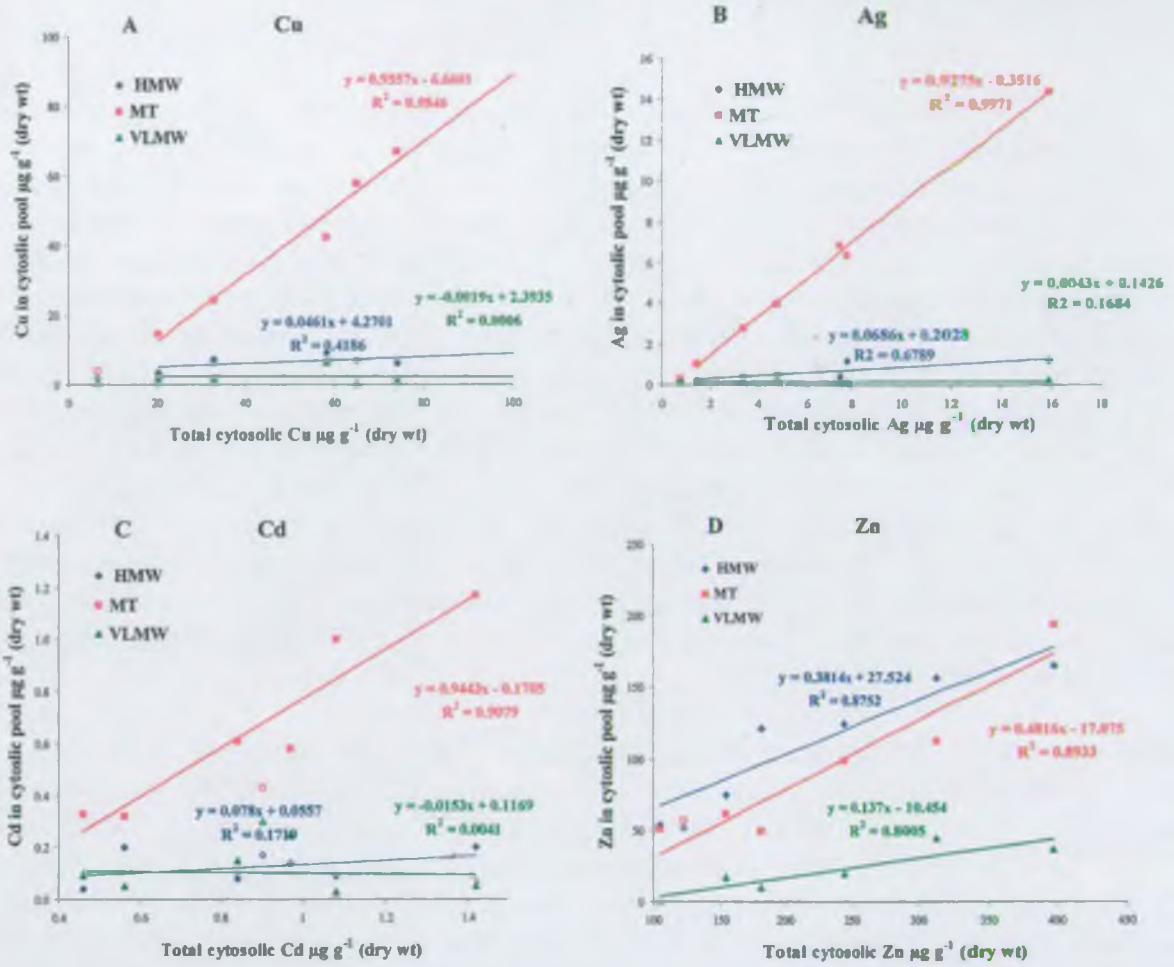

Figure 11. ***Anguilla anguilla.*** **Cu (A), Ag (B), Cd (C) and Zn (D) in high molecular weight (HMW), metallothionein (MT) and very low molecular weight (VLMW) fractions, expressed as a function of total cytosolic metals, in livers of Thames eels (August 1998 samples). Linear regressions shown. Open symbols are for Tamar reference samples (not included in regression analysis).**

High concentrations of MT in the livers of eels from the upper Thames (11.2 and 10.5 mg g^{-1} dry weight in samples from Crossness and Brentford, respectively), compared to those from the mouth of the estuary, and from the Tamar reference site (2-3 mg g^{-1}) indicates an ability to synthesise significant quantities of MT, in direct response to increasing metal burdens. Superficially, it would seem that by doing so these eel populations have become adapted to moderate levels of metal contamination, and may even be protected against more acute exposures, as demonstrated experimentally for trout by Roch and McCarter (1984a&b). However, the increased synthesis of MT and resultant adaptation in Thames eels infers a cost to the fish. The fact that they are attempting to respond to metal stress is a clear sign that the fish are affected.

In their previous laboratory study on MT in eels, Noël-Lambot *et al.* (1978) estimated a value of 0.35 mg MT g^{-1} wet weight (ww) in controls, with a tenfold increase in MT concentrations following exposure of fish to high levels of dissolved Cd. Calculated on a wet weight basis, hepatic MT concentrations in the current Thames survey ranged between 0.28 mg g^{-1} and 2.58 mg g^{-1} (in individual eels from West Thurrock and Crossness,

respectively). This is equivalent to the scale of response to Cd in Noël-Lambot's experiment.

Published values for hepatic MT levels in other teleost studies have, in the past, tended to be expressed on a wet weight basis, making comparisons somewhat difficult, as much depends on the nutritive state of the fish and the water content of the liver. Furthermore, it may be of doubtful value to extrapolate too generally between species (because of variation in individual metal requirements), or between studies: techniques used by different groups are often still in need of broader validation and inter-laboratory comparison. Nevertheless, it is interesting to put into context the current results, alongside other published data for fish, if only as a rough guide to the scale of response. Thus, MT levels in plaice (*Pleuronectes platessa*) are reported to vary between approximately 0.02 mg g^{-1} (ww) in uncontaminated fish, up to 0.3 mg g^{-1} following Zn (or Cd) injection (Overnell *et al.*, 1987; George and Langston, 1994). Similar background values (0.02 – 0.08 mg g^{-1}) and induced levels of MT (approaching 1mg g^{-1}) are encountered in flounders *Platichthys flesus* and *Psedopleuronectes americanus* (Sulaiman *et al.*, 1991; George and Langston, 1994). This would suggest that basal quantities of MT in eel (Anguillidae), and their capacity for induction when exposed to elevated metal levels, are high, relative to flatfish (Pleuronectidae).

A number of salmonids appear to occupy an intermediate position - MT concentrations presumably reflecting their substantial Cu content. Thus, basal (constitutive) hepatic MT levels are 0.14-0.24 mg g^{-1} and approximately 0.1mg g^{-1} (ww) in wild trout *Onchorhynchus mykiss* and Atlantic salmon *Salmo salar*, respectively (George and Langston, 1994). In rainbow trout *Salmo gairdneri* and *Onchorhyncus kisutch*, basal levels are even higher - between 0.35 and 0.48 mg g^{-1} (data from Roch *et al.*, 1982; Roch and McCarter, 1984a,b; McCarter and Roch, 1984) - on a par with, or even exceeding, levels in eels. It is interesting to note that an increase in MT in *S. gairdneri*, to around 1.5 mg g^{-1}, is induced by similar metal concentrations to those which elicit the onset of acute toxicity, as defined by the LC_{10} (Roch and McCarter, 1984b). The highest value of 2.58 mg MT g^{-1} (ww) in Thames eels could, therefore, conceivably herald equivalent toxic risks, although, for reasons indicated above and below, threshold data for trout cannot be considered directly comparable to those for eels, without further evaluation.

There may, in fact, be a number of endogenous and exogenous factors which modify MT levels in teleosts. *In vitro* studies have shown that MT induction can be increased by up to 2-fold by injection of 'stress' hormones such as glucocorticoids and noradrenaline (Hyllner *et al.*, 1989; George and Langston, 1994), and by 2-3 fold following administration of 17β-oestradiol (10 mg kg^{-1} body weight), the plasma steroid involved in egg development (Olsson *et al*, 1989). Baer and Thomas (1990) investigated the influence of capture stress, salinity and reproductive status, and report up to 10-fold increases in the amounts of Zn bound to hepatic MT in the striped mullet *Mugil cephalus*, as a result of capture, handling and transportation. However, in accord with stoichiometric considerations, actual protein (MT) levels, measured as acid soluble thiols, appear to have increased by less than 2-fold in *M.cephalus*. In the same study, reduced salinity and physical trauma increased the amounts of MT-bound Zn (though not necessarily MT) by approximately 25% in the red drum, *Sciaenops ocellatus*, and by 2-fold during a period of ovarian maturation in female spotted sea trout (*Cynoscion nebulosus*). Olsson *et al.* (1987,1989), also noted a rise in hepatic MT in female trout at the time of ovulation, concomitant with increased MT-bound Zn concentrations, and concluded that MT has a major involvement in Zn regulation during vitellogenesis. This rise in MT levels may coincide with a redistribution of Zn from

enzymes (present at high levels to maintain protein production in the initial stages of egg development), to MT, once vitellogenin synthesis declines. The same author subsequently observed that MT induction in rainbow trout can be affected by temperature stress, in that hepatic MT increased by approximately one third in response to a 50% drop in water temperature - however, concentrations returned to previous levels once the water stabilised at the new lower temperature (Olsson, 1996).

Not surprisingly, there are conflicting reports concerning the scale of influence of environmental stressors on MT induction and, arguably, if strictly controlled, they may be negligible in comparison to the effects of metals. For example, Overnell *et al.* (1988) investigated the effect of stress in turbot (*Scophthalmus maximus*) and concluded that neither capture, nor the injection of a variety of chemicals (eg. endotoxin, cortisol and turpentine) had any effect on hepatic MT levels (normally 0.06 mg g^{-1} ww). Similar conclusions were drawn for *P. platessa* (Overnell *et al.*,1987). In the current study, we have tried to minimise any effects from capture, handling and transportation stress. Though it may not be possible to eliminate these entirely, any effects should apply equally to all populations. The consistency in observed MT gradients in eels, and the close relationship with contamination, confirms that metals, rather than other factors, are the major determining factor in the Thames Estuary.

The purpose of sampling at three separate times of the year was to assess temporal trends in metallothionein concentrations. Variability due to season (temperature), sex and possibly reproductive status may have influenced MT levels in eels to some extent, although this did not detract from the common pattern of response to metal contamination along the Thames Estuary (decreasing seawards). The MT gradient appeared less pronounced in the November samples, partly because of the more restricted sample range, partly as a result of generally higher levels of MT, and, also, because of greater variability at each site. With regard to this point, it is worth noting that Olsson *et al.*, (1987) demonstrated an increase in hepatic MT of approx 2-fold in male rainbow trout during maturation and spawning, whilst in females much larger increases of up to 7-fold were observed at the cessation of vitellogenesis, prior to spawning. Part of the temporal and spatial variation in MT gradients in Thames eels may therefore be linked to the onset of sexual maturation (prior to migration and spawning in autumn) and also to the variable sex ratio composition of samples.

Hence, whilst MT synthesis in eels is acknowledged to be induced primarily by exposure to metals, variations caused by physiology and environmental factors need to be considered. To improve the sensitivity of this assay, such variations should be characterised further, and subsequently minimised by rigorous sampling design. A more comprehensive survey of MT fluctuations in the eel would be valuable in this respect. Allied to this, it would be useful to determine whether changes in hepatic MT and associated metals are linked to seasonal changes in metabolism (e.g. liver somatic index).

Clearly, there are a number of fundamental features associated with MT responses which have yet to be fully parameterised, though this project has made significant progress in applying metallothionein measurements in the field. The choice of *Anguilla anguilla* has proved to be highly fortuitous; this species has many useful qualities as a bioindicator, and determination of MT in liver tissue has been shown to be appropriate for the purpose of evaluating the biological significance of metal contamination. As a result, it is now possible to make informed suggestions to optimise future sampling. Notably, for example, the strongest relationship between MT and metals was found in August: this would appear

to be a suitable time of year to take samples of eels for monitoring purposes, as it is not a time of sexual maturation, the water temperature is relatively stable and the fish tend to remain in their home ranges.

In helping validate this novel approach to environmental assessment, the current work provides some of the first data on sub-lethal biological responses of fish to metals in the Thames Estuary. It identifies sites in the inner estuary as being the most impacted and establishes a useful baseline against which future spatial and temporal trends in metal pollution may be assessed.

5. Conclusions and Recommendations

The main conclusion of this project is that measurement of metallothionein in eel livers is a useful biochemical indicator of anthropogenic metal impact in the Thames Estuary.

The marked absence of information regarding sub-lethal effects of metal pollution constitutes a large gap in our knowledge. Monitoring the bioaccumulation of contaminants is increasing in frequency, but, for obvious reasons, indicator species are largely selected from invertebrate phyla. Because monitoring of fish traditionally relies to a greater extent on opportunistic collections of the more commercial species, it is generally difficult to target sampling with the specific purpose of assessing the 'health' of the environment at any given location. For inshore waters the common eel *Anguilla anguilla* has been overlooked in this respect, despite various attributes: notably, the species is found in both fresh and salt waters and is widely distributed throughout the rivers and estuaries of Europe. Additionally, the benthic habitat, feeding patterns and relatively stationary, territorial nature of the eel make it vulnerable to localised metal pollution, and as such *A. anguilla* is proposed as a suitable indicator species, particularly for waterways such as the Thames.

The use of metallothionein (MT) induction as an 'early warning system' for heavy metal pollution has begun to receive increasing attention in recent years. Detection of the subtle sublethal effects of metal exposure - including elevated MT synthesis - may be used as a precursor of more damaging contaminant stress and to provide valuable information regarding the extent, and possible consequences of prolonged chronic exposure. In laboratory experiments MT concentrations in several fish (and invertebrate) species have previously been shown to increase in direct proportion to metal levels, although application to field situations, particularly in estuaries is still in the developmental stage. This work is the first field study to investigate MT distributions in the common eel - a species which forms an important part of the Thames biota - and the first to demonstrate it's potential as an indicator of environmental quality. Measurement of MT represents a considerable refinement over the reliance on water analysis as a measure of toxicity, since it provides a more definitive measure of biologically-active metals.

Results indicate that hepatic MT levels in Thames eels are directly related to metal contamination, and are elevated upstream, particularly in response to Cu and Ag bioavailability.

Patterns of metal distribution among different cytosolic pools indicate that MT in these eels is playing an effective role in detoxification of Cu, Ag and, to a lesser extent, Cd. Nevertheless, by promoting MT induction (and hence causing fish to adapt) metal contamination must, presumably, elicit some cost to the Thames eels. As yet these costs are unknown.

It is apparent from seasonal differences in MT levels (attributed to endogenous and exogenous factors), that certain criteria must be fulfilled to obtain reliable data. Juvenile eels of a similar size (around 35-45cm) should be chosen if available. Sampling should avoid times of the year with high variations in water temperature, and also the period of sexual maturation. For the purposes of MT quantification, eels should be handled minimally and processed as soon as possible after capture to avoid unnecessary stress.

Rigorous investigations under controlled laboratory conditions, combined with further field studies, are recommended to underpin our understanding of the mechanisms of MT responses. Such work should include experiments to determine the strongest inducers of hepatic MT, coupled with identification of specific metal binding affinities. A more extensive study into subcellular partitioning of metals is also recommended, in order to determine the metal detoxification capacity of MT and the thresholds and consequences of 'spillover' in this species. It would be particularly interesting to follow the interchange between Zn in HMW and MT pools during seasonal cycles, and to establish whether, in fact, this metal is effectively regulated, or whether the presence of Zn_{HMW} in relatively high concentrations at some Thames sites signifies that the detoxification capacity of MT has been exceeded as a result of contamination.

Further work to establish accurate basal levels of hepatic MT in eels, metal uptake routes and the kinetics of MT induction is desirable, as is investigation into the influence of environmental parameters.

The data on hepatic MT and metal concentrations collected in the current survey provide a valuable benchmark against which future changes in environmental quality can be assessed. This applies not only to the Thames Estuary but also to other estuarine and freshwater sites further afield.

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8. Appendix

Data on eels (lengths, weight, and concentrations of hepatic metallothionein and metals) from the Thames Estuary collected in three surveys during 1998.

May-98 **Thames**

Site and label	Weight (g)	Length (cm)	MT mg g^{-1} (dry wt)	MT µg g^{-1} (wet wt)	Hepatic metals (µg g^{-1} dry weight) Zn	Cu	Ag	Cd
Richmond R1	325	54.1	5.03	988	224	89.4	5.61	1.91
Richmond R2	199	48	6.34	1306	220	69.1	3.70	6.06
Richmond R3	90	33.6	6.93	714	327	116.9	15.42	7.69
Richmond R4	95	38	2.24	417	180	75.4	8.79	1.39
Richmond R5	78	32.3	1.98	356	149	51.9	6.42	1.83
Richmond R6	78	34.5	1.41	495	62	25.8	2.08	3.88
Richmond R7	192	45.6	6.70	1322	276	170.1	7.85	5.87
Richmond R8	111	39.2	3.01	496	230	87.5	6.45	4.22
Brentford Bf1	240	48	4.23	749	249	68.4	1.95	21.15
Brentford Bf2	310	55	5.96	1039	275	119.0	6.08	1.18
Brentford Bf3	270	51.5	4.49	963	197	95.4	3.83	0.59
Brentford Bf4	140	42.9	3.65	887	194	79.0	5.41	3.18
Brentford Bf5	86	37.3	4.74	721	195	83.6	14.11	2.66
Brentford Bf6	139	43.6	2.43	480	216	85.9	2.84	2.99
Brentford Bf7	286	55.2	3.95	711	215	73.7	2.32	4.07
Brentford Bf8	335	56	5.12	1059	229	111.1	4.79	2.47
Battersea Bat1	205	49.1	3.11	678	180	66.2	3.04	0.30
Battersea Bat2	165	47.8	3.99	803	211	69.9	1.45	0.49
Battersea Bat3	86	36.9	3.52	896	183	79.9	3.52	0.61
Battersea Bat4	190	47.5	3.01	672	164	43.1	1.01	0.00
Battersea Bat5	98	35	3.51	763	235	113.5	3.96	1.21
Battersea Bat6	171	49.6	1.83	409	145	27.3	2.47	0.15
Battersea Bat7	94	40.4	3.09	648	209	85.7	3.99	0.56
Battersea Bat8	135	42	2.81	608	164	50.2	0.89	0.29
Woolwich W1	1033	78	6.86	1628	256	141.6	3.09	1.54
Woolwich W2	450	60	3.49	760	179	79.3	4.77	1.20
Woolwich W3	235	49	2.94	576	197	85.3	4.32	2.19
Woolwich W4	544	69.5	6.68	1567	230	54.4	1.91	0.88
Woolwich W5	110	40	2.42	540	158	50.5	2.81	0.60
Woolwich W6	111	41	1.79	384	125	32.8	2.62	1.25
Woolwich W8	120	36	3.28	698	200	106.0	6.26	0.60
Crossness Xn1	260	55	5.19	1320	224	107.6	10.89	1.77
Crossness Xn2	280	60	3.54	881	150	54.5	2.04	0.68
Crossness Xn3	120	44.5	3.38	912	154	70.2	4.76	1.04
Crossness Xn4	175	47	2.91	656	118	31.0	4.51	0.32
Crossness Xn5	115	46	3.49	767	155	43.5	1.25	0.30
Crossness Xn6	120	41.5	3.83	961	141	48.8	5.41	0.50
Crossness Xn7	70	35.5	3.34	756	139	29.7	1.55	0.94
Crossness Xn8	100	40	3.24	915	163	58.5	2.23	1.97
Greenhithe G1	90	39	2.74	510	963	38.6	12.22	1.54
Greenhithe G2	379	59	2.43	543	181	47.5	4.67	1.68
Greenhithe G3	336	66	7.10	1590	301	152.8	13.04	2.00
Greenhithe G4	451	61	6.06	1341	255	119.9	5.92	0.92
Greenhithe G5	355	58	4.53	911	201	77.7	7.42	0.73
Greenhithe G6	95	40	1.81	446	163	40.9	7.30	0.84
Greenhithe G7	170	47	7.85	1413	329	105.8	7.71	2.48
Greenhithe G8	171	46	3.19	634	186	61.0	8.13	0.82
West Thurrock WT1	213	49	1.66	432	139	32.5	4.19	0.80
West Thurrock WT2	250	57	4.16	772	252	97.5	6.14	2.05
West Thurrock WT3	390	57	4.47	1101	183	84.8	13.32	1.06
West Thurrock WT4	250	47	6.05	1107	255	114.0	10.91	1.20
West Thurrock WT5	134	43	2.80	646	176	35.2	1.80	0.88
West Thurrock WT6	292	53	1.87	468	143	29.8	4.36	0.66
West Thurrock WT7	190	44	3.07	821	169	56.3	6.86	0.89
West Thurrock WT8	218	29.5	2.69	693	143	37.8	8.17	0.59
Blythe Sands BS1	150	44.2	1.68	408	157	41.0	1.08	1.97

Appendix (cont.)

Aug-98 **Thames**

Site	Weight (g)	Length (cm)	MT mg g^{-1} (dry wt)	MT µg g^{-1} (wet wt)	Hepatic metals (µg g^{-1} dry weight) Zn	Cu	Ag	Cd
Richmond R9	323	52	5.23	961	215	81.3	2.17	1.85
Richmond R10	313	57	2.47	542	132	31.5	1.19	2.57
Richmond R11	375	60	2.68	537	183	54.6	2.50	4.19
Richmond R12	73	34	3.33	554	167	56.9	3.89	3.52
Richmond R13	77	37.8	5.41	553	319	82.8	5.88	9.11
Richmond R14	552	66.6	3.54	751	175	71.1	2.93	1.72
Richmond R15	189	47.6	2.75	539	115	29.5	13.34	0.23
Brentford Bf9	105	40	4.93	783	255	89.2	4.40	1.49
Brentford Bf10	330	60.5	6.50	825	351	82.3	2.48	1.78
Brentford Bf11	97	39.8	2.62	471	194	29.2	1.13	1.13
Brentford Bf12	105	42.2	3.54	609	180	60.0	2.71	1.04
Brentford Bf13	216	51.4	10.50	473	759	210.4	7.88	10.89
Brentford Bf14	219	50.4	3.65	590	257	89.2	3.94	1.53
Brentford Bf15	235	53.2	4.38	728	235	70.6	4.54	1.37
Brentford Bf16	96	37.3	4.08	705	218	82.4	4.14	2.42
Kew K1	123	37.5	6.45	886	308	162.4	9.09	2.21
Kew K2	64	33.8	4.96	774	242	76.8	7.57	5.69
Kew K3	105	39.7	4.04	718	201	69.8	5.61	0.62
Kew K4	113	39.5	6.77	1104	274	136.4	5.24	24.65
Kew K5	110	39.3	3.95	559	248	68.4	5.59	2.05
Kew K6	197	49	4.02	727	193	69.4	6.68	3.04
Kew K7	293	53.2	6.39	760	361	125.5	9.65	1.71
Kew K8	83	36.5	2.83	334	211	38.2	2.46	1.98
Beckton B1	94	41.3	4.65	463	352	79.2	5.50	3.76
Beckton B2	102	39	2.34	448	204	42.8	10.25	3.63
Beckton B3	50	37.6	5.69	634	333	80.5	6.57	1.80
Beckton B4	98	39.6	2.09	353	200	42.5	2.19	1.93
Beckton B5	119	42.5	2.14	537	205	56.7	1.49	0.44
Beckton B6	118	43	3.65	455	309	70.2	2.75	2.28
Beckton B7	115	43.2	2.08	451	150	35.2	1.22	0.52
Beckton B8	97	43	2.02	396	166	36.3	1.87	1.76
West Thurrock WT9	127	44	1.81	478	128	26.4	0.77	0.38
West Thurrock WT10	99	42	1.64	349	138	21.5	1.34	0.75
West Thurrock WT11	75	38.4	2.38	350	214	43.2	1.66	1.30
West Thurrock WT12	149	48	2.70	499	180	52.6	2.71	0.82
West Thurrock WT13	105	42	1.38	277	130	29.4	1.06	0.26
West Thurrock WT14	126	45.8	1.62	357	133	30.8	2.09	0.46
West Thurrock WT15	130	45.1	1.97	362	167	47.4	1.68	0.38
West Thurrock WT16	78	37.2	2.14	355	165	33.8	1.05	0.51
Blythe Sands BS2	534	61.2	2.34	675	163	46.8	0.67	1.41
Blythe Sands BS3	355	61	2.14	694	129	45.2	1.22	0.62
Blythe Sands BS4	119	43	2.13	525	144	30.6	0.05	1.82
Blythe Sands BS5	128	44	2.00	512	157	28.6	0.37	1.32
Blythe Sands BS6	316	58	2.87	706	223	70.2	3.13	1.13
Blythe Sands BS7	110	41	1.83	468	179	37.7	2.45	0.74

Appendix (cont.)

Nov-98 **Thames**

Site	Weight (g)	Length (cm)	MT mg g^{-1} (dry wt)	MT µg g^{-1} (wet wt)	Hepatic metals (µg g^{-1} dry weight) Zn	Cu	Ag	Cd
Richmond R1	109.7	41	2.56	450	163	35.8	4.83	0.51
Richmond R2	177.7	45	6.24	1182	170	84.4	6.88	3.45
Richmond R3	215.6	48	2.95	701	110	36.7	2.07	0.50
Richmond R4	224.7	41	4.52	938	151	55.1	3.77	0.75
Richmond R5	77	34	7.33	1177	230	88.5	10.02	4.42
Richmond R6	59	32	5.18	925	157	66.8	8.47	1.82
Richmond R7	46.6	31	5.01	776	173	44.3	6.89	0.89
Richmond R8	39.9	30	3.76	687	92	25.9	2.42	1.72
Crossness Xn1	261.1	55	2.80	688	127	30.8	1.47	0.26
Crossness Xn2	287	55	3.40	735	208	102.1	4.97	0.72
Crossness Xn3	164.8	45	4.85	906	172	49.5	3.94	0.39
Crossness Xn4	214.5	51	3.74	846	186	61.6	3.16	0.43
Crossness Xn5	378.1	52	11.15	2582	247	153.0	6.65	0.12
Crossness Xn6	108.2	41	5.43	1032	223	72.4	6.72	0.08
Crossness Xn7	180.3	47	2.28	453	99	18.9	2.54	0.18
Crossness Xn8	179.7	50	3.79	774	159	57.6	3.87	0.17
Brentford Bf1	113.3	41	6.13	1088	258	107.2	8.12	0.13
West Thurrock WT1	208.7	44	5.45	1269	181	74.4	6.98	1.33
West Thurrock WT2	292.8	56	2.02	643	98	23.3	1.66	0.17
West Thurrock WT3	128.6	46	3.51	629	180	54.7	9.44	0.75
West Thurrock WT4	172.3	50	5.90	1055	262	78.3	11.38	0.83
West Thurrock WT5	164.3	48	3.99	781	192	55.7	5.68	1.01
West Thurrock WT6	225.2	52	6.08	803	310	78.7	10.57	1.80
West Thurrock WT7	197.8	51	9.69	1464	355	141.5	18.37	1.62
West Thurrock WT8	366	60	5.52	1158	191	58.9	9.53	1.25
Mucking Mk1	423.4	63	7.55	1253	139	31.1	3.72	0.40
Mucking Mk2	82.7	31	1.19	310	190	48.5	3.85	0.63