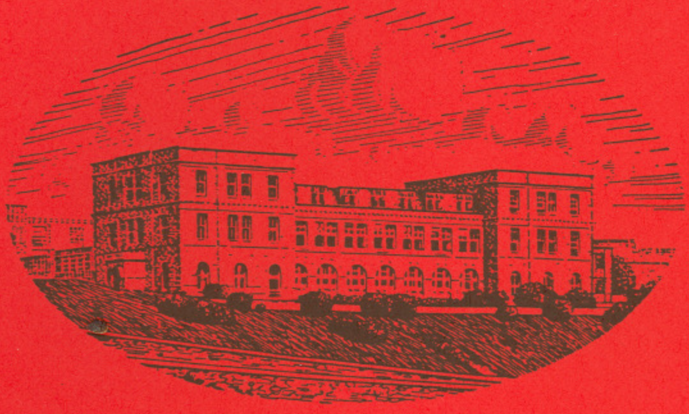
MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM



A GUIDE TO THE ASSESSMENT OF HEAVY-METAL CONTAMINATION IN ESTUARIES USING BIOLOGICAL INDICATORS.

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ABSTRACT

The rationale behind the use of analyses of estuarine organisms to assess levels of heavy-metal contamination is described and compared with alternative methods such as the analysis of waters or sediments. Based on field observations in United Kingdom estuaries and on evidence from the literature, an assessment is made of the suitability of 17 species as indicators of metals and metalloids including Ag, As, Cd, Co, Cr, Cu, Hg, Ni, Se, Sn, Pb and Zn.

Because estuarine species have distributions ranging from rocky shores to muddy sediments and absorb metals from different sources (water, sediment etc.) there is no universal indicator organism. Some species, including the seaweed Fucus vesiculosus, the clam Scrobicularia plana and the mussel Mytilus edulis can be used to monitor a range of metals or metalloids, although no species is ideal for every element. Other organisms are most useful as indicators for specific metals. Examples include the winkle Littorina littoralis for Cd, the ragworm Nereis diversicolor for Cu and the flounder Platichthys flesus for methyl mercury. Problems of variability in the use of indicator organisms can be minimised by employing procedures which are standardised with regard to time of sampling, location, size and number of organisms and method of cleaning.

A reasonable monitoring programme should involve the analysis of several species including perhaps a seaweed (e.g. Fucus vesiculosus), a suspension feeder (e.g. Mytilus edulis), a deposit feeder (e.g. Scrobicularia plana) and a carnivore (e.g. Platichthys flesus). This scheme is suggested as a means of assessing contamination in different phases (dissolved, particulate etc.) and of examining the possibility of food-chain biomagnification (e.g. of methyl mercury).

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CONTENTS

Seaweeds

SUMMARY

Because different species have distributions ranging from rocky shores to muddy estuaries and absorb metals from a variety of sources, there is clearly no universal indicator organism. Any reasonable monitoring programme should therefore include the analysis of several species (e.g. seaweed, suspension feeder, deposit feeder and carnivore) to try and assess different forms of contamination and determine the possibility of food-chain biomagnification.

Standardisation of Methods

Studies on the use of indicators have shown that none of them are completely free from problems caused by variability (see Phillips, 1980). However, many of the potential difficulties can be overcome if a few simple rules are followed. These include: 1) The sampling site should be accurately defined, particularly with regard to tidal height. 2) Samples should be collected at fixed times of year avoiding unsuitable periods such as the main breeding season of the indicator. 3) A sufficient number of relatively standard-sized organisms should be collected to ensure that individual variation is as low as practicable. This number can be found from the analysis of individuals, although ultimately the use of pooled samples is recommended. 4) Standardised methods should be employed to remove ingested sediments or surface contamination from organisms prior to analysis. Since they occur at high concentrations in sediments, analyses of Fe or Al in the organisms are useful for assessing the effectiveness of the cleaning process. Generally speaking, it is a waste of time and effort to collect estuarine organisms and simply freeze them. 5) Presentation of results on a dry weight basis is recommended, or the inclusion of the wet-dry weight ratios with results on a wet weight basis. Because both wet and dry weights are often affected by salinity, it is best to clean estuarine species in water of a fixed salinity before analysis.

Selection of Indicators

The selection of the most suitable indicators to cover a particular situation depends in part on the locality, since this determines which species are already present and which organisms might successfully be transplanted to the site. In the upper reaches of an estuary the range of potential indicators in the intertidal zone may be confined to the ragworm <u>Nereis diversicolor</u> and species of <u>Fucus</u> or <u>Enteromorpha</u>. Farther downstream the choice is increased considerably by the presence of both bivalve and gastropod molluscs. Table 1 summarises what is known about the indicator ability of 17 species towards 12 metals or metalloids. The classification of indicator ability is very subjective since it is based not

TABLE 1

THE POTENTIAL OF SPECIES AS HEAVY-METAL INDICATORS

Page No	Species	Feeding Type	Substrate	Estuarine tolerance (Spooner & Moore, 1940) Upstream limit in Tamar Estuary (km from mouth)	indicator potential () not known, (-) poor, (+) moderate, (++) good, (+++) excellent													Remarks
					Ag	As	Cd	Co	Cr(6)	Cu	Hg	Ni	Pb	Se[b]	Sn[b]	V	Zn	
	Algae																	
21	Fucus vesiculosus	-	Rock	21	+	++	++	++	-[a]	++	+	++	+[a]	+	+[a]		++	Evidence based on comparisons with water
29	Ascophyllum nodosum	-	Rock	11			++		+[b]	++	+		++				++	Based on comparisons with water
30	Enteromorpha intestinalis	-	Rock and sediment	-		+	+	+	-[a]	+	+		+[a]				+	Based on comparisons with water and other algae
	Polychaetes																	
35	Nereis diversicolor	Deposit/ omnivore	Sediment	23	+	+	+	++	+[a]	++	+	+[b]	+		+[a]		-[c]	Mainly compared with sediment, but Cd with Fucus
39	Nephtys hombergi	Carnivore	Sediment	13	+	+[b]	+	+		++			+				-[c]	Mainly compared with sediment, but Cd with Fucus
	Bivalves																	
40	Scrobicularia plana	Deposit/ Suspension	Sediment	18	++	++	++	+	+[a]	-[d]	++	+	++	++	+[a]		+[c]	Mainly compared with sediment but Cd and Zn also relate to Fucus
44	Macoma balthica	Deposit/ Suspension	Sediment	15	++	++	+	+	-[a]	-[d]	++	+	+	++			-[c]	Mainly compared with sediment but Ag, Cd and Zn also relate to Fucus
45	Mytilus edulis	Suspension	Rock	15	-/+[e]	-	++	+	++	-/+[f]	+	+[h]	++	+	+[a]	+[h]	+[cg]	Based on comparisons with water and Fucus
51	Cerastoderma edule	Suspension	Sediment	13	+	+	+		+[a]	-/+[f]		+[c]	+[a]				-/+[f]	Based mainly on comparisons with Fucus
55	Ostrea edulis and other oysters	Suspension	Sediment/ Stones	10	+	-[h]	++	+[h]	+[h]	++[g]	+[h]	+[h]	+[h]		+[hj]		++[g]	Based on comparisons with water

continued

TABLE 1

THE POTENTIAL OF SPECIES AS HEAVY-METAL INDICATORS

Page No	Species	Feeding Type	Substrate	Estuarine tolerance (Spooner & Moore, 1940) Upstream limit in Tamar Estuary (km from mouth)	indicator potential () not known, (-) poor, (+) moderate, (++) good, (+++) excellent													Remarks
					Ag	As	Cd	Co	Cr(6)	Cu	Hg	Ni	Pb	Se	Sn[b]	V[b]	Zn	
	Gastropods																	
58	Littorina littorea	Herbivore	Rock/ Sediment	11	++	+	++	-	-	+[c]	+	-	+				-/+[f]	Based largely on comparisons with Fucus
61	Littorina littoralis	Herbivore	Rock/Weed/ Sediment	11	+	++	+++	-	-	+[c]		-	+				+[c]	Based largely on comparisons with Fucus
64	Littorina 'saxatilis'	Herbivore	Rock	13			+						+					Probably most similar to L. littorea
64	Patella vulgata	Herbivore	Rock	8	+	-[c]	++			++	+	+	++				+[g]	Based largely on comparisons with Fucus
68	Nucella lapillus	Carnivore	Rock	5	+	-[c]	++			++[g]			+				+[g]	Based largely on comparisons with Fucus
	Barnacle																	
72	Semibalanus balanoides	Suspension	Rock	11			+			+							+[g]	Based on comparisons with water
	Fish																	
73	Platichthys flesus	Carnivore	Sediment	30+						-	++						-	

[a] Sometimes problems with sediment contamination; [b] some uncertainty; [c] tends to regulate metal; [d] High Cu in clams from anoxic areas; [e] may work for Ag from sewage; [f] indicates only at very high concentrations; [g] high individual variability; [h] based mainly on laboratory evidence; [j] tributyl tin oxide

only on how well concentrations in a particular species relate to those in the environment, but also on factors such as ease of analysis, the degree of individual variation and the absence of sediment contamination.

Some indicators are versatile and reflect contamination with a wide range of metals albeit not all equally efficiently. Others are very good for a few specific metals but are useless for others.

Versatile indicators

Fucus vesiculosus, Ascophyllum nodosum, Enteromorpha intestinalis. Under experimental conditions probably all three species will act as indicators of dissolved metals. In practice, contamination of the fronds by fine particles of sediment hinders the use of algae for metals which occur at relatively high concentrations in sediments: Cr is the most notable example, followed by Pb. If sediment analyses are available, the magnitude of this problem can be assessed, and it usually decreases in the order E. intestinalis > F. vesiculosus > Ascophyllum nodosum.

Nereis diversicolor, Nephtys hombergi. Both worms are fairly versatile sediment-dwelling indicators, but tend to regulate Zn (also Fe, Mn). Furthermore they are comparatively weak bioaccumulators and thus their body metal levels are generally on the low side. Both species are good indicators for Cu.

Bivalve molluscs. Of the sediment-dwelling deposit feeders, Scrobicularia plana is generally more versatile than Macoma balthica. The former species is larger and accumulates much higher concentrations of Cd and Pb than the latter. Neither organism is recommended as an indicator for Cu, and there are signs that Zn is to some degree regulated.

The suspension feeders Mytilus edulis and Cerastoderma edule are both regarded as unreliable indicators of Cu and Zn except perhaps at very high environmental concentrations. In addition, M. edulis appears unreliable for As and Ag. However, mussels are easily freed of particulate contamination and are useful indicators for Cr, Pb, Hg and Cd. Cockles appear to be better than mussels as indicators of Ag contamination: they also contain impressive levels of Ni which, however, are not directly proportional to environmental levels.

Based on laboratory experiments, oysters appear to be versatile indicators for a wide range of metals, although As is a notable exception. However, under field conditions individual variation is usually high and in the United Kingdom oysters are less readily available than other species.

TABLE 2

MOST USEFUL INDICATORS FOR SPECIFIC METALS OR METALLOIDS

Species in brackets are best of moderate indicators in Table 1

	Indicators of dissolved metals (direct or via diet)	Indicators of dissolved and particulate metals	Indicators of sediment metals (direct or via diet)
Ag	*Littorina littorea*	(*Cerastoderma edule*)	*Scrobicularia plana* *Macoma balthica*
As	*Fucus vesiculosus* *Littorina littoralis*	(*Cerastoderma edule*)	*Scrobicularia plana* *Macoma balthica*
Cd	*Littorina littoralis* *Patella vulgata* *Littorina littorea* *Nucella lapillus* *Fucus vesiculosus* *Ascophyllum nodosum*	*Mytilus edulis*	*Scrobicularia plana*
Co	*Fucus vesiculosus*	(*Mytilus edulis*)	*Nereis diversicolor*
Cr	possibly (*Ascophyllum nodosum*)	*Mytilus edulis*	(*Scrobicularia plana*)
Cu	*Fucus vesiculosus* *Ascophyllum nodosum* *Patella vulgata* *Nucella lapillus*	*Ostrea edulis*	*Nereis diversicolor* *Nephtys hombergi*
Hg	(*Fucus vesiculosus*)	(*Mytilus edulis*)	*Scrobicularia plana* *Macoma balthica* *Platichthys flesus*
Ni	*Fucus vesiculosus*	(*Cerastoderma edule*)	(*Scrobicularia plana*)
Pb	*Ascophyllum nodosum*	*Mytilus edulis*	*Scrobicularia plana*
Se	(*Fucus vesiculosus*)	(*Mytilus edulis*)	*Scrobicularia plana* *Macoma balthica*
Sn	(*Fucus vesiculosus*)	(*Mytilus edulis*)	(*Scrobicularia plana*)
Zn	*Fucus vesiculosus* *Ascophyllum nodosum*	*Ostrea edulis*	(*Scrobicularia plana*)

Gastropod molluscs, barnacles and fish Although there is some uncertainty about barnacles, none of the gastropods or the fish in Table 1 can be regarded as versatile indicators. They are, however, very useful for specific metals or metalloids.

Specific metal indicators

The indicators from Table 1 that are regarded as good or excellent for specific metals are summarised in Table 2. In some categories, no really good indicator is available and the best of the 'moderate' indicators is included in brackets. There is still some uncertainty about the best indicators of Se and Sn.

Transplantation of indicators

The brown seaweeds F. vesiculosus and A. nodosum are numbered among species that are amenable to transplantation between uncontaminated and contaminated sites.

Of the bivalve molluscs, M. edulis and O. edulis can be transplanted in cages and S. plana can be buried in sediments between marker posts. Winkles, Littorina littorea, marked with a file, and dog-whelks Nucella lapillus, marked with paint, are easily transplanted: provided that a few hundred are used, they can usually be recovered many months later even if they are not caged. Barnacles attached to stones are also easily transferred.

It should be noted that a licence for the deposition of molluscan shellfish is required from the Ministry of Agriculture, Fisheries and Food, Fisheries Division 1A, Great Westminster House, Horseferry Road, London SW1P 2AE.

GENERAL INTRODUCTION

In British waters the most obvious metallic contamination occurs in estuaries. Sources of heavy metals in estuaries include river inputs, sewage and industrial outfalls, the dumping of sewage sludge and harbour spoil, contamination from ships, and atmospheric fallout (e.g. Humber; Murray et al., 1980). In the past, metallic wastes have been discharged into estuaries based on the assumption that they would be carried to the open sea and dispersed. The truth of the matter is somewhat different and Turekian (1977) has emphasised the efficiency of estuaries as traps where high levels of heavy metals may become accumulated in the sediments. This is because interactions between sea water and natural or anthropogenic inputs generally favour the net transfer of metals from the overlying water to the sediments. Some metals including Fe and Pb are readily deposited, whereas others such as Cd remain in solution longer and are more likely to be dispersed (Sholkovitz, 1978; Sholkovitz and Copland, 1981). Consequently, the relative proportions of different heavy metals in the waters or sediments of an estuary may differ substantially from those of the original inputs (Bryan & Gibbs, 1983).

Assessment of metal contamination in estuaries

The monitoring of contamination may be based on analyses of water, analyses of sediments, analyses of biological material, or a combination of all three. However, information on the quantity and composition of the various inputs giving rise to metallic contamination is of vital importance, since ultimately it is necessary to establish the relationships between monitoring and input data.

Water analysis

Analysis of sea water is perhaps the most obvious way of assessing contamination. However, it is also the most difficult because concentrations of dissolved metals are generally very low and the possibilities of contaminating a sample during collection and analysis are appreciable. Thus, because of reduced levels of analytical contamination, the oceanic concentrations of metals given in Table 3 are in some cases 10-1000 times lower than the values accepted prior to 1975 (Bruland, 1983).

In oceanic water most metals are in solution so that a filtration step is not usually employed prior to analysis. On the other hand, particulate metals may comprise the larger fraction in estuarine waters so that a filtration step (usually 0.45 µm), with the increased possibility of contamination, becomes mandatory. The presence of higher concentrations in

Table 3

CONCENTRATIONS AND SPECIATION OF TRACE METALS IN SEA WATER

Metal	Atomic Wt	µg/litre	Trend with depth	Location	Authors	Main species in aerated water ~35‰ salinity	 <10‰ salinity[f]
Ag	107.9	0.00004-0.0025	a	N.E. Pacific	Martin et al., 1983	$AgCl_2^-$ [d]	
Al	26.98	0.162-0.864	b	N.E. Atlantic	Hydes, 1983	$Al(OH)_4^-$ [d] $Al(OH)_3^0$	
		0.218-0.674	a	N. Atlantic	Ólafsson, 1983		
As	74.92	1.27-2.10	c	Atlantic	Burton et al., 1983	$HAsO_4^{2-}$ [d]	
Be	9.012	0.000036-0.00027	a	N. Pacific	Measures & Edmond, 1982	$BeOH^+$ [d] $Be(OH)_2^0$	
Cd	112.4	0.015-0.118	a	N. Pacific	Bruland et al., 1979	$CdCl_2^0$ [e] $CdCl_3^0$ $CdCl^+$	Cd^{2+} $CdCl^+$
		0.0002-0.033	a	Sargasso Sea	Bruland & Franks, 1983		
		0.015-0.025	a	Arctic	Danielsson & Westerlund, 1983		
Co	58.93	0.007-0.0014	b	N.E. Pacific	Knauer et al., 1982	$CoCO_3^0$ [f] Co^{2+}	Co^{2+} $CoCO_3^0$
Cr	52.0	0.057-0.234	a	E. Pacific	Murray et al., 1983	CrO_4^{2-} [d] $NaCrO_4^-$	CrO_4^{2-} [g]
Cu	63.54	0.121-0.146	c	Arctic	Danielsson & Westerlund, 1983	$CuCO_3^0$ [f] Cu-organic	Cu humic $Cu(OH)_2^0$
		0.076-0.108	a	Sargasso Sea	Bruland & Franks, 1983		
Hg	200.6	0.001-0.004	c	N. Atlantic	Ólafsson, 1983	$HgCl_4^{2-}$ [f] $HgCl_3^-$	Hg humic $HgCl_2^0$
Fe	55.85	0.067-0.553	c	Arctic	Danielsson & Westerlund, 1983	$Fe(OH)_3^0$ [d] $Fe(OH)_2^+$	
Mn	54.94	0.165-0.027	b	Atlantic	Burton et al., 1983	Mn^{2+} [f] $MnCl^+$	Mn^{2+}
		0.126-0.033	b	Sargasso Sea	Bruland & Franks, 1983		
Ni	58.70	0.205-0.241	c	Arctic	Danielsson & Westerlund, 1983	$NiCO_3^0$ [f] Ni^{2+}	Ni^{2+} $NiCO_3^0$
		0.135-0.334	a	Sargasso Sea	Bruland & Franks, 1983		
Pb	207.2	0.014-0.001	b	Central Pacific	Flegal & Patterson, 1983	$PbCO_3^0$ [e] $PbOH^+$	
		0.035-0.005	b	Sargasso Sea	Schaule & Patterson, 1983		
Sb	121.7	0.092-0.141	c	N. Pacific	Andreae, 1983	$Sb(OH)_6^-$ [d]	$Sb(OH)_6^-$
Se	78.96	0.044-0.170	a	Pacific and Indian	Measures et al., 1983	SeO_4^{2-} [d] SeO_3^{2-}	
Sn	118.7	0.0003-0.0008	c	N.E. Pacific	Hodge et al., 1979	$SnO(OH)_3^-$ [d]	
V	50.94	0.83-1.57	c	N.E. Atlantic	Morris, 1975	HVO_4^{2-} [d] $H_2VO_4^-$	
Zn	65.38	0.007-0.64	a	N. Pacific	Bruland et al., 1979	Zn^{2+} [f] $ZnCl^+$	Zn^{2+}
		0.004-0.098	a	Sargasso Sea	Bruland & Franks, 1983		
		0.056-0.255	a	Arctic	Danielsson & Westerlund, 1983		

[a] Increasing with depth (nutrient type); [b] Decreasing with depth; [c] Less obvious trend; [d] Bruland, 1983;
[e] Nürnberg & Valenta, 1983; [f] Mantoura et al., 1978; [g] Cranston & Murray, 1980

TABLE 4

CONCENTRATIONS OF DISSOLVED TRACE METALS (μg/l) IN SOME EUROPEAN COASTAL WATERS AND ESTUARIES

	UK Coastal waters - surface	S North Sea - surface transect	Danish Sounds* W Baltic	Swansea Bay S Wales (range of means)	Bristol Channel SW England - S Wales	Tees Estuary NE England	Outer Firth of Forth E Scotland	Sørfjord (Norway)	Restronguet Creek SW England ($S^{o}/_{oo}$ 34-18)
Cd	0.01-0.17	0.02-0.37	0.016-0.033	0.5-0.9	0.4-9.4	0.1-4	<0.1-1.0*	0.5-9	(0.7-38 (<0.1-1.2[c]
Cu	<0.01-1.9	0.2-2.6	0.36-0.58	1.4-4.4	0.6-5.4	0.5-10	<0.1-38	1-23	(3-176 (2-26[c]
Fe	-	-	0.5-1.1	1.3-3.6	1.5-10.5	3-305	-	-	(3-2485 (4-34[c]
Hg	-	-	-	-	0.009-0.07[a]	0.03-0.46*	<0.004-0.156*	0.003-0.41[b]	-
Mn	0.1-2.3	-	-	0.8-14.1	0.4-5.9	0.6-127	-	-	(15-1513 (3-113[c]
Ni	0.01-0.5	-	0.42-0.54	1.4-6.4	0.2-3.0	-	-	-	1-18[c]
Pb	-	-	0.032-0.077	0.6-1.4	0.35-13	0.6-37*	<0.1-34	1-92	<2-4[c]
Zn	0.2-4.4	0.3-70	0.69-1.1	12.7-40.1	2.7-44	1-117	1-48*	8-900	(22-20460 (7-300[c]
	Topping et al., 1980	Duinker & Nolting, 1982	Magnusson & Rasmussen, 1982	Humphrey et al., 1980	Abdullah & Royle, 1974	Taylor, 1982	Read et al., 1982	Melhuus et al., 1978	Bryan & Gibbs, 1983
					[a] Gardner, 1978			[b] Eide et al., 1979	[c] Boyden et al., 1979 (mouth of creek only)

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*Unfiltered water

TABLE 5

CONCENTRATIONS OF METALS IN SEDIMENTS FROM SOME UNITED KINGDOM ESTUARIES (μg/g dry wt, except Al, Fe, Ti%)

Highest concentrations are underlined

	Avon (Devon) clean (typical)	Teign Barytes mining (max)	Restronguet Creek mining wastes (max)	Gannel old lead mines (max)	Poole Harbour industrial wastes (max)	Loughor tin plate manufacture (max)	Severn industrial inc. smelting (mean)	Mersey industrial inc. chlor-alkali (max)	Humber industrial inc. TiO_2 (max)
Ag	0.1	1.0	$\underline{4.1}$	2.9	3.8	0.2	0.5	–	–
Al (%)	6.21	–	$\underline{7.33}$	–	–	–	6.52	–	–
As	13	74	$\underline{2520}$	233	–	22	15	–	–
Ba	–	$\underline{2000}$ [a]	–	–	–	–	386	–	–
Be	–	–	–	–	–	–	1.6	–	–
Cd	0.3	1.8	1.2	3.0	$\underline{12}$	1.1	1.9	4.2	–
Co	10	18	22	40	16	13	16	–	$\underline{30}$
Cr	37	35	37	29	90	$\underline{799}$	145	–	201
Cu	19	68	$\underline{2540}$	217	98	47	94	–	160
Fe (%)	1.94	2.16	5.76	3.32	3.57	3.81	3.87	–	$\underline{9.2}$
Hg	0.12	0.36	0.22	0.09	–	0.13	0.15	$\underline{11.3}$ [b]	–
Mn	417	777	559	$\underline{1160}$	180	631	868	–	1098
Mo	–	–	–	–	–	–	3.8	–	–
Ni	28	30	32	49	49	31	$\underline{73}$	–	63
Pb	39	382	290	$\underline{2175}$	145	77	101	349 [c]	221
Sb	–	–	–	–	–	–	2.2	$\underline{2.9}$	–
Se	–	–	–	–	–	–	0.05	–	–
Sn	28	176	$\underline{1730}$	305	–	320	91	–	–
Ti (%)	–	–	–	–	–	–	0.4	–	$\underline{1.2}$
Tl	–	–	–	–	–	–	1.1	–	–
V	–	–	–	–	–	–	98	–	$\underline{2031}$
Zn	98	375	$\underline{3510}$	1215	386	220	296	800	433

Bryan *et al.* (1980)

[a] Merefield (1976)

Hamilton *et al.* (1979)

Leatherland and Burton (1974)

[b] Bartlett *et al.* (1978)

[c] Head *et al.* (1980)

Jaffe and Walters (1977)

coastal and estuarine waters (Table 4) would be expected to reduce the analytical problems. Even so, Jones (1982) concluded that owing to the use of different methods by different workers intercomparability of results for even the most common metals such as Zn is unsatisfactory at present.

An additional problem, particularly in stratified tidal estuaries, is the variability of metal concentrations both with locality, salinity, and depth. This, coupled with the transitory nature of metal concentrations in water, means that considerable sampling and analytical effort is necessary to obtain an integrated picture.

Sediment analysis

By comparison with water, the analysis of sediments (or of suspended particles) is relatively easy. Metal levels in sediments are a particularly useful indication of chronic contamination (Table 5) and in undisturbed sediments the history of metallic inputs can sometimes be deduced from the analysis of dated cores (Clifton & Hamilton, 1979).

In both water and sediments, metals occur in many different forms (species) only some of which are biologically available and capable of having any environmental impact. At present, however, monitoring programmes are usually based on the total analysis of water or sediments and these results may bear little relation to the levels which are biologically available. An obvious way of overcoming this problem is to use an indicator organism, the analysis of which reflects the presence of available metals.

This report describes the evidence on which the use of biological indicators is based and considers the potential possessed by different species as indicators of metal availability in waters, suspended particles and sediments in British estuaries.

BIOAVAILABILITY OF METALS

Before dealing with specific biological indicators some of the evidence as to which metal species are thought to be the most biologically available will be considered. Most of the information concerns metals dissolved in sea water. However, it should be borne in mind that, in addition to the overlying water, organisms can also absorb metals from interstitial water or ingested material, including prey organisms, detritus, or sediment particles. Thus in seeking suitable biological indicators it is necessary to consider organisms which accumulate metals from other sources in addition to sea water.

Dissolved Metals

The bioavailability of a dissolved metal is not necessarily reflected by its total concentration, nor is the most abundant chemical species (see Table 3) always the most biologically important. Copper is a good example. Zamuda and Sunda (1982) showed that Cu accumulation in the oyster Crassostrea virginica is dependent on the level of cupric ions, Cu^{++}, in the water. In these experiments the concentrations of Cu^{++} were controlled either by changing the total amount of metal or by adding different levels of the chelating agent, nitrotriacetic acid (NTA). Organic chelation, whether by a synthetic chelator like NTA or by naturally-occurring chelators such as humic materials (Table 3) can produce a situation where even at a high total concentration of dissolved Cu the availability of Cu^{++} is very low. Concentrations of humic materials are generally high in fresh water. Thus in estuaries, organic complexation is very important in controlling the amount of Cu^{++}. Other factors affecting the level of Cu^{++} are salinity and pH. In waters of different salinity having similar concentrations of total Cu and humic materials, the computed level of Cu^{++} rises with increasing salinity because the binding of Cu to humics is decreased through competition from Ca^{++} and Mg^{++} (Mantoura et al., 1978).

For Cd and Zn also, the most bioavailable forms appear to be the free ions Cd^{++} and Zn^{++} (Engel & Fowler, 1979; Anderson et al., 1978). The influence of salinity on speciation and bioavailability can be illustrated by reference to Cd. Thus, Engel et al. (1981) have shown that Cd is absorbed less rapidly by oysters when the salinity is increased. This is because the proportion of the bioavailable species Cd^{++} is reduced by the increased formation of chloride complexes at higher salinities. A similar model was used by Engel and Fowler (1979) to explain the greater toxicity of Cd to the shrimp Palaemonetes pugio at lower salinities.

The neutral monochloro complex $AgCl^0$ and the dichloro complex $HgCl_2^0$ may be the most readily available inorganic forms of Ag and Hg (Engel *et al.*, 1981; Gutknecht, 1981). However, some organic forms such as methylmercury are far more bioavailable than the inorganic species.

It is clear from the foregoing examples that, particularly in estuaries, the measurement of total dissolved metal concentrations may be an unreliable guide to their bioavailabilities. Ideally, therefore, we should be measuring the levels of readily available metal species. This is possible for some metals and metalloids, such as As, Andreae (1983); Cr, Murray *et al.* (1983); Se, Measures *et al.*, (1983), but in other cases has proved very difficult since the bioavailable species may comprise only a small percentage of an already low total concentration. For example, methyl mercury is very difficult to detect in sea water except under the most contaminated conditions (Fujita & Iwashima, 1981).

Much of the evidence cited above about the bioavailability of different metal species is based on experiments in which accumulation of the particular metal was measured in organisms exposed to a range of solutions : since the total metal concentrations, salinities, pH values and organic chelator levels of the media were known, the amounts of various metal species could be calculated based on the stability constants of the various complexes which the metal could form with inorganic and organic ligands in the water. A good fit between the degree of bioaccumulation and the concentration of a particular metal species indicates that the species is bioavailable (e.g. Engel *et al.*, 1981; Zamuda & Sunda, 1982).

Other evidence is based on direct measurements of dissolved species. For example, Crecelius *et al.* (1982) found that levels of ionic and weakly complexed Cu measured by differential pulse anodic stripping voltametry of sea water provided a better prediction of the bioavailability of Cu to the shrimp *Pandalus danae* and the bivalve *Macoma inquinata* than measurements of total Cu which included organically complexed metal.

The development of techniques for determining the levels of the most important dissolved species at low concentrations does not, however, eliminate the problem of collecting samples free of spurious contamination. In addition, although it is possible to measure particular species of some metals, routine monitoring which covers a wide range of metals is not really feasible at present. Furthermore, prediction of the biological impact of seawater from measurements of a particular metal species is complicated by the fact that competition between metals may occur at absorption sites in the organism. For example, the uptake of Cd by various species is reduced in the presence of high levels of Zn (p. 23). Other examples will be considered in the sections on indicator organisms.

Particulate metals

Concentrations of trace metals in sediments and suspended particles are so much higher than those in the overlying water (Tables 3, 4, 5) that even a minute fraction may represent an important source for uptake, especially in filter-feeding and burrowing organisms. Determining the relative importance of sediments and overlying water as sources of trace metals is sometimes difficult : methods of achieving this have been reviewed by Bryan (1985). For example, the Gannel Estuary in Cornwall receives drainage from Pb mines which ceased operation more than 100 y ago. Through changes in the course of the river, high-Pb sediments thought to have been laid down when the mines were active are being eroded at a point part way down the estuary. Presumably the less metallic present-day inputs of dissolved metals are diluted in a downstream direction, yet it is possible to find a very marked gradient for sediment Pb falling upstream over a few hundred metres. Table 6 shows that concentrations of Pb, Ag and Cu in the ragworm *Nereis diversicolor* from this locality reflect the levels in the sediments rather than the gradient of dissolved metals in the overlying water.

TABLE 6

HEAVY METAL CONCENTRATIONS IN *NEREIS DIVERSICOLOR* COMPARED WITH LEVELS EXTRACTED FROM <100 µm FRACTION OF SEDIMENT WITH 1N-HCl

Site in Gannel Estuary	Concentration (µg/g dry wt)							
	Ag		Cu		Pb		Zn	
	sed.	worm	sed.	worm	sed.	worm	sed.	worm
1) Downstream	1.20	20.7	401	108	5050	485	1020	283
2)	0.41	2.9	150	58	2200	88	746	251
3) Upstream	0.32	1.1	132	39	1750	56	1220	263
Control estuary (Avon, Devon)	0.047	0.4	10	17	34	2.1	88	197

Although this work shows that sediment metals are available to Nereis diversicolor it does not clearly identify the available fraction. Analyses of Pb in interstitial waters from the high-Pb sediments (total 8000 - 9000 µg/g) gave 68 and 168 µg/l for the oxidized surface layer and 14-43 µg/l for subsurface sediments. Experiments on the absorption of Pb from solution by Nereis suggest that if the higher interstitial concentrations were bioavailable then uptake from this source could account for much of the Pb

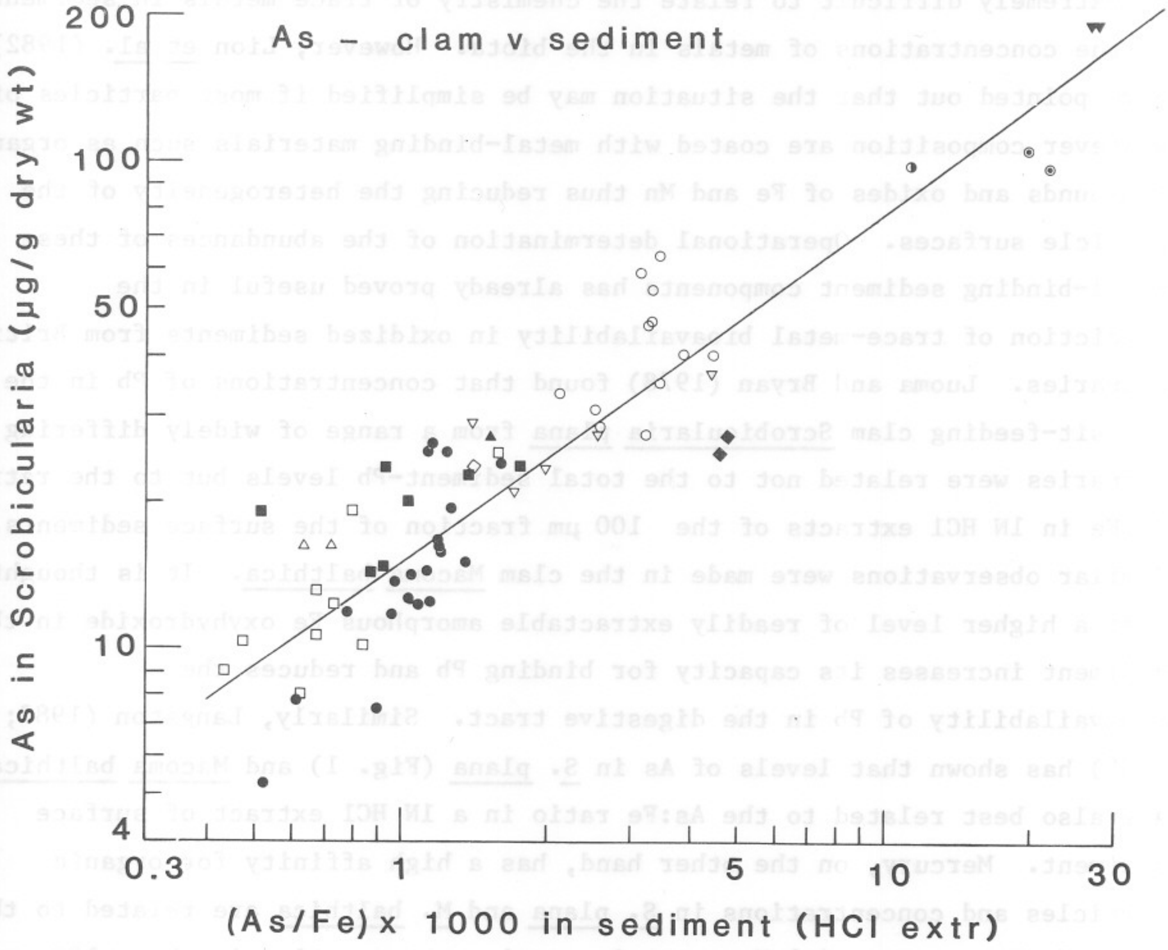


Fig. 1. Scrobicularia plana: relationship between concentration of As in clam and As/Fe ratio in 1N-HCl extract of < 100 µm fraction of surface sediment. Equation is:-

$$\log_{10} \text{As } \underline{\text{S. plana}} = 0.6767 \log_{10} \left(\frac{\text{As}}{\text{Fe}} \times 1000\right) \text{sediment} + 1.212$$

$(n = 75, r^2 = 0.82)$

(▼) Restronguet Creek/Fal, (⊙) Hayle, (◐) Gannel, (◆) Plym, (○) Tamar, (▽) Teign, (◇) Avon, (●) Severn and South Wales estuaries, (■) Northwest England, (△) Thames, (▲) Humber, (□) Southampton. (From Langston, 1980).

in the worm. However, some particulate Pb is probably also available to *Nereis*, since Pb levels in the worm *Capitella capitata* were observed to reflect those of their diet of seaweed detritus (Windom et al., 1982).

Ingested particles of sediment or suspended matter would be expected to be a major route for trace-metal uptake, especially in view of the reduced pH values sometimes encountered in digestive systems. Generally speaking, fine-grained oxidized particles probably provide the most important sources of available metals (Luoma and Davis, 1983). The composition of sediments is so complex and variable that it would appear to be extremely difficult to relate the chemistry of trace metals in sediments to the concentrations of metals in the biota. However, Lion *et al.* (1982) have pointed out that the situation may be simplified if most particles of whatever composition are coated with metal-binding materials such as organic compounds and oxides of Fe and Mn thus reducing the heterogeneity of the particle surfaces. Operational determination of the abundances of these metal-binding sediment components has already proved useful in the prediction of trace-metal bioavailability in oxidized sediments from British estuaries. Luoma and Bryan (1978) found that concentrations of Pb in the deposit-feeding clam *Scrobicularia plana* from a range of widely differing estuaries were related not to the total sediment-Pb levels but to the ratio Pb:Fe in 1N HCl extracts of the 100 µm fraction of the surface sediments. Similar observations were made in the clam *Macoma balthica*. It is thought that a higher level of readily extractable amorphous Fe oxyhydroxide in the sediment increases its capacity for binding Pb and reduces the bioavailability of Pb in the digestive tract. Similarly, Langston (1980; 1985) has shown that levels of As in *S. plana* (Fig. 1) and *Macoma balthica* are also best related to the As:Fe ratio in a 1N HCl extract of surface sediment. Mercury, on the other hand, has a high affinity for organic particles and concentrations in *S. plana* and *M. balthica* are related to the total (HNO_3 extractable) Hg : total organic matter ratios in the < 100 µm fraction of surface sediments (Langston, 1982). This agrees with the work of Breteler et al. (1981) which showed that the highest concentration of Hg in mussels *Modiolus demissus* and crabs *Uca pugnax* occurred in animals exposed to sediments having low levels of organic matter.

These results suggest that, for a few elements at least, relatively simple measurements on oxidized surface sediments can be used to assess their bioavailabilities to deposit-feeding bivalves (see Appendix).

BIOLOGICAL INDICATORS OF METAL CONTAMINATION

In view of the difficulties involved in making measurements covering a wide range of environmentally significant metal species in waters and sediments, there is a strong argument for the use of biological indicators to assess contamination.

Indicator organisms should be good accumulators of metals and their body concentrations must reflect differences in metal bioavailability. For this reason, organisms having an ability to regulate metals are clearly unsuitable. Other desirable properties are that the organism should be widely distributed, common, accessible, easily recognised, relatively stationary, available at all times of year and, for estuarine purposes, sufficiently tolerant of low salinities and high suspended solids to penetrate a reasonable distance upstream (Phillips, 1980).

Because estuarine species have distributions ranging from rocky shores to muddy sediments and absorb metals from different sources there is no universal indicator organism. Any reasonable monitoring programme should involve analyses of several species such as a seaweed, a suspension feeder and a deposit feeder to try and assess contamination in different forms. Furthermore, the analysis of carnivorous species is perhaps most likely to reveal examples of food-chain biomagnification of metals.

Seaweeds

Marine algae would be expected to be the most suitable indicators of dissolved metals since, unlike animals, the dietary route for metal uptake is not involved.

Fucus vesiculosus (Phaeophyceae) bladderwrack

The evidence for its use as an indicator is based upon both laboratory and field observations.

Laboratory experiments have been carried out to discover how well concentrations of metals in F. vesiculosus reflect those in the environment, and how the accumulation of metals (Ag, Cd, Co, Cr, Cu, Mn, Ni, Pb, Zn) is affected by other parameters. Cadmium will service as an example.

Fig. 2A illustrates the accumulation of Cd from 50% sea water (changed regularly) by small plants (3 cm long) at 13°C with continuous illumination and aeration. After 32 days an addition of 0.5 µg/l is accumulated by a concentration factor of 45 x 10^3 and an addition of 5 µg/l by a factor of 30 x 10^3. At the higher concentration equilibrium is approached more slowly, but the indications are that levels of Cd in the weed will ultimately be

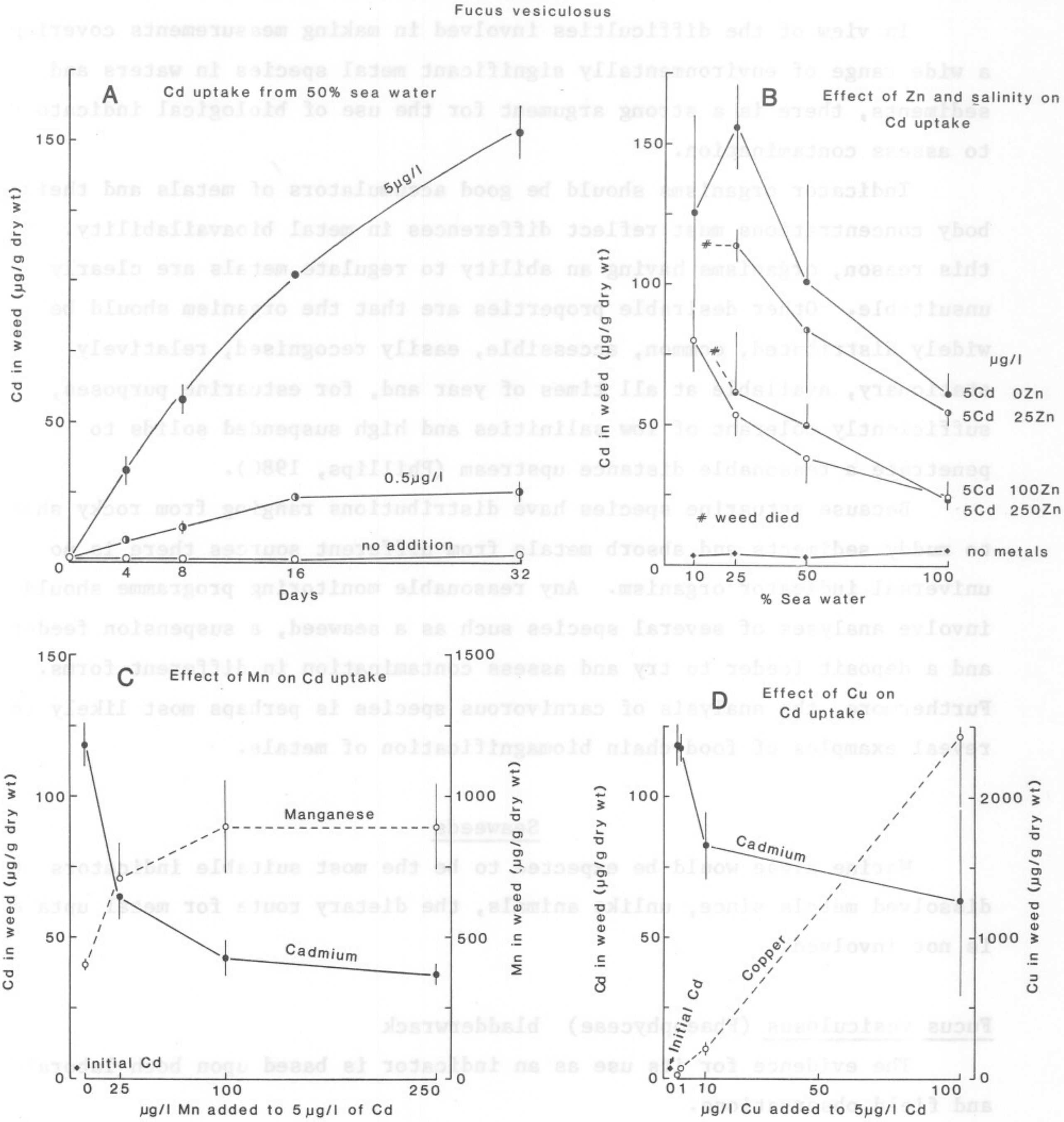


Fig. 2A. *Fucus vesiculosus*: absorption of Cd from 50% sea water by small plants in continuous light at 13°C.

2B. Effect of Zn and salinity on the accumulation of Cd after 16 days from waters containing 5µg/l of Cd.

2C. Effect of Mn on Cd accumulation after 16 days in 50% sea water containing 5 µg/l of Cd and variable levels of Mn.

2D. Effect of Cu on Cd accumulation after 16 days in 50% sea water containing 5 µg/l of Cd and variable levels of Cu.

Vertical lines are standard deviations.

almost proportional to those in the water. This suggests that the amount of bioavailable Cd^{++} in the water is a constant fraction of the total. However, as has already been discussed on p. 16, the proportion of dissolved Cd^{++} is salinity dependent and decreases with increasing salinity through the formation of less-readily-available chloro complexes : this is reflected by the increased accumulation of Cd at lower salinities (Fig. 2B).

A point that is often not appreciated is that the accumulation of one metal may be reduced through competition for uptake sites from other metals. Figures 2B,C,D show how the accumulation of Cd is suppressed by increasing concentrations of Zn, Mn and Cu. These metal-metal interactions are sometimes very important under field conditions, as can be demonstrated by comparing results from the Bristol Channel with those from Restronguet Creek in the Fal Estuary. Dissolved Cd concentrations in the Bristol Channel are quite high and so also are concentrations in F. vesiculosus (Table 7). In Restronguet Creek, however, values for dissolved Cd exceed those in the Bristol Channel but levels in F. vesiculosus are roughly an order of magnitude lower. To a large extent this anomaly can be explained by competition for Cd uptake by the weed from the high concentrations of Zn, Mn and Cu in the waters of the Creek. If this interaction between metals was confined to F. vesiculosus then its use as an indicator for Cd might be invalidated. However, the contrast between Cd levels in the biota of the two estuaries seems to be a general phenomenon. For example, concentrations

TABLE 7

COMPARISON OF METAL CONCENTRATIONS IN WATER AND SEAWEED FROM TWO ESTUARIES

Locality	Cd	Cu	Mn	Zn	Reference
	Seawater (μg/l)				
Bristol Channel	0.27-1.34	0.35-1.32	0.41-3.55	5.2-13	Morris & Bale (1975)
Restronguet Cr.	0.25-5	10-100	20-400	100-2000	Bryan & Gibbs (1983)
	Fucus vesiculosus (μg/g dry wt)				
Bristol Channel	3.8-19.5	3.8-14.3	38-89	88-262	Fuge & James (1974)
Restronguet Cr.	0.81-1.41	190-1450	61-533	2190-4200	Bryan & Gibbs (1983)

of Cd in gastropod molluscs such as *Littorina littoralis* from the Bristol Channel and Severn Estuary are also much higher than those in animals from Restronguet Creek (Bryan, 1983). These observations on Cd are very relevant to the setting of emission standards for estuaries and support the contention that if there are limits for particular metals they should not be uniform but tailored to the capacity of the individual estuary to receive them.

Experiments with other metals indicate that concentrations in *F. vesiculosus* will approach direct proportionality with those of the medium up to concentrations of 5 µg/l of Ag, 10 µg/l of Co or Pb and 100 µg/l of Cu. Experiments on As in *F. spiralis* also suggest that concentrations in the weed will come to reflect those of the water (Klumpp, 1980a). On the other hand, although the seaweed concentrations change markedly with those of the water, it is less certain that direct proportionality is ever achieved by Cr (VI), Ni, Mn or Zn.

Under experimental conditions the accumulation of Zn by *F. vesiculosus* was relatively independent of salinity : so also was that of As in *F. spiralis* (Klumpp, 1980a). In sea water diluted with distilled water, more Cu and Pb were accumulated at lower salinities : however, in reality, the presence of humic materials from fresh water would be expected to limit the availability of the free ions of Cu and Pb at lower salinities (see p. 16).

Studies on the interactions between metals in growing *F. vesiculosus* have shown that high levels of Mn or Zn (up to 1000 µg/l) in the water very markedly suppress the accumulation of low levels of Cd, Co, Ni, Zn or Mn. On the other hand, the suppression of Cu or Pb accumulation by Mn or Zn is less obvious, presumably because the affinity of the weed for Cu and Pb is particularly high (see also p. 26).

Field observations on relationships between metal concentrations in *F. vesiculosus* and those of the water have been carried out for more than 10 years. A summary of work from different sea areas is given in Table 8, the results being expressed as concentration factors (µg/g dry weed/µg/ml water) at the various dissolved seawater concentrations. The high concentration factors usually observed ($\sim 10^4$) illustrate clearly one of the advantages of analysing indicator species. However, the concentration factors do vary considerably (Cu, $3.6 \times 10^3 - 27 \times 10^3$; Zn, $7.1 \times 10^3 - 64 \times 10^3$) and there are several possible causes:-

a) Some variations undoubtedly stem from difficulties inherent in obtaining average water concentrations in estuaries for use in calculating the

TABLE 8

FUCUS VESICULOSUS: CONCENTRATION FACTORS (THOUSANDS) OBSERVED IN THE FIELD AT DIFFERENT SEAWATER CONCENTRATIONS (µg/l)

Sea area	Cd		Cu		Mn		Ni		Pb		Zn		Reference
	µg/l	(CF)	µg/l	(CF)	µg/l	(CF)	µg/l	(CF)	µg/l	(CF)	µg/l	(CF)	
Irish Sea	0.43	(2.7)	1.5	(4.5)	4.1	(23)	2.0	(2.8)	1.4	(2.4)	6.7	(20)	Preston et al. (1972)
Tamar Estuary	–	–	2.7	(25)	21	(19)	–	–	–	–	4.1	(64)	Bryan & Hummerstone (1973)
Restronguet Creek	–	–	11	(27)	28	(4.6)	–	–	–	–	113	(11)	Bryan & Hummerstone (1973)
Bristol Channel (range)	0.27 1.34	(14) (15)	0.35 1.32	(14) (11)	0.41 3.55	(220) (23)	– –	– –	– –	– –	5.2 13	(17) (20)	Morris & Bale (1975)
Menai Strait	0.20	(10.5)	1.4	(6.4)	5.3	(19)	1.2	(6.8)	1.1	(2.9)	11.3	(10)	Foster (1976)
Raritan Bay (range)	– –	– –	2 7	(3.6) (7.4)	– –	– –	– –	– –	0.4 1.8	(13) (24)	– –	– –	Seeliger & Edwards (1977)
Sørfjord (in 4 sectors of fjord)	0.85 0.81 1.2 2.2	(10) (13) (8.8) (4.2)	4.4 3.7 4.4 6.3	(4.8) (11) (19) (17)	– – – –	– – – –	– – – –	– – – –	8.6 4.3 6.4 8.9	(3.2) (22) (26) (15)	113 127 162 313	(22) (24) (18) (7.1)	Melhuus et al. (1978)

concentration factors. Fluctuating metal inputs are a particularly important source of variation.

b) The values for water in Table 8 are total dissolved concentrations and not measurements of the bioavailable species : thus the removal of Cu^{++} through binding by dissolved organic matter in the water could be responsible for the lower concentration factors for this metal observed in some sea areas. In addition, because the complexation of Cd^{++} by chloride is decreased, higher Cd concentration factors would be expected at lower salinities (Fig. 2B).

c) Organic components of F. vesiculosus, especially polyphenols, have such a high affinity for Cu and to a lesser extent Pb that it is thought that adsorbed forms of these metals can also be absorbed by the weed following contact between the surfaces of the plant and suspended material (Luoma et al. 1982). Additional uptake from particles may explain the high concentration factor for Cu in Restronguet Creek (Table 8), since the sediment levels in the estuary are around 3000 µg/g (Table 5) : this is not the same as particulate contamination of the weed surfaces, a problem that is dealt with on page 27.

d) Concentration factors will also vary if the response of the weed is not directly proportional to the increasing availability of the metal. Evidence of this can be seen in Table 8 where concentration factors for Mn in the Bristol Channel and Zn in Sjorfjord and S.W. England (Tamar and Restronguet Cr.) are lower at higher water concentrations.

e) Through competition between some metals for uptake sites in the plant, a high concentration of one metal may suppress the uptake of another. The low factor for Mn in Restronguet Creek may reflect competition from the very high level of Zn (Table 8).

f) If metals are unevenly distributed in the plant then the concentration factor will depend on the part of the frond that is analysed (Table 9).

Sampling and treatment of F. vesiculosus. Probably due to the relatively slow accumulation of metals and the synthesis of more binding sites with age, concentrations are usually lowest in the growing tips of F. vesiculosus and tend to increase for several cm before reaching a more constant level in the older tissues (Bryan, 1971). Analyses of the younger tissues at the tips will provide more recent information, whereas analyses of the older parts will give a value integrated over several months (the growth rate of F. vesiculosus is 2-3 cm/month : Knight & Parke, 1950). When using the weed as an indicator of yearly changes there is a clear need to sample at fixed times of year (i.e. similar photoperiod and temperature) and from a fairly accurately determined level in the intertidal zone (usually in the middle).

About 20 plants are collected randomly from the site and will keep for several days in plastic bags. Individual fronds are washed in 20% sea water and adhering particles removed as far as possible with a pastry brush. In the following example, the frond was separated into tips (∿1 cm), young weed (∿1-5 cm) and older weed (∿5-15 cm): the latter two sections were cut into small pieces avoiding the vesicles and damaged parts of the frond. Pieces from a large number of fronds were pooled and kept in 20% sea water for over 1 hr : this removes much of the NaCl from the weed and reduces weighing errors caused by different salt levels. After a brief wash in distilled water, the pieces were roughly dried on filter paper and approximately 4g aliquots were transferred to 100 ml conical flasks for drying at 80°C. For the analysis of Hg, Se, Sn or As the weed was not dried and for Sn and As analysis was chopped more finely prior to ashing (see Appendix).

Table 9 shows how metal levels vary between different parts of the fronds from two sites and demonstrates also the effect of shore level on concentrations in the older weed. Variations between replicate analyses are such that most coefficients of variation (standard deviations expressed as %) are less than 15%. In this instance, the lowest concentrations occur in weed from the upper shore, but this effect can vary depending on the hydrographic conditions at a particular site.

Especially in estuaries, fine particles of sediment adhering to the weed can produce errors if the fronds are not satisfactorily cleaned. If _at worst_ all the Fe in the weed comes from sediment contamination then the maximum contribution of particulate metals to the weed can be calculated by multiplying the seaweed/sediment ratio for Fe by the sediment-metal concentration. The example in Table 9 shows that, like Fe, Al in the weed may be largely particulate and there may also be significant levels of particulate Pb, Cu and Ag. Another metal which often appears in the weed largely in a particulate form is Cr (Table 10; Fig. 4A).

It is concluded that, if standardised procedures are used, analysis of _F. vesiculosus_ gives a good indication of the average bioavailabilities of Ag, As, Cd, Co, Cu, Hg, Ni, Pb, and Zn as modified by organic and inorganic complexation and interelement competition. In the older tissues, the concentrations represent several months of exposure and thus at least two and preferably four times a year would be suitable sampling intervals.

Other species of Fucus such as _F. serratus_ (Melhuus _et al._, 1978; Stenner & Nickless, 1974), _F. spiralis_ (Klumpp & Peterson, 1979; Tomlinson _et al._, 1980) and _F. ceranoides_ appear to behave qualitatively in the same way as _F. vesiculosus_.

Table 9.

FUCUS VESICULOSUS FROM TAMAR ESTUARY: MEANS AND STANDARD DEVIATIONS FOR ANALYSES OF 3 ALIQUOTS OF POOLED WEED TISSUE FROM DIFFERENT REGIONS OF FRONDS

	Concentration (µg/g dry wt)							
Part of plant	Ag*	Al †	Cd*	Cu	Fe	Mn	Pb*	Zn
	Devil's Point (mid shore weed)							
Tips	0.30±0.01	202±65	1.24±0.04	5.60±0.34	60.1±3.1	34.6±0.9	0.64±0.19	34.2±0.7
Young weed	0.19±0.01	151±8	0.59±0.02	5.47±0.39	78.0±6.0	64.5±5.7	0.58±0.10	41.8±3.2
Old weed	0.28±0.04	207±37	0.75±0.10	11.3±0.7	146±25	111±9	0.85±0.01	95.5±8.7
	Cargreen (mid shore weed)							
Tips	0.19±0.01	239±36	1.64±0.21	20.8±0.4	206±49	76.4±0.7	3.54±0.51	65.1±0.8
Young weed	0.16±0.01	448±59	1.08±0.15	29.1±2.8	412±70	182±28	6.32±2.21	109±16
Old weed	0.18±0.001	769±105	1.69±0.22	38.4±0.7	796±93	263±78	7.31±0.7	263±24
Region of shore	Cargreen (old weed)							
Upper	0.14	514	0.87	35.8	501	251	4.68	212
Mid	0.18	769	1.69	38.4	796	263	7.31	263
Lower	0.20	688	1.51	46.1	883	521	14.7	233
Particulate contribution to Lower value if all Fe is particulate	0.027 (13%)	855 (124%)	0.026 (1.7%)	9.7 (21%)	883 (100%)	19.8 (3.8%)	4.1 (28%)	9.2 (3.9%)
	Cargreen (sediment) ‡							
	1.30	40650	1.26	461	42000	941	196	439

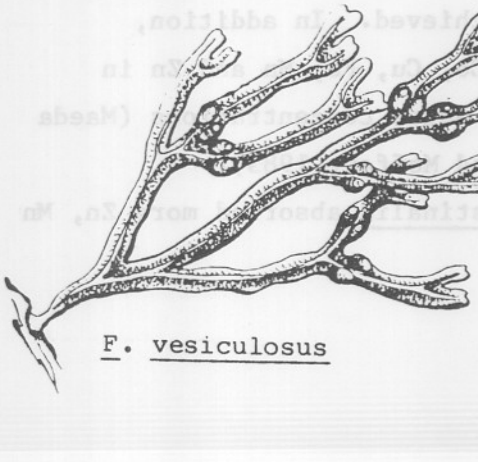
*Analysed in weed by carbon furnace AA; † analysed by flame emission; others by flame AA; ‡ digested like weed

Ascophyllum nodosum (Phaeophyceae) knotted wrack

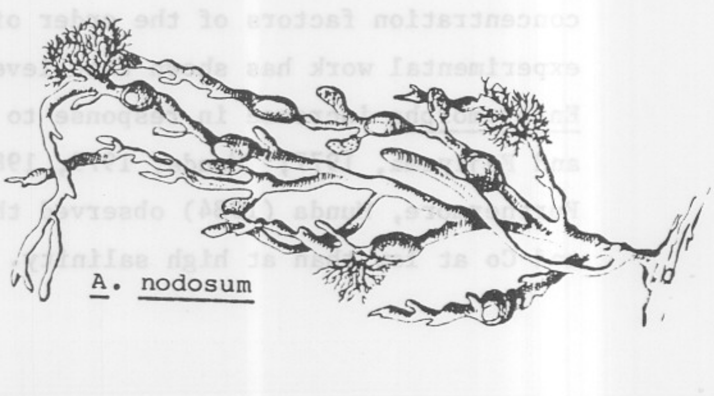
In general *Ascophyllum nodosum* responds qualitatively to metals in the same way as *F. vesiculosus*. It is less tolerant of estuarine conditions than *F. vesiculosus* but does possess some advantages over the latter. One advantage is that the fronds are less prone to contamination with particulates and therefore at the same site it usually has a much lower level of Fe than *F. vesiculosus* (Table 10). Comparisons between analyses of the whole fronds of *Ascophyllum* and *F. vesiculosus* by Stenner and Nickless (1974) and Melhuus et al. (1978) showed that concentrations of Cd, Cu and Zn were usually comparable whereas levels of Pb were appreciably lower in *A. nodosum*. Another advantage is that as *A. nodosum* grows it produces vesicles separated by internodes and this makes it easier to define which part of the plant has been analysed (Table 10). Under Norwegian conditions a vesicle is produced annually and so the tissue can be aged (Haug et al., 1974).

Experiments in which the two species were transplanted from the Tamar to the Fal Estuary indicated that As, Cu and Zn are absorbed more rapidly by the older parts of *F. vesiculosus* than by the tips and first vesicle of *A. nodosum* : local levels were reached by transplanted *F. vesiculosus* in 2-3 months, whereas equilibrium was not achieved by *A. nodosum* in 6 months (Bryan & Gibbs, 1983; Langston, 1984). *A. nodosum* has been used extensively as an indicator of contamination with Cd, Cu, Pb and Zn in Norwegian fjords (see papers by Melhuus *et al*., 1978; Eide *et al*., 1980; Julshamn, 1981a). Transplant experiments showed that the response of uncontaminated plants to contaminated conditions was very slow in the winter compared with the summer when, in the tips, concentrations of Zn and Pb reached local levels in about 3.5 months and 2 months respectively (Eide *et al*., 1980).

In conclusion, *A. nodosum* possesses an important advantage over *F. vesiculosus* by being less prone to particulate contamination. On the other hand, it is generally less readily available, particularly in the upper reaches of estuaries, and responds more slowly to changing metal levels than *F. vesiculosus*.



F. vesiculosus



A. nodosum

TABLE 10

COMPARISON OF METAL LEVELS IN A. NODOSUM and F. VESICULOSUS FROM A CONTROL SITE AND A CONTAMINATED SITE IN THE FAL ESTUARY.

Species		Concentration (µg/g dry wt)							
		Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
					Falmouth Beach				
A. nodosum	1*	0.35	N.D	3.9	36	9.9	0.37	1.12	50
	2	0.25	0.03	8.3	37	8.8	0.59	0.77	106
	3	0.19	N.D	12.5	40	11.5	0.94	0.61	179
F. vesiculosus (older tissue)		0.57	0.15	8.8	104	51.3	3.2	1.3	244
					Above Pandora Inn				
A. nodosum	1*	1.05	0.13	327	117	33.6	1.29	1.86	1371
	2	0.93	0.09	374	132	91.6	1.39	1.44	1725
	3	0.99	0.08	381	116	67.1	1.82	1.31	2081
F. vesiculosus (older tissue)		0.78	0.94	629	1249	209	3.65	11.1	1739
Particulate contribution if all Fe is particulate		0.02	0.58	50	1249	13	0.6	5.1	66
		(2.6%)	(62%)	(7.9%)	(100%)	(6.2%)	(16%)	(46%)	(3.8%)
					Sediment				
		1.1	25	2145	53900	571	26	220	2866

*1 = tip of frond and first vesicle; 2 = first internode and second vesicle; 3 = second internode and third vesicle. ND = undetectable. analyses by Y.B. Ho

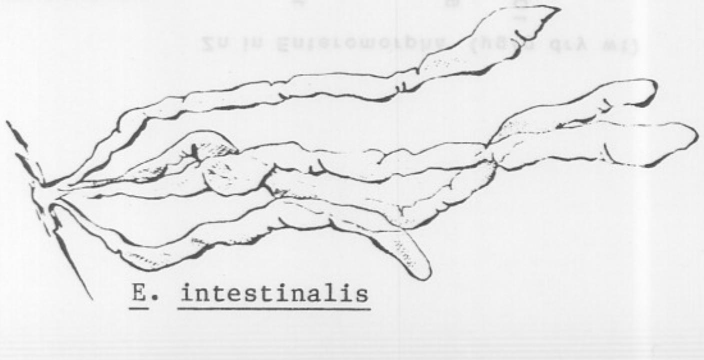
Enteromorpha species (Chlorophyceae)

There are a number of examples where species of the green alga Enteromorpha have been used as indicators of metal contamination (Hägerhäll, 1973; Stenner and Nickless, 1974; Seeliger and Edwards, 1977; Melhuus and co-authors, 1978; Klumpp & Peterson, 1979; Harding, 1982; Seeliger and Cordazzo, 1982). These field observations show that the alga responds to changes in dissolved concentrations of As, Cd, Cu, Hg, Pb and Zn and that concentration factors of the order of 10^4 are achieved. In addition, experimental work has shown that levels of Cd, Co, Cu, Fe, Mn and Zn in Enteromorpha increase in response to increasing water concentrations (Maeda and Fujiyama, 1977; Munda, 1979, 1984; Reed and Moffat, 1983). Furthermore, Munda (1984) observed that E. intestinalis absorbed more Zn, Mn and Co at low than at high salinity.

Comparisons between analyses of Enteromorpha (mainly E. intestinalis) and F. vesiculosus from the same sites indicate that for Cu and Zn both species respond to changes in the availability of the dissolved metal (Fig. 3A,B). There is some curvature of the results for Zn which could be explained by the regulation of the metal in Enteromorpha over the lower concentration ranges, or may result from differences between the rates with which the two species reflect environmental fluctuations. In general terms, levels of Pb in the two seaweeds are comparable but there is tremendous variability. One explanation of this variability is that, particularly in Enteromorpha, Pb appears to be associated with iron, a situation which often indicates contamination of the weed with fine particles of sediment. Fig. 4B shows how concentrations of Pb increase with levels of Fe in both Enteromorpha (mainly E. intestinalis) and F. vesiculosus from the Severn Estuary. The ratio of Pb:Fe in Enteromorpha is about 1:300, a value similar to that in sediments analysed in the same way. Levels of Al in E. intestinalis are comparable with those of Fe and this is further evidence for the presence of fine particulate material that is very difficult to remove (Table 11). The same conclusion applies even more clearly to Cr in both seaweeds since, unlike Pb, the Cr-Fe relationship passes almost through the origin (Fig. 4A). Again, the ratio of Cr:Fe in Enteromorpha of about 1:650 resembles that of the sediment.

Analyses of E. intestinalis and F. vesiculosus from four localities are compared in Table 11. Whole fronds of E. intestinalis were cleaned and analysed in the same way as F. vesiculosus (p. 26). Metals for which higher concentrations are found in E. intestinalis include several (Fe, Al, Cr, Pb, Sn) which are likely to be associated with particulate contamination.

Advantages of Enteromorpha over F. vesiculosus are that E. intestinalis often penetrates farther upstream, into areas of very low salinity. It may also reflect changes in ambient metal levels more rapidly than F. vesiculosus. Disadvantages include problems of obtaining good samples of Enteromorpha during the colder months, difficulties created by particulate contamination and problems of species identification in estuaries (see Fletcher, 1980 and Koeman & van den Hoek, 1982a,b for the taxonomy of Enteromorpha).



E. intestinalis

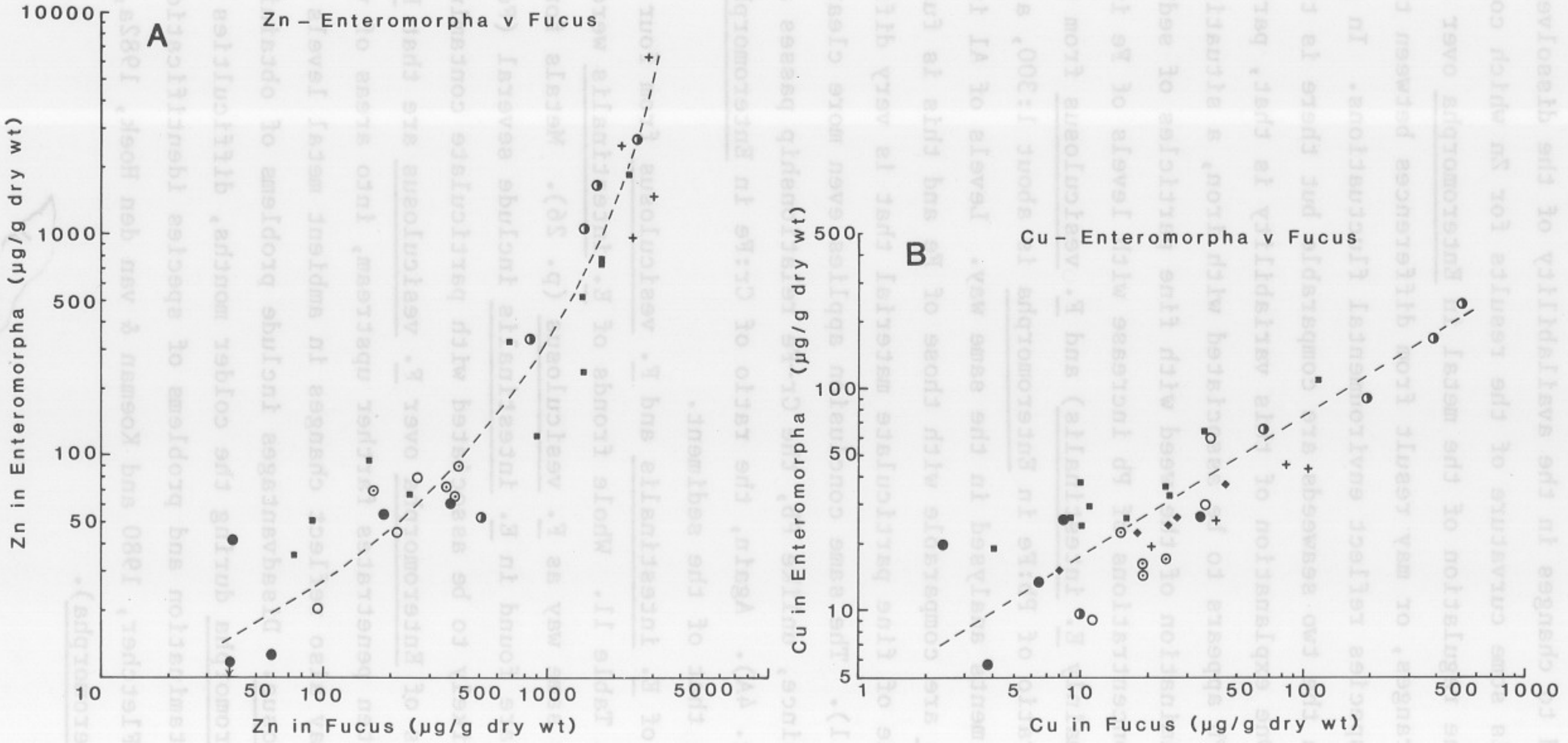


Fig. 3. *Enteromorpha* v *Fucus*. Comparisons between concentrations of Zn and Cu in *Enteromorpha* (mainly *E. intestinalis* in U.K.) and *Fucus* (mainly older tissues of *F. vesiculosus*). (◐) Restronguet Creek/Fal, (○) Tamar, (⊙) Severn, (●) other U.K. sites, (■) Norway (Stenner & Nickless, 1974), (+) Norway (Melhuus *et al.*, 1978), (◆) Raritan Bay (Seeliger & Edwards, 1977).

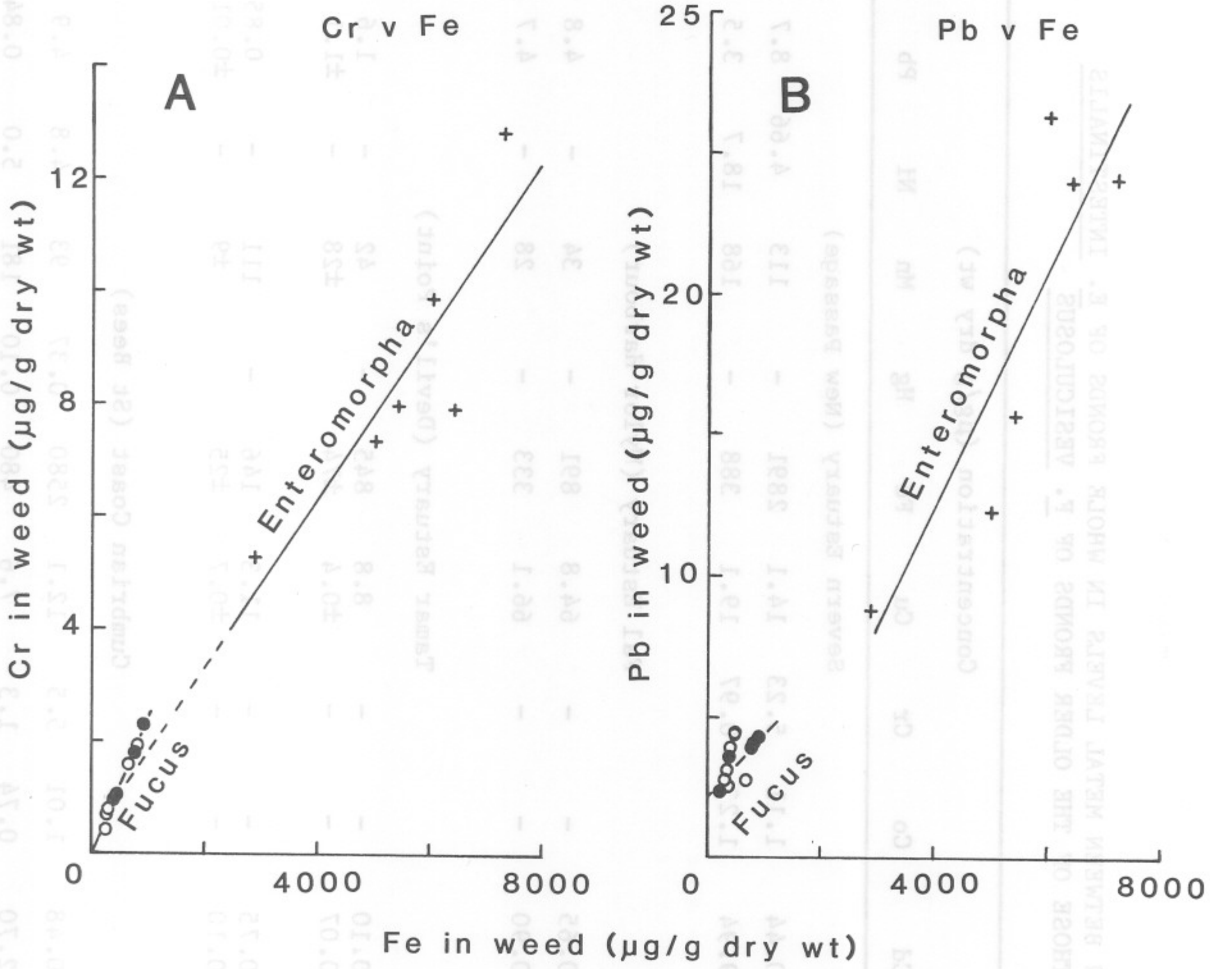


Fig. 4A. *Enteromorpha* and *Fucus vesiculosus*. Relationships between concentrations of Cr and Fe in (+) *Enteromorpha* (mainly *E. intestinalis*) and in *Fucus vesiculosus* (●) older tissues, (○) tips, from Severn Estuary.

4B. Relationships between Pb and Fe concentrations.

Table 11

COMPARISON BETWEEN METAL LEVELS IN WHOLE FRONDS OF *E. INTESTINALIS* AND THOSE OF THE OLDER FRONDS OF *F. VESICULOSUS*

Species	Concentration (µg/g dry wt)														
	Ag	As	Al	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Se	Sn	Zn
Severn Estuary (New Passage)															
E. intestinalis	0.17	–	2418	0.44	1.11	5.23	14.1	2891	–	113	4.66	8.7	–	–	71
F. vesiculosus	0.59	–	667	9.94	1.23	0.97	19.1	388	–	168	18.7	3.5	–	–	353
Fal Estuary (Mylor Harbour)															
E. intestinalis	0.22	–	963	0.65	–	–	64.8	891	–	34	–	4.8	–	–	330
F. vesiculosus	0.58	–	268	0.90	–	–	66.1	333	–	28	–	4.7	–	–	827
Tamar Estuary (Devil's Point)															
E. intestinalis (n = 3)	0.07 ±0.01	–	987 ±72	0.10 ±0.07	– –	– –	8.8 ±0.4	845 ±74	–	42 ±28	– –	1.6 ±1.1	–	–	19.8 ±1.6
F. vesiculosus (n = 3)	0.28 ±0.04	–	207 ±37	0.75 ±0.10	– –	– –	11.3 ±0.7	146 ±25	–	111 ±9	– –	0.85 ±0.01	–	–	95 ±9
Cumbrian Coast (St Bees)															
E. intestinalis	0.34	5.8	–	0.48	1.01	5.5	12.1	2580	0.37	93	4.8	4.9	0.40	0.50	31
F. vesiculosus	0.87	16.0	–	2.70	0.74	1.3	7.6	480	0.10	181	5.0	0.84	0.03	0.24	87

Polychaete worms

Nereis diversicolor (ragworm)

The ragworm is common in intertidal estuarine sediments and is usually distributed from the lower reaches into regions which, although tidal, are sometimes exposed to fresh water. It is regarded as an omnivorous species and depending on the conditions, may deposit feed, filter feed or ingest larger particles (Goerke, 1971).

Field observations: Comparisons between heavy-metal concentrations in *N. diversicolor* and those of surface sediments have demonstrated that in most cases they are significantly related. This is illustrated for Ag, Cu and Pb (Fig. 5) but applies also to As, Co and Hg (Langston, 1980; Bryan *et al*., 1980; Luoma and Bryan, 1982). Although there is good evidence that these metals are absorbed from the sediments (see also p. 18), tissue concentrations may also be influenced by those of the overlying water. Such is the situation with Cd in *N. diversicolor* from the Severn Estuary and Bristol Channel, since concentrations in the body are unrelated to those of the sediments but are very significantly related to those of *Fucus vesiculosus*, an indicator of dissolved Cd availability (Fig. 6A,B). In contrast to most other metals, concentrations of Fe and Zn in the ragworm are relatively unaffected by environmental changes (Table 6, 12, Fig. 5). The jaws of moderately-sized worms contain around 18,000 µg/g of Zn and this accounts for about 30% of the total body burden (Bryan and Gibbs, 1980).

Sampling: At least 40 worms having an average dry weight of 0.02 - 0.04 g are collected. Cleaning the worms is accomplished by keeping them in fine acid-washed sand (B.D.H. Chemicals) covered with 50% sea water for 6 days and for 1 day in water only. Pooled samples each containing 20 worms are suitable for analysis (see Appendix).

Studies on the relationships between copper concentrations in *N. diversicolor* and body weight showed them to be rather variable; sometimes size had no influence whilst at other times lower concentrations were found in large animals (Howard and Brown, 1983). In our experience, it is difficult to collect worms of equal size from all estuaries : however, apart from its inverse relationship to the body concentration of Zn (for which *N. diversicolor* is a poor indicator), size does not appear to be of overriding importance unless the differences are really extreme.

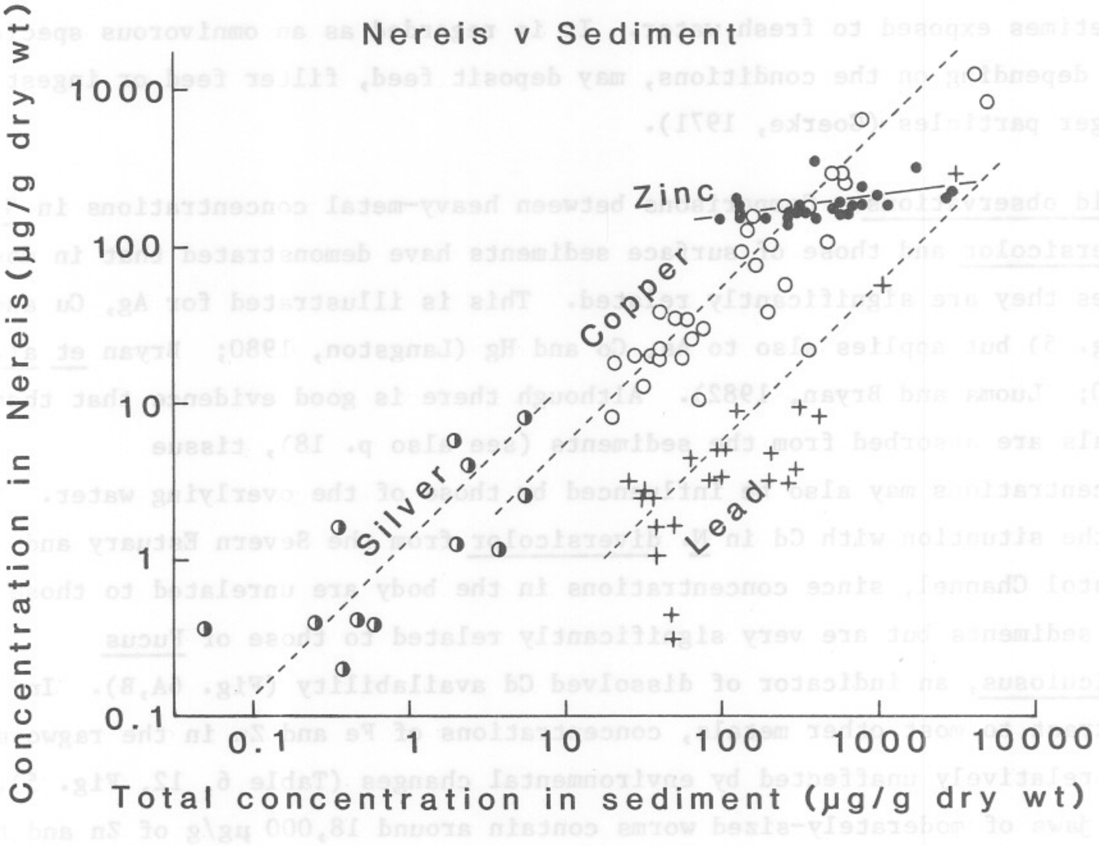


Fig. 5. Nereis diversicolor: relationships between concentrations of Ag, Cu, Pb and Zn in the ragworm and total concentrations in estuarine sediments from Southwest England. Broken lines show direct proportionality (From Bryan, 1976).

Major advantages of N. diversicolor as an indicator include its wide geographical distribution and its occurrence in some of the least saline parts of estuaries from which most other potential indicator species are excluded. By comparison with some bivalve molluscs the ragworm is not a particularly good accumulator of metals; and in uncontaminated worms, the low concentrations of some metals such as Ag, Cd, Cr and Pb may pose analytical problems (Table 10). In addition, the body levels of Fe and Zn tend to be regulated. However, N. diversicolor appears superior to bivalve molluscs as an indicator of Cu contamination.

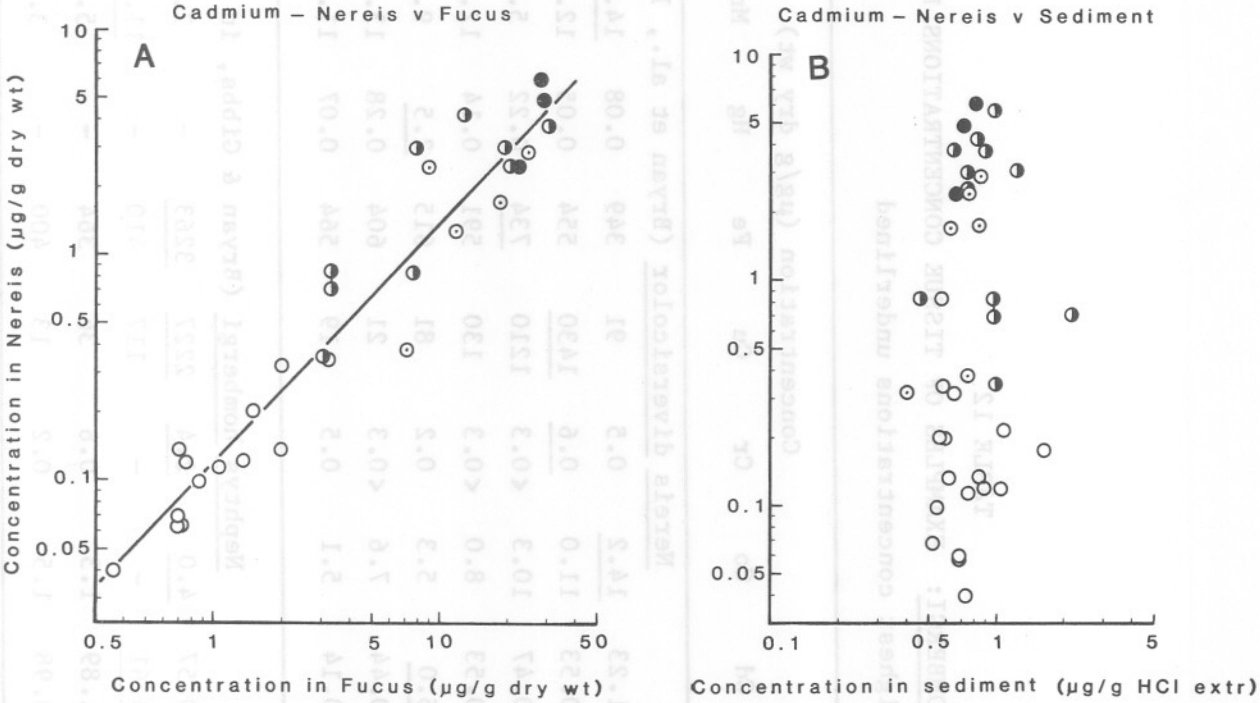


Fig. 6A. Nereis diversicolor. Relationship between concentrations of Cd in the ragworm and those in Fucus vesiculosus from sites in the Severn Estuary and Bristol Channel. (●) above Portishead, (⊙) Minehead-Portishead, (◑) Swansea Bay-Beachley, (○) Cleddau-Loughor estuaries.

6B. Relationship between concentrations of Cd in the ragworm and those in a 1N-HCl extract of the < 100 µm fraction of surface sediment. Symbols as above.

TABLE 12

NEREIS DIVERSICOLOR AND *NEPHTYS HOMBERGI*: EXAMPLES OF TISSUE CONCENTRATIONS FROM DIFFERENT SITES

Highest concentrations underlined

Estuary	Dry wt (g)	Concentration (µg/g dry wt)												
		Ag	As	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Sn	Zn
		Nereis diversicolor (Bryan et al., 1980)												
Gannel (upper)	0.020	5.3	21	1.23	*14.2*	0.5	91	349	0.08	*14.1*	*13.3*	*685*	*1.3*	*466*
Restronguet Cr. (up)	0.036	6.0	23	0.53	11.0	*0.6*	*1430*	554	0.05	12.0	2.3	5.2	0.16	262
Hayle (upper)	0.025	5.3	*84*	0.47	10.3	<0.3	1210	*734*	0.22	5.7	9.1	4.2	0.08	260
Tamar (upper)	0.027	0.6	13	0.53	8.0	<0.3	130	591	0.14	12.8	4.3	7.0	0.34	179
Severn (Sharpness)	0.011	*18.0*	16	*5.0*	5.3	0.2	81	615	*2.5*	9.6	5.4	3.3	1.0	292
Torridge (lower)	0.037	0.3	14	0.44	7.6	<0.3	21	604	0.28	13.9	4.4	2.0	0.09	163
Avon (mid)	0.034	0.1	8	0.14	5.1	0.5	19	564	0.07	11.8	3.3	5.4	0.09	197
		Nephtys hombergi (Bryan & Gibbs, in press)												
Restronguet Cr. (mid)	0.084	*3.3*	-	0.57	*4.0*	*2.4*	*2227*	*3263*	-	7.5	*4.7*	*44.7*	-	*518*
Bristol Channel (Weston)	0.056	2.3	-	*3.61*	-	-	117	410	-	*11.5*	-	4.6	-	259
Salcombe	0.154	0.5	-	1.89	1.3	0.6	36	364	-	5.4	3.0	3.7	-	268
Torre Sands	0.214	0.2	-	0.98	1.5	0.2	13	400	-	3.8	2.5	3.5	-	260

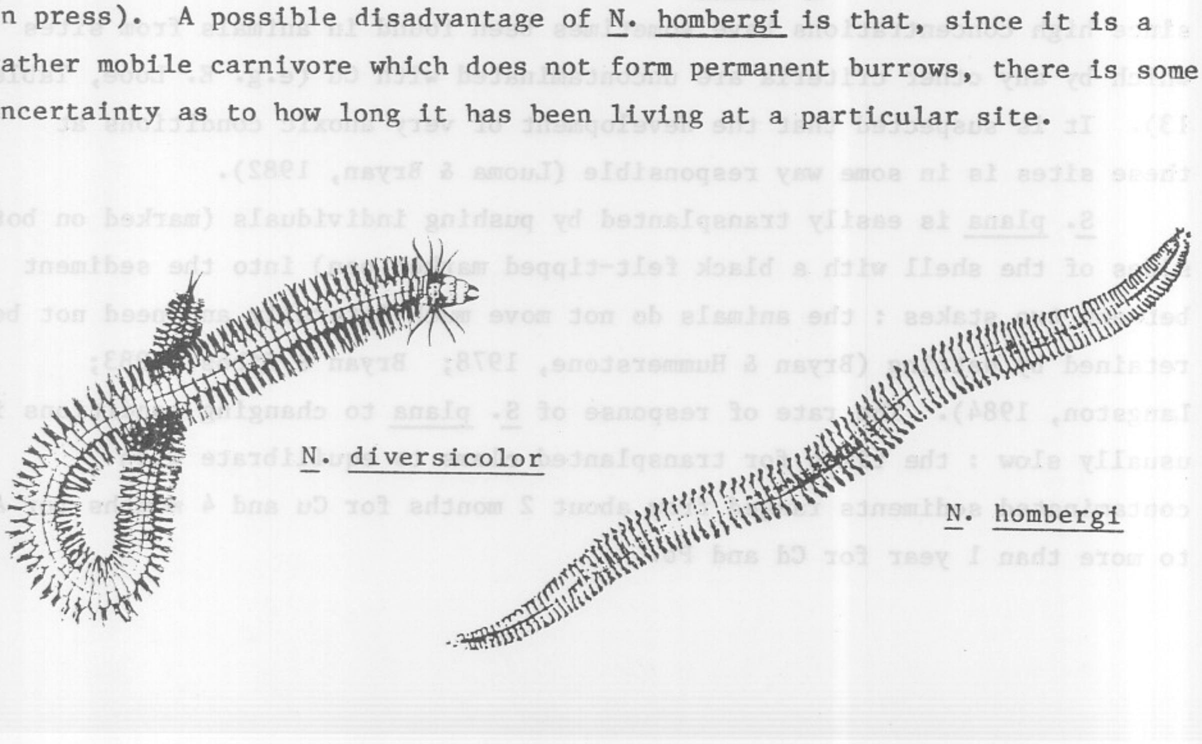
Nephtys hombergi (cat worm)

This carnivorous species may occur alongside N. diversicolor in sediments from the more saline parts of estuaries. Unlike the ragworm, however, N. hombergi is also found at offshore sites. In estuarine muds only N. hombergi is likely to be encountered but in more sandy, saline areas other species, for example Nephtys caeca and Nephtys cirrosa, may also be encountered (Fauvel, 1923).

Field observations showed that concentrations of Ag, Co, Cu, Fe, Pb and Zn in N. hombergi were very significantly related to those of the sediments (Bryan and Gibbs, in press). However, the slope for the Zn relationship was very shallow suggesting that it is fairly efficiently regulated. Unlike N. diversicolor, the jaws of N. hombergi contain a negligible amount of Zn. A significant tissue-sediment relationship for Cd was not observed, but the body concentration appears to reflect contamination in the overlying water. Examples of tissue-metal concentrations are given in Table 12.

Sampling : Compared with N. diversicolor, it is generally much more difficult to collect large numbers of N. hombergi. Samples containing 10 worms are suitable for analysis. The animals are cleaned for 2 days in acid-washed sand covered with 100% sea water and for a further day in sea water only.

Generally speaking, concentrations of metals in N. hombergi are higher than those in N. diversicolor from the same site. In addition, the concentrations are comparatively independent of body size (Bryan and Gibbs, in press). A possible disadvantage of N. hombergi is that, since it is a rather mobile carnivore which does not form permanent burrows, there is some uncertainty as to how long it has been living at a particular site.



N. diversicolor

N. hombergi

Bivalve molluscs

Scrobicularia plana

This deposit-feeding clam occurs in the majority of estuaries in England and Wales and is the most salinity tolerant of the molluscs being considered. The use of S. plana as an indicator has been described by Bryan et al. (1980).

Field observations. Oxidised surface sediment appears to be the principle source of several metals and significant organism - sediment relationships have been observed for Ag, Co, Cd (Luoma & Bryan, 1982), As (Langston, 1980), Cr (unpublished), Hg (Langston, 1982), Pb (Luoma & Bryan, 1978) and Zn (Luoma & Bryan, 1979). The best relationships have been obtained by taking into account the influence of major metal-binding sediment components such as Fe oxyhydroxide and organic matter on the availability of metals and this has already been discussed with respect to As, Hg and Pb on page 20. Metal-metal interactions have also been observed : for example, the availability of sediment Ag appears to be depressed in the presence of a high level of sediment Cu (Luoma & Bryan, 1982). Although, the sediment is a major source for most metals, the uptake of dissolved species from the overlying water also occurs and may be significant for Cd, Co and Zn, which have a greater tendency to remain in solution (Luoma & Bryan, 1982; Bryan & Gibbs, 1983). This is illustrated by the reasonably good relationships observed between concentrations of Cd and Zn in S. plana and F. vesiculosus (Figs. 7A,B). Some capacity for regulating Zn may explain why the relationship in Fig. 7B deviates from one of direct proportionality.

The behaviour of S. plana with respect to Cu is not fully understood, since high concentrations have sometimes been found in animals from sites which by any other criteria are uncontaminated with Cu (e.g. E. Looe, Table 13). It is suspected that the development of very anoxic conditions at these sites is in some way responsible (Luoma & Bryan, 1982).

S. plana is easily transplanted by pushing individuals (marked on both sides of the shell with a black felt-tipped marker pen) into the sediment between two stakes : the animals do not move much laterally and need not be retained by netting (Bryan & Hummerstone, 1978; Bryan & Gibbs, 1983; Langston, 1984). The rate of response of S. plana to changing conditions is usually slow : the times for transplanted clams to equilibrate with contaminated sediments ranged from about 2 months for Cu and 4 months for As to more than 1 year for Cd and Pb.

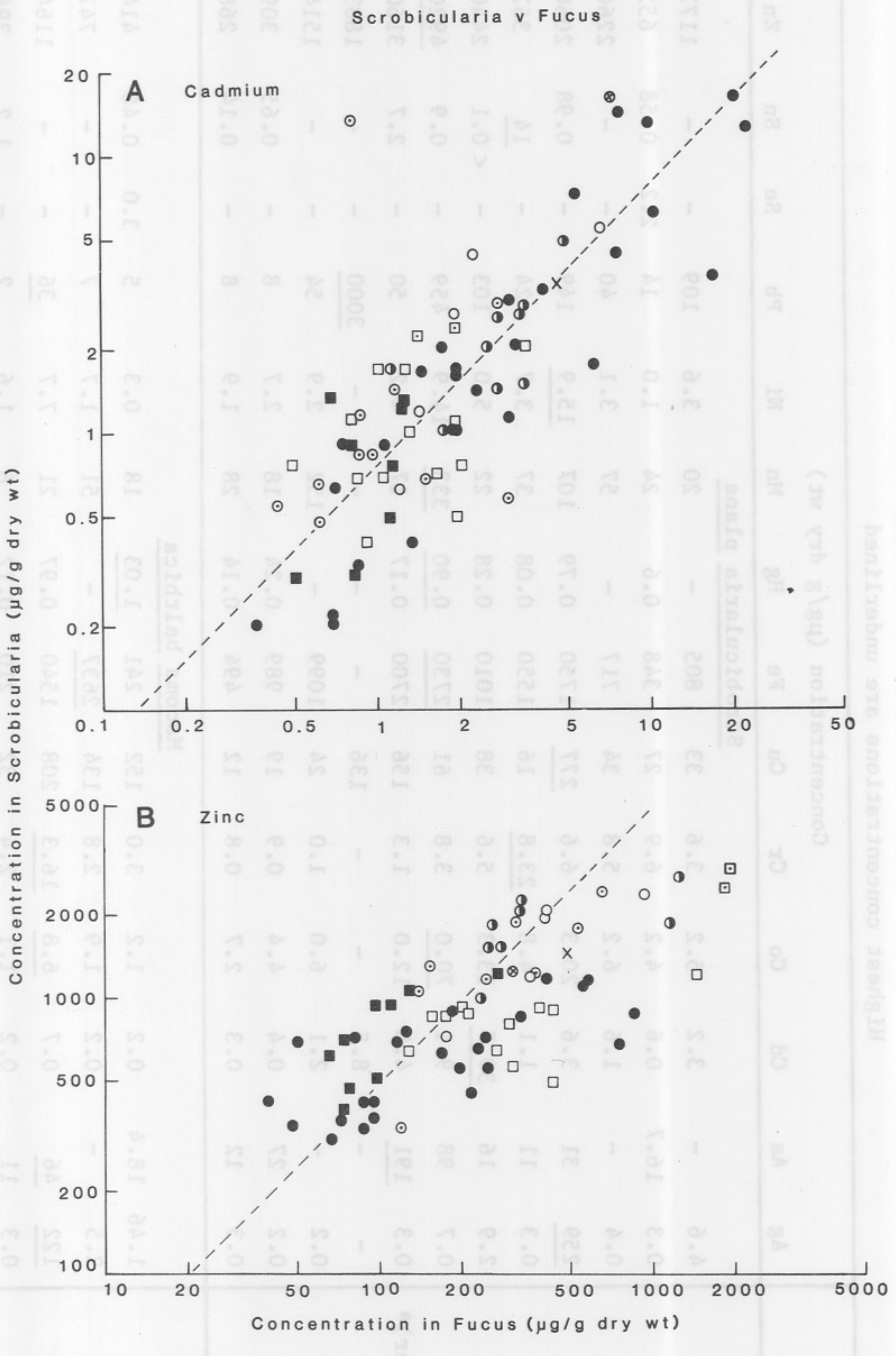


Fig. 7A,B. *Scrobicularia plana* v. *Fucus vesiculosus*. Relationships between concentrations of Cd and Zn in the two species from a range of sites. (●) Severn, Bristol Channel, South Wales, (⊗) Poole Harbour, (◑) Thames and Medway, (○) Teign, (⊙) Southampton, (X) Humber, (⊡) Mersey, (□) North and West Wales, (■) Essex and Suffolk estuaries. Broken lines show direct proportionality.

TABLE 13

SCROBICULARIA PLANA AND *MACOMA BALTHICA*: METAL CONCENTRATIONS IN CLAMS FROM DIFFERENT ESTUARIES

Highest concentrations are underlined

Estuary	Concentration (µg/g dry wt)													
	Ag	As	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Se	Sn	Zn
						Scrobicularia plana								
Tyne (Jarrow)	4.6	–	3.2	5.2	3.6	33	805	–	20	3.6	109	–	–	1177
Tees (Bran Sands)	0.3	16.7	0.6	4.2	6.9	27	348	0.6	24	1.0	14	2.2	0.58	652
Mersey (Rock Ferry)	0.4	–	1.6	6.2	5.8	34	717	–	57	3.1	40	–	–	2260
East Looe (upper)*	259	31	3.6	20.3	6.6	277	1750	0.79	107	15.9	149	–	0.98	2060
Loughor (mid)*	0.3	11	1.1	4.8	23.8	16	1550	0.08	37	3.7	24	–	14	367
Rhymney (lower)*	2.9	16	39.7	13.3	5.6	38	1010	0.28	22	5.0	103	–	< 0.1	2440
Gannel (mid)*	0.7	98	9.1	70.0	3.8	61	2730	0.90	333	14.9	459	–	0.9	4920
Restronguet Creek (upper)*	0.3	191	4.5	12.0	1.3	156	2700	0.17	17	4.2	50	–	2.7	3160
Peel (Isle of Man)+	–	–	8.5	–	–	136	–	–	–	–	3000	–	–	1883
Dovey (mid)	0.2	–	2.1	6.0	1.0	24	1099	–	152	2.9	54	–	–	1514
Camel (upper)*	0.2	27	0.4	4.4	0.9	19	989	0.24	18	2.7	8	–	0.65	309
Taf (lower)*	0.3	12	0.3	2.7	0.8	12	494	0.14	28	1.9	8	–	0.14	268
						Macoma balthica								
Tees (Bran Sands)	1.46	18.4	0.2	1.2	3.0	152	241	1.03	18	0.3	5	3.0	0.48	414
Mersey (Rock Ferry)	2.5	–	0.2	1.9	2.8	134	2637	–	51	1.7	7	–	–	747
East Looe (upper)*	122	46	0.7	6.8	16.3	208	1540	0.97	21	7.7	36	–	–	1164
Loughor (mid)*	0.3	11	0.2	1.1	2.4	32	210	0.12	8	1.6	2	–	1.2	396
Severn (Sharpness)*	100	–	9.4	3.6	4.0	224	1300	–	356	12.7	19	–	–	1510
Dovey (mid)	0.3	–	0.9	2.9	0.6	33	769	–	102	2.6	17	–	–	771
Solway (Browhouses)	0.6	–	0.6	0.7	1.1	35	612	–	25	1.5	5	–	–	365

*Bryan *et al*., 1980; +Southgate *et al*., 1983

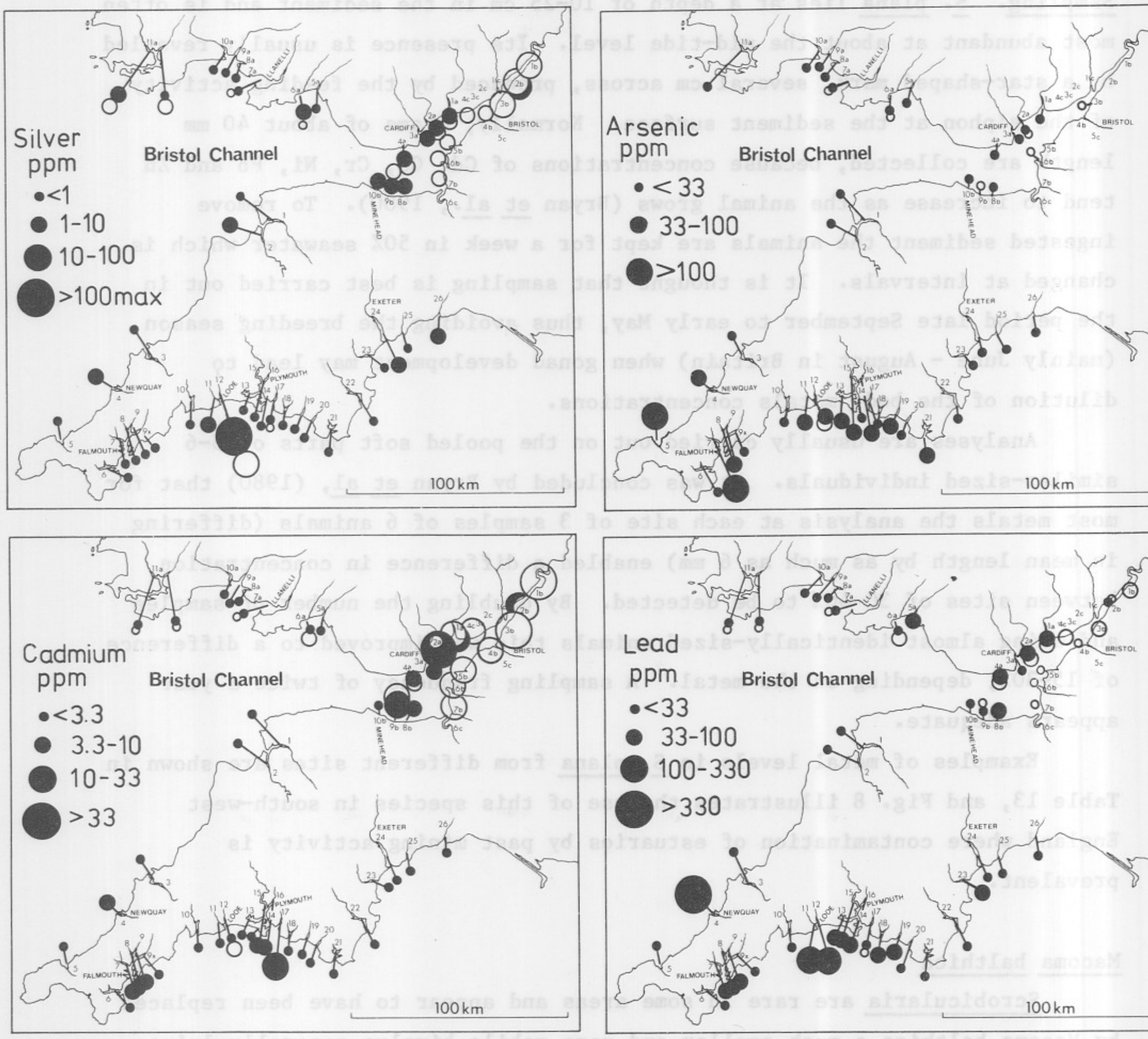
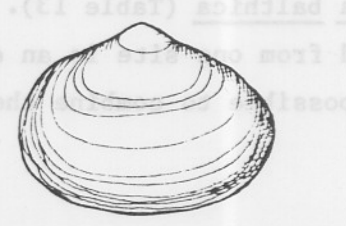


Fig. 8. Scrobicularia plana and Macoma balthica: Geographical distribution of 4 elements in S. plana from estuaries in Southwest Britain. Open circles are equivalent levels in M. balthica, concentration ranges being divided by 0.59 for Ag, 6.2 for Cd and 3.6 for Pb. Results are for individual sites in Severn (1a-4a, 1b-10b) but for other estuaries are the ranges within which most of the values for different sites occur.

M. balthica



S. plana

Sampling. S. plana lies at a depth of 10-25 cm in the sediment and is often most abundant at about the mid-tide level. Its presence is usually revealed by a star-shaped mark, several cm across, produced by the feeding activity of the siphon at the sediment surface. Normally, clams of about 40 mm length are collected, because concentrations of Cd, Co, Cr, Ni, Pb and Zn tend to increase as the animal grows (Bryan et al., 1980). To remove ingested sediment the animals are kept for a week in 50% seawater which is changed at intervals. It is thought that sampling is best carried out in the period late September to early May, thus avoiding the breeding season (mainly June - August in Britain) when gonad development may lead to dilution of the body metals concentrations.

Analyses are usually carried out on the pooled soft parts of 5-6 similar-sized individuals. It was concluded by Bryan et al, (1980) that for most metals the analysis at each site of 3 samples of 6 animals (differing in mean length by as much as 6 mm) enabled a difference in concentration between sites of 30-40% to be detected. By doubling the number of samples and using almost identically-sized animals this was improved to a difference of 15-30%, depending on the metal. A sampling frequency of twice a year appears adequate.

Examples of metal levels in S. plana from different sites are shown in Table 13, and Fig. 8 illustrates the use of this species in south-west England where contamination of estuaries by past mining activity is prevalent.

Macoma balthica

Scrobicularia are rare in some areas and appear to have been replaced by Macoma balthica a much smaller and more mobile bivalve generally lying within a few cm of the sediment surface. Pooled samples containing about 10 animals of 12-18 mm are analysed since the concentrations of some metals are influenced by size (Bryan et al., 1980). Both species seem to accumulate metals in the same way : for example concentrations of Hg in the two clams are of the same order and linearly related (Langston, 1982). Although metal levels in the two species can be related, the concentrations at the same site are not necessarily the same. For example, concentrations of Co and Ni in S. plana are roughly double and levels of Cd and Pb are about four times those of Macoma balthica (Table 13). Nevertheless provided the two species can be analysed from one site in an estuary to establish a conversion factor, it is possible to combine the results (Fig. 8).

Mytilus edulis

Because of their world-wide distribution and potential as indicators, species of Mytilus have become the subject of various monitoring programmes of the 'Mussel Watch' type (Davies & Pirie, 1980; Murray, 1982; Goldberg & Martin, 1983). In Mytilus, metals are likely to be absorbed both from solution and from ingested phytoplankton and other suspended particles (George, 1980).

Laboratory experiments, usually of a few weeks duration, suggest that for most metals and metalloids (As, Cd, Cu, Fe, Hg, Ni, Pb, Sb, Se, Tl, V, Zn) the concentrations accumulated by mussels reflect those of the water, although in some cases (As, Hg, V) direct proportionality was not observed (see reviews by George, 1980; Bryan, 1984). In addition, there is evidence for competition between metals for uptake sites. For example, the absorption of both Cd and Hg is suppressed by high levels of Zn.(Jackim et al., 1977; Gutierrez-Galindo & Breittmayer, 1981). On the other hand, the accumulation of Cu was found to be enhanced by the presence of Ag in the water (Calabrese et al., 1984).

Field observations. Evidence from the field confirming the indicator ability of mussels is not always forthcoming and is sometimes contradictory. For example, Julshamn (1981a), who compared the concentrations of metals in M. edulis and Ascophyllum nodosum with those in the contaminated waters of Sorfjorden (Norway), concluded that mussels were useless as indicators for Cd, Cu and Zn but were acceptable for Pb and probably Hg. On the other hand, Roesijadi et al. (1980) found mussels to be acceptable as indicators for Ag, Cu, Hg and Zn, but not Cd (possibly the result of competition from other metals). Comparisons between levels of Cu and Zn in M. edulis and Fucus vesiculosus from a wide range of United Kingdom sites (Fig. 9A,B) show that concentrations of both metals in mussels increase more slowly than those of the weed. Since F. vesiculosus is considered to be a good indicator of dissolved Cu and Zn (page 24), the results imply that M. edulis is partially able to regulate these metals and considerably underestimates levels of contamination with Zn and Cu, especially at moderate concentrations. On the other hand, observations for Cd indicate that M. edulis is as good an indicator as F. vesiculosus (Fig. 9c). In addition, both species have been used successfully as indicators for Hg (Forth River Purification Board, 1982).

Experimental evidence from the field suggests that M. edulis is a useful indicator for both Cr (VI) and Pb (Schulz-Baldes et al., 1983), and this is also supported by other field observations. In the U.S.A. high

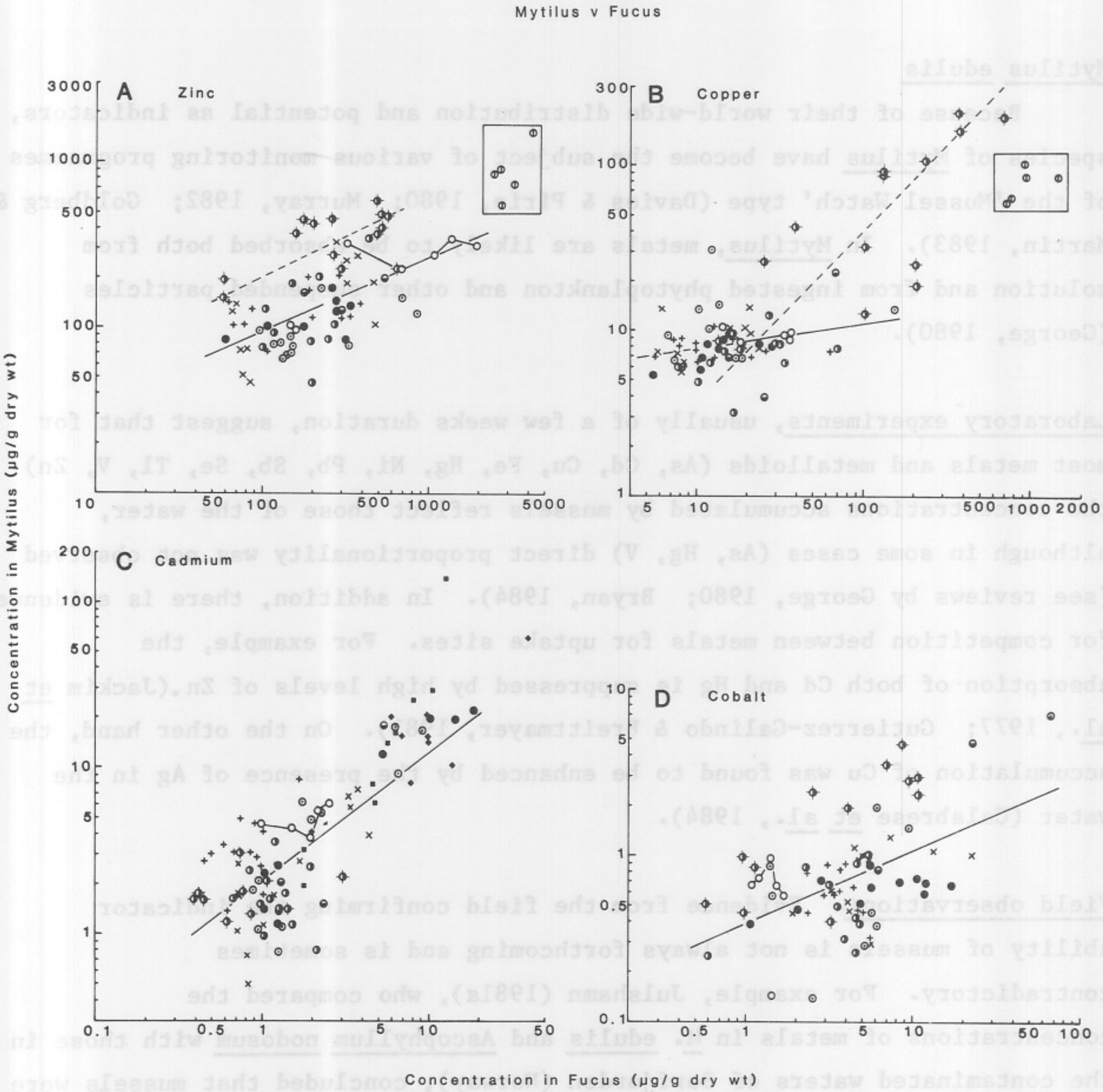


Fig. 9A. Mytilus edulis v Fucus vesiculosus. Relationship between concentrations of Zn in the two species from a range of sites. Concentrations in mussels are corrected to animals of 1 g dry weight (actual concentration/actual dry wt$^{-0.23}$). (♦) North Cornwall, (◐) Southwest England, (⊖) South Coast, (●) Northwest England, (×) East coast, (+) Scotland, (⊙) Wales, (O–O) Mersey (Egremont) on 5 occasions over 3 years to show site variability, (⦶) mussels transplanted to Restronguet Creek/Fal from Tamar for 6 months. Continuous line excludes North Cornwall (♦) and transplants (⦶): slope of log-transformed data 0.434 ($n = 68$, $P < 0.001$).

Broken line is for North Cornwall and includes many samples from exposed coasts of old mining area: slope of log transformed data 0.490 ($n = 14$, $P < 0.01$).

levels of Ag have been observed in mussels from the vicinity of sewage outfalls (Goldberg & Martin, 1983). We have occasionally found elevated levels in mussels, but based on evidence from the Ag-contaminated Looe Estuary, Bryan & Hummerstone (1977) concluded that M. edulis is an unreliable indicator for Ag. A similar conclusion was drawn by Langston (1984) for As. Examples of metal concentrations in mussels are given in Table 14.

Observations on the time taken by transplanted mussels to re-equilibrate with the new environment indicate that this is dependent on both the concentration difference and the ambient temperature. Majori et al. (1978) observed that in mussels transplanted to a contaminated area on the Adriatic Coast equilibration took 1-2 months for Hg and 3 months for Pb. Studies by Julshamn (1981b) on mussels transplanted to a contaminated site in Hardangerfjord showed that at temperatures of around 13°C equilibria for Cd and Pb were approached in 2-3 months. On returning mussels to a clean site most of the metals were lost within 2 months and normal levels were achieved within a year. On the other hand, Widdows et al. (1984) did not observe Cd equilibration in transplant experiments of 6 months duration. Concentrations of Cu and Zn in mussels transplanted to the very contaminated waters of Restronguet Creek from October to May, appeared to be equilibrating after about 6 months (Bryan & Gibbs, 1983; see also Fig.9A,B).

9B. Relationship for Cu. Size correction in mussels and symbols as above. Continuous line excludes North Cornwall (◆) and transplants (Φ) and has slope of 0.148 ($n = 67$, $P < 0.05$). Broken line is for North Cornwall and has slope of 0.735 ($n = 14$, $P < 0.001$).

9C. Relationships for Cd. Size correction and symbols as above. Additional symbols (◆) Bristol Channel and South Wales (Nickless et al., 1972), (■) Norway (Stenner & Nickless, 1974). Regression line excluding these points has slope of 0.748 ($n = 81$, $P < 0.001$)

9D. Relationship for Co. Size correction and symbols as above. Regression includes all data and has slope of 0.440 ($n = 79$, $P < 0.001$).

Sampling. Topping (1983) has recommended that at least 25 mussels of the dominant size should be collected from rocks at about the half tide level. In the United Kingdom, the best time for collection is thought to be the late winter when the body concentration is least likely to be subject to variations caused by weight changes which are related to food supply and position in the spawning cycle. For example, As levels were observed to fall by 50% after spawning (Langston, 1984). Exposure to clean sea water for about 24 h is usually sufficient to eliminate the gut contents. Since significant concentrations of metals are found in the byssal threads (Coombs & Keller, 1981) it is probably best to remove them prior to analysis of the mussels. It is intimated by Topping (1983) that by analysing homogenates of 25 pooled mussels concentration differences of 20-40% between sites can be detected (it should be noted that some stainless steel homogenisers may contaminate samples with Ni and Cr). Similarly, Gordon et al. (1980) found that for a range of metals (Cd, Cr, Cu, Ni, Pb, Zn) somewhere between 20 and 100 animals needed to be analysed at each of two sites for a concentration difference of 20% to be detectable : about three times fewer mussels allowed a difference of about 40% to be detected.

Even in mussels collected at the same time from the same site, variation between the metal concentrations of individuals may be high (Lobel & Wright, 1982a,b). A number of methods have been proposed by which this variation can be reduced. Lobel & Wright (1982b) suggested that variation in Zn levels can be reduced by normalisation to a constant flesh condition index (FC = mg dry wt flesh per 1g shell wt), since concentrations increase with decreasing condition index. In the same vein Fischer (1983), working on Cd in mussels, has shown that problems associated with changes in size and condition can be avoided by relating the amount of Cd in the soft parts to the weight of the shell. From analyses of different sized mussels (avoiding eroded shells) Fischer obtained relationships based on the equation

$$\log_{10} \text{tissue Cd content } (\mu g) = \underline{b} \log_{10} \text{shell wt } (g) + \log_{10} \underline{a}$$

where $\underline{b}$ is the slope and $\log_{10} \underline{a}$ the intercept: $\log_{10} \underline{a}$ equals $\log_{10}$ Cd content when the shell weight is 1g (since $\log_{10} 1 = 0$).

Thus the value of $\underline{a}$ is the amount of Cd (μg) in the soft body of a mussel (∿ 30 mm long) having a shell weight of 1g and is termed the Cd/shell-wt index.

One way of calculating the index is to analyse mussels covering a range of shell weights and find a from the regression equation. Alternatively, it is suggested by Fischer that 10-15 similar-sized mussels are analysed and individual results are normalised to 1g shell weight based on a slope b found from the first method. Fischer gives slopes of 1.08 for Western Baltic mussels ($S^{o}/oo = 15$) and 1.11 for Wadden Sea mussels ($S^{o}/oo = 30$). It is speculated that the method is likely to work for other metals but possibly not all of them.

Although this procedure involves the additional steps of cleaning and weighing the shells, certainly for Cd, it appears to be superior to the normalisation of the results to constant tissue weight. Topping (1983) has suggested that the use of this latter method, based on the metal content - dry tissue weight relationships of Boyden (1974), is preferable to no attempt being made to correct for size-related variations in concentration. The amount of metal in a mussel having a dry tissue weight of 1g (a) can be calculated from the equation

$$\text{metal concentration } (\mu g/g \text{ dry wt}) = a\,W^{(b-1)} \quad \text{or}$$

$$a = \frac{\text{metal concentration}}{W^{(b-1)}}$$

where W is the actual dry weight of the mussel and b-1 is the slope of the metal concentration tissue weight relationship (b is the slope of the metal content - tissue weight relationship). Values of b-1 found by Boyden are around -0.23 for Cu, Fe, Ni, Pb and Zn and zero (i.e. no effect of size) for Cd (see also Figs. 9A-D). In Fig. 9C, however, the regression for Cd was improved by correcting the values for size.

A major disadvantage of mussels as indicators is that, since they prefer a rocky substrate, they are often quite rare in many U.K. estuaries which tend to be dominated by sediments. This can be overcome by transplanting mussels to the intertidal zone in small cages or envelopes made from a $\frac{1}{2}m^2$ piece of green plastic-covered chicken wire. In large estuaries, mussel cages can be suspended below buoys and details of this and the costs involved are given by Topping (1983). Preferably, farmed mussels of uniform size and age should be used, although this is not always feasible.

The available evidence suggests that mussels are very useful as indicators of contamination with Cd, Cr, Hg, Pb and possibly several other metals. However, particularly at moderate levels of contamination, they appear to considerably underestimate contamination with Zn and Cu. Furthermore, mussels are not regarded as reliable indicators for Ag or As.

TABLE 14

MYTILUS EDULIS: METAL CONCENTRATIONS IN MUSSELS FROM DIFFERENT LOCALITIES

Highest concentrations are underlined

Locality	Concentration (µg/g dry wt)														
	Ag	As	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Sb	Se	Sn	Zn
Red River (mouth)	0.24	–	2.2	6.3	1.7	262	669	–	16	1.4	9.5	–	–	–	579
Whitehaven (harbour)	16.9	–	21.0	0.8	5.7	8	476	–	10	2.6	13.8	–	–	–	88
Poole Harbour*	–	–	65.4	–	–	11	154	–	5	12	19	–	–	–	162
West Looe (mid)	0.15	–	2.4	1.1	1.9	11	328	–	35	1.9	105	–	–	–	115
Thames (Thorpe Bay)	1.12	–	8.4	1.0	2.5	11	453	–	15	6.2	13.1	–	–	–	300
Swansea Bay	0.09	–	11.8	1.9	7.2	10	289	–	12	3.6	18.4	–	–	–	198
Walney Island	0.09	12.2	2.7	0.4	2.0	7	272	0.4	17	2.0	12.2	–	2.6	0.45	119
Boston Haven (mouth)	0.02	–	0.4	0.3	<0.2	6	64	0.09	4	0.5	1.7	–	1.6	–	45
	Examples of M. edulis surveys														
Coast of England	–	–	<1–	–	–	4–	–	0.09–	–	–	2.8–	–	–	–	54–
and Wales (range)**	–	–	36	–	–	38	–	2.3	–	–	190	–	–	–	450
Southwest Baltic (range)+	<0.03–	2.5–	0.7–	<0.2–	<0.2–	–	77–	0.07–	–	<1–	–	0.8–	1.2–	1.5–	82–
	6.6	12	3.1	1.7	7.0	–	817	0.41	–	11	–	3.2	3.7	8.2	308
Sørfjord and Hardangerfjord,	–	–	22–	0.3–	–	5–	71–	0.46–	6–	–	130–	–	–	–	380–
Norway (range) †	–	–	51	1.0	–	10	130	2.0	19	–	530	–	–	–	1400
San Francisco Bay ††	0.43	7.1	5.3	–	–	8	287	0.38	30	2.2	3.3	–	4.5	–	170

*Boyden, 1975; **Murray & Law, 1980; +Möller *et al*., 1983; †Julsham, 1981b; ††Bradford & Luoma, 1980

Cerastoderma edule

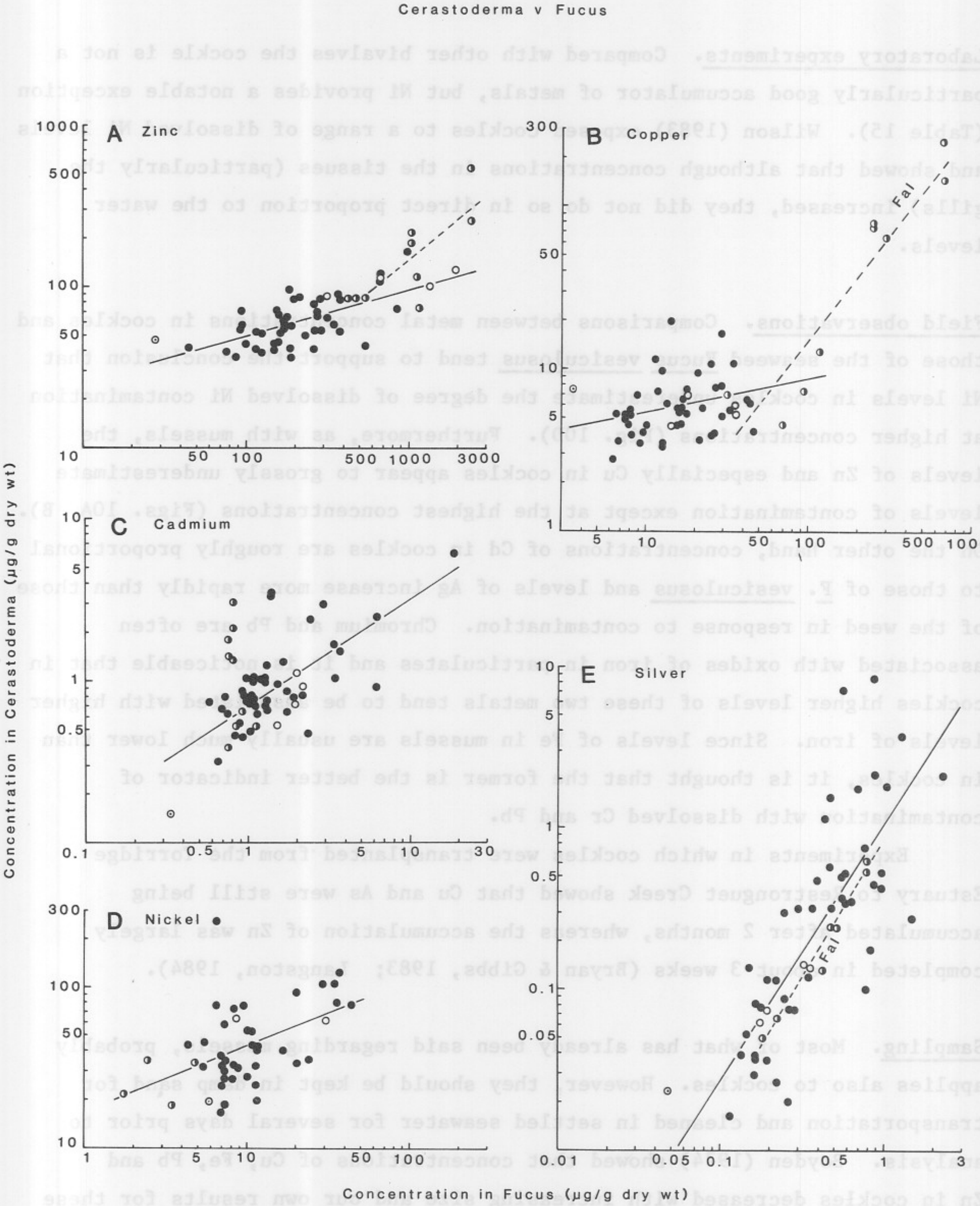
The common cockle is usually found in relatively saline areas of estuaries and tends to be most common in sediments containing a high proportion of sand : it is most likely to absorb metals from solution and from suspended particles.

Laboratory experiments. Compared with other bivalves the cockle is not a particularly good accumulator of metals, but Ni provides a notable exception (Table 15). Wilson (1983) exposed cockles to a range of dissolved Ni levels and showed that although concentrations in the tissues (particularly the gills) increased, they did not do so in direct proportion to the water levels.

Field observations. Comparisons between metal concentrations in cockles and those of the seaweed Fucus vesiculosus tend to support the conclusion that Ni levels in cockles underestimate the degree of dissolved Ni contamination at higher concentrations (Fig. 10D). Furthermore, as with mussels, the levels of Zn and especially Cu in cockles appear to grossly underestimate levels of contamination except at the highest concentrations (Figs. 10A, B). On the other hand, concentrations of Cd in cockles are roughly proportional to those of F. vesiculosus and levels of Ag increase more rapidly than those of the weed in response to contamination. Chromium and Pb are often associated with oxides of iron in particulates and it is noticeable that in cockles higher levels of these two metals tend to be associated with higher levels of iron. Since levels of Fe in mussels are usually much lower than in cockles, it is thought that the former is the better indicator of contamination with dissolved Cr and Pb.

Experiments in which cockles were transplanted from the Torridge Estuary to Restronguet Creek showed that Cu and As were still being accumulated after 2 months, whereas the accumulation of Zn was largely completed in about 3 weeks (Bryan & Gibbs, 1983; Langston, 1984).

Sampling. Most of what has already been said regarding mussels, probably applies also to cockles. However, they should be kept in damp sand for transportation and cleaned in settled seawater for several days prior to analysis. Boyden (1974) showed that concentrations of Cu, Fe, Pb and Zn in cockles decreased with increasing size and our own results for these metals were certainly improved by correcting for size (Fig. 10). Although Boyden (1974) and Wilson (1983) observed no influence of size on Ni concentrations, the application of a correction factor did improve the cockle - F. vesiculosus regression: it was also applied to results for Ag but not Cd (cf Boyden, 1974).


Cerastoderma v Fucus
A Zinc
1000
500
100
50
10
50 100 500 1000 3000
B Copper
300
50
10
5
1
5 10 50 100 500 1000
Fal
C Cadmium
10
5
1
0.5
0.1
0.5 1 5 10 30
D Nickel
300
100
50
10
1 5 10 50 100
E Silver
10
5
1
0.5
0.1
0.05
0.01
0.05 0.1 0.5 3
Fal
Concentration in Cerastoderma (µg/g dry wt)
Concentration in Fucus (µg/g dry wt)

It is concluded that cockles are not particularly useful as indicators, although they reflect environmental contamination with Ag, As, Cd and Ni. They also respond to high levels of Cu and Zn but, probably as a result of regulation, underestimate moderate levels of contamination. Particulate contamination of cockles often creates problems in their use as indicators of Cr and Pb.

Fig. 10A. *Cerastoderma edule* v *Fucus vesiculosus*. Relationship between Zn concentrations in the two species from a range of sites. Concentrations in cockles are corrected to animals of 1 g dry weight (actual concentration/actual dry wt $^{-0.23}$). (◐) Restronguet Creek/Fal, (●) other localities, (○) Mersey (Egremont) on 4 occasions over 3 years to show site variability, (⊙) *Cerastoderma glaucum* from the Fleet. Continuous line *excludes* high values from Restronguet Creek/Fal and slope of log-transformed data is 0.295 ($n = 53$, $P < 0.001$). Slope of broken line for Restronguet Creek/Fal is 0.831 ($n = 9$, $P < 0.02$).

10B. Relationship for Cu. Size correction and symbols as above. Continuous line *excludes* high values from Restronguet Creek/Fal and slope of log-transformed data is 0.199 ($n = 53$, $P < 0.05$). Slope of broken line for Restronguet Creek/Fal is 1.299 ($n = 9$, $P < 0.001$).

10C. Relationship for Cd. Symbols as above but *no* size correction. Continuous line *excludes* data from Restronguet Creek/Fal and has slope of 0.647 ($n = 53$, $P < 0.001$).

10D. Relationship for Ni. Size correction and symbols as above. Slope of line is 0.406 ($n = 44$, $P < 0.001$).

10E. Relationship for Ag. Size correction and symbols as above. Continuous line includes *all* data and slope is 1.573 ($n = 57$, $P < 0.001$). Broken line is for Restronguet Creek/Fal and slope is 1.708 ($n = 8$, $P < 0.001$).

TABLE 15

CERASTODERMA EDULE: METAL CONCENTRATIONS IN COCKLES FROM DIFFERENT LOCALITIES

Highest concentrations are underlined

Locality	Concentration (µg/g dry wt)												
	Ag	As	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Sb	Zn
Restronguet Creek (mid)	0.62	–	3.0	5.1	–	174	1692	–	14	–	7.6	–	303
Gannel (mid)	0.79	–	3.3	12.0	1.9	26	1307	–	317	126	120	–	175
Peel (Isle of Man)*	–	–	6.3	–	–	10	–	–	–	–	371	–	139
Poole Harbour+	–	5.1†	16.9	–	–	9	502	0.86†	5	174	14	0.034†	271
Loughor (mid)	0.07	–	0.8	–	20.0	14	5518	–	152	50	15.9	–	117
Swansea Bay	0.15	–	2.4	–	6.3	9	3678	–	104	107	13.3	–	100
Thames (Thorpe Bay)	10.9	–	1.6	3.5	3.2	9	819	–	11	165	3.5	–	83
Tees (lower)	0.19	7.8	0.9	2.0	4.6	15	268	0.43	19	22	3.6	–	132
Mersey (Rock Ferry)	0.52	–	1.2	1.9	–	17	1608	–	53	–	17.8	–	270
Whitehaven (harbour)	11.5	–	6.0	2.5	–	19	4085	–	56	–	18.6	–	105
Torridge (Appledore)	0.01	12.1	0.4	2.4	0.5	4	431	0.26	19	27	0.4	–	46

*Southgate *et al.*, 1983; +Boyden, 1975; †Leatherland & Burton, 1974

Ostrea edulis (native oyster); Crassostrea gigas (Pacific oysters)

Oysters of various species have been employed as indicators in the United Kingdom (Portmann, 1979), the United States (Goldberg et al., 1978), Australia (Ratkowsky et al., 1974) and in many other countries.

Laboratory experiments have shown that the levels of Cd, Co, Cr, Cu, Hg, Ni, Pb, tributyl tin oxide and Zn in oysters reflect the available concentrations in the surrounding water (Shuster and Pringle, 1969; Cunningham and Tripp, 1973; Zaroogian, 1980; Zamuda and Sunda, 1982; Watling, 1983; Zaroogian & Johnson, 1983; Waldock et al., 1983). These oyster-water relationships do not necessarily exhibit direct proportionality, although Zaroogian (1980) found that this was the case for Cd : after 40 weeks exposure to water containing 5, 10 and 15 µg/litre, Crassostrea virginica had accumulated 89, 176 and 292 µg/g dry weight of Cd. On the other hand, comparable experiments with As showed that the body concentration was unrelated to that of the water (Zaroogian & Hoffman, 1982).

Field observations have suggested that metal levels in oysters are sometimes related to those of the sediments (Ayling, 1974). Individual variability between oysters is frequently high. For example, concentrations of Zn in Ostrea edulis from the contaminated waters of Restronguet Creek varied from 1816-11185 µg/g dry weight (n = 38), the coefficient of variation being 40% (Boyden, 1977). In these oysters levels of Zn were independent of size as also were those of Cd, Mn and Cu. On the other hand, concentrations of Fe, Ni and Pb fell with increasing size, the slopes of the log-transformed data being about -0.23. Working with uncontaminated O. edulis, Julshamn (1981c) found that concentrations of Zn tended to increase with size, the slope being around +0.2, whereas levels of Cd and Cu were independent of size.

A study of Pacific oysters, Crassostrea gigas, transplanted from the Menai Strait to Restronguet Creek and the Helford River showed that small oysters seemed to equilibrate with the new, more contaminated, conditions (Cd, Co, Cu, Fe, Mn, Ni, Pb, Zn) within 4 months (Boyden and Phillips, 1981). However, Boyden (1977) reported that larger O. edulis took more than 5 months to achieve Cu equilibrium; and Julshamn (1981b), working with the same species in Norway, showed that although Pb equilibration was achieved in 2 months, Cd equilibrium was not reached, after more than one year.

Boyden and Phillips (1981) used transplanted C. gigas to investigate the reasons for seasonal variability in tissue-metal concentrations. To a large extent, maxima observed in winter were attributable to the tissue dry

TABLE 16

OSTREA EDULIS AND CRASSOSTREA GIGAS: METAL CONCENTRATIONS IN OYSTERS FROM DIFFERENT LOCALITIES

Highest concentrations are underlined

Locality	Concentration (µg/g dry wt)											
	Ag	As	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn
						Ostrea edulis						
Restronguet Cr. (lower)	0.2-	17-	3.2-	0.2-	0.2-	552-	150-	–	8-	0.1-	1.1-	4700-
(range)	0.9	39	7.9	0.7	0.6	2614	331	–	20	0.4	2.5	17080
Restronguet Cr. (lower)*	–	–	6.4	–	–	~3000	216	–	10	6.1	7.8	4358
Menai Strait*	–	–	5.2	–	–	392	223	–	18	6.0	6.2	3437
Blackwater**	–	–	1.5-	–	–	240-	–	0.15-	–	–	< 2.5-	300-
(range)	–	–	7.0	–	–	375	–	0.45	–	–	12.5	3000
Poole H. (Holes Bay)	17.2	–	29.9	0.6	–	278	219	–	10	–	1.2	3736
(Brownsea)	2.8	–	1.6	0.3	–	40	140	–	8	–	0.3	1500
Knysna (S. Africa)†	6.4	–	3.1	–	–	38	167	–	6	1.7	–	660
						Crassostrea gigas						
Restronguet Cr. (lower)*	–	–	11.8	–	–	~2500	365	–	17	5.4	9.1	7114
Menai Strait*	–	–	6.3	–	–	446	236	–	33	–	–	4293
Poole H. (Brownsea)	1.6	–	4.8	0.5	–	61	341	–	27	–	2.5	1925
Knysna (S. Africa)†	1.9	–	3.7	–	–	32	128	–	16	1.6	–	396

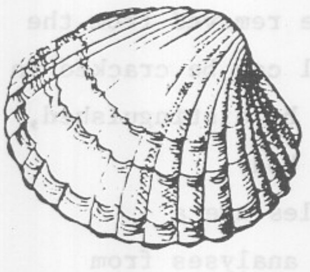
*Boyden, 1977; ** Portmann, 1979 (x5); † Watling & Watling, 1976.

weights being low, whereas the summer minima were explained in terms of dilution produced by growth and the development of gonads. However, even if these and other factors are eliminated by the use of specific sampling methods, an appreciable degree of variability remains - especially in contaminated oysters. Boyden and Phillips (1981) found that beyond a certain sample size the coefficients of variation (CV) showed little improvement. For Zn this limit occurred at about 15 individuals for O. edulis from the Menai Straits (CV = 12%), 25 for C. gigas from the same area (CV = 9%) and 20 O. edulis from Restronguet Creek (CV = 19%), the most contaminated site. Analyses of oysters from different localities are given in Table 16.

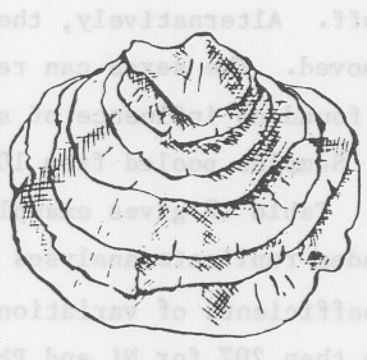
Sampling. As already discussed, 15-25 oysters are required to achieve reasonable coefficients of variation. The best time to sample oysters is during a period when the body concentrations are reasonably constant. Boyden and Phillips (1981) suggest January - March as the best period based on their results from the Helford Estuary and Restronguet Creek. However, such is the variability of oysters that these times may not apply in other areas. They suggest that some prior knowledge of the period of least weight change in the population would be useful for deciding the best time to sample. The same authors kept oysters in clean sea water for 36 hours before processing.

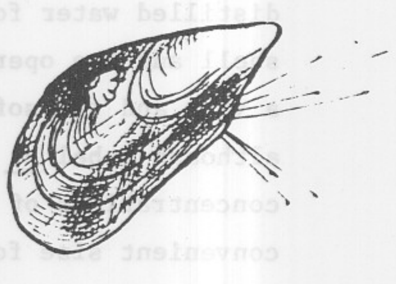
As a group, oysters are exceptional accumulators of Zn and Cu (Table 16) and their tissue concentrations appear to reflect contamination with a wide range of metals. Unfortunately, oysters are subject to seasonal fluctuations in body condition which lead to variations in concentration : in addition, individual variability tends to be high. In the United Kingdom, oysters are less widely available than many other mollusc indicators.

Ostrea edulis



Cerastoderma edule





Mytilus edulis

Gastropod molluscs

Littorina littorea (common winkle)

The winkle is usually found on rocky shores and penetrates into the middle reaches of many estuaries. In species of *Littorina* the algal diet is probably the major source of heavy metals (Young, 1975; Klumpp, 1980b) *Fucus vesiculosus* is often eaten by *L. littorea* (Fretter and Graham, 1962) and thus it is realistic to compare the concentrations of metals in the two species.

Field observations revealed very significant relationships between the winkle and *F. vesiculosus* for Ag, As, Cd and Pb, suggesting that, directly or indirectly, concentrations in the animal reflect those of the overlying water (Bryan *et al.*, 1983). However, for Ag, concentrations in the winkle were reduced relative to those in *F. vesiculosus* when concentrations of Cu in the weed were high (Fig. 11A). One possible explanation is that the absorption of Ag from the seaweed in the winkle digestive system is reduced by competition from dietary Cu. Significant relationships between the two species were also found for Cu, Fe, Hg and Zn, but the slopes were relatively shallow, perhaps reflecting regulation by the winkle : this is particularly true for Zn (Fig. 11B). Relationships of only marginal significance were found for Cr and Mn, whilst for Co and Ni concentrations seemed unrelated to environmental (seaweed) levels.

An experiment in which winkles from the Tamar Estuary were transplanted to Restronguet Creek (the most heavily contaminated part of the Fal Estuary) showed that concentrations of As, Co, Cu and Zn in the transplants approached those of the native animals in roughly 2 months (Bryan *et al.*, 1983).

Sampling. Animals of 2-2.5 cm shell length are collected and cleaned in covered beakers of low-metal sea water for 2-3 days. After boiling in distilled water for about one minute the soft parts can be removed from the shell and the operculum cut off. Alternatively, the shell can be cracked in a vice and the soft parts removed. The sexes can readily be distinguished, although Lobel *et al.* (1982) found no influence of sex on body concentrations of Cu and Zn. Samples pooled from 10 winkles are a convenient size for analysis. Table 17 gives examples of analyses from different estuaries and includes replicate analyses of 5 samples each containing 6 winkles. The coefficients of variation range from less than 10% for Cd, Fe and Zn to more than 20% for Ni and Pb.

TABLE 17

LITTORINA LITTOREA: METAL CONCENTRATIONS IN WINKLES FROM DIFFERENT ESTUARIES

Highest concentrations are underlined

Estuary	Dry wt (g)	Concentration (µg/g dry wt)											
		Ag	As	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn
Mersey (Egremont)	0.132	2.0	22	3.1	2.5	1.6	113	802	1.48	98	7.8	12.3	189
Thames (Grays)	0.246	101	11	13.2	1.9	0.7	417	322	0.88	55	5.5	4.0	141
Fal (Restronguet Pt.)	0.157	0.9	70	2.8	18.9	< 0.1	1069	492	0.22	158	7.0	3.0	956
Lune (mouth)	0.221	2.1	9	1.8	0.3	0.1	83	238	0.47	53	1.5	2.9	88
Falmouth (beach)	0.184	–	–	1.24	–	–	189	274	–	10.3	0.84	1.92	94
(n = 6 per sample)	0.172	–	–	1.10	–	–	121	240	–	11.6	1.23	3.04	99
	0.172	–	–	1.05	–	–	150	233	–	9.4	1.13	2.23	88
	0.161	–	–	0.96	–	–	157	263	–	6.4	1.57	1.81	90
	0.145	–	–	1.06	–	–	158	220	–	9.9	1.75	2.74	105
	Mean	–	–	1.08	–	–	155	246	–	9.5	1.30	2.35	95
	± S.D.			0.10			24	23		1.9	0.36	0.53	7

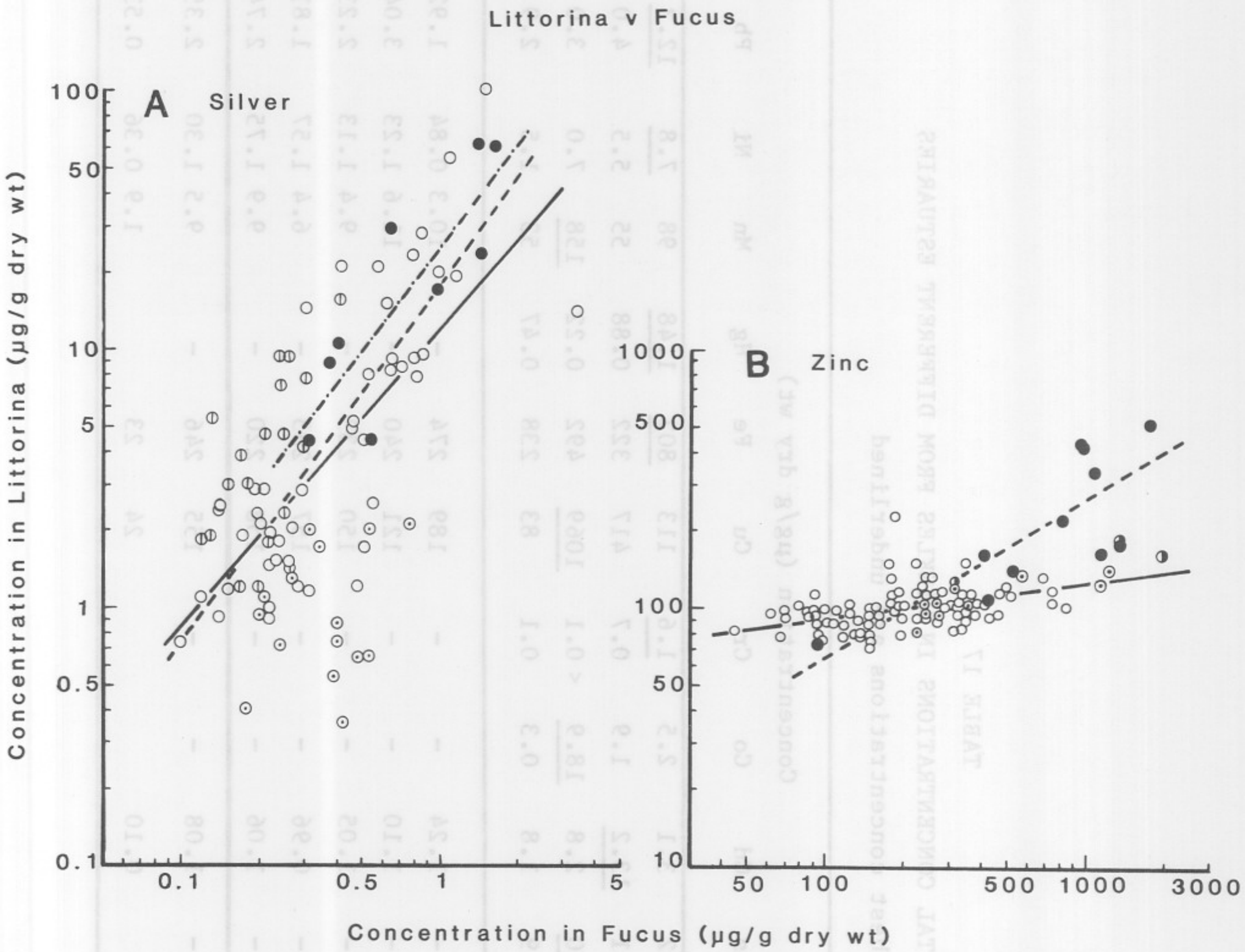


Fig. 11A. Littorina littorea v Fucus vesiculosus. Relationship between concentrations of Ag in the two species from a range of sites. Continuous line for all results has slope of 1.175 (n = 88, P < 0.001). (●) Looe Estuary; upper broken line has a slope of 1.34 (n = 9, P < 0.005), (○) seaweed contains 10-25 µg/g of Cu, for which lower broken line has slope of 1.38 (n = 51, P < 0.001), (⊙) if seaweed contains > 25 µg/g of Cu, winkle Ag is on low side, (Φ) if seaweed contains < 10 µg/g Cu, winkle Ag is on high side.

11B. Relationship for Zn. Broken line for Fal Estuary only has slope of 0.615 (n = 11, P < 0.01). (●) Fal Estuary, (◐) Mersey, (⊙) Thames and Medway, (○) other sites. Continuous line excludes Fal Estuary and slope is 0.153 (n = 114, P < 0.001).

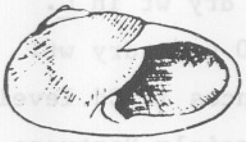
Concentrations of Ag in the winkle tend to rise with increasing body weight (slope of log-transformed data + 0.37), whereas levels of Cd, Cu, Fe, Ni, Pb and Zn usually fall (slopes approximately -0.1). A slope of -0.1 amounts to a fall in concentration of about 12% between tissue dry weight of 0.1 and 0.35 g. Thus for the best results it is important to analyse winkles within a reasonably narrow size range. Moore (1937) found that in winkles from Plymouth the dry weights of soft tissues increased gradually in relation to shell size from a minimum in March-April to a maximum in November-December. It is probably best to avoid the intervening winter-spawning period when the most rapid changes in the weight of soft tissues are likely to occur.

L. littorea seems to be a reasonable indicator of contamination with dissolved Cd (Fig. 12), Ag, Pb and perhaps As and Hg. However, it possesses no obvious advantages over *Fucus vesiculosus* for Cu and appears to be largely unsuitable as an indicator for Cr, Co, Fe, Ni and Zn.

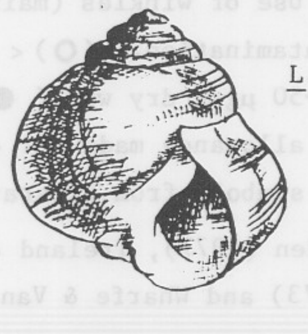
Littorina littoralis (flat periwinkle)

In estuaries this winkle often has a comparable distribution to *L. littorea* and is usually collected by shaking the fucoid seaweeds on which it grazes. Since *L. littoralis* is a smaller species as many as 20 animals can be pooled, but otherwise they are processed in the same way as *L. littorea*.

Apart from Fe and Ni, concentrations of metals in *L. littoralis* generally exceed those in *L. littorea* from the same sites. Very significant relationships were found between concentrations of Ag, Cd, Co, Cu, Mn, Pb and Zn in the two species (Bryan *et al.*, 1983). Table 18 gives the concentrations of metals in *L. littoralis* which were calculated to correspond to those of *L. littorea* under both contaminated and uncontaminated conditions. Thus in the absence of one species, the other can be used as an indicator by employing a correction factor (e.g. Fig. 12). *L. littoralis* is a particularly useful indicator for Cd since concentrations were observed to be proportional to those of *Fucus vesiculosus* but were 5 times as high (Bryan, 1983): *L. littoralis* is also superior to *L. littorea* as an indicator of As, since its body concentrations are more nearly proportional to those of *F. vesiculosus* (Langston, 1984).



L. littoralis



L. littorea

L. saxatilis

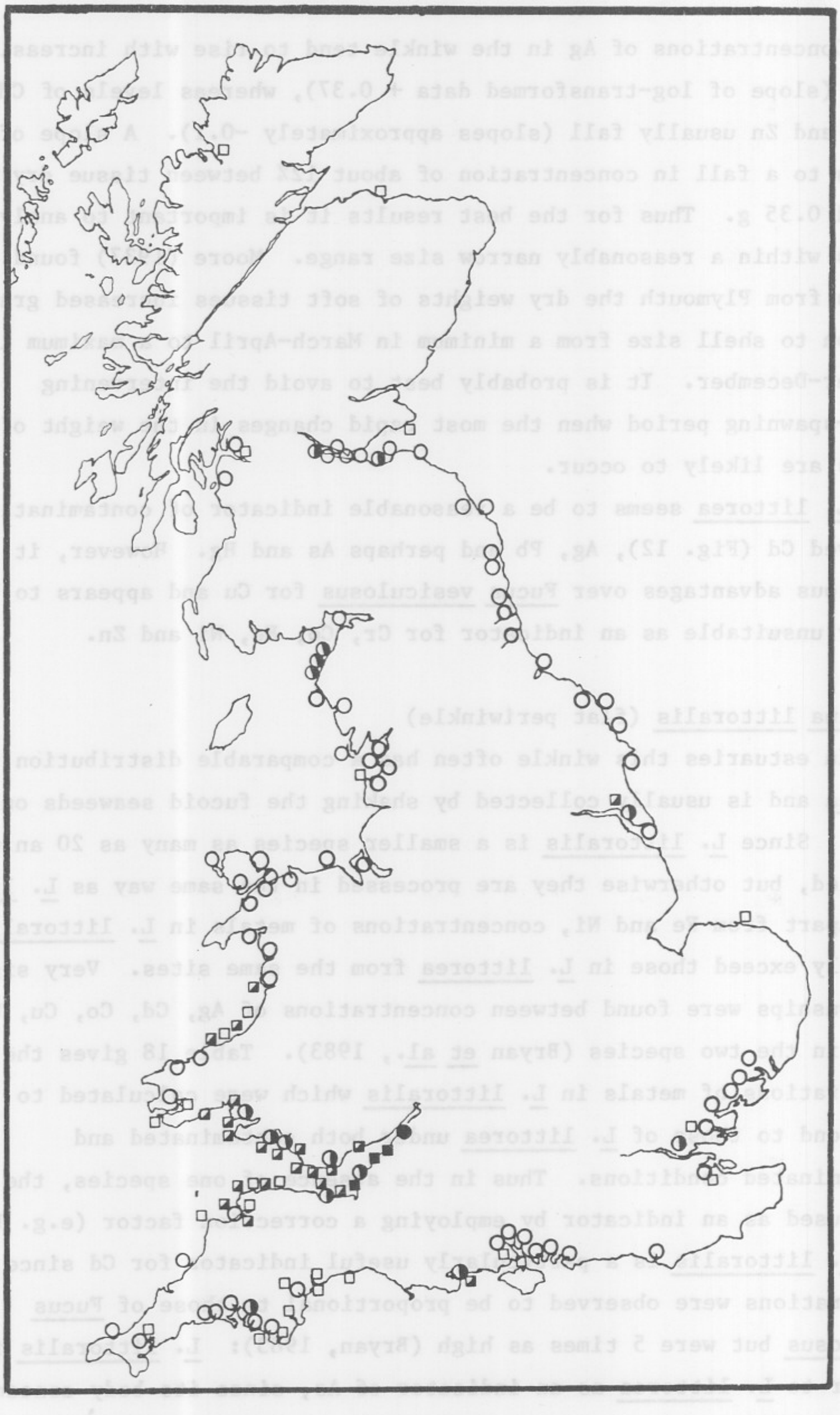


Fig. 12. Littorina species. Use of winkles (mainly L. littorea) as indicators of Cd contamination. (○) < 5 µg/g dry wt in L. littorea, (◐), 5-50 µg/g dry wt, (●) > 50 µg/g dry wt. When other species used, allowance made for differences in Cd levels (Table 18). Square symbols from literature; mainly Portmann (1979) but also Boyden (1977), Ireland & Wootton (1977), Topping (1973) and Wharfe & Van den Broek (1977).

TABLE 18

COMPARISON OF METAL CONCENTRATIONS IN THREE SPECIES OF WINKLES

Values for _L. littoralis_ and _L. saxatilis_ were calculated from regressions using typical figures for uncontaminated and contaminated _L. littorea_

Species	Approx. dry wt (g)	No. of comparisons	µg/g dry wt											
			Ag	As	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn
			Uncontaminated animals											
L. littorea	0.28	–	1	20	1	1	0.5	100	400	0.3	10	4	1	100
L. littoralis	0.055	16–21	2.1	22	3.6	1.2	0.72	241	392	0.42	18	3.8	1.9	160
L. saxatilis	0.030	6–11	0.6	16	1.0	0.8	0.67	94	333	0.28	*	3.6	2.2	101
			Contaminated animals											
L. littorea	0.28	–	10	*	10	10	*	1000	*	*	100	*	10	400
L. littoralis	0.055	16–21	12	*	24	6.3	*	1380	*	*	117	*	7.8	920
L. saxatilis	0.030	6–11	3.6	*	5.4	*	*	*	*	*	94	*	13	*

*Insufficient spread and significance of data to calculate concentrations

Littorina saxatilis (rough periwinkle)

This viviparous winkle is usually found on the upper shore and may at first be confused with small specimens of L. littorea. The L. 'saxatilis' species complex comprises L. saxatilis (usually on rocky shores), and L. rudis (usually on boulder and stone covered beaches (Smith, 1981). Concentrations of metals in L. saxatilis were found to be similar to or somewhat lower than those in L. littorea (Table 18).

Patella vulgata (common limpet)

Limpets are common on rocky shores and some penetrate into the lower reaches of estuaries. In estuaries or polluted areas only P. vulgata is likely to occur, but on some exposed coasts it is necessary to distinguish P. vulgata from P. intermedia and P. aspera (Fretter and Graham, 1962).

Field observations have demonstrated that limpets are good accumulators of Cd (Nickless et al., 1972; Portmann, 1979). Some care is needed in their use as indicators, however, because Boyden (1977) found that body levels of cadmium increased markedly with size and that the slope of the relationship was steeper under contaminated conditions (see legend to Fig. 13C). In contrast, Boyden (1974) found that concentrations of Cu, Fe, Pb and Zn tended to decrease in larger limpets (see legend to Fig. 13A). Relationships between the size-corrected concentrations of Cd, Cu, Pb and Zn in P. vulgata and those of Fucus vesiculosus (representing its algal diet) are shown in Figs. 13A-D. The results tend to support the use of the limpet as an indicator for Cd, Cu and Pb, but the evidence for Zn shows great variability. Concentrations of Ag in limpet tissues were observed to respond to contamination in the Looe Estuary (Bryan and Hummerstone, 1977). Similarly, the amount of Hg in limpets appeared to respond to contamination in the Bristol Channel, whereas the levels of As did not (Peden et al., 1973). Results from the Fal Estuary also lead to the conclusion that there is a degree of As regulation in limpets (Klumpp & Peterson, 1979).

A preliminary experiment in which uncontaminated limpets were transferred to the Bristol Channel, showed that concentrations of Cd, Cu and Zn in the transplants were approaching those of native limpets after 3-4 months (Stenner and Nickless, 1974b). Examples of metal concentrations in P. vulgata from different localities are given in Table 19.

TABLE 19

PATELLA VULGATA: METAL CONCENTRATIONS IN LIMPETS FROM DIFFERENT LOCALITIES

Highest concentrations are underlined

Locality	Dry wt (g)	Concentration (µg/g dry wt)											
		Ag	As	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn
Fal Estuary													
Falmouth Beach	0.107	-	-	3.46	-	-	17.3	1448	-	3.4	-	-	331
(n = 5)	±0.05	-	-	±2.10	-	-	±3.2	±281	-	±2.2	-	-	±76
	0.945	-	-	9.40	-	-	15.6	1494	-	9.9	-	-	177
	±0.37	-	-	±4.50	-	-	±4.3	±859	-	±8.8	-	-	±32
Mylor Harbour	-	0.75	-	7.4	0.81	1.49	64	952	-	6.2	1.85	8.9	206
St Ives Bay	0.74	0.28	-	3.3	4.09	1.08	225	2821	-	25.1	1.22	4.9	155
Bristol Channel													
Portishead	0.48	12.0	-	289	0.93	1.66	35	1304	-	102	1.07	6.2	312
Weston*	0.64	2.5	15	239	2.42	3.59	41	1738	0.12	47.2	4.50	10.3	279
Weston †	-	-	-	168	-	<7.00	59	-	1.19	-	-	7.0	476
Looe Estuary*	0.53	5.6	33	5.6	0.60	0.50	18	1160	0.26	6.0	2.30	30	145
Solent ‡	-	-	13	2.7	-	-	-	-	0.36	-	-	-	229
St Davids, Wales ‡	-	-	11	6.0	-	-	-	-	0.07	-	-	-	-
Duckpool	0.56	1.88	-	7.8	0.30	0.58	7.6	1568	-	14.0	1.12	0.4	102
Isles of Scilly	0.77	0.80	-	5.6	0.04	1.10	5.1	2361	-	7.3	3.57	2.3	81

*Bryan *et al*., 1980; †Portmann, 1979 (x7); ‡Leatherland & Burton, 1974.

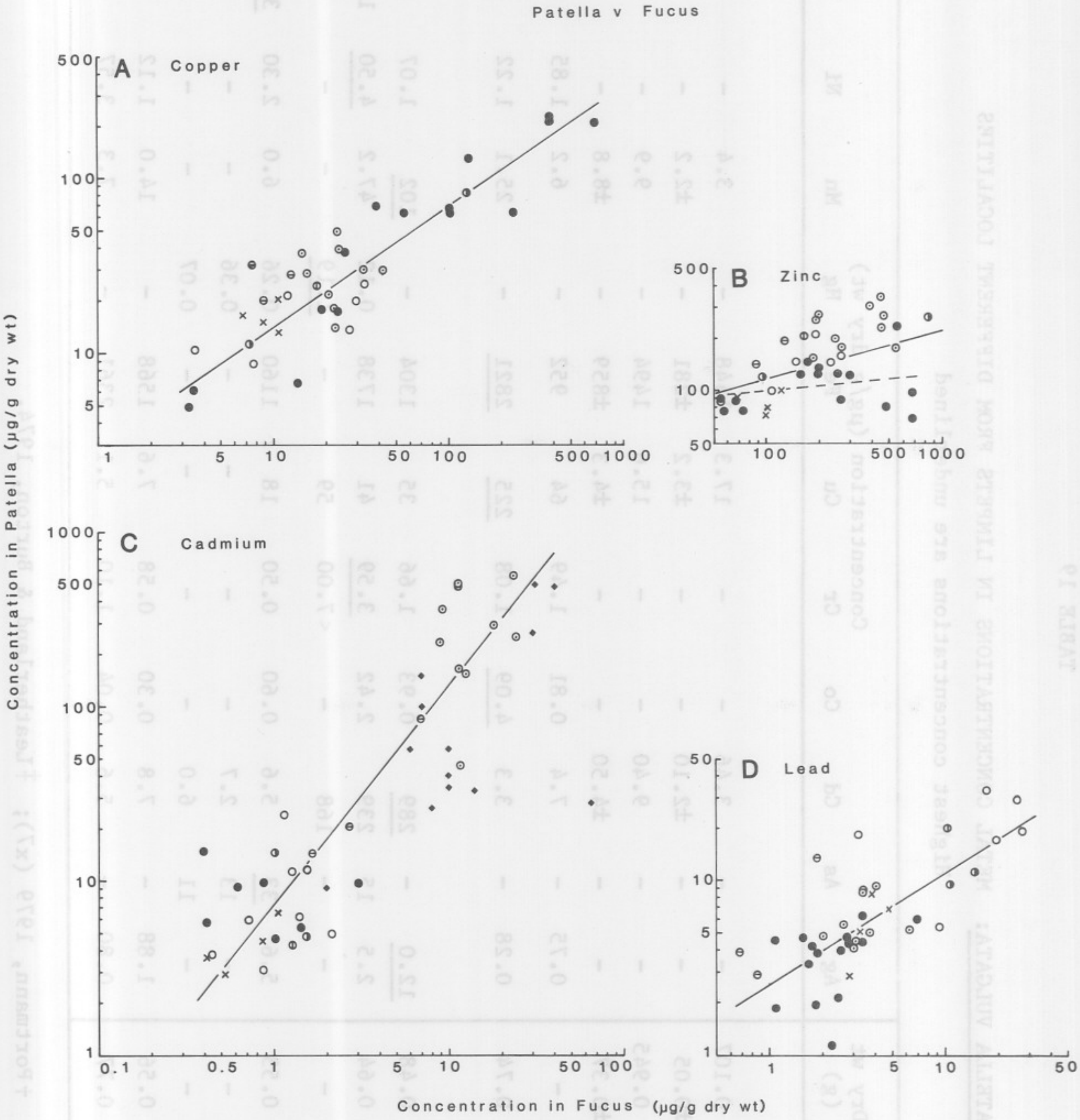
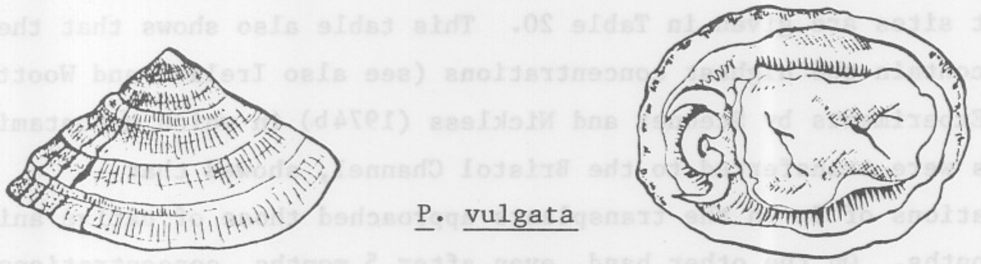


Fig. 13A. *Patella vulgata* v *Fucus vesiculosus*. Relationship between concentrations of Cu in the two species from a range of sites. Concentrations in limpets are corrected to animals of 1 g dry weight (actual concentration/actual dry wt$^{-0.23}$). (●) North Cornwall and Isles of Scilly, (◐) Fal Estuary, (○) Looe Estuary, (⦶) Gannel Estuary, (⊙) Bristol Channel, (⊖) Northwest England, (X) Eastern England. Slope of log-transformed data is 0.652 (n = 40, $P < 0.001$).

Sampling. Specimens having a shell length of about 4cm are a convenient size and samples pooled from 5 limpets provide ample material for analysis. Variation between animals appears to be rather high and Table 18 includes means and standard deviations for two groups of 5 similar-sized individuals collected in February from a relatively uncontaminated site on Falmouth Beach. The coefficients of variation are Cd 61 and 57%; Cu 18 and 28%; Fe 19 and 57%; Mn 65 and 89%; Zn 18 and 23%. Even with a sample containing 62 individuals collected in September, Lobel et al. (1982) found a coefficient of variation of 24% for Zn and, with 32 animals, a value of 18% for Fe. Samples collected during the breeding season (mainly October-December) might be expected to show even more variability.

P. vulgata is useful as an indicator of Cd and Cu contamination, and has also been observed to reflect changes in environmental levels of Ag, Hg, Pb and, to a lesser degree, Zn. By virtue of its very wide distribution, the limpet is a valuable indicator on rocky coasts. However, its occurrence within estuaries is often very limited and variation between samples tends to be rather high.



P. vulgata

13B. Relationship for Zn. Size correction and symbols as for Cu. Continuous line includes all results and slope is 0.297 ($n = 40$, $P < 0.01$). Broken line for North Cornwall and Scilly data has slope of 0.10 ($n = 15$, not significant).

13C. Relationship for Cd. Concentrations are corrected to limpets of 1 g dry weight (actual concentration/actual dry $wt^{0.4}$ for all sites except Weston and Portishead on the Bristol Channel, where formula is: actual concentration/actual dry $wt^{0.92}$. See Boyden, 1977). Continuous line applies to all points except (◆) from Bristol Channel and South Wales (Nickless et al., 1972) and slope is 1.193 ($n = 40$, $P < 0.001$).

13D. Relationship for Pb. Legend as for copper. Slope of line is 0.627 ($n = 40$, $P < 0.001$).

Nucella lapillus (dogwhelk)

Although the dogwhelk is found primarily on rocky coasts, it may be found on rocky shores in the lower reaches of estuaries. Its diet of barnacles or molluscs such as mussels or small limpets is considered to be by far the most important source of As (Klumpp, 1980b), Fe and Zn (Young, 1977). Different metal concentrations in the dietary species are likely to influence those of N. lapillus, even at a single site. For example, Peden et al. (1973) found that dogwhelks feeding on limpets contained more than twice as much Cd as those feeding on barnacles.

Field observations show that, although N. lapillus is a carnivore, its concentrations of Cd and Cu reflect environmental changes as represented by levels in Fucus vesiculosus (Fig. 14). On the other hand, levels of Zn show erratic behaviour and the accumulation of Ag appears to be suppressed in animals containing a high level of Cu. It seems likely that concentrations of Pb in the dogwhelk reflect levels of environmental contamination (Ireland & Wootton, 1977). However, As is probably regulated (Klumpp, 1980b; Bryan & Gibbs, 1983). Examples of tissue concentrations in dogwhelks from different sites are given in Table 20. This table also shows that the viscera contain the highest concentrations (see also Ireland and Wootton, 1977). Experiments by Stenner and Nickless (1974b) in which uncontaminated dogwhelks were transferred to the Bristol Channel, showed that concentrations of Zn in the transplants approached those of native animals in 2-3 months. On the other hand, even after 5 months, concentrations of Cd and Cu in the transplants were less than half those of the natives.

Sampling : When N. lapillus stops growing in its third year, the edge of the shell becomes rounded and teeth develop on the inside (Moore, 1938). It is probably best to collect dogwhelks approaching full size (∿ 2.5 cm long) in which the teeth have not yet or only just started to develop, since these animals should be 2-2.5 years old. A comparison of animals with and without teeth is shown in Table 20. In view of the effect which the type of diet may have on the accumulation of metals by N. lapillus, it is perhaps advisable to confine sampling to dogwhelks which seem to be feeding on barnacles and avoid those obviously associated with mussels, limpets etc. The animals are processed in the same way as Littorina littorea (p. 58).

Lobel et al. (1982) studied the individual variability of metal concentrations in male dogwhelks : these were of similar size and came from a small area where they were feeding on barnacles. Even with such

TABLE 20

NUCELLA LAPILLUS: METAL CONCENTRATIONS IN DOGWHELKS FROM DIFFERENT LOCALITIES

Highest whole body concentrations are underlined

Locality	Dry wt (g)	Concentration (µg/g dry wt)											
		Ag	As	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn
St Ives Bay													
Without teeth (n = 6)	0.235	1.27	–	6.8	0.96	0.44	1026	282	–	21.1	0.61	3.7	443
With teeth (n = 6)	0.193	1.75	–	10.4	0.66	0.70	1377	296	–	19.5	0.63	3.8	489
Viscera (n = 5)	0.174	2.61	–	11.3	1.61	0.94	2239	328	–	20.2	0.97	8.1	522
Foot (n = 5)	0.125	0.11	–	0.4	0.22	0.43	34	142	–	20.0	0.06	0.5	104
Bristol Channel													
Aberavon*	–	–	–	47	–	11	93	–	0.33	–	–	18	667
Penarth [‡]	–	–	–	–	–	–	458	–	–	17.3	–	19.6	2355
Amroth*	–	–	–	32	–	6.0	77	–	0.13	–	–	8.7	263
Weston	0.116	8.62	–	114	6.46	1.38	114	474	–	25.0	3.40	19.0	1836
Fal Estuary													
Restronguet Pt	0.169	1.67	48[†]	23.0	2.72	0.40	1002	383	–	36.8	1.60	5.0	3352
Falmouth Beach	0.173	2.18	–	17.4	0.84	0.89	183	184	–	12.6	1.20	34.3	571
Portland**	–	–	38	21	–	–	–	–	0.44	–	–	–	415
Exe Estuary*	–	–	–	10.3	–	3.7	43	–	0.23	–	–	3.3	–
Wembury	0.204	1.61	–	25.5	1.49	1.47	115	274	–	13.0	1.64	5.0	394
Looe Beach	0.226	1.30	–	5.5	0.67	1.57	51	270	–	11.4	2.01	1.9	235
Isles of Scilly	0.116	1.46	–	46	0.70	0.16	28	413	–	10.4	1.64	2.6	442

*Portmann, 1979 (x3.33); [‡] Ireland & Wootton, 1977; [†] Klumpp & Peterson, 1979; **Leatherland & Burton, 1974.

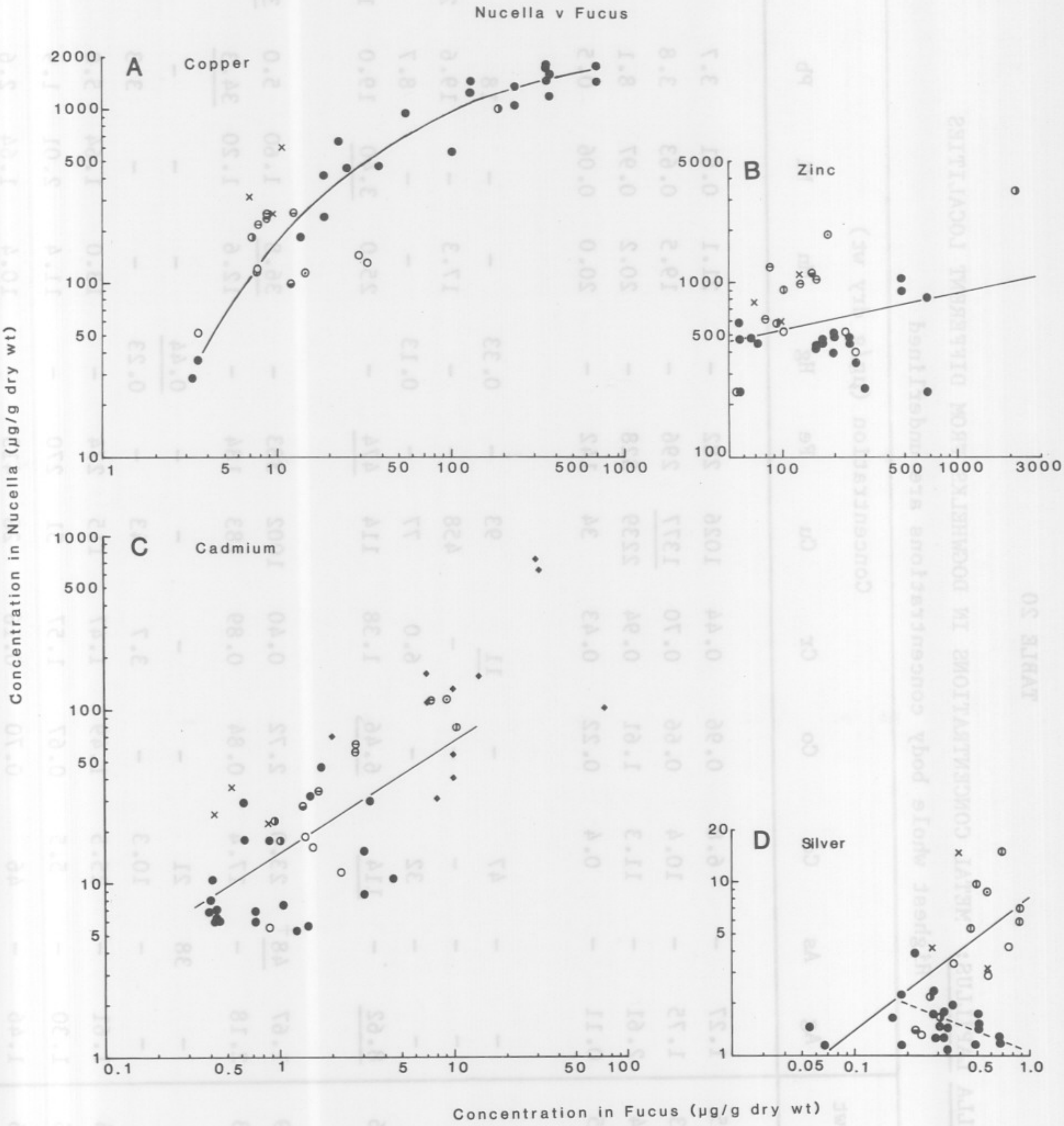
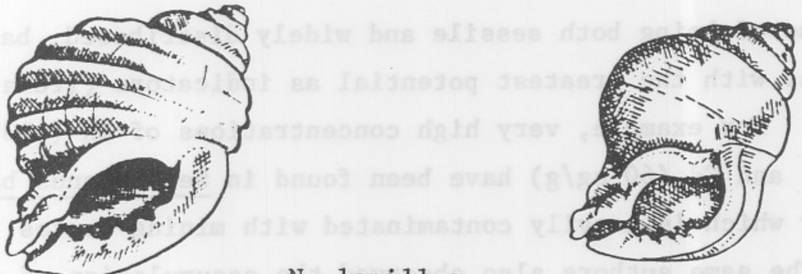


Fig. 14A. *Nucella lapillus* v *Fucus vesiculosus*. Relationships between Cu concentrations in the two species from a range of sites. (●) North Cornwall and Isles of Scilly, (◑) Fal Estuary, (○) Looe Estuary, (⊙) Bristol Channel, (⦶) Northwest England, (⊖) Anglesey, (X) Eastern England. Curve drawn by eye.

precautions the coefficients of variation for Cu, Fe and Zn ranged between 20 and 24% ($n = 48$). Analyses of individuals in groups of 5 animals from 2 sites in the Fal Estuary gave the following coefficients of variation : Cd 38 and 42%; Cu 10 and 19%; Fe 32 and 34%; Zn 37 and 76%. Against this degree of variability, the influence of size on metal concentrations in the dogwhelk remains unclear; however, in the Fal Estuary it was evident that small specimens usually contained the highest levels of Zn. A possible source of variation is the use of animals in different phases of the breeding cycle : although spawning occurs most commonly during the winter and spring, the dogwhelk appears to breed throughout the year (Fretter and Graham, 1962).

N. lapillus appears to be a useful indicator of contamination with Cd, Cu and, to a lesser degree, Ag and Pb. It has the advantages of being a fairly good accumulator of metals that is easy to clean. On the other hand, the dogwhelk is not very tolerant of estuarine conditions and individual variation tends to be high.



N. lapillus

14B. Relationship for Zn. Symbols as for copper. Slope of log transformed data is 0.21 ($n = 37$, $P < 0.1$).

14C. Relationship for Cd. Symbols as for Cu, plus (◆) from Bristol Channel (Nickless et al., 1972). These latter points not included in regression which has slope of 0.619 ($n = 37$, $P < 0.001$).

14D. Relationship for Ag. Symbols as for Cu. For animals with < 400 µg/g Cu slope of continuous line is 0.761 ($n = 19$, $P < 0.001$). For animals with > 400 µg/g Cu slope of broken line is -0.424 ($n = 18$, $P < 0.05$).

Crustaceans

Decapods

The mobility of many species of crustaceans tends to preclude their use as indicators in estuaries. Furthermore, in the most studied group, the decapod crustaceans, Zn and Cu are regulated against environmental changes: examples include the shore crab *Carcinus maenas* (Bryan & Gibbs, 1983), the prawn *Palaemon elegans* (White & Rainbow, 1982) and probably many other species (Bryan, 1968). On the other hand, Cd is accumulated under contaminated conditions by the edible crab *Cancer pagurus* (Davies *et al.*, 1981) and the shrimp *Crangon crangon* (Dethlefsen, 1977). Similarly tissue Hg levels in the lobster *Homarus americanus* reflect the degree of contamination (Thurberg *et al.*, 1977) and so also do concentrations of Pb in the crab *Macropodia rostrata* (Chaisemartin *et al.*, 1978). An added complication in crustaceans is provided by fluctuations in tissue metal concentrations, especially of Cu and Zn, which are associated with the moult cycle (Martin, 1975). Moreover, Fowler and Benayoun, (1974) observed that about 50% of the body Cd burden was removed in the shell when the shrimp *Lysmata seticaudata* moulted.

Barnacles

By virtue of being both sessile and widely distributed, barnacles are the crustaceans with the greatest potential as indicators (Ireland, 1974; Walker, 1977). For example, very high concentrations of Zn (113000 µg/g), Cu (3232 µg/g) and Cd (60 µg/g) have been found in *Semibalanus balanoides* from Dulas Bay which is heavily contaminated with mining wastes (Rainbow *et al.*, 1980). The same authors also observed the accumulation of these metals by barnacles in the laboratory.

In the field, Barber and Trefry (1981) found a linear relationship between dissolved Cu concentrations and those of *Balanus eburneus*. However, it is thought that the main source of Zn in barnacles is the diet (Young, 1974). The indicator ability of *Semibalanus balanoides* for Zn appears to be handicapped by the fact that the metal is continuously accumulated for at least the first 2 years of its life. Moreover, seasonal changes in tissue dry weight (high in September-November; low in January;March) lead to considerable fluctuations in concentration (Walker & Foster, 1979). These authors concluded that barnacles are not such useful indicators of Zn contamination as was originally thought. Certainly more information is required about the responses of barnacles to a range of heavy metals in the field before their use as indicators can be fully assessed.

Fish

Fish muscle is usually monitored in the interests of public health, but is hopelessly insensitive to most forms of metal contamination since the metals are either regulated or the tissue concentrations are inconveniently low (Phillips, 1980; Bryan, 1984). However, Hg is an important exception and the analysis of fish muscle, in which a high percentage occurs as methyl Hg, is arguably one of the best ways of monitoring mercury contamination.

Platichthys flesus

In United Kingdom estuaries the flounder *Platichthys flesus* is possibly the best indicator since it is often distributed throughout the estuary and is able to enter fresh water. An important feature of the flounder is that the larger fish (> 10 cm) occupy home feeding ranges within the estuary.

Field observations It is often found that concentrations of Hg in fish muscle increase with age or size, moreover, the concentration-size regression tends to steepen in more contaminated areas (Nuorteva & Häsänen, 1975). This is because the ability to excrete methyl mercury, the most common form in fish, is rather limited (Pentreath, 1976). The influence of body size on metal levels in flounder muscle is shown in Table 21: concentrations of Hg clearly increase with size or age.

Sampling For the purpose of comparing different sites it is necessary to use flounder of similar size or age. Since size and age are not necessarily synonymous, work has been carried out to see on which basis it is preferable to compare Hg concentrations in fish. In the ling *Molva molva*, Topping (1983) found that the best relationship was that between Hg concentration and length in cm: $\log_e$ concentration = 10.5 + 1.88 $\log_e$ length Furthermore, it was calculated that by sampling 30 ling of between 70 and 89 cm length from each of two localities it should be possible to detect a 30% difference in mercury levels. However, McKie (1983) suggests that in flounder comparisons between fish of similar age, 2 or 3 years for example, may be more reliable than those based on length.

Although flounder occupy home ranges, the mature fish move down to the sea to spawn and may also be forced downstream by high river flows - especially in the winter. It is considered that in an estuary such as the Tamar the best time for sampling is the late summer, September perhaps, when the distribution of fish will probably be at its most stable (P.R. Dando, pers. comm.).

TABLE 21

PLATICHTHYS FLESUS: METAL CONCENTRATIONS IN MUSCLE OF FLOUNDER FROM DIFFERENT ESTUARIES

Age	Size range	Mean concentration (µg/g wet[b] wt)					
(years)	(cm)	Cd	Cu	Hg	Ni	Pb	Zn
		Thames Estuary (Rickard & Dulley, 1983)					
0+[a]	4-11	0.10	1.13	0.04	0.39	0.4	22.9
1+	9-19	0.07	0.76	0.07	0.20	N.D.	17.2
2+	14-26	0.07	1.02	0.14	0.18	N.D.	10.4
3+	22-29	0.05	0.56	0.37	N.D.	N.D.	9.6
		Medway Estuary (Wharfe & Van Den Broek, 1977)					
-	0-7.9	0.06	1.3	0.10	-	0.28	18.2
-	8-13.9	0.07	0.8	0.17	-	0.39	17.2
-	14-19.9	0.07	0.3	0.46	-	0.33	12.3
-	20	0.06	0.3	0.64	-	0.33	14.5
		Inner Forth Estuary (McKie, 1983)					
2-10	12-27	-	-	0.25	-	-	-
		Outer Forth Estuary					
1-5	19-33	-	0.16	0.10	-	-	8.1
		Ythan Estuary					
2-7	18-41	-	0.16	0.10	-	-	8.1

[a] Some skin and bone included; [b] For dry wt basis multiply by 4;
N.D. = not detectable

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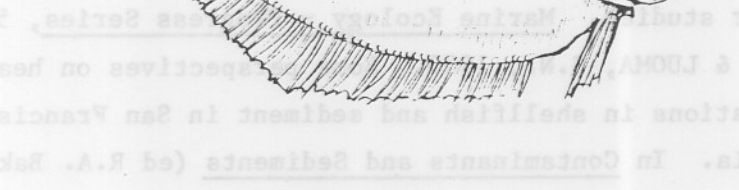

Platichthys flesus

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APPENDIX

Preparation of samples during the present work

Sediments

Sediments were collected from the surface oxidised layer of the intertidal zone and wet sieved through 100 µm plastic mesh with 50% sea water. After settling overnight, most of the water was decanted and the sediment mixed to form a slurry suitable for pipetting. Unused slurry was air dried on top of an oven.

HCl extracts 2 ml aliquots of sediment slurry were pipetted into glass liquid-scintillation-counting vials and extracted for 2 hours with 20 ml of 1 N-HCl. The acid:sediment ratio was roughly 30:1. Shaking the vials at 5-10 minute intervals gave the same results as continuous stirring. The extract was separated from the sediment by filtration under pressure through a 0.45 µm membrane filter. This was done by decanting the acid into a 10 or 20 ml glass syringe connected to a plastic membrane filter holder (Millipore, Swinnex 25 mm). Pressure was applied with the plunger and, after discarding the first few ml, the extract was collected in another vial. The apparatus and filters were all precleaned with 1 N HCl.

Other aliquots of slurry were dried and salt-free weights of sediment calculated from the salinity of water used in the sieving procedure.

HNO_3 extracts ('totals') It seems unlikely that metals remaining insoluble after this treatment are ever likely to become bioavailable. For most metals, about 1 g of air-dried sediment was digested with 20 ml of HNO_3 in the same way as the tissues (see below). Finally the extracts were decanted into test tubes and insoluble particles allowed to settle.

For As, Hg and Se, however, the sediment slurry was used: approximately 3 g were refluxed for 2 hours with 20 ml of HNO_3 and then diluted to 100 ml with distilled water. An ammonium iodide fusion followed by dissolution in 1N HCl was used to release Sn from cassiterite since this mineral is not attacked by the nitric acid digestion.

Sediment organic content This was determined from the loss in weight of dry (80°C) sediment heated at 400°C for 6 h. After both the preliminary drying and ashing, the sample was cooled for 1 h in a desiccator before weighing. Since the sediment had been treated with 50% seawater and its water content was known, the results were corrected for the loss in weight of seawater salts at 400°C.

Tissues

Dissection of biological material was carried out with plastic scalpels, having replaceable carbon steel blades, and plastic forceps. However, there is no evidence that the use of stainless steel instruments creates any major problems.

For the majority of metals (Ag, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn), the tissues were pooled in 100 ml conical flasks and dried at 80°C. About 20 ml of HNO_3 (usually 'Aristar') were added for each gram of dried tissue: the flask was then covered with a glass bubble and heated on a hot plate for 1-2 days until a pale yellow solution was obtained. After removing the bubble, the acid was slowly evaporated. The residue was dissolved in concentrated HCl (with warming) and then diluted to give 10% or approximately 1N HCl. Hydrochloric acid was used because Ag is lost from dilute solutions of HNO_3.

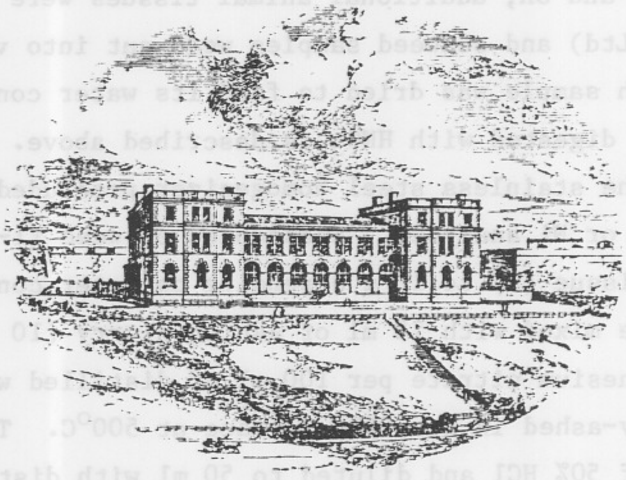
For As, Hg, Se and Sn, additional animal tissues were homogenised (Silverson Machines Ltd) and seaweed samples were cut into very small pieces. Part of each sample was dried to find its water content and, in some cases, was then digested with HNO_3 as described above. However, contamination from the stainless steel homogenizer precluded the use of these samples for Cr or Ni analyses. Subsamples of weed (1-3 g) or homogenized animal tissue (up to 10 g depending on water content) for As and Sn determination were mixed with 15 ml of ashing slurry (10 g magnesium oxide and 6 g of magnesium nitrate per 100 ml of distilled water) in 100 ml Pyrex beakers and dry-ashed in a muffle furnace at 500°C. The residue was dissolved in 25 ml of 50% HCl and diluted to 50 ml with distilled water (Langston, 1980). This solution was used for the analysis of As and Sn. For Hg and Se, the homogenate or chopped seaweed was placed in a Kjeldahl flask fitted with a condenser and digested with 20 ml of a 3:1 HNO_3/H_2SO_4 mixture at 50-60°C for 4 hours. 10 ml of 30% (w/v) H_2O_2 were added through a side arm on the condenser and the temperature raised to 80°C for 1 hour. Finally, the sample was diluted to 50 ml with distilled water (Langston, 1982).

Metal analysis

The majority of metals (Ag, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn) were analysed by flame atomic absorption (Perkin Elmer 603) and background correction was employed for Ag, Cd, Co, Cr, Ni and Pb. Levels of Ag and Cd in sediments and low concentrations of Ag, Cd, Cr and Pb in some tissues were measured by carbon furnace atomic absorption (Perkin Elmer 76B).

Al was measured by flame emission using a nitrous oxide-acetylene flame (Perkin Elmer 603).

As, Se and Sn were determined in a Perkin Elmer MHS-20 hydride system. The flameless atomic absorption method of Uthe, Armstrong and Stainton (1970) was used for Hg determinations.



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