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HEAVY METALS IN THE BURROWING BIVALVE *SCROBICULARIA PLANA* FROM CONTAMINATED AND UNCONTAMINATED ESTUARIES

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(Figs. 1–7)

Concentrations of ten metals (Ag, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn) have been analysed in the soft tissues of *Scrobicularia plana* (da Costa) from sites in the Gannel and Camel Estuaries in South-West England. In the Gannel Estuary, which receives wastes from old lead mines, the animals contained about an order of magnitude higher concentrations of lead, cobalt, cadmium and zinc than in the Camel Estuary and higher concentrations of other metals also. Differences were also observed between the sediments.

In both populations, less than 50 % of the total silver, copper and iron was found in the digestive gland. For the other metals over 50 % occurred in this organ and, in large Gannel animals, more than 90 % of the lead, cobalt, cadmium and zinc, implying that they are chiefly absorbed from ingested sediment.

In the contaminated Gannel animals, all metal concentrations, with the exception of manganese and iron, increased with increasing size. The main contrast in the Camel animals concerned the concentrations of cadmium and zinc which were independent of size. This suggests a possible relationship between the slope of the concentration/size regression and the level of contamination, although differences in growth rate may also be involved.

When animals were exchanged between the two estuaries, their metal concentrations approached those of the natives very slowly. Even after a year, concentrations of lead, cobalt, cadmium and zinc in the digestive gland were still markedly different from those of native animals.

The use of *Scrobicularia* as an indicator of metal contamination is discussed. It is concluded that it should normally be regarded as a long-term integrator of the chronic type of contamination usually associated with estuarine sediments.

INTRODUCTION

Analysis of the deposit-feeding bivalve *Scrobicularia plana* (da Costa) has been proposed as a method of assessing the biological availability of heavy metals in estuarine sediments (Bryan & Hummerstone, 1977; Bryan & Uysal, 1978). *Scrobicularia* has a number of attributes which are useful in this type of indicator: (i) it is common in many British estuaries, particularly in the south, and often penetrates much farther upstream than other common bivalves such as *Mytilus edulis*; (ii) it is a convenient size for analysis and, during its life span of perhaps 10 years, reaches a shell length of 40–50 mm (Green, 1957; Hughes, 1970); (iii) it is a good accumulator of metals and appears to reflect changes in their biological availability.

Two estuaries differing markedly in their heavy metal regimes are those of the Rivers Gannel and Camel which lie about 20 km apart on the north coast of Cornwall in South-West England (Fig. 1). The Gannel Estuary drains an old lead mining area in which the principal mine, East Rose, produced 48200 tons of 62% lead ore and much smaller amounts of silver, zinc and copper between 1845 and 1885 (Dines, 1956). Although the Camel Estuary was also associated with mining, it shows little sign of being contaminated.

In this paper, studies on *Scrobicularia* from these contrasting estuaries are described. They concern some of the factors on which metal concentrations in the animal and its use as an indicator organism depend.

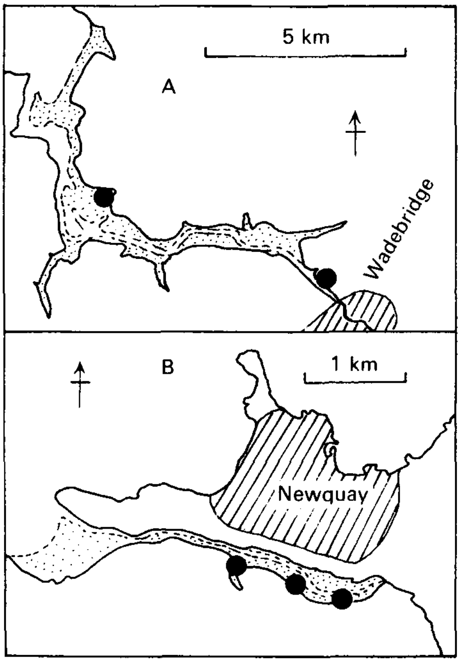

Fig. 1. Sites from which *Scrobicularia* and sediments collected in (A) Camel Estuary, (B) Gannel Estuary. Based on the 1:25000 Ordnance Survey Map with the sanction of the controller of H.M. Stationery Office.

MATERIALS AND METHODS

Scrobicularia of different sizes and samples of surface sediment were collected from the intertidal zone at low tide (Fig. 1). Prior to analysis, the animals were kept in aerated 50% sea water for 1 week and the sediment was air-dried. Samples of the whole soft parts or of individual tissues from 5–10 animals were pooled in 100 ml conical flasks and weighed before and after drying at 110 °C. Digestion of the tissues and sediments using nitric acid and their analysis for ten metals (Ag, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn) has been described by Bryan & Uysal (1978).

The empty shells were soaked in distilled water overnight and air-dried prior to being weighed and measured. A relationship between age and shell length was determined for each population by measuring the annual growth increment between growth marks on shells of different size and using the Ford–Walford plot (see Hughes, 1970 for details). It was assumed, as did Hughes, that animals are 5 mm long at the end of the first growing season; it was also assumed that about 4 months are necessary to reach this size.

To see how rapidly metals were absorbed or lost, *Scrobicularia* were transferred between the two estuaries.* About 100 animals from each were dried with paper tissues and marked on both

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* There are controls on the transfer of shellfish between different areas of the United Kingdom and a licence is required from the Ministry of Agriculture, Fisheries and Food.

sides of the shell using a fibre-tipped pen (Text Mark 700). The animals were planted within four areas, each of about 0·5 m^2, marked with stakes or stones. The exchange was accomplished on a single low tide and, at subsequent intervals, 4–5 animals were recovered for analysis.

RESULTS

Metals in whole soft parts and sediments

Concentrations in animals and surface sediments from three sites in the Gannel Estuary and two in the Camel are compared in Table 1. Levels of lead, cobalt, cadmium, zinc, manganese and nickel are markedly higher in the Gannel animals and, to a lesser extent, this contrast can also be seen in the sediments. At all sites, the sediment was sandy and therefore differences between the animals may depend on them absorbing metals from the finer sediment particles in which the contrast between the estuaries may be greater. In this paper we are mainly concerned with comparing the animals from the two estuaries and details of studies on the relationships between metals in sediments and animals will be published later. *Scrobicularia* from the Gannel Estuary are among the most contaminated we have encountered and those from the Camel among the least. The comparisons which are made below refer to animals from the middle Gannel and upstream Camel sites where they are most abundant.

Table 1. *Metal concentrations in* Scrobicularia *and sediments*

Estuary	Date	Site	Concentration (ppm dry weight) Ag	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
			Whole soft parts of *Scrobicularia*									
Gannel	Nov. 75	Upper	0·28	7·0	66	1·5	25	1240	86	11·9	828	2930
	Nov. 74	Middle	1·2	14·9	36	2·2	86	1120	87	9·8	350	2940
	May 74	Lower	0·23	1·4	19	1·5	71	1190	57	11·6	234	1470
Camel	Nov. 74	Upper	0·37	0·64	4·3	1·2	25	699	19	3·4	12	353
	Nov. 74	Lower	0·60	0·29	5·3	1·3	77	840	22	4·5	21	394
			Sediments									
Gannel	Nov. 75	Upper	0·9	0·7	12	9	24	13600	514	8·5	330	264
	Nov. 74	Middle	0·5	0·5	19	16	39	13400	606	20	260	300
	May 74	Lower	< 0·3	1·1	10	16	24	14400	346	17	147	183
Camel	Nov. 74	Upper	< 0·4	< 0·4	10	45	72	20350	423	28	51	159
	Nov. 74	Lower	0·4	0·4	5·6	19	72	9000	281	11	31	65

Metals in individual tissues

Analyses of six tissues from animals in both estuaries are compared in Table 2.

In the Gannel animals the digestive gland contains by far the highest concentrations of cadmium, cobalt, nickel, lead and zinc. It has been suggested by Bryan & Uysal (1978) that high levels in the digestive gland are an indication that metal uptake from ingested particles is very important. The contrast between concentrations in the digestive gland and those of other tissues is much smaller for chromium and copper, and values for silver, iron and manganese in the digestive gland are exceeded respectively by those of the gills/labial palps and mantle/siphons. The occurrence of relatively high concentrations in

Table 2. *Metal concentrations in individual tissues* (*October* 1975)

* Not detectable. Maximum values for different metals in italics.

Tissue	% of total dry weight excluding body fluid	% dry weight	Ag	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
			Concentration (ppm dry tissue)									
			Middle Gannel animals (six of 41 mm shell length pooled)									
Digestive gland	38·48	21·2	1·38	*39·8*	*156*	*3·56*	*92*	872	190	*43·1*	*1130*	*9630*
Mantle and siphons	31·24	11·3	0·50	0·88	6·25	2·09	29	*3480*	*360*	3·2	90	316
Foot and gonad	12·42	14·6	0·67	0·66	1·42	1·09	48	342	15	3·9	8	235
Gills and palps	9·20	13·9	*1·58*	0·85	2·26	—	36	848	—	—	29	406
Adductor muscles	6·34	16·6	*	0·98	0·53	—	5·3	236	—	—	9	132
Kidney	2·30	17·3	0·24	4·56	1·1	—	37	819	—	—	74	280
			Upper Camel animals (six of 42 mm shell length pooled)									
Digestive gland	26·99	21·7	0·75	1·70	*15·9*	*2·88*	*51*	604	*79·8*	*10·6*	*37·8*	*1104*
Mantle and siphons	33·1	13·7	0·44	0·12	1·24	1·41	30	971	14·4	1·43	3·7	170
Foot and gonad	21·66	18·3	0·34	0·12	0·83	0·57	19	298	4·0	4·04	*	149
Gills and palps	6·3	14·3	*0·81*	0·04	*	2·32	45	680	6·9	10·5	*	388
Adductor muscles	9·27	21·0	0·35	0·15	0·28	—	8·3	177	—	—	*	87
Kidney	2·65	18·3	0·35	*2·41*	4·35	—	28	*1090*	—	—	*	315

tissues such as these, which are exposed directly to the water, was thought by Bryan & Uysal (1978) to indicate that absorption from solution was becoming a significant portion of the total metal intake.

In the digestive gland of the Camel animals, concentrations of cadmium, cobalt, nickel, lead and zinc are very much lower than in the Gannel animals (Table 2). However, the contrast between the other tissues from the two populations is generally less than in digestive gland. For example, the factor separating the concentrations of zinc is 9 for the digestive glands but less than 2 for the other tissues. In the Camel animals, the highest level of silver is found in the gills/labial palps and the highest levels of cadmium and iron in the kidney. Unlike the Gannel animals, where the highest concentration occurred in mantle/siphons, the highest level of manganese was found in the Camel digestive gland, suggesting that uptake from the sediment is relatively more important in the Camel animals.

Since the bulk of most metals lies in the digestive gland, subsequent analyses of the remaining tissues were confined to combined samples.

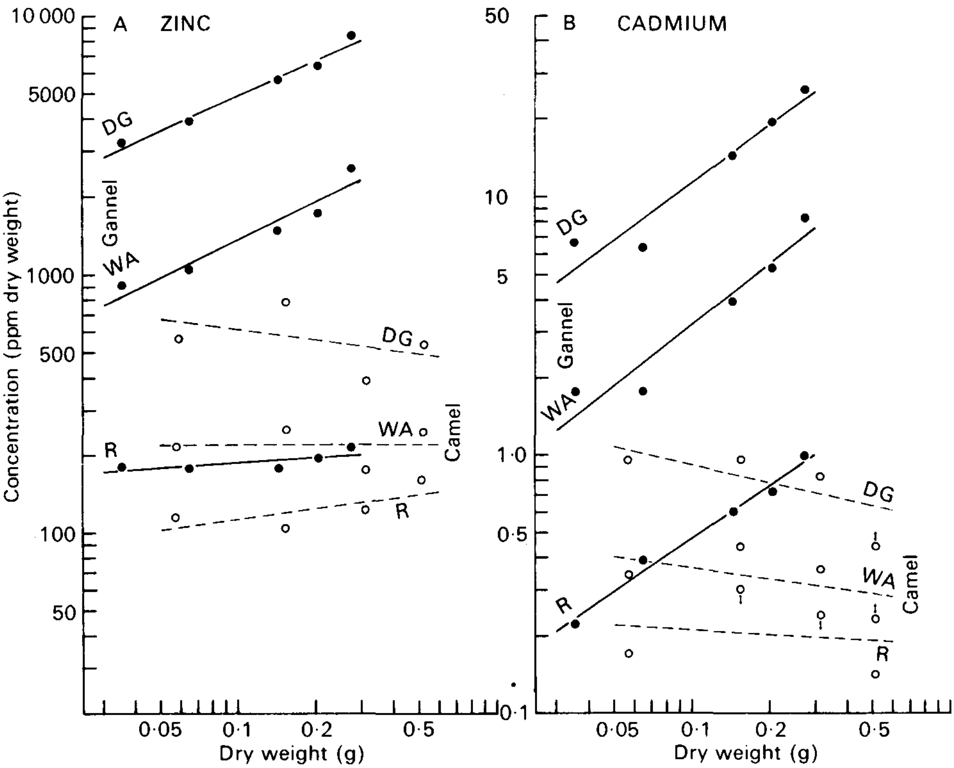

Fig. 2. (A) Influence of size of whole soft parts on concentrations of Zn in digestive gland (DG), remaining tissues (R) and whole soft parts (WA) of Gannel animals (closed symbols) and Camel animals (open symbols) in November 1975. Results for whole soft parts were obtained by adding results for other tissues. (B) Comparative results for Cd.

Table 3. *Size and metal concentration relationships in* Scrobicularia

Values of b and a from equation $\log_{10}$ concentration $= b \log_{10}$ weight $+ \log_{10} a$. Values of a are the concentrations of animals having a dry weight of 1·0 g. Since this weight is not normally reached, concentrations calculated for a dry weight of 0·3 g given.

	Whole soft parts			Digestive gland			Remaining tissues		
	Slope b	a	ppm in 0·3 g whole soft parts	Slope b	a	ppm in 0·3 g whole soft parts	Slope b	a	ppm in 0·3 g whole soft parts
				Gannel (Nov. 75)					
Ag	0·195*	1·59	1·27	0·177	2·75	2·1	0·181	1·19	0·96
Cd	0·781*	19·2	7·5	0·725*	60·2	26	0·687*	2·30	1·0
Co	0·542*	80·8	43·0	0·512*	282	155	−0·017	2·0	2·0
Cr	0·109	1·79	1·59	0·035	3·93	3·8	0·158	0·91	0·76
Cu	0·279*	161	115	0·122	156	134	0·347*	163	109
Fe	−0·015	1146	1180	0·083	1720	1580	−0·075	929	1010
Mn	−0·035	79·2	83	−0·116	161	186	−0·008	44·1	44
Ni	0·270†	13·2	9·5	0·254*	42·9	32	0·001	1·49	1·5
Pb	0·478*	586	330	0·494*	2060	1150	0·003	38·8	39
Zn	0·473*	4010	2300	0·444*	13550	8000	0·071	218	200
				Camel (Nov. 75)					
Ag	0·277	0·51	0·39	0·120	0·60	0·51	0·361	0·49	0·32
Cd	−0·152	0·26	0·31	−0·226	0·55	0·68	−0·058	0·18	0·20
Co	0·314	4·58	3·2	0·355	18·2	12	0·076	0·73	0·66
Cr	−0·098	1·05	1·2	−0·070	2·84	3·1	−0·176	0·50	0·61
Cu	0·283	30·4	22	0·328	58·1	39	0·243	22·4	17
Fe	−0·117	670	780	0·006	1010	1010	−0·166	576	700
Mn	−0·090	24·3	27	−0·062	89·5	97	−0·128	6·98	8·2
Ni	0·147	4·21	3·5	0·182	11·6	9·3	0·086	2·10	1·9
Pb	0·403	15·3	9·6	0·474	56·9	32	0·168	3·61	3·0
Zn	−0·003	219	220	−0·125	452	530	0·143	155	130

* Slope significantly different from zero at $P < 0·05$. † Significant at $P < 0·1$.

Size and metal concentrations

The influence of size on metal concentrations and content in molluscs has been described by Boyden (1974, 1977) who found that in most bivalve molluscs the concentrations either fell with increasing size or were independent of size. Bryan & Uysal (1978) found that in *Scrobicularia* from the Tamar Estuary concentrations of cadmium, cobalt, chromium, nickel, lead and zinc increased markedly with size in the whole soft parts. However, levels of iron remained relatively constant and those of silver, copper and manganese decreased with increasing size.

Table 4. *Ratio Gannel/Camel for metal concentrations of* 0·3 *g dry weight animals in Table* 3

	Whole soft parts	Digestive gland	Remaining tissues
Ag	3·3	4·1	3·0
Cd	24	38	5·0
Co	13	13	3·0
Cr	1·3	1·2	1·2
Cu	5·2	3·4	6·4
Fe	1·5	1·6	1·4
Mn	3·1	1·9	5·4
Ni	2·7	3·4	0·8
Pb	34	36	13
Zn	10·4	15	1·5

In the present work, the digestive gland and combined remaining tissues (including body fluid) have been analysed and the results added to give values for the whole soft parts. The influence of size, as represented by the dry weight of the whole soft parts, on concentrations of zinc and cadmium in animals from both estuaries is shown in Fig. 2. Logarithmic scales are used, since the concentration appears to relate to body weight as a power function, concentration = a weight^b, which may be interpreted as the linear regression, $\log_{10}$ concentration = $b \log_{10}$ weight + $\log_{10} a$, where b is the slope and a the intercept on the Y axis of the graphs when the weight is 1 g. Values of b and a and the calculated concentrations in animals having a weight of 0·3 g (approximately 40 mm shell length) are shown in Table 3.

In the Gannel animals there are three types of situation: (i) the concentrations in all tissues are independent (b = 0) or tend to fall (b negative) with increasing size (manganese, iron); (ii) concentrations in the whole animals and digestive gland increase with increasing size (b positive) but those of the remaining tissues are independent of size (cobalt, nickel, lead, zinc); (iii) the concentrations of all tissues increase with size (silver, cadmium, chromium, copper).

Concentrations of metals in the Camel animals are consistently lower than in those from the Gannel Estuary (Table 4). Where the contrast in concentration is not so great (silver, chromium, copper, iron, manganese, nickel), the Camel results, although of less significance, show a generally similar pattern to those for the Gannel. When the concentrations differ very markedly (cadmium, cobalt, lead, zinc), there appear to be two situations: (i) the pattern for cobalt and lead resembles that for the Gannel animals with concentrations in the digestive gland and whole soft parts increasing with size; (ii) unlike

Table 5. *Percentage of total metal contained by digestive gland in animals of different size (November* 1975)

Shell length (mm)	Dry weight of digestive gland as percentage of total including body fluid	Ag	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Gannel animals											
18·8	24·1	45·0	90·5	88·5	70·7	38·3	27·4	61·6	79·0	75·8	85·0
23·1	23·0	34·4	82·8	90·2	54·0	34·6	25·7	60·3	81·1	81·4	87·0
31·7	24·1	36·9	88·3	94·3	74·1	35·0	25·7	57·5	85·8	86·6	90·9
36·2	24·7	45·3	89·8	96·1	60·8	30·9	34·6	63·2	87·8	89·5	91·4
41·2	29·3	48·3	91·5	95·9	64·7	33·0	40·3	60·3	88·8	91·7	94·2
Mean	25·0	42·0	88·6	93·0	64·9	34·4	30·7	60·6	84·5	85·0	89·7
Camel animals											
21·1	22·0	40·5	61·2	78·5	56·5	38·5	25·4	72·5	57·0	68·4	58·8
28·0	21·4	34·0	46·4	76·3	55·6	33·3	21·5	79·8	49·5	62·7	68·0
36·2	20·0	29·8	46·3	80·1	44·8	37·9	28·1	76·0	53·5	74·7	44·2
44·2	23·3	29·4	54·2	88·3	69·1	44·0	33·1	76·3	64·4	81·8	50·6
Mean	21·7	33·4	52·0	80·8	56·5	38·4	27·0	76·1	56·1	71·7	55·4

the Gannel situation, concentrations of cadmium and zinc are independent of size (Fig. 2).

Size and the importance of the digestive gland

In November 1975 the dry weight of the digestive gland, expressed as a percentage of that of the whole soft parts, was relatively independent of size in both populations (Table 5). Thus, the percentage of the total metal content contained by the digestive gland depends mainly on the relative concentrations in the digestive gland and the remaining tissues. When the concentration increases more rapidly with increasing size in the digestive gland than in the other tissues, or decreases less rapidly, the percentage contained by the digestive gland increases with size. In the Gannel animals, this occurs for cobalt, iron, nickel, lead and zinc but not, for example for cadmium, where concentrations in all the tissues increase with increasing size. Definite trends are less obvious for the Camel animals, although the proportions of cobalt and lead in the digestive gland tend to increase with increasing size.

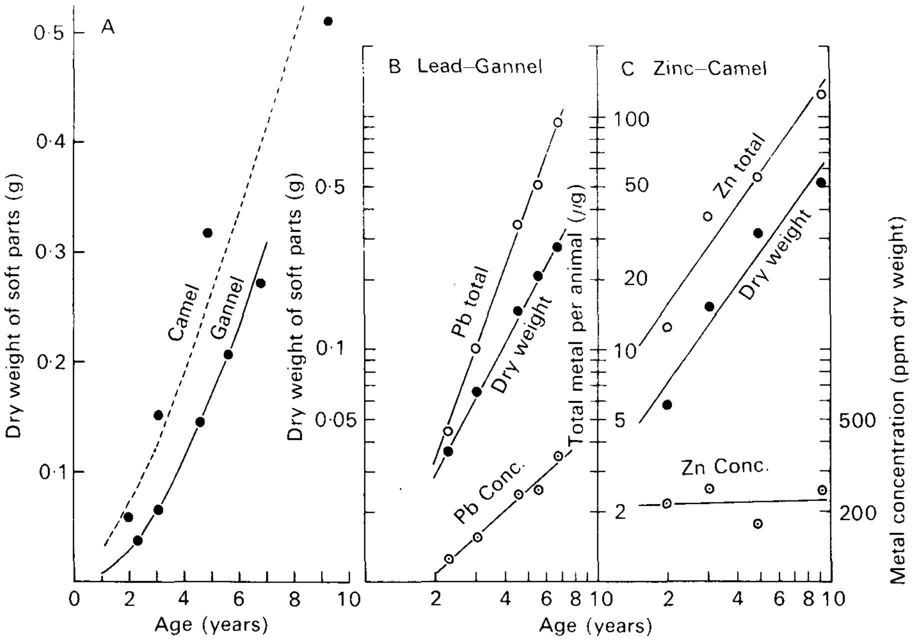

Fig. 3. (A) Relationship between dry weight of soft parts and age in both populations in November 1975. Equations for lines through points are: dry weight = 0·00775 age$^{1\cdot887}$ for the Gannel animals and dry weight = 0·0268 age $^{1\cdot4034}$ for the Camel animals. (B) Relationship of dry weight, total Pb content and Pb concentration to age in Gannel animals. Equations for lines are: $\log_{10}$ dry weight = 1·887 $\log_{10}$ age − 2·1107; $\log_{10}$ Pb content = 2·7998 $\log_{10}$ age − 0·358; $\log_{10}$ Pb concentration = 0·9126 $\log_{10}$ age + 1·752. (C) Relationship of dry weight, total Zn content and Zn concentration to age in Camel animals. Equations for lines are: $\log_{10}$ dry weight = 1·4034 $\log_{10}$ age − 1·572; $\log_{10}$ Zn content = 1·4261 $\log_{10}$ age + 0·7554; $\log_{10}$ Zn concentration = 0·0242 $\log_{10}$ age + 2·327.

In Table 5, the mean percentages for the Gannel animals are in eight out of ten cases higher than in those from the Camel, the contrast being particularly obvious for cadmium and zinc. Of the two exceptions, copper and manganese, the lower percentage of the latter in the Gannel animals was due to a high level in the mantle/siphons (Table 2, see also p. 403).

Age and metal content

The relationship between age, estimated from the shell length, and dry weight of the soft parts is shown for both populations in Fig. 3 A. The power curve, dry weight = a age^b, was fitted to the points and, over the range examined, agrees particularly well with the Gannel results. Since the curves can also be interpreted by the linear regression, $\log_{10}$ dry weight = $b \log_{10}$ age + $\log_{10} a$, the results are plotted on logarithmic scales in

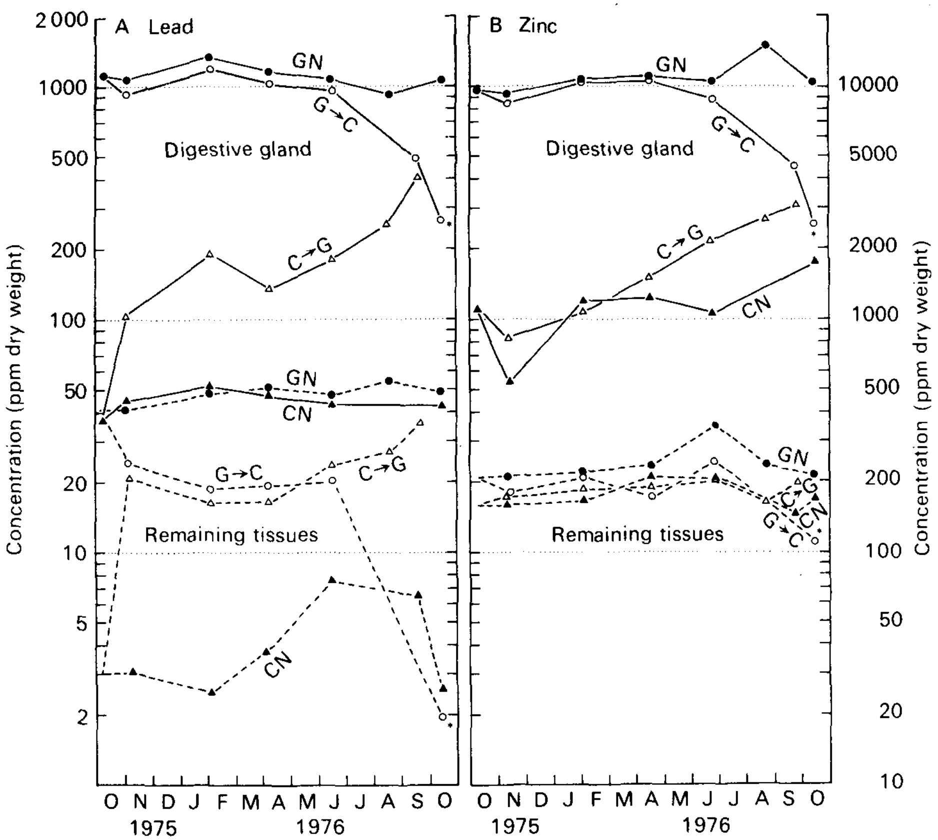

Fig. 4. (A) Concentrations of Pb in digestive gland (continuous line) and remaining tissues (broken line) from native Gannel animals (GN), Gannel animals transferred to Camel Estuary (G → C), native Camel animals (CN) and Camel animals transferred to Gannel Estuary (C → G). * One animal only recovered. (B) Comparative results for Zn.

Figs. 3 B, C and are compared with the relationships between age and both total metal content and concentration. The total lead content increases more rapidly than the animal grows, the difference in slopes being that of the increase in concentration with age (Fig. 3 B). In Fig. 3 C, the animal grows at about the same rate that zinc is incorporated and the concentration does not change with increasing age.

These results show that, particularly in the Gannel Estuary, the size and total metal content of the animal changes in a consistent manner. This may imply that conditions in the estuary have been rather stable during the life of these animals. It is also possible to see from these results that, if growth and metal intake are not closely related, growth may be an important factor controlling the size/concentration relationship. At present, we do not know to what extent the incorporation of metals and the incorporation of new tissue is related.

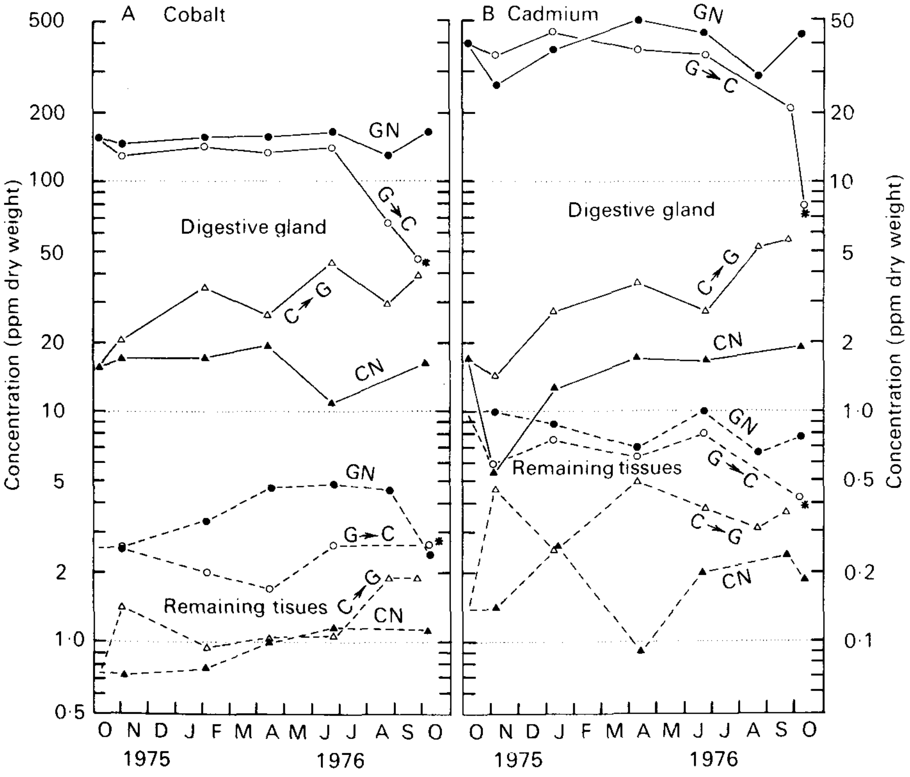

Fig. 5. (A, B) Comparative results for Co and Cd. Legend as for Fig. 4A.

Uptake and loss of metals

Preliminary attempts to study the uptake of metals in the laboratory using radioisotopes indicated that uptake was generally so slow that field experiments were a more practical proposition. These were carried out by exchanging about 100 animals between the middle Gannel and upper Camel sites. Results for lead and zinc from an experiment in which animals of about 40 mm length were exchanged are shown in Fig. 4. Similar results for cadmium and cobalt are shown in Fig. 5 and changes in the dry weight of the soft parts, corrected to a shell length of 40 mm, are shown in Fig. 6.

In the native animals from both estuaries, changes in concentrations of lead in both the digestive gland and remaining tissues were comparatively small throughout the year. Gannel animals transferred to the low-lead Camel estuary lost a little lead from the digestive gland, but retained most of it for at least 8 months. Much of the drop in concentration at 11 months can be explained by the diluting effect of growth which is shown in Fig. 6. The final drop in concentration between 11 and 12 months could not be completely explained by dilution, thus implying that there was a net loss of lead. However, there is some uncertainty because the final sample consisted of a single animal. In

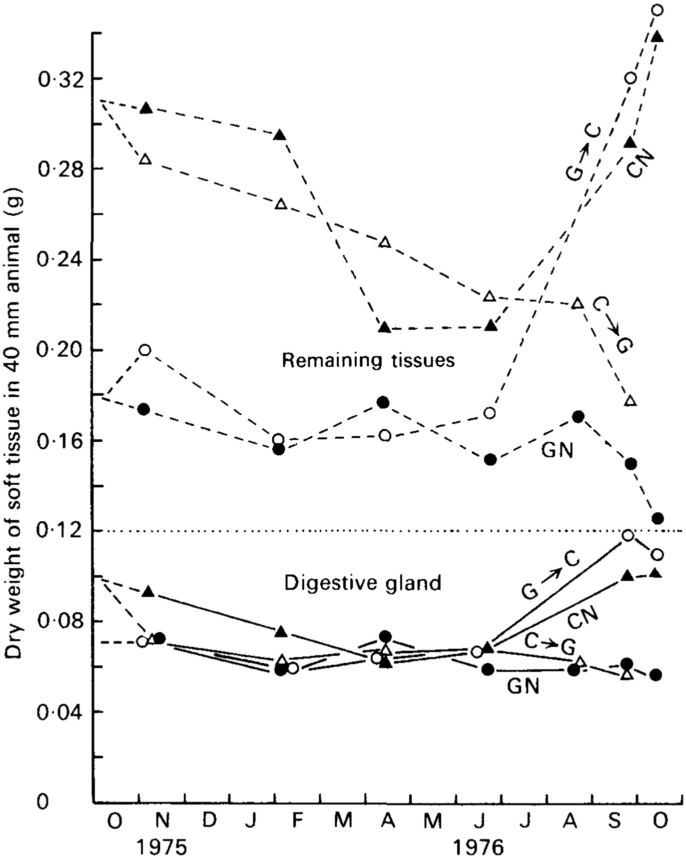

Fig. 6. Change in dry weight of digestive gland and remaining tissues of animals analysed in Figs. 4 and 5. Legend as for Fig. 4A. The tissue weights were corrected and apply to animals of 40 mm shell length.

the remaining tissues, there was some loss of lead during the first month of transfer, but the concentration then remained constant until finally a sharp drop occurred. About half of this fall can be explained by dilution, since these tissues almost doubled in weight towards the end of the experiment.

Both the digestive gland and remaining tissues of the transferred Camel animals gained lead rapidly during the first month in the high-lead Gannel Estuary. However, later changes were much slower and, although by the end of the experiment concentrations in the remaining tissues were approaching those of the native animals, the concentration in the digestive gland was still only 40% of that in the natives. The fall in the dry weight of

Table 6. *Concentrations of metals in animals transferred between two estuaries compared with those in native animals. All animals about 40 mm shell length*

	Dry weight tissue in 40 mm animal (g)	Concentration (ppm dry tissue)								
		Ag	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
DIGESTIVE GLAND											
February 1976–119 days											
Camel	0·075	0·43	1·3	17	6·7	33	995	44	12	49	1200
Camel → Gannel	0·062	0·75	2·7	35	2·9	63	1320	157	17	191	1090
Gannel → Camel	0·059	1·19	45	145	7·2	51	856	79	33	1200	11060
Gannel	0·059	2·13	37	159	16·8	96	1470	193	34	1360	10340
June 1976–236 days											
Camel	0·067	0·62	1·7	11	3·3	56	547	83	17	43	1060
Camel → Gannel	0·066	0·97	2·7	44	4·1	71	1080	153	26	179	2180
Gannel → Camel	0·066	0·68	36	141	4·7	44	531	110	32	959	8920
Gannel	0·058	1·11	44	166	5·8	65	859	164	34	1060	10460
September 1976–352 days											
Camel	0·099	0·55	1·9	16	2·7	42	697	88	12	41	1750
Camel → Gannel	0·057	1·75	5·6	39	6·3	170	1940	271	36	402	3030
Gannel → Camel	0·118	0·43	21	66	3·7	35	728	111	29	486	4605
Gannel	0·061	1·97	43	162	5·2	101	1050	182	39	1080	10400
REMAINING TISSUES											
February 1976–119 days											
Camel	0·295	0·23	0·26	0·81	1·0	15	1510	12	1·7	2·5	165
Camel → Gannel	0·264	0·51	0·25	0·98	1·1	22	903	15	2·0	16	188
Gannel → Camel	0·159	0·26	0·75	2·0	1·3	18	1820	115	1·4	19	210
Gannel	0·156	0·45	0·88	3·4	1·1	36	1980	136	0·93	48	221
June 1976–236 days											
Camel	0·210	0·44	0·21	1·2	1·5	30	1380	51	2·1	7·7	209
Camel → Gannel	0·224	0·32	0·38	1·1	1·7	25	888	30	2·0	24	202
Gannel → Camel	0·172	0·22	0·80	2·7	1·2	16	1290	36	1·9	20	249
Gannel	0·151	0·28	1·0	4·9	1·4	21	1220	166	1·4	47	357
September 1976–352 days											
Camel	0·292	0·35	0·18	1·1	0·9	21	890	7·7	1·5	2·6	170
Camel → Gannel	0·177	0·25	0·36	1·9	1·2	27	1020	134	2·3	35	199
Gannel → Camel	0·322	—	—	—	—	—	—	—	—	—	—
Gannel	0·149	0·43	0·75	2·3	1·4	3·3	1640	244	0·99	50	212

these animals during the year (Fig. 6) could have contributed to the increased concentrations, particularly in the remaining tissues.

The results for zinc in Fig. 4 are, in the case of the digestive gland, similar to those for lead. However, although in the two populations concentrations in the digestive gland differed by almost an order of magnitude, levels in the remaining tissues were very similar.

Results for cobalt and cadmium in the digestive gland are not unlike those for lead and zinc (Fig. 5). Although results for the remaining tissues are rather variable, even after a year equality between the transferred and native animals was not achieved.

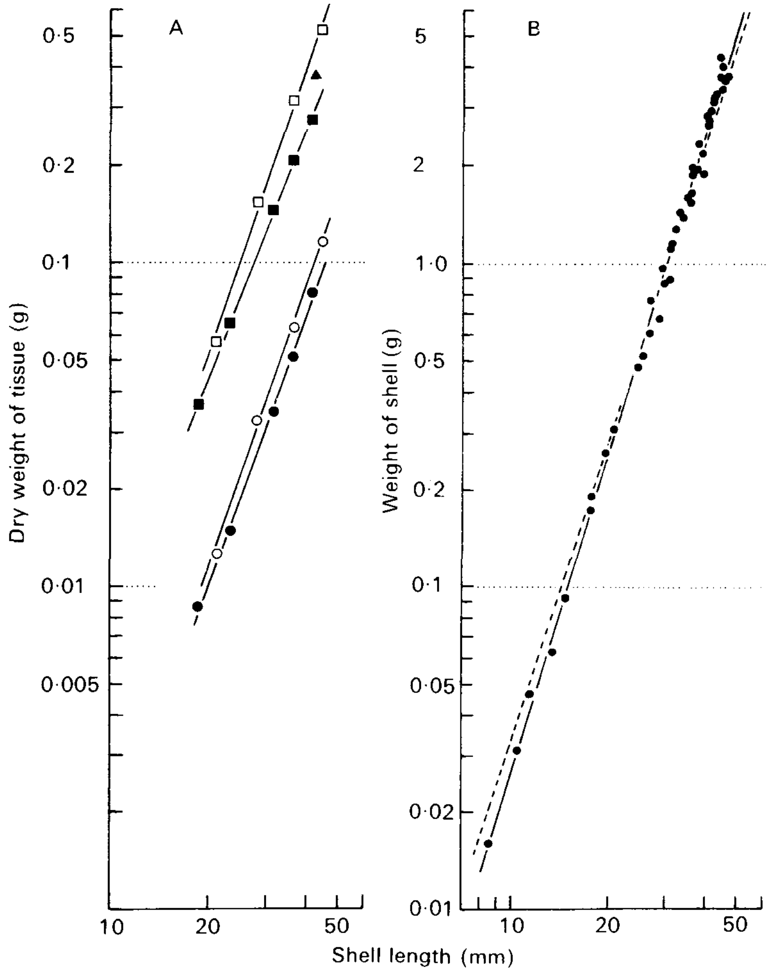

Fig. 7. (A) Relationship between dry weight of whole soft parts of Camel (□) and Gannel (■) animals and length of shell in November 1975. Equations of lines are: $\log_{10}$ dry weight = 2·9708 $\log_{10}$ shell length − 5·153 for Camel animals and $\log_{10}$ dry weight = 2·5858 $\log_{10}$ shell length − 4·726 for Gannel animals. The single point (▲) is for the Upper Gannel site. Also shown are results for digestive gland of Camel (○) and Gannel (●) animals. (B) Relationship between shell weight and shell length of Camel animals in 1975. Broken line with no points is comparable result for Gannel animals. Equations of lines are: $\log_{10}$ shell weight = 3·2822 $\log_{10}$ shell length − 4·8409 for Camel shells and $\log_{10}$ shell weight = 3·0957 $\log_{10}$ shell length − 4·5693 for Gannel shells.

Some of the results for other metals are summarized in Table 6. After 119 days, levels of iron and manganese in the digestive glands of the transferred animals approached those of the natives and after 236 days this also applies to silver and copper. At 352 days, levels of chromium and nickel in the digestive glands of Camel animals in the Gannel Estuary approached those of the natives, but their loss from the Gannel animals in the Camel Estuary was incomplete. Certainly in the digestive gland, the results suggest that, when the difference in concentration between animals from the two estuaries was large, then the time taken for transferred animals to reach equality with the natives was longer. Results for the remaining tissues are generally more variable, but the same conclusion may apply.

This experiment was carried out during an abnormally warm period when temperatures at depth in the sediment reached 20 °C during the summer, several degrees higher than usual. Lower rates of uptake and loss would be expected during a normal year and a second experiment from 1976–7 showed that this was the case.

Metals and the condition of the animals

The results in Fig. 6 show that animals from the middle Gannel site always had a lower tissue weight than those from the upper Camel. Camel animals transferred to the Gannel Estuary declined in tissue weight throughout the year and approached the condition of the native animals. In the Camel Estuary, transferred Gannel animals increased rapidly in tissue weight during the later part of the experiment and reached the weight of the native animals. This difference in condition between the populations was observed in both large and small animals (Fig. 7A). On the other hand, the relationship between length and weight of shell was similar in both populations (Fig. 7B).

It was thought that the low weight of the Gannel tissues might result from the deleterious effects of heavy metals. However, animals from the upper Gannel site which contained higher levels of lead (Table 1) were generally in a much better condition (Fig. 7A). It seems likely that factors other than metal contamination are responsible for the poor condition of the middle Gannel population. Since the estuary has been contaminated with metals for more than 100 years, it might be expected that tolerance to metals could have built up in this population (see for example, Bryan, 1976).

DISCUSSION

Comparison of metal concentrations

Concentrations of all 10 metals in large *Scrobicularia* from the contaminated Gannel Estuary exceeded those in the Camel Estuary, the differences for lead, cobalt, cadmium and zinc being approximately an order of magnitude. In both populations, the digestive gland contained the highest concentrations of most metals and contained up to 96% of the total in the body. Only for silver, copper and iron was less than 50% of the total found in the digestive gland, a similar observation to that made by Bryan & Uysal (1978) in animals from the Tamar Estuary. They suggested that a high percentage in the digestive gland indicates that uptake from ingested sediment is particularly important. In a few instances, concentrations in other tissues exceeded those of the digestive gland. This was found for silver in the gills/labial palps of both populations and for manganese and iron in the mantle/siphons of the Gannel animals and suggests that uptake of these metals from solution may be quite significant. Evidence for the uptake of manganese and iron from solution has been given by Bryan & Uysal (1978).

The influence of size on concentrations in the Gannel population was sometimes considerable. With the exception of iron and manganese, which were independent of size, all other concentrations increased with increasing size and this was particularly obvious for lead, cobalt, cadmium and zinc, the main contaminants. Bryan & Uysal (1978) obtained similar results in the Tamar Estuary, which like the Gannel receives contamination from old lead mines. However, in the Tamar animals, concentrations of copper and silver fell markedly with increasing size. In the uncontaminated Camel animals, the influence of size was much less marked and important differences from the Gannel animals for cadmium and zinc will be discussed below.

When animals were exchanged between the Gannel and Camel Estuaries, manganese and iron appeared to be absorbed or lost most rapidly, followed by silver and copper and then by chromium and nickel. Levels of lead, cobalt, cadmium and zinc, for which the contrast in concentration between the populations was greatest, approached those of the native animals very slowly and, after one year, the process of change was far from complete. In this and several other respects these four metals show similarities, but there are also distinct differences which are considered below.

Lead

This is the most notable contaminant of the Gannel Estuary and levels in the animals were the highest we have so far observed. In this population, the concentration in the digestive gland increased markedly with increasing size whereas that of the remaining tissues stayed constant. Thus, the proportion of metal contained by the digestive gland increased with increasing size and exceeded 90% of the total in large animals. Almost certainly, lead was being absorbed from ingested sediment by the digestive gland faster than it could be excreted or diluted by growth. In the other tissues, many of which are in direct contact with the water, lead may be absorbed from solution. The stable lead concentration observed in these tissues may be balanced by excretion, as was observed in *Mytilus edulis* by Schulz-Baldes (1974). On the other hand, the concentration may be kept at a constant level by transfer to the digestive gland.

In the Camel population, the level of lead in the digestive gland increased with size, although the concentration was 36 times lower than in the Gannel animals. In the remaining tissues, the concentration was independent of size and about 13 times lower than in the Gannel animals. After transfer to the Gannel Estuary, the lead level of the digestive gland of Camel animals increased by 65 ppm during the first month but subsequent increases were very slow. In the converse experiment, the digestive gland of Gannel animals lost about 100 ppm, or 10%, in the first month but very little in subsequent months. Thus, part of the digestive gland will exchange lead fairly readily, whereas the bulk of lead, at least in the Gannel animals, is stored in a very inert form. Results for the remaining tissues showed that about half of the lead was lost fairly easily from the Gannel animals whereas the remainder was relatively inexchangeable. At present, it is not known in what form the tightly bound lead in the tissues is stored.

Cobalt

Much of what has been said regarding lead applies also to cobalt. However, cobalt appeared to be absorbed or lost even less readily than lead during the exchange experiments.

Cadmium

In the Gannel animals, the concentration increased with increasing size not only in the digestive gland but also in the remaining tissues, where the concentrations of lead, cobalt and zinc were independent of size. In the Camel animals, on the other hand, the concentration of cadmium was independent of size in all tissues. These results add weight to the suggestion by Bryan (1976) that the slope relating dry weight to concentration of cadmium in *Scrobicularia* becomes steeper under contaminated conditions. Similarly, Boyden (1974, 1977) reported that, under contaminated conditions, the level of cadmium in the limpet *Patella vulgata* increased more rapidly with increasing size. Like cobalt, cadmium appeared to be absorbed and lost by *Scrobicularia* even more slowly than lead during the transfer experiments.

Zinc

The concentration in the digestive gland of the Gannel animals increased with increasing size whereas that of the remaining tissues did not. In this it resembled lead and cobalt, but differed from cadmium. Unlike lead and cobalt, but like cadmium, the concentration of zinc in the Camel digestive gland was independent of size. Thus, like cadmium there is some suggestion that the slope relating concentration to size is reduced in uncontaminated conditions. Work on other estuaries suggests that, although this trend may exist, it is not a very simple relationship, possibly because the rate of growth as well as zinc availability may influence the slope.

Although there was a 15-fold difference between levels of zinc in the digestive glands of large animals from the two populations, the difference for the remaining tissues was only 1·5 times. This suggests that zinc levels in the remaining tissues might be regulated. In the Tamar Estuary, Bryan & Uysal (1978) found zinc concentrations of the same order, but also observed changes in concentration with distance along the estuary. Thus, if zinc regulation exists, it is by no means perfect.

In the transfer experiments, zinc like cadmium was absorbed and lost very slowly by the digestive glands of the two populations.

Scrobicularia *as an indicator of the biological availability of metals*

The large concentration differences observed between animals from the two populations used in the present work, plus evidence from populations in the Tamar and Looe Estuaries (Bryan & Hummerstone, 1977; Bryan & Uysal, 1978) suggests that *Scrobicu-*

laria has considerable potential as an indicator of the biological availability of heavy metals. Its main role is probably as an indicator of the availability of metals in surface sediments, since the digestive gland, in which a high percentage of most metals is stored, probably absorbs them from ingested sediment. For this reason, analysis of the digestive gland separately may give the best indication of availability in sediments. However, for metals such as lead and cobalt, where more than 80% is contained by the digestive gland of large animals, analysis of the whole soft parts gives an equivalent result. Where the digestive gland contains less than 50% of the total metal, as has been found for silver, copper and iron, then analysis of the whole animal may reflect some intake from solution in the water also. Evidence for significant uptake of iron and manganese from solution by tissues such as the mantle/siphons was found by Bryan & Uysal (1978) in the Tamar Estuary. These observations suggest that, when using *Scrobicularia* as an indicator, it may be an advantage to analyse the digestive gland and remaining tissues separately.

The fact that concentrations of metals in *Scrobicularia* are frequently size dependent means that for comparative work it is necessary to use animals of equal size. We normally use animals of about 40 mm in length, so that the dry weight of the soft parts usually lies between 0·3 and 0·4 g. Using a constant size of animal is a compromise since, due to differences in growth rate, animals of equal size are not necessarily of the same age and age may be important. An alternative method would be to compare the largest animals in different populations and assume that they are of equal age. However, we have no evidence at present that this is better than comparing animals of equal size. It is possible to age a population by the method used in this paper, but this is a tedious process and is least reliable for the large animals where the annual growth marks are close together.

One particular feature of the effect of size, was the finding that concentrations of zinc and cadmium increased markedly with increasing size in the Gannel animals, but were independent of size in the Camel animals (Fig. 2). If the slope increases with increasing contamination, then the relationship between the concentration in a 40 mm animal and that in the environment is unlikely to be one of direct proportionality, as it would probably be if the slopes at different levels of contamination were parallel. This latter situation is more nearly approached by results for other metals such as lead, cobalt and nickel (Table 3). The fact that increasing contamination may sometimes produce a disproportionately large increase in the metal concentration of an organism does seem to be a good argument for using biological indicators in preference to direct measurements on water or sediments. After all, the object of controlling contamination is to protect the organisms and their predators.

The possibility that the regulation of zinc may occur in tissues other than the digestive gland was suggested by the comparatively small difference observed between the two populations. The evidence was not particularly strong, but does point to the apparent necessity to have relatively high concentrations of zinc in most tissues even in the absence of contamination. Whereas the minimum possible concentration of inessential metals such as lead and cadmium is likely to be zero and that for some essential metals such as cobalt may be very low, the minimum concentration of zinc seems likely to be appreciable. The possibility that there is an essential baseline level of zinc, and the variable effect of size, are both factors which may complicate the relationship between concentrations of zinc in the animals and those in the environment compared with most other metals.

When, during the transfer experiments, native animals were analysed from both estuaries, it was found that, particularly in the digestive gland, there was little seasonal variation (Figs. 4 and 5). Evidence from the transferred animals showed that a sharp fall in concentration could sometimes be explained by dilution during a period of rapid tissue growth. Changes of this type are certainly a good argument for examining results both in terms of concentration and of total body content as Boyden (1974, 1977) has done, since the growth of soft tissues can change the concentration in an animal of fixed shell length without necessarily changing the metal content.

The transfer experiments (Figs. 4 and 5) showed that although some changes in concentration in transferred animals could be detected within a month, major changes occurred very slowly. It is clear that *Scrobicularia* should be regarded as a long-term integrator of the chronic type of contamination normally associated with sediments. For this reason, there seems little advantage to be gained by frequent sampling. The most suitable times appear to be early spring or autumn, thus avoiding the possible complications of gonad development during the summer. The minimum sample is probably one pooled from 5 to 6 animals having a shell length of about 40 mm. Obviously, additional samples are a great advantage, especially if they cover a wide range of sizes. Almost certainly, changes in the availability of some metals in the environment will show up as changes in the slope of the concentration–size regression as well as in the total concentration.

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