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Studies on the Transfer of Lead in an Intertidal Food-chain

Stephen Charles Frederick Palmer

ABSTRACT

The levels of lead contamination in the intertidal sediments and flora at Lindisfarne, NE England, were studied from September 1990 to September 1991. The daily throughput of lead for Wigeon *Anas penelope* was estimated, and liver lead burdens were determined.

Around the upper shore, the mean density of spent shot in the top 10 cm of sediment was 53.1 pellets/m², the product of many seasons' wildfowling activity. The density encountered was higher than any previously reported from wildfowling sites in Britain.

An experiment was established to examine settlement and degradation rates of lead shot under natural intertidal conditions. Settlement rates were slow, except in loose sand. Degradation was also slow. Only 2% weight loss occurred in thirteen months.

The levels of chemically available lead in the sediments were determined by acid extraction. The grain size composition was the principal factor explaining variation in lead concentration between sites. The highest level recorded was 16.2 ppm. Lindisfarne is therefore considered to be a site of low to moderate lead contamination.

The lead concentrations in *Zostera* spp. were higher during Autumn 1990 than during Summer 1991. This may be due to seasonal effects or yearly differences in growth rates. During Autumn 1990, when they formed the main food of Wigeon, *Zostera* leaves contained around 20 ppm lead. *Enteromorpha* and *Salicornia* contained around 14 and 8 ppm lead respectively.

The lead content of Wigeon faeces was around 15 ppm. It was estimated that the difference between the quantity of lead ingested and excreted in the faeces for a Wigeon feeding on *Zostera* leaves was 1.06 mg per day.

Only 8% of a sample of Wigeon shot at Lindisfarne had a liver lead concentration above 5 ppm dry weight. Such low liver lead concentrations, generally considered as normal background levels, are inconsistent with the estimated lead retention rate.

**Studies on the Transfer of Lead
in an Intertidal Food-chain**

Stephen Charles Frederick Palmer

Thesis submitted for the Degree of
Master of Science
in the University of Durham

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Department of Biological Sciences

July 1992



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1 INTRODUCTION

1.1 Background

During the last thirty years there have been several investigations in Britain into acute lead-poisoning of wildfowl resulting from the ingestion of lead pellets in the form of discarded fishing weights (Birkhead, 1982; Sears, 1988) or spent gunshot (Olney, 1960; Thomas, 1975; Mudge, 1983; Spray and Milne, 1988). Lead fishing weights have been abolished as a result, but, although there has been much recent debate regarding the replacement of lead shot by non-toxic steel shot (summarised in Pain, 1992), practically no substitution has to date occurred in Britain. It has been estimated that 2000 tonnes of lead are deposited in British wetlands per year (Kear, 1990).

Shorebirds are known to accumulate toxic heavy metals from their food to a very limited extent when feeding on intertidal invertebrates on polluted estuaries (Ward, 1979; Evans *et al*, 1987). However, the possibility that wildfowl might be exposed to chronic plumbism by consuming contaminated vegetation over a lengthy period has received scant attention. Behan *et al* (1979) suggested that lead in the natural diet was unlikely to cause chronic poisoning, but only by indirect comparison of lead content in freshwater vegetation with the results of a dosing experiment (Coburn *et al*, 1951).

A number of pilot studies into lead pollution at Lindisfarne, north-east England, (Evans *et al*, 1988; Evans and Oliver, 1989; Abbott, 1990; Collins, 1990) have indicated levels of lead in the intertidal sediments, flora and invertebrates sufficiently high to cause concern that the two most numerous wintering species of wildfowl, Wigeon *Anas penelope* L. and Light-bellied Brent Goose *Branta bernicla hrota* O. F. Müller, which both feed almost exclusively on the intertidal flora, might be at risk from chronic lead-poisoning.

1.2 The Lindisfarne Study Area

The Lindisfarne National Nature Reserve was established in 1964, and covers an area of 3,300 ha of intertidal sand- and mud-flats, coastal sand-dunes and salt-marshes on the north Northumberland coast in north-east England (55° 40' N, 2° 50' W). It is of international importance for its wintering populations of six species of wildfowl and



shorebirds, including Wigeon and Brent Geese, and of national importance for a further seven species (Prater, 1981). Although formerly supporting the highest numbers of Wigeon in Britain, the peak winter count of around 20,000 to 25,000 birds has in recent years been exceeded by those at the Ouse Washes and the Ribble Estuary (Salmon and Kirby, 1990; Kirby, 1991). Wildfowling on the reserve is controlled by permit, with 500 licences issued each year plus a small number of punt-gunning licences.

It has been assumed that the primary source of lead contamination at Lindisfarne is from the erosion of spent gunshot deposited within the sediments as a result of wildfowling activity. There is no major industrial source of pollution in the area, and freshwater input is negligible. However, areas that can be considered naturally lead-free probably do not exist world-wide, and it is therefore not possible to make an accurate estimate of the proportion of the total lead burden in the sediments attributable to wildfowling.

1.3 Study Species: the Wigeon

The Wigeon is a smallish herbivorous duck which, although breeding in limited numbers in Britain, is principally a winter immigrant, breeding in Iceland and the boreal regions of Europe and Asia (Owen and Mitchell, 1988). The peak winter count in the U.K. has been about 260,000 birds in recent years (Salmon and Kirby, 1990; Kirby, 1991). Wigeon flocks are highly mobile within the British Isles, with the general pattern being of a south-westerly movement as the Winter progresses, so that numbers in northern and eastern Britain normally peak before the turn of the year (Owen and Mitchell, 1988; Mitchell and Owen, 1990), as is the case at Lindisfarne.

Traditionally Wigeon have wintered in coastal areas, although an increasing proportion are now utilising inland wetland sites (Owen and Williams, 1976; Owen and Thomas, 1979). In part the change may be due to the decline in *Zostera* stocks, a major component of the diet in coastal areas (eg. Charman, 1977), following the 'wasting disease' of the 1930's, but also the provision of refuge areas within inland wetland sites has been an important factor. Wigeon feed principally by grazing, either below the high tide mark on intertidal flora, or on short grass swards, usually in close proximity to water (Mayhew and Houston, 1989).

Wigeon annual mortality is high, as is to be expected for a quarry species. The survival rate of adults in the north-west European population was estimated from ringing recoveries to be 53% (Boyd, 1962), but this is likely to be an overestimate. There is some evidence that males may have a higher survival rate than females, presumably owing to inter-sexual competition for the best feeding sites in wintering areas (Owen and Mitchell, 1988).

1.4 Objectives

The principal objectives of the research reported in this thesis were to examine the levels of lead in the intertidal sediments at Lindisfarne, to determine the extent of lead uptake by the intertidal flora, and to estimate the transfer to and retention of lead by Wigeon, which feed solely on these flora (the Eelgrasses *Zostera angustifolia* (Hornem.) Reichenb. and *Z. noltii* Hornem., the Glasswort *Salicornia europaea* L. and the green algae *Enteromorpha* spp.) during the Autumn and early Winter months.

The study was carried out in parallel with the second year of a three-year investigation into the feeding ecology of wildfowl (concentrating primarily on Brent Geese) at Lindisfarne, by S. M. Percival of Durham University. In order to improve efficiency, and to increase sample sizes, some data were collected jointly.

2.1 Study Areas

At the outset of the project it was necessary to select a number of sites for the collection of *Zostera* samples throughout the study. Practical considerations dictated that these be readily accessible from the shore, and, in order that comparisons could be made between the two *Zostera* species, both should be present at each site. Further considerations were the nature of the substrate and the presumed variation in shooting pressure on different areas of the mud-flats. The four sites selected for *Zostera* sampling were also used for sediment sampling.

Site A	Holy Island Sands	(NU101424 (approx.))
	Substrate:	Hard sandy mud - substantial pools
	Shooting intensity:	Low (but formerly higher)
Site B	Fenham Mill	(NU092405)
	Substrate:	Soft mud overlying rocks - small shallow pools
	Shooting intensity:	Moderate - shooting butt on shoreline used occasionally
Site C	Reely Law	(NU105390)
	Substrate:	Very soft mud overlying rocks - small drainage channels
	Shooting intensity:	High - shooting butt on Harvey's Island used regularly
Site D	Stinking Goat	(NU122381)
	Substrate:	Firm mud overlying shell layer and fine gravel - intersected by channels, including Stinking Goat burn providing limited freshwater throughput
	Shooting intensity:	Moderate

Site A was situated roughly in the centre of Holy Island Sands at the edge of a small *Zostera* bed covering an area of approximately 500 m by 200 m. Sites B, C and D were all situated around the upper

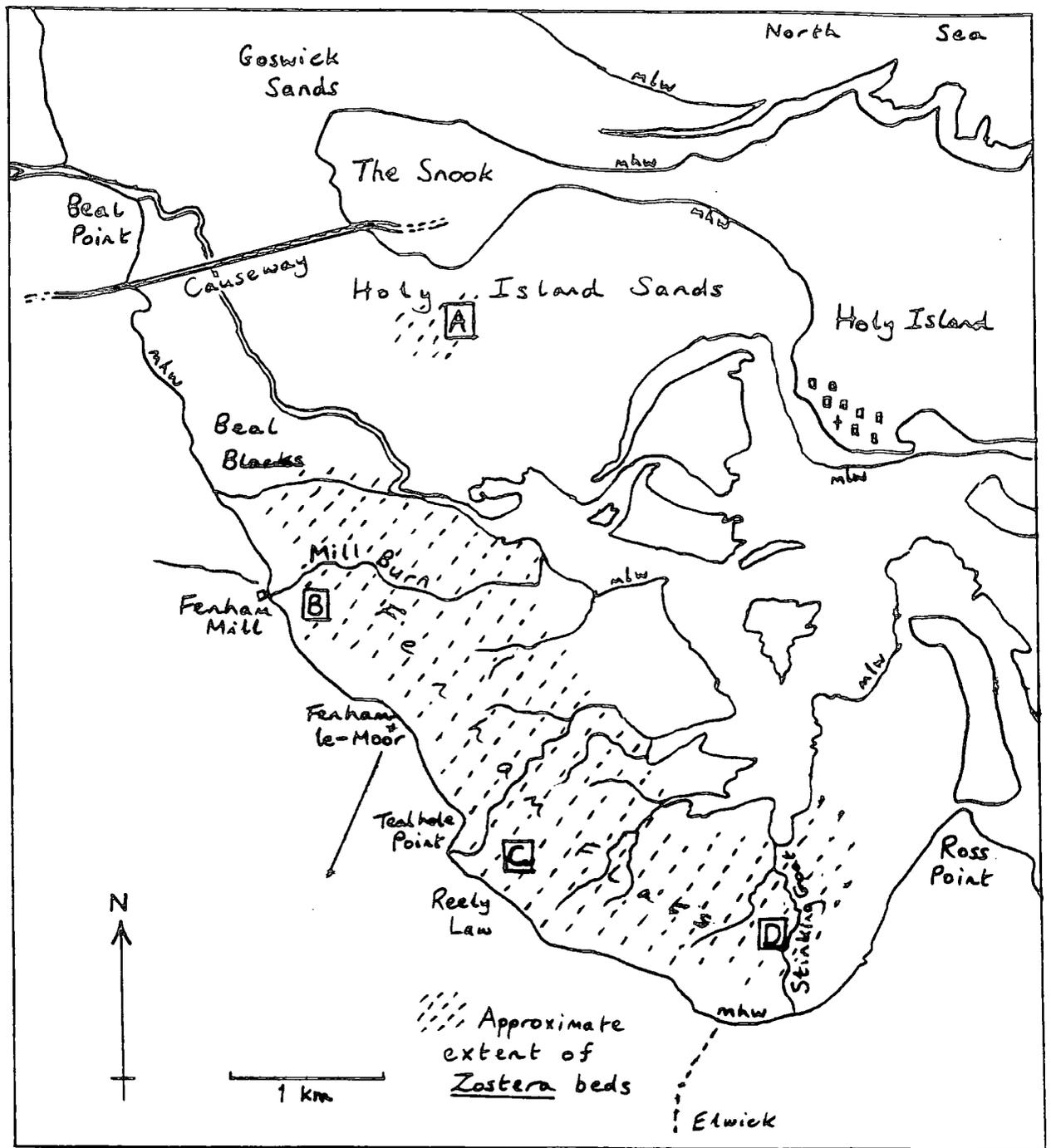


Figure 2.1 The main intertidal area of the Lindisfarne NNR (excluding Budle Bay), showing the four principal study areas: Holy Island Sands (A), Fenham Mill (B), Reely Law (C) and Stinking Goat (D). Other sites from which samples were collected occasionally are also shown. The diagonally hatched areas mark the approximate extent of the *Zostera* beds during Autumn 1990.

shore of Fenham Flats, a large area of intertidal mud-flats, mostly colonised by *Zostera*, along the mainland shore of the NNR. Site B lay at about 100 m from the mean high water level (MHW), site C at about 200 m from MHW and site D at about 250 m from MHW. The sites are shown in fig. 2.1, together with other areas from which samples were collected on an irregular basis.

2.2 Nomenclature

Previous studies at Lindisfarne have referred to the larger-leaved of the two *Zostera* species present as the Eelgrass *Z. marina* L., but more recently it has been identified as the Narrow-leaved Eelgrass *Z. angustifolia* (formerly *Z. marina* var. *angustifolia*). The two species are very closely related and similar in morphology, but *Z. marina* is usually confined to areas below the low-tide mark, whereas *Z. angustifolia* is generally found in the mid-tide region and in areas of shallow water (Clapham et al, 1987). Both species differ in ecology from the much finer-leaved Dwarf Eelgrass *Z. noltii*.

2.3 The Determination of Lead Content by Atomic Absorption Spectroscopy

Many methods have been developed for determining the concentrations of trace elements in biological materials. The technique employed during this study, flame atomic absorption spectroscopy (AAS), is well-established, but by current standards relatively crude, being limited for lead to concentrations in the range from about 1 to 5 ppm. However, once suitable extract solutions have been prepared, the actual determination of the metal concentration is rapid, permitting a large number of samples to be processed in a short time.

All lead concentrations are given in parts per million (ppm) by dry weight (dw), unless otherwise indicated.

2.3.1 Preparation of Sample Material

Vegetation samples were initially washed with water under hose pressure. Wigeon livers were washed with water, and excess blood was removed with absorbent paper. All organic samples were dried for a minimum of 48 hours in a vacuum oven at 50 °C, and then homogenised as necessary: *Zostera* and *Enteromorpha* samples were chopped finely by passing through a small mechanical 'Moulinex' grater; *Salicornia* seed-

heads were cut whole from the stalks; Wigeon livers were found to be extremely brittle after drying, and simply broken into a few pieces. Wigeon faeces were analysed individually.

A single sub-sample from each sample was weighed to an accuracy of 10 mg into a small (25 or 50 ml) conical flask. Whenever possible, the weight of sample was such that a final extract concentration in the range 2 to 4 ppm lead would be obtained (eg. about 0.8 g for *Zostera* samples). 'AnalaR' grade concentrated HNO_3 was added, sufficient to cover the sample (5 - 10 ml), a small glass funnel was placed in the neck of each flask to act as a refluxing condenser, and the samples allowed to stand overnight at room temperature to allow for the initial effervescence to subside. (Generally this was not a problem, except for the liver samples, but effervescence often occurred as soon as the flask was warmed, and care was therefore necessary at that stage.)

The samples were then warmed on a hot-plate and refluxed for about 24 hours, by which time all organic material was completely oxidised. The glass funnels were then removed, and the contents boiled until dry. In some cases (particularly for *Zostera* roots, in which very small sediment particles often remained, despite washing under pressure) bumping was a problem at this stage. Any sample believed to have lost some of the material was noted as a possible error, and reprocessed if sufficient material was available.

Once dry, the residue was re-dissolved in a known quantity of 'AnalaR' grade 3M HCl. If necessary the flask was warmed gently on the edge of the hot-plate for a short period (usually less than 30 seconds was sufficient) to ensure that any residue adhering to the base of the flask was dissolved. The resulting solutions were filtered (to remove any particles which could otherwise block the AAS capillary tube) into individual screw-cap bottles (usually plastic, but sometimes glass). Generally the volume of HCl in which the residue was dissolved was 5 ml, but for samples for which there was only a small quantity of material available (particularly *Z. noltii* leaves), or for which the lead concentration, as indicated by previous samples, was expected to be low (Wigeon livers), a smaller volume (minimum 2 ml) of HCl was used.

The preparation of sediment samples was considerably simpler, and is discussed more fully in chapter 3 and appendix 2.

Struempler (1973) showed that the loss of lead cations from a 10 ppb solution by adsorption onto the walls of the container could be complete within 4 days at low acidity (pH 6), but only 5% in that time at higher acidity (pH 4 and pH 2). To minimise possible loss by adsorption, determination of lead by AAS was always completed within 24 hours of preparing the extracts. Moreover, the concentrations of lead in this study were more than 100 times that examined by Struempler, and since all extracts were prepared in a strong acid (except for some sediment extracts in 5% acetic acid), loss by adsorption is presumed to be negligible.

All glassware was acid-washed (3M HCl) and rinsed with distilled water prior to processing each batch of samples. The screw-top bottles were scrubbed with detergent solution and rinsed with distilled water. Reagent blanks run through each batch showed no detectable levels of lead within the sensitivity limits of the AAS equipment.

2.3.2 Measurement of Lead Concentration

Determination of the lead concentration in the extract solutions was made by aspirating the extracts in a Pye-Unicam SP9 AAS machine and measuring absorption at wavelength 217 nm. A calibration curve was established for each batch from standard solutions of lead (1 to 5 ppm) in 3 M HCl. The standards were prepared afresh at least fortnightly from a master standard of 1000 ppm lead in distilled water. The standards were aspirated at least twice for each batch, and the mean absorption reading for each concentration used for calibration, thereby allowing for any slight variation in absorption during the course of processing a batch.

The calibration curve was invariably a straight line within the range of standards aspirated, and a linear regression equation was therefore calculated for each batch ($r^2 > 0.995$ for all batches). The lead concentration of the original sample material was then calculated from the absorption reading and calibration parameters thus:

$$[\text{Pb}]_{\text{sample}} = (m * \text{abs} + c) * V_{\text{HCl}} / W_{\text{sample}}$$

where

m	slope of regression of $[\text{Pb}]_{\text{standard}}$ on absorption reading
c	intercept of regression of $[\text{Pb}]_{\text{standard}}$ on absorption reading
abs	absorption reading for sample extract
V_{HCl}	volume 3M HCl used to dissolve residue
W_{sample}	weight of sample material oxidised

2.4 Reliability of Analytical Procedures

As described in section 2.3 above, the estimate of lead concentration for each sample obtained was a process incorporating several stages, at each of which inaccuracy could occur. Ideally therefore, every sample collected would have been analysed twice, to control for variations in the analytical techniques employed, but the time available did not permit such an approach, given the large number of samples analysed. Nor, in many cases (particularly for *Z. noltii* leaves), was there sufficient dried material.

The following methods were therefore adopted to check the reliability of the data obtained.

2.4.1 Matrix Interference Effects

Although AAS is a highly specific technique, problems involving chemical interference with the atomisation in the flame of the element under study may occur, especially interference by major cations present in the matrix (Cooke, 1969). In particular, high concentrations of calcium and, to a lesser extent, sodium present in samples of marine origin may distort estimations of lead concentrations by flame AAS (Waughmann and Brett, 1980)

One method of overcoming interference effects is to prepare standards containing concentrations of the background cations equal to those present in the sample matrices (Cooke, 1969), but in this study the concentrations of background cations were not known, and may, in any case, have differed between the different types of sample material. Instead, the possible effects of interference were examined in a sub-sample of the extracts using the standard additions technique (Hopkin, 1989).

From each extract solution, four aliquots of 0.75 ml were taken. These were diluted with an equal volume of blank solvent (3M HCl) and three lead standards (in 3M HCl): 1 ppm, 3 ppm and 5 ppm lead (except for sediment batches 2 and 3 (see table 2.1), when 1 ppm, 2 ppm and 4 ppm lead standards were used). The four aliquots were aspirated and the absorption reading recorded for each. The lead content of each sample was calculated from the regression line of absorption against concentration of standard, as the concentration (absolute value) of the lead standard which would give zero absorption, ie. intercept / slope. For comparison, the concentration of each original extract was

determined directly by aspirating the extract and converting the absorption reading using a standard calibration curve.

The results of the two methods are compared in table 2.1, and the percentage difference (ie. the difference between the lead concentration determined by standard addition and that by direct measurement expressed as a percentage of the former) compared with the concentration (as determined by standard addition) by the type of the original sample in fig. 2.2a, and by the site from which the sample was obtained in fig. 2.2b. (NB The concentrations shown in table 2.1 are of the extract solutions, and not of the original sample material.)

Of the 33 standard addition tests performed on four groups of extracts, 27% showed a difference of more than 10%, which is a cause of some concern. However, figs. 2.2a and b show no clear pattern to the differences; neither the type of the original sample material nor the site of origin explained the variation encountered (2-way ANOVA; $F_{3,25} = 2.03$, n/s and $F_{4,25} = 0.46$, n/s respectively; % difference (d) transformed as $(100 + d)^{0.5}$).

By virtue of the dilution with an equal part of standard solution, the two aliquots diluted with blank solvent and 1 ppm standard gave relatively low absorption readings, often less than 30 units. Since the absorption reading was accurate to 2 units, and there were only four points on each regression line, some part of the variation encountered may be due to this source of inaccuracy.

The standard addition test must therefore be regarded as inconclusive, but the absence of a trend in one particular direction indicates that matrix interference was probably not a major problem in this study.

2.4.2 Detection of Batch Effects

The equipment available permitted batches of up to sixty samples to be processed at one time. Generally most batches comprised only one type of sample, although some batches contained two or more types. The principal consideration in testing for batch-related effects was the possibility that an undetected problem had occurred in the AAS step, such as an inaccurate calibration. Batch effects were examined only within type of sample, and only for sediment, *Zostera* and Wigeon faeces.

Table 2.1 Standard Addition Analyses - Comparison with Measured Lead Concentrations

Sediment extracts (3M HCl)

Batch	Site	Plot/ Sample	Sieve mesh size	Pb (ppm) by std. addn.	Pb (ppm) measured	Diff. %
1	B	04/3	40	2.86	2.66	-7.5
1	C	02/1	40	3.40	3.42	+0.6
1	D	01/3	40	1.28	1.35	+5.4
2	A	02/1	40	1.34	1.46	+9.0
2	B	02/2	40	2.75	2.79	+1.5
2	C	02/3	40	2.79	2.84	+1.8
2	D	02/3	40	1.99	2.22	+11.6
3	A	09/1	40	1.75	1.57	-10.3
3	B	08/1	40	1.32	1.24	-6.1
3	C	12/1	40	2.17	1.84	-15.0
3	D	07/1	40	2.24	2.21	-1.0
4	A	03/3	200	2.32	2.57	+11.0
4	B	01/2	200	2.64	2.97	+12.7
4	C	01/4	200	2.61	2.75	+5.4
4	D	03/3	200	2.78	2.71	-2.6

Zostera extracts

Batch	Site	Species/ Part*	Month	Pb (ppm) by std. addn.	Pb (ppm) measured	Diff. %
12	B	a/l	7	1.82	1.87	+2.6
12	D	n/r	6	3.51	3.63	+3.4
12	A	a/l	7	1.19	1.33	+11.9
12	D	n/r	7	1.76	1.87	+6.4
13	D	a/r	5	2.49	2.54	+2.0
13	D	a/l	7	1.56	1.60	+2.1
13	B	a/l	7	1.67	1.60	-4.2
13	B	n/r	7	1.07	1.13	+5.2
15	D	a/r	6	2.51	2.16	-14.2
15	A	a/l	6	1.08	1.14	+8.6
15	A	n/r	6	1.36	1.40	+3.4
15	A	a/l	5	0.67	0.69	+3.0

* a = *Z. angustifolia*, n = *Z. noltii*, l = leaves, r = roots

(continued)

Table 2.1 (continued)

Enteromorpha extracts

Site	Month	Pb (ppm) by std. addn.	Pb (ppm) measured	Diff. %
D	6	3.03	2.90	-4.2
B	7	3.43	3.15	-8.1
A	7	2.81	2.48	-11.7

Salicornia extracts

Site	Month	Pb (ppm) by std. addn.	Pb (ppm) measured	Diff. %
Fenham Mill	7	2.04	1.85	-9.3
Beal Blacks (sand)	7	2.04	1.90	-6.8
Beal Blacks (mud)	7	2.49	2.02	-18.7

FIGURE 2.2a Standard Addition Analyses - by Type
 Difference between measured lead content
 and value obtained by standard addition

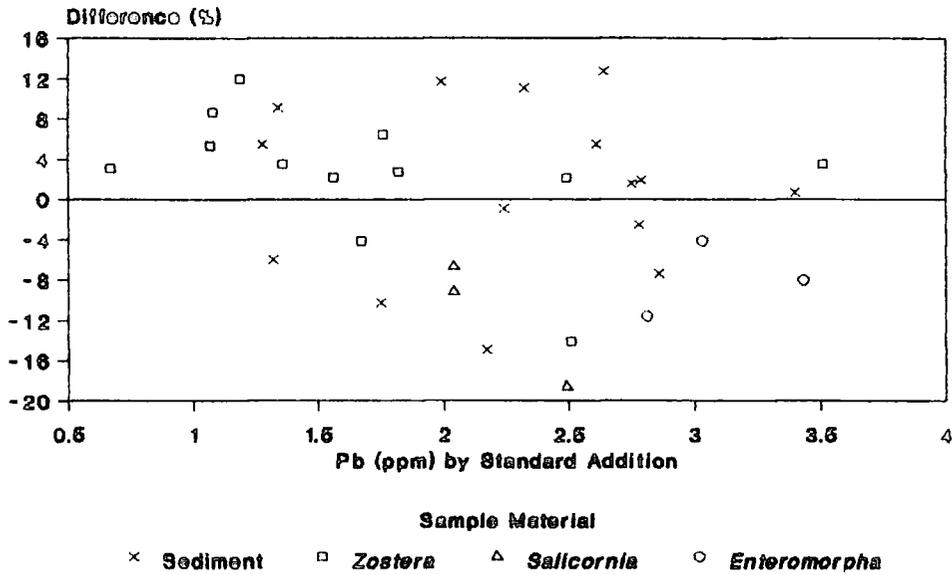
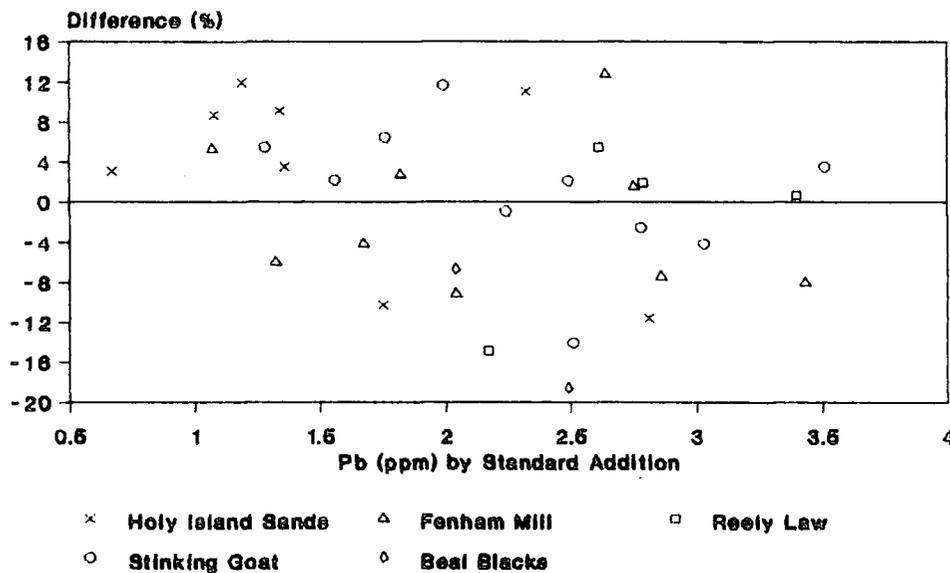


FIGURE 2.2b Standard Addition Analyses - by Site
 Difference between measured lead content
 and value obtained by standard addition



The data for undifferentiated and fine sediment fractions (as defined in chapter 3) were analysed separately. For both fractions, fifty-nine lead determinations were spread over three batches. In neither case was the batch a significant effect in explaining the variation in lead concentration (2-way ANOVA on log-transformed data of batch and site effects; $F_{2,53} = 1.58$, n/s and $F_{2,53} = 1.72$, n/s respectively).

Since all *Zostera* samples collected during Autumn 1990 and January 1991 had been analysed before any samples were collected during Spring and Summer 1991, the two periods were treated separately, but as *Z. angustifolia* and *Z. noltii* samples were intermixed within the batches all samples for each of the two periods have been analysed together. However, data for September 1991 were omitted from the second analysis as the samples had all been processed together in a single batch at the end of the study. In neither period was the batch a significant effect (5-way ANOVA on log-transformed data of batch, species, site, part and month effects; batch in Autumn 1990, $F_{7,319} = 1.33$ n/s; batch in Spring/Summer 1991, $F_{3,202} = 0.33$, n/s).

Similarly, there was no significant batch effect for Wigeon faeces (2-way ANOVA on log-transformed data of batch and sampling date effects; batch, $F_{2,99} = 1.31$, n/s).

It is therefore clear that the analytical procedures were reliable between batches.

2.4.3 Detection of Outliers

The number of samples obtained from each site on each sampling date for each of the vegetation types, on each date for Wigeon livers and faeces and from each site for sediment samples permitted a test for statistical outliers to be made on each such group of data. The statistic T of Grubbs (1969) was employed, being straightforward to calculate for large numbers of tests. Outliers were rejected at $P < 0.01$. Although such outliers could quite genuinely be due to variations in lead content, they could equally well be due to analytical errors, eg. mis-weighing a sample or undetected loss of extract due to bumping. Whenever possible a sample rejected as an outlier was re-analysed. If it was not possible to accept the repeated result, the sample was omitted from the statistical analyses of lead concentration (except for Wigeon livers - see section 5.6.2).

2.4.4 Analysis of Duplicates

One batch of duplicate sediment samples and two batches of duplicate *Zostera* samples were analysed. Some of the preliminary analyses of sediment samples (appendix 2) were also duplicated, but as these were not incorporated into the main body of data, they are not discussed here.

The lead concentration in the undifferentiated sediment (see chapter 3) from 48 cores was significantly different between the two batches (paired $t_{47} = 4.94$, $P < 0.001$). Higher lead concentrations were obtained from the second batch for all but four of the samples (91.7%), but in only two of these cases was the difference greater than 15%. The difference may have been principally due to lower weights of sediment samples analysed in the second batch. Moreover, the difference between replicates accounted for only 2.5% of the total variation in lead concentration over all the 96 samples (ANOVA on log-transformed data; residual s.s. as proportion of total s.s.), and is not therefore regarded as being of particular importance.

Of the two batches of *Zostera* duplicates analysed, one comprised samples from Autumn 1990 and January 1991, the other from Summer 1991. Only 4.8% and 5.6% respectively of the total variation observed in each case was due to differences between replicates (ANOVA on log-transformed data; residual s.s. as proportion of total s.s.). However, the difference between replicates was not significant for the former period (paired $t_{24} = 1.98$, n/s) but was for the samples from Summer 1991 (paired $t_{67} = 5.12$, $P < 0.001$). In part, this result may be a function of the number of samples analysed in each duplicate batch, but not entirely so, since for the Autumn 1990 batch, only 8% of replicate pairs showed a difference between the two replicates of more than 20%, whereas 35% of pairs did so amongst the Summer 1991 replicates.

Within the duplicate batch of Summer 1991 samples, there was evidence of elevated lead concentrations for samples which had been dissolved in a reduced volume of HCl (for the reason that only a small amount of material was available for the replicate batch). The use of a reduced volume of HCl in the duplicate batch was the only significant effect in explaining the variation in the ratio between pairs of replicates (6-way ANOVA of site, species, part, month, original batch number and volume effects; volume, $F_{1,57} = 9.17$, $P < 0.01$). 12 out of 15

pairs of replicates (80%) in which a reduced volume of HCl was used for the second replicate differed by more than 20%, but only 12 out of 53 pairs (23%) in which the full 5 ml was used did so ($\chi^2 = 14.42$, $df = 1$, $P < 0.001$).

Clearly the analysis of smaller than usual *Zostera* samples, for which the residue was dissolved in a lower volume of HCl, was very likely to result in inflated lead concentrations, although it was not the only cause of such inaccuracies. Three possible reasons were considered:

- i) Reagent contamination. Since none of the reagent blanks showed any evidence of lead, this possibility is discounted.
- ii) The concentrations recorded for large samples dissolved in 5 ml HCl were actually too low, but were more accurate for smaller samples. This could occur if oxidation of large samples (approx. 0.8 g *Zostera*) were incomplete, but there was no evidence whatsoever of incomplete oxidation of samples.
- iii) The volumes of HCl were actually less than presumed by a fixed amount. Thus all lead concentrations would be inflated, but proportionally more so when a reduced volume of acid was used. To test this possibility, the pipette used throughout the analyses was calibrated by weighing the quantity of distilled water delivered at room temperature when set at each of the three volumes used in the *Zostera* analyses, ie. 5, 3.5 and 2.5 ml. Each calibration was performed five times, and the mean taken for each volume. The mean weights of water delivered were 4.91, 3.38 and 2.40 g respectively. Thus it does appear that the pipette may have consistently delivered a volume of HCl approximately 0.1 ml less than presumed, which may explain the apparently inflated lead concentrations observed for small samples. However, since the pipette calibration was only conducted at the very end of the study, it was not possible to assess accurately the level of inaccuracies incurred in the *Zostera* analyses.

Since some of the original samples in the main *Zostera* analyses, particularly *Z. noltii* leaves, had also been dissolved in a reduced volume of HCl, there may be some over-estimates in the main dataset due to this effect. Nevertheless, it is considered that, since six plots were usually sampled for each species, and the analyses of

variance show highly significant effects, the results discussed in chapter 4 are a reliable estimation of the lead content in *Zostera* at Lindisfarne.

One further point is noted from the analysis of duplicates. Since the duplicate batch of Autumn 1990 samples was not analysed until September 1991, the possibility that the highly significant difference in lead concentration between September 1990 and September 1991 *Zostera* samples, as discussed in section 4.4.1 (a), was due to a change in the analytical technique between the analysis of Autumn 1990 samples and Summer 1991 samples, is discounted.

3.1 Introduction

The levels of lead in the intertidal sediments were examined both in terms of the distribution of spent pellets and as chemically available lead within the sediment matrix. In addition, an experiment was set up to monitor the settlement rate of pellets and the rate of degradation under natural conditions.

3.2 Distribution of Spent Pellets

In order to assess the relative shooting pressure at each of the four main study sites, an examination of the numbers of spent pellets present in the sediment was conducted.

In earlier studies, concerned with the ingestion of spent pellets by wildfowl, the numbers of pellets in sample sediment cores were determined by washing the sediment through a sieve of suitable mesh size to retain the likely range of pellets present, and then searching the remaining grit visually (eg. Mudge, 1984), a very time-consuming operation, with the likelihood of missing a proportion of pellets. More recently, sieved samples have been subjected to X-ray photography, highlighting the inefficiency of manual searching (Milne and Ramsay, 1987; Spray and Milne, 1988). Milne and Ramsay (1987) also investigated the X-ray photography of intact sediment cores, but rejected the technique as requiring long exposure times and greater quantities of X-ray paper. They were not, however, using intensifying screens in their study.

In this study a portable X-ray machine (PLH Medical K6 Electronic) was available, and preliminary trials, using intensifying screens, on cylindrical sections of sediment cores 5 cm deep produced satisfactory images from short exposure times of the order of 1 second, depending on the exact composition of the sediment. A 100% success rate was achieved in counting seeded pellets placed by a colleague in 36 cores taken from sandy and muddy Lindisfarne sediments.

3.2.1 Methods

Sediment cores were collected during the week commencing 21 January 1991, ie. effectively representing the end of the shooting

season. The number of pellets present in the sediment was therefore expected to be at its annual maximum at that time.

At each of the four study sites sixty cores of 8 cm diameter (area 50.3 cm²) were obtained in groups of four (at the corners of a square of side 1 m) at intervals of twenty paces along three parallel transects twenty paces apart. The area sampled at each site was in part coincident with the *Zostera* sampling area. The cores were placed individually in plastic bags to retain their shape, and transferred to Durham in plastic cartons.

Each core was cut into two horizontal layers: 0-5 cm and 5-10 cm depth. Material below 10 cm depth was discarded. The sections were laid out on plastic seed trays (six per tray) and the number of pellets in each section was determined by X-ray photography. The source to subject distance was 60 cm, and intensifying screens were used. Exposure times varied between 0.8 sec at 60 kV for the muddiest cores to 1.2 sec at 70 kV for the sandiest cores. If there was any doubt as to the number of pellets within a core section (generally only for the lower sections if they contained a considerable proportion of gravel), the sediment was washed through a 20-mesh sieve (or carefully ground and shaken through the sieve if already dried - see section 3.4.1) and a visual search for pellets conducted.

3.2.2 Results

A total of 53 pellets was located in the 240 cores examined. The distribution of pellets is shown in table 3.1. Over all four sites combined, a significantly greater proportion (73.6%) of the pellets was located in the upper 5 cm of sediment ($\chi^2 = 11.79$, $df = 1$, $P < 0.01$).

There was no significant difference in the number of pellets between sites B, C and D, either for the top 5 cm alone ($\chi^2 = 2.00$, $df = 2$, n/s) or for the complete 10 cm cores ($\chi^2 = 0.88$, $df = 2$, n/s). However, the number at site A was significantly lower than at the other three sites combined in both those respects ($\chi^2 = 6.23$, $df = 1$, $P < 0.02$ and $\chi^2 = 6.85$, $df = 1$, $P < 0.01$ respectively). The numbers of pellets located in the lower 5-10 cm sections were insufficient for statistical comparison.

The total area examined at each site was 0.3016 m². Since there was no detectable difference between sites B C and D, their data have been combined to yield an average estimate for Fenham Flats of

TABLE 3.1 **Distribution of spent lead pellets, January 1991**

**Number of pellets located in cores of total surface area
0.3016 m² at each site**

Site	0-5 cm	5-10 cm	Total
Holy Island Sands	3	2	5
Fenham Mill	10	7	17
Reely Law	10	3	13
Stinking Goat	16	2	18
Total	39	14	53

39.8 pellets/m² in the top 5 cm of sediment and 53.1 pellets/m² in the top 10 cm. On Holy Island Sands the corresponding estimates are 9.9 and 16.6 pellets/m² respectively.

The majority of the pellets located by X-ray were recovered, carefully washed, and weighed to an accuracy of 0.1 mg. The geometric mean weight was 128.5 mg (n = 48), and there was no significant difference between sites or depths (2-way ANOVA on log-transformed data; $F_{3,40} = 0.11$, n/s and $F_{1,40} = 0.30$, n/s respectively). Thus the mean weight of lead pellets in the top 5 cm of sediment is estimated to be 5.11 g/m² on Fenham Flats and 1.27 g/m² on Holy Island Sands.

3.2.3 Discussion

The present low level of shooting on Holy Island Sands is reflected in the low number of pellets recovered from that site. Since Brent are no longer a quarry species and Wigeon rarely feed there, the result is to be expected. In view of the low rate of pellet settlement and degradation (section 3.3), this situation has clearly pertained for a considerable number of seasons.

The similar density of spent pellets at the other three sites requires explanation, as the *a priori* assessment of shooting pressure (section 2.1) predicted a higher density in front of the shooting butt at Reely Law. Three possible explanations are offered:

- i) The shooting pressure at the three sites is in fact comparable. The butt at Fenham Mill may be used more often than was realised and there may be a considerable level of shooting from all points around the shoreline at night. Furthermore, the sea-wall at Stinking Goat used to be a popular location in heavy winds when Wigeon made regular flights between Budle Bay (3 km to the south of the study area) and Fenham Flats.
- ii) Reely Law was under-utilised during the 1990-91 season.
- iii) The shooting pressure at Reely Law is higher than at the other sites, but this was not reflected by the sampling area chosen. Fredrickson et al (1977) suggested that the majority of shot from a duck blind falls at a distance of between 100 and 200 m. The distance of the sampling area from the butt on Harvey's Island was not measured, but was certainly towards the lower end of that range, if not less.

In view of the findings regarding the settlement and degradation rates of pellets (section 3.3), a factor operating over a single season is most unlikely to have any appreciable effect on the number of pellets at a particular site, and therefore explanation (ii) is rejected. The remaining two possibilities could be examined by a more detailed investigation of shooting pressures and by the collection of further cores at differing distances from the shoreline and from shooting butts.

A previous investigation into the effects of lead shot at Lindisfarne (Evans et al, 1988) found a comparable pellet density (70.2 pellets/m²) at Reely Law and a particularly high density (127.3 pellets/m²) at Teal Hole (500 m NE of Reely Law), where there is also a frequently used shooting butt. Surprisingly, their estimate for Fenham Mill was very low (no pellets located: <6.4 pellets/m²), but the area sampled at each site was smaller than in the present study and pellets were located manually rather than by X-ray photography.

The pellet densities in the substrate at a number of wildfowling sites in Britain were compared by Mudge (1984). Although his cores were taken to a depth of 15 cm, he found that over 85% of the pellets were located within the top 10 cm of his cores, and direct comparison with the results of this study is therefore reasonable. The highest pellet density recorded by Mudge from estuarine sediments was 7.4 pellets/m², an order of magnitude lower than the 53.1 pellets/m² recorded for the upper reaches of Fenham Flats. Indeed, the density recorded in this study is higher than at all of Mudge's sites, his highest density being the 30.0 pellets/m² at a Gloucestershire flight pond.

In similar studies in Australia and North America, Kingsford et al (1989) found a density of 1.7 pellets/m² in lake sediments from a wildfowling area in New South Wales, Szymczak and Adrian (1978) estimated a figure of 0.7 pellets/m² on the ground for the area within 180 m of shooting pits in an agricultural area of Colorado, although these may have been deposited during only one season's shooting, and Fredrickson et al (1977) found 30.3 pellets/m² in uncultivated wetland soils in front of a duck blind in Missouri. In each of these studies, manual methods of searching for pellets were employed, and therefore the results should be regarded as minimum estimates, but even so, the densities recorded were considerably lower than the figure for Fenham

Flats in this study. However, much higher maximum densities have been recorded locally elsewhere, for example 2,195 pellets/m² on a Dutch clay pigeon ground (Smit et al, 1988).

It should be noted that the three sites examined on Fenham Flats all lay within 400 m of the shoreline, and extrapolation of the pellet densities recorded to the entire area of the Flats is not valid. However, in an attempt to put the densities recorded into perspective, the number of pellets shot into Fenham Flats has been estimated at 0.56 per m² per season (see appendix 1). Thus the numbers of pellets recorded apparently derive from many years' shooting (probably at least ten), a result consistent with the finding of pellets at depths below 5 cm, and in various states of decomposition. Furthermore, if the estimated input is of the correct order of magnitude, then it would appear that the densities recorded at the sampling sites are indeed considerably higher than across Fenham Flats as a whole.

If there were a real likelihood of the wildfowl at Lindisfarne ingesting pellets, then the quantities present in the sediments, at least around the upper shore, would be most alarming. Fortunately there is evidence that Wigeon, being grazers, very rarely ingest pellets in contrast to most dabbling and diving ducks (Thomas, 1975; Mudge, 1983; Butler, 1990), but there are fewer data available for Brent as they are not currently a quarry species: Mudge (1983) found no pellets in the gizzards of 25 Brent found dead. However, since Brent at Lindisfarne feed for several months principally by grubbing for *Zostera* rhizomes (pers. obs.), a technique generally not employed by Wigeon, they may be susceptible to accidentally ingesting pellets contained in the top few centimetres of the sediment, even if they do not deliberately take grit particles of similar size.

It is clear that the pellets present in the sediment form a reservoir of elemental lead, and even if the use of lead pellets were to stop immediately, there would be a release of lead compounds by degradation for several, or possibly many years. It would be instructive to determine the densities of pellets on the lower reaches of Fenham Flats, using the techniques established in this study, thereby enabling the estimate of total pellets present to be refined.

3.3 Pellet Settlement

The potential contaminating effects of spent lead shot deposited into the substrate in a wildfowling area might be considerably reduced if the pellets were to settle rapidly within the substrate before any appreciable degree of degradation could occur, so that opportunities for direct ingestion by wildfowl would be few. Also, if pellet settlement were rapid relative to degradation, then the greater part of the lead compounds derived from degraded pellets might lie at depths below the root systems of intertidal flora.

To examine the rate of settlement, Mudge (1984) conducted an experiment at eight wildfowling sites in Britain. Generally, Mudge found that settlement rates were slow, but useful results were not obtained from his two intertidal sites, and a similar experiment was therefore set up at Lindisfarne.

3.3.1 Methods

The method of Mudge (1984) was followed as closely as possible, the principal difference being that three replicate plots were established at each site in order to control for local variation. The plots were set up on 4 January 1991 near the *Zostera* sampling sites at Holy Island Sands (site A) on a firm, sandy substrate, and at Fenham Mill (site B) on a soft, muddy substrate.

At each site three rectangular plots of dimension 2 m x 1 m were placed along a north-south transect at a separation of 25 paces (and referred to as plots N - northern, C - central and S - southern). Each plot was seeded with 5000 pellets of no. 5 lead shot. The pellets were scattered from shoulder height, but on the firm sediment at site A, many did not penetrate the surface. In order to ensure that they were not immediately removed by the next high tide, these were gently pressed down into the sediment to a depth of no more than 5 mm. A large subsample (180) of the pellets actually distributed was weighed individually to an accuracy of 0.1 mg prior to seeding the plots. The mean initial pellet weight was 151.57 mg.

On each sampling visit eight cores of 8 cm diameter were obtained from each plot, using randomly generated (but not repeated) coordinates. The cores were cut into five 2 cm slices to a depth of 10 cm and the pellets recovered by washing through a sieve under hose pressure. A few spent pellets were recovered, but were obviously

separable from the seeded pellets by size and/or appearance. For the samples collected during June 1991, the lower sections of the cores (in which it was known there were unlikely to be any pellets) were examined by X-ray photography (see section 3.2.1). The pellets from all the cores within a plot were combined by layer, washed with water, carefully dried with absorbent paper, and a subsample from each layer (or all the pellets if fewer than thirty) weighed as above.

Samples were initially collected on an approximately monthly basis, during February and March 1991, but as it was apparent after the first two occasions that the settlement rate was particularly slow, and as the total number of cores which could be obtained from each plot was limited, the third sampling was postponed until June 1991. Although the period of research finished in September 1991, it was considered worthwhile to obtain a further set of samples from the settlement plots once they had been subjected to Autumn storms, which it was thought might be sufficiently severe to deposit a considerable depth of sediment onto the plots. Samples were collected during February 1992 by R. D. Evans, and processed the following month by the author. Unfortunately a few of the cores from Fenham Mill (two, two and one from the three plots) had become misshapen in the intervening period, and could not be reliably cut into 2 cm slices; the numbers of pellets shown in table 3.2 have accordingly been adjusted proportionally to the equivalent of eight cores per plot.

3.3.2 Results

The numbers of pellets recovered are shown in table 3.2, and summarised in fig. 3.1. The mean weights of pellets initially and after recovery are shown in table 3.3 and fig. 3.2.

At Holy Island Sands there was a highly significant difference amongst the three plots in the numbers recovered above and below 2 cm for each of the first three sets of samples (February: $\chi^2 = 32.3$, $df = 2$, $P < 0.01$; March: $\chi^2 = 48.0$, $df = 2$, $P < 0.01$; June: $\chi^2 = 180.4$, $df = 2$, $P < 0.01$), and by February 1992, there was a significant difference between plots in the numbers recovered from 0 - 2 cm, 2 - 4 cm and below 4 cm ($\chi^2 = 165.5$, $df = 4$, $P < 0.01$). In two of the three plots (N and C) some degree of settlement had occurred by the first sampling date, but in the third (S) no settlement had occurred by June 1991, but by February 1992 71% of the recovered pellets from that plot were below 4 cm. The numbers of pellets recovered below 2 cm at Fenham

TABLE 3.2 Pellet settlement experiment

Number of pellets recovered from plots established January 1991

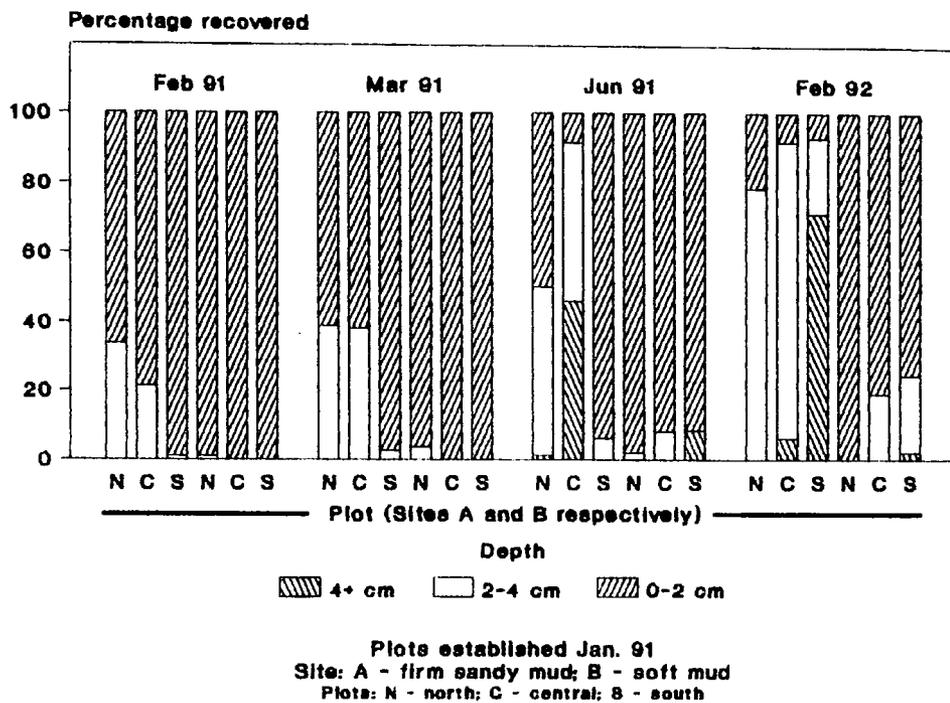
Month	Site	Plot*	0-2 cm	2-4 cm	Below 4 cm	Total
Feb 1991	Holy Island Sands	N	67	34	0	101
		C	143	39	0	182
		S	89	1	0	90
	Fenham Mill	N	99	1	0	100
		C	137	0	0	137
		S	69	0	0	69
Mar 1991	Holy Island Sands	N	55	35	0	90
		C	57	35	0	92
		S	110	3	0	113
	Fenham Mill	N	54	2	0	56
		C	128	0	0	128
		S	83	0	0	83
Jun 1991	Holy Island Sands	N	35	34	1	70
		C	9	49	49	107
		S	91	6	0	97
	Fenham Mill	N	95	2	0	97
		C	22	2	0	24
		S	53	0	5	58
Feb 1992	Holy Island Sands	N	7	25	0	32
		C	12	123	9	144
		S	11	34	110	155
	Fenham Mill	N	69	0	0	69
		C	47	11	0	58
		S	34	10	1	45

* N, C and S denote the northern, central and southern plots at each of the two sites

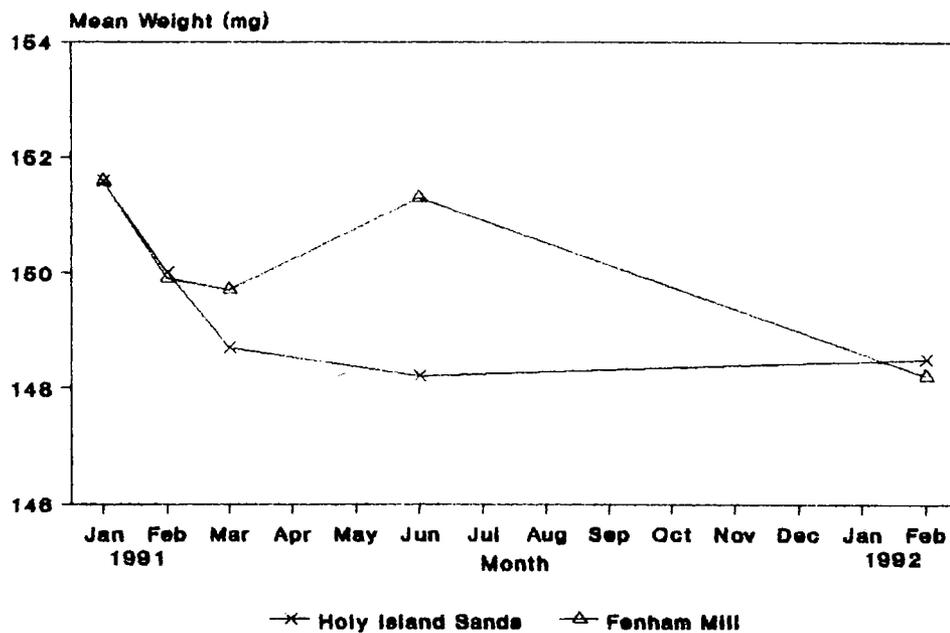
TABLE 3.3 Pellet settlement experiment - Weights of pellets recovered

Month	Site	Plot	Mean wt (mg)	s.d. (mg)	n
		Seeded pellets	151.57	5.799	180
Feb 1991	A	All plots	150.04	6.668	194
		N	150.52	6.094	74
		C	149.03	7.576	79
		S	151.10	5.586	41
	B	All plots	149.86	6.709	121
		N	149.24	6.903	41
		C	150.31	6.857	40
		S	150.04	6.478	40
Mar 1991	A	All plots	148.74	7.006	158
		N	148.28	7.168	63
		C	149.20	7.357	64
		S	148.75	6.010	31
	B	All plots	149.67	5.943	90
		N	150.83	5.173	30
		C	148.92	6.580	30
		S	149.25	6.015	30
Jun 1991	A	All plots	148.22	7.178	167
		N	148.41	6.663	61
		C	149.10	7.541	69
		S	146.29	7.128	37
	B	All plots	151.26	5.343	90
		N	150.61	6.148	32
		C	150.08	4.285	24
		S	152.69	5.016	34
Feb 1992	A	All plots	148.50	6.783	160
		N	148.46	7.191	33
		C	148.03	5.925	50
		S	148.82	7.184	77
	B	All plots	148.17	8.207	138
		N	147.03	7.313	30
		C	149.79	7.903	38
		S	147.78	8.695	70

**FIGURE 3.1 Pellet Settlement Experiment
Pellet Recovery by Depth**



**FIGURE 3.2 Pellet Settlement Experiment
Weights of Recovered Pellets**



Mill on the first three occasions were too few to permit statistical analysis, but by February 1992 there was a difference between plots in the numbers recovered above and below 2 cm ($\chi^2 = 17.6$, $df = 2$, $P < 0.01$), with no settlement having occurred in plot N, but limited settlement in each of the other two plots.

There was no difference in the mean weights of pellets between plots or by depth in any one month (ANOVA - see table 3.4). The weights of recovered pellets at each site have therefore been grouped together for comparison by month. Nor was there any difference between sites, except in June 1991, when the mean weight at site A (148.2 mg) was significantly less than at site B (151.3 mg).

The mean weight of recovered pellets in February 1992 was similar for the two sites, at 98.0% and 97.8% of the original weight (table 3.5). However, the weight loss was significant by March 1991 at Holy Island Sands, but not until February 1992 at Fenham Mill. (The change in weight between June 1991 and February 1992 at Fenham Mill was also significant (Tukey HSD test at $P < 0.05$).)

3.3.3 Discussion

On the soft mud at Fenham Mill there was no detectable settlement of pellets after five months, and only very limited settlement in two of the plots after one year. The few pellets recovered from below 2 cm up to June 1992 are most likely to be the result of disturbance during the sampling procedures, or possibly of the action of burrowing invertebrates.

On the sand at Holy Island the pattern of recovery is not straightforward. There was negligible settlement in one plot (S) over the first five months, but presumably immediate settlement in the other two (N and C). At the time of seeding the plots, the surface of plot S was, like those at Fenham Mill, covered by a growth of *Zostera noltii*, but plots N and C had a layer of sand overlying the remains of *Z. noltii* at a depth of about 4 cm. It is assumed that the *Zostera* roots and rhizomes exert an important effect in stabilising the sediment, thereby preventing rapid settlement of pellets. In the absence of plant material, pellets will settle in sand disturbed by wave action. Unfortunately no comparable vegetation-free plot on a muddy substrate was set up.

Table 3.4 Pellet settlement experiment - Weights of pellets recovered

Analysis of variance by month of recovery

February 1991

Source of Variation	Sum of Squares	df	Mean Square	F	
Site	9.29	1	9.29	0.21	n/s
Plot	55.47	2	27.73	0.62	n/s
Depth	3.54	1	3.54	0.08	n/s
Explained	71.39	4	17.85	0.40	n/s
Residual	13912.42	310	44.88		
Total	13983.81	314	44.53		

March 1991

Source of Variation	Sum of Squares	df	Mean Square	F	
Site	19.27	1	19.27	0.43	n/s
Plot	9.95	2	4.98	0.11	n/s
Depth	22.09	1	22.09	0.50	n/s
Explained	74.78	4	18.70	0.42	n/s
Residual	10823.48	243	44.54		
Total	10898.26	247	44.12		

June 1991

Source of Variation	Sum of Squares	df	Mean Square	F	
Site	286.81	1	286.81	6.53	*
Plot	55.12	2	27.56	0.63	n/s
Depth	87.63	2	43.82	1.00	n/s
Explained	644.36	5	128.87	2.93	*
Residual	10986.34	250	43.95		
Total	11630.70	255	45.61		

February 1992

Source of Variation	Sum of Squares	df	Mean Square	F	
Site	107.08	1	107.08	2.09	n/s
Plot	35.79	2	17.89	0.35	n/s
Depth	270.51	2	135.25	2.64	n/s
Explained	319.99	5	64.00	1.25	n/s
Residual	13017.90	254	51.25		
Total	13337.89	259	51.50		

* - P < 0.05

Table 3.5 Pellet settlement experiment - Weights of pellets recovered

Monthly mean weights by site

Site A - Holy Island Sands

Month	Mean	n	Std Error	95% conf int for Mean	Weight as % of seeded	
Jan 1991 (seeded)	151.57	180	0.432	150.71 - 152.42		
Feb 1991	150.04	194	0.479	149.09 - 150.98	99.0	n/s
Mar 1991	148.74	158	0.557	147.64 - 149.84	98.1	*
Jun 1991	148.22	167	0.556	147.13 - 149.32	97.8	*
Feb 1992	148.50	160	0.536	147.44 - 149.56	98.0	*

Source of Variation	Sum of Squares	df	Mean Square	F	
Month	1347.4	4	336.8	7.54	***
Residual	38173.7	854	44.70		
Total	39521.1	858			

Site B - Fenham Mill

Month	Mean	n	Std Error	95% conf int for Mean	Weight as % of seeded	
Jan 1991 (seeded)	151.57	180	0.432	150.71 - 152.42		
Feb 1991	149.86	121	0.610	148.65 - 151.07	98.9	n/s
Mar 1991	149.67	90	0.626	148.42 - 150.91	98.7	n/s
Jun 1991	151.26	90	0.563	150.14 - 152.37	99.8	n/s
Feb 1992	148.17	138	0.699	146.79 - 149.55	97.8	*

Source of Variation	Sum of Squares	df	Mean Square	F	
Month	1041.8	4	260.4	6.07	***
Residual	26332.4	614	42.9		
Total	27374.2	618			

* - P < 0.05; *** - P < 0.001

Comparison between months by Tukey's HSD test at P < 0.05

By February 1992, however, the majority of pellets recovered from plot S at Holy Island Sands were from below 4 cm, whereas in plot C, in which similar numbers of pellets had been recovered from above and below 4 cm in June 1992, very few pellets were found below 4 cm. Presumably this reflects a change in the distribution of loose sand over the site, but since it was not visited personally in February 1992, this cannot be confirmed.

In a previous analysis of the same data, but excluding those for February 1992 (Palmer and Evans, 1991), it was postulated that in a sandy sediment physical abrasion may be the main agent of degradation, but that in a muddy sediment chemical action may be more important, as by June 1991 the mean weight of pellets recovered at Fenham Mill had initially fallen but then increased. If that were so, the formation of lead compounds, eg. PbS, on the surface of the pellets under the anoxic conditions of the muddy sediment might result in an increase in weight. Many such lead compounds are of low water solubility (Fergusson, 1990), and might therefore slow the rate of degradation. However, by February 1992 the mean weight loss was almost identical at the two sites, and the possibility that the curious pattern observed for Fenham Mill was due to sampling error cannot be entirely ruled out, although the numbers of pellets weighed were considerable.

Whatever the precise mechanisms involved, it is clear that the rate of degradation of seeded pellets is particularly slow under natural intertidal conditions. Evans et al (1988) estimated the time to completely erode pellets to be 9.5 days in mud and 39 days in sand by measuring degradation under simulated wave action using a continuous magnetic stirrer. Obviously the mechanical effect of natural wave action on sediments is negligible in comparison. The question remains whether spent pellets would erode at the same rate as unused pellets. When fired, pellets are usually deformed and may also experience surface abrasion; both effects could lead to a faster, but not slower, rate of degradation than for unused shot. The rates observed must therefore be considered to be minimum rates, but most likely of the correct order of magnitude for spent pellets, as confirmed by the finding of partially eroded spent pellets below 5 cm depth (section 3.2).

3.4 Lead Compounds

The determination of heavy metal burdens in soils and sediments presents a number of problems, particularly in ensuring that results from different locations are comparable. The surface area to weight ratio of a fine-grained mud is far greater than that of a coarse sand, and the former therefore offers many more binding sites for heavy metals and their compounds than the latter; a number of standards have been proposed for reporting sediment heavy metal concentrations (de Groot *et al*, 1976, 1982). Furthermore, there exists a multitude of possible chemical extractants, masking agents and detection methods (Fergusson, 1990; de Groot *et al*, 1982)), but even so, the heavy metal concentrations of sediments as determined by laboratory techniques do not necessarily reflect specifically the bio-availability of the metals concerned (Davies *et al*, 1987).

In view of the complexities involved in determining heavy metal concentrations, a number of preliminary trials were conducted to establish a method for comparing the lead concentrations in the sample sediments from Lindisfarne. These trials are discussed in detail in appendix 2.

3.4.1 Methods

The sediment cores analysed were the top 5 cm sections of those collected from the four main study sites for the estimation of spent pellet densities (section 3.2.1). They were firstly oven-dried at 100 °C for a minimum of 48 hours, and then gently ground with a pestle and mortar and shaken through a 40 mesh/in sieve to remove gravel, invertebrate remains and plant material. Particular care was taken to remove any fragments of shell. Each sample (referred to at this stage as undifferentiated sediment) was thoroughly mixed and divided into two parts, one of which was then passed through a 200 mesh/in sieve to isolate the fine fraction.

Sub-samples of approximately 1 g weight were treated with 5 ml 'AnalaR' grade 3 M HCl at room temperature for about one hour, during which time they were frequently agitated to ensure adequate mixing of sediment and extractant. The extract solutions were filtered into screw-top plastic bottles and analysed for lead by AAS as described in section 2.3.2. Reagent blanks were analysed for each batch of samples processed.

3.4.2 Results

An initial analysis (for both undifferentiated sediment and the fine fraction) was performed on all four cores from each of four plots selected at random from the fifteen points on the grid (see section 3.2.1) at each site. For each sediment fraction within each site the variation between plots was highly significant (ANOVA on log-transformed data: $P < 0.01$ in all tests), except for the fine fraction at site A, for which the variation between and within plots was similar. The comparison between sites was therefore made on the basis of one core selected at random from each of the fifteen plots at each site.

The results of the sediment lead determinations are shown in table 3.6 and summarised in fig. 3.3. All reagent blanks were free of lead within the AAS detection limits, nor were there significant batch effects for either sediment fraction (section 2.4.2). However, one sample of the undifferentiated sediment from site A and one sample of the fine fraction from site D were rejected as outliers (section 2.4.3), although probably genuinely of higher lead concentration.

Variation in the mean lead concentration of undifferentiated sediment between sites was very highly significant (ANOVA on log-transformed data, $F_{3,55} = 9.32$, $P < 0.0001$), yet site only accounted for 33.7% of the total variation, reflecting the high degree of variation between plots at each site. Site C had a significantly higher mean lead concentration than sites A and D, and site B higher than site D (Tukey HSD test at $P < 0.05$).

Variation in the mean lead concentration of the fine fraction was also significant between sites (ANOVA on log-transformed data, $F_{3,55} = 3.03$, $P < 0.05$), but much less marked than for the undifferentiated sediment, accounting for only 14.2% of the total variation. Only sites B and D differed significantly (Tukey HSD test at $P < 0.05$).

3.4.3 Discussion

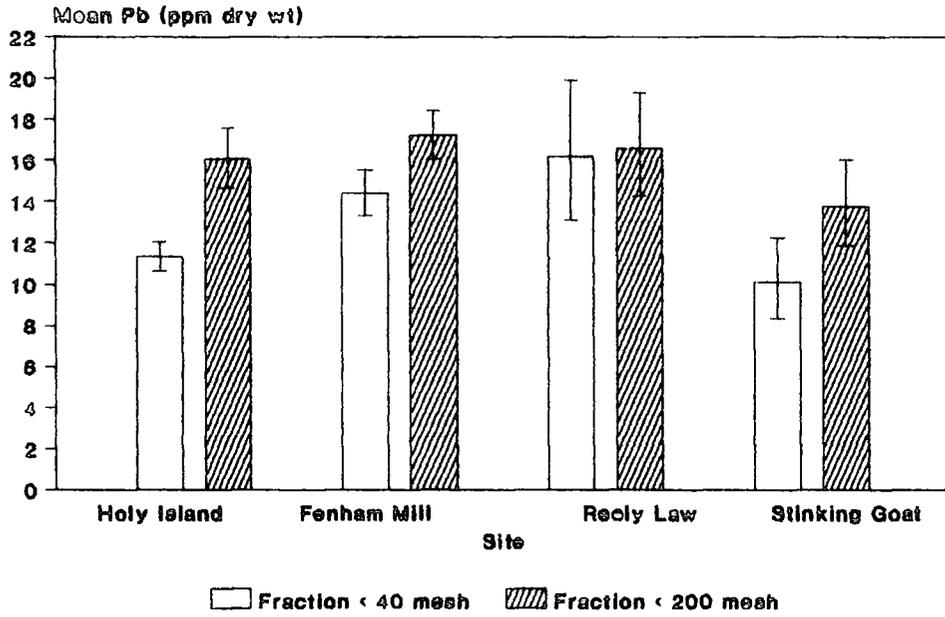
The fine fraction showed a higher mean lead concentration than the undifferentiated sediment at each site, as was to be expected. The difference was more marked at the sandier sites (D and, especially, A), again as expected from the composition of the sediments. At site C the total sediment lead content was almost as high as that of the fine fraction, reflecting the nature of the very soft mud at that site.

TABLE 3.6 Lead in sediments, January 1991

Extraction by 3 M HCl

Site	Fraction	Mean Pb (ppm dw)	n	95% conf. limits
Holy Island Sands	< 40 mesh/in	11.3	14	10.6 - 12.1
	< 200 mesh/in	16.0	15	14.7 - 17.6
Fenham Mill	< 40 mesh/in	14.4	15	13.3 - 15.5
	< 200 mesh/in	17.2	15	16.1 - 18.4
Reely Law	< 40 mesh/in	16.2	15	13.1 - 19.9
	< 200 mesh/in	16.6	15	14.3 - 19.3
Stinking Goat	< 40 mesh/in	10.1	15	8.3 - 12.2
	< 200 mesh/in	13.8	14	11.8 - 16.0

FIGURE 3.3 **Lead in Intertidal Sediments - Jan. 1991**
3M HCl Extract



Error bars show 95% confidence limits
(from log-transformed data)

Lead levels in the fine fractions show very little difference between sites, except for the lower value at site D, where rather shallow sediments overlie a layer of shells. This result may reflect a genuine difference in lead contamination at site D, but it is well known that high concentrations of calcium may interfere with the atomisation of lead in flame AAS (eg. Waughmann and Brett, 1980), and the result should therefore be interpreted with caution. Site D apart, the lead concentration in the fine fraction of the top 5 cm of sediment appears to be uniform across Lindisfarne (including Holy Island Sands); differences in the total sediment lead content between sites are principally attributable to the particle size composition of the sediments themselves.

Comparisons with other studies must be made with care, not only because of different sediment origins, but particularly in relation to differences in analytical techniques and in the extractants employed. The value of 16.2 ppm obtained from Reely Law is higher than the equivalent 'non-lithogenous' fraction from the unpolluted Ravensglass (9.9 ppm) and moderately polluted Wyre estuaries (12 ppm), but lower than the industrially polluted Mersey (20 ppm) (Badri and Aston, 1983). In the Limfjord, Denmark, Lyngby and Brix (1982) found lead levels of 0.5 and 1.1 ppm (they do not indicate the types of sediment sampled, or the fraction analysed) at two relatively unpolluted rural sites, but 16.3 ppm at a site of considerable urban and industrial pollution. In contrast, Halcrow et al (1973) found mean lead concentrations ranging from 28 ppm in sandy sediments to 67 ppm in muddy sediments from areas of the Firth of Clyde subject to sewage sludge disposal, and Bryan and Hummerstone (1977) found a lead concentration of 280 ppm in sediment from the Looe Estuary, Cornwall, which was contaminated by mining wastes.

In comparison with the estuarine sediment lead concentrations from other locations, the sediments at Lindisfarne must certainly be considered as polluted in terms of lead, but only moderately so, and the levels of contamination do not approach those encountered at some sites of high industrial contamination.

The proportion of the chemically available lead which has been derived from spent shot cannot be deduced in this study, as it was not possible to examine a comparable unshot site. Budle Bay (part of the Lindisfarne NNR lying about 3 km south-east of the main study area)

would have been the best comparison, having been free from wildfowling activity for almost thirty years, but the sediments there have been greatly disturbed by bait digging (Howell, 1985). Indeed, Evans et al (1988) and Abbott (1990) found surface sediment lead concentrations at Budle Bay to be higher than those at Lindisfarne. Since the sediments at Lindisfarne must be principally of marine origin (freshwater input is negligible), it is possible that some part of the current lead contamination may be attributable to historical industrial activity around the North Sea catchment area, but the proportion concerned is likely to be insignificant in comparison with the lead input during many decades of wildfowling.

The density of dried sediment varied between sites owing to the differences in sediment composition, but, taking the value for Fenham Mill of 1.2 g/cm^3 , and an average lead content of 15 ppm, the weight of lead in the top 5 cm of sediment is 0.9 g/m^2 . In section 3.2.2 the weight of lead present in pellet form was calculated as 5.11 g/m^2 . Thus, although these figures are extremely crude, it would appear that there is about five times the amount of lead present in pellet form compared to that chemically extractable by an acid of moderate strength. Several possible mechanisms may account for the apparent loss of lead compounds from the sediment.

- i) Lead compounds may be deposited in lower sediment strata. Abbott (1990) found some evidence of increasing lead concentration in Lindisfarne sediments down to 20 cm depth, but did not apparently allow for changes in sediment composition. However, the history of sedimentation in intertidal systems may be complex, and higher lead levels at greater depth may result from sediment redistribution or from historically higher levels of shooting activity. Moreover, in the one comparison of lead concentrations at different depths made in this study, the concentration at Fenham Mill below 7 cm was less than that above 7 cm, albeit coarser sediment (table A2.3a and section A2.4).
- ii) Lead compounds may be dissolved directly from the sediment into sea water and removed during ebb tides. The suggestion that the transfer of lead compounds from interstitial water to sea water may occur is also considered in connection with the lead levels found in *Enteromorpha* in section 4.4.2.

iii) Large quantities of *Zostera* may be washed up onto the shoreline during Autumn storms (pers. obs.), thereby removing lead from the mud-flats. Indeed a large proportion of the annual *Zostera* production, probably in excess of 95%, is removed either by that means or by grazing or natural decay.

The total standing crop of *Zostera* at Lindisfarne at the end of August 1990 (the month in which the crop is at its maximum, ie. immediately prior to grazing by wildfowl) has been roughly estimated at 140 tonnes (100 tonnes *Z. angustifolia* and 40 tonnes *Z. noltii*), of which approximately 85% was on Fenham Flats (S M Percival, pers. comm.). The annual production of *Zostera* may be estimated by doubling the maximum annual standing crop, although this may under-estimate production for *Z. angustifolia* (Jacobs et al, 1981). Given the area of Fenham Flats to be 7.5 km² (see appendix 1), then the annual production of *Zostera* on Fenham Flats during 1990 is estimated to be 32 g/m². The greater part of the annual production would have been removed during the Autumn when lead levels were relatively high although some would have been removed during the main Summer growth period when lead levels were generally lower (Collins, 1990, and by comparison with the Summer 1991 levels presented in chapter 4). It is therefore assumed that 25% of the annual production was lost in Summer with an average lead content of 10 ppm, and the remaining 75% was lost during Autumn with an average lead content of 18 ppm. These figures yield an estimate of 0.5 mg/m² for the quantity of lead removed from the sediment in *Zostera* during 1990 by all means, ie. natural decay, grazing and removal during storms.

The quantity of lead in the top 5 cm of sediment was estimated above to be 0.9 g/m², and the proportion removed in *Zostera* is therefore very small indeed, less than 0.1%. However, these figures are very crude estimates for the entire area of Fenham Flats, and the proportion of sediment lead removed through loss of *Zostera* may be considerably higher locally, perhaps up to an order of magnitude greater in small areas of dense *Z. angustifolia* growth. In other studies, Brix and Lyngby (1983) estimated that *Zostera* leaves held only 0.5 to 4.8% of the total lead content of sediments down to 0.7 cm, and Banus et al (1975) estimated the loss of lead to the sea from salt-

marsh sediments in the form of dead *Spartina* to be about 2% of the lead content of the top 10 cm of sediment per annum.

It would appear that the removal of lead in *Zostera* could not possibly account alone for the apparent loss of lead from the sediment. The possible deposition of lead within lower sediment strata could be investigated much more thoroughly, taking into account differences in sediment composition with depth, but estimation of the quantity of lead removed by tidal action would be much more difficult to achieve.

4.1 Introduction

Samples of the intertidal flora were collected during the period September 1990 to September 1991, and analysed for lead content. Since *Zostera* spp are the principal food of Wigeon at Lindisfarne, the main emphasis of the sampling regime was on the systematic analysis of *Zostera*; *Salicornia* and *Enteromorpha* were sampled on a less formal basis.

Depletion of *Zostera*, due to a combination of die-back, grazing and defoliation by strong tides during adverse weather conditions was apparent from the end of October 1990 onwards (particularly for *Z. angustifolia*). By the end of January 1991, *Z. angustifolia* was absent at all sites except Stinking Goat, and there it was no longer present in each plot. *Z. noltii* leaves were very sparse by late January, and the samples obtained from all sites were sufficient for analysis of below-ground parts only. Sampling was therefore suspended between February and April 1991 inclusive.

During May and June 1991 samples of *Salicornia* and *Enteromorpha* were collected and analysed by two Durham University undergraduate students. They used the same analytical procedures as were employed in this study, although their sampling areas were not identical. Some of their results (from Fenham Mill and Beal Blacks) have been incorporated into this chapter, with acknowledgment. Sampling of *Salicornia* and *Enteromorpha* was not conducted in my study during the period concerned, but was resumed in July 1991.

4.2 Methods

4.2.1 *Zostera*

The four *Zostera* sampling sites are described in section 1.3. At all four sites the more elevated, exposed areas of mud were colonised by *Z. noltii*; at Holy Island Sands (A) and Fenham Mill (B), *Z. angustifolia* was present in the pools and at Reely Law (C) and Stinking Goat (D), it occupied the channels, ie. it was present in the areas permanently under water at all four sites.

At each site, within an overall area of not more than 100 m x 100 m, twelve sample plots were established: six in reasonably homogeneous patches of *Z. angustifolia* and six in patches of *Z. noltii*. Sampling visits were made on a roughly monthly basis, subject to tidal conditions. On each occasion, one *Zostera* sample was collected from each plot. At sites A, B and D, random sampling was achieved by taking a random bearing from a cane marking the centre of the plot, and collecting the sample at 1 m from the cane, or the nearest point thereto if insufficient material was present at that point. It was intended to use this method at site C also, but all canes disappeared within a few days (possibly due to human intervention); samples were therefore taken at 10 m intervals along a transect out from the shooting butt on Harvey's Island (starting at a distance of 50 m). Site C was abandoned after January 1991 owing to problems of access. At sites A and B, marker canes were lost to tidal action on several occasions, and it was possible to relocate the plots only approximately. Additional samples of *Z. angustifolia* were collected from the lower reaches of Fenham Flats during a survey of the whole of the *Zostera* beds by all-terrain vehicle on 9 October 1990.

On the first visit in September 1990, samples were taken using a cylindrical corer of 20 cm diameter, but, as that method was rather awkward to use in soft mud, and the exact area or biomass sampled was not critical, subsequent samples were obtained by cutting a square turf of side 20 cm approx. with a spade. When leaves were sparse, two or three such turfs of *Z. noltii* were taken for some plots, in order to yield sufficient material for analysis.

Samples were transferred to Durham in plastic containers, where they were separated into above- and below-ground parts (hereafter 'leaves' and 'roots') by cutting the leaves from the turf with scissors. Although the method was not absolutely precise in separating leaves from roots, it was considered appropriate since it reflected the two possible feeding methods available to wildfowl: grazing the leaves or grubbing in the sediment for roots and rhizomes. The separated parts were thoroughly washed free of sediment with water under pressure, dried at 50 °C in a vacuum oven for a minimum of 48 hours and stored in individual plastic screw-top containers prior to analysis.

4.2.2 *Enteromorpha*

Enteromorpha samples were collected from the *Zostera* sites when found to be present during sampling of the latter. During Autumn 1990 *Enteromorpha* was very sparsely distributed, and one composite sample was collected from each site, except A, where replicate samples were obtained from the pools as sampling units. Additional samples were collected from a patch of anchored *Enteromorpha* by the causeway during September and November 1990. *Enteromorpha* samples were washed and dried as described in section 4.2.1.

4.2.3 *Salicornia*

During Autumn 1990 *Salicornia* samples were obtained from Beal Point, Fenham Mill and Ross Point (see fig. 2.1). The Fenham Mill patch was utilised by Wigeon at high tides during September and October 1990 (pers. obs.). By the end of November 1990 all known stands of *Salicornia* had been depleted. Samples were taken by walking a transect through the *Salicornia* bed and picking one plant intact every two paces. The seed-heads were removed from the plants with scissors, washed and dried as described in section 4.2.1. Regrettably, once dried, individual plants provided insufficient material for analysis and had to be combined into pseudo-replicates (Hurlbert, 1984).

During 1991, at Fenham Mill and Beal Blacks, sufficient plants were picked at intervals of twenty paces through the *Salicornia* beds to yield adequate samples when dried. At Beal Blacks, samples were compared from sandy and muddy sediments.

4.3 Results

In order to control for possible variations in analysis between batches, processing took place in batches formed, as far as was possible, from material taken from a number sampling dates together. No significant batch-related effects were revealed by analysis of variance (see section 2.4.2). Reagent blanks were run through all batches, and no levels of lead contamination within the sensitivity limits of the AAS equipment were detected.

4.3.1 *Zostera*

The samples from Autumn 1990 and January 1991 and those from Summer 1991 were processed as two separate groups. Both groups showed

highly significant differences in lead levels between the two species (5-way ANOVA on log-transformed data of batch, species, site, part and month effects; $F_{1,319} = 29.04$, $P < 0.001$ and $F_{1,202} = 42.55$, $P < 0.001$ respectively), as was found in a previous study of *Zostera* at Lindisfarne (Collins, 1990), and therefore the data for *Z. angustifolia* and *Z. noltii* are analysed separately. The results are shown in table 4.1 (a-d) and summarised by species for Autumn 1990 in figure 4.1 (a-b) and for Summer 1991 in figure 4.2 (a-b). The four study sites are shown individually in figure 4.3 (a-d). The results for the *Z. angustifolia* samples collected from the lower reaches of Fenham Flats during October 1990 are also listed in table 4.1a and shown in figure 4.4, together with the results from the three permanent study sites on Fenham Flats for comparison.

The differences observed in the mean lead concentrations of both *Zostera* species were complex, and clearly not due to one single factor. Analyses of variance are presented in table 4.2 (a-d). The analyses for the Autumn 1990 period are shown both excluding and including the January 1991 data, as interaction terms could not be calculated in the latter case. The differences between sites and due to month of sampling were significant in all cases; the part of the plant sampled (leaves or roots) was significant in all but one analysis, and occurred in significant interaction terms for both Autumn 1990 and the 1991 growing season.

The factor exerting the greatest effect differed between the two seasons. During Autumn 1990, site explained the greatest proportion of the total variation (32% for *Z. angustifolia*, 20% for *Z. noltii*); during 1991 the most important difference lay in the part of the vegetation (35% for *Z. angustifolia*, 13% for *Z. noltii* and 15% for part/month interaction for *Z. noltii*). The roots held higher lead levels than the leaves (but only until July for *Z. noltii*).

There was no correlation during either Autumn 1990 or Spring and Summer 1991 between the mean lead concentrations by site and month in the leaves and the roots for either *Z. angustifolia* (1990, $r = 0.301$, $n = 13$, n/s; 1991, $r = 0.134$, $n = 12$, n/s) or for *Z. noltii* (1990, $r = -0.065$, $n = 12$, n/s; 1991, $r = 0.128$, $n = 12$, n/s). Nor was there a correlation if both species were considered together (1990, $r = 0.194$, $n = 25$, n/s; 1991, $r = 0.223$, $n = 24$, n/s). These results are not



TABLE 4.1a Lead concentrations in *Zostera angustifolia*, September 1990 to January 1991

Site	Part	Month	Mean Pb (ppm dw)	s.d.	n
A	Leaves	Sep	10.3	0.36	5
		Oct	12.9	0.64	6
		Nov	14.0	1.30	6
	Roots	Sep	16.8	3.14	6
		Oct	14.7	2.63	6
		Nov	15.3	1.79	6
B	Leaves	Sep	15.2	1.47	6
		Oct	19.8	1.62	6
		Nov	21.6	1.94	6
	Roots	Sep	19.8	3.19	6
		Oct	18.0	2.78	6
		Nov	18.1	2.43	6
C	Leaves	Sep	14.0	0.74	6
		Oct	18.0	1.74	6
		Nov	21.3	1.46	6
	Roots	Sep	20.8	2.37	6
		Oct	16.0	1.64	5
		Nov	18.7	1.93	6
D	Leaves	Sep	16.8	3.00	6
		Oct	16.7	3.32	6
		Nov	20.9	3.44	6
		Jan	34.8	5.65	4
	Roots	Sep	18.4	2.41	6
		Oct	17.2	2.23	6
		Nov	17.9	1.57	6
		Jan	18.8	2.19	4

Additional results from lower reaches of Fenham Flats, 9 October 1990

Distance from Reely Law (approx)	Part	Mean Pb (ppm dw)	s.d.	n
200 m	Leaves	17.7	3.22	3
	Roots	20.1	4.21	3
400 m	Leaves	16.8	0.88	3
	Roots	16.1	0.72	3
600 m	Leaves	16.5	1.55	3
	Roots	19.7	1.72	3
800 m	Leaves	14.3	0.17	3
	Roots	16.3	0.63	3

TABLE 4.1b Lead concentrations in *Zostera noltii*, September 1990 to January 1991

Site	Part	Month	Mean Pb (ppm dw)	s.d.	n	
A	Leaves	Sep	15.1	3.36	6	
		Oct	12.4	1.66	6	
		Nov	13.1	1.35	6	
	Roots	Sep	14.7	2.30	6	
		Oct	11.8	1.85	6	
		Nov	15.6	1.22	6	
		Jan	14.4	1.53	6	
	B	Leaves	Sep	17.5	2.32	6
			Oct	20.7	2.06	6
			Nov	19.9	3.06	6
Roots		Sep	18.2	2.17	6	
		Oct	14.5	2.46	6	
		Nov	14.4	1.23	6	
		Jan	16.1	0.97	5	
C		Leaves	Sep	17.4	4.60	6
			Oct	19.5	1.58	6
			Nov	21.1	4.06	6
	Roots	Sep	19.5	1.92	5	
		Oct	12.5	1.39	6	
		Nov	12.9	0.93	6	
		Jan	17.9	3.88	6	
	D	Leaves	Sep	14.2	1.67	6
			Oct	17.3	3.47	6
			Nov	13.7	2.33	6
Roots		Sep	17.1	2.91	6	
		Oct	12.1	0.32	5	
		Nov	13.9	1.49	6	
		Jan	15.8	1.99	6	

TABLE 4.1c Lead concentrations in *Zostera angustifolia*, May to September 1991

Site	Part	Month	Mean Pb (ppm dw)	s.d.	n
A	Leaves	May	5.4	0.72	5
		Jun	6.3	1.04	6
		Jul	9.1	1.23	6
		Sep	7.7	0.59	4
	Roots	May	8.2	0.97	5
		Jun	10.6	1.10	6
		Jul	10.2	1.51	6
		Sep	9.5	1.87	4
B	Leaves	May	6.6	1.09	6
		Jun	8.6	0.78	6
		Jul	10.6	1.31	6
		Sep	8.6	0.45	4
	Roots	May	15.0	2.44	6
		Jun	18.9	7.58	6
		Jul	12.6	1.46	6
		Sep	11.1	1.40	4
D	Leaves	May	10.9	2.40	6
		Jun	7.7	0.83	6
		Jul	7.6	0.63	6
		Sep	8.3	1.24	4
	Roots	May	10.2	1.09	5
		Jun	13.1	2.35	6
		Jul	10.3	1.06	6
		Sep	10.4	0.39	4

TABLE 4.1d Lead concentrations in *Zostera noltii*, May to September 1991

Site	Part	Month	Mean Pb (ppm dw)	s.d.	n
A	Leaves	May	10.7	0.25	5
		Jun	8.9	1.66	6
		Jul	9.9	2.28	6
		Sep	9.3	1.87	4
	Roots	May	12.6	2.42	6
		Jun	13.5	2.04	6
		Jul	10.8	1.22	6
		Sep	8.9	0.99	4
B	Leaves	May	8.5	2.12	6
		Jun	9.4	1.24	6
		Jul	15.5	1.29	6
		Sep	11.7	1.17	4
	Roots	May	13.7	1.81	6
		Jun	14.3	2.77	6
		Jul	14.2	0.72	6
		Sep	10.9	0.41	4
D	Leaves	May	11.7	3.29	6
		Jun	10.5	1.43	6
		Jul	12.2	1.83	6
		Sep	14.0	1.57	4
	Roots	May	14.6	1.52	6
		Jun	13.4	3.24	6
		Jul	13.3	3.36	6
		Sep	11.4	2.04	4

FIGURE 4.1a

Lead levels in *Zostera angustifolia*
Sept. 1990 - Jan. 1991

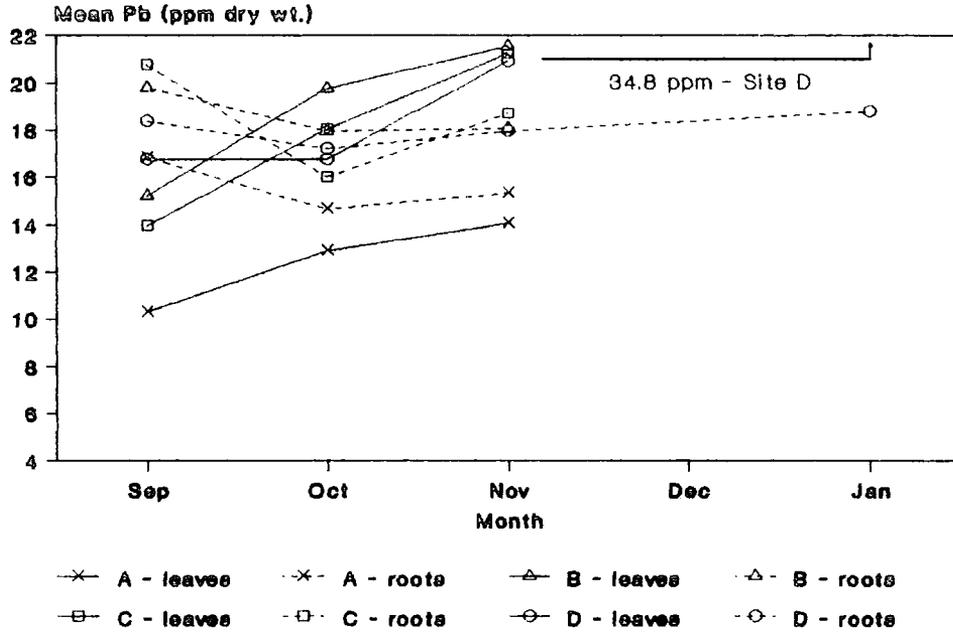


FIGURE 4.1b

Lead levels in *Zostera nolii*
Sept. 1990 - Jan. 1991

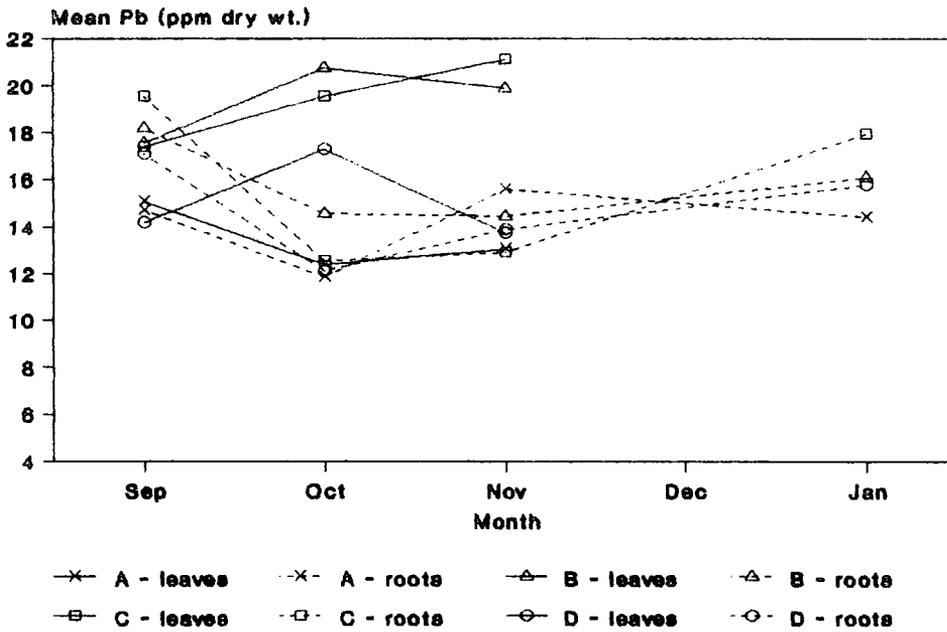


FIGURE 4.2a

Lead Levels in *Zostera angustifolia*
May - Sept. 1991

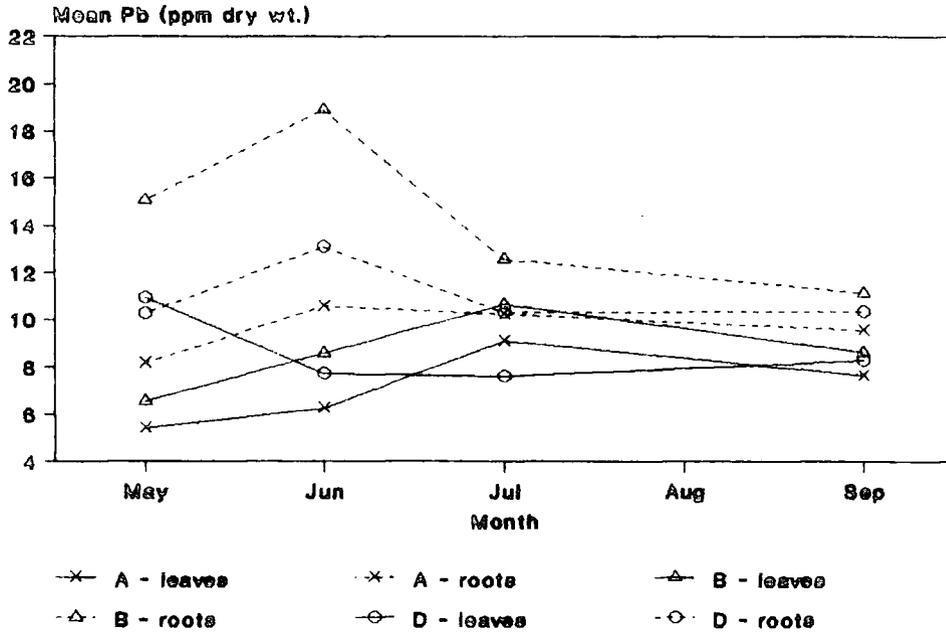


FIGURE 4.2b

Lead Levels in *Zostera noltil*
May - Sept. 1991

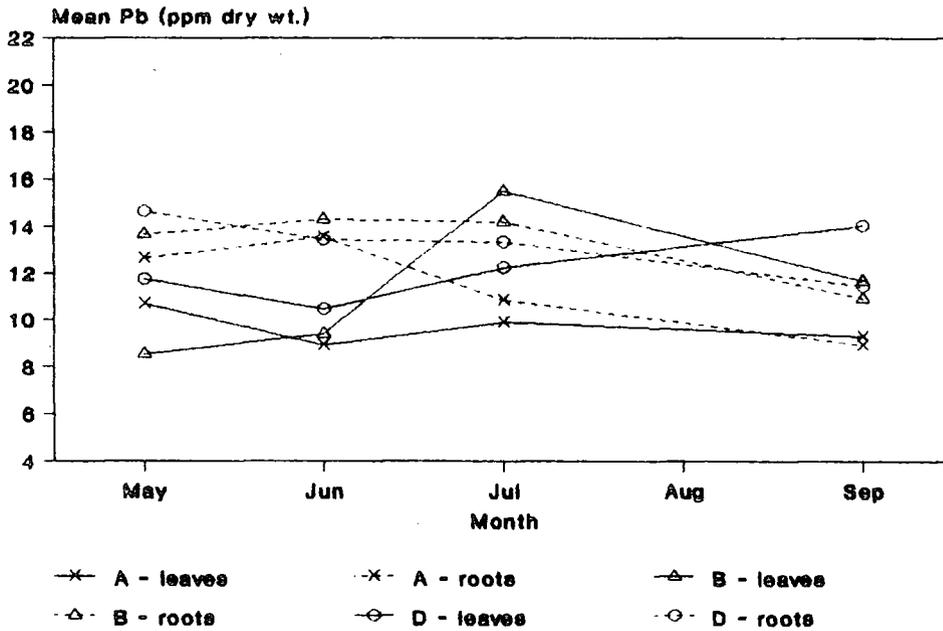


FIGURE 4.3a

Lead levels in *Zostera* 1990-91
Site A (Holy Island Sands)

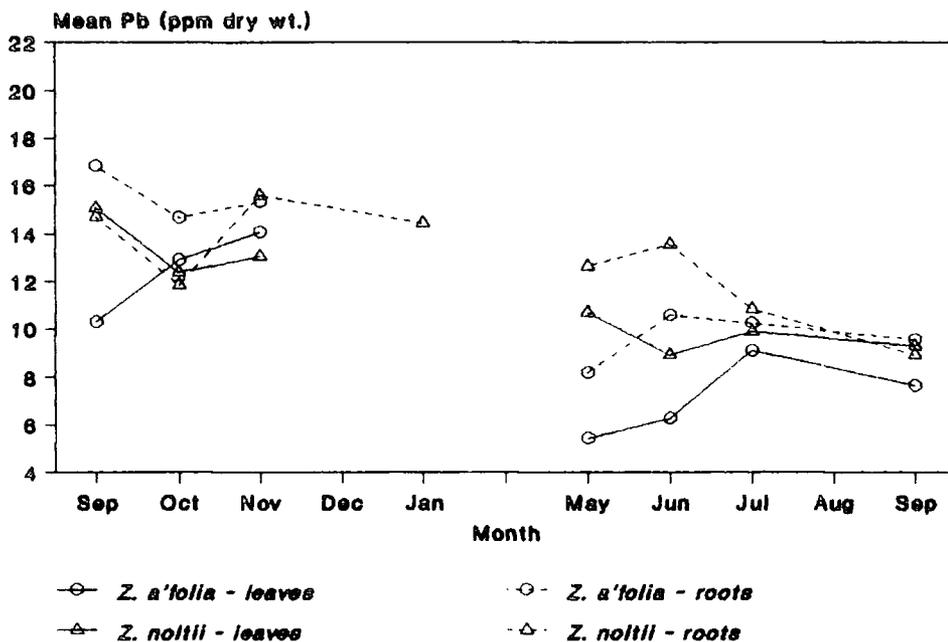


FIGURE 4.3b

Lead levels in *Zostera* 1990-91
Site B (Fenham Mill)

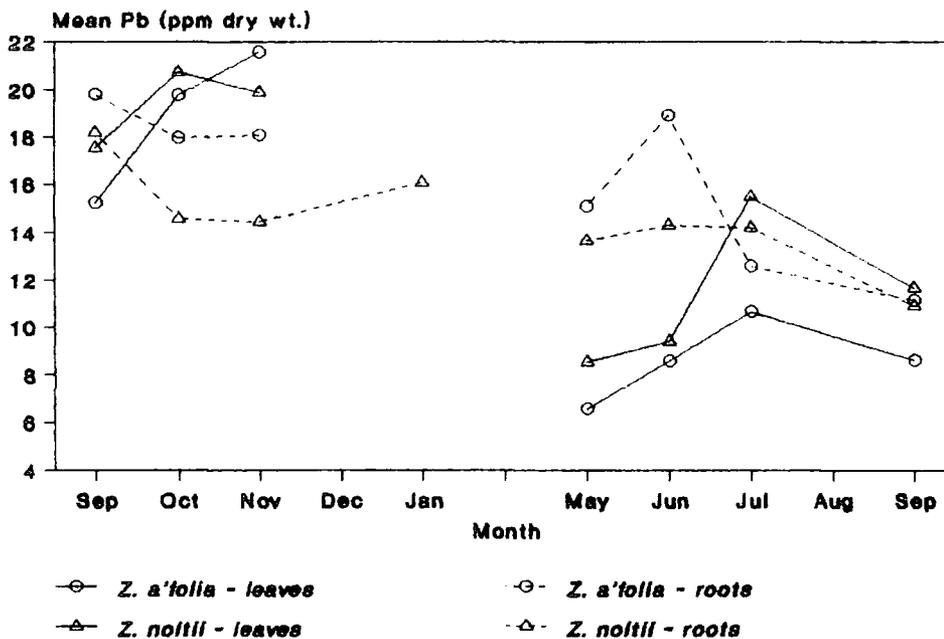


FIGURE 4.3c

Lead levels in *Zostera* 1990-91
Site C (Reely Law)

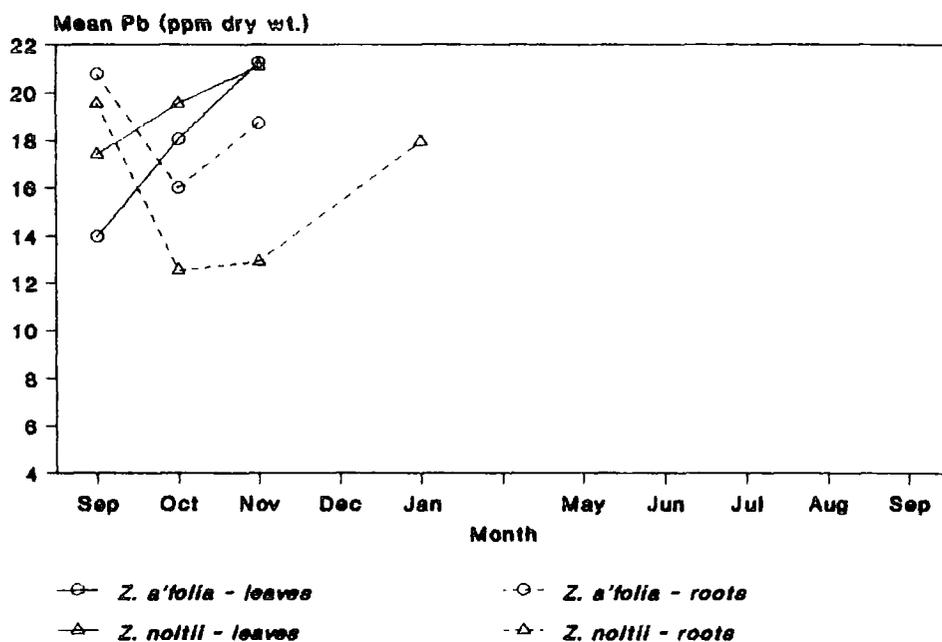


FIGURE 4.3d

Lead levels in *Zostera* 1990-91
Site D (Stinking Goat)

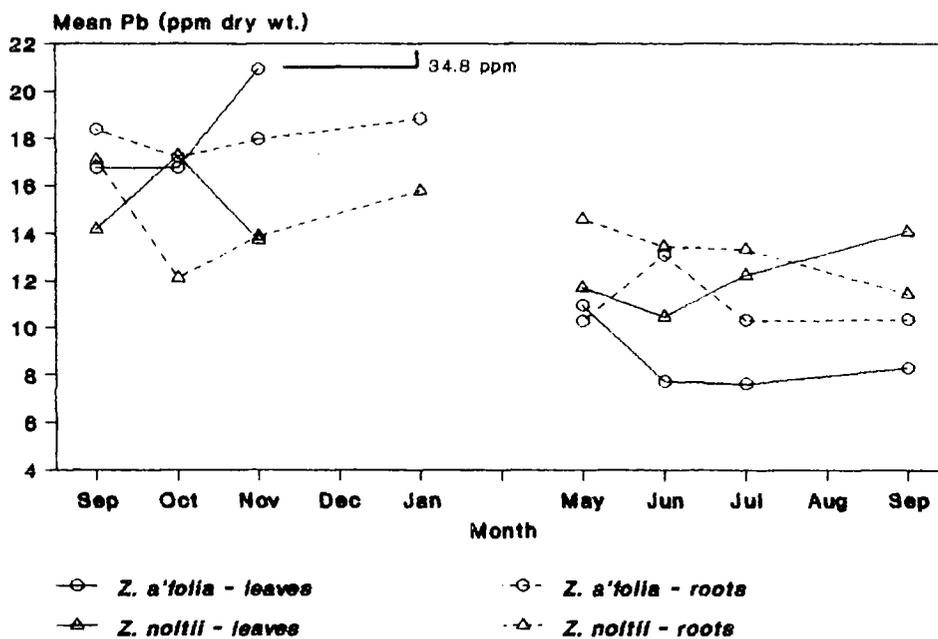
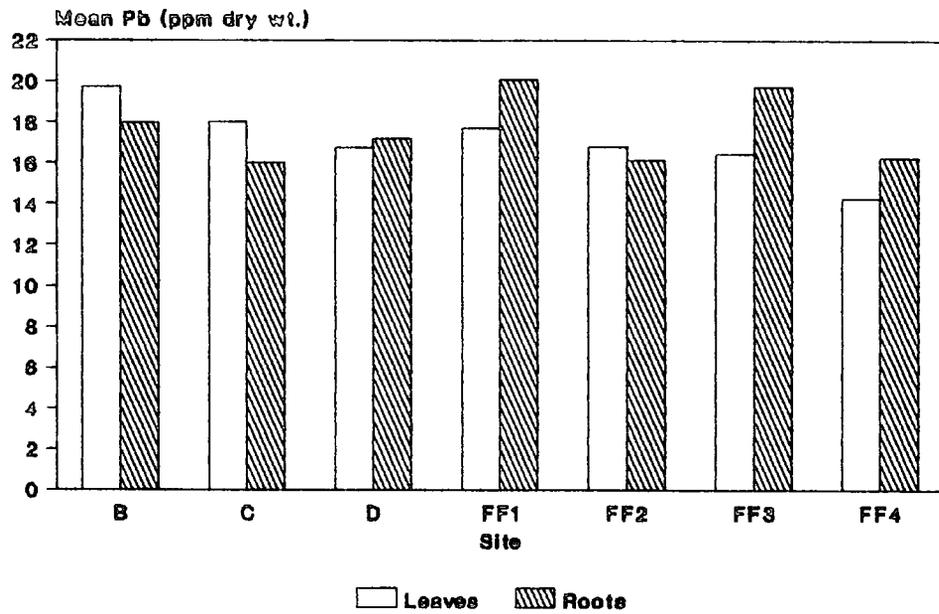


FIGURE 4.4

Lead levels in *Zostera angustifolia*
Fenham Flats - October 1990



Samples FF1 - FF4 taken from transects
at approx. 200 m intervals from site C

TABLE 4.2a Analysis of variance of mean lead concentrations in *Zostera angustifolia*, September 1990 to January 1991

Excluding data for January 1991

Source of Variation	Sum of Squares	df	Mean Square	F	
Site	1.90	3	0.63	38.87	***
Part	0.15	1	0.15	8.96	**
Month	0.40	2	0.20	12.30	***
Site/Part	0.32	3	0.11	6.51	***
Site/Month	0.07	6	0.01	0.73	n/s
Part/Month	1.05	2	0.53	32.23	***
Site/Part/Month	0.16	6	0.03	1.67	n/s
Explained	4.00	23	0.17	10.66	***
Residual	1.92	118	0.02		
Total	5.92	141	0.04		

Including data for January 1991

Source of Variation	Sum of Squares	df	Mean Square	F	
Site	1.89	3	0.63	19.94	***
Part	0.03	1	0.03	0.95	n/s
Month	1.24	3	0.41	13.06	***
Explained	3.55	7	0.51	16.03	***
Residual	4.49	142	0.03		
Total	8.04	149	0.05		

** - $P < 0.01$; *** - $P < 0.001$ (log-transformed data)

TABLE 4.2b Analysis of variance of mean lead concentrations in *Zostera noltii*, September 1990 to January 1991

Excluding data for January 1991

Source of Variation	Sum of Squares	df	Mean Square	F	
Site	1.37	3	0.46	21.18	***
Part	0.53	1	0.53	24.52	***
Month	0.29	2	0.15	6.76	**
Site/Part	0.55	3	0.18	8.57	***
Site/Month	0.21	6	0.04	1.63	n/s
Part/Month	0.85	2	0.43	19.80	***
Site/Part/Month	0.62	6	0.10	4.79	***
Explained	4.43	23	0.19	8.97	***
Residual	2.54	118	0.02		
Total	6.97	141	0.05		

Including data for January 1991

Source of Variation	Sum of Squares	df	Mean Square	F	
Site	1.43	3	0.48	14.29	***
Part	0.53	1	0.53	15.80	***
Month	0.45	3	0.15	4.49	**
Explained	2.25	7	0.32	9.66	***
Residual	5.22	157	0.03		
Total	7.47	164	0.05		

** - $P < 0.01$; *** - $P < 0.001$ (log-transformed data)

TABLE 4.2c Analysis of variance of mean lead concentrations in *Zostera angustifolia*, May to September 1991

Source of Variation	Sum of Squares	df	Mean Square	F	
Site	1.86	2	0.93	37.48	***
Part	4.31	1	4.31	173.91	***
Month	0.33	3	0.11	4.45	**
Site/Part	0.38	2	0.19	7.72	***
Site/Month	1.09	6	0.18	7.31	***
Part/Month	0.86	3	0.29	11.49	***
Site/Part/Month	0.88	6	0.15	5.89	***
Explained	9.70	23	0.42	17.01	***
Residual	2.60	105	0.03		
Total	12.31	128	0.10		

TABLE 4.2d Analysis of variance of mean lead concentrations in *Zostera noltii*, May to September 1991

Source of Variation	Sum of Squares	df	Mean Square	F	
Site	0.70	2	0.35	12.17	***
Part	0.94	1	0.94	32.90	***
Month	0.28	3	0.09	3.22	*
Site/Part	0.06	2	0.03	0.97	n/s
Site/Month	0.83	6	0.14	4.83	***
Part/Month	1.08	3	0.36	12.54	***
Site/Part/Month	0.28	6	0.05	1.64	n/s
Explained	4.15	23	0.18	6.31	***
Residual	3.06	107	0.03		
Total	7.21	130	0.06		

* - $P < 0.05$; ** - $P < 0.01$; *** - $P < 0.001$ (log-transformed data)

surprising in view of the significant interaction terms involving the part of the plants as shown in table 4.2.

It is important to consider the results from Autumn 1990 in greater detail, since that was the period when Wigeon were feeding on *Zostera*. Although site was overall the most important factor in explaining the variation in the lead concentrations of both species (tables 4.2a and b), this was principally due to lower levels on Holy Island Sands (site A) for *Z. angustifolia* (fig. 4.1a), but for *Z. noltii* the pattern is less straightforward (fig. 4.1b). Since the Wigeon rarely fed there (section 5.2.2), the data for the three months September to November 1990 have been re-analysed with site A omitted, to examine the factors effecting the mean lead concentrations on Fenham Flats, the main feeding area, during the time when Wigeon were utilising the food source. The analyses of variance are presented in table 4.3 (a, b).

For *Z. angustifolia*, the only significant effects were the month of sampling, and the interaction with the part of the plant. For both the leaves and roots separately the month remained significant; there was no difference in mean lead concentration between the three sites. Likewise, for *Z. noltii*, the month of sampling was significant for both leaves and roots, but there was also a very highly significant difference between sites in the leaves, with the concentrations at Stinking Goat (site D) being lower than at Fenham Mill and Reely Law.

Figures 4.3a, b and d clearly show higher recorded lead levels in *Zostera* during Autumn 1990 than during 1991. The data from September 1990 (excluding Reely Law) were compared with those from September 1991, regrettably not a full year later since samples were taken during the last and first weeks of the month respectively. For all but *Z. angustifolia* leaves at Holy Island Sands and *Z. noltii* leaves at Stinking Goat, the 1990 mean was at least 5.5 ppm greater than its 1991 counterpart, the maximum difference being 8.7 ppm. The difference between years was very highly significant for both species (3-way ANOVA on log-transformed data of site, part, and year effects: *Z. angustifolia*, $F_{1,47} = 234.3$, $P < 0.001$; *Z. noltii*, $F_{1,48} = 90.2$, $P < 0.001$).

4.3.2 *Enteromorpha*

The results are shown in table 4.4 and summarised for Autumn 1990 in figure 4.5a and for Summer 1991 in figure 4.5b.

TABLE 4.3a Analysis of variance of mean lead concentrations in *Zostera angustifolia* on Fenham Flats, September to November 1990

Source of Variation	Sum of Squares	df	Mean Square	F	
Site	0.03	2	0.02	1.02	n/s
Part	0.01	1	0.01	0.31	n/s
Month	0.36	2	0.18	11.14	***
Site/Part	0.02	2	0.01	0.63	n/s
Site/Month	0.06	4	0.02	0.97	n/s
Part/Month	0.79	2	0.40	24.54	***
Site/Part/Month	0.16	4	0.04	2.46	n/s
Explained	1.43	17	0.08	5.22	***
Residual	1.43	89	0.02		
Total	2.86	106	0.03		

Leaves only

Source of Variation	Sum of Squares	df	Mean Square	F	
Site	0.04	2	0.02	1.23	n/s
Month	0.99	2	0.49	31.72	***
Site/Month	0.15	4	0.04	2.44	n/s
Explained	1.17	8	0.15	9.46	***
Residual	0.70	45	0.02		
Total	1.87	53	0.04		

Roots only

Source of Variation	Sum of Squares	df	Mean Square	F	
Site	0.02	2	0.01	0.50	n/s
Month	0.16	2	0.08	4.82	*
Site/Month	0.07	4	0.02	1.04	n/s
Explained	0.25	8	0.03	1.86	n/s
Residual	0.73	44	0.02		
Total	0.98	52	0.02		

* - P < 0.05; *** - P < 0.001 (log-transformed data)

TABLE 4.3b Analysis of variance of mean lead concentrations in *Zostera noltii* on Fenham Flats, September to November 1990

Source of Variation	Sum of Squares	df	Mean Square	F	
Site	0.59	2	0.29	14.17	***
Part	0.82	1	0.82	39.71	***
Month	0.16	2	0.08	3.86	*
Site/Part	0.24	2	0.12	5.68	**
Site/Month	0.06	4	0.02	0.71	n/s
Part/Month	1.18	2	0.59	28.34	***
Site/Part/Month	0.20	4	0.05	2.44	n/s
Explained	3.26	17	0.19	9.24	***
Residual	1.83	88	0.02		
Total	5.08	105	0.05		

Leaves only

Source of Variation	Sum of Squares	df	Mean Square	F	
Site	0.78	2	0.39	13.92	***
Month	0.26	2	0.13	4.57	*
Site/Month	0.15	4	0.04	1.34	n/s
Explained	1.18	8	0.15	5.29	***
Residual	1.26	45	0.03		
Total	2.44	53	0.05		

Roots only

Source of Variation	Sum of Squares	df	Mean Square	F	
Site	0.08	2	0.04	2.98	n/s
Month	1.07	2	0.54	40.36	***
Site/Month	0.11	4	0.03	2.10	n/s
Explained	1.26	8	0.16	11.87	***
Residual	0.57	43	0.01		
Total	1.83	51	0.04		

* - $P < 0.05$; ** - $P < 0.01$; *** - $P < 0.001$ (log-transformed data)

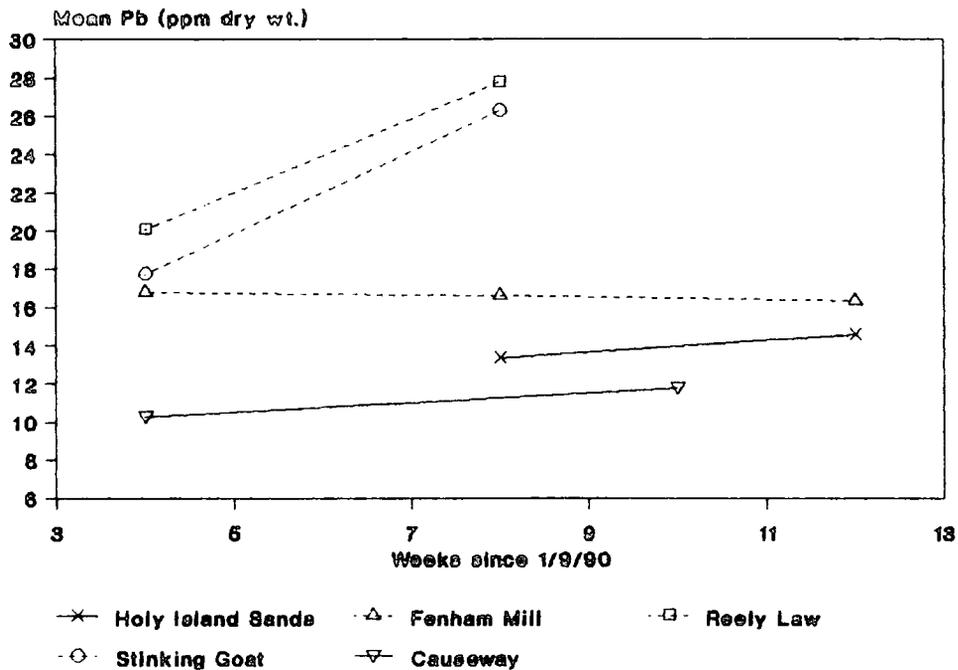
TABLE 4.4 Lead concentrations in *Enteromorpha*, September 1990 to September 1991

Site	Date	Mean Pb (ppm dw)	s.d.	n
Holy Island Sands	25 Oct 90	13.3	3.26	8
	26 Nov 90	14.6	2.50	8
	17 Jul 91	12.4	1.63	5
	02 Sep 91	10.3	1.31	5
Fenham Mill	27 Sep 90	16.8	1.26	2
	30 Oct 90	16.6		1
	26 Nov 90	16.3		1
	19 Mar 91	9.8	0.97	9
	25 Apr 91	11.7	1.29	5
	02 May 91	17.0	2.18	4
	15 May 91	19.1	0.91	3
	17 Jul 91	18.5	4.04	5
02 Sep 91	10.5	1.53	5	
Reely Law	28 Sep 90	20.1		1
	25 Oct 90	27.8		1
Stinking Goat	28 Sep 90	17.7		1
	25 Oct 90	26.3		1
	23 May 91	18.1	1.70	6
	17 Jun 91	21.3	2.63	6
	17 Jul 91	15.8	2.25	5
	02 Sep 91	11.8	1.54	5
Causeway	27 Sep 90	10.3	1.71	6
	23 Nov 90	11.8	1.39	11
	25 Apr 91	9.7	1.73	5
	06 Jun 91	23.1	2.17	4
Beal Blacks	25 Apr 91	7.9	1.09	5
	02 May 91	12.9		8
	15 May 91	15.6		8
	06 Jun 91	17.5	2.13	5

Data for May and June 1991 supplied by M. Macdonald

FIGURE 4.5a

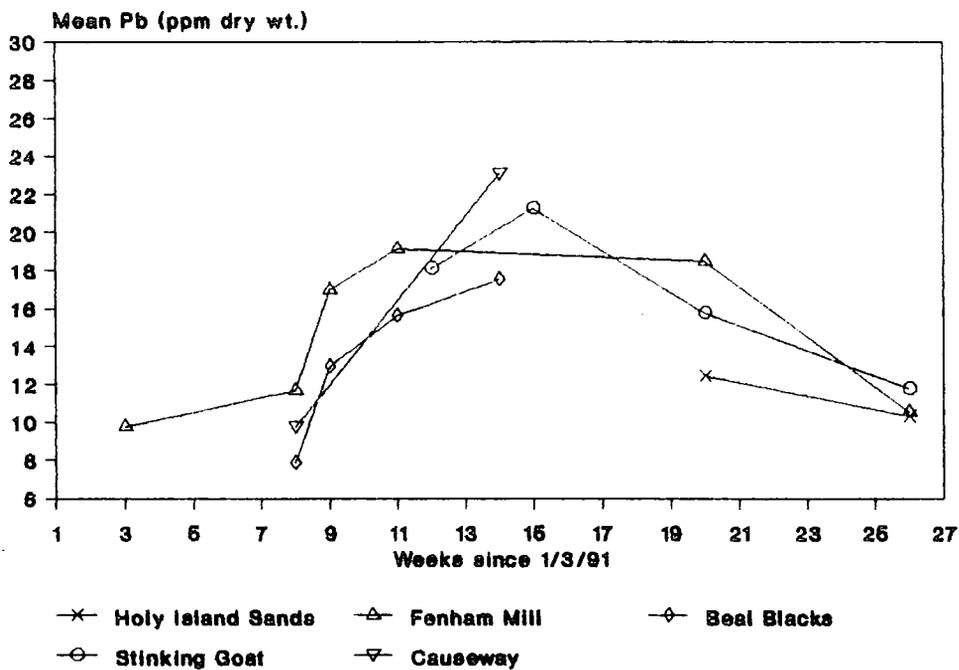
Lead in *Enteromorpha* - 1990



NB Broken line indicates single sample

FIGURE 4.5b

Lead in *Enteromorpha* - 1991



During Autumn 1990, there was no significant change in mean lead concentration at either of the two sites from which samples were collected on two occasions, Holy Island Sands and the Causeway (t test: $t_{14} = 0.86$, n/s and $t_{15} = 2.00$, n/s respectively). In only one month, November, were replicate samples obtained from both sites: the mean lead concentration at Holy Island Sands was greater than that at the Causeway (t-test: $t = 3.12$, $df = 17$, $P < 0.01$).

During Spring and Summer 1991, although there was a significant difference between the sites sampled, the principal effect was temporal, with month of sampling accounting for 55% of the overall observed variation (2-way ANOVA: site $F_{3,67} = 16.71$, $P < 0.001$, month $F_{4,67} = 38.53$, $P < 0.001$, interaction $F_{5,67} = 1.72$, n/s). In particular, at Fenham Mill, which was sampled throughout the period from 19 March to 2 September, the mean lead concentration (18.2 ppm) during the period 2 May to 17 July was significantly greater than during either the early Spring (10.4 ppm) or late Summer (10.5 ppm) (Tukey HSD test at $P < 0.05$). Similarly, a rapid increase in lead content during May was observed at both Beal Blacks, where the mean lead concentration in April was significantly lower than in May and June, and greater in June than in early May (Tukey HSD test at $P < 0.05$), and at the Causeway, where a substantial increase occurred from 9.7 ppm in late April to 23.1 ppm in early June (t test; $t = 10.38$, $df = 7$, $P < 0.001$). A less rapid decline through August was also observed at Stinking Goat, where the mean was significantly lower in July than in June, and in September compared with all previous months (Tukey HSD test at $P < 0.05$).

4.3.3 *Salicornia*

The results are shown in table 4.5 and summarised for Autumn 1990 in figure 4.6a and for Summer 1991 in figure 4.6b.

During Autumn 1990, both site and month of sampling had a significant effect on the mean lead concentration of *Salicornia* (2-way ANOVA on log transformed data: site $F_{2,74} = 63.66$, $P < 0.001$, month $F_{2,74} = 5.96$, $P < 0.01$, interaction $F_{1,74} = 7.76$, $P < 0.01$), but site accounted for the greater part (58%) of the total variation. The analysis should be treated with caution, however, as the samples were pseudo-replicates. The mean lead concentrations at Fenham Mill were higher than those at either Beal Point or Ross Point, both of which lie outwith the area of wildfowling activity. At Fenham Mill, the mean lead concentration of 12.0 ppm on 30 October 1990 was a significant increase

TABLE 4.5 Lead concentrations in *Salicornia*, September 1990 to September 1991

Site	Date	Mean Pb (ppm dw)	s.d.	n
Fenham Mill	27 Sep 90	7.8	1.20	7
	15 Oct 90	8.3	0.84	18
	30 Oct 90	12.0	1.43	13
	02 May 91	7.1	1.24	2
	20 May 91	7.3	0.11	3
	06 Jun 91	7.5	0.58	3
	17 Jul 91	6.3	0.40	5
	02 Sep 91	5.1	0.26	6
Beal Blacks (mud)	02 May 91	4.9	0.96	2
	20 May 91	6.7	0.80	2
	06 Jun 91	7.5	0.14	2
	17 Jul 91	6.5	0.25	3
	02 Sep 91	4.8	0.24	6
Beal Blacks (mud)	02 May 91	8.5	1.12	3
	20 May 91	8.4	0.40	3
	06 Jun 91	9.7	0.70	3
	17 Jul 91	6.0	0.70	4
Beal Point	30 Oct 90	6.0	0.52	14
	23 Nov 90	7.2	0.38	5
Ross Point	11 Oct 90	6.3	0.68	18
	10 Nov 90	5.6	0.46	5

Data for May and June 1991 supplied by V. Picton-Jones

FIGURE 4.6a

Lead in *Salicornia* - 1990

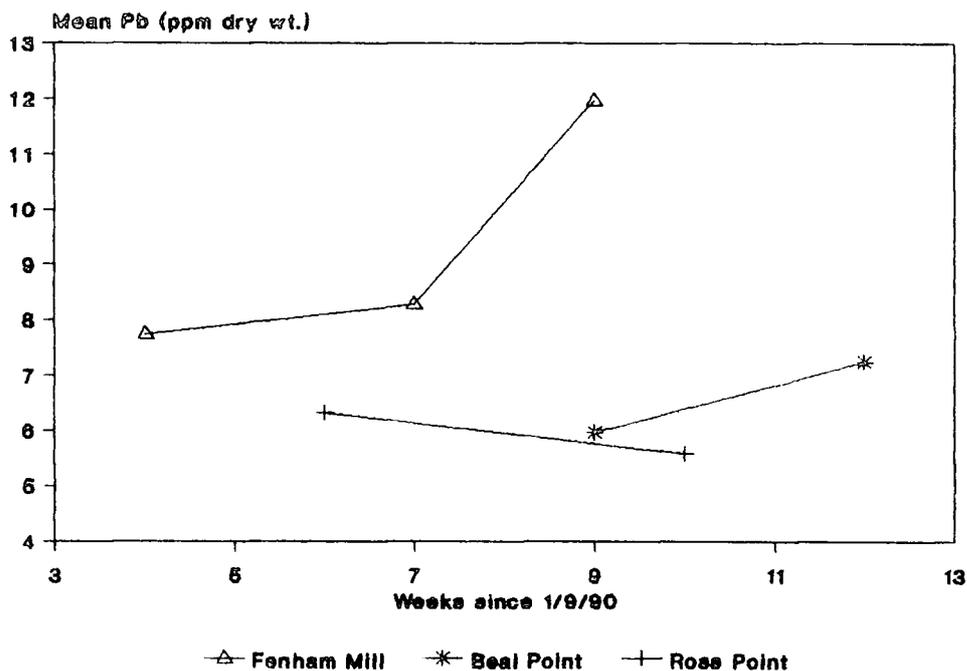
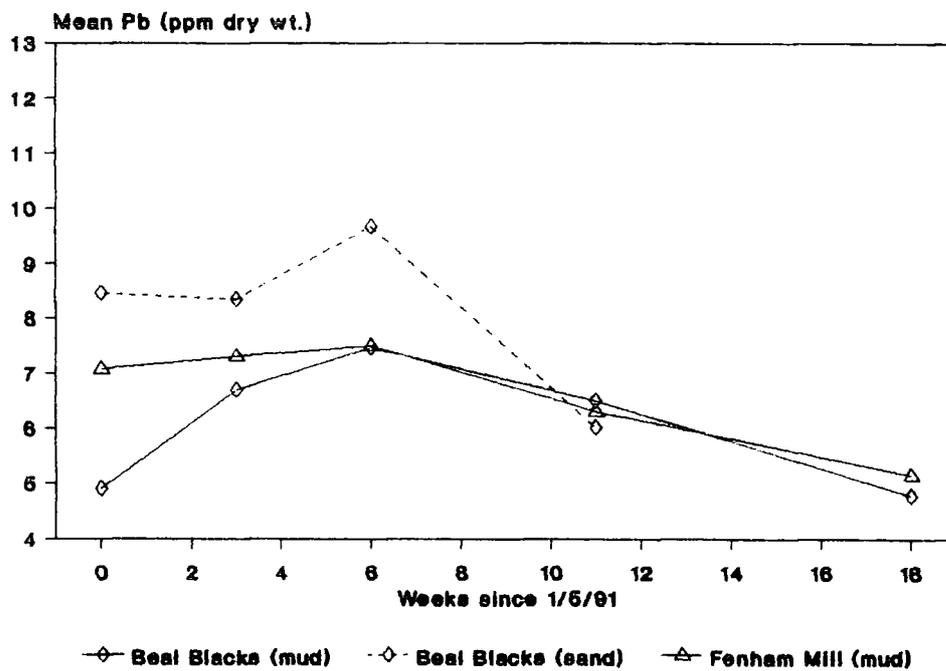


FIGURE 4.6b

Lead in *Salicornia* - 1991



on the previous means of 27 September and 15 October (ANOVA on log-transformed data: $F_{2,35} = 44.33$, $P < 0.0001$; Tukey HSD test at $P < 0.05$).

During 1991, the *Salicornia* growing on a sandy substrate at Beal Blacks had a higher mean lead concentration than that growing on mud, but by mid July the difference was no longer apparent (insufficient data for statistical comparison). On the mud of Beal Blacks and Fenham Mill, the sampling date was the most significant factor, explaining 69% of the variation in mean lead concentration, but there was also a significant difference between the two sites (2-way ANOVA on log-transformed data: site, $F_{1,24} = 8.68$, $P < 0.01$, date $F_{4,24} = 34.20$, $P < 0.001$, interaction, $F_{4,24} = 4.96$, $P < 0.01$). On Beal Blacks there was a significantly elevated mean lead concentration between 20 May and 17 July compared with early May and September (Tukey HSD test at $P < 0.05$), but at Fenham Mill the May values were relatively high, and only a significant decline was apparent, the mean of 5.1 ppm in September being lower than on all earlier dates (Tukey HSD test at $P < 0.05$).

4.4 Discussion

4.4.1 *Zostera*

a) Seasonal pattern of lead in leaves and roots

The changes in lead levels in the two species of *Zostera* showed a broadly similar pattern during Autumn 1990, as shown in figs. 4.1a and 4.1b. There was a slight decrease in the roots between September and October, followed by a very gradual increase. Comparison of the two species on a site by site basis shows the levels in *Z. angustifolia* roots to be slightly higher than in *Z. noltii* roots. *Z. angustifolia* exhibited a marked increase in lead in the leaves during the Autumn, and particularly so for the January sample from Stinking Goat. There was a less noticeable increase for *Z. noltii* leaves at Fenham Mill and Reely Law, but there was no corresponding pattern at the other two sites.

The overall effect for *Z. angustifolia* was such that in September the roots had the greater lead content, but as the Autumn progressed, greater values were observed in the leaves (except at Holy Island Sands). A similar pattern was reported for *Z. marina* in the

Limfjord, Denmark, by Lyngby and Brix (1982), although the decline in lead in the roots during the early Autumn was dramatic in their study, and the increase in the leaves rather later in the Winter, reaching a maximum during March.

The differences in foliar lead shown by the two species at Lindisfarne are consistent with their life-cycles. Widespread senescence of *Z. angustifolia* occurs during the Autumn, to the extent that there remained negligible quantities by January 1991, except at Stinking Goat. New growth commences in Spring, presumably chiefly from seed. In contrast, *Z. noltii* over-winters in the vegetative state, albeit much reduced in biomass, and Spring growth probably arises from the rhizome stock. There is little corresponding leaf senescence. Harrison and Mann (1975) detected a decrease in the proportion of organic matter in *Z. marina* leaves as they matured, and although they did not measure it, there must have been a corresponding increase in the proportion of inorganic constituents, including any trace metals present. Furthermore, Brix and Lyngby (1982, 1983) showed that the lead content of *Z. marina* leaves increased with the age of the leaf.

Similar increases in foliar lead concentrations during Autumn senescence have been reported in a variety of contexts, including roadside vegetation (Cribb, 1976), pasture herbage (Mitchell and Reith, 1966) and Spruce *Picea abies* needles (Nilsson, 1972).

The lead levels measured during Spring and Summer 1991 are considerably lower than during Autumn 1990. The general picture is of fairly consistent levels during the 1991 growing season, with the roots of both species having a higher lead content than the leaves during May and June. The roots of *Z. angustifolia* show a greater temporal variation during Spring than do those of *Z. noltii*, which may be possibly explained by the *Z. noltii* below-ground material being part new growth and part over-wintering rhizomes, whereas all the *Z. angustifolia* root material is fresh growth.

It would therefore appear that there is a transition point within a month or so after the maximum annual biomass is reached in August (Jacobs, 1979; Jacobs et al, 1983), coincident with the end of the Summer growth period, at which the mean lead levels in *Zostera* leaves begins to rise. This may be accompanied by a slight decrease in the lead levels in roots and rhizomes, particularly in *Z. noltii*, possibly due to the translocation of organic material into the rhizomes

in preparation for Winter dormancy, thereby effectively diluting the concentration of lead within the rhizomes. The differences in the changes in lead content of leaves and roots result in there being no correlation over time between the two parts of the plants, although at any one time they may be correlated between sites (Brix and Lyngby, 1984; Lyngby and Brix, 1987).

It is not clear whether the difference observed between the lead levels in late September 1990 and early September 1991 is due solely to a normal seasonal increase in lead content during September, or to a genuine difference between years. There is some evidence that seasonal differences may be involved, since the lead levels found in *Zostera* taken from Holy Island Sands and Fenham Flats during late May 1990 (Collins, 1990) were generally similar to those of May 1991, except for *Z. angustifolia* roots, which showed considerably lower levels in May 1990. Furthermore, increases of 4 ppm occurred between September and October 1990 for *Z. angustifolia* leaves at two sites, so the differences in September 1990 and 1991 lead levels could have arisen in about five or six weeks normally. However, the Summer of 1990 was particularly hot, and differences in Autumn lead levels due to contrasting Summer growth rates in 1990 and 1991 cannot be ruled out.

b) Comparison between sites

The lead concentrations in *Z. angustifolia* at Holy Island Sands were clearly lower than on Fenham Flats during Autumn 1990, but not so during Spring and Summer 1991. With regard to Fenham Flats, it is not clear why there should be a difference between sites for *Z. noltii* but not for *Z. angustifolia*.

In the Limfjord, Denmark, Lyngby and Brix (1987) found very highly significant correlations between the sediment lead levels and those of *Z. marina* leaves and roots taken during June from a large number (158) of sampling locations, but their data show a much greater range of variation in lead levels (more than an order of magnitude) than were found at Lindisfarne. With only four sites sampled, a statistical correlation between lead in the sediment and in *Zostera* is not possible in this study. Nevertheless, there is a tendency for the Autumn lead levels in *Zostera*, more notably in *Z. angustifolia*, to reflect the sediment lead content (undifferentiated fraction) from January 1991, i.e. the highest levels recorded from Fenham Mill and Reely Law, and the lowest from Holy Island Sands. The relationship at Stinking Goat may be

misleading, as the sediment lead value may possibly be reduced by matrix interference, as noted in section 3.4.3. Figure 4.4 indicates that, for *Z. angustifolia*, the lead levels across the whole of Fenham Flats are fairly uniform at any one time.

It would therefore seem that at Lindisfarne, the spatial variation in *Zostera* lead levels is not great, being slightly lower on Holy Island Sands than on Fenham Flats. The difference cannot be attributed unequivocally to variation in shooting pressure, either current or historical; differences in sediment composition between the two areas may be solely responsible.

c) Comparison with other studies

The data most comparable with this study are those on *Z. marina* from the Limfjord, Denmark. Of the three locations in the Limfjord at which Lyngby and Brix (1982) sampled *Z. marina* over the course of one year, two were unpolluted rural sites, and the lead levels recorded in both leaves and roots remained at a fairly steady low level (less than 2.5 ppm) throughout the period. At their industrially polluted site, where sediment lead levels (16.3 ppm) were similar to those at Lindisfarne, ten-fold variations in lead were observed, the maximum levels being approximately 23 ppm for leaves during November, and 25 ppm for roots in July. In a more extensive study covering forty sites sampled during August, the mean lead concentrations in *Z. marina* leaves and roots (excluding four industrially polluted sites) were 1.07 and 1.04 ppm respectively (Brix et al, 1983; Brix and Lyngby, 1984). Thus the *Zostera* lead levels recorded at Lindisfarne are greater than might be expected from a relatively unpolluted site, being similar to those from the polluted site in Denmark.

In a study carried out at a number of inland wildfowling locations in North America, Behan et al (1979) found lead levels in aquatic angiosperms ranging from 1.6 to 5.6 ppm in leaves and from 12.0 to 16.9 ppm in roots (except at one site where roots contained 41.8 ppm). In all cases, roots contained much higher levels than shoots, but the time of year when samples were collected is not stated. It may be that translocation of lead from roots to shoots occurs more readily in *Zostera* than in freshwater aquatic angiosperms.

Considerably higher lead levels have, however, been reported from terrestrial vegetation subject to high levels of pollution. Cribb (1976) found concentrations up to 68.8 ppm in roadside vegetation

contaminated by vehicle exhaust emissions. Alloway and Davies (1971) recorded a maximum of 74.2 ppm in pasture herbage at a site where the soil contamination due to mining activity was as high as 3680 ppm. Dorn et al (1975) reported a maximum of 979 ppm for the unwashed aerial parts of meadow vegetation downwind of a lead smelter. The values obtained for *Zostera* in this study clearly do not approach the levels which may arise in terrestrial vegetation from industrial activity, and in those terms Lindisfarne would be considered a site of low to moderate lead pollution.

4.4.2 *Enteromorpha*

In contrast to *Zostera*, the alga *Enteromorpha* acquires most of its lead from sea-water. That the lead levels observed in *Enteromorpha* are similar to those in *Zostera* may initially appear surprising, since the lead concentration in sea-water is normally many orders of magnitude lower than that of the intertidal sediment. Balls (1985) recorded 0.031 ppb for the northern North Sea, although lead concentrations in coastal waters may be up to two orders of magnitude above the marine levels (Förstner, 1980), eg. Preston et al (1972) reported a geometric mean of 0.21 ppb for five coastal stations in the North Sea and 1.3 ppb for seawater taken from the shoreline of the Irish Sea. However, until it is ripped up by the tide, *Enteromorpha* grows in a fine mat on the surface of the sediment, and its micro habitat may therefore subject it to lead levels closer to those of the sediment interstitial water than of the sea in general. Indeed, as mentioned in section 3.4.3, lead may possibly be removed from the sediment by transfer to sea-water. A flux of certain metals, eg. iron and manganese, between interstitial water and the overlying estuarine water, has been shown to occur under appropriate oxidation conditions (Presley and Trefry, 1980). Moreover, even when separated from the sediment, *Enteromorpha* may still absorb lead from sea-water and high concentrations result by bioaccumulation, which is well documented for marine organisms (eg. Wong et al (1978)).

Say et al (1990) determined heavy metal concentrations of *Enteromorpha* during Autumn for six British estuaries, including two in north-east England. On the relatively unpolluted Tweed, the lead concentration in *Enteromorpha* was below 10 ppm at four sampling stations and below 20 ppm at the fifth, but on the industrially polluted Tyne, seven of ten sampling stations had lead concentrations in excess of 40 ppm, and two of these exceeded 80 ppm. Say et al (1990) propose that

Enteromorpha lead concentrations in the range 20 to 60 ppm be considered indicative of moderate contamination, and above 60 ppm of high contamination. On that basis, Lindisfarne is an area of low to moderate lead contamination, as was concluded from the lead concentrations of *Zostera* discussed in section 4.4.1.

No firm conclusions can be drawn from the limited data collected during Autumn 1990, but during Spring and Summer 1991, whereas the lead levels in *Zostera* remained fairly constant, a marked rise in lead was observed in *Enteromorpha* during May, and a decline in mid Summer. Water temperature may be involved in stimulating growth, and hence uptake of nutrients and lead, in May, but is unlikely to be the sole cause since the maximum sea-water temperature is not reached until late Summer. Day-length may be important, and increased levels of solar irradiation may result in local temperature increases in pools and on the sediment surface.

4.4.3 *Salicornia*

The pattern observed in *Salicornia* during Spring and Summer 1991 is similar to that shown by *Enteromorpha*, with a peak in June, but the effect is less pronounced for *Salicornia*, and the overall lead level is much lower than for either *Enteromorpha* or *Zostera*. *Salicornia* growth commences from seed at the beginning of May, and is rapid during early Summer. Initially the rate of uptake of lead may be limited by root development, but as the root system develops, the rate of lead uptake may be expected to increase relative to plant growth. A threshold rate, dependent on the efficiency of translocation of nutrients from roots to aerial parts, may result in decreasing lead levels as growth reaches its peak in Summer. There is some evidence of an increase in lead concentration during the Autumn, but whether it is associated with seed maturation or general senescence is not clear.

5.1 Introduction

To determine the average daily lead throughput for a Wigeon wintering at Lindisfarne, it was necessary to know the quantity of food consumed in a day, the lead content of the food (discussed in chapter 4), the daily faecal output and the lead content of the faeces. Over the short term, the difference between the amount of lead ingested and excreted was taken to be the quantity retained, but the lead assimilated may not be retained indefinitely, possibly being re-mobilised at a later date, and lost by excretion, moult of feathers, or by females during egg-laying.

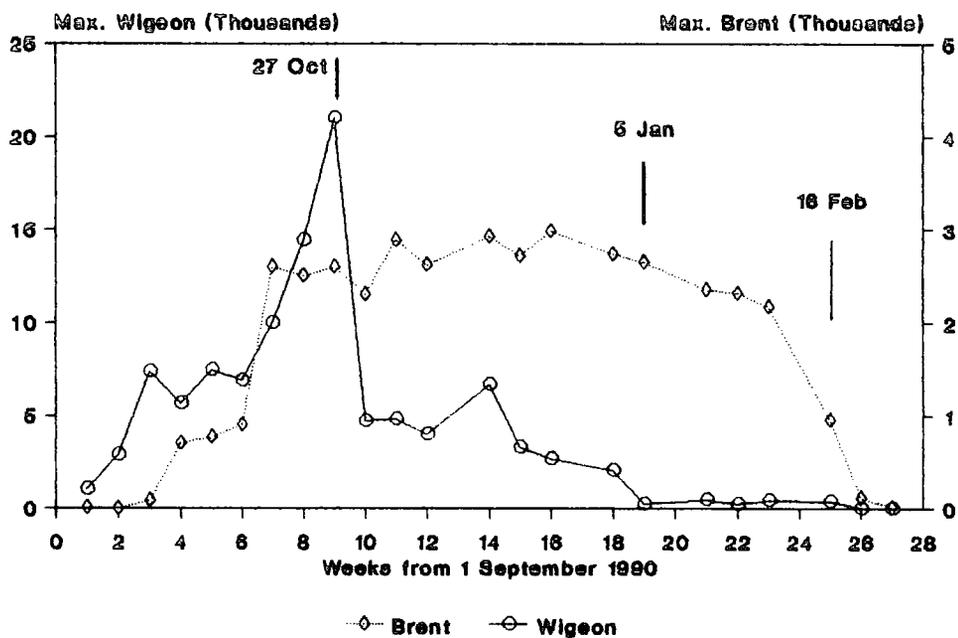
5.2 Utilisation of Vegetation

At Lindisfarne the wintering Wigeon are heavily dependent on *Zostera*. Numbers build up during September and October and decline during November and December, by which time there is virtually no *Z. angustifolia*, and the above-ground biomass of *Z. noltii* is also almost exhausted. Figure 5.1 shows the weekly maximum counts of Wigeon and Brent Geese at Lindisfarne during Autumn 1990 and the first two months of 1991. The Wigeon population peaked at the end of October 1990 at 21000 birds, but dropped to less than a quarter of that number within one week. From the start of January 1991 there were fewer than 500 Wigeon present.

The estimation of wildfowl feeding rates by observation of dropping rates and comparison of the proportions of an indigestible marker in the food and faeces is a well-established procedure (eg. Ebbinge et al, 1975). It was intended that the method be employed in this study, but problems encountered in the observation of dropping rates (as detailed in section 5.3), particularly during the first half of the Autumn, prevented the collection of sufficient data.

The Wigeon were observed to feed on *Salicornia* at high tide during September and October, but only a small proportion of the birds present did so during daylight. Nocturnal feeding habits could not be quantified. *Enteromorpha* also presumably forms part of the diet, but whether it is grazed selectively, or incidentally when grazing *Zostera* was not determined.

FIGURE 5.1 Weekly Wigeon and Brent Goose Counts
September 1990 - March 1991



Data supplied by S. M. Percival

5.2.1 Methods

Assessment of vegetation utilisation was carried out in conjunction with the concurrent Brent Goose study (section 1.4). The position and size of feeding flocks were recorded on maps at regular intervals (every 30 minutes) during periods of feeding observations. From these, the utilisation of Fenham Flats by Wigeon was compared with data on the density of both *Zostera* species and *Enteromorpha* across Fenham Flats estimated during monthly transects made by all-terrain vehicle for the Brent study.

5.2.2 Summary of Results

During Autumn 1990, the Wigeon flocks used all areas of Fenham Flats for feeding, as did the Brent Geese. However, unlike the Brent, the Wigeon were not observed to feed on Holy Island Sands, apart from infrequent visits by a small proportion of birds present on the reserve.

During September 1990 there was no correlation between the use of Fenham Flats for feeding by Wigeon and the above-ground density of either *Z. angustifolia* ($r = -0.25$, $n = 38$, n/s) or *Z. noltii* ($r = -0.23$, $n = 38$, n/s) or the density of *Enteromorpha* ($r = 0.12$, $n = 38$, n/s), but a highly significant correlation was identified between Wigeon utilisation and distance from the shore ($r = 0.50$, $n = 51$, $P < 0.001$). During October the correlation between Wigeon utilisation and distance from the shore remained, but less strongly ($r = 0.32$, $n = 51$, $P < 0.01$), there was a highly significant correlation between Wigeon utilisation and the density of *Enteromorpha* ($r = 0.60$, $n = 45$, $P < 0.001$), and a highly significant inverse correlation with the density of *Z. noltii* ($r = -0.43$, $n = 45$, $P < 0.01$). For November and December combined, there was a highly significant inverse correlation between Wigeon utilisation and distance from the shore ($r = -0.43$, $n = 51$, $P < 0.01$), such that most feeding occurred at the upper tidal levels. There was no correlation with *Enteromorpha* density ($r = 0.07$, $n = 49$, n/s), but a highly significant correlation with the density of *Z. angustifolia* ($r = 0.51$, $n = 49$, $P < 0.001$). (All analyses from S. M. Percival, pers. comm.).

5.2.3 Discussion

During the early Autumn, the Wigeon were able to satisfy their diurnal feeding requirements during the first part of the flood tide and the latter stages of the ebb tide, principally by swimming above the

Zostera beds and feeding by head-dipping or up-ending. They rarely fed near to the shore-line, presumably because of the risk of predation (ie. wildfowling). As the density of *Zostera* decreased, especially after storm-force winds and spring tides removed a large proportion of the *Zostera* leaves in early October, it became necessary to feed for longer during each tidal cycle, and by November the flocks fed close to the shore-line throughout the ebb tide (pers. obs.).

The data suggest that Wigeon may show a preference for *Z. angustifolia* over *Z. noltii*, although why this was not apparent during September is not clear, particularly as there was a definite preference for feeding at a distance from the shore-line and *Z. angustifolia* was the more abundant species in those areas. During the latter half of the Autumn, although the Wigeon fed mostly on the upper shore, which supported most of the *Z. noltii* available on Fenham Flats, there is suggestive evidence that they favoured the areas holding the limited quantities of *Z. angustifolia* still available. Since the leaves of *Z. angustifolia* are much larger than those of *Z. noltii*, feeding on the former where possible would presumably be more efficient.

The relationship with *Enteromorpha* cover during October may be spurious, given the transient distribution of much of the algae. However, van Eerden (1984) reports a higher calorific value for *Enteromorpha* than for *Zostera*. *Enteromorpha* may therefore be actively selected if present in sufficient quantities. *Salicornia* too has a greater energy value than *Zostera* (van Eerden, 1984), but is of very limited importance during daylight hours, except possibly to a small proportion of the Wigeon present, although when present in large quantities it may form the main component of the Wigeon diet (van Eerden, 1984). At Lindisfarne it is only available near the shore-line during the early Autumn, when there are ample quantities of *Zostera* available, and presumably the perceived risk of predation out-weighs the potential nutritional gains.

It is stressed that the pattern of feeding discussed relates to daylight hours only; at night the Wigeon may behave quite differently. Feeding at night is probably more important than during the day at Lindisfarne. Madsen (1988) recorded approximately equal time (6.25 hours each) spent on day and night feeding on *Zostera* during September and October in Denmark. As daylight hours decrease during the Autumn, and the total time spent feeding increases to compensate for

lower intake rates as the *Zostera* is depleted, the relative importance of night feeding is expected to increase. During September and October in the Netherlands, nocturnal feeding on *Salicornia* was particularly important for Wigeon, but by the end of October, as the *Salicornia* became depleted, they switched to feeding mainly on saltmarsh grasses, which were of lower calorific value, but more abundant (van Eerden, 1984). Nocturnal feeding on *Salicornia* may also be important at Lindisfarne during the early Autumn.

5.3 Faecal Output

5.3.1 Methods

Indirect methods of measuring faecal output, such as counting droppings in marked areas, could not be employed easily in an intertidal situation, because droppings would be redistributed by the tides and the substrate was too soft for regular access to feeding areas, particularly during the early Autumn, when most feeding took place at some distance from the shore-line.

Dropping rates were measured for Wigeon flocks feeding above the tide-line close enough to the shore-line for observations to be made through a 15-60x magnification telescope. A focal bird was picked at random and its droppings counted until it disappeared from view (behind another bird) or turned towards the observer, whereupon the nearest bird was immediately selected as the focal bird.

5.3.2 Results

Observation of dropping rates proved to be difficult for Wigeon at Lindisfarne, as the droppings are small, have a short distance to fall, and the birds usually fed in tight flocks, so that the focal bird was frequently lost to view. Moreover, during the first half of the Autumn, the principal method of feeding was by swimming above the *Zostera* beds on the flood tide; it was only after *Zostera* stocks were considerably depleted that the flocks were forced to feed above the tide-line sufficiently close to the shore to permit observations to be made.

A total of 27 droppings were recorded in 82' 24" observation time (combined data: pers. obs. and S. M. Percival, pers. comm.), giving an overall mean dropping rate of 19.7 per hour. The data were

insufficient to apply the hourly block method recommended by Bédard and Gauthier (1986), and may therefore only be regarded as a rough approximation for the whole Autumn.

5.3.3 Discussion

Madsen (1988) estimated the mean daily faecal output to be 35.0 g ash-free dry weight at a dropping rate of 16.8 per hour for Wigeon feeding on *Z. noltii* during early Autumn in Denmark. The dropping rate obtained in this study is slightly faster, but given the limitations noted above, it is reasonable to apply Madsen's results in the calculation of a daily lead budget for Wigeon (section 5.5).

5.4 Lead in Faeces

5.4.1 Methods

Wigeon droppings were collected on six occasions when flocks had been observed feeding close to the shore-line for a minimum of one hour, ie. long enough for droppings to be produced if the birds had commenced feeding on an empty gut. Additionally, samples were obtained on one occasion (27 November) by using an all-terrain vehicle.

Droppings were collected individually in stoppered glass vials, dried at 50 °C for a minimum of 48 hours in a vacuum oven, and analysed individually for lead by the procedure described in section 2.3.

The ash content of droppings was determined by ashing individual droppings in a muffle furnace for 4 to 5 hours at 500 °C.

5.4.2 Results

The lead content of the droppings analysed and the dry weights of the droppings are shown in table 5.1a and figures 5.2 and 5.3. Ash content is shown in table 5.1b and figure 5.4.

The effect of date was highly significant on the dry weight of droppings and faecal lead concentration. The droppings produced in November had a higher (geometric) mean gross dry weight of 0.279 g compared to those from September and October combined, which averaged 0.159 g (t test on log-transformed data: $t = 9.29$, $df = 106$, $P < 0.001$). The respective mean lead concentrations were 15.7 ppm and 14.5 ppm (t test: $t = 2.61$, $df = 106$, $P < 0.01$).

TABLE 5.1a Wigeon faeces - Lead content and dry weight, 1990

Site	Date	n	Mean Pb (ppm dw)	95% conf. limits	Mean wt (g)	95% conf. limits
Fenham Mill	12 Sep	5	11.4	7.9 - 15.0	0.213	0.197 - 0.238
	03 Oct	16	14.6	13.2 - 16.0	0.171	0.142 - 0.206
	11 Oct	8	15.7	13.4 - 17.9	0.151	0.111 - 0.207
	16 Oct	14	14.8	13.8 - 15.8	0.135	0.114 - 0.160
Fenham le Moor	09 Nov	10	17.0	15.5 - 18.5	0.255	0.187 - 0.348
Reely Law	23 Nov	26	15.6	14.6 - 16.6	0.276	0.250 - 0.305
Fenham Mill	27 Nov	29	15.3	14.8 - 15.8	0.290	0.263 - 0.320

TABLE 5.1b Wigeon faeces - Ash content, 1990

Site	Date	n	Mean % ash	95% conf. limits
Fenham Mill	12 Sep	2	38.2	7.5 - 68.9
	03 Oct	5	34.2	32.4 - 35.9
	11 Oct	3	47.8	40.4 - 55.1
	16 Oct	9	35.0	32.1 - 37.9
Fenham le Moor	09 Nov	3	50.9	42.6 - 59.3
Reely Law	23 Nov	7	43.2	40.7 - 45.6
Fenham Mill	27 Nov	9	42.4	37.2 - 47.6

NB Faeces collected 3 and 16 October were from *Salicornia* bed at Fenham Mill

FIGURE 5.2

Lead in Wigeon Faeces - 1990

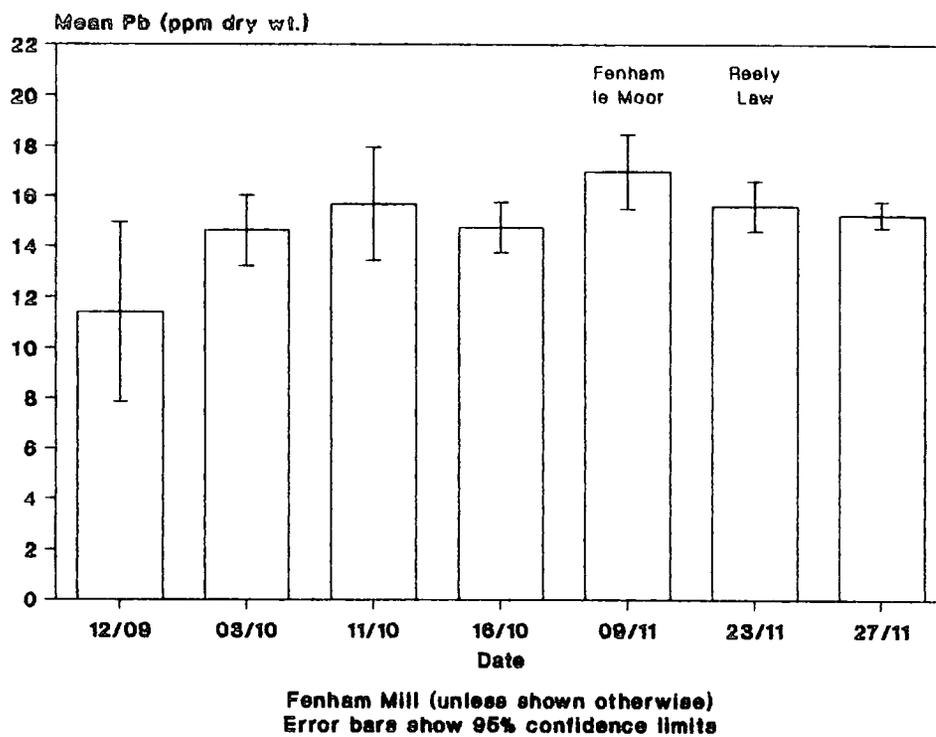


FIGURE 5.3

Wigeon Faecal Weights - 1990

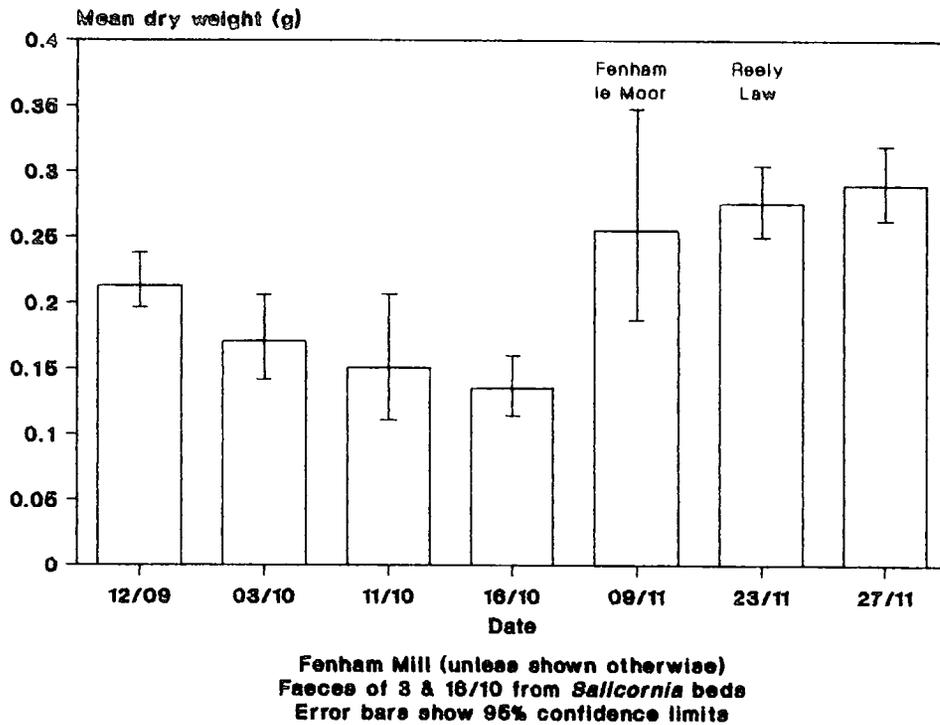
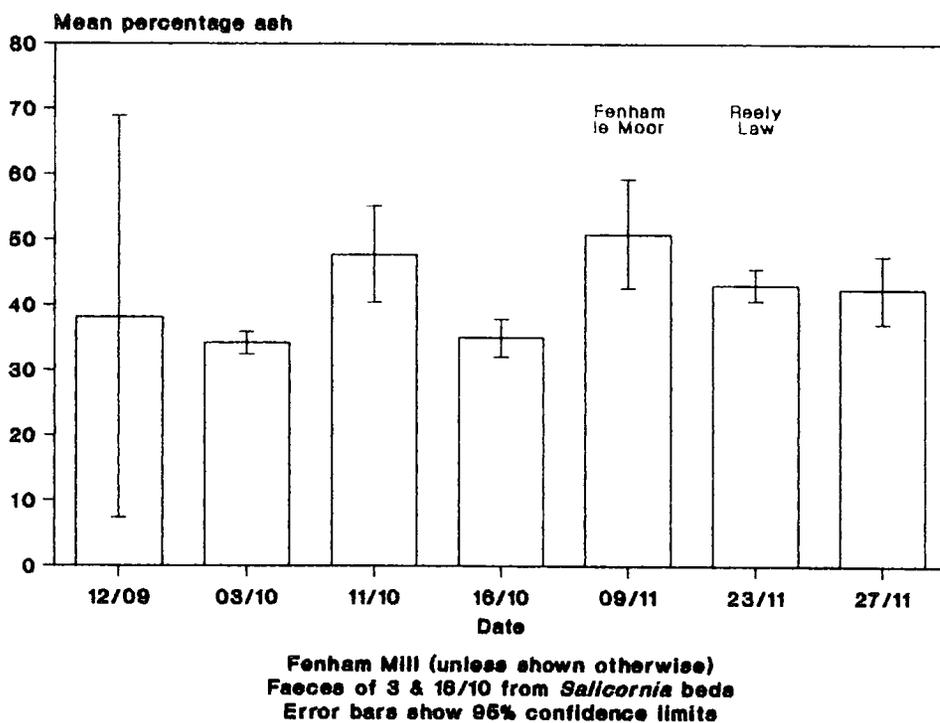


FIGURE 5.4

Wigeon Faeces - Ash Content - 1990



The droppings collected on 3 and 16 October from the *Salicornia* bed at Fenham Mill did not differ from those collected from the *Zostera* bed at the same location on 11 October in terms of either mean gross weight or lead concentration. The small sample collected on 12 September at Fenham Mill had a significantly lower lead content than each of the other samples collected from *Zostera* beds (all comparisons by Tukey HSD test at $P < 0.05$; faecal weights log-transformed).

The increase in weight of droppings in November suggested that the proportion of sediment in the droppings (and hence the proportion ingested) might have increased as the Wigeon switched from feeding mainly below to feeding above the tide-line. The percentage ash in the faeces showed a highly significant date effect (ANOVA; $F_{6,32} = 5.80$, $P < 0.001$), but this was principally due to the droppings from the *Salicornia* bed having a lower ash content than those from the *Zostera* areas (Tukey HSD test at $P < 0.05$). Droppings from the *Zostera* beds showed no significant difference between those collected in November and those from September and October (Tukey HSD test at $P < 0.05$).

5.4.3 Discussion

It would certainly appear that the November droppings contained a considerable amount of sediment, which is consistent with the feeding method employed. Wigeon must always ingest some grit for grinding food in the gizzard, but when feeding on sparsely distributed *Z. noltii* leaves above the tide-line the quantities ingested are presumably unavoidably greater than usual. In retrospect, the organic material should have been separated from the inorganic material before drying. An attempt was made to separate the two fractions of dried droppings by flotation, but it was unsuccessful. Whether the ash content of *Salicornia* is genuinely lower than that of *Zostera*, or simply the Wigeon ingest more sediment when feeding on *Zostera* leaves is not known.

Madsen (1988) reported a mean dropping weight of 0.164 g ash-free dry weight for Wigeon feeding on *Z. noltii* leaves. Since in his study the Wigeon fed only during the low water period, ie. above the tide-line, his figure is most closely comparable with the data from November at Lindisfarne (rather than those from the early Autumn, when a different feeding method was employed, and probably the main component of the diet was *Z. angustifolia*). Applying the mean November value of 44 % ash content to the mean dropping weight of 0.279 g gives a mean dropping weight of 0.151 g ash-free dry weight, which is in good

agreement with Madsen's figure. It is therefore reasonable to employ Madsen's data in the calculation of a daily lead budget (section 5.5).

The method of wet ashing the droppings by refluxing in HNO_3 will release all the lead adsorbed onto the sediment particles present in the droppings. Hence the increase in faecal lead in November may be due in part to an increase in the sediment content of the droppings, rather than to an increase in the lead content of the food, although the lead concentrations of the leaves of both *Zostera* species on Fenham Flats did increase during the Autumn.

5.5 Estimation of the Daily Lead Budget for Wigeon

For the purposes of calculating a daily lead budget for Wigeon at Lindisfarne, estimates for the mean quantity of *Zostera* ingested per day of 64.8 g ash-free dry weight and the daily defecation of 35.0 g ash-free dry weight have been taken from Madsen (1988), as the observations made during the present study did not enable food intake to be estimated. The ash content of *Zostera* was not determined in this study, but Jacobs et al give a figure of 32.3% ash for *Z. noltii*, and on that basis the mean dry weight of *Zostera* consumed by a Wigeon in a day is 95.7 g, a figure which is in close agreement with the 91.6 g dry weight of grass consumed by day reported by Mayhew (1988). It is also assumed that the Wigeon fed mainly on *Zostera* leaves, and that the roots and rhizomes formed only a minor part of the diet. Although the Wigeon may have been showing a preference for *Z. angustifolia*, they undoubtedly consumed both species, and therefore the mean leaf lead content of the two *Zostera* species is used. The calculation of daily lead throughput is shown below for two sites during November 1990. Data for October 1990 are less reliable, since the sample size of faeces (excluding those collected from the *Salicornia* beds) was small.

For November 1990 at Fenham Mill, the mean lead concentration in *Zostera* leaves was 20.8 ppm, and the total lead ingested was therefore 1.99 mg. The mean dropping weight was 0.290 g with mean ash content of 42.4%. The ash-free dropping weight was therefore 0.167 g, and the number of droppings per day 210. The mean lead content of the faeces was 15.3 ppm, giving the weight of lead in each dropping as 0.00444 mg. Thus the total quantity of lead excreted was 0.93 mg, and the weight of lead retained 1.06 mg per day.

At Reely Law, the corresponding input values are a mean lead concentration in *Zostera* leaves of 21.2 ppm, mean dropping weight 0.276 g, mean ash content 43.2% and mean lead concentration in faeces of 15.6 ppm, which give total quantities of lead ingested of 2.03 mg, excreted 0.97 mg and retained 1.06 mg per day, in exact agreement with the figure for Fenham Mill.

Approximate confidence limits may be attached to the daily lead budget by calculating best and worst case scenarios based on the 95% confidence limits of the input data. For example, for the Fenham Mill data, the best case scenario is calculated from the minimum lead concentration in *Zostera* (18.1 ppm), the maximum in the faeces (15.8 ppm) and the maximum ash content of the faeces (47.6%), which gives daily lead ingested to be 1.73 mg, excreted 1.06 mg and retained 0.67 mg. Similarly, the values for the worst case scenario are 2.24, 0.82 and 1.42 mg respectively.

Coburn et al (1951) conducted a series of dosing experiments in which captive Mallards *Anas platyrhynchos* were dosed orally with aqueous solutions of lead nitrate at various strengths. They concluded that there was a critical lead intake level at between 6 and 8 mg/kg-bodyweight/day, above which clinical symptoms of avian plumbism were observed. A Wigeon weighing 700 g ingesting 2 mg lead per day in its diet of *Zostera* at Lindisfarne would be ingesting 2.9 mg/kg-bodyweight/day, well below the critical threshold.

Scheuhammer (1991) suggests that absorption of dietary lead in the avian gut is similar to that in the mammalian, at about 5% to 10% for adults and 30% to 40% for juveniles. The evidence from this study is that absorption is considerably higher, at around 50%, although, as the composition of Wigeon feeding flocks was not recorded in this study, it is not known whether the samples of faeces collected were biased towards those produced by juveniles.

Finally, it is noted that differences in *Z. angustifolia* lead concentrations between sites on Fenham Flats were less important than the increase with month of sampling during Autumn 1990 (table 4.3a and fig. 4.1a)), and, although site was more important for *Z. noltii* (table 4.3b), the lead concentration in the leaves did increase (except at Stinking Goat) (fig. 4.1b). Thus the daily quantity of lead ingested depends more on the time of year rather than on where on Fenham Flats a bird chooses to feed, particularly if Wigeon prefer to feed on

Z. angustifolia, for which there is some evidence. If, as has been supposed, the Wigeon fed mainly on *Zostera* leaves, the quantity of lead ingested for a given quantity of food would have increased during the course of the Autumn 1990.

5.6 Analysis of Wigeon Tissues

5.6.1 Methods

The viscera of forty Wigeon shot during October and November 1990 were obtained from wildfowlers, either directly or via the warden at Lindisfarne. When shot birds were collected immediately after being killed, the sex, age and gross weight were recorded. Some of these data were also supplied by the wildfowlers themselves. An attempt was made to obtain samples by distributing collection bags to wildfowlers through a local gun-shop, but it was not fruitful. All viscera were stored frozen until analysis.

Of the samples collected, two yielded insufficient quantities of liver for analysis, and in all of the samples the kidney was either not present or badly fragmented during evisceration. Analysis was therefore limited to the liver.

The liver tissue was washed, dried of excess water using absorbent paper, weighed, dried in a vacuum oven at 50 °C for a minimum of 48 hours and then weighed again. Thus the percentage of water could be calculated. However, in many of the samples only part of the liver was present, and a comparison of liver weights of birds shot at different times could not therefore be made. The lead concentration was determined as described in section 2.3. For samples yielding enough liver tissue, two replicate analyses were conducted, and the mean of the two taken.

The gizzards and dried livers were checked for lead pellets by X-ray photography. None of the livers contained any shot. Three of the gizzards held pellets, but they were clearly shot into the muscle rather than ingested, which was confirmed by dissection. None of the gizzards showed any internal wall discolouration likely to have been associated with previous ingestion of spent shot.

5.6.2 Results

Two of the liver samples developed a peculiar oily appearance during the drying process, for reasons unknown, and were rejected. The results for the remaining thirty-six samples are listed in table 5.2. The two samples with lead concentration above 8 ppm by dry weight were each outliers (as described in section 2.4.3) when compared with the other samples from the same date. Whereas an outlying value for one of six *Zostera* samples from the same site, obtained on the same day and having experienced a similar growth history may well be the result of an analytical error, the probability of a genuine outlying value in a sample of birds which may have experienced different recent histories is considerably greater, and it would therefore be wrong to reject these data. However, in order to avoid possible bias caused by outlying values, non-parametric statistical tests were employed.

There was no significant difference in median liver lead concentration either by dry weight or by wet weight between birds shot during any pair of months October to December (Mann-Whitney U tests). The median lead concentration for females (3.0 ppm dry weight, 0.79 ppm wet weight) was significantly greater than for males (2.1 ppm, 0.61 ppm) over all months combined (Mann-Whitney U test; $U = 53.0$, $P < 0.05$ for both dry and wet weight comparisons).

For all birds, there was a significant inverse correlation between gross body weight and the liver lead concentration by dry weight, but not by wet weight (Spearman rank correlation; dry, $r_s = -0.526$, $n = 20$, $P < 0.05$; wet, $r_s = -0.360$, $n = 20$, n/s). This relationship was also present for males alone (dry, $r_s = -0.929$, $n = 7$, $P < 0.01$; wet, $r_s = -0.360$, $n = 20$, n/s), but not for females (dry, $r_s = -0.028$, $n = 13$, n/s; wet, $r_s = -0.008$, $n = 13$, n/s).

5.6.3 Discussion

The data suggest that Wigeon at Lindisfarne do not retain increasing levels of lead in the liver as Autumn progresses. Although the birds shot during October may have been recent arrivals, those shot during November and December, during the decline in numbers, are likely to have been feeding on the *Zostera* beds for at least several weeks. A number of attempts were made to catch Wigeon by cannon-netting as part of the feeding ecology project (section 1.4), but were unfortunately not successful. Identification of individually colour-marked birds would have supplied information on whether the observed rise and fall in

TABLE 5.2 Wigeon livers - Lead content, 1990

Date Shot	Sex	Age	Gross Wt (g)	Liver % Water	n	Liver Pb (ppm dry wt)	Liver Pb (ppm wet wt)
5 Oct	?			59.9	1	0.8	0.33
5 Oct	?			62.3	1	1.1	0.39
5 Oct	?			70.6	2	1.8	0.54
5 Oct	?			69.1	2	1.8	0.55
5 Oct	?			72.0	1	2.0	0.56
5 Oct	?			72.8	2	2.1	0.57
17 Oct	f			67.4	2	2.0	0.65
28 Oct	m		890	72.2	1	1.8	0.49
28 Oct	m		770	69.3	2	1.9	0.58
28 Oct	m		765	75.1	1	2.1	0.52
28 Oct	f		625	72.7	2	2.1	0.57
28 Oct	f		595	71.2	1	2.2	0.64
28 Oct	m		680	71.6	2	2.3	0.66
28 Oct	f		700	72.6	1	3.0	0.82
28 Oct	f		710	77.3	1	3.5	0.79
28 Oct	f		615	74.3	1	4.3	1.10
28 Oct	f		595	73.9	1	14.0	3.66
30 Oct	f	juv		76.7	1	5.7	1.33
3 Nov	m		880	67.8	2	1.8	0.59
3 Nov	m		810	67.6	1	2.1	0.67
11 Nov	f		655	74.1	1	2.4	0.62
11 Nov	m		715	73.9	1	2.6	0.67
11 Nov	f		625	72.8	1	2.6	0.70
11 Nov	f		775	72.7	1	2.6	0.71
11 Nov	f		540	73.7	1	3.0	0.79
14 Nov	f		625	77.3	1	2.3	0.52
14 Nov	f		625	71.3	1	2.3	0.65
14 Nov	f		655	73.2	2	3.0	0.79
16 Dec	m	adult		78.0	1	1.8	0.39
16 Dec	f	adult		70.6	1	2.3	0.66
16 Dec	m	adult		76.1	1	2.5	0.61
16 Dec	m	adult		75.2	1	3.0	0.74
16 Dec	f	adult		73.2	1	3.3	0.89
16 Dec	f	adult		76.8	1	3.6	0.84
16 Dec	f	adult		74.3	2	3.6	0.92
16 Dec	m	adult		77.0	1	8.9	2.05

Exact date of first samples not known

n denotes number of replicate liver lead analyses

Wigeon numbers during the Autumn comprised one single movement of birds or many overlapping, short duration visits by different groups of birds using Lindisfarne as a staging-post en route to wintering areas in southern England or Ireland. In the former case, some birds might be resident at Lindisfarne for three or four months, and exposed to lead contaminated food supplies throughout that period, but if the majority of birds stayed for only a few weeks, the exposure to lead would be considerably reduced. Examination of figure 5.1, which shows a sharp drop in numbers after the maximum count was recorded at the end of October, suggests that there was a substantial movement of Wigeon, numbering around 15000 birds, through Lindisfarne over a period of only four weeks during October. It is not clear, however, whether the remainder of the population comprised a series of smaller movements or a flock of up to 5000 or 6000 birds which remained at Lindisfarne for the duration of the Autumn.

Three-quarters of the Wigeon had a liver lead concentration of 3 ppm or lower. The bird with the highest concentration, 14 ppm, was a particularly light female; the weights of the other two birds above 5 ppm were not recorded. It has been shown amongst shorebirds that the concentration of lead in the liver can rise during periods of temporary starvation (Evans and Moon, 1981; Evans et al, 1987). It is possible that the birds for which high levels were recorded were in poor condition for reasons unconnected with lead ingestion, although emaciation is itself a symptom of lead poisoning in birds (eg. Forbes and Sanderson, 1978). The possibility that the birds concerned might have ingested spent shot which had been ground down by the time they were shot was unlikely, given that the gizzards did not appear discoloured or damaged internally.

In a comparable study at the principal inland wintering site for Wigeon in England, the Ouse Washes, Parslow et al (1982) found that 95% of a sample of Wigeon shot there had liver lead concentrations below 2.5 ppm dry weight, similar to the results obtained in the present study.

The lead concentrations in livers of wildfowl suffering from acute lead poisoning vary greatly. Mudge (1983) reported levels in the range 0.6 to 92.5 ppm for ducks and 1.6 to 109 ppm for swans and geese which had at least one pellet of lead shot in the gizzard. Studies on swans which had ingested lead angling weights have reported cases with

much higher liver lead concentrations: a maximum of 410 ppm was reported by Spray and Milne (1988) and 562 ppm by Birkhead (1982). The concentrations found in this study are more than an order of magnitude lower than the worst cases of acute lead poisoning, and are indeed comparable with the ranges of liver lead concentrations of wildfowl considered to be free from acute lead poisoning reported in the three studies cited. Only one bird in this study had a liver lead level above 10 ppm dw, the figure proposed by Mudge (1983) as a conservative limit for 'normal background contamination'.

The great majority of studies on lead poisoning in wildfowl, and, indeed, in other avian taxa, have concentrated on the ingestion of lead shot, and there is little information on the effects of dietary exposure to lead. Beyer et al (1988) subjected captive Mallard *Anas platyrhynchos* to a diet contaminated with lead acetate, commencing at a concentration of 20 ppm wet weight and increasing by a factor of 1.6 weekly, ie. a diet of much higher lead content than the maximum of about 20 ppm dry weight in *Zostera* available to Wigeon at Lindisfarne. The median liver lead concentration of Mallard that either died or were killed was 30 ppm wet weight (about 120 ppm dry weight), greatly in excess of any value recorded for Wigeon in this study.

There is therefore no direct evidence from the Wigeon livers examined in this study that lead levels at Lindisfarne are high enough to cause concern that mortality due to chronic lead poisoning might be a possibility. However, longer-term effects on reproduction cannot be discounted entirely. Scheuhammer (1987) found increased levels of infertile eggs and hatchling mortality for captive Ring Doves *Streptopelia risoria* maintained on a diet containing 50 ppm lead, but not at 5 ppm lead (but since in that study the diets were also contaminated by cadmium and mercury, the observed effects may not be solely due to lead).

Clearly there remains much information to be gained from experimental investigation of dietary lead exposure in herbivorous wildfowl, both in terms of likelihood of mortality and the effects on reproduction.

5.7 Comparison of Daily Lead Budget and Liver Lead Concentrations

On the basis of a daily quantity of lead retained of 1.06 mg (section 5.5), an average bird weighing 700 g would accumulate 7.4 mg

lead per week, corresponding to a weekly increase in overall lead concentration throughout the body of 10.6 ppm wet weight. If a Wigeon were present at Lindisfarne for 90 days, and fed on *Zostera* for the entire period, the accumulated lead burden would be 95.4 mg, ie. 136 ppm wet weight throughout the body. In the best and worst case scenarios outlined in section 5.5, the corresponding levels would be 86 and 183 ppm wet weight respectively. However, since the lead would not be distributed evenly throughout the bird, the levels of contamination in the organs known to accumulate the major part of the body burden, including the liver, would be considerably higher.

The maximum liver lead concentration found in a Wigeon shot at Lindisfarne during 1990 was 3.66 ppm wet weight, and the majority of birds examined held less than 1 ppm wet weight in the liver (section 5.6). These data are clearly at variance with the estimates outlined above. There is a need, therefore, to consider whether the figure of 1.06 mg for the daily weight of lead retained could be a considerable overestimate. The following possible reasons are suggested:

- i) The data obtained from other studies may not be appropriate for the situation at Lindisfarne. The ash content of *Z. noltii* should not differ greatly, but the data taken from Madsen (1988) relate to September and October rather than November. The daily feeding pattern (governed by the availability of the *Zostera* beds, and possibly to a lesser extent by day-length) may differ between the two studies. Furthermore, the energy budget of Wigeon may change as Autumn progresses. Both these factors may make Madsen's estimates of *Zostera* ingested and faeces produced inappropriate for Wigeon at Lindisfarne, though this could not account quantitatively for the large discrepancy between calculated and observed retention levels of lead.
- ii) The lead concentration in the faeces is difficult to quantify accurately because of the problem of sediment in the droppings. Moreover, a proportion of the lead adsorbed onto sediment particles ingested may be assimilated into the bloodstream during its passage through the bird's gut.
- iii) There may be an additional factor which has not been taken into account. One such possibility is that the Wigeon may be able to excrete lead together with salt from the nasal gland.

A high proportion (60-88%) of salt ingested may be excreted in this way (Sturkie, 1986), and if lead were excreted with a similar degree of efficiency, much of the difference between calculated and actual lead retention might be accounted for. This may be a continuous process occurring whilst the bird is grazing and possibly to some extent when roosting.

As a result of the wide disparity between the estimated daily lead budget and the observed liver lead concentrations, it is not possible to conclude with certainty whether Wigeon feeding at Lindisfarne are at risk from lead ingested in their food. Certainly the daily quantity of lead ingested appears to be high, but it is possible that the Wigeon may possess some physiological mechanism for coping with such levels. Further studies are necessary to clarify the possible causes of the disparity outlined above.

The following assumptions have been made:

- i) The mean number of kills over the last fifteen wildfowling seasons is 2575 per season (English Nature, unpubl. data). For practical purposes, it is assumed that these all occur across Fenham Flats.
- ii) The mean number of shots fired per kill is seven; most wildfowlingers questioned reckoned between five and ten shots per kill. Ideally this figure would be derived from a formal means of recording shots. Although this is a substantially lower success rate for shooting ducks than those reported for lead shot in recent BASC trials (59% and 38%) (Shedden, 1992), the trials were presumably not held in intertidal areas, which generally present more difficult and longer range targets than inland locations.
- iii) The number of pellets per shot is on average 235 for no. 5 shot (Mudge, 1984).
- iv) The area of Fenham Flats over which shooting occurs (including night shooting) is approx. 7.5 km² (calculated as the area of mud between the Causeway and Ross Point, as shown on O.S. 1:25000 series, 1982).

Thus the estimated number of pellets input is

$$2575 \times 7 \times 235 = 4236000 \text{ per season}$$

and the number per m² across Fenham Flats is therefore

$$4236000 / (7.5 \times 1000 \times 1000) = 0.56 \text{ per m}^2 \text{ per season.}$$

APPENDIX 2 PRELIMINARY ANALYSES OF SEDIMENT SAMPLES

A2.1 Introduction

Preliminary analyses were conducted on sediment cores obtained from the Fenham Mill site during September 1990, in order to establish the best method for comparing the sediment lead concentrations of the main study sites. Specifically, the analyses examined the effects of sediment depth and grain size, compared two methods of sieving sediment samples, and examined the suitability of three chemical extractants.

A2.2 Methods

A2.2.1 Three-stage Chemical Extraction

Twelve cores of 8 cm diameter and approximately 12 cm depth (one core from each of the *Zostera* plots) were sieved under hose pressure through a 20 mesh/in sieve to remove large particles and organic matter, and then through a 100 mesh/in sieve to separate into coarse and fine fractions. These were then dried for a minimum of 48 hours at 100 °C, and the dried sediment homogenised by gently grinding with a pestle and mortar.

One sub-sample from each fraction of between 2 and 3 g was weighed to an accuracy of 0.01 g into a 25 ml conical flask. To each was added 5 ml 5% acetic acid, and the flasks gently warmed for about 20 minutes on a hot-plate, and occasionally agitated. The acetic acid was decanted and filtered into stoppered plastic bottles (see section 2.3.1). 5 ml 'AnalaR' grade 3 M HCl was added to the sediment in each flask and the process repeated as for the acetic acid step. To the residue from the second stage was added 5 to 10 ml 'AnalaR' grade concentrated HNO₃ and the samples refluxed for 24 hours, boiled dry and the residue re-dissolved in 5 ml 'AnalaR' 3 M HCl as described for organic samples in section 2.3.1. However, in order to prevent bumping whilst the HNO₃ was being boiled away, it was necessary to first decant the nitric acid off into a separate clean flask, wash away the sediment with distilled water and then return the acid to the original flask. Finally, the lead concentrations of the three extracts from each sample were determined by AAS as described in section 2.3.2.

A2.2.2 The Reliability of Extractants

The reliability of lead concentrations obtained using the three-stage extraction described above was assessed by repeating the process on eight duplicate sub-samples of each fraction taken from a single core. In addition, six duplicate sub-samples from the fine fraction of the same core were analysed, omitting the first (acetic acid) stage, in order to compare the lead concentration determined by HCl alone with the combined concentration from the acetic acid and HCl extractions.

A2.2.3 Effect of Depth and Sieving Methods

It was noticed that when sieving wet sediment, some part of the finest fraction of the sediment was unavoidably lost, which might result in a reduced lead concentration being recorded. To test this hypothesis, and, concurrently to examine the variation of sediment lead concentration with depth, six cores were collected from Fenham Mill and separated on site into three layers: 1 - 4 cm, 4 - 7 cm and 7 - 10 cm depth (the top 1 cm was discarded). In the laboratory, each layer was cut vertically into two equal segments. One segment of each was sieved under hose pressure through a 20 mesh/in sieve and dried as above. The other segment was dried intact, homogenised with a pestle and mortar, and then sieved dry through a 20 mesh/in sieve.

Three extract treatments were performed following the above procedures: 5% acetic acid followed by 3 M HCl, 3 M HCl only and refluxing concentrated HNO₃ only.

A2.3 Results

A2.3.1 Three-stage Chemical Extraction

The mean lead concentrations determined during the three-stage process are shown in table A2.1 for the coarse and fine sediment fractions. The proportions of the total lead concentration extracted in each stage did not differ between the two fractions (paired t tests: acetic, $t_{11} = 0.27$, n/s; HCl, $t_{11} = 0.46$, n/s; HNO₃, $t_{11} = 0.13$, n/s), but the overall lead concentration in the fine fraction was more than double that in the coarse fraction.

Table A2.1 Three-stage chemical extraction of lead from two sediment fractions

Sediment cores obtained from Fenham Mill, September 1990

Sediment Fraction	Extractant	Mean Pb (ppm)	s.d.	c.v. (%)	Mean % of Total
Coarse	5% Acetic	0.6	0.28	48.3	11.7
	3 M HCl	3.3	1.07	32.7	60.8
	Conc. HNO ₃	1.5	0.52	35.6	27.5
	Total	5.3	1.53	28.8	
Fine	5% Acetic	1.3	0.50	39.7	11.3
	3 M HCl	6.9	1.87	27.2	61.5
	Conc. HNO ₃	3.0	0.79	26.0	27.2
	Total	11.2	2.91	26.0	

n = 12 for each sediment fraction

A2.3.2 The Reliability of Extractants

Table A2.2 and fig. A2.1 show the results of replicate analyses of the two fractions from a single core. At each stage of the extraction, the lead concentration of the fine fraction was significantly greater than that of the coarse fraction (Kruskal-Wallis 1-way ANOVA - see table A2.2). However, the variation between replicates, expressed as the coefficient of variation, was greater for the fine fraction at each stage except the first (acetic acid) extraction.

Omission of the acetic acid extraction resulted in no difference in mean lead concentration either after the HCl extraction ($t = 1.17$, $df = 7.53$, n/s) or overall ($t = 0.89$, $df = 8.29$, n/s), but the variation between replicates was reduced significantly at both stages ($F_{7,5} = 35.1$, $P < 0.001$; $F_{7,5} = 14.0$, $P < 0.01$ respectively).

A2.3.3 Effect of Depth and Sieving Methods

The mean lead concentrations at each depth for the two sieving methods are shown in table A2.3a. The analyses of variance for each of the three extractions performed (table A2.3b) show that the effect of sediment depth was very highly significant in all cases. The sieving method was also highly significant for the acetic acid extraction, but not for the subsequent HCl extraction. Nor was there any significant effect of method on the total lead concentration, as determined by refluxing with HNO_3 , but there was a significant difference for the extraction with HCl only.

For the extractions in which the method of sieving affected the results significantly, higher lead concentrations were found in the sediment sieved when dry (except for the lowest layer in the HCl extraction, for which the two means were nevertheless similar). The concentrations from the acetic acid extraction were rather low, and the total concentrations for the acetic acid and HCl extractions combined were consistently lower than for the corresponding extractions with HCl only (a finding at variance with the similar comparison made in section A2.3.2). Extraction with HCl alone also resulted in higher lead concentrations than were obtained by refluxing with HNO_3 (except for the lowest layer).

For both the single stage extractions (HCl and HNO_3), the mean lead concentrations in the top and middle layers did not differ, but

Table A2.2 Comparison of reliability of chemical extractants

Replicate samples from single core obtained from Fenham Mill, September 1990

Extractant	Fraction	Mean Pb (ppm)	s.d.	n	c.v. (%)
Acetic	Coarse	0.6	0.15	8) ***	22.8
	Fine	1.6	0.23	8)	14.5
HCl (after Acetic)	Coarse	2.6	0.21	7) **	7.9
	Fine	6.4	0.96	8)	15.1
Acetic + HCl	Coarse	3.3	0.21	7) **	6.2
	Fine	8.0	1.12	8)	14.0
HCl (no Acetic)	Fine	7.5	0.19	6	2.5
Nitric	Coarse	1.2	0.16	8) ***	13.4
	Fine	3.4	0.54	8)	16.1
Nitric (no Acetic)	Fine	3.3	0.37	6	11.0
Total	Coarse	4.5	0.22	7) **	4.8
	Fine	11.4	1.60	8)	14.1
Total (no Acetic)	Fine	10.8	0.43	6	4.0

Kruskal-Wallis 1-way ANOVA: ** P<0.01, *** P<0.001

Table A2.3a Effect of sediment depth and sieving method

Mean sediment lead concentrations in cores obtained from Fenham Mill, September 1990

Extraction	Depth	Sieving Method	Mean Pb (ppm)	s.d.	n
(1) 5% Acetic	1	Wet	1.5	0.42	6
		Dry	3.0	0.24	6
	2	Wet	0.9	0.11	5
		Dry	2.4	0.52	6
	3	Wet	0.6	0.35	6
		Dry	1.0	0.37	6
(2) 3 M HCl	1	Wet	8.8	1.00	6
		Dry	9.6	0.97	6
	2	Wet	7.8	0.63	5
		Dry	9.4	1.58	6
	3	Wet	5.8	1.60	6
		Dry	5.4	0.75	6
(1) + (2)	1	Wet	10.3	1.11	6
		Dry	12.7	1.01	6
	2	Wet	8.7	0.70	5
		Dry	11.7	2.07	6
	3	Wet	6.3	1.55	6
		Dry	6.4	1.09	6
3 M HCl only	1	Wet	12.9	1.45	6
		Dry	15.5	2.56	6
	2	Wet	12.4	1.20	5
		Dry	15.3	2.27	6
	3	Wet	9.2	3.17	6
		Dry	8.9	0.80	6
HNO ₃ reflux only	1	Wet	12.1	2.03	6
		Dry	12.6	0.97	6
	2	Wet	11.0	2.00	5
		Dry	13.3	1.84	6
	3	Wet	8.9	2.12	6
		Dry	9.3	0.84	6

Depths: 1 = 1 - 4 cm; 2 = 4 - 7 cm; 3 = 7 - 10 cm

NB: One sample from layer 2 (sieved wet) omitted from analyses owing to unusually high lead concentration (for all extractants)

Table A2.3b Effect of sediment depth and sieving method

Analyses of variance for sediment lead concentrations shown in table A2.3a

Source of Variation	Sum of Squares	df	Mean Square	F	
5% Acetic					
Depth	13.6	2	6.8	51.4	***
Method	11.3	1	11.3	85.0	***
Interaction	2.3	2	1.2	8.7	***
Explained	27.3	5	5.5	41.2	***
Residual	3.8	29	0.1		
Total	31.2	34	0.9		
Subsequent 3 M HCl					
Depth	91.0	2	45.5	33.4	***
Method	3.7	1	3.7	2.7	n/s
Interaction	5.4	2	2.7	2.0	n/s
Explained	100.6	5	20.1	14.8	***
Residual	39.4	29	1.4		
Total	140.0	34	4.1		
Combined: 5% Acetic followed by 3 M HCl					
Depth	172.1	2	86.0	47.4	***
Method	27.8	1	27.8	15.3	***
Interaction	14.3	2	7.2	3.9	*
Explained	215.9	5	43.2	23.8	***
Residual	52.7	29	1.8		
Total	268.6	34	7.9		
3 M HCl only					
Depth	200.5	2	100.2	22.7	***
Method	24.9	1	24.9	5.6	*
Interaction	18.3	2	9.2	2.1	n/s
Explained	246.4	5	49.3	11.2	***
Residual	128.1	29	4.4		
Total	374.6	34	11.0		
HNO₃ reflux only					
Depth	82.1	2	41.0	14.1	***
Method	9.6	1	9.6	3.3	n/s
Interaction	7.0	2	3.5	1.2	n/s
Explained	99.8	5	20.0	6.9	*
Residual	84.2	29	2.9		
Total	184.0	34	5.4		

* - P < 0.05; ** - P < 0.01; *** - P < 0.001

FIGURE A2.1

Lead Levels in Sediment
Comparison of Chemical Extractants

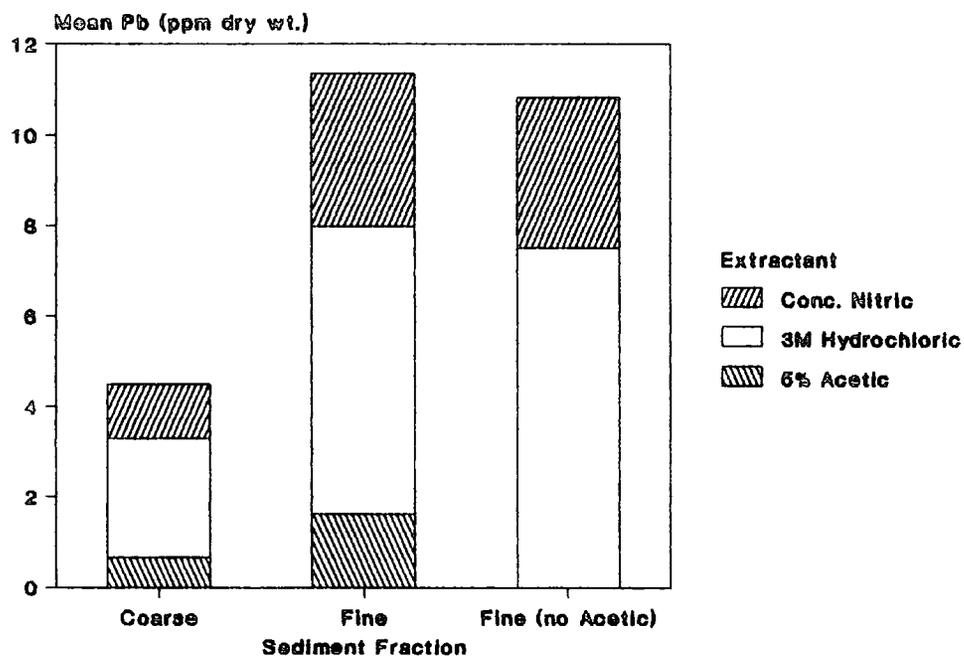
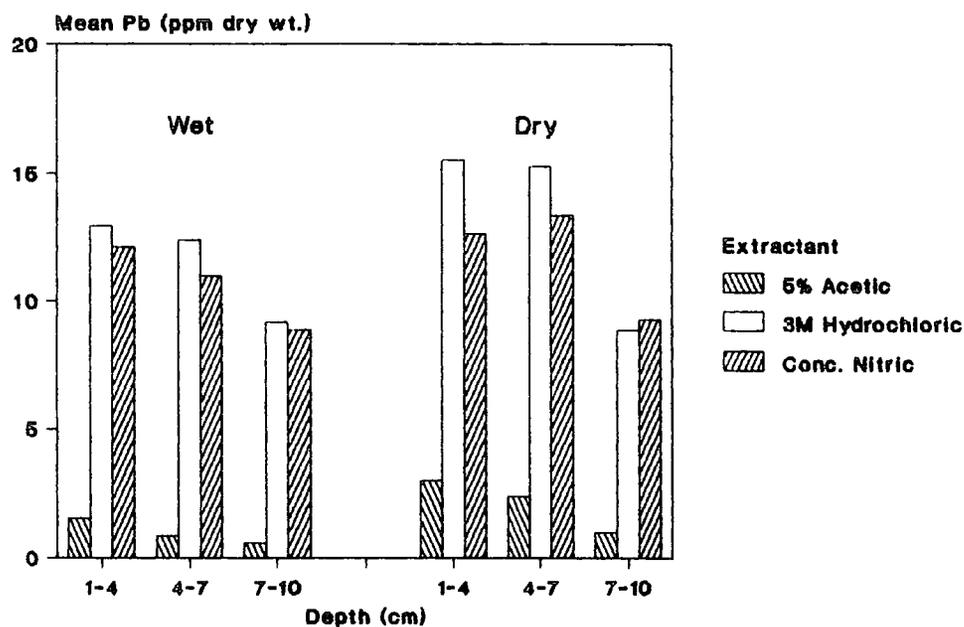


FIGURE A2.2

Lead Levels in Sediment
Effects of Depth and Treatment Method:
Sieved Wet vs. Sieved Dry



were significantly greater than in the bottom layer (Tukey HSD test at $P < 0.05$), whether the sediment was sieved when wet or dry.

A2.4 Discussion

The choice of the three strengths of chemical extractant was intended to provide estimates of the quantities of readily exchangeable lead in the interstitial water, the more strongly bound lead in inorganic salts and as organic complexes adsorbed onto particle surfaces, and the total sediment lead content. As was expected, the mean lead concentration in the fine fraction of the sediment was considerably greater than in the coarse fraction, reflecting the greater surface area per unit weight. However, the proportions extracted at each stage were identical for the two fractions, which suggests that the particle size effect was the only significant effect, and that there was not a greater proportion of lead associated with organic detritus in the finer fractions of the sediment. However, certain limitations to the analytical techniques, as discussed below, preclude the drawing of a definite conclusion.

The quantity of lead extracted by 5% acetic acid was generally low and, indeed, the extract solution obtained was, in some cases, only marginally strong enough for a reliable AAS reading to be taken. Moreover, omission of the acetic acid extraction resulted in one instance (section A2.3.3) in higher lead concentrations being obtained from a single stage HCl extraction than were obtained from the acetic acid and subsequent HCl extractions combined, and in the other (section A2.3.2) in a significant decrease in the variability. Presumably this effect was due to the retention within the flask after decanting of a proportion of the acetic acid which was of low lead concentration, and this volume of liquid reduced the lead concentration of the subsequent HCl extract. The technique could be improved by removing the acetic acid by centrifuge rather than by decanting, and then drying the sediment prior to addition of the HCl, although some lead which should have been removed with the acetic acid might still remain in the sediment and magnify the HCl extract concentration. In view of the problems encountered, and, particularly of the low and variable lead concentrations obtained, it was decided that the acetic acid extraction should be omitted for the main analysis of sediment samples.

More surprisingly, the lead concentration obtained by extraction with HCl only was higher than that from a single stage refluxing with HNO₃ (section A2.3.3), despite the latter being the much stronger extractant. Two possible reasons for this apparent anomaly are suggested. Extraction with 3 M HCl may be sufficient to remove all the lead present in the sediment (in whatever form), in which case the complexing of lead by organic ligands must presumably be unimportant. If this were so, the lead extracted during the final stage of the three-stage process (about 27% of the overall concentration) must actually have been liberated during the HCl extraction, but retained in the flask together with the sediment after decanting off the HCl extract (ie. a similar problem to the decanting of the acetic acid extract discussed above). Alternatively the problem involved the loss of some of the lead together with the sediment discarded (in order to avoid bumping) prior to boiling away the HNO₃. Whichever the cause, it is apparent that a single-stage extraction process is inherently more reliable than a multi-stage process, unless sophisticated equipment is available, in which case the numbers of samples which can be analysed may be limited.

The single-stage process is quite adequate to determine the relative lead concentrations between a number of samples, and was therefore adopted for the main samples in this study. Since extraction with HCl is much simpler than by refluxing with HNO₃, and yields comparable results, that method was chosen. However, in order to minimise potential variation in the analytical procedure itself, it was also decided to use smaller sediment samples (since the low concentrations encountered in the acetic acid step would no longer be a problem), to increase the time of the treatment from 20 minutes to one hour, agitating each flask frequently to ensure thorough mixing, and to dispense with the warming of flasks on the hot-plate, which was known to heat unevenly across its surface.

The lead concentration in sediment from Fenham Mill was clearly lower below 7 cm depth. As the examination of the effect of depth did not cover separate fractions of the sediment, it is not known whether the difference was due solely to the composition of the sediment (which was certainly coarser below this depth), or whether there was also an independent reduction in the amount of lead present once particle size was taken into account. Since the majority of *Zostera* roots and rhizomes lie in the top 5 cm of the sediment, it was decided

to limit the analysis of sediment to that depth, thereby generally avoiding problems of sediment heterogeneity with depth.

Sediment samples from different sites might well differ in composition, however, and it was therefore decided to determine the lead concentration both in the undifferentiated sediment, reflecting the overall availability of lead for uptake by the intertidal flora, and also within the finest fraction which could be isolated, which could be expected to reflect the relative amount of lead input to the site, and hence provide at least a qualitative estimate of differing levels of shooting pressure.

Finally, the lead concentrations obtained from sediment sieved when dry were slightly greater than from sediment sieved under hose pressure, and the coefficients of variation were also generally lower. This was assumed to be due to the removal of interstitial water during wet sieving, and to the loss of varying proportions of the finest sediment particles. It was therefore decided to dry sediment samples before sieving.

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