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STUDIES OF SEASONAL CHANGES IN HEAVY METAL CONTAMINATION IN THREE  
SHOREBIRD SPECIES AT TEESMOUTH, NORTHEAST ENGLAND

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SUBMITTED TO THE  
UNIVERSITY OF DURHAM

BY  
NILS CHRISTIAN HALKER II

IN FULFILLMENT OF  
THE REQUIREMENTS FOR  
THE MASTER OF SCIENCE DEGREE

FEBRUARY, 1992

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16 APR 1993

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No aspect of nature on this beach is more mysterious to me than the flights of these shorebird constellations. The constellation forms, as I have hinted, in an instant of time, and in that same instant develops its own will. Birds which have been feeding yards away from each other, each one individually busy for his individual body's sake, suddenly fuse into this new volition and, flying, rise as one, coast as one, tilt their dozen bodies as one, and as one wheel off on the course which the new group will has determined.

-- Henry Beston, *The Outermost House*

**Studies of seasonal changes in heavy metal contamination  
in three shorebird species at Teesmouth, northeast England**

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## ABSTRACT

Concentrations of lead, cadmium, copper, and zinc were measured in kidney and liver tissues of three species of shorebirds that winter on the industrial River Tees estuary: Sanderling, Calidris alba; Ringed Plover, Charadrius hiatacula; and Grey Plover, Pluvialis squatarola. Metal concentrations tended to be higher in kidneys than in livers of all three species. Seasonal variations in some metal concentrations were observed in all three species, particularly in Sanderling for which the most samples were available. Zinc levels were found to correlate with other metal levels to some degree in all three species. Few differences in metal concentrations between sexes were found. Metal concentrations were also measured in the birds' main prey, respectively the polychaete Nerine cirratulus and various Crustaceans; the crustacean Corophium volutator; and the polychaete Nereis diversicolor. Metal concentrations in the birds and their prey were compared, and it was found that only very small amounts of metals ingested were retained.

## Chapter 1 INTRODUCTION

### 1.1. Background

This thesis is concerned with contamination by heavy metals of shorebirds feeding in the River Tees estuary, northeast England. Study of heavy metal contamination of estuaries and coastal regions is of twofold interest; first from the human point of view, second from the environmental.

Indicative of human concerns are public health notices, at Teesside and at many other beaches around the world, warning that shellfish and other marine animals taken from those waters may be unsafe for human consumption. Two of the most striking effects of heavy metal contamination of humans have been seen in Japan: Itai-Itai disease and Minamata disease, cadmium and mercury poisoning respectively. In both cases, the cause of poisoning was contaminated food sources (Friberg et al., 1986; Bremner, 1974).

Environmental deterioration of estuaries and coastal waters can lead to loss of biological diversity and also can have economic repercussions, primarily on fisheries. Although shorebirds are not of economic interest, some larger species are hunted and consumed by humans. Shorebirds also are top predators and of scientific interest as important parts of the ecosystem. In many ecosystems predators contain pollutants at higher concentrations than are present in their prey. This is not always the case with heavy metals in shorebirds (Evans et al., 1987; Evans & Moon, 1981). However, if the rates of uptake, retention, and excretion of these pollutants are understood, shorebirds might be useful indicators of

environmental pollutant levels because they integrate contaminants over considerable areas of intertidal land by ranging widely while feeding.

My study concentrated on four heavy metals: cadmium, copper, lead, and zinc. Two of these metals, copper and zinc, are biologically essential. In excess, however, essential metals are toxic and so can be compared with cadmium and lead, non-essential metals. Cadmium and lead are two of the most hazardous metals after mercury in aquatic systems (Bryan, 1984).

Very little is known about the effects of heavy metals taken in or accumulated by shorebirds. Metals are found in biological systems such as metalloproteins and metal-protein complexes in many enzymes. Heavy metals exert their toxic effects by binding to enzymes or replacing normal metalloenzyme complexes (Bremner, 1974; Ashby et al., 1981; Bryan, 1984). There are many variables that affect heavy metal toxicity; most important are the particular chemical species of the metal, its availability, solubility, binding affinity and biological activity (Förstner, 1980). Presence or absence of other metals also influences the toxicity of a particular heavy metal (Bremner, 1974; Hill et al., 1963; Oh et al., 1981; Nicholson, 1981).

The toxic properties of cadmium have been recognized for over a century. Cadmium is chemically similar to zinc, and interacts with many other metals. Cadmium alters the metabolism and function of some essential trace elements such as copper, zinc, iron, manganese, selenium and calcium by competing for ligands in

biological systems (Bremner, 1974; Ashby et al., 1980; Friberg et al., 1986). Cadmium accumulates in human kidney and liver tissue, and has a half life of between 10 and 30 years. In humans, ingestion of cadmium results in gastrointestinal effects. Long term exposure leads to renal tubular dysfunction, and disturbance of the metabolism of copper, zinc and calcium (Friberg et al., 1986; Bremner, 1974).

Cadmium is also accumulated in kidney and liver tissue of many birds (Evans & Moon, 1981; Hutton, 1981; Evans et al., 1987; Stock et al., 1989). Hill et al. (1963) found that high doses of cadmium caused reduced growth rates, microcytic hypochromic anemia, atony and elongation of the gizzard, and mortality in domestic chicks. Cadmium is thought to cause liver and kidney damage in shorebirds (Nicholson et al., 1983; Stock et al., 1989). Although most shorebird species are long lived (Evans & Pienkowski, 1984) and cadmium has been found to accumulate with age, there are no records of mortalities due to cadmium poisoning in shorebirds. Di Giulio & Scanlon (1985) found that cadmium inhibited energy metabolism in Mallards Anas platyrhynchos when the ducks were placed on a restricted diet. This is of concern when considering the high energy demands migratory species are likely to encounter. Stock et al. (1989) suggest that Oystercatchers may have a cadmium regulation mechanism because, as in similar studies of Herring Gulls (Nicholson, 1981), cadmium concentrations levelled off in adult birds. Although there is no evidence, it is possible that sub-lethal cadmium accumulations could shorten the life span of

affected birds, even though the environmental dose received in any one year may not be toxic.

Lead is also toxic to many organisms. Several authors have reviewed the effects of lead poisoning in humans and mammals (Bremner, 1974; Jaworski, 1978; Chandra, 1980; Tsūchiya, 1986). An early sign of lead poisoning is gastrointestinal colic. Anaemia is common due to interference in haem synthesis, most importantly by inhibiting  $\delta$ -aminolaevulinic acid dehydratase (ALA-D). Lead will cause damage to the central nervous system causing encephalopathy and neuropathy, especially in children.

In studies of feral pigeons Columba livia in London, Hutton (1980) found high concentrations of lead in many tissues. He found several symptoms of lead toxicity, including increased kidney weight, the presence of renal intranuclear inclusion bodies, altered mitochondrial structure and function and depression of ALA-D activity in blood, liver and kidney. However, Hutton was uncertain as to how these symptoms affected the biological fitness of the birds.

In the autumn of 1979, over 1,500 waders, in addition to nearly 1,000 other birds, were found dead or sick on the Mersey estuary. The casualties were probably the result of lead poisoning through ingestion of invertebrates contaminated with organo-lead compounds, specifically alkyl leads (Bull et al., 1983). In the autumn of the following year, another 850 mortalities were noted, and in 1981, 48 dead and 67 sick birds were reported. Bull et al. concluded that the source of the pollution was industrial effluent

from petrochemical works. Shorebird deaths from inorganic lead poisoning have not yet been demonstrated.

Copper is an essential element and is important in the functioning of many enzymes (Bremner, 1974; NRC, 1977; Aaseth & Norseth, 1986). Copper is toxic in excess, but copper toxicity (and copper deficiency) is of concern mainly in sheep and cattle. Many fish and aquatic animals are also susceptible to copper poisoning; indeed copper is an effective molluscicide. Cadmium was found to be toxic to chicks in a copper deficient diet (Hill et al., 1963), but its toxic effects were reversed by the addition of copper to the diet.

Zinc is also an essential element for many biochemical processes and has been found in over 90 enzymes and proteins; its presence increases the activity of many other enzymes (Bryan, 1984). Most animals have an extremely high tolerance for zinc, and zinc toxicity is of little concern (Bremner, 1974). Zinc deficiency is easily induced in animals, and as with copper deficiency, is aggravated by the presence of cadmium (Elinder, 1986). Also like copper, zinc can prevent some symptoms of cadmium toxicity (Hill et al., 1963). Cadmium, copper and zinc are chemically similar elements, and interact biologically in many ways (Hill et al., 1963; Bremner, 1974; Nicholson, 1981), so the uptake of one of these metals may influence the uptake of the others.

Apart from the large mortality of shorebirds on the Mersey estuary, such tangible cause and effect scenarios of heavy metal

pollution in birds are the exception rather than the rule. Of perhaps greater concern are the possible effects of heavy metal contamination on longevity and breeding success. However, both are difficult to study.

Any effects on longevity are difficult to document because usually only an average value can be estimated, by recaptures of ringed birds. Many factors other than pollution already influence the results; storms, unrelated diseases, predation, disturbance by humans of breeding and/or wintering grounds, to consider but a few.

Breeding success is also difficult to study in shorebirds because most species tend to nest in remote areas, especially in the arctic. (Species such as Oystercatcher, however, can be studied directly in Britain and Europe.) The only way to monitor breeding success of most species is through population counts and estimation of the proportions of juveniles in flocks during the non-breeding season, when many shorebirds congregate in estuaries. Such counts are also sensitive to many factors unrelated to breeding success, such as mortality on migration.

Heavy metal contamination in shorebirds has been the subject of several papers, reporting studies of Dunlin Calidris alpina, Knot Calidris canutus, Redshank Tringa totanus, Sanderling Calidris alba, Oystercatcher Haematopus ostralegus, Bar-tailed Godwit Limosa lapponica and Curlew Numenius arquata. (See Table 1.1 for details.)

Studies of shorebirds have been conducted in both polluted and relatively unpolluted areas: Bull et al. (1983) worked on the

heavily polluted Mersey estuary; Ward (1979) on the industrial River Tees; Evans & Moon (1981) and Evans et al. (1987) on the River Tees and at unpolluted Lindisfarne in north Northumberland; Parslow (1973) on the agriculturally polluted Wash; Hutton (1981) on the lightly polluted Burry Inlet, Dyfed; Goede (1985), Goede & de Voogt (1985) and Stock et al. (1989) on the Dutch and German Wadden Sea; and White et al. (1980) on the industrialized Corpus Christi Bay, Texas.

Table 1.1. Species of wading birds in which heavy metals have been studied, and citation of workers.

Species	Authority
Dunlin	Bull et al., 1983; Evans and Moon, 1981; Evans et al., 1987; Parslow, 1973; Ward, 1979; White et al., 1980.
Knot	Bull et al., 1983; Evans and Moon, 1981; Evans et al., 1987; Goede, 1985; Goede & de Voogt, 1985; Goede, 1988; Parslow, 1973; Ward 1979.
Sanderling	Evans & Moon, 1981; White et al., 1980.
Redshank	Bull et al., 1983; Evans and Moon, 1981; Goede, 1985; Goede & de Voogt, 1985; Parslow, 1973.
Oystercatcher	Hutton, 1981; Stock et al., 1989.
Bar-tailed Godwit	Evans and Moon, 1981; Goede 1985; Goede & de Voogt, 1985; Parslow, 1973.
Curlew	Evans and Moon, 1981.

These workers examined variations in metal concentrations by season, sex, age, tissue (eg. kidney or liver), and concentrations of one metal in relation to another. Parslow (1973), Evans & Moon (1981), Goede (1985) and Evans et al. (1987) found variations in metal concentrations from month to month. In these studies, mercury levels increased through the winter months prior to moult, and then decreased in the spring following moult. These authors, as well as Ward (1979), suggest mercury uptake and excretion parallels the yearly zinc cycle. Zinc and cadmium concentrations also increased through the winter in Dunlin and Knot (Evans et al., 1987). It is believed that zinc and several non-essential metals, especially mercury, are excreted into feathers when birds moult into breeding plumage in the spring (Osborn, 1979; Evans & Moon, 1981).

Cadmium concentrations did not decrease, as did the other metals. Cadmium is not excreted into growing feathers (Goede & de Voogt, 1985). Evans & Moon (1981), Hutton (1981), Evans et al. (1987), and Stock et al. (1989) found that the concentrations of cadmium in the liver and kidney increased with age, while copper, lead, zinc and mercury concentrations did not.

Significant differences in cadmium concentrations between sexes were found by Evans & Moon (1981), Hutton (1981) Goede & de Voogt (1985) and Stock et al. (1989). However, their results were contradictory: Evans & Moon, Hutton, and Goede & de Voogt (working with Godwit, Oystercatcher, Knot and Redshank) found that females had higher cadmium concentrations, while Stock et al. (working with

Oystercatcher) found that females had lower concentrations than males. (Unlike the other workers, Stock et al. lumped all age classes together in calculating differences between sexes). Hutton assumed that females had higher cadmium levels due to increased intestinal calcium absorption during eggshell formation, as cadmium might displace calcium on the calcium binding protein. Evans and Moon also suggest that the difference is related to ovarian development. Stock et al. suggest that differences of metal concentrations are the result of sexual differences in feeding habits of Oystercatcher; females feeding more often on open tidal flats, while males feed more often on musselbeds.

Metals are concentrated in different tissues to different degrees. Cadmium and lead tend to be found in highest concentrations in kidney tissues (Stock et al., 1989; Evans et al., 1987; Bull et al., 1983; Ward 1979). Copper, mercury, and zinc, however, tend to concentrate at higher levels in liver (Evans et al., 1987; Evans & Moon, 1981). In a comparison between different shorebird species at Teesmouth, Evans & Moon (1981) found that species with the highest tissue concentrations of zinc and cadmium had the lowest lead concentrations, and vice versa.

## 1.2. Study Area

The River Tees estuary is on the northeast coast of England, in County Cleveland (54°37'N 1°12'W). Industrial development began on Teesside early in the nineteenth century, with the construction

of ironworks near Stockton and Middlesbrough. The demand for iron rails for the railways, and then iron for shipbuilding spurred the growth of heavy industry on Teesside. Until that time, the estuary contained about 2,400 hectares of intertidal sand- and mudflats. As heavy industries and transport facilities on the estuary expanded, silt from dredging and slag from the iron and steel works were used to fill large areas of sand- and mudflats. Today, only 140 hectares of Seal Sands, the major intertidal area on the north bank of the river, is left as a feeding area for shorebirds (Evans et al., 1979).

Both shores of the River Tees, but particularly the southern side, are crowded with an industrial complex including oil refineries, Redcar Steel Works blast furnace and Lackenby steel plant, ICI Chemicals, BASF and other chemical plants, Tioxide titanium paint pigments, the Hartlepool Nuclear Power Station, and many other industries.

Due to the high concentration of heavy industry near the mouth of the River Tees, there are correspondingly high levels of industrial and domestic effluents entering the estuary. The steelworks discharge spent acids, suspended solids, and cyanides. Zinc and cadmium, with chromium, are a byproduct of plating and galvanizing processes. Some chemical and petrochemical works discharge suspended solids, phenols, cyanides, and toxic metals (Porter, 1973). Copper contamination is possible from the power plant, due to corrosion caused by the use of seawater for cooling (Martin et al., 1977). Ships in the busy Teesport also can be a

source of pollution, from the use of copper in antifouling paints and lead and zinc preservative paints (Bellinger & Benham, 1978; Young et al., 1979). Atmospheric input also may be an important source of pollution. Burning of fossil fuels puts many metals into the atmosphere, including arsenic, zinc, cadmium, copper, nickel and vanadium (Förstner, 1980).

There are also natural sources of heavy metals. The River Tees certainly contained zinc, lead, and cadmium before industrialization, because the headwaters of the Tees drain metal-rich areas - the North Pennine orefields. Indeed, lead mining has almost as long a history as coal mining in the region (Mitton, 1924).

The birds that winter on the estuary are, therefore, exposed to high levels of contamination, primarily through feeding on the estuarine invertebrates. The Tees estuary provides winter feeding grounds for many species of shorebirds that breed in the arctic, including Dunlin Calidris alpina, Oystercatcher Haematopus ostralegus, Knot Calidris canutus, Curlew Numenius arquata, Bar-tailed Godwit Limosa lapponica, Redshank Tringa totanus, Turnstone Arenaria interpres, Grey Plover Pluvialis squatarola, Ringed Plover Charadrius hiatacula, and Sanderling Calidris alba. Species use the estuary in different ways; some species spend the entire winter there, while for others it is a "refuelling" stop on their migration routes. A few species nest on the estuary.

Feeding sites on the Tees estuary are restricted to a few suitable beaches and intertidal sand- and mudflats. On the north

side of the river these are Seaton Sands, North Gare Sands, and Seal Sands, on the south side Bran Sands, Coatham Sands, and Redcar Rocks (FIG. 1.1). My studies were concentrated on the birds using Coatham and Seal Sands...

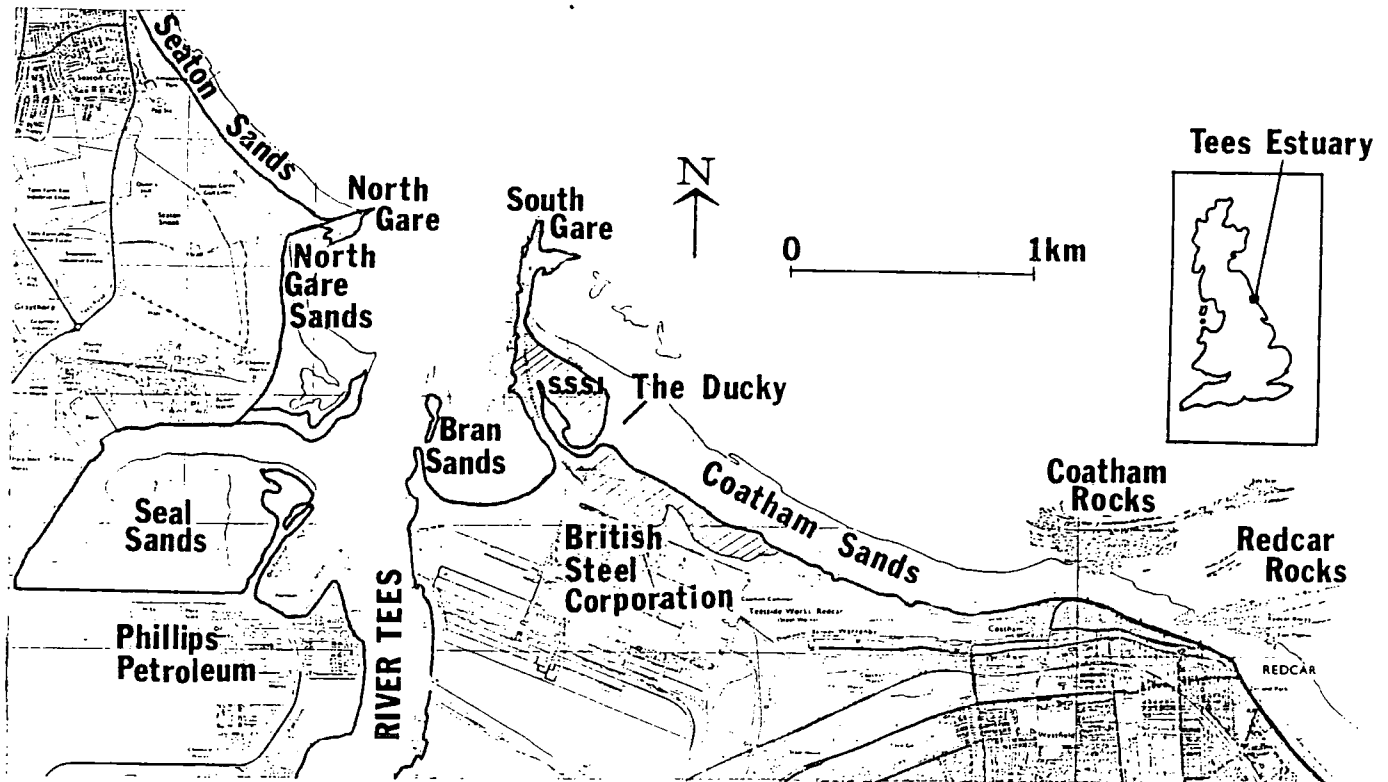


FIG. 1.1. The River Tees Estuary.

Seal Sands and Bran Sands are tidal mudflats, characterized in parts by very sticky mud. Seal Sands is very flat and drains only very slowly. Most is exposed for only seven to eight hours out of each  $12\frac{1}{2}$  hour tidal cycle. These two sites contain all that remain of the lower tidal level flats. Most of North Gare Sands lies at higher tidal levels. It is a sandflat, and is exposed for a larger part of the tidal cycle. Coatham Sands and Seaton Sands are sandy marine beaches. Redcar Rocks and Coatham Rocks are

limestone outcroppings that are exposed at low water. Towards the north end of Coatham Sands is an area of raised beach called the Ducky. From the South Gare, extending for about two kilometres southward, Coatham Sands is a "Site of Special Scientific Interest," (SSSI), designated by the Nature Conservancy Council in 1969. On the north bank of the river, Seal Sands, North Gare Sands and Seaton Sands are also SSSIs.

### 1.3. Study Species

I chose to study Sanderling, Ringed Plover, and Grey Plover because they feed on different prey in different parts of the estuary. Because of this, they are presumably exposed to varying levels of contamination within the same estuary. Sanderling typically feed on open sandy beaches or sometimes rocky shores. They feed along the tide's edge, either by probing the wet sand for their prey or by taking minute Crustaceans out of the water column. The bills of Plovers are short and not suited to the deep probing technique used by Sanderling and most other waders, so they feed by pecking at their prey on the sediment surface. Ringed Plovers feed on sand- and mudflats. Grey Plovers are typically about four times heavier than Ringed Plovers, and feed chiefly on mudflats.

### 1.3.1. Sanderling and Their Prey

Sanderling that winter at Teesmouth arrive from their breeding grounds in the arctic in August to begin moulting. Other influxes of Sanderling, birds that will fly farther south after using the estuary as a stopping point to replenish their fat reserves, also occur at this time. As many as 1,200 Sanderling use the estuary during the winter months. Many of these leave for their arctic breeding grounds in April and May (Evans & Pienkowski, 1984), but others that have wintered in southern Europe or northern Africa stop at Teesmouth in spring on their northward migrations (Breary, 1982). Teesmouth is ranked as the third most important estuary in Britain for wintering Sanderling, after the Ribble and the Uists (Prater, 1981).

Sanderling at Teesmouth change their feeding areas throughout the tidal cycle. During low tide, they feed at Redcar Rocks, chiefly on mussel spat exposed on the rocks. As the tide rises and Redcar Rocks are covered, the Sanderling work their way northward along Coatham Sands, feeding along the tide edge. An hour or two before high tide they reach the Ducky, where they roost during the day. (At night, Sanderling roost south of Redcar at Saltburn.) During the falling tide, the pattern is reversed. This pattern of movement has been observed by other workers in previous years (Evans et al., 1980; Breary, 1982).

Their primary prey along Coatham Sands are the polychaete worm Nerine cirratulus and the small Crustaceans Bathyporeia pelagica,

Eurydice pulchra, and Haustorius arenarius (Breary, 1982). Nerine are found on coastal beaches, like Coatham Sands, which are exposed for prolonged periods between tides. On Coatham Sands, the greatest densities tend to be found between 60 and 80 metres below high water mark of spring tides (i.e. just below high water mark of neap tides), especially just north of Coatham Rocks. High densities of Nerine are also found in the beaches near Redcar Rocks (See Appendix 1, Table i.1).

#### 1.3.2. Ringed Plover and Their Prey

Both migratory and resident Ringed Plover use Teesside. Some breeding birds winter locally. In the autumn, birds from Iceland stop at Teesmouth on their way to west Africa. In mid May, many hundreds of Ringed Plover pass through and refuel at Teesside, heading back to Iceland. The resident birds are already nesting when the spring passage of migrants occurs.

The main prey of Ringed Plover at Teesmouth is thought to be the Crustacean Corophium volutator, because Corophium are the predominant organism found where the Ringed Plover are known to feed on Seal Sands. Ringed Plover are also often seen feeding high above the tide edge at Redcar Beach, and occasionally on Coatham Sands, but it is unclear what they are feeding upon at those sites, although it is likely to be the same range of small crustaceans as taken by Sanderling.

### 1.3.3. Grey Plover and Their Prey

Winter populations of Grey Plover on the Tees estuary vary in number between 100 and 300 birds (Wood, 1984). Some Grey Plover use Teesmouth only as a stopover area in early autumn and late winter for replenishing their fat reserves. These birds are thought to spend the summer in Siberia, and the winter in western France. The first to return arrive in August; a few stay to moult at Teesmouth, and remain to winter there. Juveniles arrive in October but most regular adult winter residents arrive in November, after moulting in the eastern part of the Wadden Sea. Northbound migrants arrive in late January, when numbers often rise to more than 300. Most adults leave in early March, but juveniles often stay until early May. A small population of 10 to 20 birds are usually present during the summer.

Grey Plover feed primarily on Seal Sands. The main prey of Grey Plover at Teesmouth is the polychaete worm Nereis diversicolor (Evans et al., 1979; Wood, 1984).

### 1.4. Materials and Methods

Liver and kidney samples from Sanderling, Ringed Plover, and Grey Plover were analysed for metal levels. Kidney and liver tissues are preferred for analysis, because metals tend to accumulate in these tissues at higher concentrations than the average for the whole body (Evans & Moon, 1981). The samples were

collected over the past fifteen years and had been kept frozen; some birds were taken under licence from the Nature Conservancy Council, the rest were from occasional casualties from bird-catching operations. The respective tissues were excised from the birds, dried to a constant mass in a vacuum oven at 60° C, and then weighed to the nearest milligram.

Invertebrate materials were collected at Teesmouth in several ways. The polychaete worm Nerine and the crustaceans Bathyporeia, Eurydice, and Haustorius were collected by digging samples of sand and washing the sand through a 20 mpi sieve on site. The animals retained were removed and collected in plastic sample jars. The ragworm Nereis was collected by hand-picking worms from samples of mud dug up with a spade. The crustacean Corophium was collected by carefully removing the surface layer of mud with a shovel and placing the mud slab in an enamel tray. The trays were then transported to the labs at Durham, where they were placed in a 10° room. The trays were filled with clean seawater and kept over night. On the day following collection, the seawater was siphoned off and replaced with a nearly saturated NaCl solution, about three times as strong as seawater. The NaCl solution caused the Corophium to swim out of their burrows, and they were then collected with forceps.

Nereis and Nerine were stored for 24 hours after collection in clean seawater in a 10° degree room to allow them to clear their guts of particulate matter.

The invertebrate materials were handled in the same way as the

bird materials; i.e. dried in a vacuum oven at 60° and weighed to the nearest milligram.

Thereafter the procedure for preparing invertebrate and bird materials was identical. After weighing, samples were placed into individually numbered flasks, oxidized with concentrated nitric acid, and evaporated to dryness. The residue was redissolved in a known volume of 3 M HCl, and metal levels were measured with a Pye-Unicam SP 9 Atomic Absorption Spectrophotometer, re-calibrated with a range of concentrations of each metal analysed on each occasion the machine was used.

Because sample sizes were often small, making it difficult to assume that the population distributions were normal, metal concentrations in bird and invertebrate material were compared by Mann-Whitney and Kruskal-Wallis non-parametric tests where appropriate. Pearson product-moment coefficients of correlation were calculated to establish if there were any relationships between individual metals and between the same metals in different tissues.

#### 1.4.1. Chemical Analyses - Limits of Accuracy

The flame atomic absorption spectrophotometry (AAS) method is most sensitive for detection of zinc, less so for cadmium and copper, and least for lead. Because cadmium, copper, and especially lead AAS readings from samples tended to fall at the lower end of the calibration curves relating absorption values to

metal concentrations (see examples in Appendix 2), concentrations of cadmium and copper calculated from AAS readings have been rounded to the nearest 0.5 ppm, lead concentrations to the nearest 1 ppm, and zinc concentrations to the nearest 0.1 ppm. To provide readings which lay on the calibration curve for zinc, sample solutions were diluted 10 times more than other samples. (Cadmium, copper and lead samples were dissolved in 5 ml HCl, zinc samples in 50 ml HCl.) Metal concentrations for bird and invertebrate tissues were calculated by the formula:

$$\text{Concentration in Tissue (ppm)} = \frac{5^* \times \text{Conc. Measured in Solution (ppm)}}{\text{Dry Sample Mass (g)}}$$

\*In the case of zinc, 50

As an example, the AAS absorption reading for copper in the liver sample of Grey Plover GP502 was 163. Using the calibration curve in Appendix 2, Figure ii.2 for copper:

$$\text{Concentration Measured in Solution} = \frac{163 + 17.9}{60.7} = 2.98$$

The mass of the sample was 0.860 g, so it follows:

$$\text{Concentration in Tissue} = \frac{5 \times 2.98 \text{ ppm}}{0.860 \text{ g}} = 17.326 \text{ ppm}$$

Rounded to a concentration of 17.5 ppm.

Not all of each tissue was used for analysis, hence a wide range of masses was analysed. Sample mass might affect the accuracy of calculations: small samples could contain quantities of metals which, after dilution in 3 M HCl, would be present at concentrations only just above the limits of sensitivity of measurement (referring to the tables and figures in Appendix 2, these would be AAS readings of about 20, 25 and 6 for cadmium, copper and lead respectively). Any errors would be magnified by the calculation involved (multiplying by 5, or 50 for zinc, and divided by a very small mass) and from problems in sample preparation.

Testing of "blanks" (a sample prepared without any tissue, i.e. nitric acid evaporated to dryness, residue dissolved in 3 M HCl) yielded virtually undetectable levels of cadmium, copper and zinc. Background interference or trace levels of lead were detectable but correspond to < 1 ppm. Because of the low level of lead in many tissue samples, it should be noted that lead concentrations might be slightly inflated by including background levels. This is another reason for conservative rounding of lead concentrations. (But as all tissues, bird and invertebrate, were tested on the same equipment, any error would be relatively consistent throughout, except in the very smallest samples, and not alter comparisons between tissues.)

Many livers were large enough to yield two samples (some Grey Plover livers yielded three or four) per liver. Kidney tissues were too small to allow more than one sample to be analysed.

Presumably, metal concentrations should be the same in replicate samples taken from the same tissue. As previously discussed, variations might result from differences in sample mass, metal tested for, low metal concentrations and machine sensitivity (lead concentrations are the most affected by these factors). Examination of metal concentrations in multiple samples of the same tissue helped establish the reliability of the analytical technique. Table 1.2 gives two examples of livers from which multiple samples were taken.

Table 1.2. Examples of livers from which multiple samples were taken. Concentrations expressed as ppm ( $\mu\text{g}/\text{g}$  dry weight).

Bird number	Sample mass (grams)	Concentrations (ppm)			
		Cadmium	Copper	Lead	Zinc
GP501	0.698	0.5	20.5	2	92.8
	0.850	1.0	20.0	2	106.2
	0.859	1.0	20.0	2	96.7
	0.890	1.0	20.5	2	90.3
	Mean $\pm$ S.D.	0.9 $\pm$ 0.22	20.3 $\pm$ 0.25	2.0 $\pm$ 0.0	96.5 $\pm$ 6.05
GP504	0.489	1.0	21.5	3	97.7
	0.692	1.0	21.5	3	115.6
	0.907	1.0	19.5	3	82.8
	0.973	1.0	19.0	3	86.8
	Mean $\pm$ S.D.	1.0 $\pm$ 0.00	20.4 $\pm$ 1.14	3.0 $\pm$ 0.0	95.7 $\pm$ 12.70

They show how large sample masses yield consistent results for cadmium, copper and lead, but not such high reproducibility for zinc, for which serial dilution had to be performed.

Multiple samples were taken from 41 birds (14 Sanderling, 11 Ringed Plover and 16 Grey Plover). A total of 96 liver samples were taken from those 41 birds. For each set of multiple samples, the mean, standard deviation and coefficient of variation of metal concentrations was determined. Then the mean coefficient of variation for each metal was calculated (see Table 1.3).

Table 1.3. Variability of AAS determination of four metals.

Number of samples	Number of birds	Mean of the coefficients of variation			
		Cadmium	Copper	Lead	Zinc
96	41	0.32	0.07	0.18	0.06

Cadmium and lead had the highest coefficients of variation, most likely the result of rounding concentrations to the nearest 0.5 or 1 ppm when concentrations were low. Copper and zinc AAS readings were higher, and so relatively unaffected by variations due to rounding.

## **Chapter 2 HEAVY METAL CONCENTRATIONS IN INVERTEBRATE AND BIRD TISSUES.**

### 2.1. Introduction

Many chemical forms of most heavy metals that enter a river system tend to precipitate and become concentrated in estuarine sediments when they encounter saline water. Invertebrates that live in the sediments, especially filter-feeding organisms, take in and may accumulate heavy metals in their bodies (Bryan, 1984). In their turn, organisms such as shorebirds that prey upon benthic invertebrates ingest metals contained in those invertebrates.

### 2.2. Heavy Metal Concentrations in Invertebrates

Table 2.1 summarizes the levels of four metals, determined by AAS, in the six invertebrate species (divided into four groups) sampled in this study that formed the main prey of the three shorebirds studied. Complete data (mass of the individual samples, AAS results, and dates and locations of collection) are given in Appendix 3.

Table 2.1. Heavy metal concentrations (ppm dry mass) in invertebrates collected at Teesmouth.

Species groups	Number of samples	Concentration $\pm$ Standard Error			
		Cadmium	Copper	Lead	Zinc
<u>Nerine cirratulus</u>	12	1.1 $\pm$ 0.06	43.8 $\pm$ 1.67	14.1 $\pm$ 3.0	238.5 $\pm$ 13.13
Crustaceans*	3	1.3 $\pm$ 0.14	78.0 $\pm$ 3.97	20.3 $\pm$ 1.9	188.4 $\pm$ 8.89
<u>Nereis diversicolor</u>	6	0.5 $\pm$ 0.00	39.2 $\pm$ 1.86	17.2 $\pm$ 1.6	172.3 $\pm$ 6.35
<u>Corophium volutator</u>	3	1.0 $\pm$ 0.00	68.5 $\pm$ 14.38	33.3 $\pm$ 2.4	111.4 $\pm$ 6.20

\*Species included: Bathyporeia pelagica, Eurydice pulchra and Haustorius arenarius.

Nerine and the Crustaceans were collected from the sea beaches on the south side of the river, where Sanderling feed on them. Nereis and Corophium were collected from the mudflats within the estuary on the north side where Grey Plover and Ringed Plover, respectively, feed on them. Collection of invertebrates was extremely time consuming, yielding small samples sizes. Consequently, the number of samples of the most difficult to collect invertebrates (Bathyporeia, Eurydice, Haustorius and Corophium) was small.

Taking all invertebrate species together (Kruskal-Wallis non-parametric test), there were significant differences in all four metal concentrations between species (see Table 2.2). Of the invertebrates from the north side of the river, cadmium, copper and lead levels were significantly higher in the amphipod crustacean Corophium than in the polychaete Nereis (Kruskal-Wallis protected pairwise test). In comparing metal levels in the mixed Crustaceans and the polychaete Nerine from the south side, copper and lead were found at significantly higher levels in Crustaceans (see Table 2.2).

These patterns can be related to the physiology of the invertebrates. Nerine and Nereis are soft-bodied polychaete worms, while Corophium and the Crustaceans Bathyporeia, Eurydice, and Haustorius are hard-shelled crustaceans. Certain metals tend to be found at higher concentrations in jaws and exoskeletons of invertebrates than in the soft tissues (Evans et al., 1987). In agreement with this, cadmium, copper and lead were found at higher

levels in Corophium than in Nereis, and to a less significant extent at higher levels in the Crustaceans than in Nerine. Zinc, however, an essential metal, was most concentrated in the polychaete Nerine. The concentration of zinc was significantly higher in Nerine than in Corophium or Nereis (Kruskal-Wallis pairwise test respectively: 5.291,  $p < 0.02$ ; 4.172,  $p < 0.05$ ). The concentration of zinc is known to be regulated in at least some marine invertebrates (Bryan, 1984)

Table 2.2. Differences between metal concentrations in invertebrates collected at Teesmouth.

Groups compared	n	Cadmium	Copper	Lead	Zinc
Kruskal-Wallis all-groups test					
All invertebrates	24	17.222**	12.556**	19.095**	14.848**
Kruskal-Wallis protected pairwise test					
<u>Nerine</u> /Crustaceans	15	1.690	3.123°	2.760°	1.744
<u>Nereis</u> / <u>Corophium</u>	9	3.909*	3.440*	3.782*	1.880

° $p < 0.10$ ; \* $p < 0.05$ ; \*\* $p < 0.01$

### 2.2.1. Heavy Metal Concentrations in Nerine

Most of the Nerine analysed were taken from Redcar Sands, which is about three kilometres south of the mouth of the Tees. Nerine were more difficult to obtain in quantity from Coatham Sands than from Redcar Sands, possibly because Nerine population densities on Coatham Sands were reduced by the effects of close

proximity to pollutants discharged from the river's mouth. Nerine were sorted into three size classes to test if size, and therefore age, of the worms and metal concentrations were correlated. This is of interest because birds might select larger worms over smaller worms, or vice versa, thus influencing the concentrations of metals they ingest. The size classes (less than 2.5 cm, 2.5 cm to 3.5 cm, and greater than 3.5 cm.) were chosen to provide samples of similar mass and number from the material available. A "mixed size" sample was also analysed. It contained fragments of broken worms whose unbroken size were unknown. Results are shown in Table 2.3.

Table 2.3. Mean heavy metal concentrations in Nerine collected at Teesmouth. Concentrations expressed as ppm ( $\mu\text{g}/\text{g}$  dry weight).

Size class	Number of samples	Concentration $\pm$ Standard Error			
		Cadmium	Copper	Lead	Zinc
Mixed	1	1.0	40.0	32.0	282.2
<2.5cm	3	1.0 $\pm$ 0.00	46.5 $\pm$ 2.83	17.0 $\pm$ 5.4	275.6 $\pm$ 20.09
2.5-3.5cm	4	1.1 $\pm$ 0.12	45.6 $\pm$ 2.54	7.5 $\pm$ 0.5	235.1 $\pm$ 24.88
>3.5cm	4	1.3 $\pm$ 0.15	41.0 $\pm$ 3.25	14.0 $\pm$ 5.5	203.2 $\pm$ 13.05

There were no significant differences in metal concentrations between the largest size class and the two smaller size classes combined (Mann-Whitney test  $p > 0.10$ ). Lead levels were subject to wide variation with large standard errors: in the less-than 2.5 cm size the concentration of lead ranged from 9 ppm to 30 ppm; in the greater-than 3.5 cm size class, the concentration of lead ranged

from 6 ppm to 33 ppm; and the single sample in the mixed-size class had a concentration of 32 ppm. In contrast, lead concentrations in the other invertebrates were much more consistent from sample to sample (See Appendix 3, Tables iii.1, iii.2, iii.3, & iii.4).

### 2.3. Heavy Metal Concentrations in Sanderling

A total of 31 kidney samples and 57 liver samples were analysed. A number of livers were large enough to provide two samples; the 57 samples were taken from 43 individuals. Kidney tissues were too small to yield more than one sample, so the 31 samples represent 31 individuals. 31 of the livers, yielding 45 samples, came from the same individuals as those from which the kidneys were taken, while 12 livers lacked matching kidneys. Most samples were taken from adult birds; one kidney and two liver samples were taken from a juvenile; and a number were taken from birds whose ages were unknown, so all age classes were lumped together. In two cases, sample solutions were exhausted before zinc could be tested by AAS, so 29 pairs of kidneys and livers were tested for zinc. The mean and median concentrations of heavy metals in Sanderling tissues are summarized in Table 2.4. Complete data, including mass of the individual samples, AAS results, dates and locations of capture (where known) are given in Appendix 4, Tables iv.1 and iv.2.

Table 2.4. Heavy metal concentrations (ppm dry mass) in Sanderling kidneys and livers.

Number of samples	Number of birds	Concentrations $\pm$ Standard Error (median in parentheses)				
		Cadmium	Copper	Lead	Zinc	
Kidney and Liver pairs						
Kidneys	31	31	18.1 $\pm$ 2.64 (15.0)	22.6 $\pm$ 0.63 (22.5)	10.4 $\pm$ 0.8 (11.0)	171.2 $\pm$ 14.27 <sup>1</sup> (138.3)
Livers	45	31	3.6 $\pm$ 0.44 (3.0)	23.3 $\pm$ 0.63 (22.5)	6.5 $\pm$ 0.5 (6.5)	108.2 $\pm$ 3.72 <sup>2</sup> (104.4)
Ratio Kidney:Liver			5:1	0.97:1	1.6:1	1.6:1
All Liver samples*						
Liver	57	43	3.3 $\pm$ 0.38 (2.5)	25.1 $\pm$ 0.96 (24.0)	6.3 $\pm$ 0.4 (6.5)	115.1 $\pm$ 3.25 <sup>3</sup> (110.4)

<sup>1</sup>n<sub>s</sub>=29, n<sub>b</sub>=29; <sup>2</sup>n<sub>s</sub>=42, n<sub>b</sub>=29; <sup>3</sup>n<sub>s</sub>=55, n<sub>b</sub>=42

(n<sub>s</sub>=number of samples, n<sub>b</sub>=number of birds)

\*These values are not strictly comparable with the subset, as the extra data could come from different months

Concentrations of cadmium, lead, and zinc were significantly higher in kidneys than in livers of the 31 birds for which both tissues were analysed (see Table 2.5). There was no significant difference in copper concentrations between tissues. There were significant positive correlations between kidney and liver concentrations of cadmium and of lead (see Table 2.5, and Figures 2.1 - 2.4).

Table 2.5. Comparisons between metal concentrations in paired kidney and liver tissue of Sanderling.

n	Mann-Whitney test, Kidney v. Liver			
	Cadmium	Copper	Lead	Zinc
31	586.5**	937.5	698.0**	561.5**
	Correlation Coefficients, Kidney v. Liver			
31	0.892***	0.001	0.630***	-0.101

\*\* $p < 0.01$ ; \*\*\* $p < 0.001$

### 2.3.1. Seasonal Variations of Metal Concentrations in Sanderling Kidney and Liver Tissues

Because the birds are exposed to different physiological demands at different times of the year, seasonal differences in metal concentrations might be expected.

There were data from Sanderling captured during four periods: in the autumn months of September, October and November, when Sanderling return from their breeding grounds; February and March, the late winter; May, when moulting into breeding plumage and preparing for spring migration; July, just before the complete moult. Except for one bird captured in March and one captured in July, all birds for which there were data were adults. The concentrations of metals in kidney and livers are listed in Table 2.6.

Table 2.6. Monthly heavy metal concentrations (ppm dry mass) in Sanderling kidneys and livers.

Month	Number of samples	Number of birds	Concentrations $\pm$ S.E. (median in parentheses)			
			Cadmium	Copper	Lead	Zinc
Kidneys						
Autumn*	4	4	3.1 $\pm$ 0.99 (2.3)	25.1 $\pm$ 1.24 (24.5)	2.8 $\pm$ 1.4 (2.5)	211.4 $\pm$ 13.00 (209.3)
March	13	13	13.8 $\pm$ 1.79 (15.0)	20.8 $\pm$ 1.01 (22.0)	11.5 $\pm$ 1.2 (12.0)	177.3 $\pm$ 24.41 (133.9)
May	9	9	22.8 $\pm$ 3.44 (23.0)	23.8 $\pm$ 1.02 (23.0)	11.1 $\pm$ 0.9 (11.0)	163.4 $\pm$ 28.25 (136.1)
July	5	5	32.6 $\pm$ 10.56 (23.0)	22.9 $\pm$ 1.01 (22.5)	12.4 $\pm$ 1.2 (12.0)	129.3 $\pm$ 6.98 <sup>1</sup> (127.6)
Livers						
Autumn*	4	4	1.0 $\pm$ 0.18 (1.0)	22.9 $\pm$ 1.28 (23.3)	3.8 $\pm$ 0.5 (4.0)	144.3 $\pm$ 7.36 (149.4)
February	8	8	1.9 $\pm$ 0.14 (2.0)	29.9 $\pm$ 0.96 (29.3)	6.3 $\pm$ 0.5 (6.0)	131.7 $\pm$ 4.01 (136.5)
March	21	14	2.7 $\pm$ 0.21 (2.9)	22.7 $\pm$ 0.61 (22.2)	6.3 $\pm$ 0.7 (6.8)	100.9 $\pm$ 2.65 <sup>2</sup> (98.5)
May	13	9	4.7 $\pm$ 0.43 (4.5)	24.0 $\pm$ 1.75 (25.5)	8.2 $\pm$ 0.7 (8.0)	97.1 $\pm$ 3.88 (100.6)
July	8	5	6.1 $\pm$ 1.86 (5.3)	24.5 $\pm$ 0.77 (25.0)	6.5 $\pm$ 0.7 (6.5)	126.8 $\pm$ 5.07 <sup>3</sup> (130.0)

<sup>1</sup>n=4; <sup>2</sup>n<sub>s</sub>=20, n<sub>b</sub>=14; <sup>3</sup>n<sub>s</sub>=7, n<sub>b</sub>=4

\*September, October and November

Figure 2.1. Correlations between Cd concentrations in Sanderling kidney and liver tissues

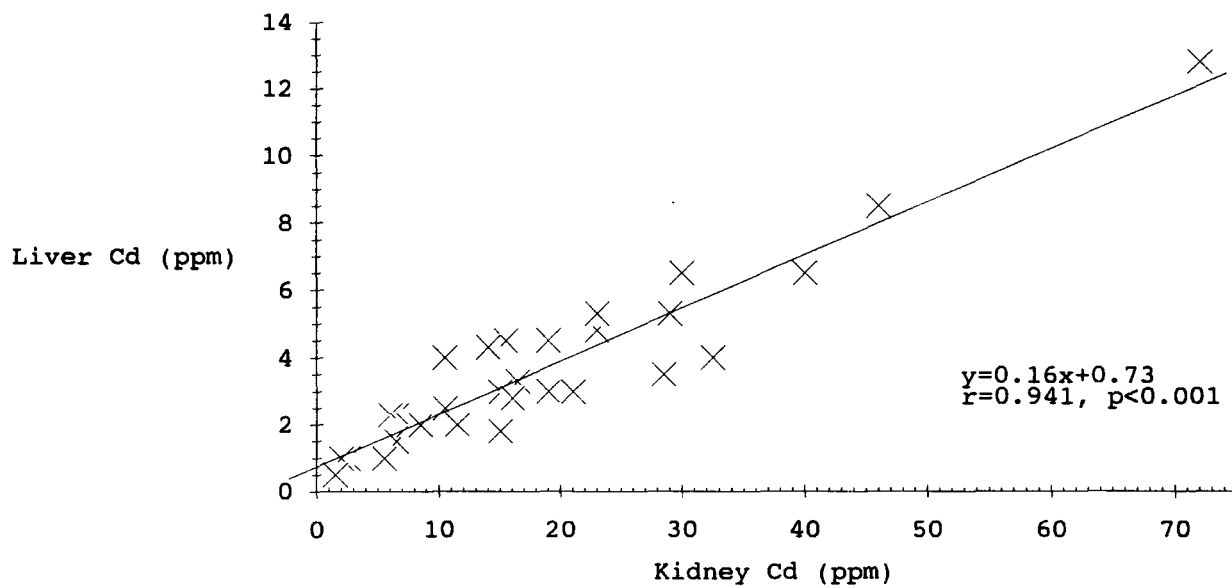


Figure 2.2. Correlations between Cu concentrations in sanderling kidney and liver tissues

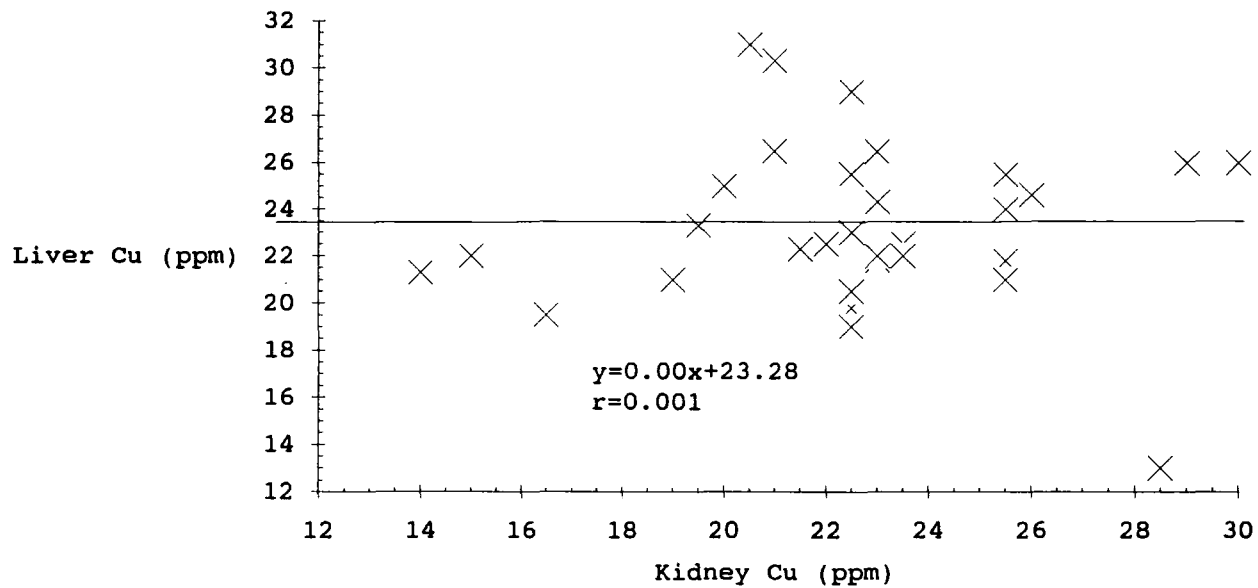


Figure 2.3. Correlations between Pb concentrations in Sanderling kidney and liver tissues

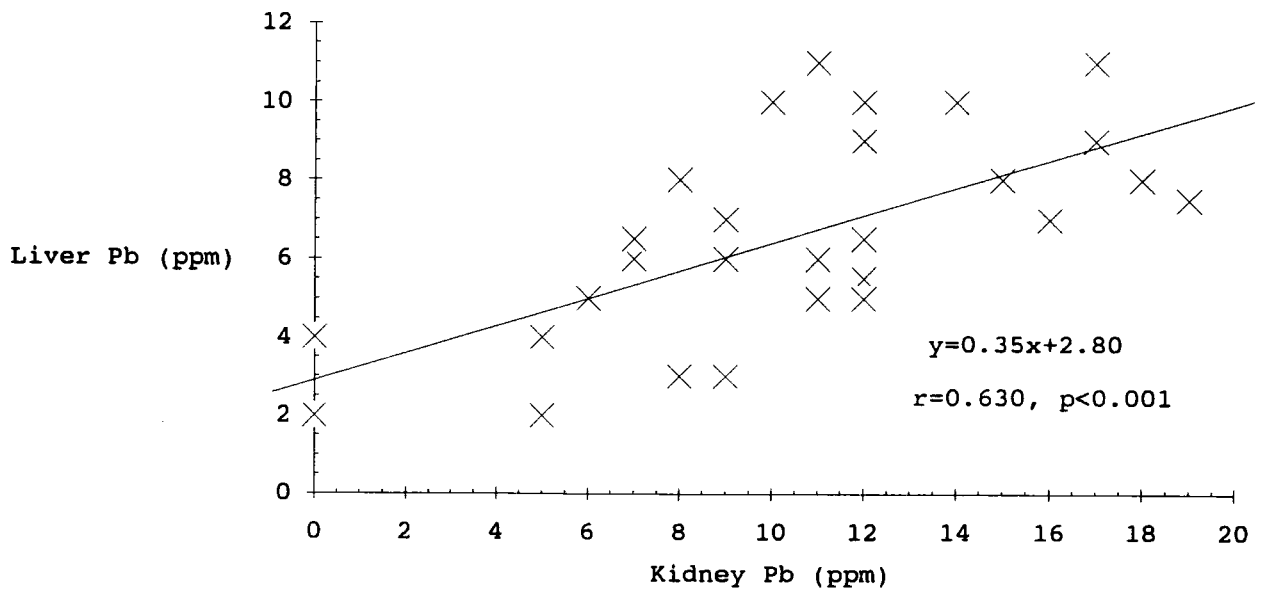


Figure 2.4. Correlations between Zn concentrations in sanderling kidney and liver tissues

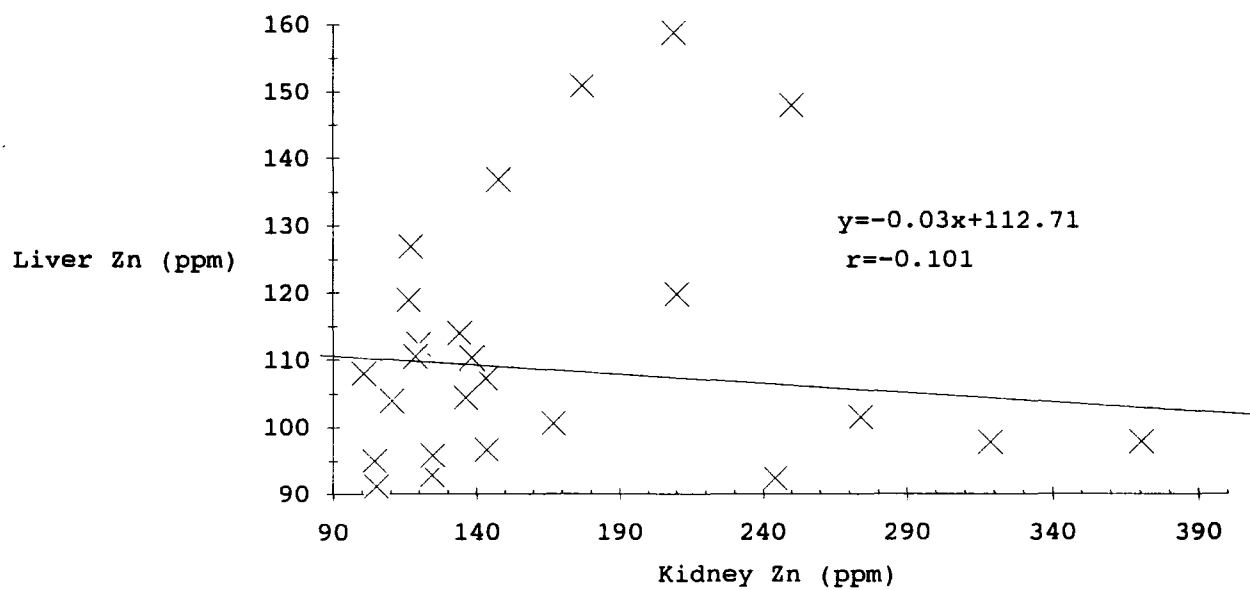


Table 2.7 lists the results of Kruskal-Wallis tests between various months for both Sanderling kidney and liver tissues. Taking all months together, kidney tissue cadmium and lead concentrations had significant seasonal variations. In liver tissues, all four metal concentrations had significant seasonal variations.

Table 2.7. Significance of monthly differences of metal concentrations in Sanderling kidney and liver.

Months compared	n	Cadmium	Copper	Lead	Zinc
Kidneys - Kruskal-Wallis all-groups test					
All months	31	13.003**	5.100	10.422*	4.375 <sup>1</sup>
Kidneys - Kruskal-Wallis protected pairwise test					
Autumn/March	17	2.731°		3.325*	
March/May	22	2.098		0.183	
May/July	14	0.163		0.958	
Autumn/May	13	4.113*		3.032°	
Autumn/July	9	3.820*		3.513*	
March/July	18	1.902		0.865	
Livers - Kruskal-Wallis all-groups test					
All months	40	21.807**	15.382**	10.033*	25.360 <sup>2**</sup>
Livers - Kruskal-Wallis protected pairwise test					
Autumn/February	12	1.814	3.258*	1.980	1.162
February/March	22	2.386°	4.978**	0.259	5.569**
March/May	23	2.994*	1.251	1.801	0.643
May/July	14	0.849	0.312	1.383	4.048**
Autumn/March	18	3.825*	0.373	2.341°	5.608**
Autumn/May	13	5.737**	0.537	3.489*	5.748**
Autumn/July	9	4.433*	0.741	1.976	1.445
February/May	17	4.809**	3.441*	1.820	5.644**
February/July	13	3.268*	2.628°	0.198	0.506
March/July	19	1.546	1.359	0.004	3.806*

<sup>1</sup>n=30; <sup>2</sup>n=39

°p<0.10 \*p<0.05; \*\*p<0.01

For cadmium, the pattern of concentrations is very similar between kidneys and livers; concentrations were lowest in the autumn months (September, October and November) and rose through July (see Figure 2.5). In kidneys, autumn concentrations were significantly lower than the other months. In livers, autumn and February concentrations were significantly lower than the other months. There was a significant increase in cadmium concentrations from February to March and March to May.

Copper concentrations did not vary significantly by season in kidney tissues. In livers, February levels were significantly higher than the other months (see Figure 2.6).

Lead concentrations followed similar patterns in both tissues; concentrations were significantly lower in autumn but did not vary significantly the rest of the year (see Figure 2.7).

Zinc concentrations in kidney were the reverse of cadmium; highest in autumn and diminishing through July, although not significantly. In liver tissues, concentrations were highest in autumn, and were lowest in May, rising again in July. March concentrations were significantly lower than February concentrations, and July concentrations were significantly higher than May concentrations (see Figure 2.8).

The presence or uptake of one metal might influence the uptake of another. Of special interest are zinc/lead and zinc/cadmium interactions. It is possible that when there is an increased uptake of the essential metal zinc, small amounts of toxic metals in the birds' food, specifically cadmium and lead, are also

accumulated. Table 2.8 gives the results of comparisons between concentrations of metals by season for Sanderling kidney and liver tissues.

Table 2.8. Seasonal correlation between metal concentrations in Sanderling kidney and liver samples.

	n	Metals correlated, correlation coefficient					
		Cd/Cu	Cd/Pb	Cd/Zn	Cu/Pb	Cu/Zn	Pb/Zn
Kidneys							
Total	31	-0.012	0.160	-0.379 <sup>1*</sup>	-0.273	0.470 <sup>1**</sup>	-0.256 <sup>1</sup>
March	13	-0.242	0.069	-0.451	-0.278	0.555 <sup>*</sup>	-0.479 <sup>°</sup>
May	9	-0.224	-0.796 <sup>*</sup>	-0.461	0.483	0.520	0.713 <sup>*</sup>
July	5	0.307	-0.195	0.927 <sup>2*</sup>	-0.592	-0.006 <sup>2</sup>	-0.548 <sup>2</sup>
Livers							
Total	43	0.320 <sup>*</sup>	0.286 <sup>°</sup>	0.095 <sup>3</sup>	0.244	0.647 <sup>3***</sup>	-0.018 <sup>3</sup>
February	8	-0.181	-0.086	0.014	0.452	0.573	0.313
March	14	0.146	0.650 <sup>*</sup>	0.173	0.302	0.559 <sup>*</sup>	0.204
May	9	0.212	-0.068	0.492	0.069	0.679 <sup>*</sup>	-0.068
July	5	0.213	-0.279	0.478 <sup>2</sup>	0.405	0.785 <sup>2</sup>	-0.395 <sup>2</sup>

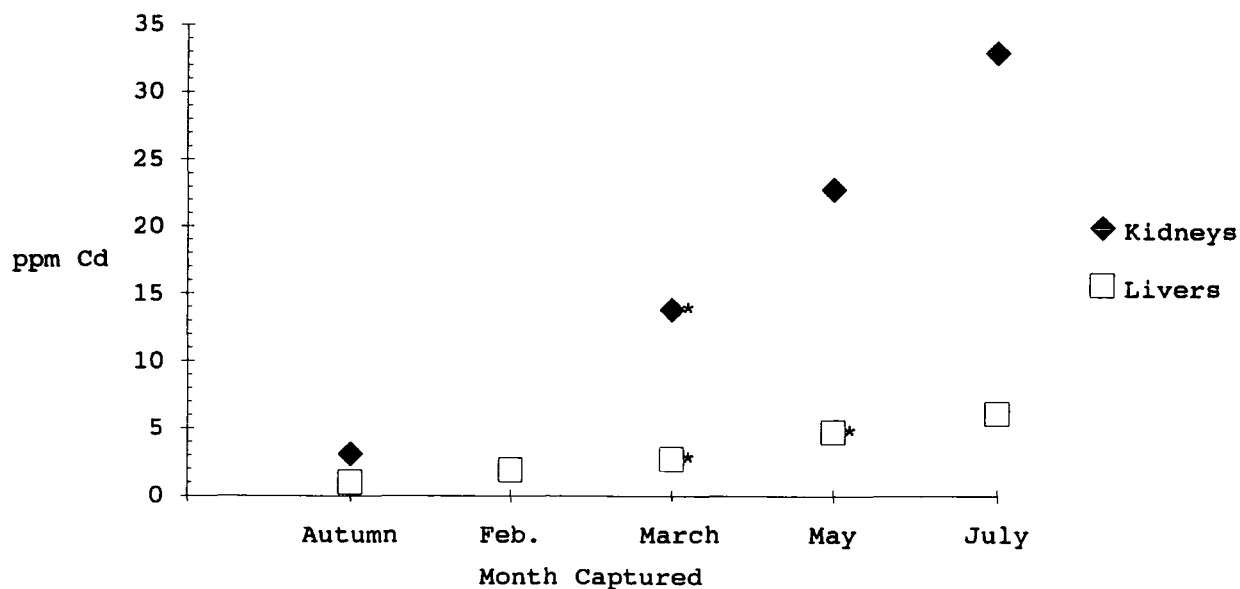
<sup>1</sup>n=30; <sup>2</sup>n=4; <sup>3</sup>n=42

<sup>°</sup>p<0.10; <sup>\*</sup>p<0.05; <sup>\*\*</sup>p<0.01; <sup>\*\*\*</sup>p<0.001

Taking all kidneys together, there was a significant negative correlation between cadmium and zinc concentrations, while in July there was a significant positive correlation (see Figure 2.9). There was a significant positive correlation between lead and zinc in May kidney samples (see Figure 2.10), and a significant negative correlation in March. There were also significant correlations between cadmium and lead in May; and copper and zinc in total kidneys and March kidneys.

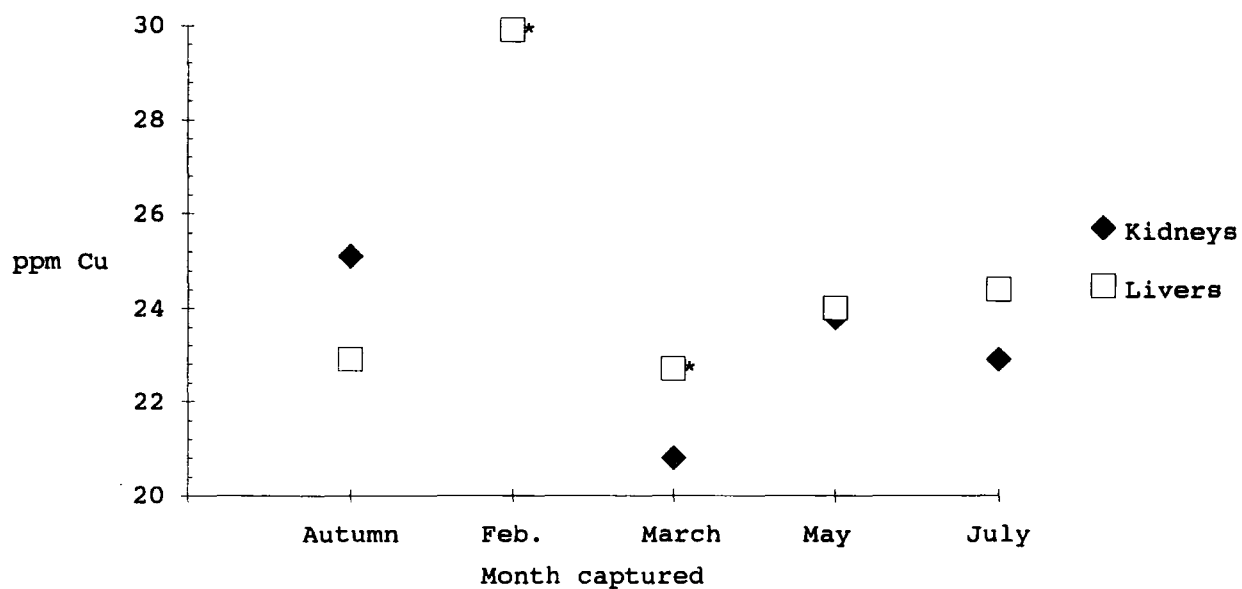
In total liver tissues, there were significant correlations between cadmium and copper, cadmium and lead, and copper and zinc. Significant correlations between cadmium and lead were also found in March, and between copper and zinc in March and May.

Figure 2.5. Monthly concentrations of cadmium in Sanderling kidney and liver samples



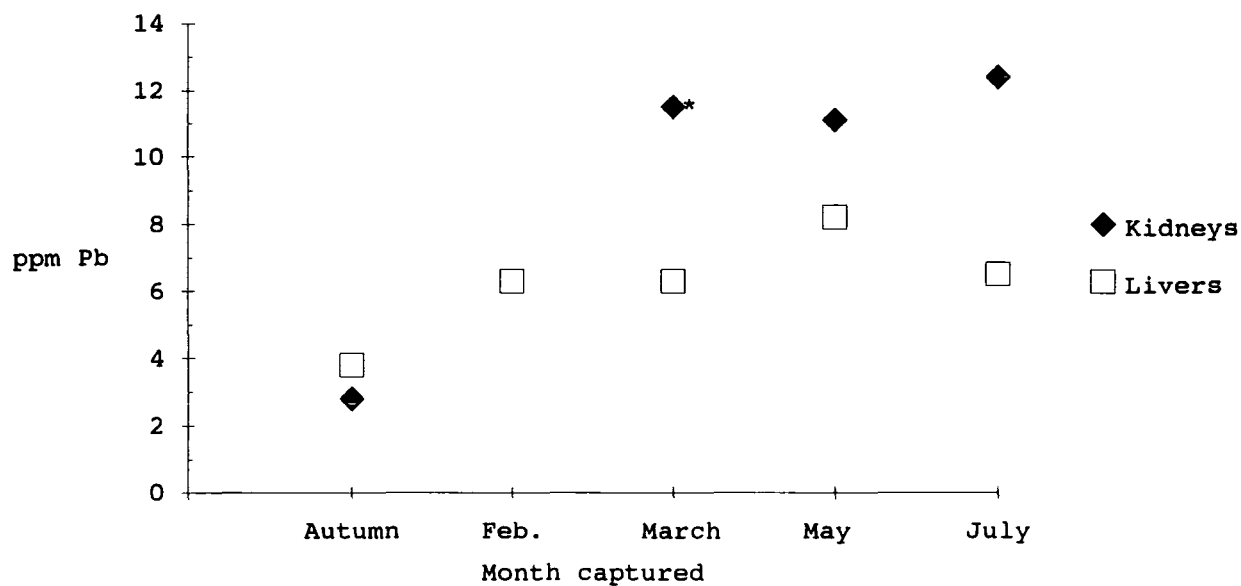
\*Indicates that metal concentration in that month were significantly different from metal concentrations in the previous measurement period.

Figure 2.6. Monthly concentrations of copper in Sanderling kidney and liver samples



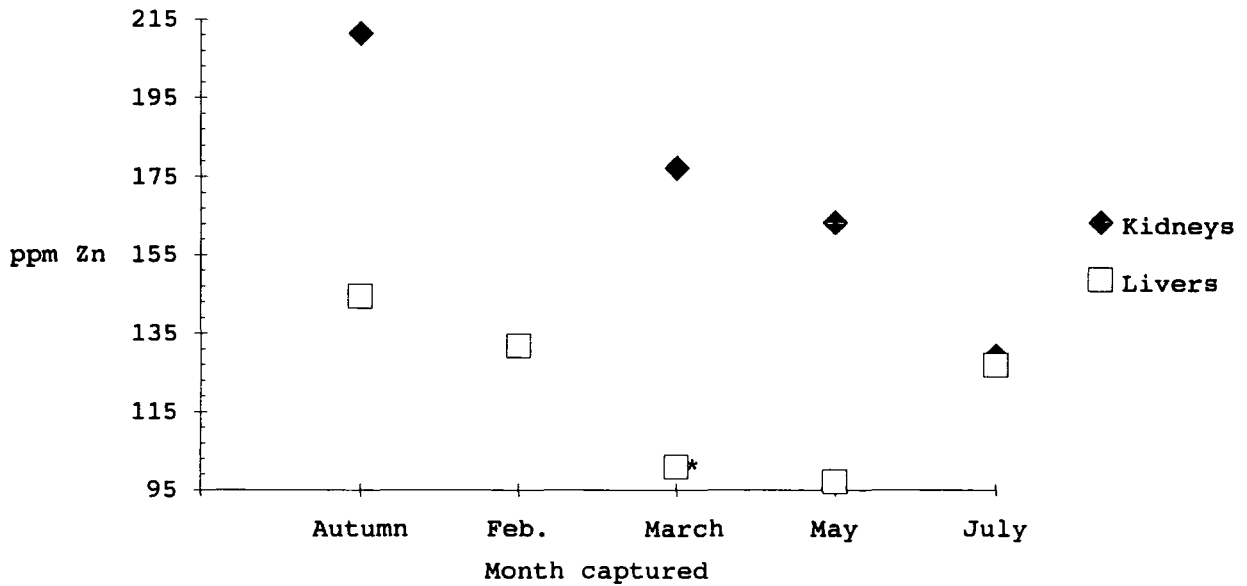
\*Indicates that metal concentration in that month were significantly different from metal concentrations in the previous measurement period.

Figure 2.7. Monthly concentrations of lead in Sanderling kidney and liver samples



\*Indicates that metal concentration in that month were significantly different from metal concentrations in the previous measurement period.

Figure 2.8. Monthly concentrations of zinc in Sanderling kidney and liver samples



\*Indicates that metal concentration in that month were significantly different from metal concentrations in the previous measurement period.

Figure 2.9. Correlations between Cd and Zn concentrations in sanderling kidney tissues

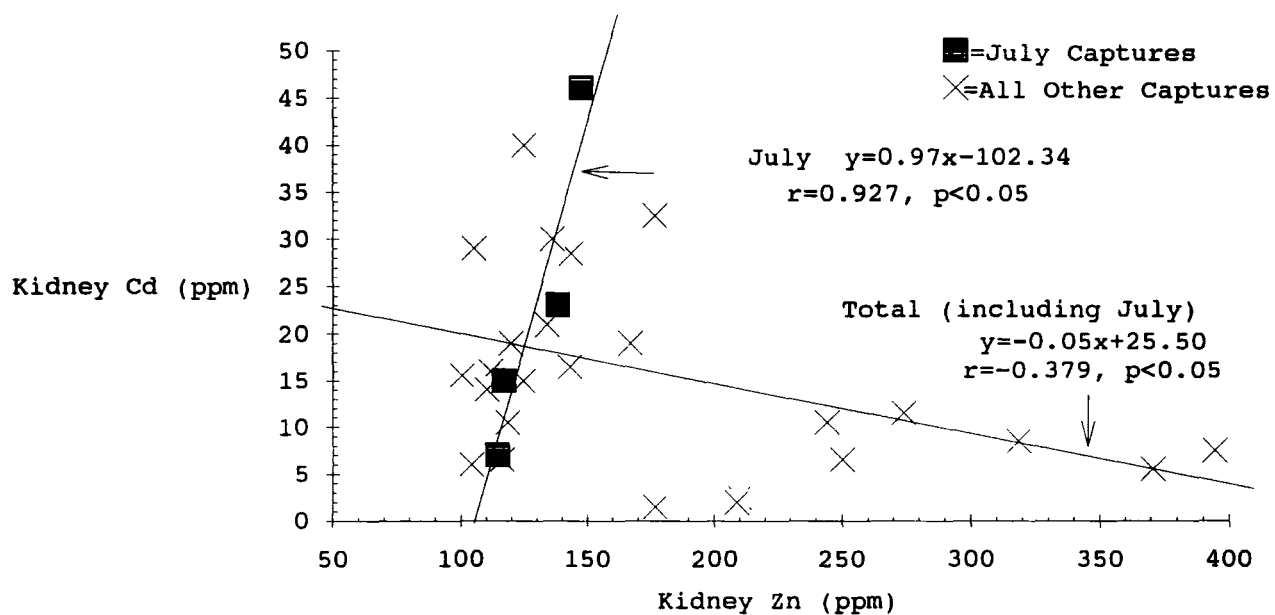
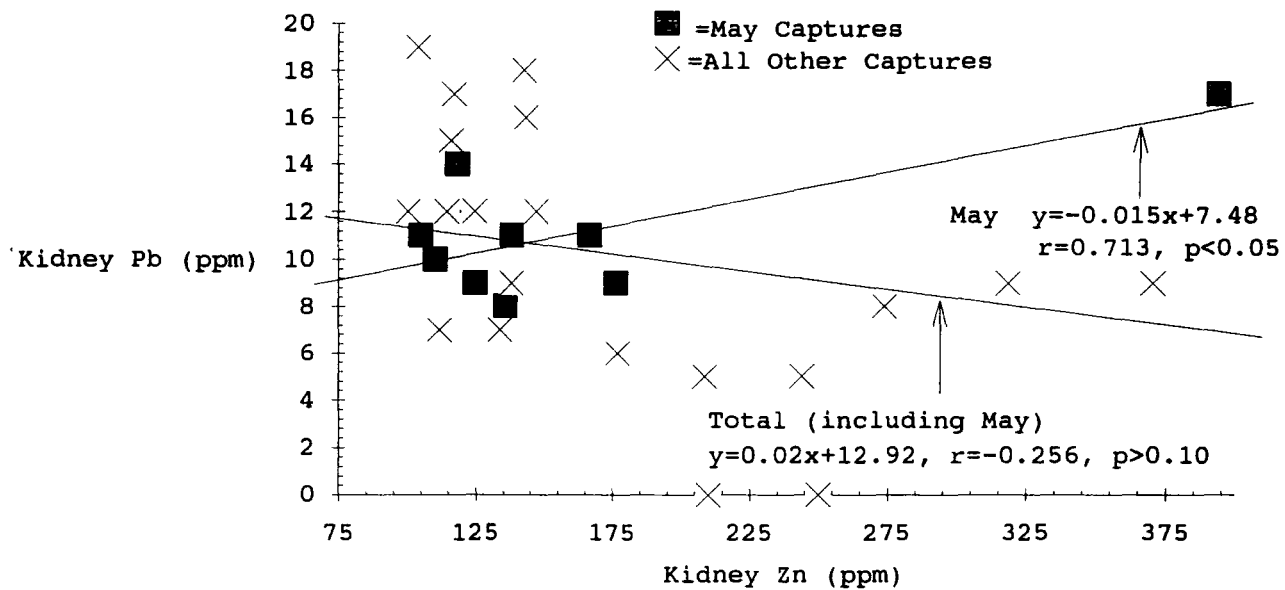


Figure 2.10. Correlations between Pb and Zn Concentrations in Sanderling Kidney Tissues



Sex might have an influence on metal concentrations, as a result of different physiological demands for nutrients, especially egg production (however, none of the birds was captured during the breeding season). Table 2.9 gives results of Mann-Whitney tests for differences between sexes.

Table 2.9. Differences in metal concentrations between sexes, Sanderling kidney and liver samples

	n		Mann-Whitney test, male v. female			
	♂	♀	Cadmium	Copper	Lead	Zinc
Kidneys						
Total	9	8	77.0	68.0	67.0	37.0
March	3	5	9.0	16.0	13.5	15.0
July	4	1	5.0	4.5	3.0	-
Autumn	2	2	6.0	6.0	4.5	6.0
Livers						
Total	9	12	81.5	109.5	95.5	108.0
February	4	4	14.0	24.5 <sup>*</sup>	19.0	17.0
March	3	6	12.0	9.0	13.0	11.0
Autumn	2	2	5.5	6.0	5.0	6.0

<sup>\*</sup>p<0.10

The only apparently significant difference at the 10% (1 in 10) level between sexes was found in livers during February, males having higher concentrations of copper, but this could have been chance, since Table 2.9 includes 31 comparisons.

Differences due to proximity to the mouth of the river might be expected to influence the concentration of metals found in bird tissues if invertebrates were contaminated less in one area than another. Assuming Sanderling are site-faithful, with individuals

feeding exclusively to the north or south of the river (Breary, 1982), known differences in pollution levels on the respective sides of the river might be detected in the birds. March was the only month for which birds were captured on both sides of the river, 13 on North Gare Sands, and four on Coatham Sands (see Figure 1.1). Table 2.10 lists the results of Mann-Whitney tests for differences in metal concentrations between north and south side birds.

Table 2.10. Differences in metal concentrations in Sanderling livers collected in March in relation to location of capture.

	n	Cadmium	Copper	Lead	Zinc
North means $\pm$ S.D.	13	3.0 $\pm$ 0.8	22.2 $\pm$ 2.3	7.2 $\pm$ 2.0	99.6 $\pm$ 12.2
South means $\pm$ S.D.	4	2.8 $\pm$ 0.6	25.8 $\pm$ 4.8	8.0 $\pm$ 2.2	115.0 $\pm$ 10.3 <sup>1</sup>
Mann-Whitney values		30.0	46.0	41.5	38.0°

<sup>1</sup>n=3

°p<0.10

The results are rather unexpected, although the sample size was quite small. Zinc was found at significantly higher levels in birds captured on the sea beaches at Coatham Sands than in those captured on North Gare Sands, which is within the estuary. Perhaps zinc stays in solution or suspension longer and is carried out of the river mouth, before being washed along Coatham Sands by sea currents.

#### 2.4. Heavy Metal Concentrations in Ringed Plovers

A total of 10 kidney samples and 72 liver samples were analysed. As with Sanderling, several livers provided two samples; 72 samples were taken from 60 individuals. 9 livers came from the same individuals as those from which kidneys were taken. Again, as with Sanderling, most samples were taken from adult birds. All kidney samples were from adult birds. Eight liver samples were taken from juveniles, and many livers were from birds of unknown age. Table 2.11 summarizes mean concentrations of heavy metals in Ringed Plover livers and kidneys. Complete data are listed in Appendix 4, Tables iv.3 and iv.4.

Table 2.11. Heavy metal concentrations (ppm dry mass) in Ringed Plover kidneys and livers.

Number of samples	Number of birds	Concentrations $\pm$ S.E. (median in parentheses)				
		Cadmium	Copper	Lead	Zinc	
Kidney and Liver Pairs						
Kidneys	9	9	9.2 $\pm$ 2.31 (7.0)	33.3 $\pm$ 2.31 (31.5)	8.1 $\pm$ 1.1 (9.0)	122.0 $\pm$ 4.24 (123.3)
Livers	10	9	1.3 $\pm$ 0.30 (1.0)	19.2 $\pm$ 0.91 (18.5)	3.3 $\pm$ 0.5 (3.5)	90.9 $\pm$ 6.24 (81.5)
Ratio Kidney:Liver			7.1:1	1.7:1	2.5:1	1.3:1
All Kidney and Liver samples*						
Kidneys	10	10	9.6 $\pm$ 2.11 (9.8)	33.3 $\pm$ 2.08 (32.0)	8.4 $\pm$ 1.0 (9.0)	121.6 $\pm$ 3.84 (120.5)
Livers	72	60	2.0 $\pm$ 0.25 (1.3)	25.8 $\pm$ 1.67 (23.5)	5.3 $\pm$ 0.3 (5.0)	107.6 $\pm$ 2.61 <sup>1</sup> (106.6)

<sup>1</sup>n<sub>s</sub>=68, n<sub>b</sub>=60: sample solutions of four livers samples were exhausted before zinc was measured.

\*These values are not strictly comparable with the subset or with each other, as extra data could come from different months.

Concentrations of cadmium, copper, lead and zinc were significantly higher in kidneys than in livers of the 9 birds for which both tissues were available (see Table 2.12). As in Sanderling, cadmium was more concentrated in Ringed Plover kidneys than the other metals. Also as in Sanderling, there were significant positive correlations between kidney and liver concentrations of cadmium and of lead (see Table 2.12 and Figures 2.11 - 2.14).

Figure 2.11. Correlations between Cd concentrations in Ringed Plover kidney and liver tissues

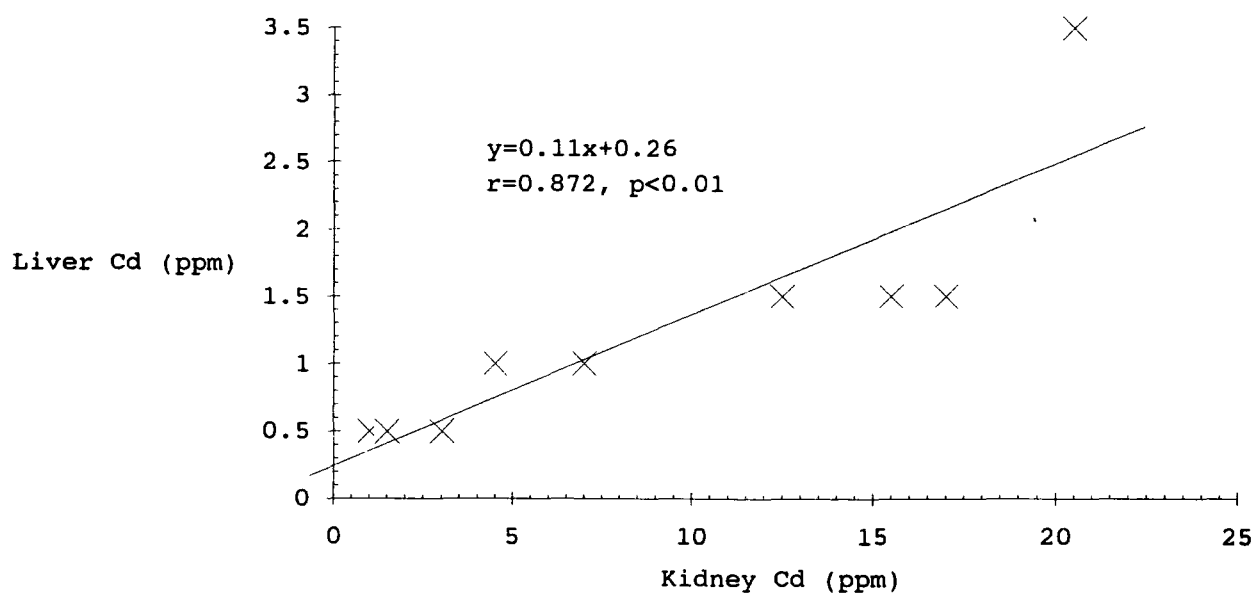


Figure 2.12. Correlations between Cu concentrations in Ringed Plover kidney and liver tissues

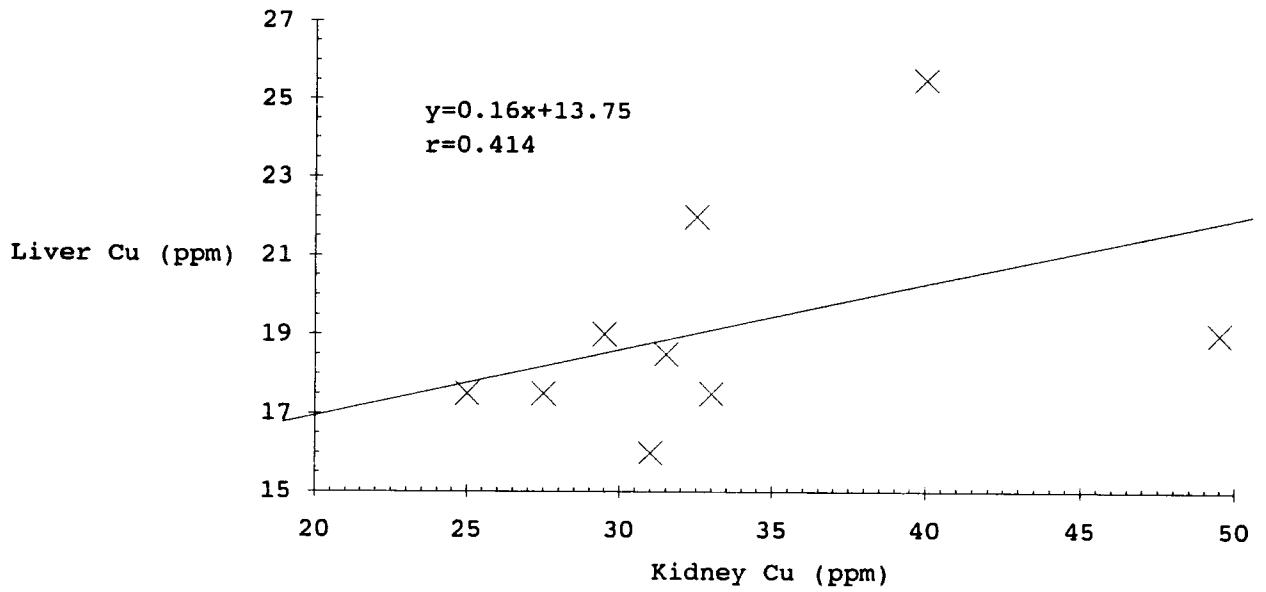


Figure 2.13. Correlations between Pb concentrations in Ringed Plover kidney and liver tissues

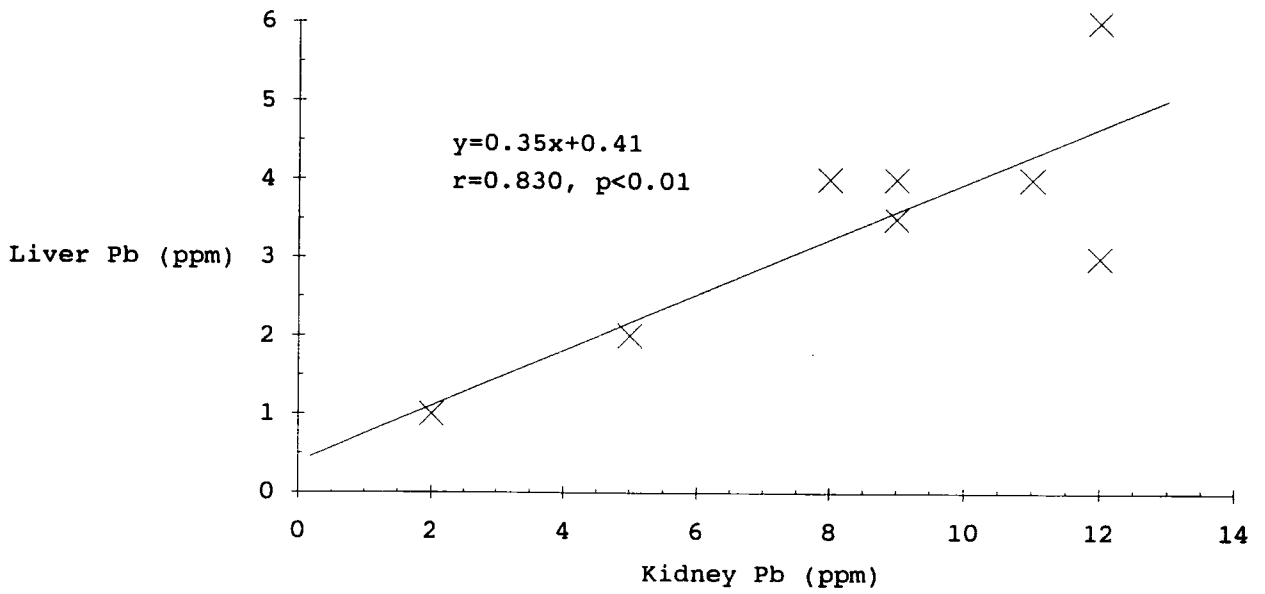


Figure 2.14. Correlations between Zn concentrations in Ringed Plover kidney and liver tissues

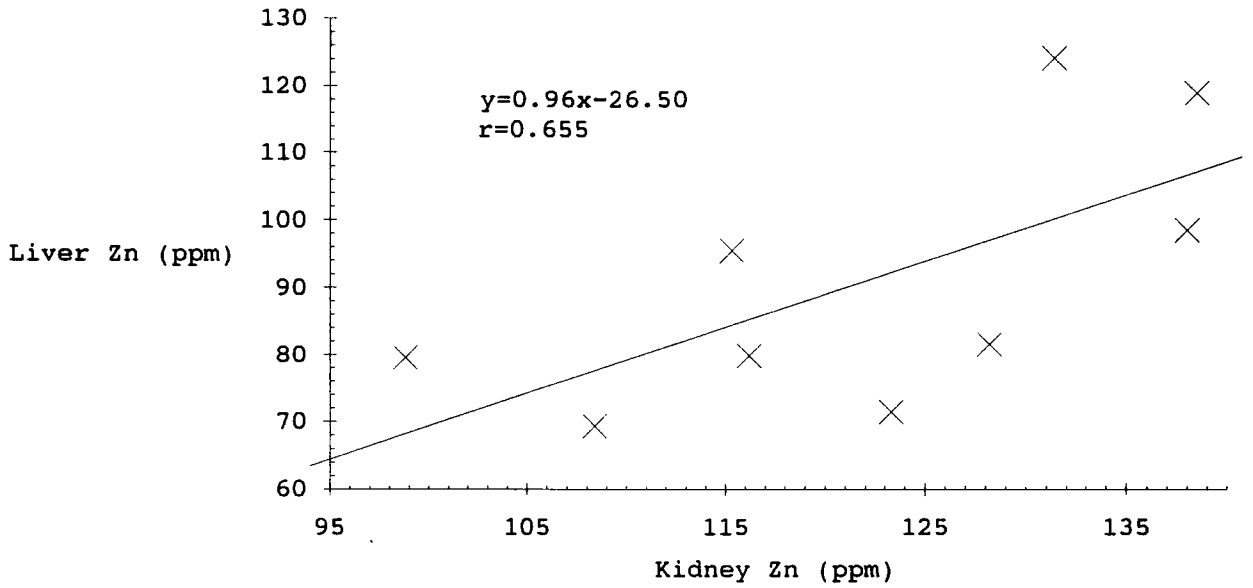


Table 2.12. Comparisons between metal concentrations in paired kidney and liver tissue of Ringed Plover.

n	Mann-Whitney test, Kidney v. Liver			
	Cadmium	Copper	Lead	Zinc
9	117.5**	125.0**	117.0**	117.0**
	Correlation Coefficients, Kidney v. Liver			
9	0.872**	0.414	0.830**	0.655

\* $p < 0.05$ ; \*\* $p < 0.01$

2.4.1. Seasonal Variations of Metal Concentrations in Ringed Plover Kidney and Liver Tissues

There were data on Ringed Plover captured during three periods: the passage of spring migrants in April and May; post-breeding moult in August; and late summer in September. The concentrations of metals in kidney and livers are listed in Table 2.13.

Table 2.13. Monthly mean heavy metal concentrations (ppm dry mass) in Ringed Plover kidneys and livers.

Month	Number of samples	Number of birds	Concentrations $\pm$ S.E. (median in parentheses)			
			Cadmium	Copper	Lead	Zinc
			Kidneys			
Spring*	6	6	5.9 $\pm$ 2.03 (3.8)	32.0 $\pm$ 1.85 (32.0)	8.3 $\pm$ 1.1 (8.5)	118.3 $\pm$ 4.79 (116.9)
August	4	4	15.0 $\pm$ 2.48 (16.3)	35.3 $\pm$ 4.22 (32.0)	8.5 $\pm$ 2.0 (10.0)	126.5 $\pm$ 5.52 (129.8)
			*April & May			

Table 2.13. Continued.

Month	$n_s$	$n_b$	Cd	Cu	Pb	Zn
			Livers			
May	30	23	2.2 ± 0.35 (1.5)	23.1 ± 0.75 (23.5)	5.1 ± 0.5 (5.0)	104.2 ± 4.81 <sup>1</sup> (102.9)
August	12	10	2.1 ± 0.54 (1.8)	25.6 ± 3.41 (22.0)	4.0 ± 0.4 (4.0)	102.4 ± 7.32 <sup>2</sup> (96.7)
Sept	16	14	0.7 ± 0.10 (0.5)	24.5 ± 1.42 (23.0)	6.3 ± 0.7 (6.3)	112.8 ± 4.51 <sup>3</sup> (115.0)

<sup>1</sup> $n_s=28$ ,  $n_b=23$ ; <sup>2</sup> $n_s=11$ ,  $n_b=10$ ; <sup>3</sup> $n_s=15$ ,  $n_b=14$

Comparisons between the months are listed in Table 2.14.

Table 2.14. Monthly differences of metal concentrations in Ringed Plover kidney and liver.

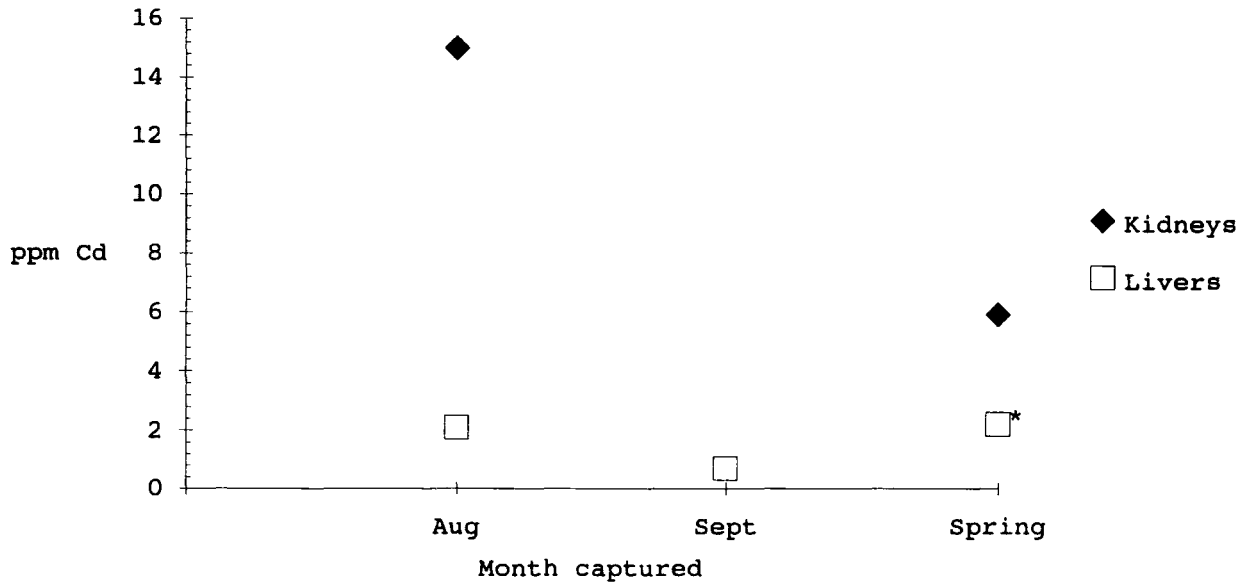
Months compared	n	Cadmium	Copper	Lead	Zinc
Kidneys - Mann-Whitney test					
Spring/August	10	32.0*	23.0	23.5	26.0
Livers - Kruskal-Wallis all-groups test					
All months	47	12.763**	1.227	5.950°	2.286
Livers - Kruskal-Wallis protected pairwise test					
May/August	33	0.248		1.205	
August/September	25	2.967°		2.480	
September/May	47	3.900°		1.683	

° $p < 0.10$ ; \* $p < 0.05$ ; \*\* $p < 0.01$

In kidneys, spring migrants had significantly lower cadmium concentrations than resident birds captured in August. In contrast, liver cadmium concentrations were significantly higher in

May than in September, although there was no significant difference between May and August concentrations (see Figures 2.15 - 2.18).

Figure 2.15. Monthly concentrations of cadmium in Ringed Plover kidney and liver samples



\*Indicates that metal concentration in that month were significantly different from metal concentrations in the previous measurement period.

Figure 2.16. Monthly concentrations of copper in Ringed Plover kidney and liver samples

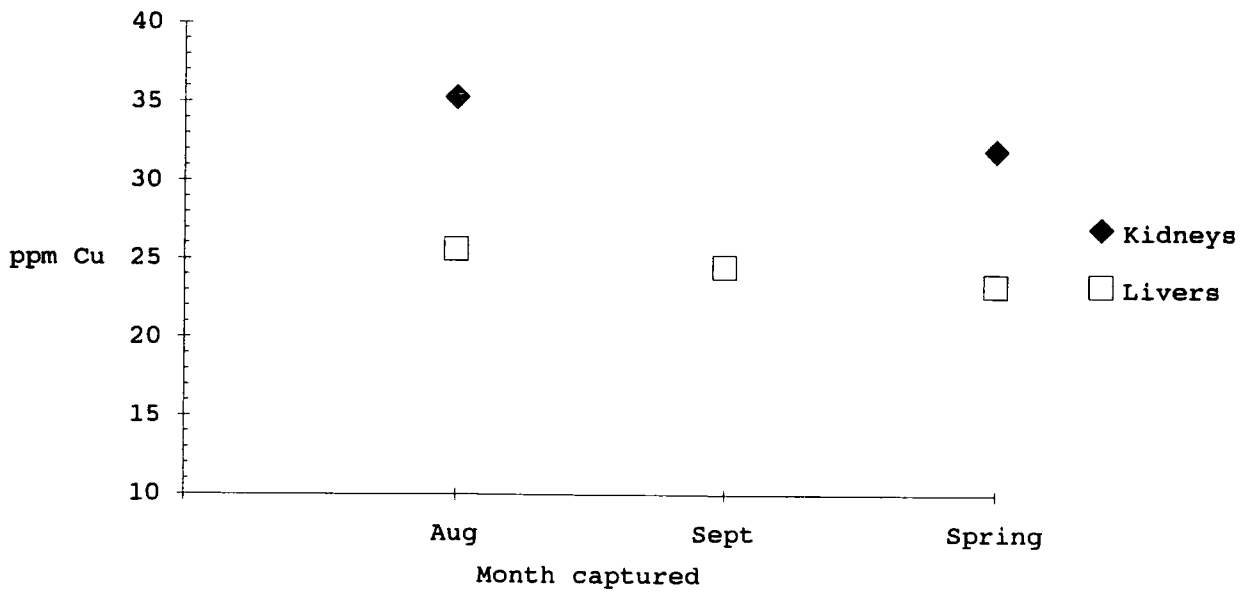


Figure 2.17. Monthly concentrations of lead in Ringed Plover kidney and liver samples

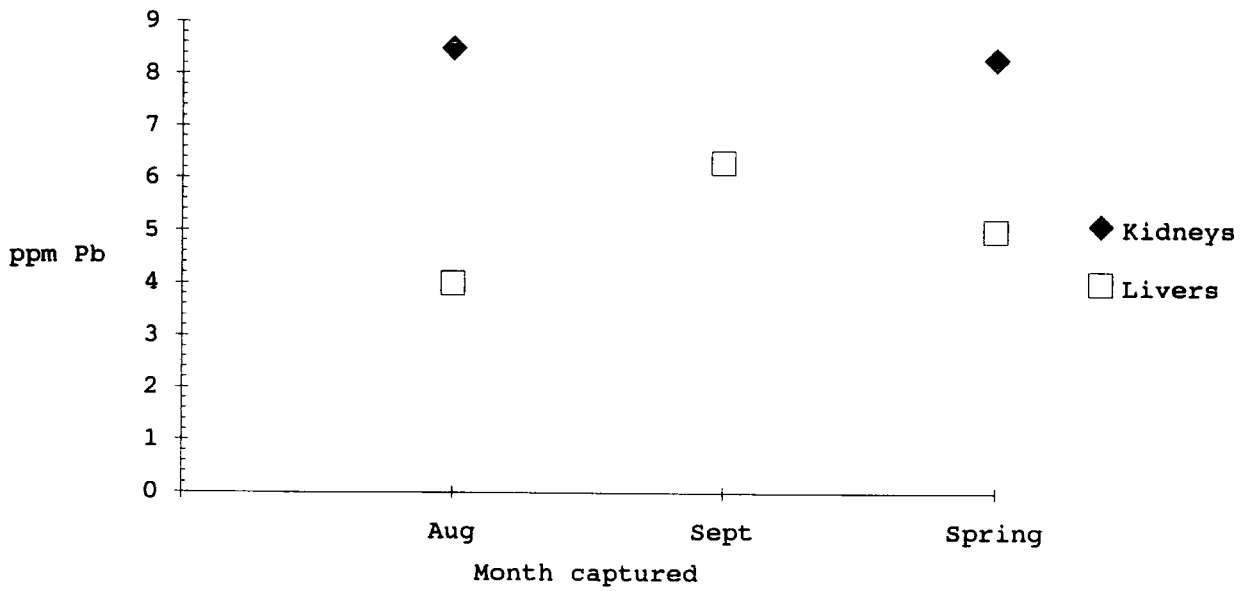
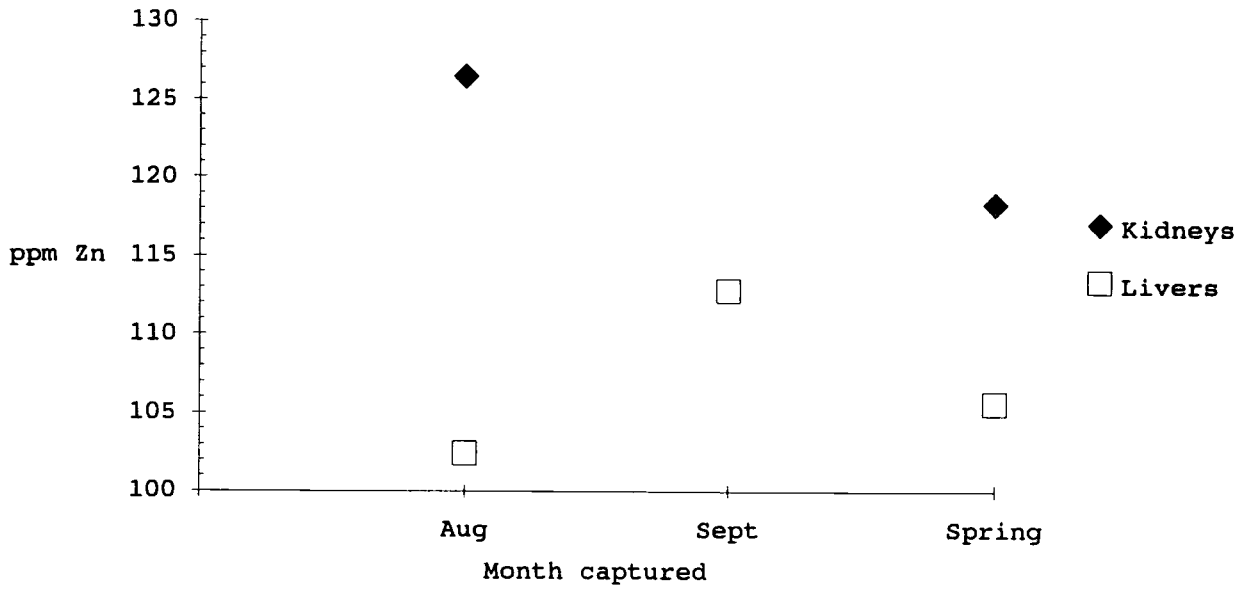


Figure 2.18. Monthly concentrations of zinc in Ringed Plover kidney and liver samples



Correlations between metals in Ringed Plover kidney and liver samples are listed in Table 2.15.

Table 2.15. Seasonal correlation between metal concentrations in Ringed Plover kidney and liver samples.

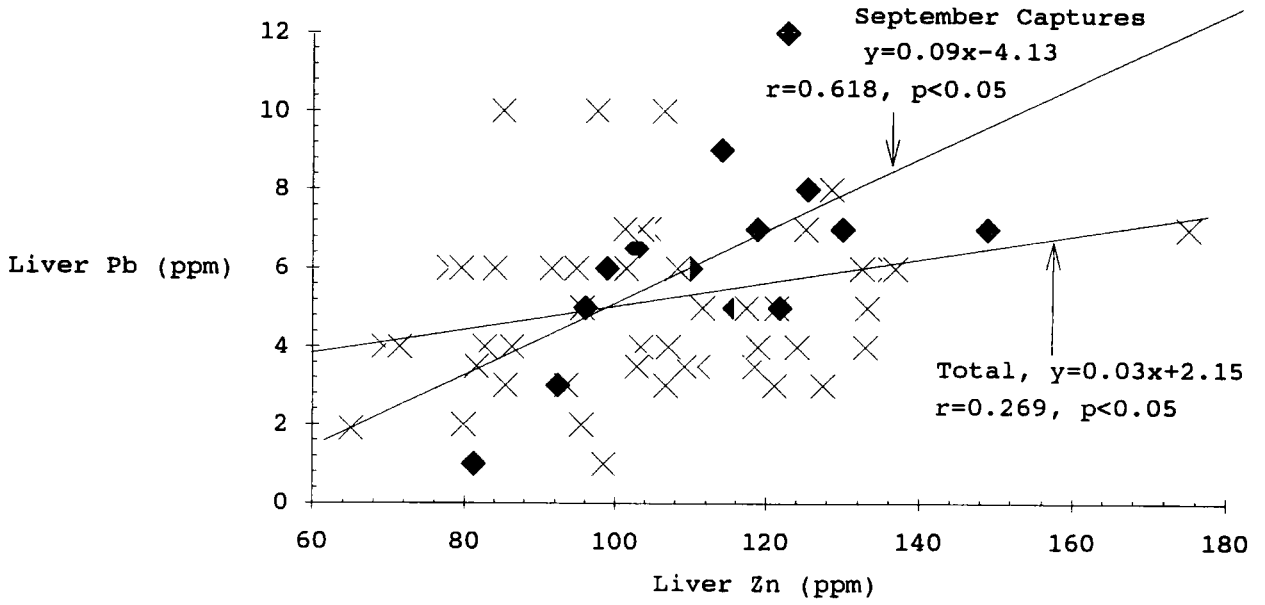
	n	Metals correlated, correlation coefficient					
		Cd/Cu	Cd/Pb	Cd/Zn	Cu/Pb	Cu/Zn	Pb/Zn
Kidneys							
Total	10	0.025	-0.215	0.491	0.399	0.473	-0.404
Spring	6	0.622	0.146	0.572	0.410	0.474	-0.316
August	4	-0.060	-0.793	0.084	0.406	0.436	-0.592
Livers							
Total	60	0.106	0.075	0.342**	0.379**	0.221	0.269*
May	23	0.338	0.346	0.685***	0.485*	0.336	0.057
August	10	0.739*	-0.038	0.447	0.246	0.700*	0.368
September	14	-0.116	0.222	0.002	0.390	0.442	0.618*

\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$

No metal/metal correlations in kidneys were significant, although the  $r$ -values were sometimes high. This was probably because of the small number of samples. In livers, zinc concentrations were significantly correlated with both lead and cadmium; cadmium and zinc were correlated in total livers and in the spring sample, and lead and zinc were correlated in the September sample. Copper concentrations were correlated with concentrations of cadmium (in August), lead (total livers and May), and zinc (in August). See Figures 2.19 - 2.20.



Figure 2.20. Correlations between Pb and Zn concentrations in Ringed Plover liver samples



Results of tests for differences between metal levels in the two sexes are listed in Table 2.16.

Table 2.16. Differences in metal concentrations between sexes, Ringed Plover kidney and liver samples.

	n		Mann-Whitney test, male v. female			
	♂	♀	Cadmium	Copper	Lead	Zinc
Kidneys						
Apr & May	3	3	13.0	9.0	8.0	9.0
Livers						
Total	21	19	335.0	339.5	361.0	311.0*
May	14	9	102.0	97.0	110.5	99.0
August	3	5	16.0	16.5	11.5	21.0*
September	4	5	27.0°	22.5	28.0*	27.0°

° $p < 0.10$ ; \* $p < 0.05$

Male livers had significantly higher concentrations of cadmium and lead than female livers during September. Male livers also had significantly higher levels of zinc in August and September.

## 2.5. Heavy Metal Concentrations in Grey Plovers

Six kidney samples and 56 liver samples were analysed. Five liver and kidney samples were taken from the same individuals. Because of the large size of Grey Plover livers, as many as four samples could be taken from the same tissue. As a result, the 56 liver samples represent only 26 individual birds. 21 livers and one kidney lacked matching samples. Unlike the Sanderling and

Ringed Plover tissue samples, most Grey Plover tissue samples came from either juveniles or birds of unknown age. The results of the analyses are summarized in Table 2.17. Complete data is listed in Appendix 4, Tables iv.5 and iv.6.

Table 2.17. Heavy metal concentrations (ppm dry mass) in Grey Plover kidneys and livers.

Number of samples	Number of birds	Concentrations $\pm$ Standard Error (median in parentheses)				
		Cadmium	Copper	Lead	Zinc	
Kidney and Liver Pairs						
Kidneys	5	5	3.8 $\pm$ 1.72 (2.0)	23.5 $\pm$ 0.62 (24.0)	10.0 $\pm$ 3.2 (8.0)	109.9 $\pm$ 15.84 (97.7)
Livers	14	5	0.7 $\pm$ 0.26 (0.5)	21.3 $\pm$ 2.23 (19.2)	4.7 $\pm$ 0.8 (3.7)	104.2 $\pm$ 9.10 (88.4)
Ratio Kidney:Liver			5.4:1	1.1:1	2.1:1	1.1:1
All Kidney and Liver Samples*						
Kidneys	6	6	3.3 $\pm$ 1.50 (1.5)	23.0 $\pm$ 0.69 (23.3)	9.7 $\pm$ 2.7 (8.0)	111.0 $\pm$ 13.24 (100.9)
Livers	56	26	0.8 $\pm$ 0.10 (0.5)	22.8 $\pm$ 0.73 (21.6)	3.7 $\pm$ 0.3 (3.0)	109.7 $\pm$ 2.97 (107.4)

\*These values are not strictly comparable with the subset or with each other, as extra data could come from different months.

Although all four metals were present at higher levels in kidney tissues than in liver tissues, only cadmium and lead were significantly higher at the 10% level (see Table 2.18). This was probably the result of the small number of samples. As in Sanderling and Ringed Plover, there was a significant positive correlation between kidney and liver concentrations of cadmium, but although the correlation between kidney and liver lead

concentrations was high, it was not significant. The correlation between kidney and liver copper concentrations however, was significant (see Table 2.18 and Figures 2.21 - 2.24).

Figure 2.21. Correlations between Cd concentrations in Grey Plover kidney and liver tissues

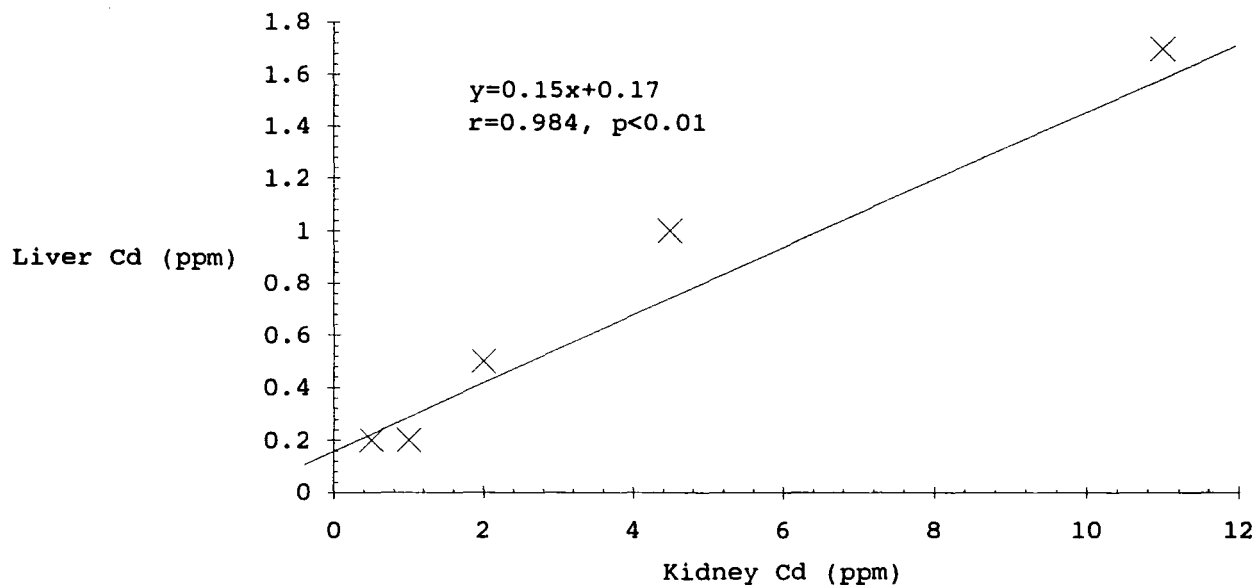


Figure 2.22. Correlations between Cu concentrations in Grey Plover kidney and liver tissues

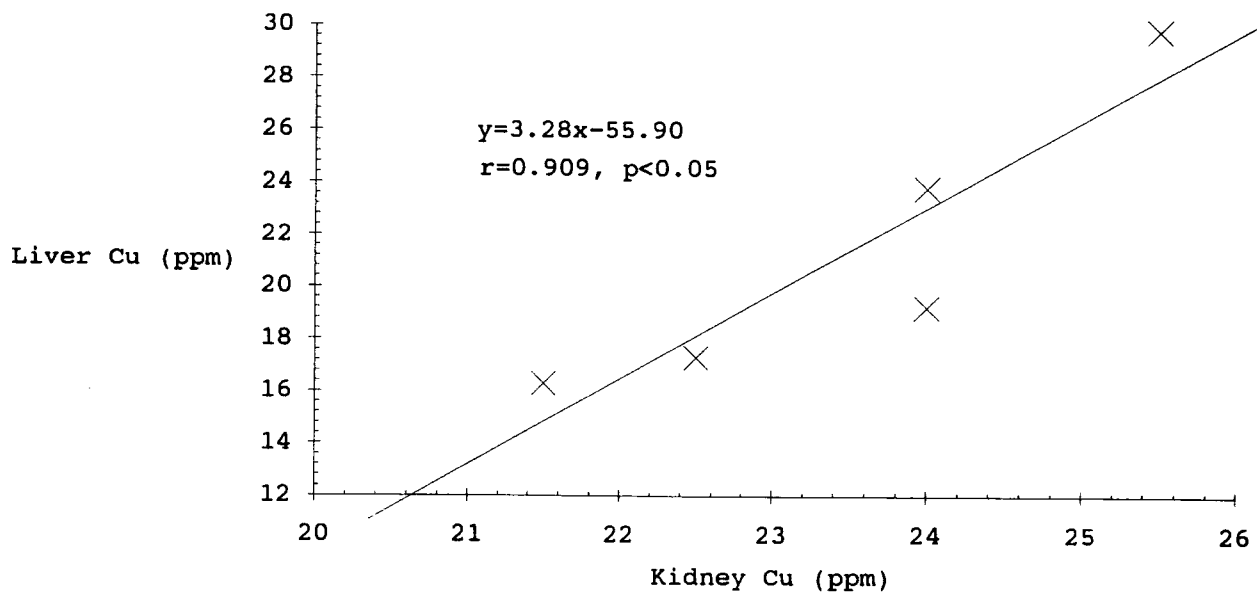


Figure 2.23. Correlations between Pb concentrations in Grey Plover kidney and liver tissues

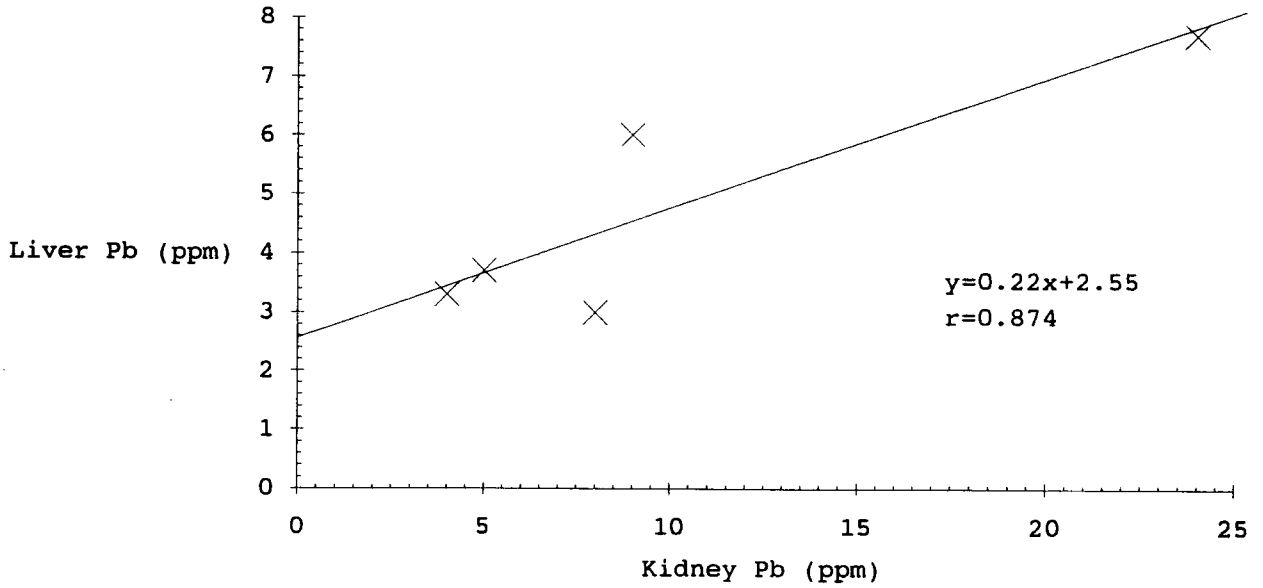


Figure 2.24. Correlations between Zn concentrations in Grey Plover kidney and liver tissues

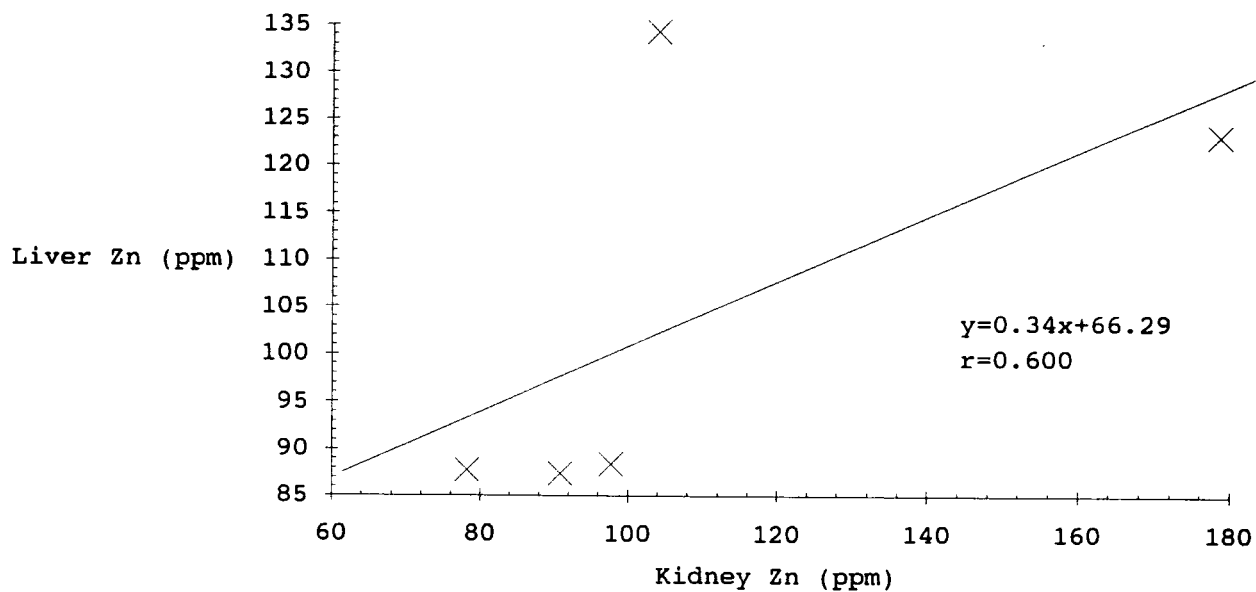


Table 2.18. Comparisons between metal concentrations in paired kidney and liver tissue of Grey Plover.

n	Mann-Whitney test, Kidney v. Liver			
	Cadmium	Copper	Lead	Zinc
5	36.0°	33.0	36.0°	29.0
Correlation Coefficients, Kidney v. Liver				
5	0.984**	0.909*	0.874	0.600

°p<0.10 \*p<0.05; \*\*p<0.01

2.5.1. Seasonal Variations of Metal Concentrations in Grey Plover Kidney and Liver Tissues

There were data from small numbers of both adult and juvenile Grey Plovers captured in five periods: mid- and late winter in January and March; early returning adults in September; early returning juveniles in October and November; and midwinter in December. The concentrations of metals in kidney and livers are listed in Table 2.19. Comparisons between months are listed in Table 2.20.

Table 2.19. Monthly mean heavy metal concentrations (ppm dry mass) in Grey Plover kidneys and livers.

Month	Number of samples	Number of birds	Concentrations ± S.E. (median in parentheses)			
			Cadmium	Copper	Lead	Zinc
			Kidneys			
Sep & Oct	4	4	3.6 ± 2.15 (1.5)	22.1 ± 0.65 (22.0)	6.3 ± 0.9 (6.5)	95.9 ± 6.97 (94.3)
November	2	2	2.8 ± 1.24	24.8 ± 0.53	16.5 ± 5.3	141.3 ± 26.38

Table 2.19. Continued

Month	n <sub>s</sub>	n <sub>b</sub>	Livers			
			Cd	Cu	Pb	Zn
Jan & Mar 8	3		1.1 ± 0.43 (1.0)	26.2 ± 2.38 (28.5)	3.9 ± 0.9 (3.0)	109.8 ± 6.21 (111.9)
September 9	4		0.9 ± 0.28 (0.8)	18.7 ± 1.09 (18.3)	4.0 ± 0.6 (3.5)	89.8 ± 1.67 (88.1)
October 8	6		0.6 ± 0.06 (0.5)	23.2 ± 1.22 (23.5)	2.8 ± 0.3 (2.8)	125.8 ± 1.97 (125.1)
November 8	3		0.7 ± 0.20 (0.8)	24.9 ± 2.08 (23.8)	5.7 ± 1.0 (6.0)	119.3 ± 8.15 (123.2)
December 10	4		0.5 ± 0.15 (0.4)	23.0 ± 1.33 (22.7)	3.0 ± 0.5 (3.0)	115.9 ± 3.93 (118.8)

Table 2.20. Monthly differences of metal concentrations in Grey Plover kidney and liver.

Months compared	n	Cadmium	Copper	Lead	Zinc
Kidneys - Mann-Whitney tests					
Sept & Oct/Nov	6	7.5	10.5	11.0°	10.0
Livers - Kruskal-Wallis all-groups test					
All months	20	1.363	6.665	6.260	12.399*
Livers - Kruskal-Wallis protected pairwise test					
Sept/Oct	10				5.236**
Oct/Nov	9				0.683
Nov/Dec	7				1.078
Dec/Jan & Mar	7				0.589
Jan & Mar/Sept	7				2.126
Jan & Mar/Oct	8				2.483°
Jan & Mar/Nov	6				1.559
Sept/Nov	7				3.793*
Sept/Dec	8				2.933*
Oct/Dec	10				2.023

°p&lt;0.10 \*p&lt;0.05; \*\*p&lt;0.01

In kidneys, birds captured in November had significantly higher lead concentrations than birds captured in September.

In livers, zinc concentrations varied significantly between months. Concentrations of zinc in September were significantly lower than all other months for which there were data (see Figures 2.25 - 2.28).

Figure 2.25. Monthly concentrations of cadmium in Grey Plover kidney and liver samples

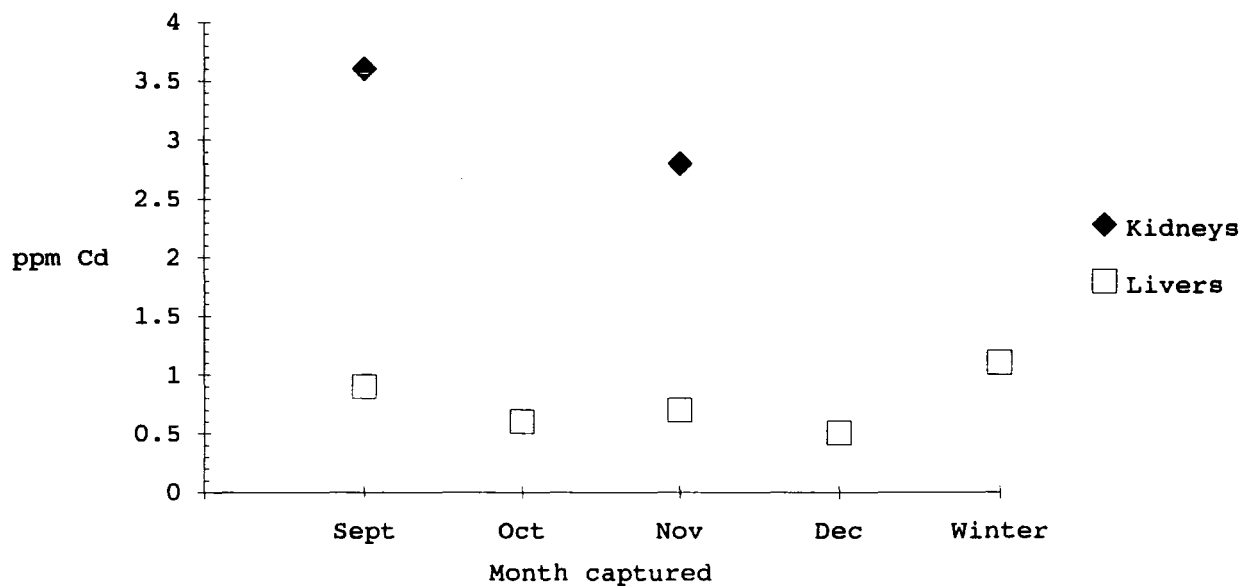


Figure 2.26. Monthly concentrations of copper in Grey Plover kidney and liver samples

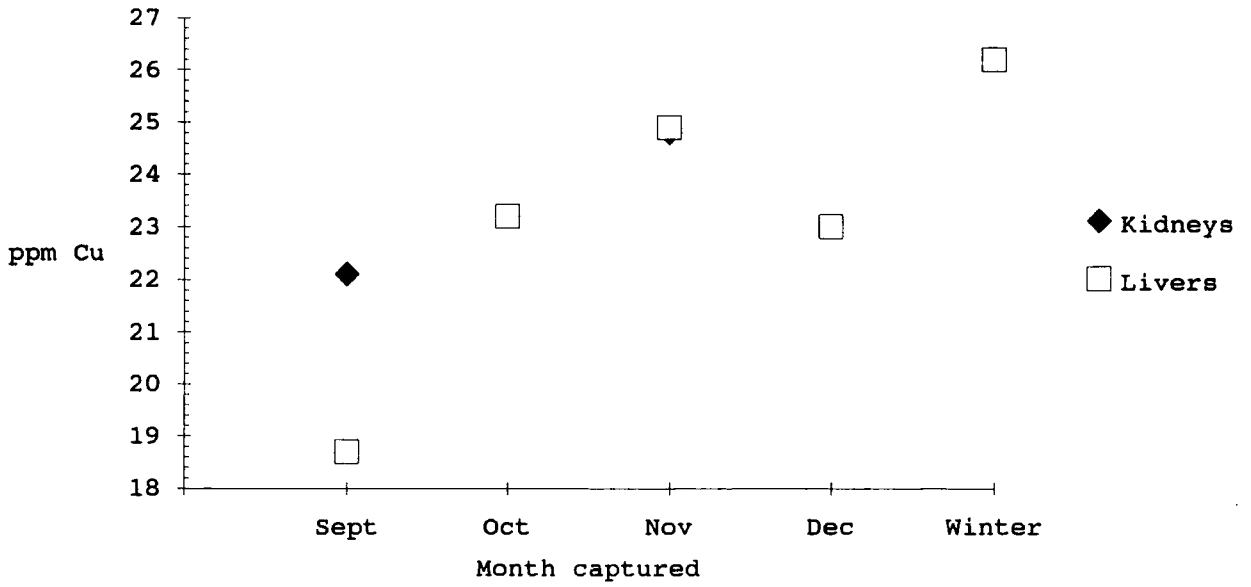
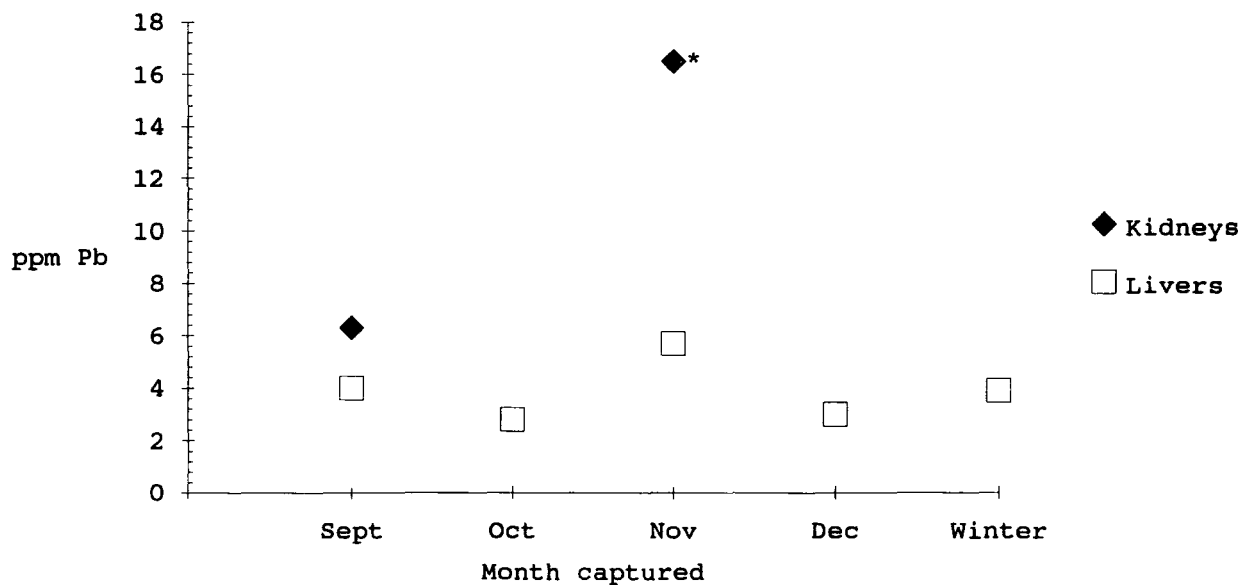
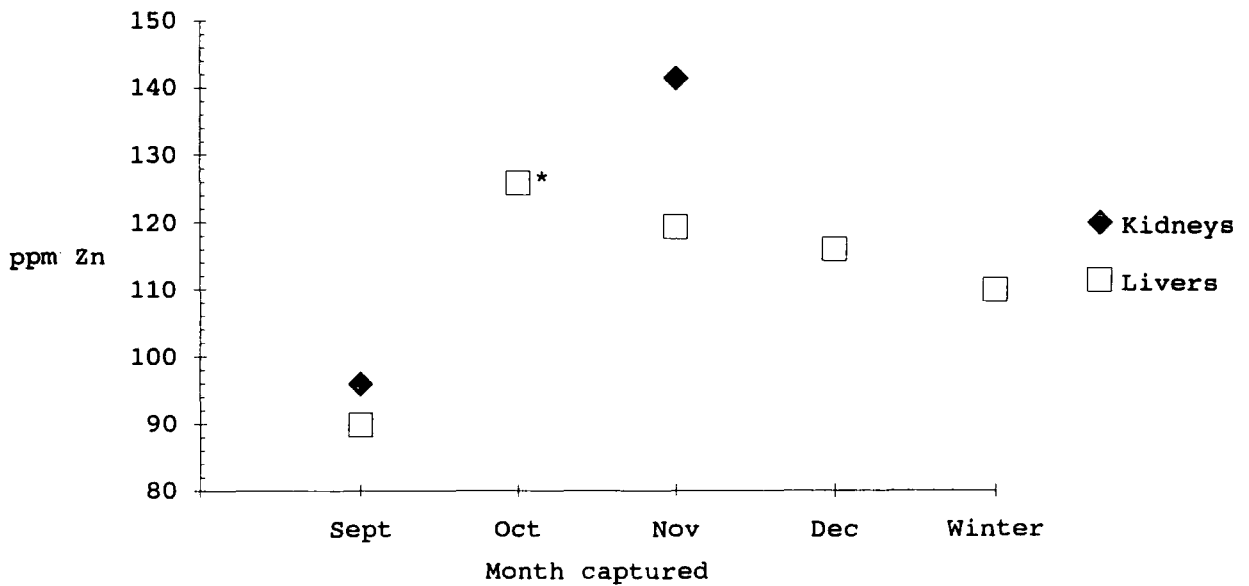


Figure 2.27. Monthly concentrations of lead in Grey Plover kidney and liver samples



\*Indicates that metal concentration in that month were significantly different from metal concentrations in the previous measurement period.

Figure 2.28. Monthly concentrations of zinc in Grey Plover kidney and liver samples



\*Indicates that metal concentration in that month were significantly different from metal concentrations in the previous measurement period.

Correlations between metals in Grey Plover kidney and liver samples are listed in Table 2.21.

Table 2.21. Seasonal correlation between metal concentrations in Grey Plover kidney and liver samples.

	n	Metals correlated, correlation coefficient					
		Cd/Cu	Cd/Pb	Cd/Zn	Cu/Pb	Cu/Zn	Pb/Zn
Kidneys							
Autumn <sup>1</sup>	6	-0.277	-0.429	-0.353	0.655	0.463	0.968**
<sup>1</sup> September, October & November							
Livers							
Total	26	-0.019	0.385*	-0.100	0.397*	0.540**	0.027
Jan & March	3	-0.055	0.930	0.434	0.317	0.876	0.736
September	4	-0.164	0.171	0.061	0.929	0.878	0.958*
October	6	0.568	0.808	0.277	0.252	0.451	0.149
November	3	-0.857	-0.624	0.046	0.949	0.476	0.752
December	4	-0.152	0.487	0.334	0.000	-0.113	-0.655

\* $p < 0.05$ ; \*\* $p < 0.01$

In kidneys, there was a significant positive correlation between lead and zinc concentrations.

In total livers, there were significant positive correlations only between cadmium and lead, copper and lead, and copper and zinc. There was also a significant correlation between liver lead and zinc concentrations in September, but in 30 comparisons one would be expected to be significant at the 5% level by chance (see Figures 2.29 - 2.30).

Results of tests for differences between sexes are listed in Table 2.22.

Table 2.22. Differences in metal concentrations between sexes, Grey Plover kidney and liver samples,

	n		Mann-Whitney test, male/female			
	♂	♀	Cadmium	Copper	Lead	Zinc
Kidneys						
Sep, Oct & Nov	3	2	7.0	9.0°	7.0	5.0
Livers						
Total	11	5	45.5	37.0	43.0	33.0
Sep & Oct	7	3	13.5	15.5	15.0	13.0
Nov & Dec	4	2	9.5	5.0	7.0	4.0

° $p < 0.10$

The only significant difference in 16 comparisons between sexes was in copper concentrations in kidneys, male copper concentrations being significantly lower than females. This could have been a chance result at the low level of significance found.

Figure 2.29. Correlations between Pb and Zn concentrations in Grey Plover kidney tissues, autumn

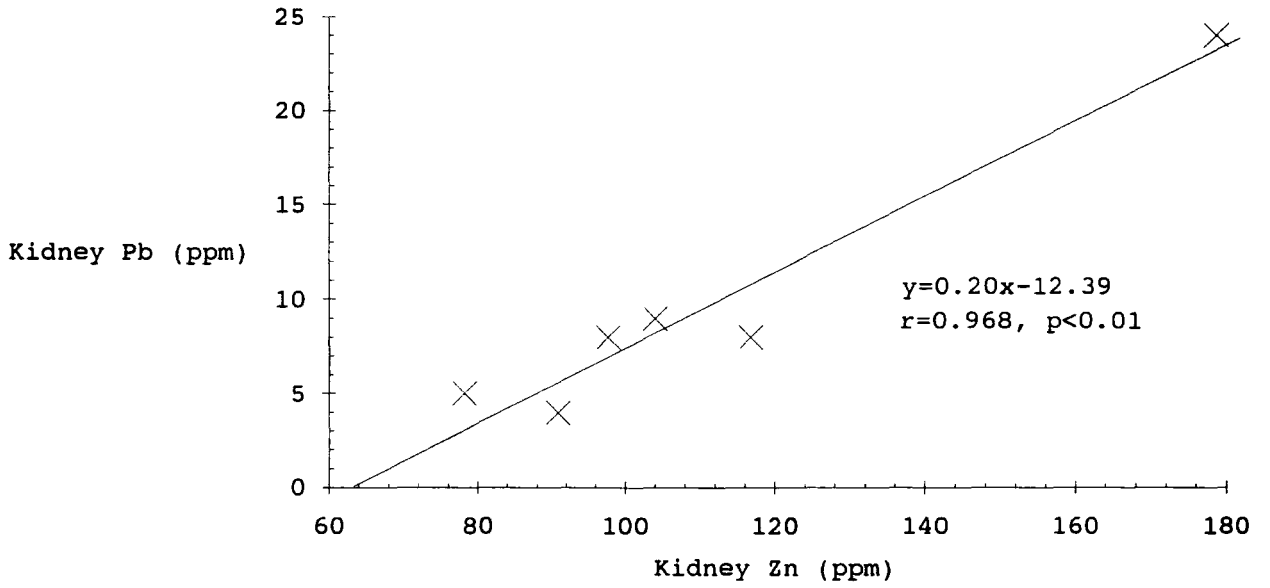
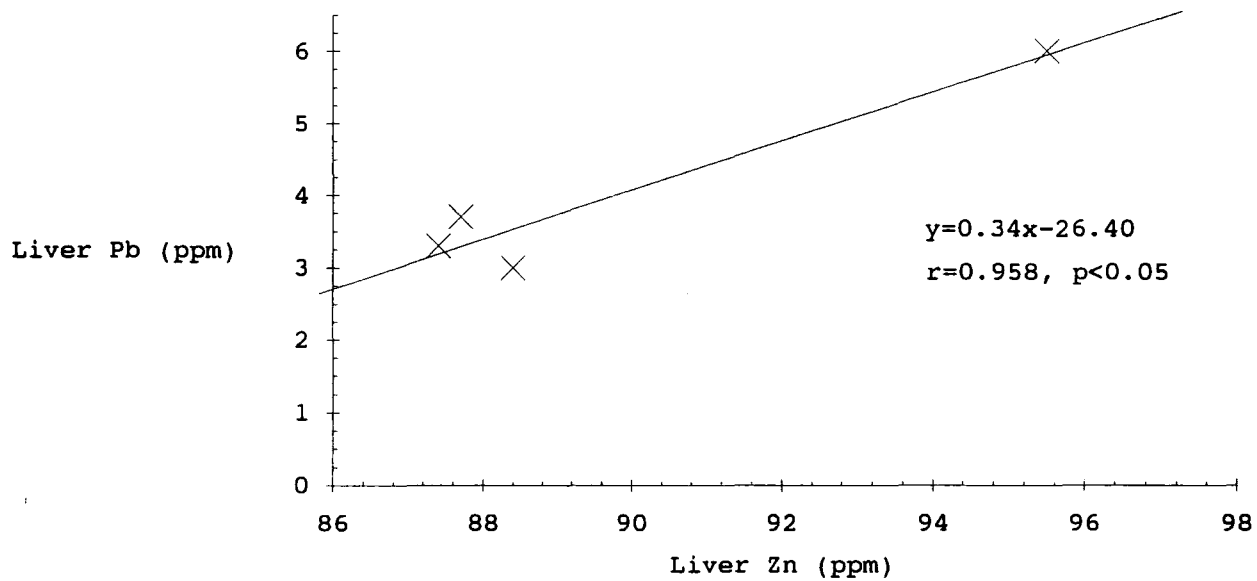


Figure 2.30. Correlations between Pb and Zn concentrations in Grey Plover liver samples, September



## 2.6. Interspecific Comparisons

Although all three species feed on the same estuary, several parameters that influence metal concentrations vary amongst the respective species. Each uses a different part of the estuary, and so may be exposed to different degrees of habitat contamination; these lead to differences in contamination of their respective prey, as discussed in section 2.2. There are also physiological differences between the species - they have differing daily energy requirements, as will be discussed in Chapter 3, as well as (perhaps) differences in how efficiently that energy is assimilated.

Only a small number of adult birds of each species were captured in the same month, and only liver samples were available from those birds. Although the month (September) was the same for the three species, it is difficult to be sure the birds were in the same physiological state. Both Sanderling and Ringed Plover arrive and begin post-breeding moult in August at Teesside. A few of the earliest returning adult Grey Plover also arrive in August and moult at Teesmouth, but most arrive in September and some do not stay long. Thus it may well be that the September-captured Grey Plover had only just arrived in Teesmouth and had not fed extensively on metal-contaminated prey at Teesmouth. Two Sanderling, 13 Ringed Plover and three Grey Plover held the mean and median concentrations of heavy metals in their livers in September summarized in Table 2.23.

Table 2.23. Heavy metal concentrations (ppm dry mass) in adult Sanderling, Ringed Plover and Grey Plover livers captured in September

	Number of samples	Number of birds	Concentrations ± Standard Error (median in parentheses)			
			Cadmium	Copper	Lead	Zinc
Sanderling	2	2	1.3	22.5	3.0	133.8
Grey Plover	15	13	0.7 ± 0.10 (0.5)	25.0 ± 1.43 (23.0)	6.7 ± 0.59 (6.5)	115.2 ± 4.15 (115.8)
Ringed Plover	7	3	1.1 ± 0.28 (1.0)	19.2 ± 1.34 (19.2)	4.3 ± 0.69 (3.7)	90.2 ± 2.16 (87.7)

Table 2.24 details the results of the Kruskal-Wallis test for differences between species in metal concentrations in livers of adults captured in September.

Table 2.24. Differences between metal concentrations in adult Sanderling, Ringed Plover and Grey Plover liver samples from birds captured in September

	Kruskal-Wallis all-groups test				
	n	Cadmium	Copper	Lead	Zinc
Sanderling/Grey/Ringed	18	3.487	4.886°	6.209*	7.518*
	Kruskal-Wallis protected pairwise test				
	n	Cadmium	Copper	Lead	Zinc
Sanderling/Ringed	15		0.598	2.370	1.233
Sanderling/Grey	5		1.162	0.627	3.034°
Ringed/Grey	16		2.365	1.917	2.861

°p<0.10; \*p<0.05

Zinc concentrations were significantly lower at the 10% level in livers of Grey Plover than in Sanderling. Lower levels of zinc in Grey Plover may also indicate that the Grey Plovers were recent

arrivals at Teesside and had only just gone through moult, because tissue concentrations of zinc are known to decrease after moult in other shorebirds, such as Dunlin and Knot (Evans et al., 1987; Ward, 1979).

## 2.7. Discussion

Metal concentrations found in one of the three shorebird species are not directly comparable to those in the other two, except in livers collected in September, assuming the birds were in the same physiological state during that month. However, the ratios of concentrations of metals in kidneys as compared to livers can be compared in other months (see Tables 2.4, 2.11 & 2.17). In all three species, significant correlations and high ratios were found between cadmium concentrations in kidneys and livers, with levels of cadmium being significantly higher in kidneys (see Tables 2.5, 2.12 & 2.18). This selective retention is perhaps due to binding of cadmium to metallothionein (discussed below), which is stored in kidneys (Hutton & Goodman, 1980; Friberg et al., 1986). A similar relationship was found in many shorebirds and Herring Gulls (Evans & Moon, 1981; Evans et al., 1987; Hutton, 1981; Nicholson, 1980; Stock et al., 1989).

Metallothioneins are low molecular-weight proteins that bind with certain heavy metals. Chelation of cadmium to metallothioneins probably prevents cadmium toxicity (Hutton & Goodman, 1980). Cadmium, zinc and mercury ingestion may induce

metallothionein synthesis in the liver. Metallothionein-bound cadmium is filtered through the kidneys, where the bond is broken down, which in turn stimulates new metallothionein production. In human kidneys, increased cadmium concentrations are accompanied by increased zinc concentrations. This is thought to be due to metallothionein stored in kidneys, which contains equimolar amounts of both metals (Friberg, et al., 1986). The toxic effects of cadmium apparently occur when the binding capacity of this new metallothionein is exceeded (Friberg, et al., 1986; Nicholson, 1981).

A parallel increase of cadmium with zinc in the shorebirds I studied might be expected, as it has been observed in kidneys of Oystercatcher, Herring Gull and Great Skua by Hutton (1981), and in other seabirds, pigeons, rats, horses and humans (Hutton & Goodman 1980, and Friberg et al., 1986).

Zinc is thought to have a protective influence against cadmium toxicity. Cadmium exerts toxic effects possibly through the replacement of zinc by cadmium in zinc metallo-enzymes (Friberg, et al., 1986). Nicholson (1981) suggests that the positive correlation between zinc and cadmium in Herring Gulls guards against the possible toxic effects of low level cadmium. The protective influence of zinc is thought to be partly related to competition for ligand sites on target tissues, and partly related the induction of enhanced synthesis of metallothioneins (Hutton & Goodman, 1980).

Hutton & Goodman (1980) report co-accumulation of zinc with

lead in bone tissue of urban pigeons. Evans & Moon (1981) found that in Curlew, Redshank, Knot and Dunlin, those species with the highest concentrations of both zinc and cadmium had the lowest concentrations of lead, and vice versa. Ward (1979) reported that changes in mercury and cadmium concentrations paralleled seasonal changes in zinc concentrations in livers of Dunlin and Knot.

The relationship between zinc and lead toxicity is less clear. Zinc has been shown to reduce tissue lead concentrations and to reactivate lead-inhibited ALA-D (Jaworski, 1978; Hutton & Goodman, 1980). Hutton & Goodman (1980) suggest that a competitive interaction between zinc and lead in bones of pigeons may arise during egg formation, when there is increased alkaline phosphatase activity, a zinc metallo-enzyme known to be inhibited by lead.

While there are examples of correlations between zinc/cadmium concentrations and zinc/lead concentrations, there was no consistent pattern (see Tables 2.8, 2.15, & 2.21). In both cases, depending on tissue (liver or kidney) and season, there were both negative and positive correlations. This could be the result of the small number of samples taken from each month, because half of the significant correlations were found when samples from all months were lumped together. Indeed, there were examples of correlations between cadmium/copper, cadmium/lead, and copper/lead, again with no apparent pattern. The most frequent significant positive correlations were found between the two essential metals, copper and zinc, which would seem to indicate that copper and zinc cycles might be similar.

A hypothetical annual cycle for liver zinc concentrations in shorebirds predicts two peaks per year; one in late winter/early spring, prior to pre-nuptial moult, the other in late summer prior to post-nuptial moult (Ward, 1979). Females could have a third peak early in summer, prior to egg laying. After moult, zinc concentrations drop to lows in July and December. Although there are not data for every month of the year for any species in this study, and no data during the breeding season, this hypothetical pattern can be superimposed on and is not inconsistent with the data for all three species (see Figure 2.31).

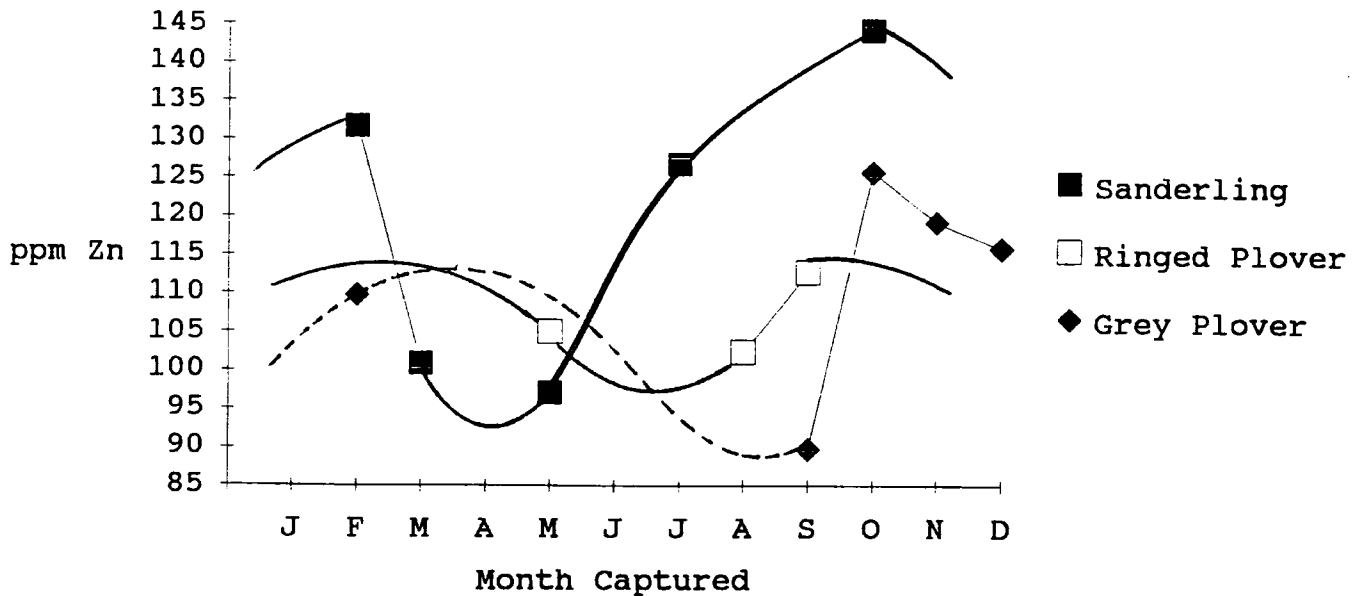
Sanderling was the only species for which there were large enough numbers of kidneys to provide samples from more than two months. The pattern of zinc concentrations in Sanderling kidneys is similar to Sanderling livers; peaks in March and Autumn, and a trough in the summer.

Sanderling was also the only species for which there was a significant increase in cadmium concentrations from arrival in the autumn to departure in spring, indicating that cadmium might be accumulated while at Teesside. However, cadmium concentrations tended to decline in the autumn in the other two species.

Ages of many birds were determined at capture. However, only for Ringed Plover livers were sufficient numbers available from the same month (August) to make a comparison between metal levels in different age classes. Juveniles had significantly higher concentrations of lead than adults (Mann-Whitney test: 24.0,  $p < 0.10$ ). However, adults in this test were captured six years

earlier than the juveniles, so the difference might be due to a factor peculiar to year, rather than (or as well as) to age.

Figure 2.31. Hypothetical annual cycles of zinc concentrations in Sanderling, Ringed Plover and Grey Plover livers



## **Chapter 3 DAILY FOOD REQUIREMENTS AND CALCULATED METAL INTAKE AND RETENTION.**

### **3.1. Introduction**

Apart from Evans & Moon (1981), little information is available on the proportions of the metal content of different invertebrates species that are retained by their predators in estuarine ecosystems, but similar studies exist for terrestrial situations, e.g. roadsides (Williamson, 1979).

From the measured metal concentrations in the invertebrate prey of Sanderling, Ringed Plover, and Grey Plover (Chapter 2), an estimate can be made of the amount of each metal consumed by these birds during the time they are at Teesmouth, if their mean daily food requirements are known. Metal intakes have been estimated from the published information on the birds' daily energy requirements, measurement of the energy content of the prey species, and hence calculations of the birds' daily food intakes.

### **3.2. Methods and Results**

The calorific values of thirteen samples of Nerine, mixed small Crustacea, Corophium, and Nereis were determined with a miniature bomb calorimeter (Phillipson, 1964). Results of these determinations are summarized in Table 3.1. (Complete results are listed in Appendix 5.)

Table 3.1. Mean calorific value (per gram dry mass) of invertebrate foods of shorebirds at Teesmouth.

Prey	Number of samples	kcal/g $\pm$ S.E.	kJ/g $\pm$ S.E.
<u>Nerine</u>	4	4.36 $\pm$ 0.08	18.19 $\pm$ 0.34
<u>Crustacea</u> *	2	3.37 $\pm$ 0.12	14.07 $\pm$ 0.53
<u>Corophium</u>	2	2.55 $\pm$ 0.13	10.63 $\pm$ 0.56
<u>Nereis</u>	5	4.87 $\pm$ 0.31	20.84 $\pm$ 0.25

\*Species included: Bathyporeia pelagica, Eurydice Pulchra and Haustorius arenarius.

Estimates taken from the literature of mean daily energy requirements (DER) of the birds are given in Table 3.2.

Table 3.2. Estimated mean daily energy requirements of Sanderling, Ringed Plover, and Grey Plover outside the migration season.

Species	Locality	DER (kcal)	Period	Authority
Sanderling	New Jersey	47.85	winter	Castro 1988
Ringed Plover	Lindisfarne	45.65	August	Pienkowski 1982
Grey Plover	Lindisfarne	98.66	September	Pienkowski 1982

Using the doubly-labeled water method, Castro (1988) estimated that Sanderling in New Jersey, U.S.A., with a mean wet body mass of 50.4 g required  $47.85 \pm 2.99$  SE (n=10) kcals of food per day in winter.

Pienkowski (1982) studied Ringed and Grey Plover at Lindisfarne, Northumberland, about 160 km north of Teesmouth. He estimated the Plovers' daily food intake through direct observation of birds feeding on the sandflats at Lindisfarne during August and September, when the Plovers were not preparing for migration.

During those months there was almost no night feeding and the Plovers probably took all they needed during the day, so Pienkowski suggested that estimates for these months were reasonable ones for total daily intake. These figures do not, however, allow for extra energy requirements during cold weather in winter.

Estimates of daily food intakes needed to provide the energy required have been made using the formula:

$$\text{Mean Daily Food Intake (g dry mass)} = \frac{\text{Mean Daily Energy Requirement (kcal)}}{0.85 \times \text{Calorific Value of Prey (kcal/g dry mass)}}$$

where 0.85 is the proportion of food energy that is assimilated by shorebirds (a mean of figures calculated by Evans et al., 1979). Results are given in Table 3.3.

Table 3.3. Estimated daily food intake by Sanderling, Ringed Plover and Grey Plover at Teesmouth.

Bird species	Prey	Mean daily food intake (g dry mass)
Sanderling	<u>Nerine</u>	12.9
	Crustacea	16.7
Ringed Plover	<u>Corophium</u>	21.1
Grey Plover	<u>Nereis</u>	23.8

From these, mean daily metal intakes have been calculated by the formula:

$$\text{Mean Daily Metal Intake } (\mu\text{g}) = \left( \text{Mean Daily Food Intake (g dry mass)} \right) \times \left( \text{Metal Concentration in Prey Species } (\mu\text{g/g dry mass}) \right)$$

Metal concentrations in prey species have been taken from Table 2.1. Results are given in Table 3.4.

Table 3.4. Estimated daily metal intake by Sanderling, Ringed Plover and Grey Plover at Teesmouth.

Bird species	Prey	Mean daily metal intake ( $\mu\text{g}$ )			
		Cd	Cu	Pb	Zn
Sanderling	<u>Nerine</u>	14.2	565	182	3,080
	Crustacea	21.7	1,300	339	3,150
	mean	18.0	934	261	3,110
Ringed Plover	<u>Corophium</u>	21.1	1,450	703	2,350
Grey Plover	<u>Nereis</u>	11.9	933	409	4,100

These results were then adjusted to provide estimates of amounts ingested over the seven to ten month period the birds are at Teesside during the non-breeding season:

$$\text{Total Metal Intake at Teesmouth } (\mu\text{g}) = \left( \frac{\text{Mean Daily Metal Intake } (\mu\text{g})}{\text{Duration of Stay at Teesmouth}} \right) \times \left( \text{Duration of Stay at Teesmouth} \right)$$

Results are given in Table 3.5.

Table 3.5. Estimated intake of heavy metals by Sanderling, Ringed Plover, and Grey Plover during the non-breeding season at Teesmouth

Bird species	Prey	Mass of metal ingested ( $\mu\text{g}$ )			
		Cd	Cu	Pb	Zn
Sanderling, ten months (August - May inclusive)					
	<u>Nerine</u>	4,260	170,000	54,600	924,000
	Crustacea	6,510	390,000	102,000	945,000
	mean	5,400	280,000	78,300	933,000

Table 3.5. Continued.

Ringed Plover, nine months (March - November inclusive)

<u>Corophium</u>	5,700	392,000	190,000	635,000
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Grey Plover, seven months (September - March inclusive)

<u>Nereis</u>	2,500	196,000	85,900	861,000
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Detailed explanations are given in the following sections.

### 3.3. Estimated Amounts of Metal Retained by Sanderling, Ringed Plover, and Grey Plover

#### 3.3.1. Sanderling

Applying Castro's (1988) results to Teesmouth, Sanderling require each day as little food as:

$$\frac{47.85 \text{ kcal}}{(0.85) \times (4.36 \text{ kcal/g dry mass Nerine})} = 12.9 \text{ g dry mass Nerine}$$

or as much as 16.7 g dry mass of Crustacea each day (Nerine is the more calorifically valuable prey). If Sanderling consumed only Nerine, they would ingest 14.2  $\mu\text{g}$  cadmium per day, since

$$\text{Daily Metal Intake} = (12.9 \text{ g Nerine}) \times (1.1 \text{ } \mu\text{g/g Cd}) = 14.2 \text{ } \mu\text{g Cd}$$

Assuming that most Sanderling stay at Teesmouth from early August to late May, a period of about ten months, a Sanderling of mean wet body mass 50.4 g would consume between

$$(300 \text{ days}) \times (14.2 \text{ } \mu\text{g Cd/day}) \approx 4,260 \text{ } \mu\text{g Cd}$$

(as Nerine) and 6,500  $\mu\text{g}$  (as Crustacea) of cadmium. From field observations (Breary, 1982), it is known that the diet of Sanderling at Teesmouth is a mix of both Crustaceans and Nerine. Assuming a 50:50 mix of Crustaceans and Nerine, the mean estimated intake would be 5,400  $\mu\text{g}$  cadmium during the non-breeding season. If there were 100% retention of cadmium, there would be an annual increase of

$$\frac{5,400 \mu\text{g Cd}}{50.4 \text{ g (wet mass of bird)}} = 107 \text{ ppm Cd } (\mu\text{g/g wet mass})$$

if it were to be distributed evenly throughout the body. The mean level of cadmium found in dry kidney tissues of Sanderling was  $16.3 \pm 2.64$  ppm, and in dry livers  $3.2 \pm 0.34$  ppm (see Table 2.4.). However, because these figures represent ppm dry mass, they must be adjusted to be compared to the wet mass of the birds. Di Giulio & Scanlon (1984) determined that in Mallard drakes the average dry mass/wet mass percentages were 32.7 for livers and 23.4 for kidneys. Metal concentrations in Sanderling tissues adjusted to ppm wet mass are listed in Table 3.6.

Table 3.6. Mean heavy metal concentrations in Sanderling kidneys and livers. Concentrations expressed as parts-per-million ( $\mu\text{g/g}$  wet mass).

	cadmium	copper	lead	zinc
Kidneys	3.8	5.3	2.4	40.5
Livers	1.0	8.2	2.1	37.6

If the concentration of cadmium measured in the birds' tissues is expressed as a percent of the estimated annual increase, the result is the maximum annual percent retention (MAPR). The MAPR is the largest possible proportion of metal retained in one season at Teesmouth. The actual percent retention is many times lower. Because most birds in this study were adults, concentrations of metals measured in their livers and kidneys probably result from retention over several years of feeding rather than after only one winter at Teesmouth. Further, metal concentrations summarized in Tables 2.4, 2.17 & 3.8 were obtained from birds captured at different times during the year, not simply the end of the winter period.

Mean MAPR in Sanderling for cadmium was 3.5% in kidneys and 0.9% in livers. Table 3.7 details mean MAPR of the four metals in kidney and liver tissues. Table v.5 in Appendix 5 lists the ranges of MAPR for all four metals.

Table 3.7. Mean expected annual increase in concentrations and maximum annual percent retention of heavy metals in Sanderling.

Metal	Mean expected annual increase in concentrations (ppm wet mass)	Mean maximum annual percent retention	
		kidney	liver
Cd	107	3.6	0.9
Cu	5,560	0.1	0.1
Pb	1,550	0.2	0.1
Zn	18,500	0.2	0.2

As would be expected from other studies of birds in which cadmium has been accumulated over time, a higher proportion of cadmium is retained than the other three metals. Cadmium appears to be retained more effectively in the kidney, while copper and zinc retention is similar in both tissues.

### 3.3.2. Ringed Plover

From Pienkowski's estimates, Ringed Plovers of mean wet body mass 72.4 g require 21.1 g dry mass of Corophium per day. A few resident Ringed Plover remain at Teesmouth through the winter, while others disperse or pass through on migrations to winter elsewhere. Assuming most resident birds (thus excluding migrants passing through) feed on the estuary for nine months (270 days), a Ringed Plover would consume 5,700 g dry mass of Corophium during that time. Table 3.5 lists the amount of each metal ingested during nine months at Teesside. Mean metal concentrations in resident birds, excluding migrants, are listed in Table 3.8. Metal concentrations adjusted to ppm wet mass are listed in Table 3.9.

Table 3.8. Mean heavy metal concentrations in resident Ringed Plover kidneys and livers. Concentrations expressed as parts-per-million ( $\mu\text{g}/\text{g}$  dry mass).

	Number of samples	Number of birds	Concentrations $\pm$ S.E. (median in parentheses)			
			cadmium	copper	lead	zinc
Kidneys	6	6	10.3 $\pm$ 3.16 (11.3)	31.1 $\pm$ 3.78 (29.5)	7.7 $\pm$ 1.4 (8.0)	129.7 $\pm$ 8.95 (129.8)
Livers	29	25	1.9 $\pm$ 0.50 (1.0)	28.3 $\pm$ 3.80 (23.0)	5.3 $\pm$ 0.5 (5.0)	106.5 $\pm$ 3.62 (102.8)

Table 3.9. Mean heavy metal concentrations in Ringed Plover kidneys and livers. Concentrations expressed as parts-per-million ( $\mu\text{g}/\text{g}$  wet mass).

	cadmium	copper	lead	zinc
Kidneys	2.4	7.3	1.8	30.3
Livers	0.6	9.3	1.7	34.8

The expected annual increase of metal concentrations and MAPR for Ringed Plover are listed in Table 3.10. Data for birds captured during spring migration and birds for which there is no date of capture have been excluded.

Table 3.10. Mean expected annual increase in concentrations and maximum annual percent retention of heavy metals in Ringed Plover.

Metal	Expected annual increase in concentrations (ppm wet mass)	Maximum annual percent retention	
		kidney	liver
Cd	79	3.0	0.7
Cu	5,410	0.1	0.2
Pb	2,620	0.1	0.1
Zn	8,770	0.3	0.4

As in Sanderling, a higher proportion of cadmium is retained than the other three metals, and it is stored at higher concentrations in kidney than in livers.

### 3.3.3. Grey Plover

Using Pienkowski's calculations, daily energy requirements for a Grey Plover with a wet body mass of 242.9 g is 98.66 kcal, which translates to 23.8 g dry mass of Nereis. Assuming Grey Plover feed on the estuary for seven months (210 days), from September to May, they would ingest 5000 g dry mass of Nereis. Estimated amount of metals ingested during seven months are listed in Table 3.5. (See Table 2.17 for heavy metal concentrations in Grey Plover.) Metal concentrations in Grey Plover tissues adjusted to ppm wet mass are listed in Table 3.11. Expected annual increase in concentrations and MAPR are listed in Table 3.12.

Table 3.11. Mean heavy metal concentrations in Grey Plover kidneys and livers. Concentrations expressed as parts-per-million ( $\mu\text{g}/\text{g}$  wet mass).

	cadmium	copper	lead	zinc
Kidneys	0.8	5.4	2.3	26.0
Livers	0.3	7.5	1.2	35.9

Table 3.12. Mean expected annual increase in concentrations and maximum annual percent retention of heavy metals in Grey Plover.

Metal	Expected annual increase in concentrations (ppm wet mass)	maximum annual percent retention	
		kidney	liver
Cd	10	8.0	3.0
Cu	807	0.7	0.9
Pb	354	0.6	0.3
Zn	3,540	0.7	1.0

As with Sanderling and Ringed Plover, a higher percentage of cadmium is retained than the other three metals.

Cadmium MAPR in kidneys was remarkably high. However, referring to cadmium concentrations found in the kidney tissues (Appendix 4, Table iv.5), there is a large range of concentrations. While the mean was 3.3 ppm ( $\mu\text{g}/\text{g}$  dry mass), the median was 1.5 ppm ( $\mu\text{g}/\text{g}$  dry mass) (equivalent to 0.8 and 0.4 ppm wet mass, respectively). If the median value is used instead of the mean, the MAPR in kidneys becomes 4.0%, which is similar to MAPR in the other two species.

### 3.4. Discussion

These calculations provide only the most general indication of actual metal retention rates, but clearly demonstrate that a very small proportion of ingested metals are retained. This is consistent with the findings of Evans & Moon (1981), who concluded that Curlews at Teesmouth retain much less than 1% of the metals they ingest.

Expected annual increases in concentrations of metals if all materials ingested had been retained were quite high, especially in Sanderling and Ringed Plover. Lead levels would be potentially lethal if there were retention of perhaps 2% in Sanderling and Ringed Plover. Bull et al. (1983) in their report on mortality in the Mersey Estuary of shorebirds and Black-headed Gulls found mean lead concentrations in liver of 21 ppm (wet mass) and 30.4 ppm in kidneys of dead Dunlin. However, a little more than half of the

lead was in the alkyl form, and Bull et al. specifically cite alkyl-leads for toxicity. At Teesmouth, most lead is thought to be inorganic, and not in the alkyl forms.

Evans et al. (1987) suggested two possible reasons for low retention of heavy metals in Curlew and other shorebirds: first, assuming that metals are digested along with the organic components of their food, low metal retention could have been due to efficient excretion of digested materials through urine; second, it is possible that metal granules in the prey may not be digestible, and are excreted in faeces.

Another path of metal excretion might be into feathers at moult. Because zinc is required for feather synthesis, a number of researchers have examined the possibility that toxic metals might accompany the zinc and become incorporated into feathers during moult. Zinc and mercury concentrations in livers have been found to be highest just before moult and lowest just after moult (Parslow, 1973; Osborn, 1979; Evans & Moon, 1981; Parslow et al., 1982). Goede (1988) found in Knot that mercury accumulated in the body was incorporated into keratin during feather formation at moult. Selenium and arsenic were also detected in some individuals (Goede, 1985). Goede & de Voogt (1985) found elevated lead concentrations in feather vanes, but suggest that it might have been partly the result of external contamination due to lead elimination from the salt glands. Although cadmium was found in measurable amounts in some feathers, it resulted from external contamination rather than excretion into growing feathers (Goede & de Voogt, 1985; Stock, et al., 1989).

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**Appendix 1 INVERTEBRATE DENSITIES ON  
COATHAM SANDS AND REDCAR SANDS.**

On 31 October, 1988, Gilbert Roberts and I undertook a survey of invertebrate densities on Coatham and Redcar Sands. The purpose of this survey was to relate Sanderling feeding areas to relevant invertebrate densities, and then to compare metal levels in Sanderling to metal levels in these invertebrates at the favoured feeding sites. We sampled six transects on Coatham Sands, and two on Redcar Sands. Eight stations were sampled per transect, at 30, 40, 50, 60, 70, 80, 100, and 150 metres below high water mark (HWM), with two samples taken at each station. On Coatham Sands, the transects began just north of the northern tip of Coatham Rocks (see fig. 1.1) and were spaced at intervals of about 500 metres to the Ducky. The transects at Redcar Sands were also about 500 metres apart. Samples were taken with a 10cm x 10cm x 10cm corer, returned to the University of Durham labs in polythene bags, where they were washed through a 30 mpi sieve. The animals retained were counted, and the mean densities calculated.

Table i.1. Mean Densities of  
Nerine cirratulus (<sup>number</sup>/<sub>sq. metre</sub>)

metres below HWM	North<----->South							
	Transect						Redcar Sands	
	Coatham Sands						8	7
	6	5	4	3	2	1		
30	-	-	-	-	-	-	-	-
40	-	-	-	-	-	-	150	150
50	-	-	-	-	200	-	-	650
60	-	-	350	100	4900	4050	1750	2900
70	-	-	1400	2600	300	250	1750	2759
80	-	-	650	4050	300	250	2400	300
100	350	100	50	250	150	-	1050	250
150	50	200	50	-	50	-	-	-

Table i.2. Mean Densities of  
Bathyporeia pelagica (<sup>number</sup>/<sub>sq. metre</sub>)

metres below HWM	North<----->South							
	Transect						Redcar Sands	
	Coatham Sands						8	7
	6	5	4	3	2	1		
30	-	-	-	-	-	-	-	-
40	50	50	-	-	-	-	300	1500
50	-	-	-	-	100	-	150	1950
60	-	-	350	-	200	200	400	650
70	500	50	1000	400	700	300	1700	550
80	2200	-	950	600	500	500	1150	700
100	3650	1300	800	1400	2500	500	2350	450
150	850	1550	700	1350	1000	700	1050	400

Table i.3. Mean Densities of Eurydice pulchra  
and Haustorius arenarius (<sup>number</sup>/sq. metre)

metres below HWM	North<----->South								
	Coatham Sands				Transect		Redcar Sands		
	6	5	4	3	2	1	8	7	
30	-	-	-	-	-	-	-	-	<u>Eurydice</u>
	-	-	-	-	-	-	-	-	<u>Haustorius</u>
40	-	-	-	-	-	-	-	-	<u>Eurydice</u>
	-	-	-	-	-	-	-	-	<u>Haustorius</u>
50	-	-	-	-	150	-	-	-	<u>Eurydice</u>
	-	-	-	-	250	-	100	-	<u>Haustorius</u>
60	-	-	50	150	200	200	-	-	<u>Eurydice</u>
	-	-	-	50	-	400	-	50	<u>Haustorius</u>
70	-	-	200	100	450	50	-	-	<u>Eurydice</u>
	-	-	250	100	-	100	50	-	<u>Haustorius</u>
80	-	-	100	100	430	-	-	-	<u>Eurydice</u>
	400	-	50	150	-	-	150	50	<u>Haustorius</u>
100	300	50	300	100	300	400	50	-	<u>Eurydice</u>
	250	400	-	-	-	-	-	-	<u>Haustorius</u>
150	-	450	-	300	100	50	-	-	<u>Eurydice</u>
	-	800	-	-	-	-	-	-	<u>Haustorius</u>

**Appendix 2 EXAMPLE AAS CALIBRATION CURVES  
FOR CADMIUM, COPPER, LEAD AND ZINC.**

Table ii.1. Example of calibration solutions (ppm) and AAS readings for cadmium.

Calibration solution (ppm)	AAS reading
0.2	53
0.4	80
0.6	122
0.8	166

Figure ii.1. Example calibration curve for cadmium

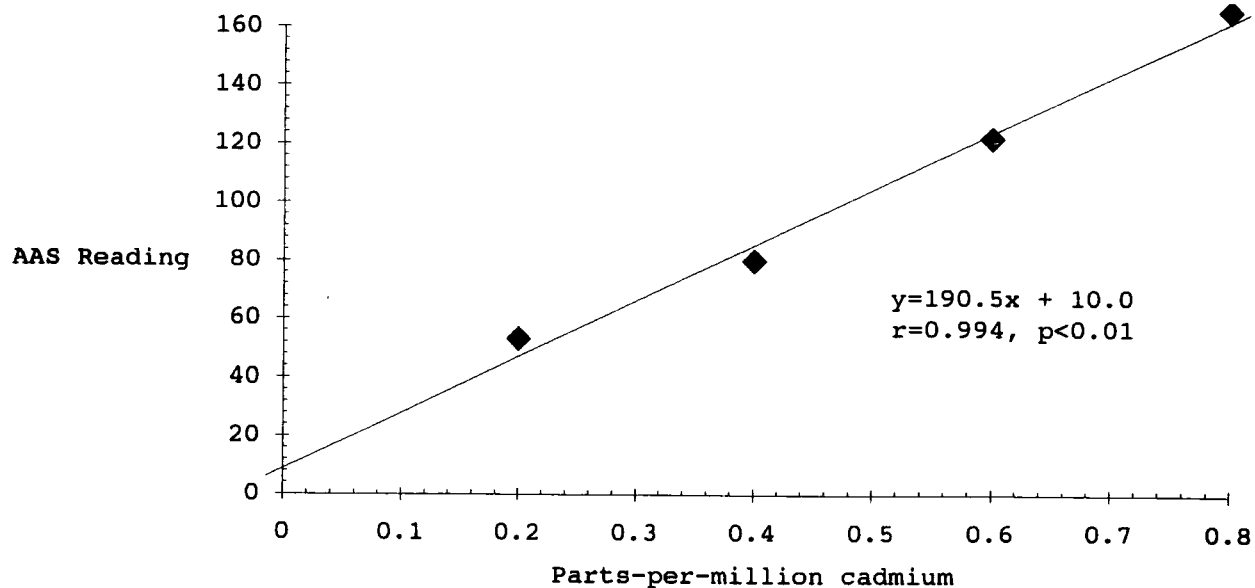


Table ii.2. Example of calibration solutions (ppm) and AAS readings for copper.

Calibration solution (ppm)	AAS reading
1	50
2	103
3	153
4	220
5	295

Figure ii.2. Example calibration curve for copper

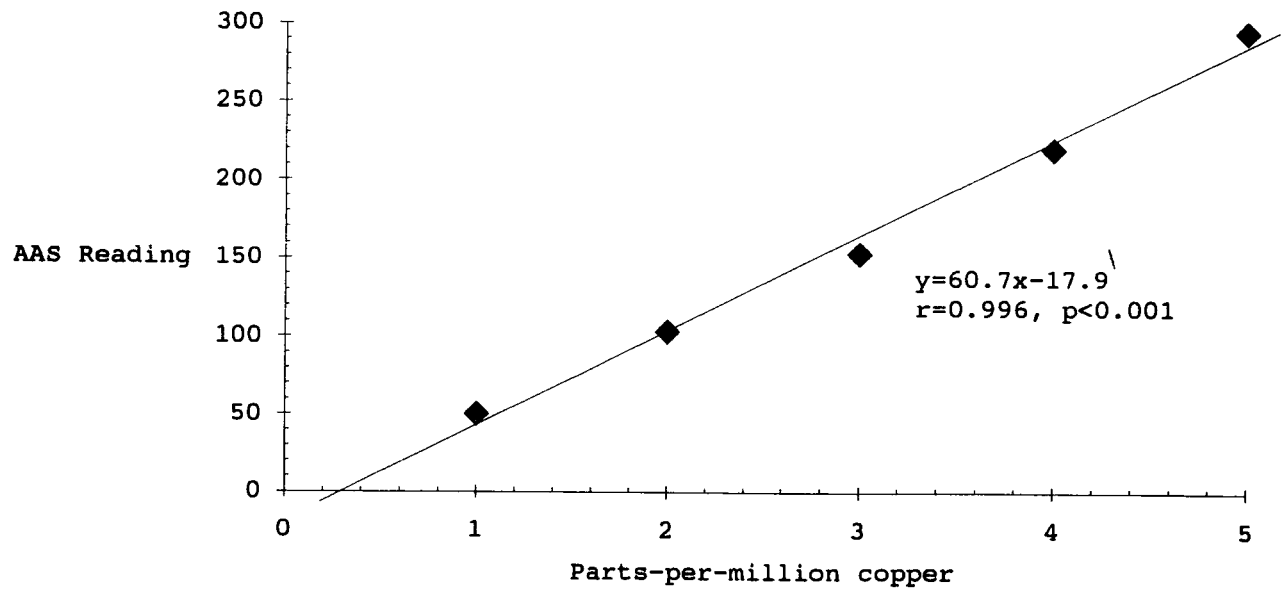


Table ii.3. Example of calibration solutions (ppm) and AAS readings for lead.

Calibration solution (ppm)	AAS reading
1	12
2	24
3	48
4	70
5	90

Figure ii.3. Example calibration curve for lead

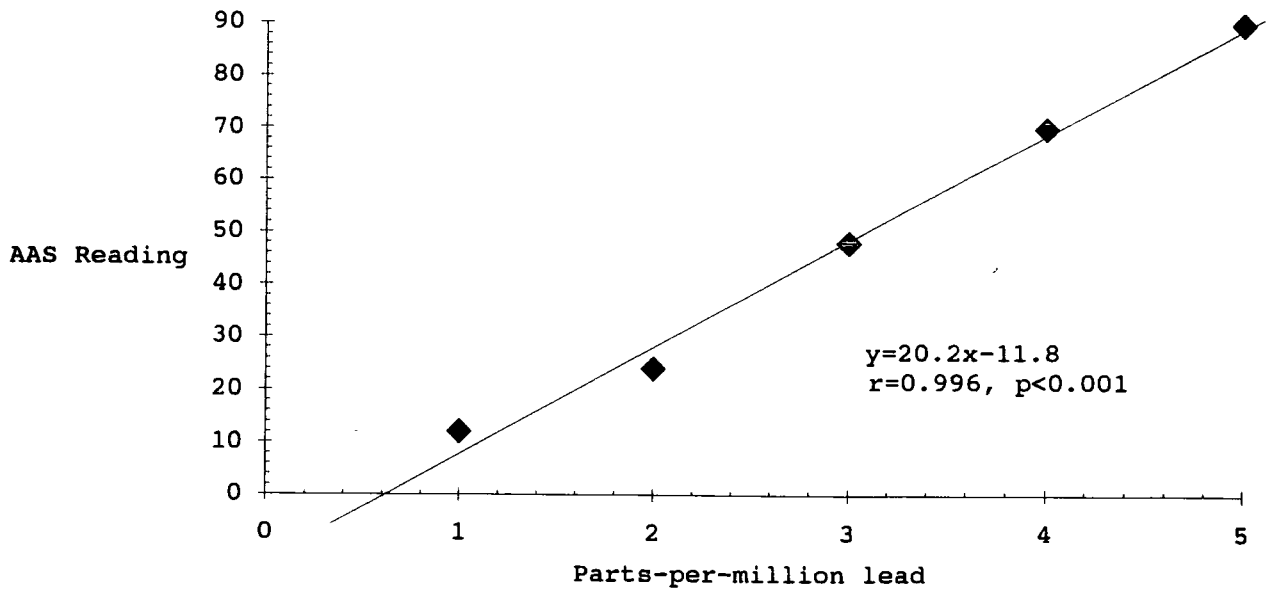
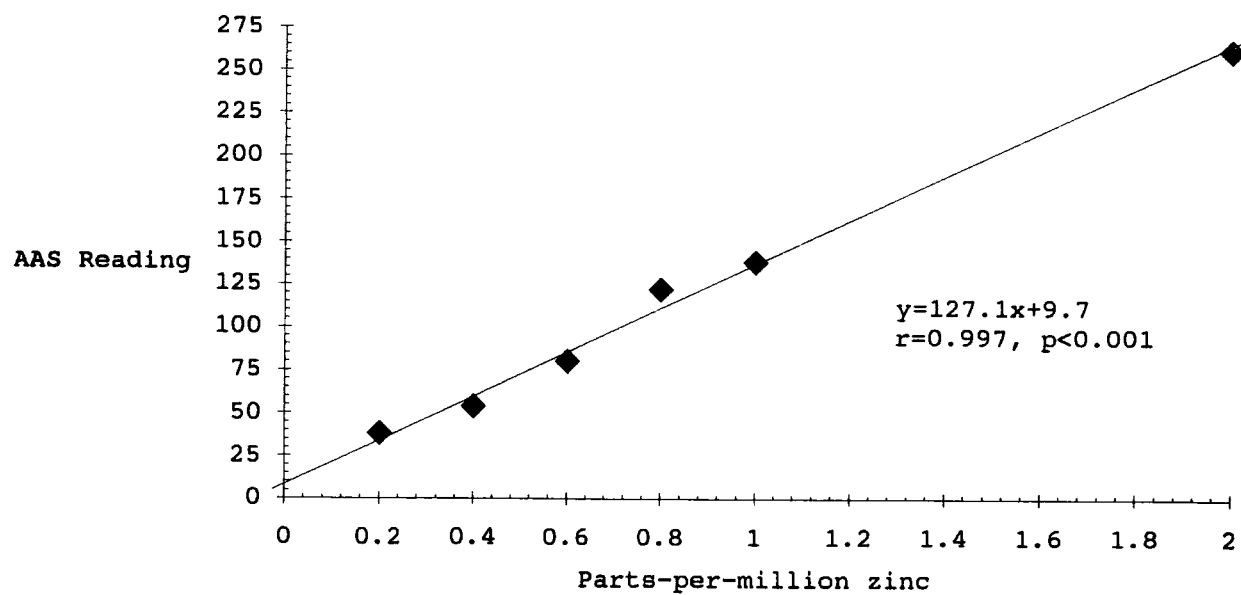


Table ii.4. Example of calibration solutions (ppm) and AAS readings for zinc.

Calibration solution (ppm)	AAS reading
0.2	38
0.4	54
0.6	80
0.8	122
1.0	138
2.0	262

Figure ii.4. Example calibration curve for zinc



**Appendix 3 INVERTEBRATE COLLECTION SITES AND ANALYTICAL RESULTS FROM ATOMIC ABSORPTION SPECTROPHOTOMETRY**

Key for Location  
of Collection:

C = Coatham Sands, ca. 1.5 km south  
of South Gare  
RC = Redcar Sands  
SS = Seal Sands

Table iii.1. Heavy Metals in Nerine cirratulus.

Size classes: M = Mixed  
A = <2.5cm  
B = 2.5-3.5cm  
C = >3.5cm

Size Class	Location & Date of Collection Dy/Mn/Yr		Dry Sample Mass (g)	Concentration ppm ( $\mu\text{g/g}$ )			Zinc
				Cadmium	Copper	Lead	
M	RC	13 03 89	0.980	1.0	40.0	32	282.2
A	C	14 12 88	0.683	1.0	45.5	9	299.2
A	RC	09 02 89	0.869	1.0	41.0	12	226.4
A	RC	13 03 89	0.530	1.0	53.0	30	301.2
B	RC	09 02 89	1.000	1.0	38.5	7	185.9
B	RC	09 02 89	0.583	1.5	45.5	9	199.7
B	RC	13 03 89	0.660	1.0	49.5	7	287.4
B	RC	13 03 89	0.640	1.0	49.0	7	267.2
C	RC	09 02 89	0.678	1.5	31.5	9	166.7
C	RC	09 02 89	0.884	1.5	41.0	8	194.7
C	RC	13 03 89	0.998	1.0	50.0	33	238.3
C	RC	13 03 89	1.018	1.0	41.5	6	213.1
Total Mean				1.1	43.8	14.1	238.5
Std. Dev.				0.2	5.8	10.3	45.5
Std. Error				0.06	1.67	3.0	13.13
Median				1.0	43.5	9.0	232.4
Mean of Size A				1.0	46.5	17.0	275.6
Std. Dev.				0.0	4.9	9.3	34.8
Std. Error				0.00	2.83	5.4	20.09
Median				1.0	45.5	12.0	299.2

Mean of Size B	1.1	45.6	7.5	235.1
Std. Dev.	0.2	4.4	0.9	43.1
Std. Error	0.12	2.54	0.5	24.88
Median	1.0	47.3	7.0	233.5
Mean of Size C	1.3	41.0	14.0	203.2
Std. Dev.	0.3	6.5	11.0	26.1
Std. Error	0.15	3.25	5.5	13.05
Median	1.3	41.3	8.5	203.9

Table iii.2. Heavy Metals in Bathyporeia,  
Eurydice, and Haustorius.

Location & Date of Collection	Dry Sample Mass (g)	Concentration ppm ( $\mu\text{g}/\text{g}$ )			Zinc
		Cadmium	Copper	Lead	
C 14 12 88	0.525	1.0	81.0	18	176.0
C & RC 12 04 89	0.514	1.5	68.5	18	179.1
C 09 05 89	0.457	1.5	84.5	25	210.1
	Mean	1.3	78.0	20.3	188.4
	Std. Dev.	0.2	6.9	3.3	15.4
	Std. Error	0.14	3.97	1.9	8.89
	Median	1.5	81.0	18.0	179.1

Table iii.3. Heavy Metals in Corophium.

Location & Date of Collection	Dry Sample Mass (g)	Concentration ppm ( $\mu\text{g}/\text{g}$ )			Zinc
		Cadmium	Copper	Lead	
SS 26 04 89	0.457	1.0	102.5	29	120.5
SS 27 05 89	0.533	1.0	59.5	39	117.3
SS 27 05 89	0.599	1.0	43.5	32	96.3
	Mean	1.0	68.5	33.3	111.4
	Std. Dev.	0.0	24.9	4.2	10.7
	Std. Error	0.00	14.38	2.4	6.20
	Median	1.0	59.5	32.0	117.3

Table iii.4. Heavy Metals in Nereis.

Location & Date of Collection Dy/Mn/Yr	Dry Sample Mass (g)	Concentration ppm ( $\mu\text{g}/\text{g}$ )			Zinc
		Cadmium	Copper	Lead	
SS 26 04 89	0.572	0.5	48.5	19	165.3
SS 26 04 89	0.519	0.5	39.0	10	170.9
SS 26 04 89	0.671	0.5	38.5	-	153.8
SS 27 05 89	0.688	0.5	36.0	19	204.6
SS 27 05 89	0.962	0.5	39.0	20	169.6
SS 27 05 89	0.900	0.5	34.0	18	169.7
	Mean	0.5	39.2	17.2	172.3
	Std. Dev.	0.0	4.6	3.7	15.5
	Std. Error	0.00	1.86	1.6	6.35
	Median	0.5	38.8	19.0	169.7

**Appendix 4 DATA ON SANDERLING *Calidris alba*, RINGED PLOVER *Charadrius hiaticula*, AND GREY PLOVER *Pluvialis squatarola* USED FOR HEAVY METAL ANALYSES; AND A.A.S. RESULTS.**

Locations:	1 North Gare Sands	Sex:	1 Male
	2 Seaton Snook		2 Female
	3 Peninsular		
	6 The Ducky	Amt. of Tissue:	1 All (N.B. Kidneys are all whole)
	7 Coatham Sands	(For liver only)	2 Samples together make whole liver
	8 Redcar		3 Samples together make part of liver;
	9 South Gare		no mark means fragment of liver

Table iv.1. Sanderling Kidneys Samples.

Bird Number	Month & Year of Collection	Location Captured	Age	Sex	Total		Concentrations in Parts per Million					
					Wet Body Mass (g)	Dry Sample Mass (g)	Cadmium	Copper	Lead	Zinc		
TS001K	3	83	7	6	64.0	0.149	16.5	26.0	18	143.0	} Late Winter	
TS002K	3	83	7	6	56.0	0.211	6.5	22.5	15	116.2		
TS003K	3	83	1	6	57.0	0.250	15.5	15.0	12	100.4		
TS004K	3	83	1	6	62.0	0.223	19.0	14.0	12	119.7		
TS005K	3	83	1	5	66.0	0.239	6.0	16.5	19	104.2		
TS006K	3	83	1	6	1	58.0	0.194	15.0	19.0	12		124.5
TS007K	3	83	1	6	2	62.0	0.211	28.5	19.5	16		143.5
TS009K	3	83	1	6	2	60.0	0.222	16.0	21.5	7		111.7
TS010K	3	83	1	6	2	62.0	0.265	21.0	23.0	7		133.9
TS012K	5	83	6		2	91.0	0.192	10.5	23.0	14		118.4
TS013K	5	83	6		2	88.0	0.196	14.0	21.0	10	110.2	
TS014K	5	83	6		2	95.0	0.208	23.0	30.0	11	138.3	
TS016K	5	83	7	6	2	92.0	0.252	40.0	23.0	9	124.7	
TS017K	5	83	7		2	91.0	0.178	32.5	22.5	9	176.5	
TS018K	5	83	7		2	91.0	0.186	19.0	20.5	11	166.9	
TS019K	5	83	7	6	2	91.0	0.237	30.0	23.0	8	136.1	
TS020K	5	83	7	6	2	78.0	0.293	29.0	22.5	11	105.0	
TS021K	7	83	2	4	1	48.0	0.179	23.0	25.5	9	138.1	} Before Moulting (?)
TS022K	7	83	2		1	45.0	0.140	46.0	20.0	12	147.5	
TS023K	7	83	2		2	57.0	0.190	72.0	25.5	12		
TS024K	7	83	2		1	48.0	0.141	15.0	21.0	17	117.0	
TS025K	7	83	2		1	58.0	0.196	7.0	22.5	12	114.5	
TS1K	9	79	6	2	58.0	0.260	2.5	22.5	0	209.8	} Pre-Winter	
TS2K	10	80	6	1	51.5	0.211	2.0	23.5	5	208.7		
TS3K	11	80	6	2	56.0	0.261	1.5	25.5	6	176.8		
TS5K	9	80	6	1	54.4	0.195	6.5	29.0	0	250.1		
TS6K	5	78	6			0.169	7.5	28.5	17	394.3	} Spring	
TS7K	3	81	6	1	49.8	0.204	5.5	25.5	9	370.5	} Late Winter	
TS8K	3	81	6	2	57.0	0.224	8.5	22.5	9	318.6		
TS9K	3	81	6	1	51.1	0.204	10.5	23.5	5	244.2		
TS10K	3	81	6	2	57.7	0.249	11.5	22.0	8	274.0		

Number of Samples			Cadmium	Copper	Lead	Zinc
31	Total Mean		18.1	22.6	10.4	171.2 <sup>1</sup>
	Std. Dev.		14.7	3.5	4.5	78.2
	Std. Error		2.64	0.63	0.8	14.27
	Median		15.0	22.5	11	138.2
13	March Captures		13.8	20.8	11.5	177.3
	Std. Dev.		6.4	3.6	4.3	88.0
	Std. Error		1.79	1.01	1.2	24.41
	Median		15.0	22.0	12	133.9
9	May Captures		22.8	23.8	11.1	163.4
	Std. Dev.		10.3	3.1	2.6	84.7
	Std. Error		3.44	1.02	0.9	28.25
	Median		23.0	23.0	11	136.1
5	July Captures		32.6	22.9	12.4	129.3 <sup>2</sup>
	Std. Dev.		23.6	2.3	2.6	14.0
	Std. Error		10.56	1.01	1.2	6.98
	Median		23.0	22.5	12	127.6
4	Autumn Captures		3.1	25.1	2.8	211.4
	Std. Dev.		2.0	2.5	2.8	26.0
	Std. Error		0.99	1.24	1.4	13.00
	Median		2.3	24.5	2.5	209.3

<sup>1</sup>n=30; <sup>2</sup>n=4

Table iv.2. Sanderling Liver Samples.

Bird Number	Month & Year of Collection	Location Captured	Age	Sex	Total		Concentrations in				Amt of Tiss	
					Wet Body Mass (g)	Dry Sample Mass (g)	Parts per Million	Cadmium	Copper	Lead		Zinc
TS001L	3 83	7	6		64.0	0.432	3.5	24.5	5		2	} Late Winter
TS001L	3 83	7	6		64.0	0.321	3.0	20.5	11	107.3	2	
TS002L	3 83	7	6		56.0	0.254	2.5	24.5	9	129.6	2	
TS002L	3 83	7	6		56.0	0.579	2.0	33.5	7	108.2	2	
TS003L	3 83	1	6		57.0	0.214	4.5	22.0	9	108.0		
TS004L	3 83	1	6		62.0	0.223	3.5	20.0	7	119.3	2	
TS004L	3 83	1	6		62.0	0.767	2.5	22.5	4	105.2	2	
TS005L	3 83	1	5		66.0	0.160	3.0	21.5	10	95.4	2	
TS005L	3 83	1	5		66.0	0.764	1.5	17.5	5	94.8	2	
TS006L	3 83	1	6	1	58.0	0.204	3.0	21.0	10	92.9		
TS007L	3 83	1	6	2	62.0	0.248	4.0	21.0	8	96.2	2	} Late Winter
TS007L	3 83	1	6	2	62.0	0.641	3.0	25.5	6	97.1	2	
TS008L	3 83	1	6	2	61.0	0.257	3.0	24.0	9	99.1		
TS009L	3 83	1	6	2	60.0	0.251	3.5	19.5	8	85.3	2	
TS009L	3 83	1	6	2	60.0	0.697	2.0	25.0	4	73.5	2	
TS010L	3 83	1	6	2	62.0	0.169	3.5	23.0	7	118.3	2	} Late Winter
TS010L	3 83	1	6	2	62.0	0.800	2.5	25.5	6	109.5	2	

TS012L	5	83	6	2	91.0	0.245	4.0	26.5	10	110.5		
TS013L	5	83	6	2	88.0	0.347	5.0	24.5	7	104.4	2	
TS013L	5	83	6	2	88.0	0.463	3.5	36.0	13	103.9	2	
TS014L	5	83	6	2	95.0	0.314	5.0	21.5	5	104.9	2	
TS014L	5	83	6	2	95.0	1.287	4.5	30.5	5	115.3	2	
TS016L	5	83	7	6	2	92.0	0.368	8.0	15.0	6	83.3	2
TS016L	5	83	7	6	2	92.0	0.758	5.0	28.5	8	108.2	2
TS017L	5	83	7	2	91.0	0.266	4.0	25.5	6	83.3		
TS018L	5	83	7	2	91.0	0.210	4.5	31.0	11	100.6		
TS019L	5	83	7	6	2	91.0	0.310	6.5	22.0	8	104.4	
TS020L	5	83	7	6	2	78.0	0.286	6.0	17.0	8	99.3	2
TS020L	5	83	7	6	2	78.0	0.926	4.5	22.5	4	83.0	2
TS021L	7	83	2	4	1	48.0	0.165	6.5	22.5	8	118.3	2
TS021L	7	83	2	4	1	48.0	0.638	4.0	21.0	6	102.2	2
TS022L	7	83	2	1	45.0	0.247	8.5	25.0	5	136.8		
TS023L	7	83	2	2	57.0	0.165	15.5	27.0	8	143.5	2	
TS023L	7	83	2	2	57.0	0.449	10.0	24.0	5	122.6	2	
TS024L	7	83	2	1	48.0	0.213	2.5	26.5	8	144.4	2	
TS024L	7	83	2	1	48.0	0.519	1.0	26.5	10	109.4	2	
TS025L	7	83	2	1	58.0	0.266	2.0	23.0	5			
TS071L						0.271	1.0	17.0	2	137.6	1	
TS072L						0.669	1.5	21.0	3	111.9	1	
TS073L						0.287	10.0	55.0	8	157.7	1	
TS401L	2	89	7	6	2	66.0	0.847	2.0	26.0	5	106.5	1
TS402L	2	89	7	6	1	49.0	0.444	2.0	34.5	9	136.3	1
TS403L	2	89	7	6	2	49.0	0.556	1.5	29.0	7	143.3	1
TS404L	2	89	7	6	1	49.0	0.560	1.5	31.5	5	137.4	1
TS405L	2	89	8	6	2	55.0	0.469	2.5	29.5	7	140.7	1
TS406L	2	89	8	6	2	51.0	0.580	2.5	27.0	5	131.1	1
TS407L	2	89	8	6	1	49.0	0.572	1.5	29.0	7	121.8	1
TS408L	2	89	8	6	1	47.0	0.573	2.0	33.0	5	136.7	1
TS1L	9	79	6	2	58.0	1.022	1.0	19.0	2	119.7	1	
TS2L	10	80	6	1	51.5	0.622	1.0	22.5	4	158.7	1	
TS3L	11	80	6	2	56.0	0.801	0.5	24.0	5	150.8	1	
TS5L	9	80	6	1	54.4	0.575	1.5	26.0	4	147.9	1	
TS6L	5	78	6			1.169	2.0	13.0	11	74.1	1	
TS7L	3	81	6	1	49.8	0.686	1.0	21.0	3	97.9	1	
TS8L	3	81	6	2	57.0	0.778	2.0	20.5	3	97.8	1	
TS9L	3	81	6	1	51.1	0.630	2.5	22.0	2	92.4	1	
TS10L	3	81	6	2	57.7	0.838	2.0	22.5	3	101.5	1	

Preparing for Spring Migration

Before Molt (?)

Mid Winter

Pre-Winter

} Spring

Late Winter

Number of Samples	Number of Birds		Cadmium	Copper	Lead	Zinc
57	43	Total Mean	3.3	25.1	6.3	115.1 <sup>1</sup>
		Std. Dev.	2.5	6.3	2.4	21.3
		Std. Error	0.38	0.96	0.4	3.25
		Median	2.5	24.0	6.5	110.4

<sup>1</sup> n<sub>a</sub> = 55, n<sub>b</sub> = 42

8	8	February Captures	1.9	29.9	6.3	131.7
		Std. Dev.	0.4	2.7	1.4	11.3
		Std. Error	0.14	0.96	0.5	4.01
		Median	2.0	29.3	6.0	136.5
21	14	March Captures	2.7	22.7	6.3	100.9 <sup>2</sup>
		Std. Dev.	0.8	2.3	2.5	9.9
		Std. Error	0.21	0.61	0.7	2.65
		Median	2.9	22.2	6.8	98.5

<sup>2</sup> n<sub>a</sub> = 20, n<sub>b</sub> = 14

Number of Samples	Number of Birds		Cadmium	Copper	Lead	Zinc	
13	9	May Captures	4.7	24.0	8.2	97.1	
		Std. Dev.	1.3	5.3	2.2	11.6	
		Std. Error	0.43	1.75	0.7	3.88	
		Median	4.5	25.5	8.0	100.6	
8	5	July Captures	6.1	24.5	6.5	126.8 <sup>3</sup>	
		Std. Dev.	4.2	1.7	1.5	10.1	
		Std. Error	1.86	0.77	0.7	5.07	
		Median	5.3	25.0	6.5	130.0	
<sup>3</sup> n <sub>a</sub> =7, n <sub>b</sub> =4							
4	4	Autumn Captures*		1.0	22.9	3.8	144.3
		Std. Dev.	0.4	2.6	1.1	14.7	
		Std. Error	0.18	1.28	0.5	7.36	
		Median	1.0	23.3	4.0	149.4	
* September, October, and November							

Table iv.3. Ringed Plover Kidney Samples.

Bird Number	Month & Year of Collection		Location Captured	Age	Sex	Total Wet Body Mass (g)	Dry Sample Mass (g)	Concentrations in Parts per Million					
								Cadmium	Copper	Lead	Zinc		
TRP1K	5	82		6	1	68.0	0.216	3.0	31.0	5	116.2	} Migrant	
TRP2K	8	80		6	1	77.2	0.239	20.5	31.5	2	138.0	} Molt	
TRP4K	5	80		6	1	58.0	0.204	4.5	25.0	5	115.3	} Migrant	
RP11K	8	77	1	6	2	78.0	0.221	7.0	49.5	12	131.4	]	
RP12K	8	77	1	6	2	71.0	0.251	15.5	27.5	11	108.4	Post-Breeding Molt	
RP13K	8	77	1	6	2	66.0	0.287	17.0	32.5	9	128.2	]	
RP14K	4	78		6	1	68.0	0.243	13.0	33.5	11	117.6	]	
RP15K	4	78		6	2	65.0	0.246	12.5	40.0	8	138.5	Spring Migrants	
RP16K	5	79		6	2	65.0*	0.169	1.0	29.5	9	123.3	]	
RP17K	5	79		6	2	79.1	0.354	1.5	33.0	12	98.8	]	
Number of Samples								Cadmium	Copper	Lead	Zinc		
10								Total Mean	9.6	33.3	8.4	121.6	
								Std. Dev.	6.7	6.6	3.2	12.1	
								Std. Error	2.11	2.08	1.0	3.84	
								Median	9.8	32.0	9.0	120.5	
6								April & May Captures	5.9	32.0	8.3	118.3	
								Std. Dev.	5.0	4.5	2.7	11.7	
								Std. Error	2.03	1.85	1.1	4.79	
								Median	3.8	32.0	8.5	116.9	
4								August Captures	15.0	35.3	8.5	126.5	
								Std. Dev.	5.0	8.4	3.9	11.0	
								Std. Error	2.48	4.22	2.0	5.52	
								Median	16.3	32.0	10.0	129.8	

Table iv.4. Ringed Plover Liver Samples.

Bird Number	Month & Year of Collection	Location Captured	Age	Sex	Total Wet Body Mass (g)	Dry Sample Mass (g)	Concentrations in Parts per Million				Amt of Tiss	
							Cadmium	Copper	Lead	Zinc		
TRP000L						1.270	2.0	10.0	3	93.5		
TRP001L	5	83	1		59.0	0.855	7.0	22.5	7	175.1	1	
TRP002L	5	83	1		59.0	0.385	1.5	23.5	6	84.0	1	
TRP003L	5	83	1			0.377	2.5	24.5	7		2	
TRP003L	5	83	1			0.469	2.0	23.5	5	91.5	2	
TRP004L	5	83	1		79.0	0.318	1.0	23.5	5		2	
TRP004L	5	83	1		79.0	0.598	0.5	21.5	3	86.3	2	
TRP006L	5	83	3		80.0	1.076	1.5	20.5	3	106.7	1	Spring Migrants
TRP007L	5	83	3		76.0	0.295	4.0	32.0	8	128.6		
TRP008L	5	83	3		70.0	0.376	1.5	26.5	4	118.4	2	
TRP008L	5	83	3		70.0	0.566	1.5	25.5	3	103.4	2	
TRP010L	5	83	3		61.0	0.590	1.5	28.0	3	121.1	1	
TRP011L	5	83	2	6	86.0	0.353	1.5	25.0	10	85.1		
TRP012L	5	83	2	6	87.0	0.473	3.0	23.0	4	121.9	2	
TRP012L	5	83	2	6	87.0	0.599	2.5	22.5	3	115.0	2	
TRP013L	5	83	2	6	91.0	0.965	1.0	22.0	5	121.4	1	
TRP014L	5	83	2	6	86.0	0.315	4.5	25.0	10	97.5		
TRP015L	8	83		4	43.0	0.347	2.0	41.0	4	133.0		
TRP017L	8	83		4	55.0	0.383	0.5	18.0	6	94.8		
TRP018L	8	83		4	49.0	0.356	2.0	23.0	4	82.6	1	Post-Breeding Moul
TRP019L	8	83		4	55.0	1.018	6.5	51.5	5	133.2	1	
TRP020L	8	83		4	58.0	0.438	0.5	21.0	4		2	
TRP020L	8	83		4	58.0	0.523	0.0	23.0	3	109.2	2	
TRP070L	5	84	3		77.0	0.348	4.5	23.5	4	103.4		
TRP071L	5	84	3		82.0	0.438	1.0	23.5	4	106.1	2	
TRP071L	5	84	3		82.0	0.681	0.5	24.0	3	99.7	2	
TRP072L	5	84	3		84.0	0.324	4.0	26.0	7	87.7	2	
TRP072L	5	84	3		84.0	0.529	4.0	26.5	3	103.4	2	Spring Migrants
TRP073L	5	84	3		83.0	0.291	1.5	27.5	9	99.9	2	
TRP073L	5	84	3		83.0	0.619	0.5	25.5	5	109.1	2	
TRP074L	5	84	3		81.0	0.310	1.5	22.5	6	77.9		
TRP075L	5	84	3		88.0	0.942	4.0	19.5	3	127.5	1	
TRP076L	5	84	3		85.0	0.571	3.0	25.0	6	132.6		
TRP083L	8	84		4	63.0	0.462	2.0	23.0	6	136.2		Moul
TRP084L	9	84	7	6	58.0	0.689	0.5	23.0	5	121.8		
TRP085L	9	84	7	6	76.0	0.365	0.5	40.5	7	130.0		
TRP086L	9	84	7	6	65.0	0.311	2.0	21.5	9	90.3	2	
TRP086L	9	84	7	6	65.0	0.475	1.5	23.0	4	115.2	2	
TRP087L	9	84	7	6	65.0	0.374	1.0	23.0	7	148.9		Late Summer
TRP088L	9	84	7	6	64.0	0.305	1.5	31.5	8		2	
TRP088L	9	84	7	6	64.0	0.548	0.5	29.5	4	109.9	2	
TRP089L	9	84	7	6	61.0	0.583	1.0	19.5	5	115.8	1	
TRP090L	9	84	7	6	87.0	0.852	0.5	21.5	3	92.4	1	
TRP092L	9	84	8	6	71.0	0.799	0.5	22.0	5	96.0	1	
TRP201L						0.889	1.0	22.5	4	107.0	1	
TRP1L	5	82		6	68.0	1.165	0.5	16.0	2	79.8	1	Spring
TRP2L	8	80		6	77.2	0.991	3.5	18.5	1	98.5	1	Moul
TRP4L	5	80		6	58.0	0.747	1.0	17.5	2	95.5	1	Spring
TRP5L	9	80		4	64.0	1.119	0.5	17.5	1	81.2	1	Summer
RP1L	11	76	9	6	89.7	0.291	1.0	27.0	4	124.1	1	Winter

RP11L	8	77	1	6	2	78.0	1.056	1.0	19.0	3	85.4	1	] Post-Breeding ]
RP12L	8	77	1	6	2	71.0	1.123	1.5	17.5	4	69.3	1	
RP13L	8	77	1	6	2	66.0	0.572	1.5	22.5	3	80.3	2	
RP13L	8	77	1	6	2	66.0	0.495	1.5	21.5	4	82.6	2	
RP15L	4	78		6	2	65.0	0.789	1.5	25.5	4	118.9	1	] Spring ]
RP16L	5	79		6	2	65.1*	0.809	0.5	19.0	4	71.4	1	
RP17L	5	79		6	2	79.1	1.014	0.5	17.5	6	79.5	1	
RP201L							0.843	8.0	28.0	5	111.5	1	
RP203L							0.768	8.0	28.0	7	125.2	1	
RP204L							0.691	1.0	29.5	5	117.4	1	
RP205L							0.706	2.0	23.5	6	101.4	1	
RP206L							0.751	1.0	113.0	10	106.4	1	
RP207L							0.956	7.5	31.0	6	108.2	1	
RP208L							0.848	1.0	24.5	7	103.5	1	
RP209L							0.810	0.5	24.5	7	101.3	1	
RP210L							1.068	2.5	30.0	6	136.9	1	
RP212L	9	84					0.708	0.5	23.0	6	98.9	1	] Late Summer ]
RP213L	9	84					0.675	0.5	24.0	8	125.4	1	
RP214L	9	84					0.623	0.5	24.5	9	114.1	1	
RP215L	9	84					0.673	0.5	26.0	7	118.8	1	
RP216L	9	84					0.324	1.0	25.5	12	122.7	1	

Number of Samples	Number of Birds		Cadmium	Copper	Lead	Zinc
72	60	Total Mean	2.0	25.8	5.3	107.6 <sup>1</sup>
		Std. Dev.	2.0	12.9	2.2	20.2
		Std. Error	0.25	1.67	0.3	2.61
		Median	1.3	23.5	5.0	106.6
			<sup>1</sup> n <sub>a</sub> =68, n <sub>b</sub> =60			
30	23	May Captures	2.2	23.1	5.1	104.2 <sup>2</sup>
		Std. Dev.	1.7	3.6	2.2	23.1
		Std. Error	0.35	0.75	0.5	4.81
		Median	1.5	23.5	5.0	103.2
			<sup>2</sup> n <sub>a</sub> =28, n <sub>b</sub> =23			
12	10	August Captures	2.1	25.6	4.0	102.4 <sup>3</sup>
		Std. Dev.	1.7	10.8	1.4	23.2
		Std. Error	0.54	3.41	0.4	7.32
		Median	1.8	22.0	4.0	96.7
			<sup>3</sup> n <sub>a</sub> =11, n <sub>b</sub> =10			
16	14	September Captures	0.7	24.5	6.3	112.8 <sup>4</sup>
		Std. Dev.	0.4	5.3	2.5	16.9
		Std. Error	0.10	1.42	0.7	4.51
		Median	0.5	23.0	6.3	115.0
			<sup>4</sup> n <sub>a</sub> =15, n <sub>b</sub> =14			

Table iv.5. Grey Plover Kidney Samples.

Bird Number	Month & Year of Collection		Location Captured	Age	Sex	Total Wet Body Mass (g)	Dry Sample Mass (g)	Concentrations in Parts per Million				
								Cadmium	Copper	Lead	Zinc	
TGP1K	9	81		4	1	220	0.599	0.5	22.5	8	97.7	} Early Return
TGP2K	10	80		4	1	171	0.528	1.0	20.5	8	116.8	
TGP3K	11	80		6		253	0.658	1.0	25.5	24	178.6	} Winter
TGP4K	11	80		6	2	195	0.874	4.5	24.0	9	104.0	
GP12K	9	78		6	1	230	0.942	11.0	21.5	4	90.9	} Early Return
GP13K	9	78		6	2	236	0.867	2.0	24.0	5	78.2	

Number of Samples		Cadmium	Copper	Lead	Zinc
		6	Total Mean	3.3	23.0
	Std. Dev.	3.7	1.7	6.6	32.4
	Std. Error	1.50	0.69	2.7	13.24
	Median	1.5	23.3	8.0	100.9
4	Sep & Oct Captures	3.6	22.1	6.3	95.9
	Std. Dev.	4.3	1.3	1.8	13.9
	Std. Error	2.15	0.65	0.9	6.97
	Median	1.5	22.0	6.5	94.3
2	November Captures	2.8	24.8	16.5	141.3
	Std. Dev.	1.8	0.8	7.5	37.3
	Std. Error	1.24	0.53	5.3	26.38

Table iv.6. Grey Plover Liver Samples.

Bird Number	Month & Year of Collection		Location Captured	Age	Sex	Concentrations in Parts per Million						Amt. of Tiss		
						Total Wet Body Mass (g)	Dry Sample Mass (g)	Cadmium	Copper	Lead	Zinc			
TGP1L	9	81		4	1	220	0.625	0.5	17.0	5	97.0	3	} Early Return	
TGP1L	9	81		4	1	220	0.448	0.0	18.5	2	88.1	3		
TGP1L	9	81		4	1	220	0.961	0.0	16.5	2	80.0	3		
TGP3L	11	80		6		253	0.621	0.5	40.0	10	134.6	3	} Winter Return	
TGP3L	11	80		6		253	0.984	0.0	26.0	6	109.1	3		
TGP3L	11	80		6		253	0.446	0.0	23.5	7	125.8	3		
TGP4L	11	80		6	1	195	1.099	1.0	24.0	6	132.1	2	}	
TGP4L	11	80		6	1	195	0.988	1.0	23.5	6	136.4	2		
TGP5L	3	82		4	1	222	0.879	2.0	28.5	6	121.8		} Spring	
TGP6L				6	2	249	0.620	2.0	24.5	7	102.8			
TGP7L	1	82		3	1	210	0.603	0.5	30.0	6	117.9	3	} Midwinter	
TGP7L	1	82		3	1	210	0.755	0.0	28.5	3	104.1	3		
TGP7L	1	82		3	1	210	0.359	0.0	30.5	2	113.8	3		
TGP9L	9	81		6	1	192	0.859	1.0	22.0	6	95.5		} Early Return	
TGP12L				6	2	255	0.673	0.5	25.5	7	98.6			
TGP14L	12	82		3	1	240	0.580	0.5	25.5	6	116.2	3	} Midwinter	
TGP14L	12	82		3	1	240	0.444	0.0	25.0	3	101.4	3		
TGP14L	12	82		3	1	240	0.899	0.0	23.0	3	90.1	3		
GP12L	9	78		6	1	230	0.951	1.5	17.5	3	89.1	2	} Early Returns	
GP12L	9	78		6	1	230	1.019	1.5	15.5	3	84.9	2		
GP12L	9	78		6	1	230	1.027	2.0	16.0	4	88.3	2		
GP13L	9	78		6	2	305	1.269	0.5	19.5	4	70.0	2		
GP13L	9	78		6	2	305	0.936	0.5	18.5	4	88.1	2		
GP13L	9	78		6	2	305	1.006	0.5	19.5	3	105.1	2		
GP501L				4	1	252	0.850	1.0	20.0	2	106.2	2	}	
GP501L				4	1	252	0.890	1.0	20.5	2	90.3	2		
GP501L				4	1	252	0.859	1.0	20.0	2	96.7	2		
GP501L				4	1	252	0.698	0.5	20.5	2	92.8	2		
GP502L				4	1	270	1.020	0.5	17.0	2	98.5	3		
GP502L				4	1	270	0.860	0.5	17.5	3	78.1	3		
GP502L				4	1	270	0.413	0.5	21.0	3	89.7	3		
GP503L				4	1	241	1.273	0.5	23.5	2	102.7	2		
GP503L				4	1	241	0.998	0.5	23.5	2	103.0	2		
GP504L	1	85	3	6	1	264	0.692	1.0	21.5	3	115.6	2		} Mid Winter
GP504L	1	85	3	6	1	264	0.907	1.0	19.5	3	82.8	2		
GP504L	1	85	3	6	1	264	0.973	1.0	19.0	3	86.8	2		
GP504L	1	85	3	6	1	264	0.489	1.0	21.5	3	97.7	2		
GP505L				3	1	254	0.794	1.0	20.5	3	98.6		}	
GP506L	11	85		4	2	292	0.764	1.0	21.5	3	112.9	3		
GP506L	11	85		4	2	292	0.361	0.5	22.5	4	103.6	3		
GP506L	11	85		4	2	292	0.929	1.0	19.5	3	84.6	3		
GP507L	10	84		4	1	243	0.710	1.0	27.5	4	129.5			} Early Returns
GP508L	10	84		3	2	232	0.970	0.5	19.0	3	119.5			
GP509L	10	84		3	2	244	0.929	0.5	26.0	2	119.2		}	
GP510L	12	84	1	4	1	242	0.805	0.5	20.0	2	109.3	2		
GP510L	12	84	1	4	1	242	0.879	0.5	20.0	2	129.2	2		
GP510L	12	84	1	4	1	242	0.919	0.5	19.0	2	117.9	2	} Midwinter	

GP511L	12	84	1	3	1	264	0.853	0.5	26.5	2	123.2		Midwinter
GP512L	12	84	1	4	2	218	0.865	1.0	20.5	4	113.6	2	
GP512L	12	84	1	4	2	218	1.026	1.0	20.0	4	115.7	2	
GP512L	12	84	1	4	2	218	0.548	1.0	22.0	4	124.2	2	
GP513L	10	84		3	1	290	0.918	0.5	21.0	3	123.1		Early Return
GP514L	10	84		3	1	232	0.796	0.5	19.0	2	130.6	2	
GP514L	10	84		3	1	232	0.753	0.5	20.5	2	123.6	2	
GP515L	10	84		3	1	271	0.949	0.5	26.5	2	137.1	2	
GP515L	10	84		3	1	271	1.065	0.5	25.5	3	135.5	2	

Number of Samples	Number of Birds		Cadmium	Copper	Lead	Zinc
56	26	Total Mean	0.8	22.8	3.7	109.7
		Std. Dev.	0.5	3.7	1.7	15.2
		Std. Error	0.10	0.73	0.3	2.97
		Median	0.5	21.6	3.0	107.4
8	3	Jan. & Mar. Captures	1.1	26.2	3.9	109.8
		Std. Dev.	0.7	4.1	1.5	10.8
		Std. Error	0.43	2.38	0.9	6.21
		Median	1.0	28.5	3.0	111.9
9	4	September Captures	0.9	18.7	4.0	89.8
		Std. Dev.	0.6	2.2	1.2	3.3
		Std. Error	0.28	1.09	0.6	1.67
		Median	0.8	18.3	3.5	88.1
8	6	October Captures	0.6	23.2	2.8	125.8
		Std. Dev.	0.2	3.0	0.6	4.8
		Std. Error	0.06	1.22	0.3	1.97
		Median	0.5	23.5	2.8	125.1
8	3	November Captures	0.7	24.9	5.7	119.3
		Std. Dev.	0.3	3.6	1.8	14.1
		Std. Error	0.20	2.08	1.0	8.15
		Median	0.8	23.8	6.0	123.2
10	4	December Captures	0.5	23.0	3.0	115.9
		Std. Dev.	0.3	2.7	1.0	7.9
		Std. Error	0.15	1.33	0.5	3.93
		Median	0.4	22.7	3.0	118.8

**Appendix 5 INVERTEBRATE ENERGETICS DATA.**Table v.1. Nerine energetics data.

Sample mass	kcal	kJoules	kcal/g	kJ/g
0.328	1.38	5.77	4.21	17.59
0.324	1.47	6.15	4.54	18.98
0.432	1.94	8.11	4.50	18.77
0.544	2.31	9.66	4.17	17.43
		mean	4.36	18.19
		standard error	0.083	0.344

Table v.2. Crustacea energetics data

Sample mass	kcal	kJoules	kcal/g	kJ/g
0.332	1.06	4.43	3.19	13.34
0.356	1.26	5.27	3.54	14.80
		mean	3.37	14.07
		standard error	0.124	0.526

Table v.3. Corophium energetics data.

Sample mass	kcal	kJoules	kcal/g	kJ/g
0.310	0.73	3.05	2.36	9.84
0.472	1.29	5.39	2.73	11.42
		mean	2.55	10.63
		standard error	0.131	0.559

Table v.4. Nereis energetics data

Sample mass	kcal	kJoules	kcal/g	kJ/g
0.304	1.51	6.31	4.97	20.76
0.322	1.63	6.81	5.06	21.15
0.302	1.56	6.52	5.17	21.59
0.350	1.72	7.19	4.91	20.54
0.336	1.62	6.77	4.82	20.15
		mean	4.87	20.84
		standard error	0.312	0.248

Table v.5. Range of estimated expected annual increase in concentrations and maximum annual percent retention of heavy metals in Sanderling.

Metal	Estimated expected annual increase in concentration (ppm) (wet mass)		Maximum annual percent retention			
	(prey taken)		Kidney		Liver	
	<u>Nerine</u>	Crustacea	Min.	Max.	Min.	Max.
Cd	85	129	2.9%	4.8%	0.8%	1.2%
Cu	3,370	7,740	0.1%	0.2%	0.1%	0.2%
Pb	1,080	2,020	0.1%	0.2%	0.1%	0.2%
Zn	18,300	18,800	0.2%	0.2%	0.2%	0.2%

