Aspects of the heavy metal budgets of common tern, Sterna hirundo(L.), chicks on the Tees estuary

Tony Quirke

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Aspects of the heavy metal budgets of common tern, *Sterna hirundo(L.)*, chicks on the Tees estuary.

by

Tony Quirke

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University of Durham
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Summary

- Common terns, *Sterna hirundo,* breed on the Tees estuary during the summer months. They feed their chicks on a diet of sprat, *Sprattus sprattus* and herring, *Clupea harengus,* which accumulate high levels of zinc, lead, copper and cadmium from the grossly polluted Tees estuary. Intake and excretion rates along with the storage of each metal by the common tern chicks were investigated in an attempt to derive their heavy metal budgets, all aspects of each metal budget being discussed.

- The metal concentrations of the fish varied significantly with time and differences were found between the two fish species which may have been due to many factors. Significant negative correlations were also found between the length of the fish and the zinc, lead and cadmium concentrations. The results for the pooled June fish samples were used in the derivation of the budgets since most feeding of the chicks took place during this period.

- A significant difference was found between the individual and latrine faecal samples but there was no significant temporal variation found in any metal concentration in either set of faecal samples. It was decided to use the individual faecal sample results in the calculation of the excretion rate since these samples more accurately reflect the metal concentration voided normally from individual chicks.

- Samples of the carcass had high levels of all metals. Zinc and lead had their highest concentrations in the bone, whereas copper concentrations were greater than lead in most tissues. Cadmium was not detected in the majority of samples probably due to the low levels in their diet. Zinc and copper are essential elements and, as such, this may account for the high accumulation in the tissues of these chicks.

- Elevated levels of all four metals were found in the eggs of these common terns on the Tees estuary.

- The accuracy of the metal budgets was greatly affected by the numerous errors which had to be taken into account at every stage, in the estimation of the feeding and the excretion rates.

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1. Introduction

1.1 The ecology of the common tern, *Sterna hirundo* (L.).

The common tern, *Sterna hirundo* (L.), is found globally, from North America to the Caribbean, Europe to North and West Africa and from the middle East to western Siberia. It is a slim, lightweight bird measuring 33-35 cm from bill tip to tail tip and weighs approximately 100-140 g. Characteristic features of the common tern include pointed bill, evenly curved line to the upper head, long wings, slim oval body and an unmistakable forked tail. The adults in summer show a black tipped red bill, neat black crown, soft pale grey upper parts and mostly white underparts. Immature common terns have less uniform upperparts, dusky outer tail feathers with a brown saddle in juvenile common terns and also a broad leading edge to the upper wing. Young common terns also have fleshy pink (sometimes orange-yellow) bills, with a dark tip which gradually darkens and they have a dull pinkish-red or yellow-orange legs.

Common terns breed over a wider variety of habitats than other tern species. They breed from the Arctic fringes to the boreal, temperate, steppe, Mediterranean and semi-desert zones to the tropics. The coastline is the preferred breeding and feeding area but inland freshwater areas are also utilised (Cramp, 1985). However its breeding distribution does not completely encircle the north pole but is broken in western North America and in Europe and Asia (Hume, 1993). The numbers of common terns in many countries did previously decline due primarily to increasing anthropogenic disturbance and exploitation, but most have subsequently increased under protection (Cramp, 1985)

The distribution of breeding common terns in Britain and Ireland is very widespread and in 1976 the total population was estimated at between 15-20,000 pairs. They are found throughout Scotland and the whole Irish coast with concentrations of common terns found throughout England and Wales. Migrating common terns are also widespread in Britain and Ireland in the spring, late summer and autumn. Common terns are migratory almost throughout their Pale Arctic and Nearctic ranges. Most western Pale Arctic terns move south to spend the winter around Africa where they can be found anywhere around the vast coastline of Africa. However an interesting contrast can be found between common terns which breed in Southern and Western Europe but winter north of the equator, while those common terns from the north and east of Europe go much farther south involving a much longer migration (Hume, 1993).

Common terns have not got very specific breeding or feeding requirements and are found in a wide variety of habitats from inland flooded areas, freshwater rivers and to the coastline where the majority are to be found. They avoid icy waters, exposed sites, tall and dense vegetation but also dislike sheer cliffs. They prefer coasts with flat rock surfaces, sand and shingle particularly on the upper beaches or in dunes but they also use mainland peninsulas, spits and salt marshes (Cramp, 1985 & Hume, 1993).

Common terns nest in colonies usually on uniform ground but interestingly the individual pairs are quite territorial. The nest itself is usually only a shallow depression in the sand or soil and it is always on the ground. Nests are primarily to be found in the open but are sometimes found in amongst dense vegetation or among broken rocks and stones. Common terns are primarily a social species due to the obvious benefits associated with flocks and breeding groups including the increased feeding success within flocks and better protection against predators. This is particularly important given that common terns are particularly vulnerable, at the breeding areas, to predators aswell as inclement weather, flooding but also human disturbance.

1.2 Feeding behaviour of common terns.

Common terns primarily feed on marine fish and/or crustaceans but they are generalist feeders, switching prey or changing their feeding methods depending on the particular circumstances encountered. The species of marine fish eaten by common terns is very widespread but consists primarily of sprat, *Sprattus sprattus* (L.), herring, *Clupea harengus* (L.)or sand-eels, *Ammodytes* spp. but also pollack, *Pollachius pollachius* (L.) , haddock, *Melanogrammus aeglefinus* (L.), whiting, *Merlangius merlangus* (L.), and cod, *Gadus morhua* (L.), amongst many others which are less commonly used. Common terns breeding inland may also choose to feed on many other species of freshwater fish. Crustaceans, like shrimps, prawns and crabs, can make up a large proportion of a common terns diet in some areas, while insects, small squid, worms, leeches, berries and vegetation fragments have also been occasionally eaten (Cramp, 1985 & Hume, 1993).

The feeding strategy employed by common terns changes depending on the various conditions encountered such as the weather, water quality, abundance of prey and Erwin (1977) refers to this as a "jack of all trades" strategy, which he suggests is an attempt to decrease the uncertainty of locating food in a varying environment. However it has also been suggested that common terns concentrate on the most abundant small inshore species (Pearson, 1968). Common terns feed in flocks when prey is abundant, like, for example, when a shoal of fish is discovered, but otherwise they are mainly found feeding dispersed along the shoreline. Dispersed feeding on invertebrates at the surface of the water, catching insects in the air and kleptoparasitism on other terns has also been recorded with common terns (Nisbett, 1983). At times of food scarcity, common terns often resort to stealing food off other terns or other bird species, but have also been known to move inland and feed on freshwater food in some areas (Becker & Frank, 1990).

Common terns, when feeding, will initially search for prey over the water and when prey is spotted they will pause momentarily before plunge-diving head first into the water. This may often be preceded by hovering. Common terns dive usually from a height of 1-6 m and depending on the height of the dive, it will be either completely or only partially submerged. Once the prey is caught it is brought to the surface held crosswise in the bill and is either swallowed or carried back to the colony to be fed to its mate or to the chicks. The density of the prey distribution greatly influences common tern feeding activity as a high prey density will result in more changes inflight direction, more 180° turns, slower searching speed and a tendency to search into the wind. Furthermore the success rate of diving terns is influenced by the size, abundance, depth, visibility of the prey as well as the age and experience of the bird. Generally common terns bring more food into the colony at low or falling tides but in estuaries, where marine fish advance upriver on incoming tides, the fishing success increases with rising tides, as shown in the Ythan estuary by Taylor (1975). Wind speed and consequently water surface conditions also affects the feeding ability of common terns. It is also thought that the tidal cycle has a greater influence on the common tern feeding activity, rather than the diurnal cycle. It has also been shown that, not surprisingly, the fishing success improves where there is a shoal of fish close to the surface (Cramp, 1985 & Hume, 1993).

1.3 Feeding of common tern chicks.

During the first few days after hatching of a chick, the male will do most of the fishing to catch food for the chicks. It will bring back a fish of a size roughly appropriate for the size of the chick and, therefore, as the chick grows, the size of the prey being fed to the chicks will increase. By the second day after hatching, the parents can easily recognise its own young and *vice versa*, with the chick quickly learning the individual qualities of the parents' calls. After being fed, the chicks will hide in the nearby vegetation or rocks, or alternatively they will shelter in shallow scrapes. Once the parent returns it will immediately come out into the open to await its food. There is, however, a lot of competition among chicks for food, with it commonplace to observe larger chicks attempting to rob smaller chicks of their food, to supplement their own food intake. Sometimes, when the parent is feeding its young, the fish is dropped. The parent will then take the fish and clean it either by dipping it into the water while flying or simply by washing it at the waters edge, before bringing it back and trying again.

As mentioned, to ensure the best diet for its young, the parents will increase the size of fish being fed to the chicks as they grow older, and as such the parents will feed themselves only on small fry or fish for the duration of this period. However the number of feeds received by chicks very much depends on the size of the brood. Larger broods of 2 and 3 chicks will receive more feeds per day but, in general, each chick in the brood will receive less food, compared to single chicks which only require the parent to visit the nest maybe once an hour. Therefore the parents of larger broods must work much harder to enable it to make these frequent visits back and fro to the nest (Cramp, 1985 & Hume, 1993).

1.4 The Tees estuary.

This estuary has been radically altered and reclaimed since the 1850's, from a vast expanse of inter-tidal mud and sandflats, to land which now supports agriculture but, more particularly, heavy industry. The estuary is now one of the largest ports in the UK and has the largest concentration of chemical industries in Europe (Davies *et al.*, 1991). Petrochemical, engineering and other manufacturing industries, such as Teesside Steel works, are also found along the River Tees and the estuary. The most seaward section of the estuary, where the common tern colony to be studied in this investigation is found, is very heavily industrialised and contains a continuously dredged channel. This is bonded on both sides by port and storage facilities and also industrial installations. The area of Teesside itself, as well as being heavily industrialised, also has a large expanding population. The estuary receives much municipal waste, aswell as the large volumes of industrial effluents and is classed as grossly polluted. Indeed over the years, the quantities of some pollutants discharged into the estuary have reached toxic levels but more recently, due to E.C. legislation, the discharges of sewage and industrial effluent are controlled and this has probably improved the pollution status of the estuary a little (Davies *et al.*, 1991).

1.4.1 Aspects of heavy metals in estuarine ecosystems.

Estuaries can be referred to as a transition zone between land, freshwater and the sea and are subject to gradients of salinity, oxygen availability, temperature, exposure to wave action, particle size and also tidal height (Raffaelli, 1992). As such there is great short term variability in estuaries which can have a marked effect on the two primary forms of heavy metals found in the estuarine environment, which are the dissolved and suspended particulate metal concentrations. Increased freshwater run-off can either concentrate metals leached by terrestrial weathering or alternatively it may dilute metals discharged from point sources. Concentrations of biochemical reactive elements can also be affected by the time, magnitude and position of any biological productivity maximum but also the tidal cycle and the increased tidal current stress may lead to increased sediment re-suspension and injection of interstitial waters into overlying water (Laslett, 1995).

It can, however, be stated emphatically that estuaries are sinks for heavy metals with long term partitioning to the sediments resulting in high concentrations (Davies *et al.*, 1991). The heavy metal bound particulate matter flocculates and precipitates thus resulting in these elevated sedimentary heavy metal levels (Evans & Moon, 1981). Also the overlying water column can contain elevated heavy metal levels due to the fact that the water circulation pattern in estuaries traps suspended particulate matter bound with heavy metals but also because many dissolved metals are found in the water column (Laslett, 1995).

Heavy metals can originate from many different sources: geological weathering and the natural biogeochemical processes, burning of fossil fuels, agricultural run-off, industrial effluents, leaching of metals, contribution from sewage and air pollution fall-out. Estuaries receive inputs from all of the above major sources and the Tees estuary contains very high heavy metal concentrations. The four heavy metals under investigation in this study, i.e. zinc, lead, copper and cadmium, vary greatly in their toxicity which can generally be ranked as follows (from McLusky, 1989):

$$\text{Cd (most toxic)} > \text{Cu} > \text{Pb} > \text{Zn}$$

Cadmium is the most toxic, persistent and bioaccumulative but the others can have deleterious effect on organisms depending on the form and concentration of the metal. However, the heavy metals zinc and copper are physiologically important elements in most organisms being essential for efficient metabolism, while lead and cadmium are non-essential metals.

1.5 Common terns on the Tees estuary

Common terns are migratory birds and they are not resident at the Tees estuary but use it exclusively as a breeding ground arriving around mid-May and begin to breed by the end of May / beginning of June. Most eggs hatch by the end of June and usually by the end of July / beginning of August, all the juveniles have fledged and have left the colony deserted. However they remain at the Tees estuary until the beginning of September when they begin their winter migration to Africa (Cramp, 1985). Therefore, throughout this breeding season, the common terns forage and feed primarily on sprat and herring on the Tees estuary but they may also feed out to sea and up the River Tees (Ward, pers. comm.). The diet of the common terns, i.e. the sprat and herring, are also non-residents in the Tees estuary and they use the estuary as a nursery ground. Given that the estuary is heavily polluted with high levels of numerous metals including zinc, lead, copper and cadmium, these fish may accumulate relatively high heavy metal levels. Therefore the primary source heavy metal intake in the common terns is through ingestion of these sprat and herring, with external atmospheric pollution contributing very little.

The potential for significant bioaccumulation of these metals in the common terns at this high trophic level, due to the high levels in their diet, is very strong unless they have the capacity to efficiently excrete or detoxify the metals. Therefore, taking into account the above factors, the derivation of a heavy metal budget for the common terns, on the highly polluted Tees estuary, was thought to warrant investigation.

1.6 Outline and objective of the project

There are many different components in the dynamics of any particular metal with respect to any organism. Even so, most previous studies have concentrated mainly on one particular component but usually with reference to a few or many metals. Many studies have looked at the tissue distributions of heavy metals in birds, the levels in the eggs, bioaccumulation of heavy metals in birds but none have actually endeavoured to investigate the actual intake, excretion and storage of any heavy metal in any bird species. The project aimed to provide, firstly, an estimate of the intake rate of each metal using observations of the feeding rates accompanied by analysis of the heavy metal levels in fish samples from the estuary. An excretion rate was estimated for each of the four heavy metals by measuring the levels in the faeces of the common terns and storage of the metals will be measured from heavy metal analysis of the tissues of the common terns. The chemical form in which the metal is ingested will determine the distribution among the tissues but in this investigation only the level for the total metal was obtained and as such no differentiation can be made between the different forms of each metal. Excretion of heavy metals via the faeces is of undigested metals whereas excretion in the urine is of absorbed and metabolised metals but it will be impossible to separate both fractions, as they are both voided together from the cloaca. It is also possible that heavy metals are also being excreted in nasal secretions of birds and any relevant information from a project currently investigating this phenomenon will be discussed

Therefore, the objective of this project was to attempt to derive budgets for the heavy metals, zinc, lead, copper and cadmium for the common terns on the Tees estuary.

It was extremely difficult in the preliminary investigations to estimate the food intake of the adult common terns, the collection of adult faecal samples was not guaranteed and attempts to obtain the samples was not going to take place until late

July or early August, due to the fact that the routine marking programme was only due to begin at that time. Therefore given the convenient access to the colony which enabled faecal samples to be obtained from the chicks as well as reasonably accurate estimates of the food intake of the chicks, it was decided to shift the emphasis of the project to estimation of the heavy metal budget of common tern chicks.

2. Materials and Methods

2.1 Site description.

The common tern colony investigated was located on the larger of two man-made islands on the 200 square metre Nature Conservancy Area (NCA) on the site of ICI Wilton adjacent to the Tees estuary (see Fig.1). This year the smaller of the two islands contained approximately 60 nests whereas the larger island, under investigation, supported approximately 130 nests. The nests on the larger colony were found amongst the tall growing vegetation on the island but there were also some found in the open at the edges of the island, close to the water. The island itself is approximately 20 m^2 and was dominated by tall growing hogweed, *Heracleum sphondylium* (L.), and had a hard substrate of rock and soil. The NCA is situated adjacent to the River Tees and Teesmouth estuary. It is surrounded by heavy oil and chemical industries and the Teesside steel works borders it to the north.

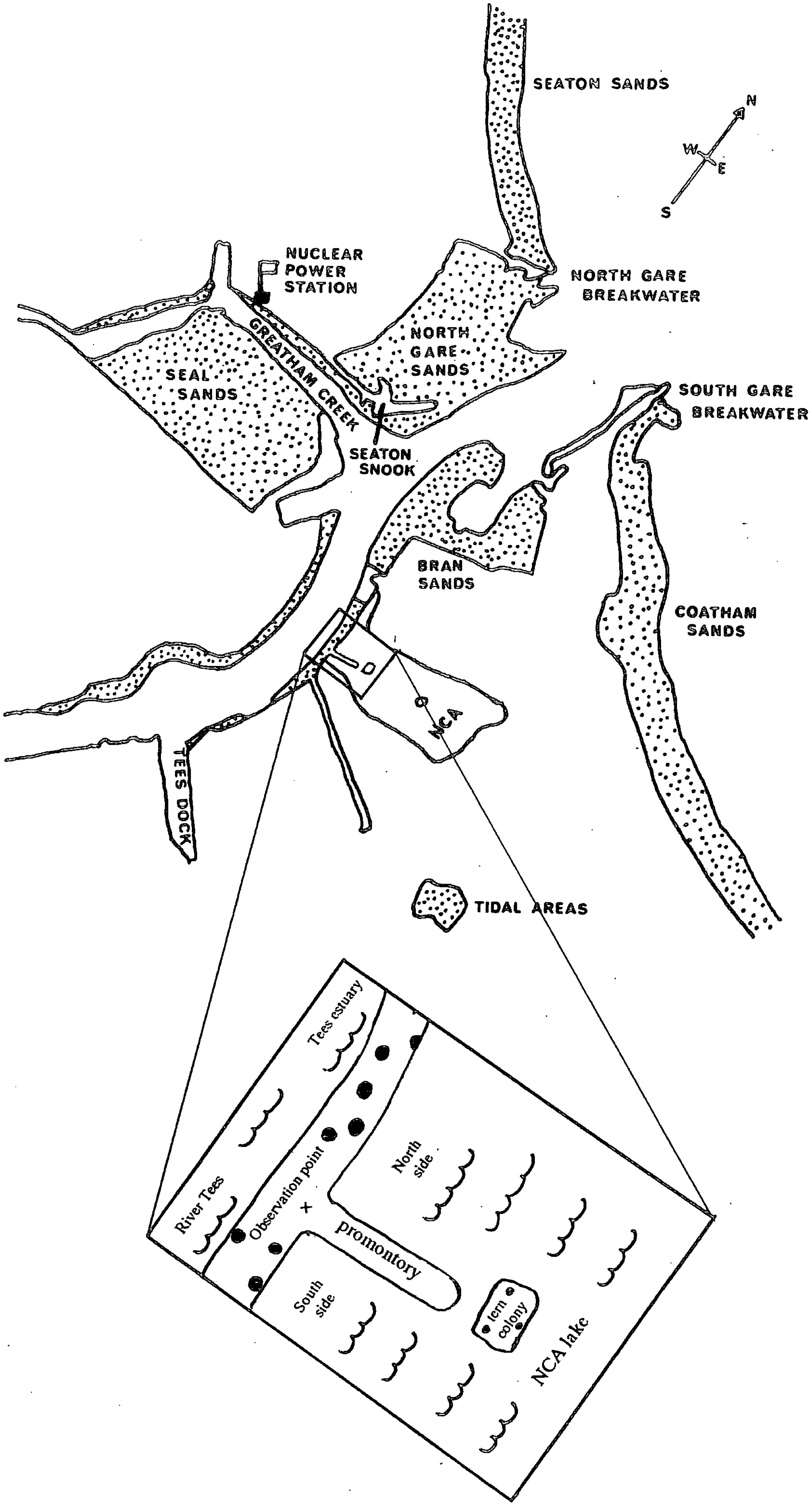
This site was chosen for two reasons: 1) the presence of the highly polluted Teesmouth estuary which is the primary feeding ground for the common terns during the breeding season and 2) the convenient presence of a common tern colony which was relatively easy to access and study.

2.2 Collection of samples.

2.2.1 Fish samples.

The two fish species sprat and herring, were collected monthly from the intake screens of Hartlepool Power Station. A sample representative of the size range present, of approximately 150 fresh fish in total between the two species was taken, brought back to the lab, placed into individual plastic bags, length and weight measurements were recorded and finally the fish were frozen until analysis. Abandoned fish samples were also collected from the colony to enable comparison with the fish samples collected at Hartlepool Power Station. These fish were probably either dropped by the parents before reaching the nest or were probably dropped and left during unsuccessful attempts to feed the chicks.

Fig.1 A map(1:25000) of the study area at the Tees estuary.



2.2.2. Faecal samples.

As part of a research contract being undertaken by Robinson, fifteen nests were enclosed on the larger of the two islands containing common tern colonies. For a period of 4 weeks the colony was visited every second day and during this period the chicks, in each enclosure, were weighed three times a day. Two types of faecal samples were collected from the colony. Initially faecal samples were obtained from individual chicks by placing the chicks in plastic bags before being put into the weighing bag. Then after it became noticeable that the chicks in the enclosures were defecating in certain areas of the enclosures, it was decided to attempt to collect faeces by placing small thin plastic sheets over these "latrines", in the enclosures where it was most feasible. Every second day these latrines were collected, marked and new plastic was laid down. All faecal samples were subsequently brought back to the lab and frozen.

2.2.3 Chicks and eggs.

Dead chicks only were collected from the enclosures on the island or the adjacent mainland when found during the four week period. Eggs were taken only when they were clearly deserted and cold but also only if there was no other chick alive in the enclosure as it was felt that this might affect the survival chances of the remaining chicks.

2.3 Feeding observations.

Given the fact that the adults foraged for food primarily on the estuary but also upriver, it was decided to observe the frequency of adults returning to the colony with fish from two directions. This was observed every 10 minutes and was repeated for both those adults returning to the colony from the north side of the promontory and to the south side of the promontory (see Fig.1). The size of the fish being brought back to the colony was also recorded at regular periods of 10 minutes by noting the size of the fish in relation to the bill length of the returning bird. Using the mean bill length for adult common terns, the size of the fish was estimated.

On some of the mornings spent at the colony, a period of between 1.5 and 3 hours was also spent in a hide which was located approximately 30 feet from the island and with a good view of four of the enclosures. While inside the hide, the number and size of the fish being fed to each brood in the enclosures under observation was noted.

2.4 Preparation of samples.

The fish samples were prepared in the following manner. Each individual fish sample was removed from the freezer and placed into a petri dish. Approximately thirty samples at a time were then oven dried at 40°C for 48 hours or until a constant dry weight was reached and were then placed in a vacuum oven for 24 hours. The whole dry fish (as fed to the chicks) was then ground up, using a pestle and mortar, into a fine homogenous powder. Between 1.0 and 1.5g dry weight of each sample was then taken for analysis.

The faecal samples were also removed from the freezer but were then simply oven dried in their plastic bags as above. All of the dried individual faecal samples weighed less than 1.0g, so the whole sample was taken but a weight of approximately 1.0g was taken from the latrine faecal samples since these usually weighed a couple of grams when dry. Each faecal sample was scraped off the plastic using a stainless steel spatula with the fragments of mud and other non-faecal material separated away from the latrine faecal samples.

The kidney, liver, bone and a sample of breast muscle was dissected from all the chicks where applicable, as a sample of bone or breast muscle could not be obtained from some of the smaller chicks. All tissues and the remaining carcasses including the feathers were oven dried again at 40°C until a constant dry weight was reached and were then placed in a vacuum oven for 24 hours. A sample of between 1.0 and 1.5g, where possible, was taken from each tissue but a larger sample of between 2-3g was taken for the carcasses and eggs since these samples were sometimes quite large.

Therefore each fish, faecal, tissue, carcass and egg sample were then placed in pre-weighed clean conical flasks, 30 samples in total being done at any one time. Each sample was then placed in a pre-weighed clean conical flask. Analytical grade concentrated nitric acid of between 5 and 10 millilitres was then added to each flask and the samples were left overnight in a fume cupboard at room temperature with a glass funnel in the neck of each flask. The following day the samples were refluxed by warming gently on a hot plate and were left to simmer for between 12 - 16 hours until a clear, light coloured liquid remained. After removal of the glass funnels the nitric acid was then evaporated off in a fume cupboard and when the sample was just dry, it was removed from the hot plate and allowed to cool before re-dissolving in exactly 5 ml 3N hydrochloric acid. Each sample was then filtered into a clean polythene bottle and stored at 4°C prior to analysis. Blanks of de-ionised water and the 3N acid were also prepared as above.

2.5 Prevention of contamination.

Trace metal investigation requires precise measurements and thus contamination will adversely affect the final results. Therefore it is vitally important to prevent contamination throughout the investigation. As such, all glassware and apparatus which was to come into contact with the samples, were thoroughly cleaned and acid-washed. Acid-washing involved an initial clean with a solution of concentrated nitric acid to remove any metal traces and then rinsed thoroughly twice with de-ionised water and left to dry. Dust (which may contain metal traces) accumulation was prevented by covering the samples if they were to be left standing for a long period of time.

During the dissection of the chicks, all instruments were cleaned between each dissection.

Splattering of the samples during refluxing and evaporation was difficult to prevent but was achieved usually by keeping those samples which began to splatter on a cooler area of the hot plate.

2.6 Metal analysis.

All four metals were analysed on a Pye Unicam SP9 series atomic absorption spectrophotometer (AAS). The instrument used has a mixture of air and acetylene for the flame and the radiation source is a hollow cathode lamp. For the analysis of each metal, the instrument was calibrated to five working standards and then each sample was aspirated into the flame and the mean of two readings was taken.

2.7 Calculations and statistics.

A regression was calculated between the five standards and their corresponding readings on the AAS. Subsequently each sample reading was converted into μg/g dry weight using this calculated slope and intercept as follows:

metal conc.($\mu g\ g^{-1}$ dry wt.) = ((AAS reading x slope) ± intercept) x 5 / dry wt.
(5 = volume of acid)

Zinc concentration was calculated as above but the figure obtained from the above equation was further multiplied by 20 which was its dilution factor.

Data was found to be, in general non-normal after visual observation of a histogram plot of the data and comparison of the mean to variance ratio. Therefore the data was normalised using the log transformation and subsequently back transformed to give means and different upper and lower standard errors which were subsequently used in the presentation of the results. Two-way analysis of variance were carried out to test for any significant time, metal or time-metal interactions in the fish, faecal and tissue samples. A t-test was performed between the latrine and individual faecal samples as well as the sprat and herring fish samples, to see if there was a significant difference between the two sets of data. Correlations were then performed on all data sets to explore for any significant metal correlations.

The body burdens of each metal were calculated from the mean concentration of each metal in each of the tissues, eggs and carcass which were then multiplied by the total dry weight of each of the samples. The age of each chick (in days) was estimated from the wet weight when collected and the growth rate of the chicks from data collected by J. Robinson (pers. comm.), see Appendix 1.

All statistics were performed using Microsoft Excel and SPSS (for Windows). Graphs were drawn using Microsoft Excel

3. Results

All data was log transformed and subsequently back transformed to give a mean but different upper and lower standard errors. Therefore all tables and graphs are drawn up using these back transformed values.

3.1 Temporal variation in fish heavy metal concentrations.

All four metals were detected in every one of the 123 fish sampled. Zinc levels ranged from 66.5 μg g^{-1} - 192.7 μg g^{-1} with the highest levels being found in the May fish samples and the lowest levels found in the June fish samples. Lead levels ranged from 4.05 μg g^{-1} - 14.14 μg g^{-1} and the highest levels were found in the June samples and the lowest found in the April samples. Copper levels ranged from 1.15 - 7.87 μg g^{-1}, March having the highest and June having the lowest values. Finally cadmium had a range of 0.378 - 2.213 μg g^{-1}, with May having the highest values and the lowest values found in the March samples.

Table 1 gives a summary of the concentrations of all four metals for each monthly sampling period (March, n = 33; April, n = 40; May, n = 19; June; n = 23; colony fish, n=10).

Table 1. Metal concentrations (mean ± s.e.) in the fish samples from the four consecutive monthly sampling periods expressed as μg g^{-1} dry weight.

		Zn			**Pb**			**Cu**			**Cd**	
	+ s.e.	**mean**	**- s.e.**	**+ s.e.**	**mean**	**- s.e.**	**+ s.e.**	**mean**	**- s.e.**	**+ s.e.**	**mean**	**- s.e.**
March	4.08	126.3	3.96	0.21	5.82	0.21	0.15	3.02	0.14	0.03	0.78	0.02
April	5.46	122.6	5.22	0.17	5.52	0.17	0.08	2.13	0.08	0.05	0.87	0.05
May	7.75	141.8	7.35	0.28	6.40	0.27	0.08	2.54	0.08	0.07	1.53	0.07
June	2.86	105.4	2.78	0.58	7.08	0.54	0.08	1.81	0.07	0.03	0.83	0.03
Colony fish	2.73	107.9	2.65	0.92	7.28	0.68	0.07	1.73	0.09	0.03	0.77	0.04
June + colony fish	2.68	106.7	2.61	0.89	7.19	0.83	0.07	1.77	0.07	0.03	0.80	0.03

A significant difference in the metal concentrations was detected (F =2108.13, P<0.001, 3 d.f.,), a significant difference was found in each metal with time (F=7.253; P<0.001, 3 d.f.,) aswell as a significant interaction (F=7.187, P<0.001, 3 d.f.) between the metals and time (see Figs.1-4).

A comparison of the metal levels in herring and sprat showed no significant difference in the Zn concentration but a highly significant difference was found in the lead (t = -4.59, P<0.0001, 25.6 d.f., n =32), in copper (t = -3.8, P<0.001, 17.8 d.f., n = 32) and also in the cadmium levels (t = -2.76, P<0.012, 21.08 d.f., n = 32).

Fig.2 Temporal variation in the zinc concentration(mean ± s.e.) of the fish.

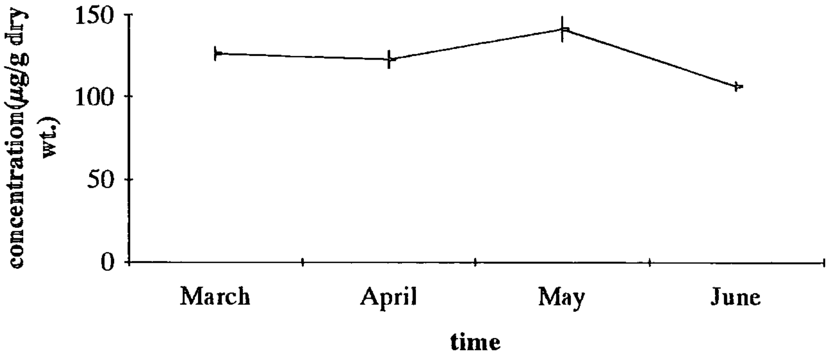
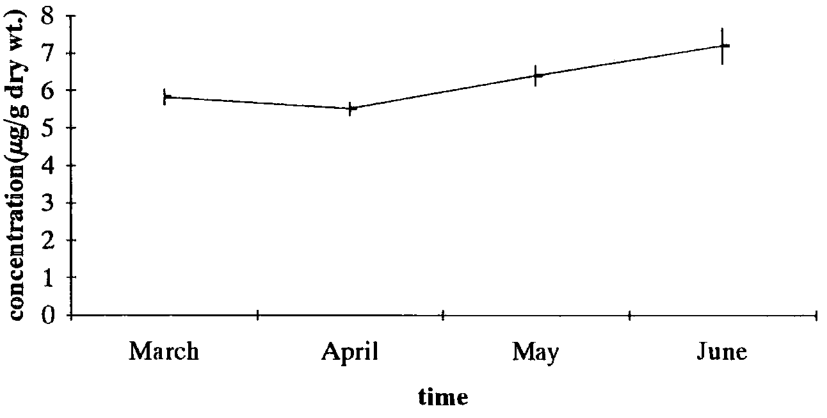


Fig.3 Temporal variation in the lead concentration(mean ± s.e.) of the fish.



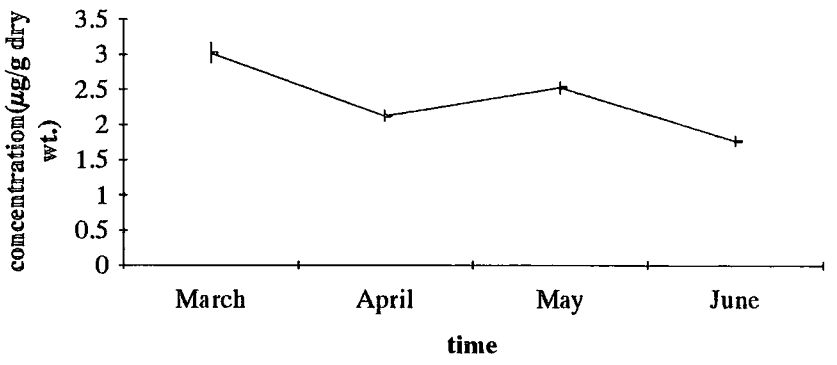


Fig.4 Temporal variation in the copper concentration(mean ± s.e.) of the fish.

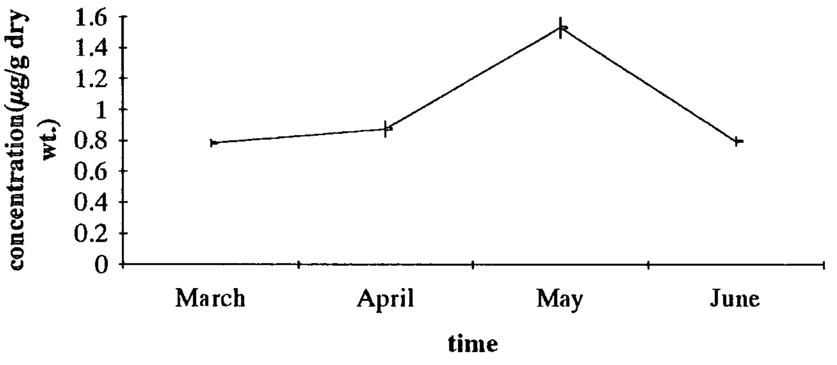


Fig.5 Temporal variation in the cadmium concentration(mean ± s.e.) of the fish.

A comparison between the June fish samples and the fish collected from the colony showed no significant differences in any metal concentration (see Table 1). There was also no significant difference in the percentage dry weight of the June fish samples (21.6% ± 1.3) and the fish collected from the colony (22.3 ± 2.4). Therefore it was decided to pool these two sets of values for use in the estimation of the heavy metal budgets.

There were significant negative associations between length of the fish and the Zn, Pb and Cd concentration but no significant correlation with Cu levels (Table 2).

Table 2. Correlation co-efficients and the significance values for each metal against the length of the fish.

	Herring		**Sprat**	
	r value	**significance(P=)**	**r value**	**significance(P=)**
Zinc	-0.2783	0.038	-0.5127	0.001
Lead	-0.2731	0.042	-0.6288	0.000
Copper	0.0173	0.899	-0.0578	0.730
Cadmium	-0.3561	0.007	-0.5704	0.000

3.2 Variation in the faecal samples.

Significant differences were found between the individual and latrine faecal samples (Table 3) for three of the four metals, Pb (t = -4.59, P<0.0001, 25.6 d.f., n=32), Cu (t = -3.8, P<0.001, 17.8 d.f., n=32) and Cd (t = -2.76, P<0.012, 21.1 d.f., n=32). However there was no significant difference found in the Zn concentration of the individual and latrine faecal samples.

Table 3. Heavy metal concentrations(mean ± s.e.) of the latrine and individual faecal samples expressed as $\mu g\ g^{-1}$ dry weight.

		Latrine			**Individual**	
	mean	**+ s.e.**	**- s.e.**	**mean**	**+ s.e.**	**- s.e.**
Zinc	312.3798	21.23173	19.8805	186.64	33.15	28.15
Lead	13.98472	1.0673	0.991621	55.21	9.95	8.44
Copper	6.074929	0.484664	0.448854	14.4	2.04	1.79
Cadmium	1.522399	0.161216	0.145779	3.853	0.912	0.738

There was, however, no significant temporal variation in any metal concentration, in either the individual ($n_1=7$, $n_2=5$, $n_3=5$, $n_4=2$, $n_5=3$, $n_6=6$, $n_7=3$) or latrine ($n_1=6$, $n_2=6$, $n_3=5$, $n_4=6$, $n_5=5$, $n_6=4$, $n_7=4$, $n_8=5$, $n_9=4$, $n_{10}=3$) faecal samples (see Figs. 6 - 9). Figures 6 - 9 also illustrate the significant differences between the individual and latrine faecal samples.

Fig.6 Temporal variation in the zinc concentration(mean ± s.e.) of the individual and latrine faecal samples.

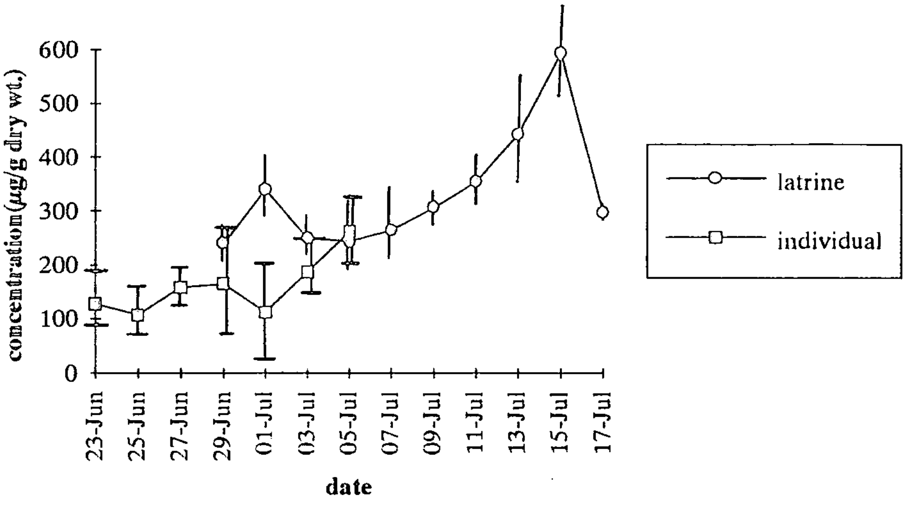


Fig.7 Temporal variation in the lead concentration(mean ± s.e.) of the individual and latrine faecal samples.

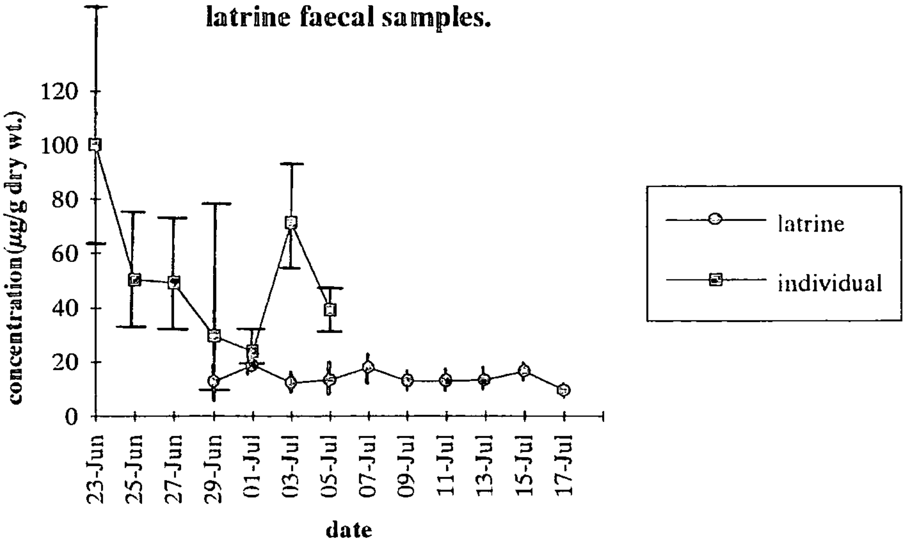


Fig.8 Temporal variation in the copper concentration(mean ± s.e.) of the individual and latrine faecal samples.

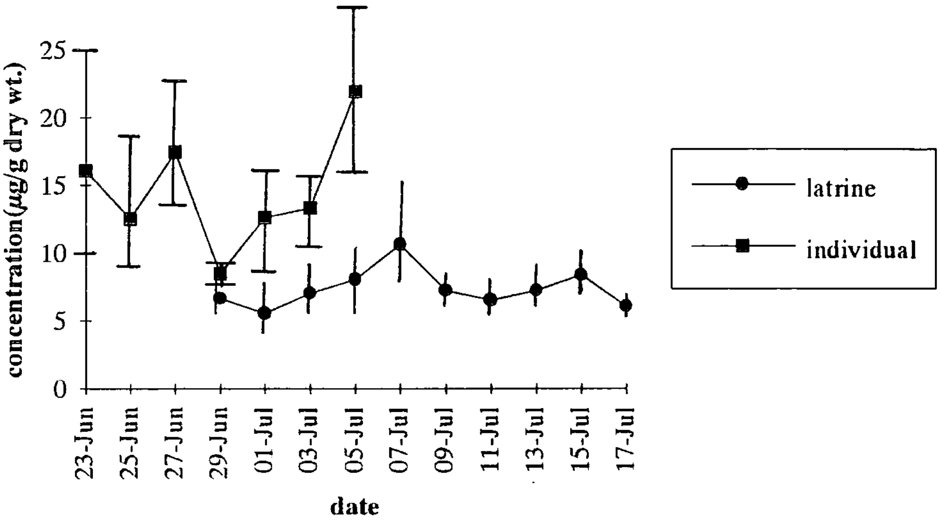
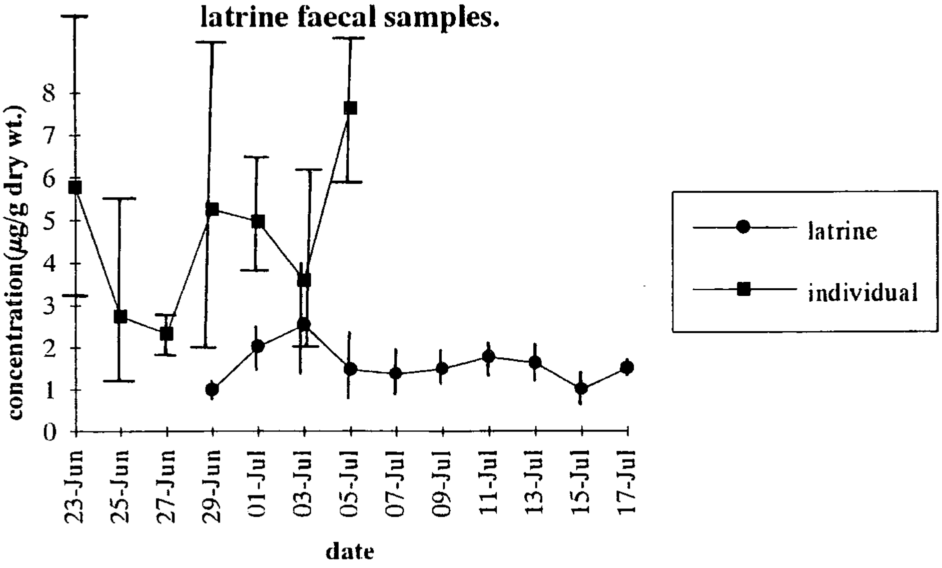


Fig.9 Temporal variation in the cadmium concentration(mean ± s.e.) of the individual and latrine faecal samples.



3.3 Variation in tissue heavy metal concentrations

There was significant differences between the metal concentrations (F=303.8, P<0.001, 3 d.f.), each metal concentration varied significantly (F=9.617, P<0.001, 3 d.f.) between tissues and there was also a significant metal-tissue interaction(F=8.7, P<0.001, 12 d.f.) (see Figs.10-13)

Fig.10 Zinc concentration(mean ± s.e.) in the carcass and tissues of the common tern chicks

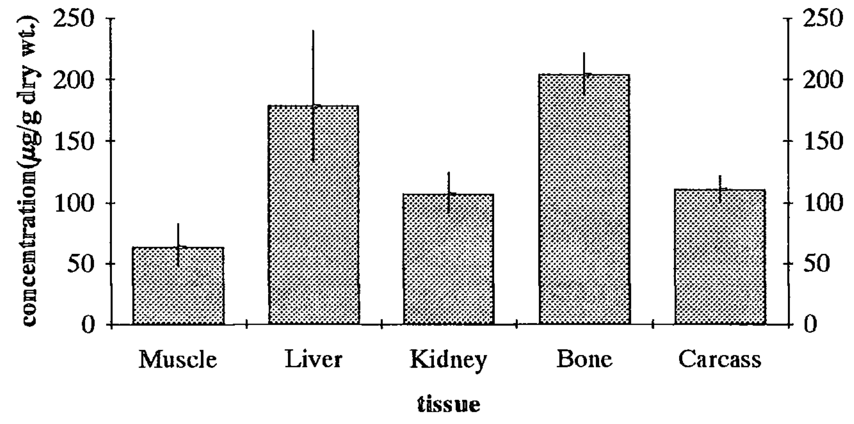
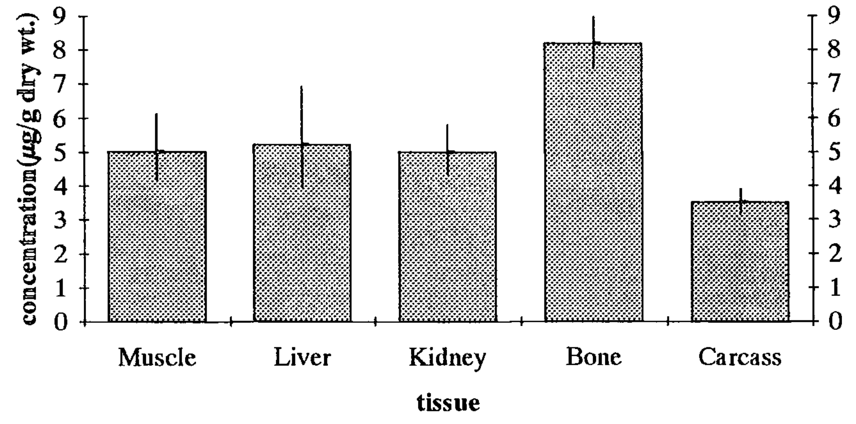


Fig.11 Lead concentration(mean ± s.e.) in the carcass and tissues of the common tern chicks



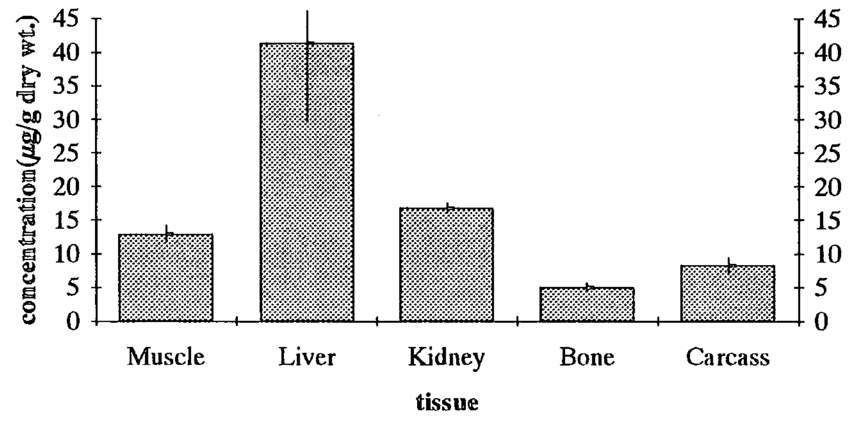


Fig.12 Copper concentration(mean ± s.e.) in the carcass and tissues of the common tern chicks

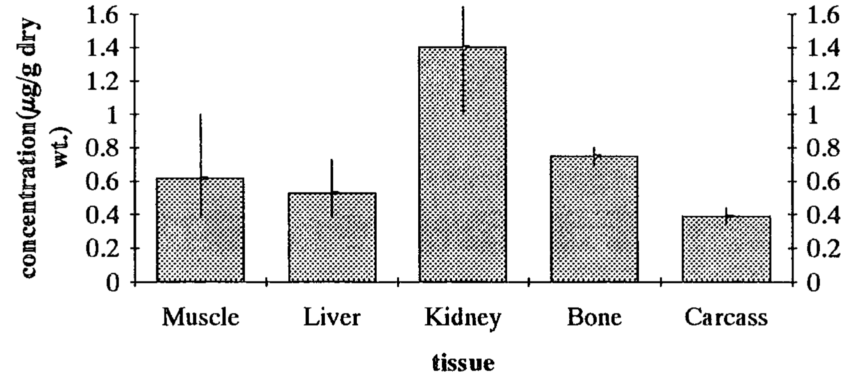


Fig.13 Cadmium concentration(mean ± s.e.) in the carcass and tissues of the common tern chicks

Zinc, lead and copper were detected in all tissues, carcasses and eggs sampled but cadmium was only detected in 24 of the 38 samples. Zinc levels were highest in the liver and bone and lowest in the muscle. Lead levels were highest in the bone and lowest in the carcass. Liver copper concentrations were the highest with copper levels lowest in the bone while cadmium levels were highest in the muscle and lowest in the liver. Table 4 displays the means and standard errors of each tissue, egg and carcass sample.

The metal concentrations in the eggs also varied significantly (Kruskal Wallis X^2 = 16.303, 3 d.f., P=0.001, n=6) with the Zn concentration highest and cadmium lowest, as expected.

There was no significant correlations between the tissues, eggs or carcasses for each of the metal concentrations nor was there any significant correlations found between metals for any of the tissues, eggs or carcasses.

Table 4. Metal concentrations (mean ± s.e.) of the carcass, tissues and eggs of the common tern chicks collected on the tern colony at the Tees estuary, expressed as $\mu g\ g^{-1}$ dry weight.

		Muscle			Liver			Kidney			Bone	
	+ s.e.	mean	- s.e.	+ s.e.	mean	- s.e.	+ s.e.	mean	- s.e.	+ s.e.	mean	- s.e.
Zn	82.5	63.5	48.9	239.9	178.4	132.7	124.8	107	91.7	221.6	203.6	187
Pb	6.13	5.03	4.13	6.93	5.24	3.96	5.8	5.01	4.33	8.98	8.19	7.46
Cu	14.19	12.92	11.77	57.48	41.35	29.76	17.44	166.77	16.11	5.64	4.95	4.34
Cd	0.14	0.14	0.0	0.156	0.153	0.15	1.967	1.402	1.0		0.75	

		Carcass		Egg	
	+ s.e.	mean	- s.e.	mean	s.e.
Zn	122.2	110.7	100.3	264.7	62.5
Pb	3.92	3.52	3.15	8.54	1.72
Cu	9.42	8.22	7.18	9.8	1.0
Cd	0.44	0.39	0.35	0.9	0.08

3.4 Feeding observations.

The average number of birds returning to the colony and the average size of the fish is displayed in Table 6.

Table 5. Estimates of feeding parameters of the common terns at the Tees estuary based on approximately 40 hours of observations

Average no. of birds returning to the colony per hour.	**277 ± 14**
Initial no. of hatched chicks on the colony.	**360**
Estimate of no. of fish given to each chick per hour based on the two estimations above.	**0.77 ± 0.05**
Observed size of fish brought back to the colony measured relative to size of adult bill (mean ± s.e.).	**1.69 ± 0.15**
Observed no. of chicks being fed by the adults per hour (mean ± s.e.).	**0.93 ± 0.08**
Observed no. of fish fed to the enclosed chicks per hour (mean ± s.e.).	**0.7 ± 0.1**

Given that the average bill size of an adult common tern is 36 mm and using the values obtained above, a value of 60 mm was estimated for the average size of the fish being fed to the chicks. From this it was further estimated that sprat of this size weigh approximately 2.1g (estimated using data from the pooled June fish samples). It was then calculated, again using the more applicable pooled June fish samples, that the average percent dry weight was approximately 22 % of the wet wt.

There fore using the following, the rate of fish intake by the chicks was estimated:

1) an average no. of fish fed to the chicks of 0.8 ± 0.1 g/hour.
2) a 15 hour feeding activity period (Kaye, 1989).
3) an average dry wt. of fish given to the chicks of 0.462g.

0.462 x 0.8 x15 = 5.544 g / day
0.462 x 0.7 x15 = 4.851 g /day
0.462 x 0.9 x15 = 6.237 g /day

Therefore the average intake rate = 5.544 ± 0.693 g dry wt. of fish / day.

This figure was used in the estimation of the rate of intake of each of the 4 metals. Furthermore the values for the pooled June fish samples were used in the estimation of the metal intake (Table 1).

3.5 Estimation of the heavy metal budgets.

The body burdens of each metal in each of the chicks is presented in Table 6 and a combination of both the intake and excretion rates accompanied by these body burdens of the chicks, with their estimated age, is presented in Tables 7-10.

Table 6. The estimated heavy metal body burdens(μg dry wt.) and the estimated age of each chick.

Chick no.	Wet weight(g)	Dry weight(g)	Age(days)	Zinc	Lead	Copper	Cadmium
1	7.88	2.9	0	203.91	6.77	14.4	1.41
2	10.68	3.4	0	270.38	9.77	18.01	ND
3	18.8	6.3	2	459.461	11.83	29.87	0.55
4	22.53	6.8	3	962.313	39.06	51.64	ND
5	28.8	9.3	4	643.524	15.14	73.63	6.438
6	29.6	9.6	4	1842.091	42.06	101.57	1.946
7	75.4	25.4	10	3453.65	74.61	459.122	9.203
8	84.1	27.3	11	1912.622	108.034	223.012	14.613
9	113.1	36.5	20	3752.18	117.41	691.942	19.847
10	120.6	40.3	22	4642.507	200.275	1017.226	19.315

ND = Not detected.

Table 7. Budget for zinc with the cumulative intake and excretion rates and the calculated zinc loading in chicks of that estimated age in days.

		Intake (μg)				Excretion (μg)			Storage (μg)		Intake: excretion	Intake: storage
Day	**lower**	**mean**	**upper**		**lower**	**mean**	**upper**					
0	202	265	327						204			1.3
1	505	591	682		97	164	251		270		3.60	2.2
2	1009	1183	1364		195	327	501		459		3.62	2.58
3	1514	1774	2046		292	491	752		962		3.61	1.84
4	2019	2365	2728		389	655	1002		644		3.61	3.67
5	2524	2956	3410		487	818	1253		1842		3.61	3.61
11	5552	6504	7501		1184	2342	3873		3454		2.84	1.71
12	6057	7096	8183		1296	2604	4336		1913		2.77	3.71
20	10094	11826	13638		1606	5690	11173		3752		2.08	3.15
22	11104	13008	15002		1606	6574	13255		4643		1.98	2.8

Table 8. Budget for lead with the cumulative intake and excretion rates and the calculated lead loading in chicks of that estimated age in days.

		Intake (µg)				**Excretion (µg)**			**Storage (µg)**		**Intake: excretion**	**Intake: storage**
Day	**lower**	**mean**	**upper**		**lower**	**mean**	**upper**					
0	6.82	8.54	10.26						6.77			1.26
1	30.87	39.87	50.38		28.72	48.42	74.28		9.77		0.82	4.08
2	61.74	79.74	100.75		57.43	96.84	148.56		11.83		0.82	6.74
3	92.61	119.61	151.13		86.15	145.26	222.85		39.06		0.82	3.06
4	123.47	159.48	201.50		114.87	193.68	297.13		15.14		0.82	10.53
5	154.34	199.35	251.88		143.58	242.10	371.41		42.06		0.82	4.74
10	308.69	398.70	503.76		316.54	615.21	1011.15		74.61		0.65	5.34
11	339.55	438.57	554.14		349.47	692.72	1148.25		108.03		0.63	4.06
20	617.37	797.40	1007.52		474.06	1683.08	3312.34		117.41		0.47	6.79
22	679.11	877.14	1108.27		474.06	1944.55	3929.54		200.275		0.45	4.38

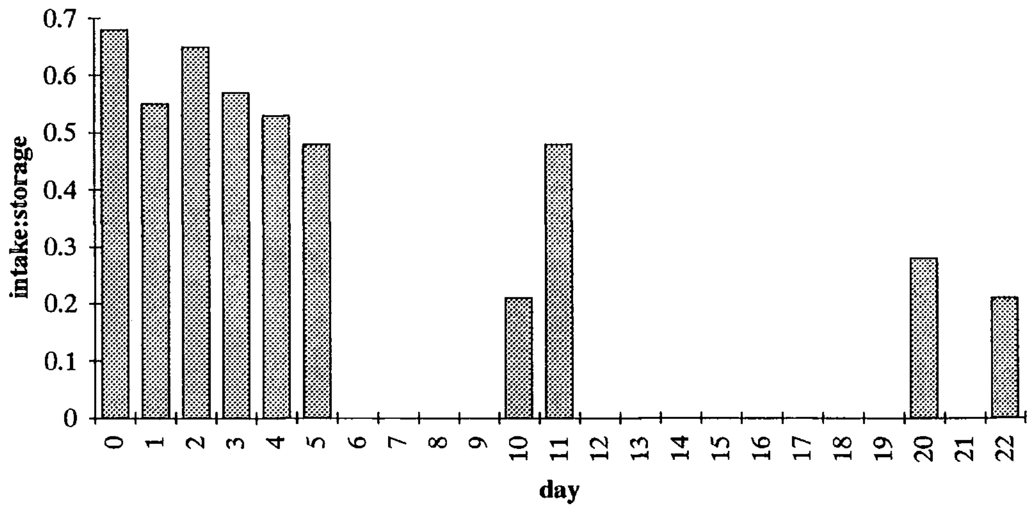
Table 9. Budget for copper with the cumulative intake and excretion rates and the calculated copper loading in chicks of that estimated age in days.

		Intake (µg)				**Excretion** (µg)			**Storage** (µg)		**Intake: excretion**	**Intake: storage**
Day	**lower**	**mean**	**upper**		**lower**	**mean**	**upper**					
0	8.80	9.80	10.80						14.4			0.68
1	8.28	9.82	11.48		14.38	12.63	18.74		18.01		0.78	0.55
2	16.56	19.65	22.95		28.75	25.26	37.48		29.87		0.78	0.65
3	24.84	29.47	34.43		43.13	37.89	56.22		51.64		0.78	0.57
4	33.12	39.30	45.91		57.50	50.52	74.97		73.63		0.78	0.53
5	41.39	49.12	57.38		71.88	63.14	93.71		101.57		0.78	0.48
10	82.79	98.25	114.8		195.7	160.46	255.1		459.12		0.61	0.21
11	91.07	108.07	126.2		222.2	180.68	289.7		223.01		0.6	0.48
20	165.6	196.49	229.5		641.0	438.98	835.7		691.942		0.45	0.28
22	182.1	216.14	252.5		760.5	507.18	991.4		1017.23		0.426	0.21

Table 10. Budget for cadmium with the cumulative intake and excretion rates and the calculated cadmium loading in chicks of that estimated age in days.

		Intake (µg)				**Excretion** (µg)			**Storage** (µg)		**Intake: excretion**	**Intake: storage**
Day	**lower**	**mean**	**upper**		**lower**	**mean**	**upper**					
0	0.82	0.90	0.98						1.41			0.64
1	3.72	4.42	5.16		1.91	3.38	5.43		ND		1.31	
2	7.45	8.84	10.32		3.83	6.76	10.86		0.55		1.31	16.07
3	11.17	13.26	15.49		5.74	10.14	16.30		ND		1.31	
4	14.90	17.68	20.65		7.65	13.52	21.73		6.438		1.31	1.31
5	18.62	22.10	25.81		9.56	16.90	27.16		1.946		1.31	11.36
10	37.25	44.20	51.62		21.08	42.93	73.94		9.203		1.03	4.8
11	40.97	48.62	56.78		23.28	48.34	83.97		14.613		1.00	3.32
20	74.49	88.39	103.2		31.57	117.46	242.2		19.847		0.75	4.45
22	81.94	97.23	113.6		31.57	135.71	287.4		19.315		0.71	5.03

Fig.14 Variation in the copper intake:storage ratio with time in the common tern chicks on the Tees estuary.



4. Discussion

4.1 Variation in the fish samples.

There was, as expected, a highly significant difference in metal concentrations of the fish samples but also a significant temporal variation for each metal.

There was a distinct pattern in the metal concentrations within both sprat and herring in the Tees estuary. Zinc was found in the highest concentrations and cadmium found in the lowest concentrations, with however the lead levels significantly greater that the copper levels. This pattern agrees with the findings of Hardisty *et al.*, (1975) with all the fish species investigated in the Severn estuary, having the zinc concentration highest, the cadmium concentration lowest and the lead found in intermediate concentrations. This disagrees slightly with the findings of Casson (1993) who found copper levels, again in both sprat and herring, significantly greater than lead levels. There are many factors which cause vast differences in metal concentrations within organisms such as the levels found in the surrounding environment, i.e. the water and sediment within the estuary, the uptake, regulation and excretion of each metal but also whether the specific metal is essential or non-essential for metabolic function of the particular organism. Therefore one possibility is that the heavy metal levels in the estuary have changed due to the short-term variability in estuarine environments which can greatly affect metal concentrations (Laslett, 1995). This could have caused a change in the lead and copper concentrations in the Tees estuary resulting in greater concentrations of lead being found in the sprat and herring compared to the copper levels. The copper levels may have decreased in the Tees estuary, which was found in 1990 by Davies *et al.* in the Tees estuary, rather than increasing lead levels, therefore suggesting that there has been a decline in the levels of copper within the estuary since this previous investigation. Results from the NRA in a study completed in 1991 (Casson, 1993), showed that metal concentrations (total and dissolved) in the power stations uptake water showed lead levels to be quite significantly higher than the copper levels but Casson's results did not reflect this pattern. However if this was due to a recent change in the lead and copper levels in the estuary, then the lack of a similar pattern in his results may have been because there was not sufficient time for this difference in the environmental levels to be reflected in the levels in those fish samples. It may be only now that we are seeing this pattern reflected in the fish from the estuary.

The zinc and cadmium levels found in this investigation seem to be similar to those found by Casson (1993), despite the fact that the tissues of the fish were analysed separately in that study as opposed to the whole fish analysis necessary for this investigation.

The bioavailability of each metal will ultimately determine the level within an organism. Therefore the differences between the metal concentrations within the fish are related to the preferential uptake of each metal, its bioavailability, aswell as its requirement in metabolism. Also the shift between the lead and copper levels over the past few years since the results of Casson's (1993) study may be due to a change in the chemical form (or proportions of the different forms of lead and copper) of lead or copper in the estuary, resulting in a change in its bioavailability and subsequent uptake by the fish.

There was a significant temporal variation in the metals with, however, no set pattern. The presence of the significantly different results in June is probably due to the fact that the fish sampled in this month were much smaller than the other months. The higher values of zinc and other metals could be due to a change in of diet, differences in feeding intensity or movements into the area from other regions (Hardisty *et al.*, 1995). They also found a distinct seasonal variation in the zinc concentration in the flounder from the Severn estuary, where they found a decreased zinc concentration with increasing length(age) and it was suggested that this may have been due to metabolic factors.

It has been suggested by Phillips (1980) that there are three primary factors contributing to the seasonality of pollutants:

1) The delivery of the pollutant to the aquatic environment.
2) the physiology of the organism.
3) Changes of ambient water quality parameters such as temperature and salinity.

Therefore the temporal variation in the metal concentrations in the Tees estuary may have been caused by one or all of the above factors.

Each monthly fish sample collected from the intake screens of the power station was a random sample of that contained in the bins. Since most fish in good condition were found in the top layers of the intake screens/bins, most fish were taken from this area. This may have resulted in a biased sample but this would have occurred at each of the four months, so this effect should have been nullified

However it is likely that there was significant variation between these samples due to differences in the fish populations dominating the area next to the intake screens at the end of each month. These samples could also have differed, like in the case of the June sample, due to the fact that there may have been a large shoal of similarly sized fish sucked onto the intake screens just prior to the sample being taken.

There is also variation to be found between individual fish of the same species but also between different populations and this may contribute to the overall temporal variation in each metal concentration between the four months. These varying temporal patterns of metal accumulation may reflect the varying periods, in which the sampled sprat and herring, had actually spent within areas of high/low metal concentrations within the estuary (Hardisty *et al.*, 1975). It was also found by Davies *et al.*, (1990) that the concentration of all four metals were lower at the seaward end of the estuary relative to concentrations found adjacent to Seal Sands and in waters upriver. Therefore variation in the metal concentration of the water may have contributed to the variation in fish samples and the differences between the sprat and herring.

Another significant result is the difference in the lead, copper and cadmium concentrations between sprat and herring. This generally agrees with the findings of Casson (1993) where there was also significantly higher concentrations of all metals in the sprat. This difference in metal concentration between species may be the main factor causing the temporal variation in metal concentration because of the fact that fish samples in March were a mixture of sprat and herring, the fish samples from April were composed nearly entirely of herring whereas the May and June fish samples were composed completely of sprat. Therefore given the fact that the fish samples from May and June both contained only sprat, the significant differences between the two time periods may be accounted for with the difference in the size of the fish, with the June samples, as mentioned previously, much smaller than the other samples. The actual difference in heavy metal concentration between the two species, taking into account that they are both clupeids and therefore have similar lifestyles and activities, may be attributable to minor differences in their diet, age of the fish or the metabolic requirement for the metal. It may be that the sprat arrived in the estuary before the herring and therefore have been exposed to the high heavy metal levels for a longer period of time. It may also be due to efficiency of excretion in both species. The herring may be able to excrete a higher proportion of the metal ingested under the contaminated conditions in the Tees estuary thus having lower levels when compared to sprat (Bryan, 1976). Another possibility is that the channel on the Tees estuary was dredged at some time between March and July, and this may have contributed to the temporal variation in metal concentrations through mixing and the re-suspension of metals into the water column (Davies *et al.*, 1990).

The correlation results show that, for both species, as the fish get bigger, the heavy metal concentration decreases, (except for copper which shows no correlation) thus indicating the absence of metal bioaccumulation in these two species. This disagrees with Marchovecchio *et al.* (1988) who found evidence for bioaccumulation of zinc and cadmium in the sole, *Paralichthys* sp. Therefore given that the sprat are much smaller than the herring (range of 79-112 mm, as opposed to 100-162 mm), it may be that this size difference is causing the higher metal levels in the sprat. This may be due to a dilution of the metal concentration with increasing growth, as suggested by

Stronkhorst (1992) when looking at mussels. Alternatively it may be due to the presence of a lower fat content resulting in this higher metal load (Grimas *et al.*, 1985).

4.2 Variation in the faecal samples.

From the results it is evident that the zinc concentration is significantly greater in the latrine faecal samples whereas the concentrations of lead, copper and cadmium are significantly greater in the individual faecal samples. Since the individual faecal samples were collected directly from the chicks while they were being weighed, it was decided to use these results, as opposed to the latrine faecal samples, when attempting to derive the metal budgets. It was felt that these results were a more accurate reflection of the heavy metal concentration voided normally from the cloaca given that both faeces and urine are voided together in birds. Therefore two types of metal elimination is occurring in these chicks, that of the excretion of undigested metal in the faeces but also metals that have been absorbed through the gut wall, metabolised in the kidney and then excreted in the urine. Thus in the individual faecal samples both types of excretion were collected when the chicks defecated during weighing and therefore it is probable that the samples collected from the latrines differed in some way in it's actual composition. It could be that some of the urine voided with the faeces was lost, maybe by running off the plastic after defecation. It is quite possible that some faeces was lost by chicks walking across the plastic and inadvertently picking up some faeces, thus affecting the sample taken for analysis. Alternatively it may have been due to the fact that some of the latrine faecal samples may have been contaminated with mud and even though great care was taken in separating out the mud, it is quite likely that some samples remained contaminated.

Also the latrines were placed in enclosures containing two, three or four chicks and thus the faecal samples collected could not differentiate between chicks and thus the individual variation in the heavy metal concentration of the faeces and urine excreted by each of the chicks could have resulted in these significantly different metal concentrations in the latrine samples.

Each piece of plastic was laid down at approximately 15.30 during the final weighing of the chicks and they were not collected until 2 days later during the first weighing. Therefore there is a period of approximately 42 hours between collection of each latrine sample. It may be that during this relatively long period that some of the metals may have leached from the faeces or somehow metals were lost from these samples.

There was also quite a large amount of faeces collected from most of the latrines, and since only a small sample of this could be taken for analysis, this could have resulted in a biased sample being taken giving these markedly different results.

4.3 Variation in tissue heavy metal concentrations.

The metal concentrations in the liver of the chicks in this investigation may have been over-estimated. The time period between collection of the chicks and its time of death meant that it is likely that the liver volume of the chicks had decreased thus resulting in over-estimated liver concentrations.

Levels of all metals were quite high in the carcass samples and this is not unexpected. The carcass contains everything except for the sample of muscle, liver, kidney and bone which was previously dissected from the chicks and given the fact that the feathers may contain relatively high levels of some metals (Burger *et al.*, 1994a; Burger & Gochfeld, 1992; Burger *et al.*, 1994b), it is not surprising that the carcass contains high levels of the four metals.

Since we do not actually have any tissue metal levels from adult common terns on the Tees estuary, any accurate comparisons with adults in other investigations from other areas, may be somewhat misleading. However comparison with levels of metals in adult common terns by Connors *et al.*(1975), may be sufficient given the fact that they used both an unpolluted site and a polluted site, where there were no significant differences between the two.

4.3.1 Zinc

Zinc concentrations are the highest of the four metals and this is primarily because it is found in the highest concentrations in their diet but maybe also because zinc is an essential element being necessary for metabolism and cellular functions. Zinc is found in the highest concentration in the bone and then the liver with the lowest concentration in the muscle. The liver concentration being greater then the kidney concentration was also found by Muirhead and Furness (1988), in five adult seabird species but, more applicably, the levels of zinc found in this investigation agree quite well with the findings of Connors *et al.*, (1975). The zinc concentration in the bone, kidney and muscle are very similar with however a greater concentration of zinc found in the livers of these chicks. This may indicate that the chicks do, indeed, reach the levels of the adults relatively quickly and then begin to efficiently regulate the concentration of zinc, or they are simply less efficient in excreting the metal (Gochfeld & Burger, 1987).

4.4.2 Lead

The highest concentration of Pb is found in the bone and this is as expected considering the fact that lead primarily accumulates in bone tissue and also is quite stable once deposited there (Elliot *et al.*, 1992; Custer *et al.*, 1992; Howarth *et al.*, 1981). The levels of Pb for all tissues, being between 3 and 8 ppm were far greater than those reported by Howarth *et al.*, (1981) for adult common terns from an industrialised area, where they found concentrations of <1 ppm for all tissues. This may be an indication of the extent of Pb pollution in the Tees estuary but the results from Gochfeld & Burger (1989) suggest that common tern chicks rapidly accumulate Pb on the first two days after hatching, peak Pb levels are found on the third day and then there is a significant decrease in chicks aged 20 days. Therefore it may be that the high concentrations of Pb in the chicks in this investigation could be rapidly lost with time. However Connors *et al.* (1975) also found the highest level of lead in the bone but the figure in this investigation was much greater than the level found in the chicks, indicating the possibility that lead accumulates with time.

4.4.3 Copper

These are the most interesting since all the copper concentrations for each tissue, except the bone, are far greater than that for lead, even though lead is ingested in far greater concentrations. It may be that since copper is an essential element, it is being accumulated and stored in these high levels to be used in metabolism and development of the chicks. The trend in the tissue distribution is quite similar to that found by Connors *et al.* (1975) for adult common terns. In that investigation the bone copper levels were significantly lower than the other tissues and the liver concentration was the highest, which was found in this investigation. Similarly, it was also found in that investigation that the breast muscle had, in general, a lower copper concentration than the kidney.

The actual concentration of some tissues however differs significantly between these two investigations with the disputable liver concentration far greater in this investigation, and the mean bone concentration, fractionally lower but quite similar when the errors are taken into account. However the breast muscle and the kidneys in the adult terns investigated by Connors *et al.* (1975) had greater copper concentrations than the chicks in this investigation. This may indicate that as the terns get older they begin to regulate the copper concentration in the liver but may accumulate the metal in the muscle and kidney.

There is also a very similar pattern found in the copper levels and the zinc levels where all tissue copper and zinc concentrations are similar in the adults and chicks, with the possible exception of the liver which has a greater copper and zinc concentration in the chicks. It is likely that the heavy metals in the liver are over-estimates but to what extent is unknown. It may be that the levels are only slightly over-estimated and as such the levels may be still quite high and accurate. Since these two metals are the two essential elements it is likely that the chicks are absorbing large amounts from the food into the bloodstream which subsequently has resulted in these high liver concentrations. It may be that since these chicks must develop and grow rapidly, the requirement for these two metals during this period may be very high, which may account for these high liver levels.

4.3.4 Cadmium

The figure for kidney cadmium concentration is likely to be an overestimate, as it was based on only one surprisingly high reading which could have occurred due to experimental error. In adult seabirds it seems to be that kidney Cd levels are usually much greater than liver levels (Elliot *et al.*, 1982; Howarth *et al.*, 1981) but in juveniles this pattern may not be the same. Elliot *et al.*, (1982) found that in juvenile cormorants, the liver Cd levels were significantly higher than the kidney and since cadmium levels were detected in most chick liver samples but only one kidney sample, that trend may be the same here.

Since cadmium levels in adults exceed those of juveniles for most species studied (Ferns & Anderson, 1984 ; Maedgen *et al.*, 1982), it can be said that Cd seems to be accumulated with time. Therefore it is not surprising that Cd was not detected in 24 of the 38 samples given the fact that the sample size taken may have been too small coupled with the fact that the levels in these young chicks must have been quite low.

The lack of a significant difference in Cd concentration from the youngest to the older chicks would tend to suggest that the expected accumulation is not occurring. It has been shown that Cd accumulation is related to its binding to special low molecular weight proteins called metallothioniens (Gochfeld & Burger, 1982) and therefore it may take a long period of time for the development of these proteins, so that they may not have yet developed even in the larger chicks.

This quite stable and low concentration of Cd in the very young and prefledging chicks agrees well with the findings of Maedgen *et al.* (1982) who found little change in the average values of Cd between downy young and prefledglings.

The cadmium concentration in the tissues of these common tern chicks are much lower than the adult common tern levels found by Connors *et al.*(1975). All tissues, except for the muscle which had a similar level, had much lower concentrations which may show that cadmium does tend to accumulate with time in common terns.

Finally the results of the correlations between tissues showing no relationships among any of the tissues or between metals may be indicative of the "correlational chaos" suggested by (Gochfeld and Burger, 1987), probably reflecting the immaturity of their physiological defence mechanisms and the absence of a dynamic equilibrium. Also the differences in metal levels in the chicks could have arisen because of the way that the chicks process and store the particular metals.

4.4 Feeding rates.

The size of the fish being brought back to the chicks on the colony did increase slightly by the end of the investigation but rather than incorporate these slight changes into the budget calculation it was decided to use the mean size of the fish. Further, the rate of feeding of the chicks also varied due to factors such as the weather , wind speed, tidal cycle and again it was decided to use the mean number of fish being brought back to the colony in the calculation of the budget. It was thought that the incorporation of these changes into the budget calculation would have proved too complex and unnecessary and it was felt that the errors used were sufficient in taking into account the many differences in feeding rates.

This data is probably more accurate than the rest of the data in this investigation but, however, it too is open to substantial variation. The final figure of 5.54 g ± 0.69 g dry weight of fish per day is likely to be quite valid but unfortunately it doesn't take into account all possible errors. There is an error associated with every variable in this investigation and for the feeding rate, errors could have been used in the estimation of the fish size, the estimation of the average wet wt. calculated from this fish size and the percentage dry weight conversion factor of the fish. However it was decided to use the calculated average figure for the above variables and use the errors associated with the amount of fish fed to the chicks per hour. This figure of 0.8 ± 0.1 agrees quite well with other investigations (Courtney & Blokpoel, 1980; Langham, 1972; Erwin, 1977) and should be reasonably accurate particularly given the data found by Robinson (pers. comm.). Therefore the use of only this error should be sufficient but, of course, it may have resulted in an inexact intake rate.

The actual feeding rate of the chicks varied quite significantly which was reflected in the growth rates of the chicks, which showed numerous peaks and troughs on different days (Robinson, pers. comm.) and this is due to a number of factors previously mentioned. Wind speed, clear or dull days, the tidal cycle, the diurnal cycle, the density, abundance, visibility etc. of the prey (Cramp, 1985; Dunn, 1975; Hume, 1975), all affect the feeding success of the common terns and therefore the number of fish fed to the chicks varied a lot. Therefore even though there was significant variation in the feeding rates of the chicks it was decided to take the average number of fish fed to the chicks and use this in the budget. The calculated value in this investigation, as mentioned, agreed quite well with other studies, and so this seems to be valid.

4.5 Heavy metal levels in the eggs.

The level of all metals in the eggs sampled in this investigation were quite similar to levels found by Burger & Gochfeld (1988) in their eggs sampled in the New York Bight region in 1982. Given that their values are in ppb, based on a fresh wet wt. basis, an approximate dry weight conversion was obtained by multiplying by three. This shows very comparable levels of copper and cadmium with the values obtained for zinc and lead slightly lower than that obtained in this investigation. However taking the values obtained from the eggs sampled in 1971, we find a much different situation. The levels of all four metals, in the NY Bight, have drastically decreased since 1971 but the levels at that time were much greater than those obtained in this investigation. The values obtained by Connors *et al.*, (1975) for common tern eggs, are also quite similar to those obtained in this investigation. Two other studies by Burger & Gochfeld (1991 & 1993) showed significantly lower lead and cadmium concentrations in common tern eggs, again from the NY Bight area. The study in 1991 had a mean lead level of 0.89 ppm and a mean cadmium level of 0.004 ppm which is much lower than found in this investigation. The other study in 1993 from two other areas of the NY Bight had lead levels of < 1 ppm and cadmium levels < 0.02 ppm. This shows that relative to their recent studies from this NY Bight area, the lead and cadmium levels have fallen significantly and since the levels found in the eggs in this investigation were now far greater than those values, it can be said that the level of heavy metal pollution in the Tees estuary may, at present, be much greater than the NY Bight. They suggested that the decreased lead levels in the eggs may have likely been as a result of the decreased lead emission from petrol thus decreasing the atmospheric lead pollution. It is quite likely that this is a primary source of lead deposition given the high volume of traffic at Teesside and this may contribute significantly to these significantly high lead levels in the common tern eggs at the Tees estuary.

Females sequester heavy metals in their eggs in order to reduce their body burden even though it is suggested that this may have detrimental effects on the developing embryo (Burger & Gochfeld, 1993). The levels of heavy metals deposited in the eggs is determined primarily by the heavy metal levels in their food during courtship and breeding but also from stored body burdens. It is thought that the nutrition of the female at the time of egg-laying makes the more significant contribution to the heavy metal levels in eggs, since Petering (1978) postulated that nutritional status influences the uptake and metabolism of metals. The main problem associated with heavy metal levels in eggs is the difficulty in assigning metal burdens to the immediate dietary intake rather than to mobilised tissue stores. Therefore given the relatively high levels of heavy metals in the diet of the common terns from the Tees estuary, it is not surprising to find the elevated levels of all 4 metals in the eggs.

However taking into account the amount of heavy metals in the food, it can be said that the females here sequester a relatively small percentage of the total heavy metal ingested, into the eggs. Therefore most of the metals must be either excreted or stored and since only a relatively small percentage of heavy metals seems to be stored, the vast majority of heavy metals ingested at the time of egg-laying must be excreted.

Laboratory experiments have shown that very little cadmium and lead is transferred to the eggs regardless of the amount consumed. This has been confirmed by seabird data with concentrations usually less than 0.7 mg g^{-1} fresh weight in eggs despite high levels of cadmium being found in the kidneys of the adult seabirds (Furness, 1993).

Overall the variation in the heavy metal levels between the eggs was quite large and the sample size was quite small (n=5) but the values obtained were still adequate to draw the above comparisons.

4.6 The heavy metal budgets.

Unfortunately the discussion of each heavy metal budget is made more difficult given the very wide errors found with each excretion rate. This could not be overcome since the excretion rates were derived from the results of an experiment which itself had very large errors. However the budgets will be discussed primarily in relation to each mean value, but where applicable, the associated errors may have to be accounted for.

The age of each chick collected for the purposes of storage of the metals, was estimated from the growth rate (Robinson, pers. comm.) and the wet weight on collection of the chick (Appendix 1). Obviously this is a rough estimate with the possibility that the weight on collection was different than that at the time of death of the chick.

Some of these chicks may have died due to a disease or possibly a bacterial infection because there seemed to be no significant loss of weight before dying, being indicative of starvation nor was it likely to have been due to stress as the other chicks in the broods survived. However it is known that the three largest chicks sampled died because they got caught in a thick mat of algae and were unable to fly out of it.

The great variation in the excretion rate based on the very variable individual faecal samples may be due to the fact that these young chicks have yet to reach an equilibrium between intake and excretion. These chicks are ingesting much greater quantities of food relative to their body mass and as such they are undergoing rapid growth (Gochfeld and Burger, 1987).

4.6.1 Zinc

The two smallest chicks (1 and 2) with wet weights of approximately 7.88 and 10.7g respectively suggests that these were not fed any fish at all given the mean wet weight of a new-born chick was approximately 13g. Therefore it could be that these chicks are the third or possibly fourth egg to hatch from a brood and maybe were neglected when the parents were feeding the brood. Since the average zinc loading of the eggs, taking into account the standard error, was very similar to the body burden found in the these chicks, the non-feeding of these chicks seems to be a feasible explanation.

It is apparent that there was an accumulation of zinc with increasing age of the chicks. There were two anomalous readings for chick number 4 and 7, which had a body burden greater than the next biggest chick. This could be merely a reflection of the great variation in metal levels of individuals within a population. It may also be that the wet weight on collection of the chick were much lower than their actual wet weight at the time of death thus underestimating the age of the chick.

The overall large accumulation of zinc in the early days of chick growth may be because zinc is an essential element for metabolism and as such the chicks are accumulating a greater proportion of zinc to use in their development. As they reach prefledging age, the zinc requirement may have decreased giving rise to the lower percentage accumulation of the zinc and possible regulation of the metal.

All in all, when one looks at the budget of each chick using each mean value there is a significant shortfall between the intake and the storage and excretion. Therefore if we assume that the calculated body burden of the chicks was quite accurate, this may be an indication that either the intake rate of zinc is being overestimated or that the excretion rate is underestimated. However one other possibility is that zinc was being excreted through their nasal salt glands. It was noted during the weighing of these chicks that liquid exuded from this area of the chicks and from the findings of Mercer (pers. comm.) it is likely that some zinc may be excreted from these glands. High levels of the four metals, zinc, lead, copper and cadmium were found in the salt glands of the crested tern (Howarth *et al.*, 1981) and therefore it is highly likely that the salt glands are probably involved in the attempted elimination of zinc and the other metals via the nasal secretions.

4.6.2 Lead

These results, unfortunately, are where the errors for intake and excretion have to be taken into account. If only the means were looked at, then one would see that the excretion rate exceeds the intake rate on every day. Therefore either the intake rate is underestimated or the excretion rate is overestimated. As mentioned, because of the large errors associated with these values it is more likely that the excretion rate is overestimated since the intake rate is probably more accurate. There is an accumulation of the metal with time, but again , we have an anomalous reading for chick number 4. It, therefore, is highly likely that this chick's weight had decreased between its time of death and collection. Therefore it may be that this chick is slightly older than its estimated age.

The proportion of lead accumulated is lower than that for zinc. It may be that since lead is a non-essential element, the chicks are attempting to excrete it in a greater amount. Therefore since the mean excretion rate is probably an overestimate it is hard to discuss these results with much degree of accuracy but it can be said that a significant proportion of the lead ingested is subsequently excreted, something of the order of 80 %, given that the approximately 20% of the lead is stored in the chicks.

4.6.3 Copper

Again these results are the most interesting given that even the lower value for the excretion rate is greater than its corresponding upper intake value. For the first 4-5 days it is only slightly higher but from days 6 onwards, the difference becomes increasingly more marked with a difference of approximately 344 mg on day 26. Storage of the copper is markedly greater than the upper copper intake value aswell with a far greater value for the chick aged approximately 22 days, compared to the upper copper intake value. Therefore one possible reason for these storage results is that the chicks are getting a large input of copper from some other source. It could be that the soil used to make the island has a large concentration of copper and that the chicks are pecking at the soil thus ingesting more copper.

It may also be due to significant variation in the copper levels of the food brought to the chicks at this time. It may be that the copper levels in the fish were much higher than the fish samples analysed thus giving rise to an under-estimated intake rate and the relatively high storage of copper. However this is unlikely since the fish samples collected from the colony did not differ from the other fish samples analysed.

It was found that the liver concentration contributes the highest copper levels to the overall body burdens and it was found by Lock *et al.* (1992) that the copper levels in the livers of the some juvenile seabird species were far greater than that of the adults.

Again we see a similar trend for copper accumulation over time with the larger chicks containing higher levels of cadmium compared to the younger chicks.

4.6.4 Cadmium

As with lead, we find a similar trend here with the same approximate proportion of cadmium accumulated but however the cadmium levels are much more variable in the younger chicks. In the four older chicks we see an accumulation of between 20-28% which is quite similar to lead. This may have been expected since cadmium and lead are both the non-essential elements.

However again we see an overestimate of the mean excretion rate with higher cadmium levels being excreted from days 12 onwards, therefore the errors must be taken into account.

4.7 Error associated with rate of intake and excretion of the heavy metals.

The most important point to note is that the intake values are more accurate than the values for excretion. Since there was adequate data on feeding rates which gives a relatively accurate estimate of the number of fish being fed to the chicks and given the relatively large sample size of the fish taken for heavy metal analysis enabling an estimate of the levels to be found in the diet of the chicks, it can be said that the accuracy of the rate of intake, albeit with some degree of variation itself, is superior to the estimation of the excretion rate. The excretion rate was estimated using two quite variable components with the main one being the weight of the faeces dropped by common tern chicks per day, when fed herring, by Massias and Becker (1990). Their experiment is beneficial in giving a rough estimate of the rate of excretion and the results obtained were quite valid and applicable to this investigation but the error associated with the values obtained were quite large. This was primarily due to the small sample size in the experiment. Three values were obtained, one for chicks 3-5 days old, one for chicks 8-12 d and the other for chicks > 18 days old. However the error associated with each one was of the order of approximately a third, a half and for >18 d, the error was equal, to the mean. It is clear that the error involved was great and, as such, the error values given here are also very large since the excretion rate was interpolated from the results of Massias and Becker. These chicks were also fed *ad libitum* for 15 hours of the day and hence this is likely to be a much better feeding rate than found in their natural environment and as such, it is likely to have been superior to the chicks in this investigation. Therefore it is quite likely that the excretion rate given here may be an over-estimate.

4.8 Intake:excretion ratios.

All metals displayed the exact same trend albeit on different scales but this is simply due to the fact that all the variables used to calculate both the intake and excretion had fixed means and standard errors. Since there was no significant temporal variation in the metal concentration of the individual faecal samples, one figure was used to calculate the cumulative rate of excretion and also the one figure from the pooled June fish samples was used in the intake rate calculation, thus giving the same trend for each metal. The decrease in the intake:excretion ratio in the four older chicks was due to the fact that there was three different figures used for the actual rate of excretion, which were interpolated from Massias and Becker (1990) using their three estimations of the number of faeces dropped in chicks from three different age brackets. The chicks aged 10 and 11 days fell into the second of the age brackets and, as such, have a different intake:excretion ratio and the two oldest chicks had their excretion rate interpolated from the third different age bracket, thus giving rise to this further decreased ratio.

Therefore even though these ratios are calculated from fixed variables which gave rise to these identical trends for all metals, it does seem to be that the older chicks are excreting more than the younger chicks but again this is based on the results of Massias and Becker which had quite large errors associated with them.

4.9 Intake:storage ratio

These results were quite variable for all metals again showing the great individual variation in excretory efficiency and/or metal storage. The copper results seem to indicate that the older chicks were storing more copper compared to the younger chicks (Fig.14).

5. Conclusions

1) The heavy metal concentration of the June fish samples was significantly lower than the previous three months due primarily to the smaller size of the fish.

2) The heavy metal concentration of the individual faecal samples were significantly different to the latrine faecal samples but were a more accurate reflection of the metal concentration voided normally in chicks.

3) The concentration of lead in the fish was much higher than the copper concentration but a greater concentration of copper was accumulated by the chicks possibly due to its essential requirement in metabolism.

4) Elevated levels of all metals were found in the common tern eggs.

5) The very high copper body burden of the common tern chicks is worthy of future study to ascertain the exact reason for this unusual accumulation.

6) Interpretation of the heavy metal budgets was difficult due to the large errors associated with the intake rate and more notably, the excretion rate.

7) There is much scope for further ecological research in the estimation of the excretion rate of heavy metals in chicks.

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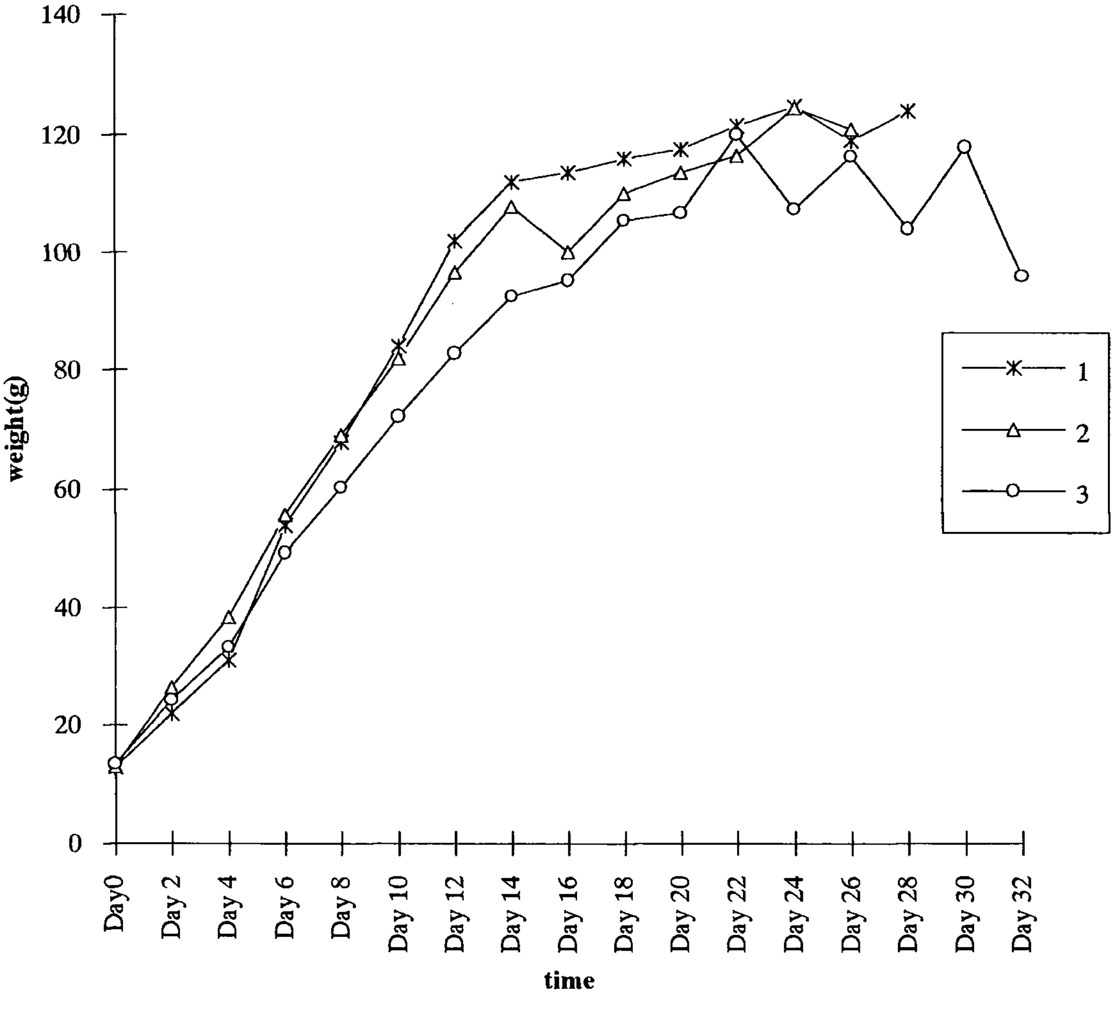
Appendix 1

Growth rates of the common tern chicks on the Tees estuary using mean weights over time (1 = First chick to hatch in brood, etc.)



Appendix 2

Calculation of the heavy metal budgets of the common tern chicks on the Tees estuary.

Estimation of the intake rates.

Zinc

5.544g= mean no. of fish eaten by a chick per day.
106.6547 μg g^{-1} = mean zinc concentration of the pooled June fish samples.

(5.544 ± 0.693) x 106.6547(+ 2.68: -2.61) for each day.
Day 1 ==> mean = 591.29 μg Zn on day 1 (upper s.e. = 681.9: lower s.e. = 504.71)
Day 2 ==> mean = 1182.6 μg Zn on day 2 (upper s.e. = 1363.8: lower s.e. = 1009.42)

Lead

(5.544 ± 0.693) x 7.1915416 (+ 0.89: - 0.83) etc. etc.

Estimation of the excretion rates

The cumulative excretion rate was estimated from Massias and Becker(1990) given their values of :-

Age of chicks in days, 3 - 5 d ==> 0.877 ± 0.263 g faeces dropped per day
8 - 12 d => 1.404 ± 0.700 g
>18d => 2.368 ± 2.368

The mean ± s.e. for 6-7 d was averaged from the means and s.e.'s for 3-5 d and 8-12 d and the mean ± s.e. for 13-17 d was averaged from the figures for 8-12 d and the >18 d.

Therefore a cumulative excretion rate was estimated for each day of the approximately 26 days to fledging (Tables 7-10). Each cumulative daily value of the weight of faeces excreted was multiplied by the mean ± s.e. value for each metal obtained from the individual faecal samples mentioned above (Table 3)

e.g. Day 2 ==> 1.754 ± 0.526 g faeces excreted / day
186.64(+ 33.15: -28.15) mean ± s.e. conc. of Zn excreted / day

mean ====> 1.754 x 186.64 = 327.37 μg Zn excreted on day 2
upper s.e. => (1.754 + 0.526) x (186.64 + 33.15) = 501.12
lower s.e. => (1.754 - 0.526) x (186.64 - 28.15) = 194 .63 etc. etc.