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TRACE ELEMENTS IN DEEP-WATER FISH SPECIES FROM THE ROCKALL TROUGH

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SUMMARY

Samples of three species of deep water fish (*Nezumia aequalis*, the smooth grenadier, *Lepidion eques*, the largeye lepidion, and *Raja fyllae*, the round ray) were obtained from 850-950 m depth on the continental slope of the Rockall Trough west of Scotland (58-59°30'N) in March 1998. Muscle, liver and gill tissues were analysed for cadmium, copper, lead, zinc, mercury, nickel and chromium by various atomic absorption techniques. Median concentrations in muscle tissue were 0.004, 0.21, 0.005, 0.150, and 3.91 mg/kg respectively for the grenadier; 0.005, 0.17, 0.002, 0.077 and 2.62 mg/kg respectively for the lepidion and 0.012, 0.33, 0.027, 0.129 and 5.53 mg/kg respectively for the ray (nickel and chromium were not detectable). In general, the concentrations are similar to those previously reported in other species from this area. Differences in accumulation patterns between species and elements are described using univariate and multivariate statistics (discriminant analysis).

INTRODUCTION

Deep-water fisheries are growing in importance, particularly in the North Atlantic. In comparison to shelf species, relatively little is known about the "natural" levels of trace metal in deep-water species and there is increasing interest in the degree of contamination of the deep-sea environment. Significant effort has been applied recently to the study of deep-sea fishes. Work on the biology and ecology of these species is leading to a better understanding of stock size, production and behaviour (eg Gordon *et al.*, 1987, 1994; Savvatimsky *et al.*, 1993).

The main concerns over chemical contamination arise because deep-water species are long-lived and tend to feed at higher trophic levels than their shallow water counterparts (Gordon *et al.*, 1995), allowing a potentially higher level of accumulation of trace metals, with particular concern for mercury levels. Some of these species are commercially exploited and fisheries scientists and consumers need information on the levels of contamination in these fishes and whether they meet the statutory levels recognised as safe for human consumption. Few data are currently available on levels of trace metals in deep-sea fish (Windom *et al.*, 1987; Vas *et al.*, 1993; Cronin *et al.*, 1998), and in some cases appear to be contradictory. Most of these data concentrate on levels of metals in muscle tissue only, without any broader picture of accumulation processes and distribution of metals between organ tissues in these species.

This paper reports trace metal (Cd, Cu, Pb, Zn, Ni, Cr and Hg) concentrations in gill, muscle and liver tissue in three species of fish from the Rockall Trough.

MATERIALS AND METHODS

Species Studied

All fish were collected at the beginning of March 1998 from the discards of a single commercial vessel working out of Lochinver, Wester Ross, Scotland. About 30 individuals of three different species were caught at 850-950 m depth between 58°N and 59°30'N in the Rockall Trough, off the west coast of Scotland.

The following description of the species is based on Gordon *et al.* (1996), Whitehead *et al.* (1984) and Scott and Scott (1988). None of the species studied are currently of commercial importance.

Nezumia aequalis - Smooth grenadier

The Smooth grenadier, *Nezumia aequalis*, belongs to the macrourid family, whose closest shallow water relatives are the gadids such as cod, haddock and whiting. There are at least 15 species of grenadier in the waters to the west of the British Isles. The smooth grenadier's habitat is benthopelagic at 200 to 2,300 m. The diet consists of a wide variety of organisms dominated by amphipods. Benthic polychaetes, isopods, decapods and brittle stars are minor but significant contributors to the diet, eaten in greater quantities by larger fishes. All organisms eaten are epibenthic or closely associated with the sediment surface (Mauchline and Gordon, 1984).

The tail of grenadiers is very fragile and often not fully recovered. Fish size is expressed as the length of the head. The relationship between total length and head length is not known for this species of grenadier.

Lepidion eques - Largeye lepidion

The largeye lepidion, *Lepidion eques*, is also a cod-like fish belonging to the closely related family of morid fishes. Its habitat is benthopelagic, caught between 127 and 1,850 m, with smaller individuals at the shallower depths. It is caught singly and in aggregations of more than 100 individuals. The diet is mainly crustaceans but also polycheates. The principal dietary components are epibenthic and hyperbenthic decapod crustaceans, although a wide variety of other organisms also occur in the stomach contents as minor components (Mauchline and Gordon, 1984). The largeye lepidion is found in the Bay of Biscay, along the western slopes of the British Isles, west from the Faroes long the Iceland-Faroe ridge to Iceland, Greenland, Davis Strait, northern Labrador and the Grand Banks of Newfoundland.

Raja fyllae - Round ray

There are many different skates and rays living at all depths on the continental slope. In the Atlantic waters the two species which are most likely to be encountered are the Norwegian skate (*Raja nidarosiensis*) and the round ray (*Raja fyllae*). The round ray's habitat is benthic in about 170 to 2,050 m, restricted to waters of 1 to 7 °C; common between 300 and 800 m. The diet is varied with a preference for bottom-living invertebrates. The round ray is found off the Atlantic coasts southwards from Spitsbergen to southern Norway, and westwards to Shetlands, Faroe, Iceland and southern Greenland; also off the western coast of the British Isles southwards to the Bay of Biscay (southern limit at about 45°N, but single questionable records exist south to Morocco); elsewhere, western North Atlantic south to Nova Scotia.

Methods

Twenty-five to 30 individuals of each species were collected, representing a range of weights and sizes. The fish were stored on ice without freezing and were weighed, measured and dissected soon after the landing. Flesh (muscle), liver and gills were sampled. The samples dissected were wrapped in aluminium foil and put in plastic bags to allow analysis for trace metals (excluding aluminium) and subsequently for organic contaminants. Samples were then kept frozen before being digested. Homogenised sub-samples of muscle tissue (*ca* 10 g), liver (*ca* 1 g) and gills (*ca* 4 g) were digested in boiling nitric acid and made up to 25 ml with distilled water.

Copper and zinc were analysed by flame atomic absorption spectroscopy with deuterium background correction; the limits of detection obtained were both of 0.02 ppm. Lead and cadmium were analysed by graphite furnace atomic absorption spectroscopy with L'Vov platform, Zeeman background correction and respectively palladium and magnesium nitrate matrix modifiers; the limits of detection were both of 5 ppb. Mercury was analysed by continuous flow hydride generation with potassium permanganate and stannous chloride; the limit of detection was 5 ppb, expandable to 1 ppb for the low concentrations found in gill samples.

The quality control of the data was provided through the analysis (Table 1) of certified reference materials Dorm-2 (dogfish muscle, National Research Council, Canada), the use of laboratory reference materials (LRM), of repeated samples and the recovery of spikes.

RESULTS

The median concentrations of trace metals for each species and each organ are summarised in Table 2 and Figures 1 and 2. Levels of nickel and chromium were under the limits of detection (0.04 and 0.06 mg/kg respectively) for all samples analysed.

Median cadmium concentrations varied from 0.004 to 0.012 mg/kg in muscle, from 0.040 to 0.094 mg/kg in the gills and from 0.268 to 0.509 mg/kg in the liver, with highest concentrations being found in the ray including a surprisingly high value of 4.40 mg/kg in one of the livers. Median copper concentrations varied from 0.17 to 0.33 mg/kg in the muscle, 0.47 to 0.93 mg/kg in the gills and 1.92 to 3.07 mg/kg in the muscle, the maximum being in the ray's liver at 15.90 mg/kg. Median lead levels ranged from 0.002 to 0.027 mg/kg in the muscle, 0.012 to 0.093 mg/kg in the gills and 0.026 to 0.048 mg/kg in the liver. Median mercury concentrations ranged from 0.033 to 0.101 mg/kg in the gills, 0.050 to 0.083 mg/kg in the liver and 0.077 to 0.150 mg/kg in the muscle tissue. Median zinc concentrations varied from 2.62 to 5.53 mg/kg in the muscle, 15.05 to 17.97 mg/kg in the liver and 12.89 to 29.88 mg/kg in the gills.

Matrices of Pearson's correlation coefficients were calculated for each of the three species for all the variates, using Bonferroni's probabilities for the confidence level of the correlations. The significant correlations are listed in Table 3.

In *Nezumia aequalis*, both mercury in muscle and in liver were very strongly positively correlated with the weight, the levels of cadmium in liver, with one another, and strongly positively correlated with the length. Cadmium in liver was itself very strongly positively correlated with lead in liver. Mercury in gills was positively correlated with mercury in liver. Copper in muscle was negatively correlated with the weight.

In *Lepidion eques*, all correlations were positive: cadmium in liver was strongly correlated with copper and zinc in liver. Zinc in liver was very strongly correlated with copper in liver, which was strongly correlated with mercury in liver. Mercury in liver was very strongly correlated with mercury in muscle.

In *Raja fyllae*, both copper and mercury in gills were positively correlated with the length. Mercury in gills was also positively correlated with the weight, and very strongly positively correlated with mercury in muscle and in liver.

DISCUSSION

Comparison with the European Dietary Standards and Guidelines

Dietary standards and guidelines for trace metals in fish have been summarised by MAFF (1995). The relations of our data to these guidelines is as follows:

Cadmium: In the liver, the median levels were higher in the ray (0.5 mg/kg) than in the grenadier and the lepidion (respectively 0.29 and 0.22 mg/kg), and all three medians were above the European guideline (0.2 mg/kg). The maximum concentrations found were 0.84, 0.86 and 4.3 mg/kg for respectively the grenadier, the lepidion and the ray. Three individuals of the ray presented levels above 2 mg/kg. The maximum levels found in muscle tissue were ten times lower than the guideline, and the consumption of flesh should not be a problem. It is unlikely that the livers of these species are consumed.

Copper: All levels are under the limits for consumption (20 mg/kg); the medians being about 10 times lower. The maximum was 15.9 mg/kg in liver from a ray.

Lead: All medians are under 0.1 mg/kg whereas the guideline limit for this element is 2 mg/kg. Even the highest concentrations were found less than 0.6 mg/kg. The levels of lead in these deep-sea fishes would be unlikely to be of concern in relation to human consumption.

Mercury: The median concentrations of mercury are quite high, but still below the acceptable limits for consumption. However one of the individuals of the grenadier presented a mercury concentration in the muscle tissue above the limit: 0.53 mg/kg instead of 0.5 mg/kg. Similar results were obtained by Cronin (1998) on a different species of grenadier. Care may be necessary in the use of grenadiers' flesh for human consumption.

Zinc: The maximum regulation limit is 50 mg/kg. All but one sample were under this limit, the gills of a grenadier. The highest concentrations were normally found in the gills, which are not used for human consumption.

Comparison with Published Data

The most closely related publications found about deep-sea fishes are: Windom *et al.* (1987), Vas *et al.* (1993) and Cronin *et al.* (1998). Among these, Vas *et al.* (1993) are the only authors who studied the liver and gills as well as the muscle tissue, but copper is the only element common to both studies. The two other papers were only related to concentrations in muscle.

The concentrations found in this study are similar to those found by Cronin *et al.* (1998) (*cf* Table 4), and by Windom *et al.* (1987), after applying a correction factor of 6 (unpublished data) for dry to wet weight conversion.

The range of median concentrations of Cd, Cu and Zn found in the current study are similar to those reported by Cronin *et al.* (1998). Mercury values tend to be lower than those in Cronin *et al.* (1998), possibly reflecting the lack of large predatory fish in the present study. For lead, concentrations were an order of magnitude less than reported by Cronin *et al.* (1998).

The concentrations of zinc and mercury (*cf* Table 4) were similar to those published for shallow water species (eg Brown and Balls, 1997). The lead levels were slightly lower in the shallow water species than in deep-water ones, but with higher maxima in the shallow water individuals. The cadmium concentrations were much lower in shallow water species than in the deep-water studies. Deep-water species are long lived and tend to feed at higher trophic levels than their shallow water counterparts (Gordon *et al.*, 1995), and this may be reflected in cadmium levels. Copper levels were more scattered in shallow water species than in the deep-water species, and tended to be higher.

The distribution of copper between the organs found by Vas *et al.* (1993) was:

- *Coryphaenoides rupestris*: muscle > liver >> gills
- *Coryphaenoides guentheri*: liver = gills >> muscle
- *Antimona rostrata*: liver > gills > muscle
- *Synaphobranchus bathybius* and *Nematonurus armatus*: exclusively in the gills (less than the detection limits in other organs).

The tendency found during the present study was that in all three species, liver > gills > muscle. Thus the accumulation pattern is very much species dependant even between deep-water species coming from the same sea area.

Other differences in the accumulation patterns were found. Lead was mainly accumulated in muscle, then in liver with very low concentrations in gills for the *Nezumia aequalis* and *Lepidion eques*, whereas it was mainly accumulated in liver in the *Raja fyllae*. Mercury concentrations were highest in muscle tissue for all three species, but levels were higher in liver than gills for the two first species, and higher in gills than liver for the ray. Zinc concentrations were higher in the gills than the liver for the two first species, and in liver for the ray. Thus any accumulation pattern between organs for elements other than copper is also very highly species-dependent. The bi-variate relationships were not in agreement between all three studies: copper in muscle was positively correlated to length by Windom *et al.* (1987), negatively by Vas *et al.* (1993) and negatively correlated with weight in this study. Previous studies have tended to find rather more correlations between metal concentrations and age/length than in the present study. This study was carried out on discarded fishes of fairly narrow size range. Differences between individuals may have masked some underlying correlations with length.

Differentiation of the Species

Discriminant analysis was used to find a linear combination of the variates that best classifies or discriminate among the three species. In the initial analysis, using all the variates in the model, the differentiation of the species was not very good. The F-statistics (Table 5) were used to test the equality of group means and indicated that the ray was well separated from the other two species. However, less than 70% of the individuals were correctly classified.

The classification functions for all three species contained the same six most important variables, namely the constant, copper and cadmium in muscle, cadmium and zinc in gills and mercury in liver.

A backwards stepwise modelling approach was used to eliminate variables that did not assist in the separation of species. At the end of the stepping process, once all non essential variates have been taken out, the canonical discriminant functions (*cf* Table 6) represent the two best functions to separate the three groups. The coefficients represent what variables "drive" each canonical variable, and therefore best separate the groups. The separation of the groups became more complete: the distances between groups means increased appreciably compared to the previous functions (*cf* Tables 5 and 7).

The efficiency of the discriminant functions was tested through a jackknife count (Systat, 1996). The classification was completely correct for both the lepidion and the ray, and 88% correct for the grenadier (*cf* Table 8).

The ray is primarily separated from the two other species by the first factor (*cf* Fig. 3) ie based on the concentrations of cadmium in muscle and of mercury and cadmium in gills. The lepidion and the grenadier are separated by the second factor mainly, that is upon the concentration of cadmium in the muscle. The clearest separation is between the ray and the other two species. This might be expected for phylogenetic reasons. However, there are also clear differences in mode of life. The lepidion and the grenadier are benthopelagic species, with a diet of epibenthic or hyperbenthic decapod crustaceans; whereas the ray is a benthic fish, eating all kinds of bottom animals. The differences in diet may lead to the ray being exposed to larger amounts of trace metals through diet than is the case for the two other species.

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TABLE 1

Quality control

Element	Cadmium	Copper	Lead	Mercury	Zinc
limit of detection	5 ppb	20 ppb	5 ppb	5 ppb 1 ppb (expanded)	20 ppb
dorm mg/kg quoted value	0.048±0.004 0.043±0.008	2.31±0.12 2.34±0.16	0.066±0.007 0.065±0.007	4.63 4.64 ± 0.26	25.1 ± 0.8 25.6 ± 2.3
LRM mg/kg (rsd)	uld	0.70 (13%)	uld	0.23 (6%)	14.78 (2%)

uld: under limit of detection

TABLE 2

Concentration ranges and medians of the three fish species (expressed in mg/kg)

	length (cm)	weight (g)	Cd musc	Cd liv	Cd gil	Cu musc	Cu liv	Cu gil	Pb musc	Pb liv	Pb gil	Hg musc	Hg liv	Hg gil	Zn musc	Zn liv	Zn gil
Nezumia aequalis																	
max	9.5	177.0	0.010	0.841	0.108	0.37	7.49	1.47	0.024	0.128	0.233	0.532	0.221	0.117	5.79	35.08	50.95
min	6.0	40.0	0.002	0.143	0.011	0.17	0.90	uld	uld	0.014	uld	0.035	0.015	0.025	3.06	10.52	16.78
median	7.5	93.5	0.004	0.292	0.045	0.21	1.92	0.87	0.005	0.048	0.093	0.150	0.075	0.058	3.91	16.18	29.88
std	1.1	44.2	0.002	0.177	0.020	0.05	1.34	0.33	0.006	0.029	0.067	0.144	0.058	0.022	0.65	4.79	6.69
Lepidion eques																	
max	37.0	343.0	0.013	0.863	0.099	0.24	8.54	1.43	0.011	0.084	0.516	0.398	0.276	0.107	3.56	47.08	39.11
min	24.0	67.8	0.003	0.146	0.013	0.13	1.53	0.39	uld	0.001	0.002	0.038	0.016	uld	2.16	9.29	17.80
median	30.2	158.4	0.005	0.268	0.040	0.17	3.07	0.93	0.002	0.033	0.039	0.077	0.050	0.033	2.62	15.05	24.32
std	3.1	68.5	0.003	0.142	0.017	0.03	1.55	0.25	0.003	0.027	0.113	0.074	0.053	0.020	0.28	9.10	5.31
Raja fyllae																	
max	54.0	709.3	0.027	4.399	0.187	0.83	15.90	0.66	0.044	0.128	0.096	0.410	0.288	0.327	6.15	32.67	21.07
min	38.0	234.3	0.008	0.228	0.044	0.22	1.12	0.20	uld	uld	uld	0.044	0.024	0.037	4.57	9.67	7.82
median	49.5	567.5	0.012	0.509	0.094	0.33	1.98	0.47	0.027	0.026	0.012	0.129	0.083	0.101	5.53	17.97	12.89
std	3.9	124.0	0.004	0.928	0.036	0.10	2.79	0.08	0.011	0.039	0.024	0.867	0.061	0.059	0.41	5.31	3.67

uld: under limit of detection

TABLE 3

Summary of Pearson's correlation coefficients

Species			Pearson coefficient	Bonferroni's probability
Nezumia aequalis	Cu muscle	Weight	- 0.645	0.051
	Pb liver	Cd liver	0.823	0.000
	Hg muscle	Length	0.727	0.004
		Weight	0.833	0.000
		Cd liver	0.752	0.001
		Hg liver	0.930	0.000
	Hg liver	Length	0.712	0.004
		Weight	0.788	0.000
		Cd liver	0.742	0.001
		Hg gills	0.646	0.037
Lepidion eques	Cu liver	Cd liver	0.713	0.006
		Hg liver	0.700	0.009
		Zn liver	0.876	0.000
	Zn liver	Cd liver	0.720	0.005
	Hg liver	Hg muscle	0.933	0.000
	Cu muscle	Zn muscle	0.691	0.004
Raja fyllae	Hg gills	Length	0.645	0.028
		Weight	0.620	0.050
		Hg muscle	0.807	0.000
		Hg liver	0.766	0.000
	Cu gills	Length	0.210	0.030

The correlations confidences are based on Bonferroni's probabilities (SYSTAT, 1996):

$p < 0.001$	very highly correlated
$p < 0.01$	highly correlated
$p < 0.05$	correlated

TABLE 4

Comparison with previous data on metal concentration in fish muscle tissue (mg/kg) from the North East Atlantic

		Cronin *et al.*, 1998 (mg/kg)	Brown and Balls, 1997 (shallow water)	This study (mg/kg)
Cd	Range of medians	0.002-0.02	<0.001-0.003	0.0041-0.0053-0.0121
	Maximum value	0.41	0.014	0.027
Cu	Range of medians	0.01-0.47	0.07-0.92	0.10-0.21-0.33
	Maximum value	0.89	2.11	0.83
Pb	Range of medians	0.004-0.72	<0.01-0.014	0.0016-0.0046-0.0275
	Maximum value	2.4	0.34	0.044
Hg	Range of medians	0.06-0.42	0.02-0.11	0.0741-0.1293-0.1500
	Maximum value	0.88	0.23	0.53
Zn	Range of medians	2.2-6.7	3.32-5.17	2.62-3.91-5.53
	Maximum value	10.6	8.36	6.15

TABLE 5

Between groups F-matrix using Mahalanobis D^2 statistics

	Nezumia aequalis	*Lepidion eques*	*Raja fyllae*
Nezumia aequalis	0.0		
Lepidion eques	9.117	0.0	
Raja fyllae	42.934	63.941	0.0

TABLE 6

Canonical discriminant functions

	First factor	Second factor
Constant	- 6.199	- 4.977
Zn muscle	1.803	1.300
Cd muscle	26.466	- 114.246
Zn gills	- 0.129	0.081
Cd gills	11.635	- 11.169
Hg gills	15.875	- 3.039
Zn liver	0.031	- 0.028
Hg liver	- 11.508	2.169

TABLE 7

Between groups F-matrix for the canonical scores

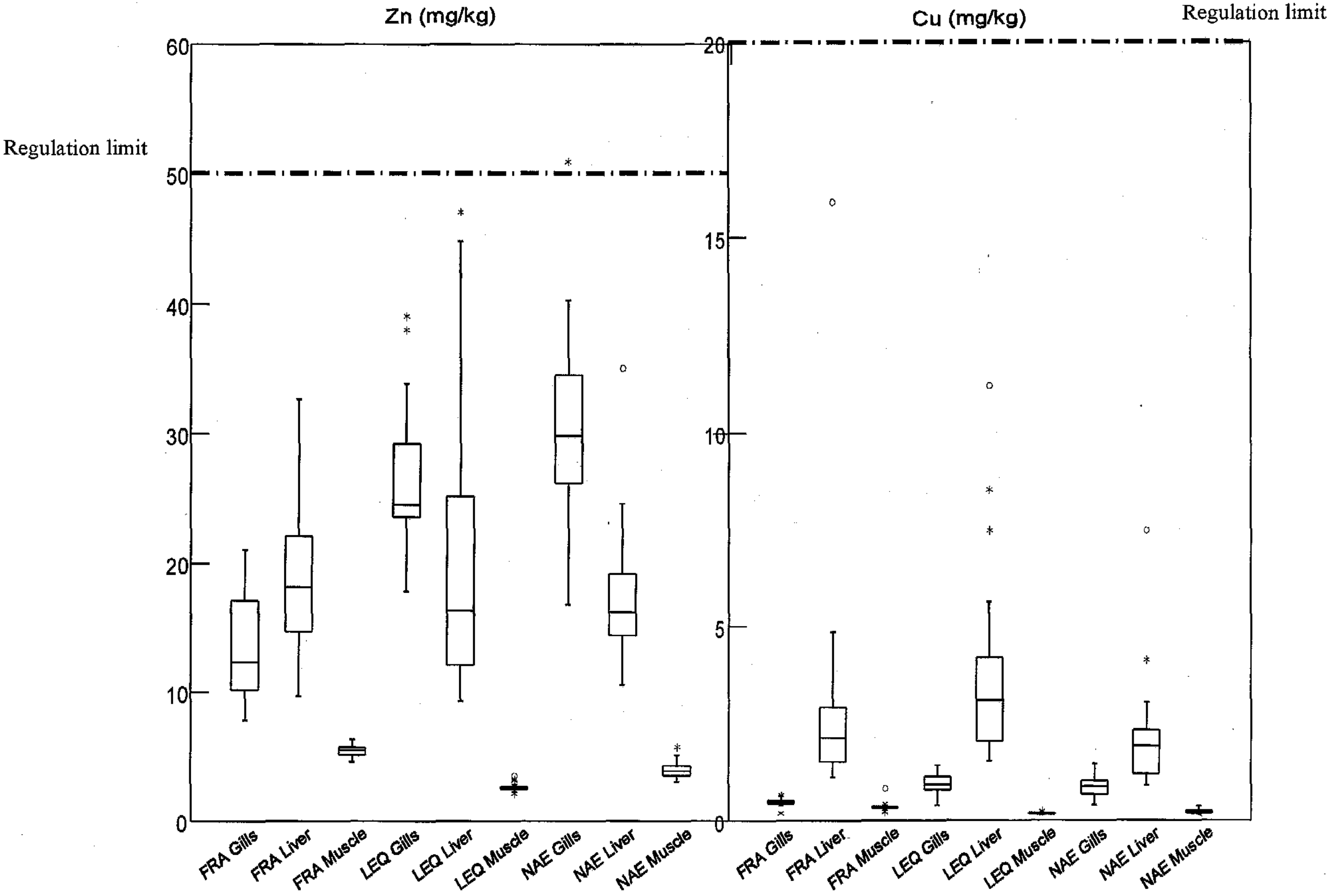
	Nezumia aequalis	*Lepidion eques*	*Raja fyllae*
Nezumia aequalis	0.0		
Lepidion eques	15.581	0.0	
Raja fyllae	82.139	121.730	0.0

TABLE 8

Classification matrix

Classified in ⇨	*Nezumia aequalis*	*Lepidion eques*	*Raja fyllae*	% correct
Nezumia aequalis	22	3	0	88
Lepidion eques	0	23	0	100
Raja fyllae	0	0	28	100

Fig 1: Cu and Zn (mg/kg) DISTRIBUTION IN THE THREE SPECIES



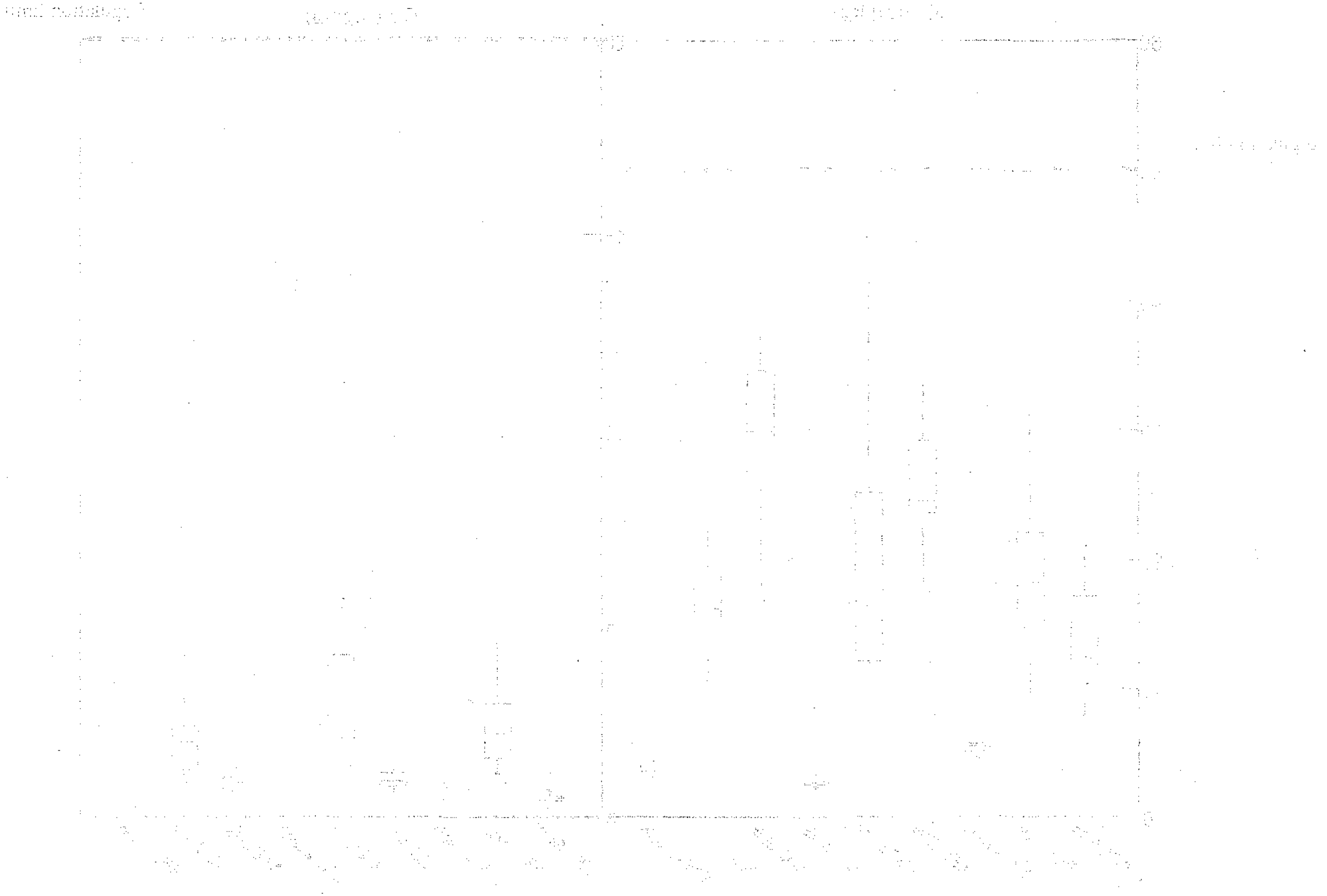
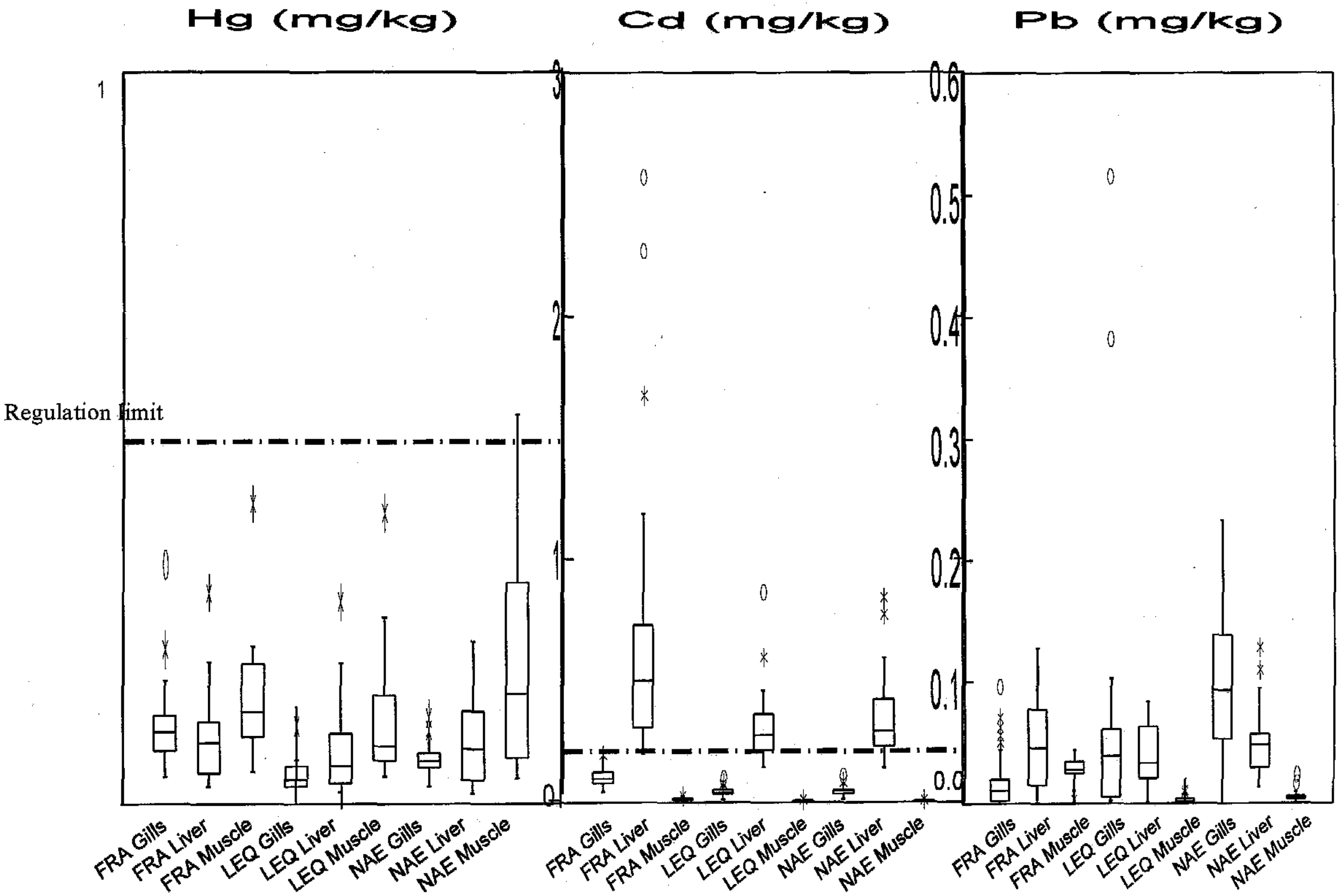


Fig 2: Cd, Hg and Pb (mg/kg) DISTRIBUTION IN THE THREE SPECIES



Hg (mg/kg)

Cd (mg/kg)

Pb (mg/kg)

Fig 3: Canonical Scores Plot