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Cadmium, copper and zinc in octopuses from Kerguelen Islands, Southern Indian Ocean

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ABSTRACT: Concentrations of cadmium, copper and zinc were measured in 34 octopuses over a large range of size and weight caught in the Kerguelen shelf waters. Compared with levels normally encountered in European cephalopods, Cd concentrations in both species were very high: from 30.7 to 47.1 and from 27.3 to 54.4 µg/g dry weight in *Graneledone* sp. and *Benthoctopus thielei*, respectively; Cu concentrations were generally low while Zn concentrations exhibited similar levels. Distribution of Cd in tissues showed that the high levels of Cd in Kerguelen octopuses resulted from very high levels of the metal in the digestive gland (369 and 215 µg/g dry wt in *Graneledone* sp. and *Benthoctopus thielei*, respectively). Digestive gland accumulated about 90 % of the total Cd in the whole animal. Due to the very high concentrations of Cd in the Kerguelen octopuses, we hypothesize that these species play an important role in the process of Cd transfer throughout the food chain to top vertebrate predators in this area.

KEY WORDS: Cephalopods . Octopus . Sub-Antarctic . Heavy metals . Cadmium . Digestive gland . Detoxification processes.

INTRODUCTION

Recent studies have shown elevated concentrations of Cd in several marine invertebrates from Antarctic areas, mostly in crustaceans (Hennig et al. 1985; Mauri et al. 1990; Petri & Zauke 1993; Zauke & Petri 1993; Ahn et al. 1996; Bargagli et al. 1996). Data on Cd levels in mollusks from this area are few. Especially, there are no data available regarding cephalopods. First, cephalopods occupy a predominant niche in the trophic chains (Rodhouse & Nigmatullin 1996), particularly in the Antarctic Polar Frontal Zone in the South Atlantic Ocean, where they, especially the ommastrephid *Martialia hyadesi*, occupy the ecological niche of epipelagic fish (Rodhouse & White 1995). Cephalopods are predominant in the trophic system, because they are eaten by many oceanic animals like marine mammals (Clarke 1985; Clarke 1996), and seabirds (Croxall & Prince 1996; Cherel & Klages 1997). In the Southern Ocean, benthic octopuses were found in the food of Rockhopper and Gentoo penguins (Brown & Klages 1987; Adams & Klages 1989), royal albatrosses (Imber 1991) and Weddell seals (Clarke & McLeod 1982). They were also reported as minor but regular prey of southern elephant seals (Rodhouse et al. 1992) and albatrosses (Cherel & Klages 1997). In Kerguelen waters, octopuses are common items of black-browed albatrosses during the chick-rearing period (Y. Cherel and H. Weimerskirch, unpublished data) and they were found in the diet of gentoo penguins (Bost 1991). Second, some studies on elemental bioaccumulation show that cephalopods accumulate high levels of trace elements, particularly Cd and Cu (Ghiretti-Magaldi et al. 1958; Rocca 1969; Renzoni et al. 1973; Martin & Flegal 1975; Smith et al. 1984; Miramand & Guary 1980; Miramand & Bentley 1992). Thus, cephalopods represent important species for studying the transfer of Cd into marine food webs in Antarctic and sub-Antarctic areas.

This paper describes the bioaccumulation and tissue distribution of Cd, Cu and Zn in two octopus species living in the Kerguelen shelf waters: *Graneledone* sp. and *Benthoctopus thielei*. The Kerguelen Islands constitute a small archipelago in the South Indian Ocean, at the Antarctic Polar Frontal Zone (Fig. 1), where release of heavy metals is presumably negligible or non-existent. Moreover, the Kerguelen Islands are of special interest because there is no information available on heavy metal levels in invertebrates from sub-Antarctic areas.

MATERIALS AND METHODS

Octopuses were collected from catches of a trawling fishery for Patagonian toothfish *Dissostichus eleginoides* in the upper slope (400-600 m) of the northern Kerguelen shelf, located between 47°00' and 47°20'S, and 69°00' and 69°20' E. They were immediately frozen aboard and kept at -20°C. Two groups of cephalopods were used: some were collected in February 1994, the others in May/June 1995. In order to calculate allometric equations between body mass, body length, hood, and crest lengths of beaks (Y. Cherel, unpublished data), the first group was thawed one time and re-frozen before heavy metal analysis. Thus, due to possible metal diffusion during a thawing/freezing cycle (Martin & Flegal 1975), the distribution of heavy metals in various tissues was only measured in the latter group. Digestive gland, branchial hearts, gills, digestive tract, genital tract and ink sac were totally removed from the dissected individuals. In addition, pieces of mantle muscle and of the skin were sampled to determine metal concentrations. This implies that the remains of the animal were composed of arms, the rest of the mantle, muscle and skin. For all animals, stomach contents were removed before metal analysis. Our laboratory participate to European

intercalibration exercises which allowed to quantify the internal analytical variability about 12.5 %.

For the nomenclature of the two octopus species commonly found in Kerguelen waters, i.e. *Benthoctopus thielei* and *Graneledone* sp. (a still undescribed species closely related to *G. antarctica*) we followed Nesis (1987). Seventeen individuals of each species were used: 11 females (mean wet wt = 123 ± 89 g) and 6 males (mean wet wt = 121 ± 75 g) of *Benthoctopus thielei* and 11 females (mean wet wt = 85 ± 93 g) and 6 males (mean wet wt = 208 ± 98 g) of *Graneledone* sp. Each individual has been weighted and measured (mantle length, total length).

Tissue samples (whole individual regarding 1994 samples and the different organs regarding 1995 samples) were dried for several days at 80°C until constant weight. Two aliquots of approximately 300 mg of each homogenised dry sample were digested with 4 ml of 65 % HNO₃ and 1 ml of HClO₄ 70 % during 24 hours at 80°C. After evaporation, the residues were dissolved in nitric acid 0.3 N. Blank were carried through the procedure in the same way as the sample. Cu and Zn were determined by flame and Cd by flameless atomic absorption spectrophotometry using a Varian spectrophotometer Vectra 250 Plus with Deuterium background correction.

All glassware and plastic was cleaned with HNO₃ / HCl 1N and rinsed with deionized water. Reference material, Orchard-Leaves standard (NBS) and MA-A-2 fish-flesh standard (IAEA) were treated and analysed in the same way. The results for standards are shown in Table 1. All the results are given in micrograms of metal per gram of the dry weight tissue (µg/g dry wt). For each element, the mean \pm standard deviation and the coefficient of variation are

given. Distribution percentages of metals are given in percent of the total fresh weight of the individual.

RESULTS

Our sampling included males and females and represented a large range of size and weight: between 14 g and 279 g for *Benthoctopus thielei* and between 6 g and 343 g for *Graneledone* sp. Metal concentrations ($\mu\text{g/g}$ dry wt) in *Benthoctopus thielei* and *Graneledone* sp. from the Kerguelen Island waters are reported in Table 2. In both species, Cd, Cu and Zn were concentrated in similar amounts. Mean Cd concentrations were $39.1 \pm 5.3 \mu\text{g/g}$ in *Graneledone* sp. and $38.2 \pm 7.6 \mu\text{g/g}$ in *Benthoctopus thielei*. Mean Cu concentrations were $68 \pm 30 \mu\text{g/g}$ and $68 \pm 29 \mu\text{g/g}$ and corresponding Zn concentrations $131 \pm 19 \mu\text{g/g}$ in *Graneledone* sp. and $166 \pm 39 \mu\text{g/g}$ in *Benthoctopus thielei*.

For both species, heavy metal concentrations were similar in small and large individuals. The analytical results for males and females were compared. ANOVA test have shown no significant differences between metals concentrations by sex ($p < 0.05$). For both elements, no correlation were found for *Graneledone* sp. while in *Benthoctopus thielei*, Cd and Zn were correlated positively ($p < 0.05$) and Cu and Zn were correlated negatively ($p < 0.05$). Figure 2 show relationships between metal concentrations and mantle length by sex. Correlation coefficients were significant in *Graneledone* sp. for Cu and Zn in females and in *Benthoctopus thielei* for Cd in males and Zn in females. The coefficients of variation (CV) were less than 25 % for Cd and Zn, and near to 40 % for Cu (Table 2).

The distribution of heavy metals in one individual of each species are reported in Table 3. Most of the Cd (91 % and 89 %, respectively in *Graneledone* sp. and *Benthoctopus thielei*) was accumulated in the digestive gland with concentrations as high as 369 and 245 $\mu\text{g/g}$ in

Graneledone sp. and *Benthoctopus thielei*, respectively. Zn concentration was higher in *Benthoctopus thielei* digestive gland (416 µg/g) and represented 53 % of the total amount, whereas in *Graneledone* sp. it was close to the concentrations found in the other organs (102 µg/g), and represented only 16 % of the total amount. In both species, the remains contained high percentages of Zn with 67 % for *Graneledone* sp. and 44 % for *Benthoctopus thielei*. Total Cu concentrations (165 µg/g for *Graneledone* sp. and 25 µg/g for *Benthoctopus thielei*) as well as distribution were quite different between both individuals. The digestive gland of *Graneledone* sp. exhibited high Cu concentration (1092 µg/g) and corresponded to the major site of Cu storage with 79 % of the total body burden. In contrast, the digestive gland of *Benthoctopus thielei* contained only 25 % of the total Cu body burden and displayed a very low concentration (42 µg/g).

DISCUSSION

Metal Levels

Despite the analysis of males and females over a large range of size and weight, the variability of the metal concentrations was relatively small. The most striking feature was the higher values for the coefficient of variation for Cu compared to that of Cd and Zn.

The homogeneity of metal concentrations found in the 34 individuals was particularly remarkable. We hypothesise that the concentrations measured in this study were a relatively good representation of the concentrations encountered in specimens subject to similar ambient water composition and probably similar diets. Nevertheless, the coefficient of variation of Cu was particularly interesting. This coefficient was higher in both species sampled from the Kerguelen waters than in other octopuses species: 27 % in *Octopus*

vulgaris (Miramand and Guary 1980) and 11 % for *Eledone cirrhosa* (Miramand and Bentley 1992). Moreover the Cu variability was higher than Cd variability. This was surprising when one considers that Cu and Zn (unlike Cd) are essential elements which implies that they are maintained at a fairly constant concentration. The high Cd concentration might disrupt Cu homeostasis.

In order to compare the concentration of trace elements with other cephalopod species, Table 4 shows Cd, Cu and Zn concentrations in the digestive gland and in whole individuals of cephalopods from the European waters where industrial releases of pollutants occur. In both species from Kerguelen waters, Zn concentrations were in the same range than those found in other species (Table 4).

In contrast, Cd and Cu concentrations found in octopuses caught from Kerguelen waters were noteworthy. Cu concentrations were low except for one individual for *Graneledone* sp. (165 µg/g) and two individuals of *Benthoctopus thielei* (114 and 149 µg/g) (Table 2 and Table 4). Cd concentrations in both species were very high (Table 2). These concentrations in Kerguelen Islands octopuses were one order of magnitude greater than those found in others cephalopods (Table 4).

Such high Cd concentrations were unexpected for mollusks coming from a presumably unpolluted area far from any anthropogenic source of contaminants. Recently, Bargagli et al. (1996) have measured Cd concentrations in various organisms caught in Terra Nova Bay (Antarctica). All organisms collected in this area showed relatively elevated concentrations, but in most groups, concentrations were lower than 10 µg/g dry wt or close to 15 µg/g dry wt. Only some samples of the sponge *Porifera* exhibited higher Cd concentrations (i.e. 10-80 µg/g dry wt). In Antarctic areas, such high Cd concentrations only occurred in the scallop *Adamussium colbecki* (Berkman & Nigro 1992), and in some zooplanktonic crustaceans,

particularly in the hyperiid amphipod *Themisto gaudichaudii*. These species accumulated Cd to levels similar or greater than Kerguelen octopuses (Hennig et al. 1985). Compared with Cd concentrations in other mollusk species, these concentrations were also remarkable. Thus, most of the mollusks species (bivalves and gastropods) caught in unpolluted areas contained Cd concentrations lower than 5 µg/g dry wt (Eisler 1981, Bryan 1984). Only some Pectinidae species which are well known for their ability to accumulate Cd, exhibited concentrations nearer to those found in the Kerguelen octopuses (Bryan 1973; Berkman & Nigro 1992; Francesconi *et al.* 1993). For comparison, Cd concentrations similar to those found in whole Kerguelen octopuses (excluding Pectinidae) have only encountered in filter-feeders caught in a Cd polluted area (Boutier & Chiffolleau 1986).

The particular strong accumulation of Cd in octopuses from Kerguelen, an area without anthropogenic inputs of this metal, is difficult to explain. The Kerguelen Islands are located at the Antarctic Polar Frontal Zone (Fig. 1), and Cd enrichment in surface waters may occur due to upwelling of deep nutrient-rich waters. Unfortunately, we lack measurements of dissolved Cd in Kerguelen sea water and this hypothesis cannot be tested. Nevertheless, metal analyses of the stomach content of some Kerguelen octopuses showed elevated Cd concentrations greater than 30 µg/g dry wt (unpublished data). This suggests elevated Cd concentrations in octopus prey which could be an indicator of a Cd enrichment in Kerguelen waters or bottom fauna. In this context, food could be an important pathway of Cd uptake for Kerguelen Octopodidae. Octopus eat many prey species, mainly crustaceans, mollusks and fishes (Boyle 1990; McQuaid 1994; Laidig et al. 1995) but it has been impossible to determine clearly the diets of Kerguelen octopuses from stomach contents and no fish otoliths was found. The carnivorous diets of octopuses rely on the hypotheses of low supply of Cd by the soluble phase, unlike suspensivores species which filter important volumes to feed on

micro-organisms. Moreover, gills in cephalopods shown low Cd concentrations and present less than 1 % of the total Cd body burden (Table 3, Miramand & Bentley 1992).

Distribution of metals and implication in detoxification process

The dissection of a single individual of *Graneledone* sp. and one individual of *Benthoctopus thielei* (Table 3) indicate that these Kerguelen octopuses exhibit a Cd distribution in tissue similar to that found in octopuses from the Mediterranean Sea (Miramand and Guary, 1980), or in the English Channel (Miramand & Bentley 1992), with about 90 % of the metal contained in the digestive gland. Digestive gland of Kerguelen octopuses contained very high concentrations (Table 3). In contrast, muscle concentrations are close to or slightly greater than those encountered in other octopus species (0.1-0.5 µg/g dry wt) (Miramand & Guary 1980; Miramand & Bentley 1992; Barghigiani et al. 1993). Thus, the high concentrations measured in whole Kerguelen octopuses were due to the high concentrations in the digestive gland.

The high Cd concentrations in the digestive gland were greater than those usually found in others cephalopods (Table 4). Only two species caught in the Pacific Ocean exhibited similar or higher concentrations: Martin and Flegal (1975) found 287 µg/g dry wt in the squid liver of *Ommastrephes bartrami* and 782 µg/g dry wt in the squid liver of *Symplectoteuthis oualaniensis*. High concentrations of Cd in the digestive gland of cephalopods suggested that most of the detoxification processes occurred in this organ. However, studies on these mechanisms are scarce and concern only two squids species. Tanaka et al. (1983) and Finger and Smith (1987) have shown an association of Cd with high molecular weight material in the digestive gland of the squids *Todarodes pacificus* and *Nototodarus gouldi*. Nevertheless Cd detoxification processes in octopuses are not known and more studies are needed to

clarify this mechanism. In this context, Kerguelen octopuses appear as good species for future investigations.

Branchial hearts of octopuses, which have polyhedral cells containing granules with brown pigments (adenochromes) rich in iron (Fox & Updegraff 1943; Ghiretti-Magaldi et al. 1958; Nardi & Steinberg 1974; Schipp & Hevert 1978), are certainly also engaged in storage and detoxification processes for some trace elements such as cobalt, copper, vanadium (Ueda et al. 1979; Nakahara et al. 1979; Miramand & Guary 1980), or transuranic elements (Guary et al. 1981; Miramand & Guary 1981; Guary & Fowler 1982). However, in European octopuses with relatively low Cd concentrations in whole tissues, branchial hearts (concentrations $< 0.3 \pm 0.1 \mu\text{g/g}$) do not seem to be implicated in Cd detoxification processes (Miramand & Guary 1980; Miramand & Bentley 1992). In octopuses from Kerguelen, branchial hearts exhibited high Cd concentrations (Table 3). A possible hypothesis could be a is a threshold effect, that is, beyond a certain Cd concentration the digestive gland would not be sufficient for the entire detoxification processes and branchial hearts would then be used.

The ink sac of cephalopods contains melanin, a macromolecule with the ability to act as a cation-exchange resin (Larsson & Tjalve 1978). It is known that tissues containing melanin pigments are often rich in some trace elements such as manganese, copper and zinc. The binding affinity of melanin towards certain metal ions has been investigated in some detail (Aime et al. 1989). The interaction is essentially electrostatic and its strength is therefore expected to be dependent on the charge to mass ratio of the metal ion. However, Sarzanini et al. (1992) have found in *Sepia officinalis* that the ability to concentrate metals by melanin is significantly lower than that observed for melanin sampled from other marine organisms,

such as bivalves (Simkiss et al. 1982). The ink could be an excretion pathway for Cd while its missing in Kerguelen octopuses exclude Cd excretion by this way.

Conclusion

Due to the very high concentrations of cadmium in both *Benthoctopus thielei* and *Graneledone* sp., these cephalopods probably play an important role in the process of Cd transfer to top vertebrates predators in this region. Cd is well known for its accumulation in kidneys of vertebrates, where at high doses it causes renal diseases (Nicholson et al. 1983; Nogawa 1984; Lauwerys 1990). For human consumption, a “provisional tolerable weekly intake” of 400-500 µg Cd per person has been proposed by the World Health Organisation (1972). This approximates 1µg/kg body weight for most individuals or 55-70 µg/day. In Kerguelen, the daily consumption by marine predators of one small octopus (about 10 g wet weight) is sufficient to reach the equivalent of this dose. Thus, Kerguelen top predators, which have cephalopods in their diets, could achieve high levels of Cd in their tissues, like in the South Atlantic Ocean where cephalopod-eating albatrosses have high levels of cadmium in kidney and liver (Muirhead & Furness 1988). In this context, research of Cd levels in tissues of top marine vertebrates from Kerguelen Islands, with particular attention to kidney, is of particular interest.

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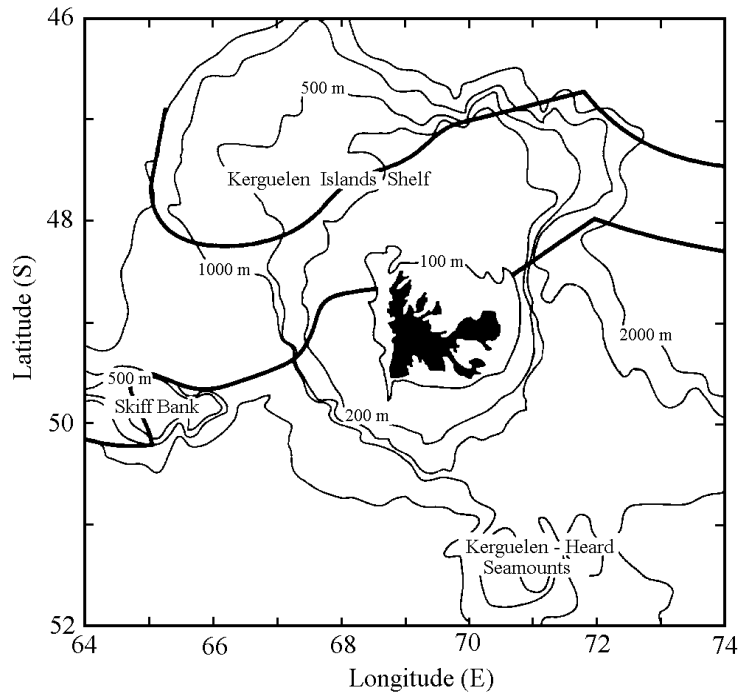


Fig 1 Location of the Kerguelen Archipelago and the southern and northern limits of the Antarctic Polar Front derived from joint French-Soviet oceanographic cruises in 1987 (Koubbi et al. 1991).

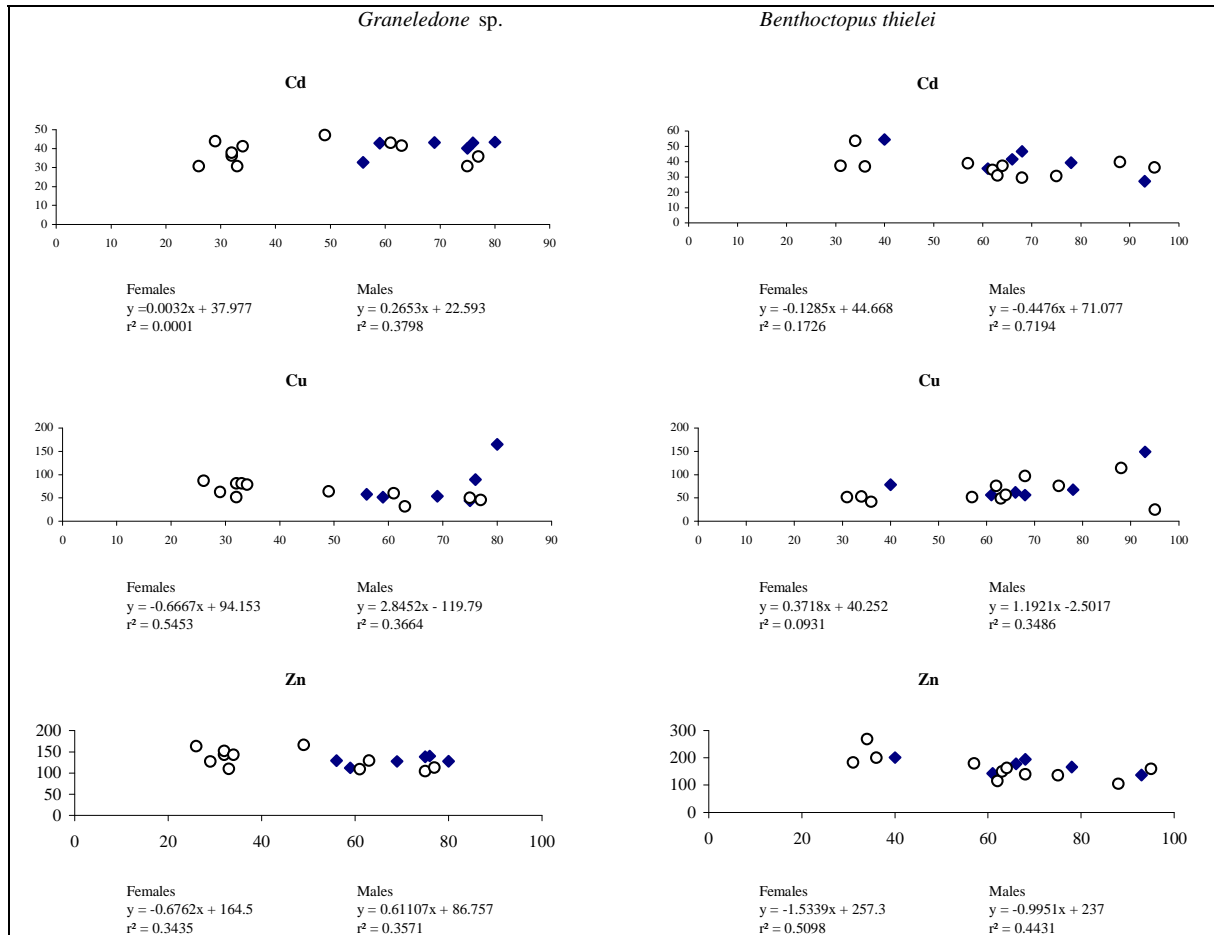


Fig. 2 Variations of metal concentrations ($\mu\text{g/g}$ dry wt) in Kerguelen octopuses with mantle length (mm) by sex. \diamond : males; \circ : females

Table 1 Concentrations ($\mu\text{g/g}$ dry wt) of cadmium, copper and zinc in MA-A-2 fish flesh homogenate (mean \pm standard deviation) and Orchard Leaves (mean \pm confidence interval 95 %).

Standards	Cd	Cu	Zn
Orchard Leaves			
Present study	0.13 ± 0.03	13 ± 1	25 ± 2
Certified value	0.11 ± 0.02	12 ± 1	25 ± 3
MA-A-2			
Present study	0.064 ± 0.001	4.0 ± 0.1	34 ± 2
Certified value	0.066 ± 0.004	4.0 ± 0.1	33 ± 1

Table 2 Metal concentrations ($\mu\text{g/g}$ dry weight) in 17 whole *Benthoctopus thielei* and 17 whole *Graneledone sp.* caught in the Kerguelen shelf waters in February 1994 and May/June 1995. The standard deviation (SD) and coefficient of variation (CV) are given for each element.

Species	Sample size	Mantle length (mm)	Fresh weight (g)	Cd	Cu	Zn	Water content (%)
<i>Graneledone sp.</i>							
Males	6						
Mean		69	208	40.9	77	129	84
SD		10	97	4.2	46	10	1
CV (%)		14	47	10	59	8	1
Range		56-80	96-343	32.8-43.5	44-165	112-140	81-85
Females	11						
Mean		46	85	38.1	63	133	84
SD		19	93	5.8	17	22	1
CV (%)		42	110	15	28	17	1
Range		26-77	6-232	30.7-47.2	32-87	104-166	83-86
All specimens	17						
Mean		54	128	39.1	68	131	84
SD		20	110	5.3	30	19	1
CV (%)		36	86	14	44	14	1
Range		26-80	6-343	30.7-47.2	32-165	104-166	81-86
<i>Benthoctopus thielei</i>							
Males	6						
Mean		68	121	40.8	78	170	83
SD		18	75	9.3	36	26	2
CV (%)		26	62	23	46	16	2
Range		40-93	21-223	27.3-54.4	56-149	137-201	82-86
Females	11						
Mean		61	123	36.8	63	163	82
SD		21	89	6.5	26	45	4
CV (%)		34	72	18	41	28	5
Range		31-95	14-279	29.4-53.4	25-114	105-269	76-89
All specimens	17						
Mean		63	122	38.2	68	166	82
SD		20	82	7.6	29	39	3
CV (%)		31	67	20	43	23	4
Range		31-95	14-279	27.3-54.4	25-149	105-269	76-89

Table 3 Metal concentrations ($\mu\text{g/g}$ dry wt) and percentage distribution (wet wt) in the organs of one male *Graneledone* sp. (fresh weight = 343 g) and one female *Benthoctopus thielei* (fresh weight = 279 g) caught in the Kerguelen shelf waters in May/June 1995. Skin and muscles are included in remainder.

Organs	Cd	%	Cu	%	Zn	%	Water content (%)
<i>Graneledone</i> sp.							
Digestive gland	369	91.2	1092	79.9	102	15.8	68
Branchial hearts	24.6	0.11	465	0.61	126	0.35	84
Gills	22.6	0.52	530	3.59	98	1.41	84
Digestive tract	90.5	1.96	354	2.27	92	1.25	85
Genital tract	5.57	0.41	31	0.68	306	1.4	84
Remainder	6.64	5.76	50	12.9	121	67.0	86
Skin	1.94	-	67	-	121	-	90
Muscles	0.37	-	15	-	113	-	87
Whole individual	43.5	100	165	100	128	100	85
<i>Benthoctopus thielei</i>							
Digestive gland	215	88.6	42	24.8	416	52.6	69
Branchial hearts	31.5	0.15	306	3.46	172	0.26	85
Gills	49.1	0.89	168	7.07	147	0.82	84
Digestive tract	85.1	1.30	35	1.24	202	0.95	86
Genital tract	10.3	0.47	34	3.59	101	1.41	84
Remainder	7.06	8.54	22	59.8	118	43.9	85
Skin	0.81	-	18	-	95	-	90
Muscles	0.21	-	3	-	138	-	82
Whole individual	36.2	100	25	100	159	100	84