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Cadmium, zinc and copper accumulation in the squid *Illex argentinus* from the Southwest Atlantic Ocean

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Abstract The levels and burdens of Cd, Zn, Cu and Hg were measured in the dorsal mantle, digestive gland and gonads of the squid *Illex argentinus*, from the Southwest Atlantic Ocean. Mature and immature individuals of both sexes were analysed. Correlations of heavy metal concentrations and burdens with sex and food habits were studied. The highest metal levels were found, particularly for Cd, in the digestive gland, which attained a concentration of up to 270 $\mu g\ g^{-1}$ (wet weight). Dorsal mantle and gonads exhibited the same order of metal enrichment: Zn > Cu > Cd. Zinc and Cu levels were higher in ovaries than in testes and varied with the stage of maturation. The dorsal mantle exhibited the lowest heavy metal concentrations. Mercury levels were below the detection limit of the method in all the tissues analysed.

Introduction

Trace metals are accumulated by marine invertebrates from both water and food. Trace metal accumulation processes vary within the same invertebrate species, according to the metal concerned. Body metal concentrations and burdens may be influenced by changes in body weight, e.g. with age, seasonal cycles, the depletion of food reserves, gamete emission or the brooding of juveniles (Rainbow 1996). Some metals, such as Cu and Zn, are essential for proper metabolic functioning, whereas others, like Cd and Hg, are highly toxic to aquatic organisms and have unknown biological activities. Copper is an active centre of both the metal enzymes and the oxygen-transporting proteins, e.g. hemocyanin superoxide dismutase. Zinc is a cofactor of several enzymes, such as carbonic anhydrase, alcalin phosphatase and DNA and RNA polymerase. In spite of its biological function, both Zn and Cu could produce toxic problems at very high levels.

In the southwestern Atlantic Ocean, between 27 and 55°S, *Illex argentinus* (the Argentine short-finned squid) is the most important cephalopod species to play a major role in the ecosystem (Brunetti 1990). This squid is the most important commercial mollusc exploited in Argentina and exported to different countries. Moreover, it is a food item for several species of fish (Prenski and Angelescu 1993), seabirds (Coria et al. 1995) and marine mammals (Bastida et al. 1992). Squid naturally accumulates high levels of heavy metals in the digestive gland (in particular Cd), regardless of their levels of exposure to anthropogenic contamination (Martin and Flegal 1975; Honda and Tatsukawa 1983; Smith et al. 1984; Finger and Smith 1987; Kurihara et al. 1993).

The aim of the present study was to correlate the concentrations and body burdens of Cd, Zn, Cu and Hg in *Illex argentinus* with sex and food composition in mature and immature organisms. The effects of sexual maturation on heavy metal levels in the gonads and digestive gland were also examined.

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Materials and methods

The squid collected were pre-reproductive individuals from stocks of Buenos Aires Province, northern Patagonian subpopulation (BNPS, located between 36 and 40°S) and summer spawning stock

(SSS, located between 42 and 44°S) (Brunetti et al. 1991). Samples were transported to the laboratory on ice, and the sex and stage of sexual maturity were determined for each individual. Sexual maturity was based on histological criteria and microscopic characteristics of the gonads. The life cycle of *Illex argentinus* consists of three phases: the immature to maturing stage (S II to III), mature phase (S IV to V) and the spawning to postspawning stage (S VI to VIII; classification according to Brunetti 1990). This species only spawns once. Total body weight, dorsal mantle length and weight, the digestive gland weight, ovaries (including oviducts) and testes were measured.

Total mercury was determined by flameless atomic absorption spectrophotometry (AAS), after wet mineralisation of the samples with nitric and sulphuric acids (1:4) and potassium permanganate digestion. Permanganate excess was reduced with hydrogen peroxide (30%), and ionic mercury was reduced to Hg° with tin (II) chloride (10%). The method employed has been described by Moreno et al. (1984). Cadmium, Zn and Cu concentrations were determined by AAS with air–acetylene flame, using a deuterium lamp for the background correction. The samples were digested with perchloric and nitric acids (1:3) according to the method of the FAO/SIDA (1983). Determinations were made with a Shimadzu AA-640-13 spectrophotometer. Analytical grade reagents were used to prepare samples, blanks and calibration curves. In order to assure quality control, a Certified Reference Material No. 6 (mussel) from the National Institute for Environmental Studies (NIES, Tsukuba, Japan), Japan Environmental Agency, was analysed. The values obtained were in agreement with the certified concentrations ($P < 0.05$). The detection limit of the method for Cd and total Hg was 0.05 $\mu g\ g^{-1}$ wet weight. Metal concentrations were expressed in micrograms per gram and organ burden in micrograms (gonads and dorsal mantle) or milligrams (digestive gland). Organ burdens were estimated using the BNPS organ weights (from the same squid sample) reported by de Moreno et al. (1998).

The differences between mature and immature organisms and between sexes were tested by the Kruskal–Wallis non-parametric test, as variances were not normally distributed. The BMDP Statistical Software System was used for statistical analyses.

Results and discussion

Digestive gland

The digestive gland of *Illex argentinus* varies from 5 to 10% of the total body weight (de Moreno et al. 1998). This organ supplies most of the digestive enzymes, playing an important role in the absorption and storage of nutrients and excreting digestive residues (Miramand and Bentley 1992).

The concentrations of Cd, Zn and Cu found in the digestive gland of *Illex argentinus* from BNPS are shown in Table 1. Mercury levels were below the detection limit of the method in all the tissues analysed. The highest metal concentrations were found in the digestive gland of both males and females. Moreover, the Cd levels and contents (up to 2.5 mg) were higher than those of the essential elements studied here. Similar data have been reported by other authors (Table 2). We found a great individual variability in the concentration and burden of each metal, which is indicated by the high CVs (Table 1). Smith et al. (1984) found in *Nototodarus gouldi* that Cu had the greatest variability (CV > 100%). Other authors also reported high CVs for Cd and Zn (Finger and Smith 1987). This variability is usual in squid species, probably as a consequence of a high accumulation of metals in its short life cycle (about 1 year, Brunetti et al. 1991).

In cephalopods, the ratio between heavy metal concentrations in the digestive gland and those in the muscle has been used to distinguish three groups of elements (Miramand and Bentley 1992): poorly concentrated metals (ratio < 10), such as Zn, Cr, Mn, V and Ni; moderately concentrated elements (ratio 10 to 50), such as Co, Cu and Fe; and highly concentrated metals (ratio > 50), such as Ag and Cd. Cadmium, Zn and Cu ratios found in the present study for the tissues of *Illex argentinus* from BNPS are in close agreement with these trends.

Table 3 shows the body burdens of metals in the studied tissues of male and female squid. In both sexes the digestive gland contains almost 99% of the total body burdens (considering digestive gland + dorsal mantle + gonads = 100%) of Cd. This percentage is similar to Cu (about 90%), but is twofold that of Zn (about 50%). Cadmium, Zn and Cu burdens do not show significant differences ($P < 0.01$) between sexes for mature or immature organisms.

The presence of high levels of the essential elements, Cu and Zn, in the digestive gland of *Illex argentinus* is not surprising, but the very high levels of Cd are

Table 1 Heavy metal concentrations (mean ± SE $\mu g\ g^{-1}$ wet wt) in the digestive gland of *Illex argentinus* from BNPS and SSS stocks, and dorsal mantle length (*BNPS* Buenos Aires Province and northern Patagonia subpopulation; *SSS* summer spawning stock; *CV* coefficient of variation; *MS* maturity stage; *M* mature; *I* immature)

Stock, sex	MS	Mantle length	(*n*)	Cd		Zn		Cu	
				Mean ± SE	CV (%)	Mean ± SE	CV (%)	Mean ± SE	CV (%)
BNPS									
Male	M	239 ± 19	(7)	194.30 ± 63.53	32.7	83.29 ± 13.14	15.8	167.06 ± 32.38	19.4
	I	218 ± 24	(16)	263.04 ± 100.54	38.2	89.06 ± 28.10	31.6	73.69 ± 33.07	45.2
Female	M	272 ± 22	(5)	163.96 ± 21.83	13.3	100.50 ± 42.16	42.0	112.96 ± 60.87	53.9
	I	217 ± 34	(18)	255.95 ± 131.52	51.4	94.02 ± 21.27	22.6	61.75 ± 26.64	43.1
SSS									
Male	M	183 ± 19	(3)	339.00 ± 129.10	38.0	149.33 ± 35.83	24.0	236.83 ± 120.65	50.9
Female	M	223 ± 19	(3)	485.01 ± 118.74	24.5	161.10 ± 13.48	8.4	233.40 ± 173.26	74.2

Table 2 Cadmium concentrations (mean ± SD, *n* in parentheses, μg g^{-1} dry wt, except as noted) and burdens (g) in cephalopods from different areas (*F* female; *M* male; *NA* not analysed)

Species	Area	Tissue	Cd	Zn	Cu	References
Loligo opalescens	North Pacific	Digestive gland	85.0 ± 5.2	NA	NA	Martin and Flegal (1975)
Ommastrephes bartrami	North Pacific	Digestive gland	287.0 ± 202.0	247 ± 131	5350 ± 3210	Martin and Flegal (1975)
		Digestive gland	211.0[a]	163 ± 55	195 ± 212	Castillo and Maita (1991)
		Digestive gland	826.5 ± 369.1 (10)	NA	NA	Kurihara et al. (1993)
Loligo forbesi	North Atlantic	Body burden	134.0[b]	NA	NA	Caurant and Amiard-Triquet (1995)
Illex argentinus	Argentina	Digestive gland	144.8 ± 65.0	NA	NA	Kurihara et al. (1993)
Octopus vulgaris	Mediterranean Sea	Digestive gland	50.0 ± 10.0 (3)	1450 ± 400	2500 ± 700	Miramand and Guary (1980)
		Mantle	0.08 ± 0.04 (3)	70 ± 30	26 ± 2	
		Ovary	0.02 ± 0.01 (3)	100 ± 60	60 ± 6	
		Testis	0.10 ± 0.04 (3)	360 ± 200	50 ± 8	
Nototodarus gouldi	Australia	Digestive gland (M)	58.0 ± 28.0 (10)	799 ± 325	230 ± 211	Smith et al. (1984)
		Digestive gland (F)	45.0 ± 21.0 (15)	596 ± 295	258 ± 356	
	Australia	Digestive gland	33.0 ± 30.0 (6)	830 ± 335	363 ± 338	Finger and Smith (1987)
Eledone cirrosa	English Channel	Digestive gland	24.0 ± 1.8 (2)	646 ± 86	456 ± 11	Miramand and Bentley (1992)
		Mantle	0.24 ± 0.01 (2)	105 ± 4	17 ± 1	
		Testis	0.24 ± 0.02 (2)	110 ± 1	60 ± 2	
		Ovary	0.14 ± 0.01 (2)	149 ± 3	66 ± 1	
Onychoteuthis borealijaponica	North Pacific	Digestive gland	154 ± 37	143 ± 116	99 ± 55	Castillo et al. (1990)
		Mantle	1 ± 0	589 ± 520	7 ± 4	

[a] μg g^{-1} wet wt
[b] μg

Table 3 Metal burdens of *Illex argentinus* from Buenos Aires Province and northern Patagonian subpopulation. Values for digestive gland (in mg), gonads (in μg) and dorsal mantle (in μg) are given (*MS* maturity stage; *M* mature; *I* immature)

Tissue	Sex	MS	(*n*)	Cd	Zn	Cu
Digestive gland	Male	M	(7)	4.31 ± 1.71	1.80 ± 0.33	3.71 ± 1.13
		I	(16)	3.26 ± 0.75	1.17 ± 0.31	1.14 ± 0.83
	Female	M	(5)	7.01 ± 2.33	4.54 ± 2.64	5.12 ± 3.58
		I	(18)	3.78 ± 1.18	1.64 ± 0.82	1.31 ± 0.18
Gonads	Male	M	(7)	1.29 ± 0.55	202.80 ± 26.38	14.59 ± 5.66
		I	(11)	0.91 ± 0.39	177.56 ± 32.83	8.24 ± 4.51
	Female	M	(5)	4.55 ± 2.10	1055.97 ± 72.55	265.71 ± 98.61
		I	(12)	0.53 ± 0.39	147.64 ± 86.60	66.61 ± 39.25
Dorsal mantle	Male	M	(7)	21.80 ± 10.99	1681.58 ± 347.02	221.37 ± 74.27
		I	(11)	9.22 ± 4.15	1403.21 ± 397.40	196.23 ± 46.32
	Female	M	(5)	13.38 ± 2.69	2162.26 ± 428.18	265.93 ± 99.19
		I	(20)	10.51 ± 9.98	1289.76 ± 511.44	250.97 ± 91.13

certainly noteworthy, especially considering the short life span of this species (1 year). The accumulation of Cd is presumably a function of the metal's affinity for organic ligands, in combination with the very high feeding rates and the habits of the squid. *I. argentinus* is a voracious feeder, juveniles preying mainly on amphipods and euphausiids, whereas both fish and other squid are the most important food items of adults (Ivanovich and Brunetti 1994). Amphipods, in particular, accumulate high Cd levels in their tissues, e.g. in the Bering Sea (Hamanaka and Ogi 1984; 3.3 to 10.5 μg g^{-1} dry weight) and in the southwestern Atlantic Ocean (Gerpe and Moreno 1992; 3.0 to 9.0 μg g^{-1} wet weight). By contrast, high Cd bioaccumulation is not noted amongst teleosts (Honda et al. 1983; de Moreno et al. 1997).

At the biochemical level, various insoluble and soluble metabolic products play a detoxification role by binding toxic metals in unavailable forms. Metallothioneins (soluble metal-binding proteins), bind certain metals strongly via thiolate bonds (e.g. Zn, Cu, Cd, Hg and Ag) (George 1990; Moksnes et al. 1995). These proteins are widespread amongst eukaryotes, and can be induced by metal exposure. Information about metal-binding proteins in squid is scarce. Several authors reported that Cd, Cu and Zn are distributed in three types of proteins: high molecular weight (HMW > 70 000), intermediate molecular weight (IMW 30 000 to 40 000) and low molecular weight (LMW < 3000) (Tanaka et al. 1983; Finger and Smith 1987; Castillo et al. 1990; Castillo and Maita 1991). The molecular weight fraction that binds the highest percentage of metals varies among species. The highest proportion of Cd was found in the LMW and HMW fractions (Tanaka et al. 1993). The high heavy metal accumulation found in squid, especially Cd, may be due to the wide range of proteins that can bind metals, in addition to the metallothioneins. Evidently, it appears to be necessary to extend the study of heavy metal metabolism in squid.

Mean Cd levels in the digestive gland of larger squid (mantle length >200 mm) were lower than those of smaller individuals, although the Kruskal–Wallis test revealed significative differences only for males ($P < 0.05$). A dietary change takes place in squid of the BNPS stock at about 200 mm dorsal mantle length. Specimens below that size eat only crustaceans, whereas larger specimens feed mainly on fish (dominant) and juvenile squid (Ivanovic and Brunetti 1994). As mentioned, crustaceans are likely to be a major Cd source for the smaller squid. Moreover, small squid exhibit longer and more intense feeding activity than the mature squid. Perhaps, this is the true reason why small squid accumulate more Cd than mature ones. On the other hand, the difference in metal accumulation between large and small squids may be also a function of size, i.e. a dilution of metals with growth (Boyden 1977; Najdek and Sapunar 1987). Mature squids from the SSS population presented higher Cd levels in the digestive gland than the BNPS organisms (Table 1). Unfortunately, it was not possible to test these differences statistically, due to the low number of organisms sampled in the SSS population. This stock does not exhibit a dietary change with increasing size; crustaceans comprise the main food item throughout its life cycle.

Copper levels were higher in mature (larger) squid at above 100 μg g^{-1}, than in immature individuals (smaller), displaying <106 μg g^{-1} ($P < 0.05$ for females, $P < 0.01$ for males). However, Zn concentrations were similar between the two groups, at approximately 90 μg g^{-1} (mean value). In squid, Zn and Cu would be bound to both metallothioneins and high and very low molecular weight proteins (Finger and Smith 1987). Copper would be concentrated in spherulae (Martoja and Marcaillou 1992), and this element is also a component of the respiratory pigment hemocyanin (Miramand and Guary 1980).

No differences between sexes were found in metal concentrations for either immature or mature individuals ($P > 0.05$). This result was expected, as there are no differences in feeding habits between males and females (Ivanovic and Brunetti 1994).

Gonads

Heavy metal concentrations in the ovaries and testes of *Illex argentinus* from BNPS decreased in the order Zn > Cu > Cd (Table 4), and the levels were lower than those in the digestive gland. Similar results were reported by Miramand and Guary (1980) and Miramand and Bentley (1992). The Zn concentrations in gonads of squid (20 to 40 μg g^{-1}) are probably related to the high levels of Zn-containing enzymes and metalloproteins in these tissues.

Zinc and Cu levels were significantly higher in ovaries than in testes, in both mature and immature squid. Zn levels were significantly higher in mature than in immature ovaries ($P < 0.01$), whereas Cu concentrations, although with no significant differences ($P > 0.05$), were lower in the former tissues. The ovary mass of *Illex argentinus* increases >20-fold during sexual development, while the testes are much less modified (de Moreno et al. 1998). Mature ovaries contain 13.6% of the total body burden of Zn, while the immature gonads contain only 4.8% ($P < 0.05$, Table 3). The Cu burden was approximately doubled in mature ovaries and testes compared to immature tissues of the same type. Cadmium, Cu and Zn gonad burdens showed significant differences ($P < 0.01$) between sexes. These differences between mature and immature females are also found in gonad burdens for the three metals analysed.

As seen for the digestive gland, the gonads of mature squid from the SSS population exhibited higher Cd and Cu levels than BNPS organisms. However, Zn levels were similar in the two stocks (Table 4).

Table 4 Heavy metal concentrations (mean ± SE, μg g^{-1} wet wt) in the gonads of *Illex argentinus* from BNPS and SSS stocks (*BNPS* Buenos Aires Province and northern Patagonia subpopulation; *SSS* summer spawning stock; *MS* maturity stage; *M* male; *I* immature; *CV* coefficient of variation)

Stock, sex	MS	(*n*)	Cd		Zn		Cu	
			Mean ± SE	CV (%)	Mean ± SE	CV (%)	Mean ± SE	CV (%)
BNPS								
Male	M	(7)	0.14 ± 0.05	35.7	22.67 ± 0.74	3.3	1.64 ± 0.63	38.4
	I	(12)	0.10 ± 0.04	40.0	21.97 ± 2.65	12.1	2.29 ± 1.66	72.5
Female	M	(5)	0.17 ± 0.08	47.1	35.86 ± 3.22	9.0	8.98 ± 2.03	22.6
	I	(13)	0.13 ± 0.08	61.5	30.64 ± 2.67	8.7	15.48 ± 5.08	32.8
SSS								
Male	M	(3)	0.23 ± 0.06	26.1	22.40 ± 1.59	7.1	2.05 ± 0.62	30.2
Female	M	(3)	0.66 ± 0.25	37.9	35.45 ± 5.60	15.8	11.55 ± 4.31	37.3

Table 5 Heavy metal concentrations (mean ± SE, μg g^{-1} wet wt) in the dorsal mantle of *Illex argentinus* from BNPS and SSS stocks (*BNPS* Buenos Aires Province and northern Patagonia subpopulation; *SSS* summer spawning stock; *MS* maturity stage; *M* mature; *I* immature; *CV* coefficient of variation)

Stock, sex	MS	(*n*)	Cd		Zn		Cu	
			Mean ± SE	CV (%)	Mean ± SE	CV (%)	Mean ± SE	CV (%)
BNPS								
Male	M	(7)	0.16 ± 0.02	12.5	12.67 ± 1.00	7.9	1.71 ± 0.43	25.1
	I	(11)	0.08 ± 0.02	25.0	12.49 ± 1.06	8.5	1.83 ± 0.73	39.9
Female	M	(5)	0.08 ± 0.02	25.0	13.18 ± 0.98	7.4	1.60 ± 0.41	25.6
	I	(20)	0.09 ± 0.07	77.8	12.42 ± 1.91	15.4	2.45 ± 0.55	22.5
SSS								
Male	M	(3)	0.80 ± 0.17	21.3	11.80 ± 1.41	12.0	3.63 ± 1.46	40.2
Female	M	(3)	0.74 ± 0.40	54.0	12.79 ± 1.19	9.3	5.14 ± 1.40	27.2

Mantle

The dorsal mantle of cephalopods is consumed by humans and is among the tissues with the lowest heavy metal concentrations (Miramand and Guary 1980; Miramand and Bentley 1992). Levels decrease in the order Zn > Cu > Cd (Table 5). Zinc in the mantle nevertheless represented the major body burden (1000 to 2000 μg; Table 3) as the mantle weight accounted for 43 to 53% of the total body weight (de Moreno et al. 1998). No significant differences were noted in Zn levels between sexes for immature or mature individuals. Significant differences in Cu concentrations existed between the sexes only for immature organisms ($P < 0.05$), and Cu levels in immature females were significantly higher than those of mature females ($P < 0.01$). This negative correlation between Cu levels and squid size may be attributable to a higher growth rate and faster metabolism in younger organisms. Noteworthy, Zn, Cu and Cd concentrations of the dorsal mantle were lower than maximum values for human consumption (Nauen 1983), indicating that consumption does not represent a risk to humans.

As for the other tissues analysed in this study, the dorsal mantle of mature squids from the SSS population exhibited higher Cd and Cu levels than BNPS individuals, while Zn levels were similar in the two stocks (Table 5).

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