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Copper, Zinc, Cadmium, and Mercury in Southern Sea Lions (*Otaria flavescens*) from Argentina

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Abstract

Copper, zinc, mercury, and cadmium were studied in the muscle, liver, and kidney of three adults and one juvenile Southern sea lion (*Otaria flavescens*) found dead on the beaches of Argentina. Heavy metal concentrations were determined by Atomic Absorption Spectrophotometry; cold vapor and air/acetylene flame techniques were applied for mercury and for the rest of the metals, respectively. Previous acid digestion was made with nitric/sulphuric (Hg) and perchloric/nitric (Cd, Zn, Cu) mixtures. Quality was checked with a Certified Reference Material. Mercury concentrations were highest in the liver, whereas cadmium levels were highest in the kidney. The juvenile and adults presented the same tissue distribution pattern for all studied metals. Hepatic mercury concentrations ranged from 23.3 µg/g (juvenile female) to 47.6 µg/g (adult male), with renal cadmium concentrations between 0.8 µg/g and 5.7 µg/g, respectively.

Key Words: Heavy metals, distribution, food habit influence, Argentina, Southern sea lion, *Otaria flavescens*

Introduction

Marine mammals, as top predators of marine food webs, generally present high levels of heavy metals in their organs, even in muscle where the lowest levels are usually found (Law, 1995; Gerpe et al., 2002). The levels in each species are mainly associated with their trophic habits and geographical distribution. Coastal species usually present higher levels than oceanic ones. Moreover, diet plays an important role as the main metal source and prey preferences can influence the heavy metal burdens of marine mammals. It is known that cephalopods and fish are natural accumulators of cadmium (Miramand & Bently, 1992; Caurant & Amiard-Triquet, 1995; Gerpe et al., 2000) and mercury (Zhang et al., 2001; Jewett et al., 2003), respectively. As a consequence, squid and fish-eating marine mammals are selectively exposed to these heavy metals. This characteristic, coupled with their longevity and diverse foraging ecology, place marine mammals as an interesting group in which to study heavy metals.

Otaria flavescens is distributed along the Atlantic and Pacific coasts of South America, being the most frequently found pinniped along the Argentinian coast. It is not a migratory species and remains somewhat concentrated on the coastal zone, though some specimens may reach the edge of the continental shelf. Their foraging distribution makes them good indicators of the heavy metal contaminants in this region.

Although heavy metal studies were performed in several marine mammal species, information on the South American sea lion is scarce (Peña et al., 1988; Gerpe, 1996). *O. flavescens* is legally protected and entanglement is uncommon; sampling is restricted to those fresh specimens found stranded in accessible areas. Consequently, heavy metal studies in the Southern sea lion are very limited in contrast with incidentally killed or culled marine mammal species such as spinner or spotted dolphins and Arctic phocids.

The aim of the present paper was to study the levels and distribution of cadmium, zinc, copper, and total mercury in the liver, kidney, and muscle of juvenile and adult Southern sea lions from Argentina.

Materials and Methods

The studied Southern sea lions were obtained from beaches of the Buenos Aires Province (36° 14' to 38° 32' S), Argentina (Figure 1). Total

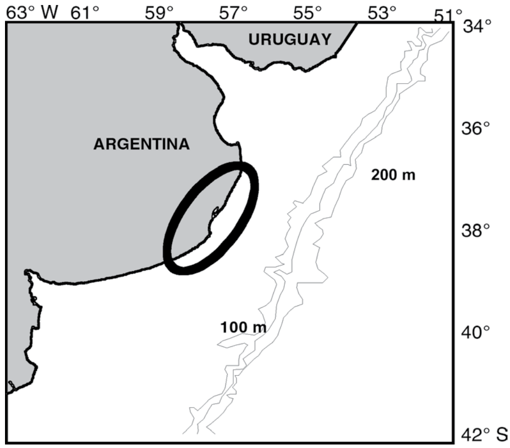

Figure 1. Sampling areas of *Otaria flavescens*, Buenos Aires province (36° 14' to 38° 32' S), Argentina

length and weight, sex, and age class were determined for each specimen (Table 1). Liver, muscle, and kidney samples were taken to analyze total mercury, zinc, copper, and cadmium in fresh tissue. Only very fresh carcasses were sampled to prevent further biases in analytical procedures due to post-mortem decomposition.

Cadmium, copper, and zinc concentrations were determined by Atomic Absorption Spectrophotometry (AAS) with air-acetylene flame, using a deuterium lamp for background correction. Samples were digested with perchloric and nitric acids (1:3), according to the method described by FAO/SIDA (1983). Total mercury was examined following the cold vapor method of Moreno et al. (1984). Mineralization was made with sulphuric and nitric acids (4:1) in a thermostatic bath (T < 60° C); the oxidation was completed with potassium permanganate and hydrogen peroxide. Both 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 ensure quality control, a Certified Reference Material No. 6 (mussel) from the National Institute for Environmental Studies (NIES, Tsukuba, Japan), Japan Environmental Agency, was analyzed with the samples. The values obtained were in agreement with the certified concentrations ($p < 0.05$). The detection limit of the method was 0.05 µg/g wet weight. Metal concentrations were expressed in µg/g (wet weight) and determinations were by duplicates.

Statistical differences in tissue concentrations were tested by parametric (Student's *t* test and one-way analysis of variance [ANOVA]) or nonparametric (Mann Whitney and Kruskall-Wallis tests) procedures, with an initial test of homoscedasticity (Levene test; $p < 0.05$). Post-hoc comparisons were performed by Scheffé test. Statistical analyses were performed using *Statistica* (1999) software.

A statistical comparison between age classes could not be performed because of small sample size.

Results

Heavy metal concentrations found in *O. flavescens* are presented in Table 2. No significant differences in concentrations were found between tissues for both zinc (ANOVA F = 0.064; $df = 2, 9$; $p = 0.939$) and copper (ANOVA F = 0.669; $df = 2, 9$; $p = 0.536$). In contrast, significant differences in concentrations between tissues were found in both cadmium (Kruskall-Wallis H = 7.138 [2; $n = 8$]; $p = 0.028$) and mercury (ANOVA F = 42.093; $df = 2, 9$; $p < 0.001$). The liver presented the

Table 1. Biological data of *Otaria flavescens*; ND: not determined.

Specimen #	Total length (cm)	Total weight (kg)	Sex	Age class
1	146	67	Female	Adult
2	230	300	Male	Adult
3	137	ND	Female	Adult
4	121	ND	Female	Juvenile

Table 2. Heavy metals concentrations (µg/g, wet weight, mean ± SD) in muscle, liver, and kidney of *Otaria flavescens*

Tissue	Mercury	Cadmium	Zinc	Copper
Muscle	1.42 ± 0.53	0.12 ± 0.21	7.74 ± 5.74	0.83 ± 0.50
Liver	33.90 ± 10.09	0.63 ± 0.47	8.31 ± 9.05	1.26 ± 0.57
Kidney	0.75 ± 0.47	2.16 ± 2.39	6.45 ± 7.47	0.92 ± 0.57

highest levels of mercury (Scheffé test, $p < 0.001$), whereas cadmium was mainly concentrated in the kidney. Although limited by the small sample size, the distributional pattern for Hg (liver > muscle > kidney) and cadmium (kidney > liver > muscle) seem to be consistent with lower values in the younger animal (Table 3).

The resulting distributions of heavy metals in each organ of all specimens were liver (Cd < Cu < Zn < Hg), kidney (Cu ~ Hg < Cd < Zn), and muscle (Cd < Cu < Hg < Zn).

The Liver/Muscle ([L]/[M]) ratio was above 1 for all heavy metals, with the exception of zinc in a single animal (Table 4). The Kidney/Muscle [K]/[M] ratio was above 1 for cadmium and below 1 for mercury, with fluctuating values for zinc and copper (Table 4). This pattern was consistent in both the single juvenile and the adults.

Discussion

The liver and kidney are considered the target organs for mercury and cadmium, respectively. This trend was confirmed in pinnipeds from different areas by Law et al. (1991, 1992), Malcolm et al. (1994), Sydeman & Jarman (1998), Woshner et al. (2001), and Riget et al. (2005). This metal accumulation surely is related to the presence of metallothioneins to bind these metals. Unfortunately, metallothionein information on marine mammals is scarce and is absent for sea lions. Tohyama et al. (1986) reported a correlation between these proteins and age in *Phoca vitulina*, and Das et al. (2000, 2002) found high cadmium and mercury levels in liver and kidney related with metallothioneins in marine mammals, particularly for *Lagenorhynchus acutus*. So, the high levels of non-essential metals found in the liver and kidney of *O. flavescens* could be associated with the presence of those proteins.

Metal accumulation in the liver and kidney of *O. flavescens* could be evaluated by [L]/[M] and [K]/[M] ratios, as the relation between target organs to muscle levels, the latter usually considered close to the background ones. They could be used as good indicators to measure the accumulation of non-essential metals in those organs. The high cadmium and mercury [L]/[M] and cadmium [K]/[M] ratios found in *O. flavescens* reflect the high capacity to accumulate these metals in the species.

The principal source of metals in Southern sea lions is food, and particular prey can determine the contribution of specific metals. Vaz Ferreira (1982), Rivero et al. (1999), Koen Alonso et al. (2000), and Naya et al. (2000) reported that Southern sea lions prey on fish, crustaceans, and cephalopods. The specimens analysed here could belong to Patagonian or Buenos Aires Province populations. Both groups show some different prey preferences: in Patagonia, sea lions frequently prey on fish (Frequency of Occurrence [FO] = 3.8 to 72.7%) and cephalopods (squid and octopuses: FO = 3.8 to 54.5%) (Koen Alonso et al., 2000), whereas in Buenos Aires Province, fish are more frequently preyed upon (FO = 100%) than cephalopods (only octopuses: FO = 23%) (Rivero et al., 1999). The higher level of hepatic mercury compared with renal cadmium found in the studied specimens may suggest that they belong to the Buenos Aires Province stock. Sea lions have higher exposure to mercury than cadmium via food because fish are good accumulators of that metal (Zhang et al., 2001; Jewett et al., 2003). Fish accumulate mercury in the methylated form, presenting higher bioavailability and toxicity than inorganic mercury. Southern sea lions accumulate mercury from food at a high ratio, surpassing concentrations reported in fish (Table 5), and revealing a biomagnification process. In

Table 3. Range of heavy metal concentrations (µg/g, wet weight) found in juvenile and adult Southern sea lions in study

	Mercury		Cadmium		Zinc		Copper	
Tissue	Juvenile	Adults	Juvenile	Adults	Juvenile	Adults	Juvenile	Adults
Muscle	0.85	0.99-2.09	< 0.05	< 0.05-0.43	3.54	4.24-16.03	0.37	0.79-1.54
Liver	23.26	32.31-47.59	0.27	0.30-1.27	4.12	1.89-21.69	1.00	0.79-2.09
Kidney	0.47	0.42-1.43	0.75	0.93-5.73	3.97	2.62-17.57	0.64	0.42-1.73

Table 4. Liver/Muscle ([L]/[M]) and Kidney/Muscle ([K]/[M]) ratios found in *Otaria flavescens*

Ratios	Mercury	Cadmium	Zinc	Copper
[L]/[M]	18.74-32.64	2.95-68.00	0.26-1.35	1.00-2.70
[K]/[M]	0.39-0.68	13.33-122.00	0.23-1.12	0.53-1.73

Table 5. Cadmium and mercury concentrations (μg/g, wet weight) in prey species of *Otaria flavescens*; ND: not detectable, NA: not analyzed.

Prey species	Tissue	Mercury	Cadmium	Reference
Cynoscion guatucupa (Striped weakfish)	Muscle	0.23-0.42	NA	Pérez et al., 1986
	Muscle	0.14 ± 0.08	NA	Marcovecchio
	Liver	0.16 ± 0.07	NA	et al., 1989
Micropogonias furnieri (Whitemouth croaker)	Muscle	< 0.05-0.25	NA	Pérez et al., 1986
	Muscle	0.11 ± 0.04	NA	Marcovecchio et al., 1989
	Liver	0.13 ± 0.04	NA	
Conger orbignyanus (Argentine conger)	Muscle	0.29 ± 0.07	0.22 ± 0.07	Marcovecchio
	Liver	0.34 ± 0.11	2.16 ± 0.66	et al., 1989
Loligo brasiliensis (Longfin inshore squid)	Hepatopancreas	0.06 ± 0.02	NA	Marcovecchio et al., 1988
Illex argentinus (Shortfin Argentine squid)	Muscle	< 0.05-0.41	NA	Pérez et al., 1986
	Muscle	ND	0.03-0.18	Gerpe et al., 2000
	Hepatopancreas	ND	142.00-363.00	
	Gonads	ND	0.06-0.25	
Pleoticus muelleri (Red Argentine shrimp)	Muscle	NA	ND	Jeckel et al., 1996
	Hepatopancreas	NA	3.00-11.80	
	Gonads	NA	0.08-1.1	

contrast, no biomagnification occurred in cadmium because the levels in Southern sea lions were lower than those reported in their prey. Crustaceans (Jeckel et al., 1996), mainly cephalopods (Miramand & Bently, 1992; Gerpe et al., 2000), are accumulators of cadmium, being transferred to top predators (Bustamante et al., 1998). In the case of the studied Southern sea lions, these items could have been preyed upon at a low frequency and thus reflected in the lowest cadmium levels.

The essential role of zinc and copper complicate the evaluation of their accumulation and establishment of the level at which the normal physiological concentrations is surpassed. Based on [L]/[M] and [K]/[M] ratios, we suggest that the concentrations found are near background concentrations because they fluctuate slightly above and below 1. Essential metals are under metabolic homeostatic control, which are usually maintained at physiological levels. Law et al. (1991) postulated a range for liver concentrations where zinc and copper could be regulated at < 100 μg/g (wet weight) and < 30 μg/g (wet weight), respectively. *O. flavescens* presented significantly lower levels than these values. Further studies on a higher number of specimens could confirm if these low values are normal for this species.

In the only juvenile analysed in the present study, all the tissue concentrations were lower than the adults' range or close to the minimum value. Although not conclusive, a slight tendency to accumulate mercury and cadmium with age was recorded, suggesting that Southern sea lions have physiological mechanisms that allow its concentration throughout life. Bioaccumulation of these toxic metals could affect the survival or behavior of Southern sea lions but, unfortunately, related literature is null. Moreover, levels found here were below the maximum hepatic levels of tolerance reported by Wagemann and Muir (1984) for mercury (100 to 400 μg/g wet weight) and Law et al. (1996) for cadmium (20 to 200 μg/g wet weight). So, levels of *O. flavescens* may not represent a severe problem for its health, probably associated with high levels (natural and/or induced) of metallothioneins, detoxifying both toxic metals.

Mercury and cadmium in *O. flavescens* were previously studied only by Peña et al. (1988). Although no body length or age class was reported, we found a similar distribution pattern with the highest mercury levels in the liver and cadmium in the kidney. Maximum hepatic mercury concentrations (47.59 μg/g wet weight) were slightly higher than those in the kidney, and significantly higher for renal cadmium levels (1.40 to 8.60 μg/g). Southern sea lions studied by Peña et al. belonged to a male haulout located inside of the Mar del Plata harbor. The sea lions from this colony feed mainly on discarded fish and squid (Baldás et al., 1987; Rodríguez et al., 1992; Chaijale, 1999), with a high frequency of squid in their diet. As mentioned above, squid may have contributed to higher cadmium levels found by Peña et al. (1988).

Finally, the Southern sea lions could be considered a potential biomonitor of its environment. Top predator information could provide valuable insight about bioavailability of metals, principally toxic ones; bioaccumulation processes; and biomagnification through marine food webs.

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