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Note

Hepatic and renal metallothionein concentrations in Commerson's dolphins (*Cephalorhynchus commersonii*) from Tierra del Fuego, South Atlantic Ocean

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ABSTRACT

The Commerson's dolphin is the most common endemic odontocete of subantarctic waters of Tierra del Fuego, Argentina incidentally caught in fishing nets. The species is classified as "Data Deficient" by the IUCN. Metallothioneins (MTs) are considered as suitable biomarkers for health and environmental monitoring. The aims of the study were to assess MT concentrations in the liver and kidney of bycaught specimens. Moreover, correlations with Zn, Se, Cd, Ag and Hg, and the molar ratios of MT:metals were estimated to evaluate if there is an indication of their respective protective role against metal toxicity in tissues. Hepatic and renal MT concentrations were similar, ranging from 11.6 to 29.1 $\text{nmol}\cdot\text{g}^{-1}$ WW, and Kidney/Liver ratios ranging from 0.73 to 1.93 corresponded to normal ranges. Results suggest that MTs are related to physiological ranges for the species. This information constitutes the first MT report on Commerson's dolphins and possibly considered as baseline for species' conservation.

High marine vertebrates exhibit physiological mechanisms to prevent the toxic effects of metals in their diet, including intracellular sequestration as non-toxic granules and the synthesis of metallothioneins (MTs) (Bebianno et al., 2007; Raymond and Ralston, 2009). Mammalian MTs are cytosolic metal–proteins with low molecular weight and high cysteine content, and they are one of the primary proteins for preventing the harmful effects of toxic metals in tissues (Amiard et al., 2006; Thirumoorthy et al., 2011). MTs are involved in the homeostasis of essential metals such as copper (Cu) and zinc (Zn), and they also can bind toxic metals (e.g., cadmium, Cd, and mercury, Hg) (Klaassen et al., 1999; Das et al., 2000, 2002; Amiard et al., 2006). The induction of MT has been considered as one of the most important detoxification processes against metal toxicity (Roesijadi, 1994). An influx of metals into the cells can induce MT synthesis de novo, a direct biochemical response to metal exposure (Cosson, 2000). The capacity for MT induction is greatest in tissues that are active in uptake, storage and excretion of metals, such as the liver and kidney (Company et al., 2010; Siscar et al., 2014). Currently, MTs are considered as suitable biomarkers for health and monitoring programs (Decataldo et al., 2004; Carpenè et al., 2007; Sonne et al., 2009; Polizzi et al., 2014).

Trace elements in the marine environment come from natural and anthropogenic sources. They are concentrated through the food chain and accumulated in the tissues of various species of marine mammalian predators at the top of the food web. Essential elements which are vital to life include Zn, Fe, Cu and Se, as they are co-factors for many enzymes involved in physiological functions (Das et al., 2003). Nevertheless, they become toxic when accumulated at high concentrations in tissues and organs. Non-essential metals include toxic elements such as Cd, Ag and Hg, which can be tolerated by living organisms at low levels, but also become toxic at high concentrations and adversely affect mammalian health (Das et al., 2003). The formation of Se–Hg–protein complexes has an important role in preventing Hg toxicity in the liver of marine mammals (Koeman et al., 1973; Ikemoto et al., 2004) because Hg has a greater affinity for Se than it has for sulfur groups of proteins (Ikemoto et al., 2004; Kunito et al., 2004; Raymond and Ralston, 2009). Excess Se reduces Hg toxicity, and a Se:Hg molar ratio of 1:1 or

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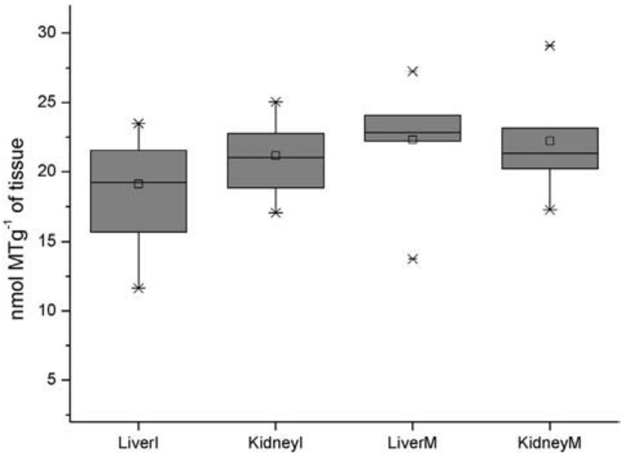

Fig. 1. Distribution pattern by age groups (I, immature and M, mature) of metallothionein (MT) concentrations in liver and kidney of Commerson's dolphins. Inside the box, the horizontal line represents the mean, the bottom and top of the box constitute the interquartile range (25% and 75%) distribution, and the lines extending vertically indicate the minimum and maximum values.

greater prevents the adverse effects of Hg consumption (Raymond and Ralston, 2009; Sørmo et al., 2011). Moreover, the molar ratios of both MT:metal and Se:metal are a better measure of the metal detoxification capacity than MT and Se concentrations alone (Company et al., 2010; Sørmo et al., 2011).

To date, most studies of MT in marine mammals have been based on tissues from dead animals (Tohyama et al., 1986; Kwohn et al., 1986; Das et al., 2000, 2002, 2006; Decataldo et al., 2004) including sperm whales (*Physeter macrocephalus*) (Holsbeek et al., 1999), narwhals (*Monodon monoceros*) (Wagemann et al., 1984), long-fined pilot whales (*Globicephala melas*) (Caurant et al., 1996), striped dolphins (*Stenella coeruleoalba*) (Kwohn et al., 1986, 1988; Decataldo et al., 2004), harbor porpoises (*Phocoena phocoena*) (Das et al., 2000, 2006), bottlenose dolphins (*Tursiops truncatus*) (Decataldo et al., 2004) and white-sided dolphins (*Lagenorhynchus acutus*) (Das et al., 2002). Nowadays, such analysis remains limited for species at South Atlantic Ocean. In Argentina, only one study has been performed with the Franciscana dolphin (*Pontoporia blainvillei*) (Polizzi et al., 2014). The Franciscana together with the Commerson's dolphin (*Cephalorhynchus commersonii*) comprises the smallest cetaceans which are affected by anthropogenic pressure (e.g. by-catch and marine pollution) due mainly to their coastal habits (Goodall et al., 1994; Goodall et al., 1988; Polizzi et al., 2014; Negri et al., 2014; Cáceres-Saez et al., 2015). The Commerson's dolphin is a coastal species distributed from 41°30′S to 55°S in the Southwestern South Atlantic Ocean, including the Malvinas Islands (Goodall et al., 1988). Throughout its range, these dolphins are vulnerable to entanglement in artisanal fishery nets (Goodall et al., 1988; Cáceres-Saez et al., 2015). The International Union for Conservation of Nature (IUCN) has classified the species as "Data Deficient" — DD (Reeves et al., 2013). Despite conservation concerns for this species, there is little information on the health status. As a result, research on biomarkers such as MT and toxic metals, particularly in coastal species that are sentinels for environmental pollution, can provide important information on environmental stress and conservation status. In this study, we measured MT in the liver and kidneys of different age classes of Commerson's dolphins, and evaluate their relationship with trace elements (Ag, Hg, Cd, Zn and Se). We also calculated the molar ratios of MT:metals to evaluate if there was a protective role against metal toxicity in tissues.

Commerson's dolphins were collected as bycatch from artisanal fishing nets in coastal areas of Tierra del Fuego, Argentina (S53°17′–W68°28′ to S54°25′–W66°33′) as a part of the AMMA project (Aves y Mamíferos Marinos Australes) during the austral summers of 2009–2014. All dolphins were given a RNP number (corresponding to abbreviation's Rae Natalie Prosser) which is the code that identifies specimens in the collection of the Museo Acatushún, located at the Estancia Harberton, Tierra del Fuego, Argentina. Data on sex, total body length and body weight were recorded during external examination before necropsy. Tissue samples were wrapped in individual polyethylene bags, chilled in a portable refrigerator during transport, and then kept frozen at −20 °C until analysis. In total 14 liver and 16 kidney samples from 16 individuals were analyzed for MT concentrations. Age was determined using Growth Layers Groups (GLGs) in dentine, for that, teeth were removed and processed following the methodology described by Dellabianca et al. (2012) and assuming that one GLG represents one year.

MT quantification was performed according to the spectrometric method described by Viarengo et al. (1997). The absorbance was read at 412 nm, and MT concentration was quantified using reduced glutathione (GSH) as a standard (see Polizzi et al., 2014). All samples were analyzed in duplicate and with blanks. The concentrations were reported as nmol MT gram^{-1} wet weight tissue (WW). The concentrations of essential (Zn and Se) and non-essential (Cd, Ag and Hg) elements have already been measured in the liver ($n = 9$) and kidney ($n = 6$) of these species (Cáceres-Saez et al., 2013a, 2013b). Here, we assessed the relationships between MT and trace element concentrations to evaluate whether there was a potential protective role of MT to metal toxicity. Molar ratios (MT:metal) were calculated as described by Company et al. (2010). A molecular weight of 6000 Da for MT and the respective atomic weights for metals were used for the conversion of MT and metal mass concentrations (μg g^{-1} WW) into molar concentrations (mol·g^{-1} WW). Statistical analyses were performed using the software package STATISTICA 6.0 (Statsoft, Inc.) and OriginPro 8. Data were checked for normality (Kolmogorov–Smirnov test) and homogeneity (Levene's test). One way ANOVA and Mann Whitney (non-parametric) tests were used to assess statistical differences between tissues of mature and immature specimens. Spearman correlation coefficients (r) were used to assess the relationships between concentrations of MTs in the liver and kidney, and the toxic metal (Cd, Hg and Ag) and essential element (Zn and Se) concentrations that were previously reported (Cáceres-Saez et al., 2013a, 2013b). Differences at the 5% level were considered statistically significant.

Biological parameters of the studied specimens are detailed in Table 1. Individuals were grouped as juveniles less than two years of age, including the suckling, still nursing and immature (I; $n = 12$) dolphins, and the other group as adults, sexually mature (M; $n = 4$) individuals older than five years (Goodall et al., 1988). MT concentrations in the liver and kidney of Commerson's dolphins and the tissue ratios (K/L) are also showed in Table 1. Hepatic MT concentrations ranged from 11.6–27.2 nmol·g^{-1} WW, and renal MT concentrations from 17.0–29.1 nmol·g^{-1} WW. The highest concentrations in the liver occurred in a four year old female (RNP 2844), whereas the highest concentrations in the kidneys occurred in a seven year old female adult (RNP 2670). Mean hepatic MT concentration increased slightly from 19.1 nmol·g^{-1} WW for immature animals to 22.3 nmol·g^{-1} WW for adults, and a similar increase from 21.2 nmol·g^{-1} WW for immature and 22.2 nmol·g^{-1} WW in adults was observed in renal MT. (Fig. 1). However, the small and unequal sample size for each age classes was insufficient to determine statistical significance.

Concentrations of MTs in this study were generally low relative to that in coastal Franciscana dolphin from Buenos Aires Argentina, which ranged from 20 to 60 nmol·g^{-1} WW in both, the liver and kidneys (Polizzi et al., 2014). Das et al. (2000) observed that the MT concentrations in the liver and kidney of stranded cetaceans and pinnipeds varied from 58 to 1200 μg·g^{-1} WW. Among them, higher concentrations were found in renal tissues of pilot whales (751 ± 213 μg·g^{-1} WW; Caurant et al., 1996), sperm whales (468–951 μg·g^{-1} WW; Bouquegneau et al., 1997; Holsbeek et al., 1999) and narwhal (1200 μg·g^{-1} WW; Wagemann et al., 1984). Furthermore, MT

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Table 1
Biological data, metallothionein concentrations (MT in wet weight) in the liver and kidney and the ratio of MT K/L in Commerson's dolphins.

Specimen	Sex	Age		Total length	Body weight	Liver	Kidney	K/L
RNP number		Years (GLGs)	Group	(cm)	(kg)	(nmoles de MT gr^{-1})		
2628	Male	1	I	118.9	27	n.a.	22.76 ± 0.3	-
2629	Male	0	I	79.4	n.r.	n.a	18.52 ± 0.6	-
2669	Male	2	I	121	27.25	21.57 ± 5.2	22.04 ± 2.3	1.02
2671	Male	<1	I	109	18.1	23.48 ± 4.5	25.04 ± 4.3	1.07
2701	Male	<1	I	117.4	27	11.65 ± 3.0	22.48 ± 3.7	1.93
2724	Male	14	M	136.9	35	23.73 ± 4.2	17.25 ± 0.9	0.73
2725	Female	11	M	135.1	37.5	13.76 ± 1.5	21.33 ± 0.9	1.55
2727	Female	<1	I	99.3	25	19.22 ± 0.63	23.62 ± 0.0	1.23
2728	Male	<2	I	116.6	28.5	17.08 ± 2.4	21.02 ± 3.7	1.23
2834	Female	<1	I	119.2	n.r.	21.54 ± 4.0	17.02 ± 0.6	0.79
2839	Female	<1	I	122.5	32	22.65 ± 2.7	18.83 ± 4.2	0.83
2843	Male	5	M	130.7	34.5	22.83 ± 9.3	23.15	1.01
2844	Female	4	I	132.3	39.5	27.24 ± 0.1	20.20 ± 0.0	0.74
2856	Female	4	I	134.7	37	22.21 ± 1.0	22.27 ± 1.8	1.00
2857	Female	2	I	135	39	15.65 ± 3.0	20.25	1.29
2670	Female	7	M	139	31.2	24.08 ± 3.2	29.10 ± 0.1	1.21

I, immature; M, mature; n.r. — not registered; n.a. — not analyzed.

concentrations in the kidney were 1.2 to 1.6-fold higher than in the liver (Das et al., 2000). The tissue ratios Kidney/Liver (K/L) of our specimens (Table 1) were within the range reported from previous studies, taking into account biological variation due to age and physiological status (e.g. pregnancy and moulting) and stress (Das et al., 2000). The regulation of MT as well as metal concentrations will be influenced by local environmental concentrations and differences in prey preference. Other environmental variables such as water temperature and salinity could also be factors (Siscar et al., 2014).

Previous studies have used the relative concentration of MT and toxic metal as evidence for the role of MT in Cd, Hg and Ag detoxification in higher marine predators (Cosson, 1994; Das et al., 2006; Bebianno et al., 2007; Siscar et al., 2014). The concentrations of Cd, Hg, Ag, Zn and Se that were previously reported in the liver and kidney of some of the specimens studied here (Cáceres-Saez et al., 2013a, 2013b), were used to assess the potential relationships between them. Regarding measurements of essential elements, Zn concentrations were similar between both tissues (liver, 33.4 ± 3.4 $\mu g \cdot g^{-1}$ WW and kidney, 31 ± 2.4 $\mu g \cdot g^{-1}$ WW), as well as concentrations of Se (liver, 5.3 ± 2.5 $\mu g \cdot g^{-1}$ WW and kidney, 3.4 ± 0.7 $\mu g \cdot g^{-1}$ WW) (Cáceres-Saez et al., 2013a, 2013b; Cáceres-Saez, 2014). The concentration of Cd was higher in the kidney (9.0 ± 6.2 $\mu g \cdot g^{-1}$ WW), however no differences were found compared to those in the liver (2.6 ± 1.8 $\mu g \cdot g^{-1}$ WW). Among the non-essential elements, concentrations of Hg and Ag were higher in the liver (4.5 ± 4.4 and 2.0 ± 2.1 $\mu g \cdot g^{-1}$ WW; respectively), although no differences were found to those in the kidney (Hg, 0.92 ± 0.51 and Ag, 0.37 ± 0.67 $\mu g \cdot g^{-1}$ WW; respectively) (Cáceres-Saez et al., 2013a, 2013b). At this time, relationships between MT and the known MT inducers, Cd, Hg, Ag and Zn (Cosson, 1994; Bebianno et al., 2007; Company et al., 2010) were evaluated separately. Concerning that, no correlations of hepatic and renal MT concentrations to those of Zn ($r_{liver} = -0.17$, $r_{kidney} = -0.08$, $p > 0.05$) and Se ($r_{liver} = 0.24$, $r_{kidney} = 0.18$, $p > 0.05$) were found. Also no significant correlations were found between concentrations of MTs and toxic metals, such as Cd ($r_{liver} = 0.33$, $r_{kidney} = 0.14$, $p > 0.05$), Hg ($r_{liver} = 0.55$, $r_{kidney} = 0.08$, $p > 0.05$) and Ag ($r_{liver} = 0.14$, $r_{kidney} = -0.03$, $p > 0.05$). As in other report, our results did not show any relationship between MT with Cd and Zn in hepatic and renal tissues (Polizzi et al., 2014). The lack of relationship between both variables could be due since these values possible corresponds to the background of physiological levels of the organisms. Likewise this suggests a similarity between specimens, independently from the age or growth, or a narrow range variation for MT within those individuals. The essential elements, such as Zn and Cu, are basically related to MT and involved in their homeostasis, which would indicate that there are no excess of essential metal (Zn) in specimens analyzed. As quoted above MT concentrations in tissues are generally low, consequently no induction of MT or their synthesis de novo of these proteins will be expected, then background level is enough to reach their union. Cadmium is the main inducer of MT (even more than Zn), therefore if there is no connection between MT–Cd it can be said that there is no excess of free toxic metal. Only one study, conducted in harbor porpoise, detected a significant relationship between MT levels and Cd and Hg contents (Das et al., 2006), certainly with higher concentrations of both toxic metals. This is in agreement with the information provided by Decataldo et al. (2004) for stripped dolphins and bottlenose dolphins, and even with those for pinnipeds such as the California sea lion (*Zalophus californianus*) (Lee et al., 1977) and grey seal (*Halichoerus grypus*) (Teigen et al., 1999).

As commented before, two main mechanisms have been indicated to prevent the toxic effect of metals: first, the protective role of Se to prevent Hg and Ag toxicity has been widely described for aquatic vertebrates (Koeman et al., 1973; Cuvin-Aralar and Furness, 1991; Das et al., 2000; Nakazawa et al., 2011). The formation of Se–Hg complex has an important role in Hg detoxification by the liver of marine mammals (Caurant et al. 1996; Ikemoto et al., 2004), indeed Se binds several other cations such as Ag (Sasakura and Suzuki, 1998) and their protection seen would be relative. Evidence was also found that Se protects against toxicity of Cd and Ag by storing these toxic metals alone and/or by forming a complex with Zn (Ikemoto et al., 2004; Kunito et al., 2004; Nakazawa et al., 2011). Particularly, molar ratios of Se:Hg and Se:Ag have been proposed as a measure of Se to face toxic effects of both metals (Raymond and Ralston, 2009; Nakazawa et al., 2011). In second instance, the MTs are involved in the homeostasis of essential metals, such as Cu and Zn, and play a role as detoxifying mechanism when their levels are higher than those required, and also for those non-essential metals, such as Cd, Hg and Ag (Das et al., 2000; Amiard et al., 2006; Bebianno et al., 2007).

Since the molar ratios of MT:metal were considered as a proxy of metal saturation of the protein (Company et al., 2010; Siscar et al., 2014), we calculated the molar ratios between MT and metals *per tissue* across all specimens studied, and summarized in Table 2. The highest mean value of MT:Cd was observed in the liver, although the highest ratio of MT:Hg and MT:Ag was attained in renal tissue (see Table 2). Molar ratios showed that MT proteins in Commerson's dolphins analyzed are possibly not saturated for any of the toxic metals considered, since MT is much more abundant than these elements. In general, the molar ratios of MT:Hg and MT:Ag were higher suggesting a higher buffering capacity for metal binding of MT proteins. Few data concerning metal to thionein molar ratio are available for other species, for example the striped dolphins with renal values of 2.93 for Cd/MT, 0.04 for Hg/MT

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Table 2
Molar ratios between metallothionein (MT) and selected elements (Zn, Se, Cd, Ag and Hg) in liver and kidney of Commerson's dolphins.

Specimen	Liver					Kidney				
RNP number	MT:Zn	MT:Se	MT:Cd	MT:Hg	MT:Ag	MT:Zn	MT:Se	MT:Cd	MT:Hg	MT:Ag
2628	n.a.	n.a.	0.86	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
2669	0.04	1.10	0.50	1.28	4.65	0.04	0.55	0.15	3.85	152.9
2671	0.04	1.75	0.69	4.12	6.51	0.05	0.61	0.18	6.53	79.5
2701	0.02	0.75	1.55	2.84	1.63	0.05	0.52	0.34	9.92	186.9
2724	0.05	0.44	2.10	0.36	0.40	n.a.	n.a.	n.a.	n.a.	n.a.
2728	0.03	0.57	2.43	1.65	0.41	0.05	0.50	0.81	4.51	1.3
2725	0.03	0.37	1.14	0.35	1.45	n.a.	n.a.	n.a.	n.a.	n.a.
2727	0.04	0.86	6.19	10.07	1.65	0.05	0.38	1.35	11.37	170.9
2670	0.05	0.80	0.65	0.54	2.31	0.07	0.83	0.34	3.24	6.9
Mean	0.04	0.83	1.79	2.65	2.37	0.05	0.57	0.53	6.57	99.73
SD	0.01	0.44	1.78	3.28	2.14	0.01	0.15	0.47	3.38	82.70

n.a. — not analyzed.

and 2.86 for Zn/MT (Kwohn et al., 1986) and also for the white-sided dolphins with values of 2.99 for Cd/MT, 0.22 for Hg/MT and 3.88 for Zn/MT (Das et al., 2006). As we seen in our previous study, the Se:Hg molar ratios in all of this specimens were equal or greater than 1, ranging from 3.90–29.6 in the kidney and from 1.71–29.6 in the liver (Cáceres-Saez et al., 2013b; Cáceres-Saez, 2014) suggesting that Se and also MT are in excess and still provide spare binding capacity. Apparently, MTs only respond to the presence of Hg in the case of Se deficiency (Sørmo et al., 2011) and this does not seem to be the case for Commerson's dolphins under study. It is possible that toxic metals are being detoxified by another mechanism. Therefore, it is expected a Hg detoxification with greater affinity of Se as compared with MT, attributable to the lower induction of Hg on these proteins. Though, relationship between Hg and Se could be stronger to detoxify Hg than those binding to MT.

This study constitutes the first information about MT in liver and kidney tissue of the Commerson's dolphin, and provided us with the first data on MT:metal molar ratios in dolphins. This study therefore can be considered as baseline data for future research aiding to delineate adequate strategies for the species conservation.

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