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Baseline

Trace metal concentrations in Southern Right Whale (*Eubalaena australis*) at Península Valdés, Argentina

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ABSTRACT

The presence of essential (Fe, Mn, Zn, Cu, Ni and Al) and non-essential trace metals (Cd and Pb) was tested in liver ($N = 26$) and kidney ($N = 42$) from dead Southern Right Whale (SRW – *Eubalaena australis*) calves found beached in Península Valdés, Argentina. Essential metals were detected in all samples, particularly in hepatic tissue; though Ni and Al were accumulated mainly in the kidney. Cd and Pb were not detected in any samples. Sex and length of calves did not influence metal levels found, nor did the geographic location of carcasses. Our findings for essential metals were similar to those reported for mysticetes in other parts of the world. Except for a previous report on one SRW calf, this is the first data on trace metals for this species in Patagonia. This information is vital for SRW management considering increasing human pressures impacting their feeding and breeding grounds.

The coastal zone of Patagonia in Argentina is under increasing development pressure. This area presents a rich biological diversity that supports a flourishing tourism industry, which in turn favors urban and industrial growth in the region. The main environmental problems are related to effluent discharge, together with oil extraction and transport, and fishing. Previous studies on trace metals in abiotic compartments and invertebrates are available for this region (Harvey and Gil, 1988; Gil et al., 1989, 1999; Giarratano et al., 2002; Vázquez et al., 2007). However, information related to seabirds and marine mammals is very limited (Marcovecchio et al., 1994; Pérez et al., 2005; Gil et al., 2006).

Because of their biological features and longevity, and their vast geographic ranges, baleen whales (mysticetes) are considered sentinels of marine ecosystem health. As such, they reflect environmental changes in feeding, reproduction and migration areas that, in turn, could affect their survival (Moore, 2008). Right whales occupy two wide ocean strips between 20° and 60° latitude, in the Northern and Southern Hemispheres. *Eubalaena glacialis* is located in the Northern Hemisphere while *Eubalaena australis,* the Southern Right Whale (SRW), is in the Southern Hemisphere (Payne, 1986). SRW regularly visit high latitudes of the Antarctic Ocean during the feeding period of their life cycle. This period includes the austral summer months when krill, its main food item, is more abundant. In autumn, as water solidifies and daylight decreases, reduced photosynthesis and subsequent lower phytoplankton and zooplankton biomasses trigger SRW migration to warmer waters (Best and Schell, 1996). Even though migration implies substantial energy losses affecting blubber storage, whales do not commonly feed during this time (Payne, 1986). In northern Patagonia, the relatively warm and protected waters of Península Valdés offer a favorable and sheltered location for SRW reproduction and calving, which occurs every year from May to December (Payne, 1986).

As occurs with all species, whale mortalities also occur at Península Valdés. To record mortality numbers, identify causes of mortality and determine the overall SRW population's health the Southern Right Whale Health Monitoring Program performs post-mortem analysis on dead SRW since 2003, making a large number of samples available for research (Uhart et al., 2009). In recent years, the SRW population that visits Península Valdés has experienced alarming increased mortality rates of unknown origin (Werner et al., 2011). This study provides information on trace metals in SRW calves which could potentially be affecting their fitness and survival.

The aim of this paper was to test the levels of essential (Fe, Mn, Zn, Cu, Ni and Al) and non essential trace metals (Cd and Pb) in liver and kidney of dead SRW calves beached in San José and Nuevo Gulfs at Península Valdés, Argentina (Fig. 1). Moreover, these levels were compared to previous published data, and metal concentration differences between Gulfs, age, sex and organs were evaluated.

Between 2003 and 2009, 373 SRW carcasses stranded on the shores of Nuevo and San José Gulfs, in Península Valdés. In each case, location, date, sex and total length (as an indicator of

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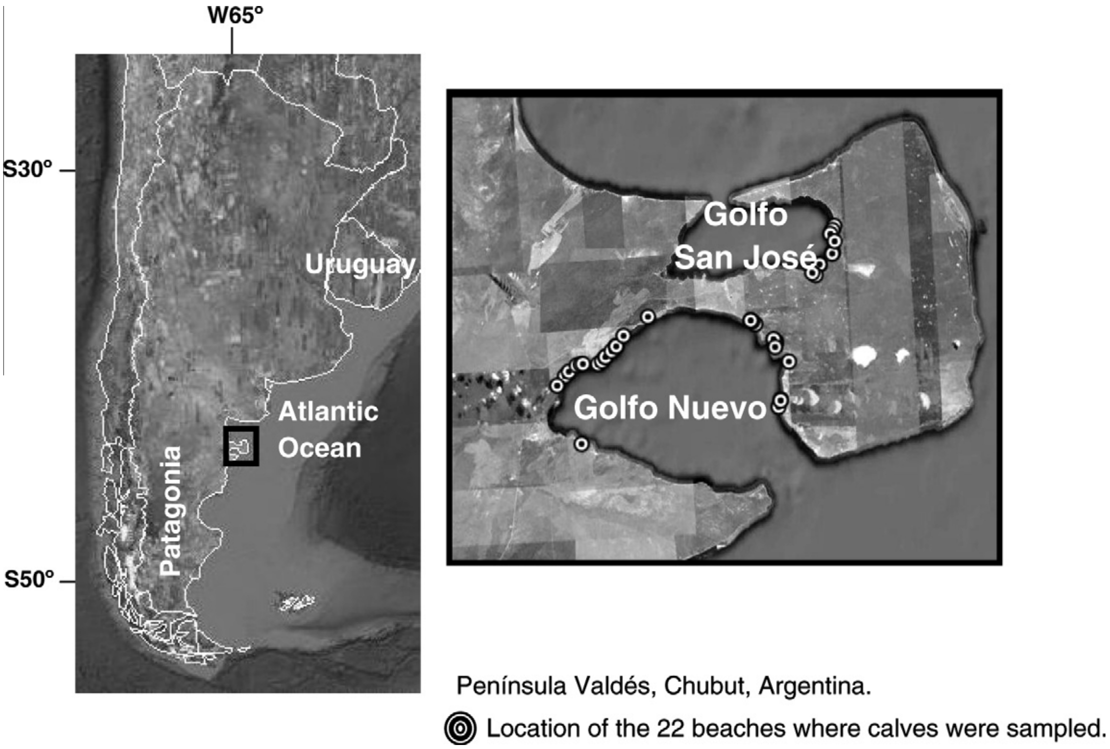

Fig. 1. Location of the 22 beaches where SRW calves included in this study were sampled (Península Valdés, Chubut, Argentina).

age – Best and Ruther, 1992) were registered and a post-mortem analysis was performed. Carcass condition was established by examination of external and internal organs and a 2 (fresh) to 5 (skeletal remains) code was assigned, following Geraci and Lounsbury (2005).

For this study, samples from 45 calves were selected on the basis of carcass condition (condition codes 2 ($N = 12$), 3 ($N = 16$) and 4 ($N = 17$)), and stranding location to ensure equal representation between the Península's two Gulfs (Table 1). Nuevo Gulf is much larger than San José Gulf, and hosts the city of Puerto Madryn (80,000 inhabitants), an active port, and industries (including an aluminium plant). San José Gulf on the other hand is smaller, has no urban settlements, and is a protected area with no activities beyond extraction of shellfish by coastal diving (SPPC, 2000). While the distribution of live whales has been almost equal between both bays over all years, more calves have consistently died in Golfo Nuevo than in Golfo San José (Uhart et al., 2008, 2009).

Samples from liver ($N = 26$) and kidney ($N = 42$) tissues were collected with stainless steel knives for trace metal analysis, and stored in sealed bags at −20 °C. Four replicas of about 10 g were separated from each sample. Two were used for calculating sample humidity and the others were used to determine metal concentrations. Tissue mineralization was performed according to SENASA (1991). Briefly, two homogenate replicas of about 10 g were weighed in porcelain crucibles and then placed in an oven at 450 °C for 6 h. After cooling, 2 ml of concentrated HNO_3 were added. Samples were evaporated until total dryness on a heating plate at 80 °C. These steps were repeated until white ashes were obtained. Ashes were then dissolved with 4 ml of a solution containing HNO_3 (3% v/v) and HCl (6% v/v) and were brought to a final volume of 10 ml with the same solution. Two blanks were run with every sample set and were processed in identical manner than tissue samples. Measurements were made by flame Atomic Absorption Spectrometry. Reagents of analytical grade were used for the blanks and for calibration curves. Quality assurance was done through analysis of standard reference oyster tissue (NIST 1566) provided by the National Institute of Standards & Technology. The recovery was between 90% and 99% for all metals. Detection limits of the method used were (µg/g dry weight): Fe: 2.00, Mn: 0.13, Zn: 0.05, Cu: 0.05, Ni: 0.50, Al: 5.00, Cd: 0.05, Pb: 0.55. The variation coefficients tested for 5 replicates of the same samples were always below 10%.

All results are reported as µg/g per wet weight of tissue (µg/g ww). Reference data from literature informed as "per dry weight of tissue" was transformed by multiplying it by a factor of 4 (Méndez et al., 2002). To test the differences between metal concentrations by tissue, carcass location, sex and age of whale calves, a multifactorial ANOVA ($p < 0.05$) was used. Moreover, a correlation analysis was done for each metal between tissues and within each tissue between metals ($p < 0.05$).

A summary of the results is shown in Table 2. Except Pb and Cd, all metals were detected in SRW calve liver and kidney tissues. Concentrations of Fe, Mn, Zn, Cu, Ni and Al were found within reported ranges for mysticetes in other geographical areas (Table 3). In relation to the Patagonian coastal area in particular, the only previous record corresponds to a three-month-old SRW calf found dead in Golfo Nuevo (Gil et al., 1996). The mentioned report was similar to those found in this study (Table 3), although Cd and Pb were detected. Unfortunately, there are few reports on trace metals in mysticete whales and the age of tested individuals is not always mentioned, therefore limiting potential comparisons.

No significant correlation between metal levels in tissues, location and calf sex were found, and no significant differences between location and calf sex were detected. However, significant differences were found between tissues, with higher average of Fe, Mn, Zn and Cu concentrations in liver and higher average of Ni and Al in kidney.

During the time *E. australis* mothers and calves spend at Península Valdés, they have coastal habits remaining in waters approximately 5 m deep (Payne, 1986) where fine sediment particles are

Table 1
Southern Right Whale calves examined in this study (Animal ID, Tissue, Carcass condition, Sex, Length, Location, Year, and Latitude/Longitude).

Animal ID[a]	Tissue[b]	Carcass condition	Sex	Length (m)	Location[c]	Year	Latitude (S)/longitude (W)
080403Pv-Ea03	L/K	2	Female	4.75	GN	2003	42°45′/64°1′
081603Pv-Ea07	K	2	Female	4.68	GN	2003	42°37′/64°55′
092503Pv-Ea20	L	4	Female	4.55	GSJ	2003	42°22′/64°3′
081104Pv-Ea05	K	3	Female	3.80	GSJ	2004	42°25′/64°7′
090904Pv-Ea06	K	4	Female	4.75	GSJ	2004	42°24′/64°6′
092104Pv-Ea11	L/K	2	Male	4.95	GN	2004	42°34′/64°15′
071605Pv-Ea01	L/K	2	Male	5.40	GSJ	2005	42°24′/64°6′
081805Pv-Ea04	L/K	2	Female	4.91	GN	2005	42°36′/64°54′
092005Pv-Ea13	L/K	3	Female	5.41	GN	2005	42°46′/65°1′
092105Pv-Ea14	L/K	4	Male	4.90	GN	2005	42°35′/64°48′
101205Pv-Ea18	K	3	Male	4.87	GSJ	2005	42°20′/64°59′
112205Pv-Ea42	L/K	2	Male	6.61	GN	2005	42°39′/64°59′
081206Pv-Ea02	K	3	Female	5.43	GN	2006	42°43′/64°15′
080108 Pv-Ea06	L/K	4	Female	4.10	GN	2008	42°35′/64°15′
080108Pv-Ea08	K	4	Male	5.54	GN	2008	42°36′/64°49′
081308Pv-Ea14	L/K	3	Male	5.46	GN	2008	42°37′/64°57′
081408Pv-Ea15	L	4	Male	6.03	GN	2008	42°36′/64°55′
081708Pv-Ea17	L/K	3	Female	5.15	GN	2008	42°35′/64°15′
081908Pv-Ea20	K	4	Male	6.18	GN	2008	42°36′/64°50′
082008Pv-Ea21	K	4	Male	5.40	GN	2008	42°35′/64°15′
082208Pv-Ea25	L/K	3	Male	5.58	GN	2008	42°36′/64°54′
082408Pv-Ea27	L/K	4	Female	5.53	GN	2008	42°18′/64°2′
082508Pv-Ea29	L/K	4	Female	5.53	GN	2008	42°32′/64°20′
082808Pv-Ea33	K	3	Male	5.55	GN	2008	42°30′/64°40′
083108Pv-Ea38	K	4	Male	6.32	GN	2008	42°37′/64°55′
090508Pv-Ea41	K	3	Male	5.78	GSJ	2008	42°25′/64°6′
090908Pv-Ea45	L/K	4	Male	5.32	GN	2008	42°32′/64°19′
090908Pv-Ea48	L	4	Female	6.44	GN	2008	42°32′/64°19′
091408Pv-Ea52	K	4	Male	5.60	GN	2008	42°43′/64°15′
091908Pv-Ea58	L/K	3	Male	4.70	GSJ	2008	42°19′/64°3′
092108Pv-Ea59	K	4	Male	5.42	GSJ	2008	42°22′/64°3′
092908Pv-Ea63	K	4	Female	6.25	GN	2008	42°34′/64°47′
092908Pv-Ea64	K	2	Female	4.13	GN	2008	42°34′/64°47′
101008Pv-Ea85	L/K	3	Female	4.00	GN	2008	42°38′/64°13′
102408Pv-Ea90	K	3	Female	4.75	GN	2008	42°33′/64°45′
120208Pv-Ea97	K	3	Female	5.40	GN	2008	42°48′/64°55′
062909Pv-Ea01	L/K	2	Male	5.50	GN	2009	42°37′/64°56′
070209Pv-Ea02	L/K	3	Male	5.15	GN	2009	42°42′/64°14′
071609Pv-Ea06	K	2	Male	5.05	GN	2009	42°46′/65°00′
072309Pv-Ea07	L/K	2	Male	5.90	GN	2009	42°36′/64°49′
080209Pv-Ea13	L/K	2	Male	5.30	GN	2009	42°36′/64°52′
080409Pv-Ea15	L/K	3	Female	4.50	GSJ	2009	42°16′/64°4′
081009Pv-Ea16	K	4	Male	4.65	GSJ	2009	42°25′/64°6′
081809Pv-Ea21	L/K	2	Male	5.80	GN	2009	42°35′/64°47′
082309Pv-Ea25	L/K	3	Male	5.05	GN	2009	42°47′/64°57′

[a] Animal ID: month, day, year Pv-Ea (number of animal).
[b] Tissue: L = Liver, K = Kidney.
[c] Location: GN = Golfo Nuevo, GSJ = Golfo San José.

resuspended. Furthermore, they rarely move from one to another (Rowntree et al., 2001). Even though it has been informed that particulate Fe, Zn, Cu and Pb levels are higher in Golfo Nuevo than in Golfo San José, the fact that there were no differences between metal levels in animals from either gulf suggests that they are likely not bioavailable for calves. The main route by which metals could accumulate in calve tissues would be via the placenta during gestation and/or through milk during lactation (Das et al., 2003). Further investigation is needed to test the probable influence of the life history of whale mothers on metal concentrations in their calves.

Higher trace metal bioaccumulation in sexually mature males rather than females (at similar sexual stages) has been reported for a number of mammals (Monteiro-Neto et al., 2003). This suggests that females might eliminate metals by transferring them to their calves through the placenta or milk (Das et al., 2003). Denton et al. (1980) reported differences between both sexes for Zn in liver and kidney of *Dugong dugong* (Orden Sirenia), without providing any possible explanation. Nevertheless, other authors found similar concentrations between sexes in many cetaceans (Honda et al., 1983; Sanpera et al., 1996; Krone et al., 1999; Gerpe et al., 2002; Monteiro-Neto et al., 2003; Rosa et al., 2008; Seixas et al., 2009) and polar bears (*Ursus maritimus*) (Dietz et al., 1996). The short age of individuals analyzed in this study rules out the main factors that could determine differences in metal accumulation due to sexual immaturity or diet similarity, which in this case is restricted to their mother's milk.

Absence of correlation between liver and kidney for all metals tested show differential metal behavior in each tissue. On the other hand, a positive significant correlation between Cu-Zn, Zn-Mn and Ni-Mn was found in hepatic tissue and between Cu and Zn in renal tissue (Tables 4 and 5). This suggests a similar pathway for these metals in each tissue and confirms what Rosa et al. (2008) reported for Bowhead whales, *Balaena mysticetus.* In any case, metal concentrations found in this study were not associated to carcass condition (Table 1), which is expected since trace metals are not biodegradable. It is known that Zn and Cu mostly accumulate in hepatic tissue (Woshner et al., 2001; Méndez et al., 2002). According to Das et al. (2006) no clear explanation was found for this fact, although it could be related to the different storage abilities of each organ, the species, and the metal involved (Phillips, 1995). On the other hand, organisms can regulate, in a homeostatic manner,

Table 2

Concentrations of trace metals Fe, Mn, Zn, Cu, Ni and Al (µg/g ww), ranges and min–max found in beached Southern Right Whale calves at Península Valdés, Argentina.

	Sex	Fe		Mn		Zn		Cu		Ni		Al	
		Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney
Golfo Nuevo	♀	99.82 ± 57.33 25.50–176.47 *N* = 8	60.26 ± 26.12 17.90–120.60 *N* = 13	1.46 ± 0.61 0.39–2.11 *N* = 8	0.43 ± 0.29 0.19–1.29 *N* = 13	122.95 ± 60.25 43.63–224.06 *N* = 8	19.44 ± 8.36 29.89–5.04 *N* = 13	98.86 ± 58.61 15.78–198.77 *N* = 8	3.13 ± 0.98 1.67–5.06 *N* = 13	0.17 ± 0.04 0.23–0.10 *N* = 8	0.24 ± 0.21 0.10–0.94 *N* = 13	7.29 ± 7.96 1.80–26.50 *N* = 8	7.25 ± 4.04 1.10–17.30 *N* = 13
	♂	112.87 ± 47.13 44.40–184.48 *N* = 13	50.37 ± 15.20 23.17–80.55 *N* = 19	1.47 ± 0.97 0.09–2.90 *N* = 13	0.53 ± 0.74 0.15–3.33 *N* = 19	138.14 ± 80.87 12.94–303.01 *N* = 13	21.38 ± 5.67 11.42–35.89 *N* = 19	88.82 ± 88.13 2.29–264.41 *N* = 13	3.56 ± 1.01 1.93–5.65 *N* = 19	0.17 ± 0.03 0.13–0.22 *N* = 13	0.17 ± 0.03 0.10–0.23 *N* = 19	5.03 ± 2.67 2.20–10.30 *N* = 13	7.43 ± 4.9 2.90–22.70 *N* = 19
Golfo San José	♀	93.25 ± 65.54 46.91–139.60 *N* = 2	52.41 ± 25.34 36.89–81.65 *N* = 3	1.13 ± 0.09 1.07–1.19 *N* = 2	0.29 ± 0.13 0.19–0.44 *N* = 3	111.22 ± 70.05 61.68–160.75 *N* = 2	19.00 ± 5.05 13.97–24.06 *N* = 3	60.66 ± 6.52 56.05–65.27 *N* = 2	2.84 ± 1.01 1.70–3.57 *N* = 3	0.16 ± 0.04 0.14–0.19 *N* = 2	0.12 ± 0.03 0.10–0.15 *N* = 3	5.03 ± 5.69 1.00–9.05 *N* = 2	5.35 ± 1.70 3.50–6.85 *N* = 3
	♂	121.93 ± 22.17 108.10–147.50 *N* = 3	48.15 ± 23.43 16.10–89.40 *N* = 7	0.43 ± 0.24 0.16–0.62 *N* = 3	0.49 ± 0.40 0.19–1.37 *N* = 7	32.75 ± 45.93 0.23–85.28 *N* = 3	24.27 ± 9.54 19.01–45.67 *N* = 7	54.37 ± 80.89 1.88–147.53 *N* = 3	3.02 ± 0.73 2.19–4.12 *N* = 7	0.16 ± 0.00 0.16–0.17 *N* = 3	0.22 ± 0.13 0.15–0.50 *N* = 7	4.47 ± 1.71 2.50–5.60 *N* = 3	5.95 ± 2.89 1.00–9.50 *N* = 7

Table 3

Review of reported Fe, Mn, Zn, Cu, and Ni values (µg/g ww) in baleen whales.

Species	Age	Site features	Fe_L[b]	Fe_K	Mn_L	Mn_K	Zn_L	Zn_K	Cu_L	Cu_K	Ni_L	Ni_K	Al_L	Al_K	References
Eschrichtius robustus (*N* = 10)	Calf, juvenile and adult whales	Low anthropic activity	2300	100	3	NA[c]	1.60–160	32–110	0.63–25	0.45–4.90	0.23	0.210	32	5	Varanasi et al. (1994)
Balaena mysticetus (*N* = 20)	Calf, juvenile and adult whales	Possible influence of urban, industrial and oiling activities	1005	NA	0.5–1.9	NA	22–65.25	NA	3–10	NA	NA	NA	<10	NA	Krone et al. (1999)
Balaena mysticetus (N_l = 55, N_k = 48)[a]	Calf, juvenile and adult whales		NA	NA	2.27 *N* = 20	0.53 *N* = 20	34.94	27.72	11.11	3.28	NA	NA	NA	NA	Woshner et al. (2001)
Balaena mysticetus (N_l = 110, N_k = 108)	Juvenile and adult whales		NA	NA	NA	NA	6.99–135.11	9.07–56.31	1.09–203.81	0.76–7.94	NA	NA	NA	NA	Rosa et al. (2008)
Balaena mysticetus (N_l = 34, N_k = 33)	Calf, juvenile and adult whales		72.2–3690	26.9–110	0.45–2.43	0.20–0.55	23.6–65.1	12.7–57.2	3.08–8.9	1.13–2.2	NA	NA	NA	NA	O'Hara et al. (2003)
Eubalaena australis ($N_{l\text{-}k}$ = 1)	Calf	Golfo Nuevo (possible influence of Puerto Madryn city)	NA	NA	NA	NA	54	NA	18.60	5.70	NA	NA	NA	NA	Gil et al. (2006)

[a] N_l: number of liver samples, N_k: number of kidney samples, $H_{l\text{-}k}$: number of liver and kidney samples.

[b] L: Liver, K: Kidney.

[c] NA: No data available.

Table 4
Correlation matrix between trace metals and hepatic tissue of *E. australis* calves beached at Península Valdés, Argentina (significant correlations highlighted in bold).

	Cu	Zn	Fe	Mn	Ni	Al
Cu	1.00					
Zn	**0.65**	1.00				
Fe	0.19	0.15	1.00			
Mn	**0.45**	**0.66**	0.32	1.00		
Ni	**0.54**	**0.43**	**0.51**	**0.61**	1.00	
Al	0.12	0.15	0.15	0.33	**0.41**	1.00

Table 5
Correlation matrix between trace metals and renal tissue of *E. australis* calves beached at Península Valdés, Argentina (significant correlations highlighted in bold).

	Cu	Zn	Fe	Mn	Ni	Al
Cu	1.00					
Zn	**0.38**	1.00				
Fe	0.09	0.07	1.00			
Mn	0.14	**0.36**	0.12	1.00		
Ni	−0.07	0.25	0.09	0.06	1.00	
Al	−0.11	0.27	0.23	**0.56**	0.12	1.00

essential metals such as Zn and Cu (cytosolic "pool") that are released according to enzymatic and metabolic demands. When not needed, metals are kept linked to low-molecular-weight non-enzymatic proteins (6–8 kDa) with a high cysteine content (30–35%) called metallothioneins (Amiard et al., 2006).

Basic SRW life history parameters such as size and growth rates are hard to obtain. Therefore, we used the parameters established by Best and Ruther (1992). In our study, the length of analyzed individuals varied from 4 to 6.61 m, but no significant correlation between metal concentrations and length was found (Table 1). This suggests that essential metals are under osmotic regulation, and it is therefore not expected that this concentration would be influenced by length or age under normal internal and environmental conditions (Sanpera et al., 1996).

Data obtained in this study on trace metals in *E. australis* calves provides baseline information for this species at Península Valdés and suggests that trace metals are probably not a significant cause of concern in relation to the recent increased calf mortality observed (Werner et al., 2011). Furthermore, information from this study is vital for SRW management considering increasing human pressures largely impacting their feeding and breeding grounds.

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