Metal levels in southern right whale (*Eubalaena australis*) skin biopsies from Península Valdés, Argentina

Julieta Martino[1,2,4], Sandra S. Wise[1,2,3,6], Christopher Perkins[5], Iain Kerr[2,6], Victoria J. Rowntree[6,7], Mariano Sironi[4,8] and John Pierce Wise, Sr[1,2,3,6,**]

[1]Wise Laboratory of Environmental and Genetic Toxicology, [2]Maine Center for Toxicology and Environmental Health, [3]Department of Applied Medical Sciences, University of Southern Maine, Portland, ME 04104, USA

[4]Instituto de Conservación de Ballenas, Capital Federal, Argentina

[5]Center for Environmental Sciences and Engineering, University of Connecticut, Storrs, CT 06269, USA

[6]Ocean Alliance, Lincoln, MA 01773, USA

[7]University of Utah, Department of Biology, Salt Lake City, UT 84112, USA

[8]Cátedra de Diversidad Animal II, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Argentina

** Corresponding Author:
Dr. John Pierce Wise Sr.,
University of Southern Maine,
478 Science Building
96 Falmouth Street
Portland, ME 04103
207-228-8050
John.Wise@usm.maine.edu

ABSTRACT

The southern right whale population from Península Valdés, Argentina has experienced unusually high calf mortalities in the last five years. In view of these deaths, it seems that this population and its ecosystem may be less healthy and robust than previously thought. Possible causes for the high mortalities include biotoxins, infectious diseases and reduced availability of food for females. Exposure to chemical pollutants such as metals can also affect reproduction as well as development and thus could play a role in cetacean mortality. However, little is known about the metal levels in the tissues of the living whales in this population. In this study we measured nonessential and essential metals/elements in skin biopsies from ten living, free ranging female southern right whales. Samples were analyzed for total levels of antimony (Sb), arsenic (As), barium (Ba), beryllium (Be), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), gold (Au), iron (Fe), lead (Pb), lithium (Li), magnesium (Mg), manganese (Mn), mercury (Hg), molybdenum (Mo), nickel (Ni), selenium (Se), silver (Ag), strontium (Sr), tin (Sn), titanium (Ti), uranium (U) and zinc (Zn) using inductively coupled plasma-mass spectrometry (ICP-MS). Aluminum (Al) was measured using inductively coupled plasma-optical emission spectrometry (ICP-OES). For nonessential metals, Al had the highest value with an average of 9.75 ± 2.7 ug/g tissue (w/w). Tissue levels of As, Pb, Ni, Ba, Cr, Sr and Ti ranged from 0.11 to 3.95 ug/g tissue (w/w). Au, Sn, Cd, Li, Sb, Ag, Be and Hg had average levels below 0.1 ug/g tissue (w/w). U and Co were not detected in any of the samples. For essential metal/elements, Mg had the highest value with an average of 187.02 ± 9.1 ug/g tissue (w/w). Tissue levels of Mo, Mn, Cu, Se, Fe and Zn ranged from 0.02 to 14.71 ug/g tissue (w/w). The levels of nonessential and essential metals in the skin of these animals are on the low end of the spectrum of measured concentrations when compared to other studies. These metal levels do not reflect substantial amounts of accumulation and serve as a baseline for the population.

INTRODUCTION

The waters around Península Valdes (PV), Argentina, are a nursing ground for a population of southern right whales (*Eubalaena australis*). The population has been increasing at a rate of approximately 6.9% from the early 1970s to 2000 (Cooke *et al.*, 2001, 2003). However, between 2003 and 2011, 482 animals were found dead along the coast of PV and surrounding area (Sironi *et al.*, 2012), with extremely high mortalities from 2007 through 2011 (Uhart *et al.*, 2009; Rowntree *et al.*, 2011). Eighty-nine percent of the dead animals were calves in their first three months of life. In the three years with the highest mortalities (2007-2009) a mean of 79 calves died each year at PV. This represents 170% more than expected using historical projections of the death rate over the preceding three decades (1971-2000) (Sironi *et al.*, 2012). The causes for most of the mortalities remain unknown (IWC, 2010). Leading hypotheses of causation include biotoxins, infectious diseases and reduced availability of food for females (IWC, 2010).

Another possible cause could be exposure to chemical pollutants such as metals. Although historically it was thought that baleen whales did not accumulate metals, recent data suggest they can, depending on location. For example, North Atlantic right whales (*Eubalaena glacialis*) accumulate Cr (Wise *et al.*, 2008). Metals are common environmental pollutants that can affect reproduction and development (Apostoli *et al.*, 2007; Foster and Gray, Jr.; 2008). In cetaceans, metal exposure has been correlated with infectious disease mortality, parasitic infections and pneumonias and histopathological changes in lung and kidney tissues (Bennett *et al.*; 2001, Siebert *et al.*; 1999, Rosa *et al.*; 2008). Cetacean cell culture studies show that metals can induce cytotoxic, immunotoxic and genotoxic effects (De Guise *et al.*, 1996; Betti and Nigro, 1996; Gauthier *et al.*; 1998; Cámara Pellissó *et al.*, 2008; Wise *et al.*, 2008, 2011; Li Chen *et al.*, 2009, 2012).

At PV, Argentina sources of metals include mining, storage and transport of petroleum, harbor activities and cities that have settled in the area and are expanding (Marcovecchio *et al.*, 1994). The only aluminum smelter in the country (ALUAR) is located in the city of Puerto Madryn on Golfo Nuevo, the southern gulf of the Peninsula. In addition, sea currents moving in north-south direction bring waters from the Buenos Aires coast which is the most populated and industrialized area of the country with numerous metallurgical, petrochemical, textile and pharmacological industries (Bisbal, 1995). This scenario suggests that exposure to metals from contaminated water and air is possible for southern right whales that use the PV nursery ground annually from June to December. Currently, the only data regarding metal levels in tissues of this species comes from dead calves (Gil *et al.*, 2006; Rosas *et al.*, 2012). Here we present results from a study in which we collected skin biopsies from living female southern right whales at Península Valdés and analyzed them for levels of nonessential and essential metals and one essential element (selenium).

METHODS

Study Area and Biopsy Sampling

Biopsies were collected from ten apparently healthy, free ranging adult female southern right whales in San José Gulf, Península Valdés, Argentina (42º30'; S, 64º 00'; W; Figure 1), during the months of September and October of 2011. Adult females were recognized by the close proximity of a calf over an extended period of time. Animals were approached slowly in a small rubber boat. Prior to biopsying, the callosity patterns on the head of each whale were photographed to avoid re sampling the same individual (Payne *et al.*, 1983). Whales were biopsied on their flanks using a crossbow and a dart, following standard methods described in Brown *et al.* (1991). A 60mm x 6mm stainless steel cylindrical biopsy dart was used. All tissue samples were immediately frozen after collection, stored in a liquid nitrogen dewar and shipped frozen to the laboratory.

Metal Analyses

Whale skin samples were analyzed for total levels of antimony (Sb), arsenic (As), barium (Ba), beryllium (Be), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), gold (Au), iron (Fe), lead (Pb), lithium (Li), magnesium (Mg), manganese (Mn), mercury (Hg), molybdenum (Mo), nickel (Ni), selenium (Se), silver (Ag), strontium (Sr), tin (Sn), titanium (Ti), uranium (U) and zinc (Zn) using a Perkin–Elmer/Sciex ELAN inductively coupled plasma-mass spectrometer (ICP-MS) following methods in Wise *et al.* (2008). Aluminum (Al) was measured using inductively

coupled plasma-optical emission spectrometry (ICP-OES) according to standard methods. Standard quality assurance procedures were employed. Instrument response was evaluated initially, after every 10 samples, as well as the end of each analytical run using a calibration verification standard and blank. Interference check solutions were analyzed with all sample runs to compensate for any matrix effects that might be interfering with sample analysis. Results are reported in ug/g per wet weight of tissue (ug/g ww). Levels in the literature are mostly reported as per dry weight of tissue and do not include information on water content. Hence, in order to compare our data the literature values were transformed to per wet weight values by multiplying them by a factor of 0.25, which corresponds to the dry/wet weight ratio of most tissues.

RESULTS

Average tissue levels for each element are summarized in Table 1. Among nonessential metals, Al had the highest value with an average of 9.75 ± 2.7 ug/g tissue (w/w). Tissue levels of As, Pb, Ni, Ba, Cr, Sr and Ti ranged from 0.11 to 3.95 ug/g tissue (w/w). Au, Sn, Cd, Li, Sb, Ag, Be and Hg had average levels below 0.1 ug/g tissue (w/w). Ti, Sr and Ni were detected in all of the samples. U and Co were not detected in any of the samples. Nonessential metal levels in decreasing order of concentration are: Al > Ti > Sr> Cr > Ba > Ni > Pb >As >Au > Sn > Cd, Li, Sb > Ag > Be, Hg. Within the essential metals/element (i.e. selenium), Mg had the highest value with an average of 187.02 ± 9.1 ug/g tissue (w/w). Tissue levels of Mo, Mn, Cu, Se, Fe and Zn ranged from 0.02 to 14.71 ug/g tissue (w/w).

Table 1. Levels of nonessential and essential metals/elements in skin biopsies from ten adult female southern right whales in decreasing order of concentration.

Element	Tissue Levels (ug/g ww)	N(Nd)*	Detection Limit (ug/g)	Element	Tissue Levels (ug/g ww)	N(Nd)*	Detection Limit (ug/g)
Mg	187.02 ± 9.1	10 (10)	2.3	**As**	0.11 ± 0	10 (3)	0.09
Zn	14.71 ± 0.6	10 (10)	0.18	**Au**	0.09 ± 0	10 (2)	0.09
Al	9.75 ± 2.7	10 (9)	2.3	**Sn**	0.07 ± 0	10 (7)	0.05
Fe	7.24 ± 3	10 (7)	2.3	**Cd**	0.04 ± 0	10 (6)	0.02
Ti	3.95 ± 0.2	10 (10)	0.05	**Li**	0.04 ± 0	10 (3)	0.05
Se	2.06 ± 0.4	10 (10)	0.05	**Sb**	0.04 ± 0	10 (2)	0.05
Sr	0.87 ± 0.1	10 (10)	0.02	**Mo**	0.02 ± 0	10 (6)	0.02
Cr	0.64 ± 0.2	10 (9)	0.18	**Ag**	0.02	10 (1)	0.02
Cu	0.35 ± 0.1	10 (10)	0.05	**Hg**	0.01 ± 0	10 (2)	0.02
Ba	0.32 ± 0.1	10 (9)	0.09	**Be**	0.01	10 (1)	0.02
Mn	0.27 ± 0.1	10 (10)	0.02	**U**	ND**	10 (0)	0.02
Ni	0.19 ± 0	10 (10)	0.05	**Co**	ND**	10 (0)	0.02
Pb	0.15 ± 0	10 (8)	0.05				

*N(Nd) = Total number of samples (number of samples with detectable levels); **ND = Not detected

DISCUSSION

Our study is the first to report metal levels in southern right whale skin from apparently healthy, free ranging adult female animals. Overall, the levels of the metals were generally low. Two previous studies investigated metal levels in southern right whales from PV. Both focused on calves found dead on the beach in PV. One study investigated metals in liver, kidney and muscle in a single calf (Gil *et al.*, 2006). The second study (Rosas *et al.*, 2012) investigated levels in liver and kidney tissues from 45 dead calves. Table 2 compares the results presented here with these previous studies. Overall our levels of Al, Cd, Hg, Ni and Pb are similar to what they found. However, we found lower levels of Cu, Fe, Mn and Zn.

The lower levels found are probably due to the different tissues measured. We measured skin samples while previous studies focused on liver, kidney and muscle. Metals are known to preferentially accumulate in these internal organs more than skin (Law, 1996). In minke whales (*Balaenoptera acutorostrata*), Cr and Cd skin levels correlate with those found in the liver (Kunito *et al.*, 2002) suggesting that skin levels can reflect levels of internal organs. However, there is no data available on metal levels for internal organs in adult southern right whales to determine whether this correlation holds true for southern right whales as well.

Aluminum (Al) levels were of particular interest because of the presence of an Al smelter in the nearby city of Puerto Madryn, located on Golfo Nuevo, the southern Gulf of PV (Figure 1). Nine out of ten animals had detectable levels of Al. The levels found were comparable to those measured in liver and kidney of dead calves from PV (Rosas *et al.*, 2012). They were also similar to those reported in livers of bowhead whales (*Balaena mysticetus*) (Krone *et al.*, 1999). Lower or similar levels of Al to those measured in our study were also found in liver, kidney and brain of gray whales (*Eschrichtius robustus*) stranded on the west coast of North America (Varanasi *et al.*, 1994; Tilbury *et al.*, 2002). These data suggest the southern right whales are not experiencing higher Al as a result of the nearby smelter.

Previously we investigated Cr levels in skin biopsies from North Atlantic right whales, a species closely related to southern right whales (Wise *et al.*, 2008). The average Cr levels in North Atlantic right whales were approximately ten times higher (7.1 ug/g ww) than those we found in southern right whales (0.64 ug/g ww). Similar protocols were used in each study. The explanation for this difference is uncertain, but is likely due to the fact that North Atlantic right whales live along one of the most industrialized coasts in the world while there is no significant chromium industry around PV.

Only a few studies have reported metal levels from skin biopsies of other baleen whales. De Luna and Rosales-Hoz (2004) measured As, Fe, Mn, Pb, Se and Zn in skin of calf, juvenile and adult gray whales at Ojo de Liebre Lagoon, Mexico. Levels of As were similar to those in our study but the Pb level they report for adult gray whales is much higher than our study (~3.75 ug/g ww vs. 0.15 ug/g ww). This could be explained by differences in feeding behavior between gray and southern right whales. Gray whales are benthic bottom feeders that filter sediments in order to obtain their prey (Rice *et al.*; 1984). Higher levels of Pb could be due to a higher exposure resulting from the ingestion of sediments, which contain metals from natural or anthropogenic sources. Fe, Mn, Se and Zn levels in our study were higher than the levels found in these gray whales. This difference could be due to a low sample number (N=3) in the Luna and Rosales-Hoz (2004) study but could also reflect differences among sampling sites, ingested prey or species-specific differences in the toxicokinetics of these elements.

Ba, Cu, Cd, Cr, Mn, Hg, Sr and Zn were measured in skin of 161 (39 females and 122 males) minke whales in the southern hemisphere (Kunito *et al.*, 2002). Our data are consistent with these results with the exception of Ba which was found at almost ten-fold higher levels in our study (0.004 ug/g ww vs. 0.35 ug/g ww). Barium is widely used to make drilling fluids, a product used in oil and gas extractions (ATSDR, 2007), an activity that is very prevalent in the Patagonia region surrounding PV. The higher levels of Ba in southern right whale could reflect a higher exposure to these Ba-containing compounds.

In summary, this is the first study to report metal levels in living southern right whales from PV. Overall, our data suggest that metal levels in southern right whales from PV are low and provide a valuable baseline for metals in tissues of this species. These low levels should not necessarily be interpreted as being safe since the effects of metals in marine mammals are largely unknown. Future work is aimed at providing more data from living southern right whales, including females, males and other age classes and reproductive status.

Table 2. Summary of reported metal levels in tissues of southern right whales.

	This Study N=10 Adult Females	**Gil *et al.* (2006) N=1 Calf**	**Rosas *et al.* (2012) N=45 Calves**
Metal	**Tissue Level (ug/g ww)**	**Tissue Level Range (ug/g ww)**	**Tissue Level Range (ug/g ww)**
Al	9.75	-	4.47-7.43
Cd	0.04	0.04	ND**
Cu	0.35	2.6-18.6	2.84-98.86
Fe	7.24	-	48.15-121.93
Hg	0.01	0.04	-
Mn	0.27	-	0.29-1.47
Ni	0.19	-	0.12-0.24
Pb	0.15	0.12-0.13	ND**
Zn	14.71	54-83	19.00-138.14

*- = not measured

**ND = not detected

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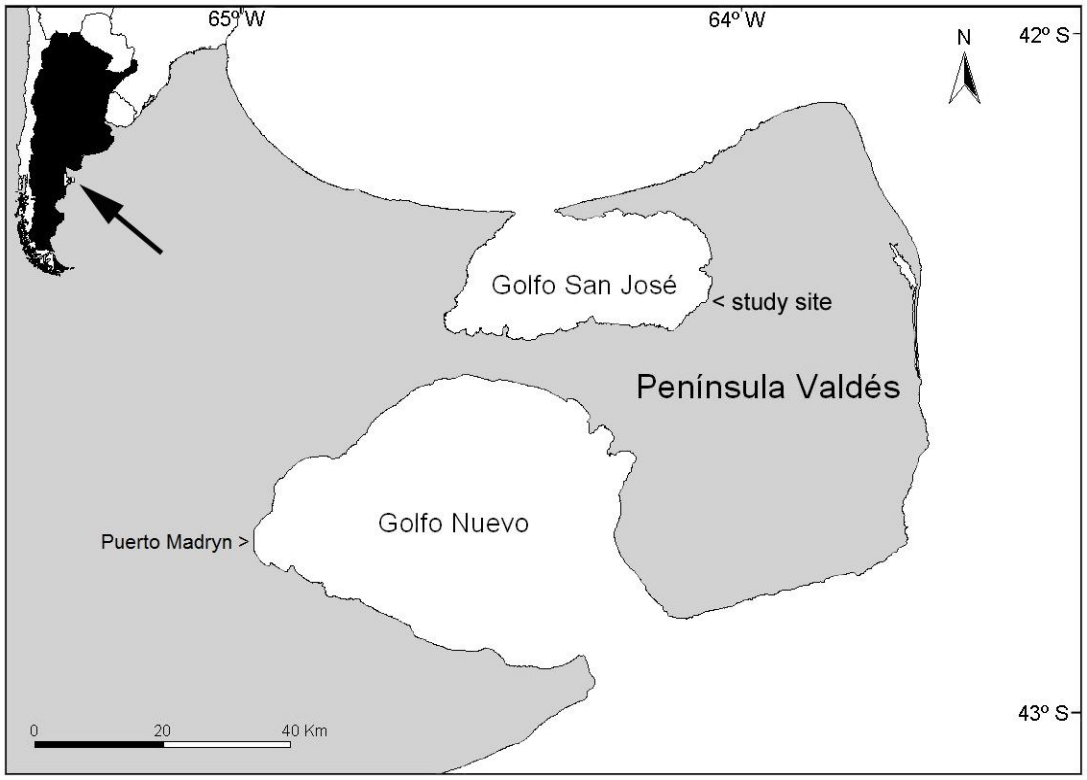

Figure 1: Map of Península Valdés, Argentina showing the San José Gulf, and the study site where the samples were collected as well as Puerto Madryn, located on Golfo Nuevo. Inset shows the position of Península Valdés on the South American coast.