Chemosphere 199 (2018) 637–646

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

High status of mercury and selenium in false killer whales (*Pseudorca crassidens*, Owen 1846) stranded on Southern South America: A possible toxicological concern?

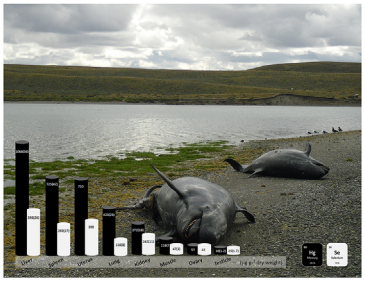
Iris Cáceres-Saez [a, *], Daniela Haro [b, c], Olivia Blank [d], Anelio Aguayo Lobo [e], Catherine Dougnac [f], Cristóbal Arredondo [f], H. Luis Cappozzo [a], Sergio Ribeiro Guevara [g]

[a] Consejo Nacional de Investigaciones Científicas y Técnicas, Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Avenida Ángel Gallardo 470 (C1405DJR), Buenos Aires, Argentina
[b] Laboratorio de Ecofisiología y Ecología Isotópica, Facultad de Ciencias, Universidad de Chile, Las Palmeras 3425, Ñuñoa, Santiago, Chile
[c] Centro Bahía Lomas, Universidad Santo Tomas, Punta Arenas, Chile
[d] Clínica Veterinaria Timaukel y Centro de Rehabilitación de Aves Leñadura (CRAL), José Pithon 01316, Punta Arenas, Chile
[e] Instituto Antártico Chileno (INACH), Plaza Muñoz Gamero 1055, Punta Arenas, Chile
[f] Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Av. Santa Rosa 11735, La Pintana, Santiago, Chile
[g] Laboratorio de Análisis por Activación Neutrónica, Centro Atómico Bariloche, Av E. Bustillo km 9.500, Bariloche, Argentina

HIGHLIGHTS

- Hg and Se were measured in internal tissues of false killer whale stranded in Southern South America.
- Bioaccumulation of Hg and Se was found in tissues.
- Se-to-Hg correlations in tissues suggest the formation of mercury-selenium complexes.
- Hg values exceeded in all specimens the toxic thresholds for hepatic damage in marine mammals.
- Molar Se:Hg ratio in liver, lung and muscle were <1 indicating a possible toxicological risk of Hg.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 2 November 2017
Received in revised form
26 January 2018
Accepted 7 February 2018
Available online 10 February 2018

Handling Editor: Martine Leermakers

Keywords:
Trace elements
Toxic metal
Odontocetes

ABSTRACT

The study was carried out to determine Hg and Se concentrations in false killer whales stranded on the Estrecho de Magallanes, Chile, South America. Tissue samples of five mature specimens were analyzed (two females and three males). Mean Hg concentration in liver 1068 (234) μg g^{-1} dry weight (DW) (standard deviation in parenthesis) was markedly higher than those in kidney 272 (152) μg g^{-1} DW, lung 423 (325) μg g^{-1} DW, spleen 725 (696) μg g^{-1} DW, muscle 118 (94) μg g^{-1} DW and testicle 18.0 (2.8) μg g^{-1} DW. Mean Se concentration in liver, 398 (75) μg g^{-1} DW, was higher than those in kidney 162 (69) μg g^{-1} DW, lung 128 (84) μg g^{-1} DW, spleen 268 (245) μg g^{-1} DW, muscle 47 (38) μg g^{-1} DW and testicle 25.4 (2.1) μg g^{-1} DW. Positive correlations were found between Hg and Se molar concentrations in muscle, lung, spleen and kidney. Molar ratio of Se/Hg in liver, lung and muscle were <1, but those in kidney and testicle were markedly >1 suggesting a Se protection against Hg toxicity. In all the examined specimens Hg values exceeded the toxic thresholds defined for hepatic damage in marine mammals, with Se/Hg molar ratios below 1 implying limited protective action of Se. Generally, our results showed

* Corresponding author.
E-mail addresses: caceres.saez@gmail.com, caceres-saez@macn.gov.ar (I. Cáceres-Saez).

https://doi.org/10.1016/j.chemosphere.2018.02.046

Strandings
Estrecho de magallanes
Southwestern South Atlantic Ocean

that individuals are carrying a significant burden, reflecting a high exposure to this toxic metal. This constitutes the first report on Hg and Se levels for a large subantarctic odontocete in South America region, providing insights into their contamination status and with information to the understanding of possible impacts on wild populations.

1. Introduction

Mercury (Hg) is a highly toxic and persistent pollutant of high concern due to its accumulation in organisms with serious risk to marine wildlife (UNEP, 2002). In vertebrates, Hg accumulates principally from dietary sources and causes numerous sub-lethal effects including impaired reproduction, reduced hepatic metabolism, and altered behavior (UNEP, 2002). Mercury can also lead to neurological damage and mortality (Clarkson and Magos, 2006). Evidence suggests that Hg may compromise immune function of marine mammals, therefore heightening their infection and disease susceptibility (Kannan et al., 2006). Marine mammals are typically high trophic level feeders and represent apex species for methylmercury (MeHg) biomagnification in food webs. Hence, exposure to Hg in those organisms is significantly higher than in other marine vertebrates (Kehrig et al., 2013; Law et al., 1991; Wagemann et al., 2000). Marine mammals take up Hg mostly as MeHg from muscle of their prey, and dietary MeHg is bioaccumulated in their skeletal muscle (Gui et al., 2014; Sakamoto et al., 2015; Wagemann et al., 2000). Consequently, it is extremely important to monitor Hg in cetaceans, and one way is by measuring their occurrence in stranded individuals. Mass-mortality events have led to increased awareness of the potential toxicity of Hg pollution on stranding species.

Marine mammals use a variety of homeostasis and biotransformation processes to limit the accumulation of trace elements that could cause toxic effects. As the main storage and detoxification organ, liver is important in the sequestration of toxic elements and the homeostatic regulation of essential ones. As previous studies have shown, MeHg is transformed into less toxic inorganic forms in liver (Kunito et al., 2004; Wagemann et al., 1998). Selenium Se as an essential element is under homeostatic control, and is critical for antioxidant defense mechanisms, enzyme function and also Hg detoxification (Khan and Wang, 2009). Selenium occurs within all animal body in concentrations that vary with tissue types, and the level and chemical form of Se. It is noteworthy that Se as a micronutrient can protect marine mammals from Hg toxicity through a number of mechanisms, including antioxidant properties, competition for binding sites, and the formation of non-toxic inert complexes (Cuvin-Aralar and Furness, 1991). The Se:Hg molar ratio is considered to be a critical parameter for Hg toxicity assessment, provided that ratios over 1 imply Se availability to combine with Hg to form stable mercury selenide crystals (HgSe) (Berry and Ralston, 2008).

The false killer whale (*Pseudorca crassidens*) occurs in socially cohesive herds (~20–50 animals) mainly associated with foraging habits (Stacey and Baird, 1991), and occupies high trophic positions in its respective marine food web. Because false killer whales are top predators with a large body mass, their potential to accumulate contaminants against the slow rate elimination, make them useful "indicator species" of pollutants bioaccumulation in marine ecosystem. Only limited information of trace elements has been reported for hepatic and skin tissues of this top predator (Endo et al., 2006; Hansen et al., 2016; Kemper et al., 1994; Mouton et al., 2015). Quantifying baseline concentrations and patterns of Hg together with Se, due to its Hg toxicity neutralization role, is critical for the risk assessment and monitoring over time for these species.

The main objectives of this study were (a) report Hg contamination status of false killer whales from subantartic region by study stranded individuals; (b) evaluate Se-to-Hg molar ratios in different organs to determine possible correlation between those elements for aiding potential protective effect of Se regarding Hg toxicity, and (c) assess the risk of Hg toxicity by comparing observed values with already known threshold benchmarks for marine mammals.

2. Material and methods

2.1. Specimens, necropsies and tissue collection

On 24 February 2013 a group of 46 specimens of false killer whales was reported as stranded or attempting to strand on shores of the Estrecho de Magallanes, Chile (Susana cove, 52°39′12″S - 70°19′57″W; Fig. 1) (Haro et al., 2015) resulting in the death of 29 of these toothed whales, while the other were rescued and moved offshore. The Estrecho de Magallanes is a navigable sea route separating mainland South America to the north, and Tierra del Fuego, to the south. The strait is the most important natural passage between Atlantic and Pacific oceans.

The stranding was attended by experienced veterinarians and marine biologists coordinated by the Servicio Nacional de Pesca y Acuicultura from Chile (SERNAPESCA). Detailed necropsies were performed on-site according to standard protocols (Geraci and Lounsbury, 2005) and depending on the animal's condition. Otherwise, basic measurements/information (i.e. length, sex, decomposition state) was collected. To prevent biases associated with decomposition state of animals, only samples of recently dead individuals were used. Tissue samples were recovered from five specimens including two females and three males (Table 1). The sex was determined by external examination (protrusion of the penis and mammary glands) during field necropsy. Sexual maturity was established based on the total body length (TBL) considering that females reach maturity between 3.40 and 3.80 m while males between 3.96 and 4.30 m (Kasuya, 1986). Both sexes reach sexual maturity between 8 and 14 years (Ferreira et al., 2014). Based on these criteria, the specimens were classified as individuals (Table 1). Tissue samples including liver, kidney, muscle, lung, spleen, and gonads of false killer whales were excised and immediately wrapped in plastic bags (Haro et al., 2015). After field-dissection samples were frozen at −80 °C and then freeze-dried at the Laboratorio del Instituto Antártico Chileno (INACH), Punta Arenas, Chile. Lyophilized tissues were stored in individual plastic vials and transferred from INACH, to the Laboratorio de Análisis por Activación Neutrónica, Centro Atómico Bariloche, Argentina; where analyses were carried out.

2.2. Analytical methods

Tissues samples were prepared for Hg and Se determinations by placing aliquots of lyophilized material (100–150 mg) in Suprasil

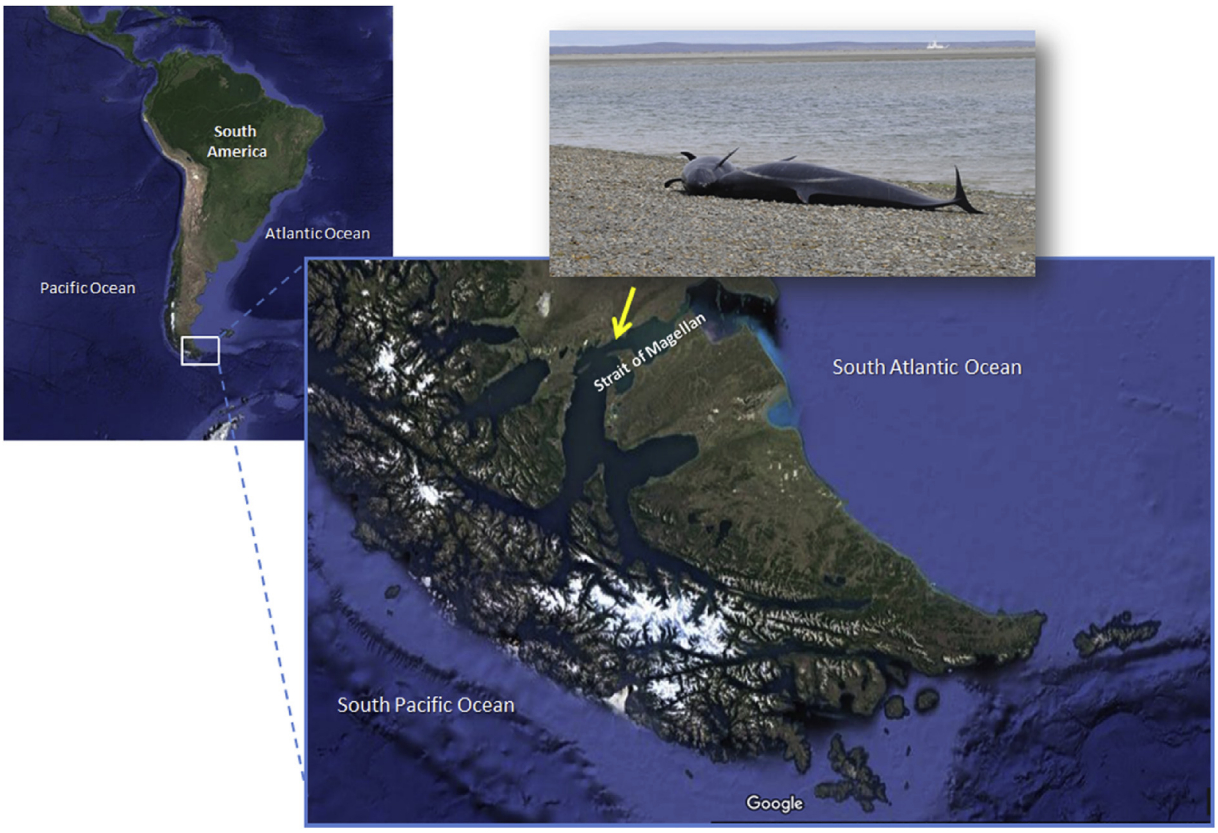


Fig. 1. Study area and stranding sampling location of false killer whales (*P. crassidens*) in the Strait of Magellan, Chile; Southern South America.

Table 1
Stranded false killer whales (*P. crassidens*) at Southern South America, together with Hg and Se concentration (μg g^{-1} DW) in tissues.

Specimen code	Body length (cm)	Element	Organ/tissue[a]							
			Muscle	Liver	Kidney	Lung	Spleen	Testicle	Ovary	Uterus
FKW#1	5.29	Hg	50.9 ± 2.8	801 ± 45	181 ± 15	131.3 ± 7.8	113.9 ± 6.9	16.8	–	–
♂		Se	17.1 ± 1.2	315 ± 22	135 ± 10	56 ± 4	66.2 ± 4.2	25.5 ± 1.8		
		Se/Hg	0.85	1.00	1.90	1.09	1.48	3.87		
FKW#2	4.43	Hg	48.8 ± 1,8	*sna*	291 ± 17	57.8 ± 4.8	*sna*	–	56,9	*sna*
♀		Se	21.1 ± 1.2		181 ± 12	37.0 ± 2.4			42.1 ± 1.9	
		Se/Hg	1.10		1.58	1.63			1.88	
FKW#3	4.10	Hg	86.7 ± 4.8	1168 ± 63	171.8 ± 9.6	754 ± 48	510 ± 35	16.1	–	–
♂		Se	34.3 ± 2.3	461 ± 30	103.7 ± 6.7	205 ± 12	192 ± 13	27.5 ± 1.8		
		Se/Hg	1.01	1.00	1.53	0.69	0.96	4.34		
FKW#4	4.96	Hg	127 ± 7.3	1236 ± 66	188 ± 10	444 ± 25	550 ± 30	21.2	–	–
♂		Se	51.4 ± 3.3	418 ± 26	116.8 ± 7.3	122.0 ± 7.7	187 ± 12	23.3 ± 1.5		
		Se/Hg	1.03	0.86	1.58	0.70	0.86	2.79		
FKW#5	4.25	Hg	276 ± 16	*sna*	530 ± 30	729 ± 40	1727 ± 96	–	*sna*	710
♀		Se	109.5 ± 7.3		275 ± 18	221 ± 14	625 ± 40			308 ± 21
		Se/Hg	1.01		1.32	0.77	0.92			1.10
Mean (SD)		Hg	118 (7)	1068 (58)	272 (16)	423 (25)	725 (42)	18.0 (1.2)		
		Se	47 (3)	398 (26)	162 (11)	128 (8)	268 (17)	25.48 (1.7)		
		Se/Hg	1.0 (0.09)	0.95 (0.08)	1.58 (0.21)	0.98 (0.40)	1.06 (0.29)	3.67 (0.79)		

sna, sample not available.
The analytical uncertainty is reported after '±' Se/Hg, Se to Hg molar ratio.
[a] Values of the relative coefficient of variation (CV%) of Hg in spleen 96.0%, muscle79.7%, lung 76.8%, kidney 55.8%, liver 21.9% and testicle 15.4%, and of Se in spleen 91.7%, muscle 80.5%, lung 65.3%, kidney 42.8%, liver 18.9% and testicle 8.3%.

AN quartz ampoules in a laminar flow hood and sealed afterwards. The concentration of total Hg ([Hg]) and Se ([Se]) was determined by Instrumental Neutron Activation Analysis (INAA). The absolute parametric method was used to determine the elemental concentrations (Cáceres-Saez et al., 2015). The analytical quality control was performed by the analysis of the Certified Reference Materials NRCC DORM-2 (dogfish muscle) and ERM- BB422 (fish muscle); the results showed good agreement with the certificate

(listed in Supplementary Table S1). The concentrations are presented in dry weight (DW) basis.

2.3. Calculation of Se/Hg molar ratio

The molar ratio of Se to Hg was calculated as:
Se/Hg = ([Se]/78.96)/([Hg]/200.59), where 200.59 and 78.96 $gmol^{-1}$ are the atomic weight of Hg and Se; respectively.

2.4. Data analysis

Pearson correlations (r) were calculated to evaluate relationships between Hg and Se per tissue. Due to small sample size differences between sexes were not assessed. A comparison of Hg levels in liver and muscle with other species worldwide were conducted, and a discussion concerning Hg risk toxicity with toxicological benchmarks was included.

3. Results and discussion

Bioaccumulation of trace elements in marine mammals is mainly dependent on diverse biotic (i.e. age, sex, feeding habits, nutritional condition, and physiological status) and abiotic factors (i.e. contamination level and physico-chemical parameters of the marine environment) (Caurant et al., 1996; García-Alvarez et al., 2015; Lahaye et al., 2007; Law et al., 1997; Palmisano et al., 1995; Seixas et al., 2008; Wagemann et al., 1983). Feeding preferences are key factor controlling Hg levels because upper-level predators are mainly exposed to metals through their food. Considering the high mobility of cetaceans, levels of Hg concentrations in their tissues reflect the general contamination of a broad and poorly defined area in which cetaceans live. In Southern South America, recent studies on trophic ecology of false killer whales through stable isotopes suggest similarities in the long-term foraging strategies between sex such as type of prey ingested and feeding areas (Haro *comm. pers.*; Riccialdelli and Goodall, 2015). Along the western and southwestern South Atlantic Ocean this species may have a flexible foraging behavior, as analyses on stomach contents have showed (i.e. coastal fishes, epipelagic and oceanic-squids, and demersal-benthic fishes) (Koen Alonso et al., 1999). In this study, the evaluation of specimens and further comparison to other studies discussed below is qualitative rather than statistically-based.

3.1. Comparison of Hg status in toothed whales

A comparison of Hg and Se concentrations in stranded false killer whales on the shores of the Estrecho de Magallanes, Chile, was established with other odontocetes stranded, caught and/or hunted from diverse marine areas worldwide (listed in Supplementary Table S2). Hepatic Hg concentrations were among the highest when compared with other cetacean species stranded or caught in the Atlantic Ocean, namely pilot whale (*Globicephala melas*) from the Faroe islands (128 ± 175 $\mu g\,g^{-1}$ WW, Caurant et al., 1996; 54–351 $\mu g\,g^{-1}$ WW, Sonne et al., 2010) (WW: wet weight; water contents depend on tissues, but the estimated conversion factor from WW to DW could range from 3 to 5, since water contents may range from 60 to 80%), short-finned pilot whale (*Globicephala macrorhynchus*) from New Caledonia (1411 $\mu g\,g^{-1}$ DW, Bustamante et al., 2003), striped dolphin (*Stenella coeruleoalba*) from the Strait of Gibraltar (5.92–1078 $\mu g\,g^{-1}$ DW, Rojo-Nieto-Fernández Maldonado, 2017). Also, other species in the Pacific Ocean, such as killer whale (*Orcinus orca*) from the Hawaiian islands (263.7 $\mu g\,g^{-1}$ WW, Hansen et al., 2016); melon-headed whale (*Penonocephala electra*) (16–322 $\mu g\,g^{-1}$ WW, Endo et al., 2008), spotted dolphin (*Stenella attenuata*) from coasts of Japan (205 ± 102 $\mu g\,g^{-1}$ WW, Itano et al., 1984), and harbor porpoise (*Phocena phocena*) from Portland, USA (1.4–380 $\mu g\,g^{-1}$ WW, Mackey et al., 1995). In other cases, lower hepatic Hg concentrations have been reported for stranded odontocetes such as the Indo-Pacific humpback dolphins (*Sousa chinensis*) from China (32.3 ± 59 µg g-1 DW, Gui et al., 2014), harbor porpoises from the North sea, Netherlands (0.88–139 $\mu g\,g^{-1}$ WW, Lahaye et al., 2007), East Scotland (0.38–31 $\mu g\,g^{-1}$ WW, Lahaye et al., 2007), and the Baltic Sea species such as the white-beaked dolphin (*Lagenorhynchus albirostris*) (5.7–220 $\mu g\,g^{-1}$ DW, Siebert et al., 1999), sperm whale (*Physeter macrocephalus*) from Belgian coasts (8.7–132 $\mu g\,g^{-1}$ DW), narwhal (*Monodon monoceros*) from West Greenland (0.39–32 $\mu g\,g^{-1}$ WW, Sonne et al., 2013) and common dolphin (*Delphinus delphis*) form Irish coasts (0.7–150 $\mu g\,g^{-1}$ WW, Law et al., 1991). Comparisons suggest that stranded false killer whales could be exposed to Hg accumulation in the South Atlantic Ocean. Moreover, Hg concentration of individuals analyzed in the present study, were higher than those observed in liver of species from northern areas of Brazil, South Atlantic Ocean, such as tucuxi (*Sotalia fluviatilis,* 77 ± 107 $\mu g\,g^{-1}$ DW, Kunito et al., 2004), Guiana dolphin (*S. guianensis*, 0.53–132 $\mu g\,g^{-1}$ WW, Lailson-Brito et al., 2012; 12.7 ± 7.1 $\mu g\,g^{-1}$ WW, Kehrig et al., 2016), franciscana dolphin (*Pontoporia blainvillei*, 0.66–51.6 $\mu g\,g^{-1}$ DW, Seixas et al., 2008; 1.6 ± 1.0 $\mu g\,g^{-1}$ WW, Kehrig et al., 2016), rough-toothed dolphin (*Steno bredanensis*, 595 ± 200 $\mu g\,g^{-1}$ DW, Lemos et al., 2013) and *D. delphis* (290 $\mu g\,g^{-1}$ DW, Lemos et al., 2013). Besides, Hg concentrations of false killer whales analyzed were lower than in the same odontocete species stranded in Australia, Pacific coast of British Columbia, Canada and the Hawaiian Islands, NE Atlantic Ocean. Baird et al. (1989) reported hepatic Hg concentration of 728 $\mu g\,g^{-1}$ WW in an adult male; Langelier et al. (1990) found a concentration of 614 $\mu g\,g^{-1}$ WW in an adult female; while Kemper et al. (1994) presented a range of 41–479 $\mu g\,g^{-1}$ WW in specimens from Australian waters. More recently, Hansen et al. (2016) described a concentration of 1572 $\mu g\,g^{-1}$ WW in an adult female. A single contrast with muscle Hg concentration of false killer whale was made by the report of (Endo et al., 2005) who found a range of 17.4–81.0 $\mu g\,g^{-1}$ WW comparable to our levels.

Even higher Hg concentrations were measured in stranded odontocetes from the Mediterranean Sea (Roditi-Elasar et al., 2003) and the Adriatic Sea (Bilandžić et al., 2012; Frodello et al., 2000). In Mediterranean waters there are naturally occurring Hg deposits that cause resident wildlife an extreme Hg bioaccumulation (Andre et al., 1991; Augier et al., 1993). Concentrations reaching 80 $\mu g\,g^{-1}$ WW in muscle and about 1500 $\mu g\,g^{-1}$ WW in liver were informed in striped dolphins from French coasts (Andre et al., 1991), and a maximal Hg concentration of 4250 $\mu g\,g^{-1}$ WW was reported in liver of a bottlenose dolphin (*Tursiops truncatus*) from the Thyrrhenian Sea (Leonzio et al., 1992). Nevertheless, some species from this area showed hepatic Hg concentrations that were comparable or varying in the same range to those observed in false killer wales from our study. For instance, spotted dolphins (1.4–550 $\mu g\,g^{-1}$ WW, Roditi-Elasar et al., 2003) and bottlenose dolphins (0.97–491 $\mu g\,g^{-1}$ WW, Roditi-Elasar et al., 2003) from Mediterranean coast and from Adriatic Sea (44.7–295 $\mu g\,g^{-1}$ WW, Bilandžić et al., 2012). Overall, comparison suggests that stranded false killer whales could be exposed to Hg accumulation in the South Atlantic Ocean. Therefore, our results are important for monitoring purposes and for conservation issues of these species in an area where surveillance regarding chemical contaminants might be occur.

3.2. Mercury concentration and distribution

Mercury distributes across the body more evenly compared to other metals, which concentrate in detoxifying organs before excretion. Following digestion, the pathway for Hg circulation is through the mucosa of gastrointestinal tract, transferred via lymph and blood vascular portal systems into the liver, and reaching therefore all organs (Basu and Head, 2010; Dietz et al., 2013). In the false killer whales studied, Hg concentration was higher in liver, as expected, since it is the main organ of protein metabolism and recipient of amino acids from the intestinal adsorption and other organs. This is the most important site for redistribution, detoxification or transformation of MeHg. Once mobilized to the intestine, MeHg can be excreted in bile, reabsorbed by the gut, or accumulated by kidneys for further redistribution (Frodello et al., 2000; Wagemann et al., 1998, 2000). This is due to the fact that kidney stores a significant fraction of metal and is also involved in excretion routes (Augier et al., 1993; Leonzio et al., 1992). Low percentages of MeHg in kidneys suggest that demethylase processes occur also in this organ (Leonzio et al., 1992; Wagemann et al., 1983). In the false killer whale, the kidney samples presented low Hg concentrations in relation to other tissues such as spleen and lung (Table 1), in agreement with other studies of odontocetes (Andre et al., 1991; Augier et al., 1993; Cardellicchio et al., 2002; Frodello et al., 2000; Itano et al., 1984). Additionally, a wide range of variation (CV%) in Hg concentrations for both tissues were found; the spleen with 96% and lung with 76.8%. A significant positive correlation in Hg concentrations was found between liver and spleen ($r = 0.99$, $p = 0.03$), and spleen and muscle ($r = 0.99$, $p = 0.01$), indicating a proportional accumulation of Hg between tissues. Positive correlation tendency was observed between liver and muscle, and kidney and muscle, although there were not significant ($r = 0.68$, $p = 0.20$ and $r = 0.61$, $p = 0.07$; respectively). But no correlation was observed between lung and the other tissues, inferring that there might be a different Hg dynamic process increasing Hg accumulation in this organ. Augier et al. (1993) hypothesized that Hg enter from the atmosphere into the lungs via alveoli, which could partly explain the relatively high values found in this organ. Likewise, other authors while evaluating Hg accumulation in odontocetes, found higher contents of Hg compounds in respiratory system of striped dolphins ($17.1 \pm 14.0\ \mu g\ g^{-1}$ WW, Itano et al., 1984; $3.0–396.8\ \mu g\ g^{-1}$ DW, Augier et al., 1993; $0.41–36.2\ \mu g\ g^{-1}$ WW, Cardellicchio et al., 2002), bottlenose dolphins ($264 \pm 22\ \mu g\ g^{-1}$ DW, Frodello et al., 2000), pilot whales ($200 \pm 9.1\ \mu g\ g^{-1}$ DW, Frodello et al., 2000) and Risso's dolphins (*Grampus griseus*, $301 \pm 64\ \mu g\ g^{-1}$ DW, Frodello et al., 2000). Mercury concentrations in lung of false killer whales ranged from 57.8 to $729\ \mu g\ g^{-1}$ DW, being higher than levels compared to those previously published. Lung appears to store a substantial portion of Hg; then, it is an organ which probably involved the process of Hg uptake and bioaccumulation through direct inhalation. Recognized differences between marine mammals (i. e. odontocetes accumulate Hg to levels 10–100 times higher than fishes) and vertebrates occupying similar trophic level like pelagic predatory fishes could be related on their different respiratory system (Leonzio et al., 1992). Passive diffusion of MeHg through gill may be an important pathway for Hg accumulation from water (Hall et al., 1997), although fish gills also have an excretory function. Mammals are air-breathing organisms and lack this, and the other systems are unable to prevent rapid accumulation to levels which are higher than those in fish. Furthermore, throughout histochemical studies Rawson et al. (1995) found amounts of HgSe in lung and hilar lymph nodes of bottlenose dolphins and short-finned pilot whales, which suggest that HgSe compounds are associated with particles from airborne. Rawson et al. (1995) suggest that the respiratory system inhaled already preformed HgSe, instead of hepatic HgSe that is end-product storage of Hg demethylation. Body tissues are specific in their ability to accumulate Hg and transfer to other tissues, which involves different Hg species (Wagemann et al., 1998). Mercury in skeletal muscle is largely accumulated in the form of MeHg which is thought to have a half-life about 1000 days in mammals (Stavros et al., 2011). The MeHg accumulation in muscle likely due to its high affinity for sulfhydryl groups associated with the thiol-containing amino acids in the myoglobin proteins (Castellini et al., 2012). Therefore, muscle Hg burden has been pointed to be the best indicator of accumulation (Wintle et al., 2011), representing MeHg that readily crosses the gastrointestinal tract and deposits in vascular tissues (Basu and Head, 2010). Consequently, analyzing muscle tissue for Hg is toxicologically significant because unlike liver, skeletal muscle accumulate the most toxic form as MeHg (Wintle et al., 2011). In the false killer whales analyzed, muscle tissue showed lower Hg concentrations in relation to other organs (i.e. liver, lung, kidneys, spleen and uterus), although they level are among the highest mean concentration ($118\ \mu g\ g^{-1}$ DW), being higher than those in other species, striped dolphins, $15.2 \pm 8\ \mu g\ gr^{-1}$ WW (Itano et al., 1984), pilot whales *Globicephala melas*, $2.68 \pm 0.18\ \mu g\ gr^{-1}$ WW and harbor porpoise *Phocoena phocoena*, $0.10–3.16\ \mu g\ g^{-1}$ DW (Wintle et al., 2011), sperm whales, $2.4–4.6\ \mu g\ gr^{-1}$ WW (Squadrone et al., 2015) and Guiana dolphins, $0.6 \pm 0.1\ \mu g\ gr^{-1}$ WW (Kehrig et al., 2016). This value is in the range of studies on odontocetes contamination in Mediterranean Sea such as striped dolphins $7.4–155.4\ \mu g\ gr^{-1}$ DW (Augier et al., 1993); $36.8–168\ \mu g\ gr^{-1}$ DW (Leonzio et al., 1992); $0.44–28.0\ \mu g\ gr^{-1}$ WW (Cardellicchio et al., 2002); $0.94–116.5\ \mu g\ gr^{-1}$ DW (Bellante et al., 2012), and bottlenose dolphins $38–292\ \mu g\ gr^{-1}$ DW (Leonzio et al., 1992). On the basis of toxicological studies in mammals, such levels could be expected to produce evident, if not poisonous toxic effects. Mercury levels in odontocetes have been found to be higher than those of other long-lived marine predators such as tunas or sharks (Storelli et al., 2002), most likely due as a result of their peculiar sequestration of Hg into inert forms and the comparatively poorer excretion capacity of this organisms.

3.3. Selenium and mercury interaction

Selenium is an essential element of major metabolic significance. Many factors, including dietary intake or natural sources and differences in physiologic needs or retention of Se for detoxification processes, may influence its tissue-concentrations (Caurant et al., 1996; Daniels, 1996; Kehrig et al., 2013). There is no evidence of a specific storage form or site; concentrations per weight of tissue are higher in liver and kidney but these organs contain only a relatively small proportion of total body Se. Therefore, the metabolic fate of Se varies according to the form ingested and the overall Se status of individuals (Daniels, 1996). Accordingly, Se is under homeostatic control and has tissue-specific regulation to some extent in mammalian systems. Reference levels in liver tissues of marine mammals range from 0.1 to $10\ \mu g\ g^{-1}$ WW, reaching in some cases a limit up to $100\ \mu g\ g^{-1}$ (Mackey et al., 1996). Accumulation of Se in liver occurs when the element is present at levels exceeding physiological requirements. Mean levels found in tissues of false killer whales (Table 1), exceed the limit of Se homeostatic control suggested for marine mammals (range only reported for liver $0.4–40\ \mu g\ g^{-1}$ DW, Mackey et al., 1996). Bioaccumulation of Se in hepatic tissue has also been described for marine mammals (Correa et al., 2014; Mackey et al., 1995). The anomalously high hepatic levels of Se are typically observed in cases in which levels of other toxic metals like Hg are also relatively high, due key-role of Se in detoxification processes.

Positive correlations of Hg and Se molar concentrations were

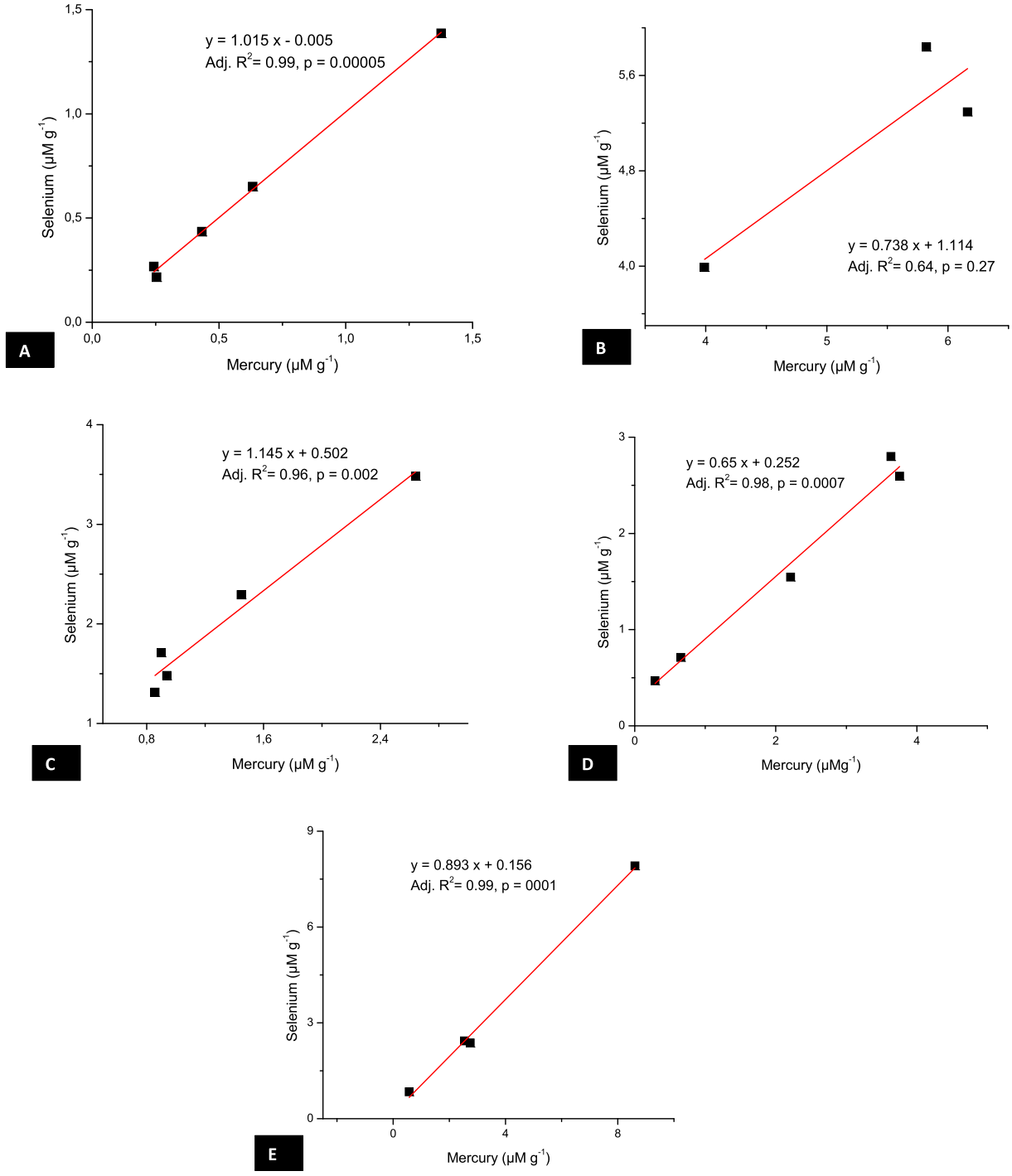


Fig. 2. Correlation between Hg and Se concentrations (µM g^{-1} DW) with a graphic representation of potential regression linear (R^2) in the A) muscle, B) liver, C) kidney, D) lung and e) spleen tissues of stranded false killer whales (*P. crassidens*) from Southern South America.

observed in muscle ($r = 0.99$, $p = 0.00004$), lung ($r = 0.99$; $p = 0.0007$), spleen ($r = 0.99$; 0.001) and kidney ($r = 0.98$; $p = 0.002$) (Fig. 2). In the liver positive correlation was observed, but due the small sample size ($n = 3$) no significant difference was obtained ($r = 0.91$; $p = 0.27$) (Fig. 2). The consistent positive relationship between Hg and Se accumulation suggests that possible interaction occurs between elements in these tissues, as in agreement with other authors (Caurant et al., 1996; Gui et al., 2014; Palmisano et al., 1995). Tissue differences in Hg-Se relationships suggests that interaction and distribution of elements can be different in organs, especially which are closely connected to the blood system and require a large blood supply (Kehrig et al., 2013), such as lung, spleen, and also skeletal muscle (which showed the strongest correlation) (Fig. 2). A positive association of Hg and Se concentrations in muscle of dolphins has already reported (Endo et al., 2005; Leonzio et al., 1992; Sakamoto et al., 2015).

It is also needed to consider direct interactions between Se and Hg (covalent linkages, Hg–Se). Selenium competes with Hg for its various physiological functions, which contributes to lowering the potential Hg toxicity (Khan and Wang, 2009). Selenium can

increase Hg half-life in liver and blood, making it less reactive, with a significant effect in organ distribution and excretion of Hg (Khan and Wang, 2009). The false killer whales are mainly piscivorous diving mammals and tend to have higher Se concentrations than non-diving mammals through their marine fish diet. Selenium, as the major component of Se-dependent GPX (Glutathione peroxidase), helps to alleviate the production of reactive oxygen species (ROS) caused by episodes of ischemia and reperfusion (restriction and return of blood flow; respectively) that occur in diving mammals (Vázquez-Medina et al., 2007). Consequently, high Se intake is an advantage for odontocetes because it can play a physiological role as antioxidant for diving features, as well as the essential role in ameliorating Hg toxicity (Correa et al., 2014; Khan and Wang, 2009).

3.4. Selenium-to-mercury relationship and toxicity risk assessment

The role of Se in prevention and inhibition of Hg toxicity has been reported for diverse marine vertebrates (Cuvin-Aralar and Furness, 1991; Frodello et al., 2000); however physiological differences may partially explain different abilities to store and eliminate Hg among species. The implications of Hg toxicity modifications by Se remain unclear because of variability in toxicokinetic (Watanabe, 2002) and the mechanisms are considered species- and tissue-specific (Cuvin-Aralar and Furness, 1991). All these mechanisms involve the formation of inert HgSe complexes (Cardellichio et al., 2002; Endo et al., 2005), the binding of Hg with selenoproteins (Palmisano et al., 1995) and the indirect action of Se in preventing oxidative damage by Hg through the increase of GPX activity (Cardellicchio et al., 2002). Wagemann et al. (1998) indicate that hepatic Hg concentrations largely reflect the predominant proportion of biologically unavailable Hg bound to Se. Demethylation of MeHg followed by formation of HgSe compounds was linked to successful detoxification in organic Hg-exposed humans (Korbas et al., 2010). In marine mammals, HgSe has been shown to be present in liver as small, round black, inert granules, aggregated into clusters in macrophagues or Kupffer cells (Lailson-Brito et al., 2012; Nigro and Leonzio, 1996). Additionally, HgSe has been found in other organs therefore the process may not be inherent to liver (Leonzio et al., 1992). Here, our results were insufficient to elucidate the type of mechanism, but it appears that toxicity of Hg in false killer whales could be counteracted by presence of Se in tissues.

A common approach to assess the risk of Hg exposure is to determine the Se to Hg molar ratio in body (Se/Hg) (Cuvin-Aralar and Furness, 1991; Sørmo et al., 2011). This ratio was widely used among diverse odontocetes (Caurant et al., 1996; García-Alvarez et al., 2015; Gui et al., 2014; Itano et al., 1984; Squadrone et al., 2015) and pinnipeds (Correa et al., 2014; Dietz et al., 2013) to evaluate Hg toxicity risk. It has been hypothesized that an abundance of Se as compared to Hg on a molar basis - where Se:Hg molar ratio is well above 1 - is important for potential amelioration of adverse effects due to MeHg exposure and the maintenance of Se dependent processes (Cuvin-Aralar and Furness, 1991; Khan and Wang, 2009). For stranded false killer whales analyzed, the Se/Hg molar ratios showed variation among tissues (Table 1). Kidney and testicle tissues presented Se/Hg molar ratios that were higher than 1 and particularly all testicle values were considerably higher than 1 indicating an effective protection against Hg toxicity. Molar ratios of Se/Hg in muscle range around 1; so there is no Se excess, and there would be no relevant protective action against Hg. This is particularly important in muscle, which most Hg is as MeHg, and would be indicative of the toxicological impact on individuals. In addition, Sakamoto et al. (2015) recently published a study addressing that demethylation of MeHg occurs in muscle of odontocetes. Therefore they can accumulate inert forms of Hg within skeletal muscle, where an equimolar ratio of 1 is found. The MeHg is demethylated in muscles and total Hg maintain their increase as HgSe within it, and the principal form of inert complexes was HgSe as confirmed by XAFS analysis (Sakamoto et al., 2015).

In liver of false killer whale Se/Hg molar ratio were lower to or equal to 1 (Table 1). The molar ratios of Se/Hg ≤ 1, suggest a limit protective effect of Se to Hg toxicity, aside of the liver, which is well known that mayor fraction of Hg corresponds to possible immobilization of Hg as HgSe stable compounds, in particular at higher Hg concentrations. Despite there being a great deal of variation in concentrations of Hg and Se between individuals and tissues, data showed Se to Hg ratios >1 in spleen, kidney and testicle, which suggest in that case a possible Se protective effect. Nevertheless, further analysis are required to assess the individual effects of high loads of Hg and either large amounts or deficiency of Se.

3.5. Mercury exposure and toxicological benchmarks in cetaceans

Odontocetes have a natural tendency to accumulate large quantities of Hg, although their trophic position and physiology must have provided a series of adaptations during evolution. Many anthropogenic factors, like presence of synthetic compounds and Hg rise in ecosystems, could seriously affect the balance upon which animals are based. Nowadays, elevated concentrations of Hg raise a significant health concern for wildlife and humans in general, exerting toxic effects in organs (i.e. liver, kidneys, and brain). The adverse toxic effects of Hg include neurotoxicity, reduced reproductive success, hepatic and renal damage, impaired development and immune-modulation (UNEP, 2002). Mercury ecotoxicology in odontocetes has been highlighted as a need to better assess potential causes contributing to mass mortalities. Owing to absence of data pertaining effect thresholds, the toxicological benchmarks of concentrations in critical organs at which effects occur, are useful key in evaluating potential risks and their toxicological impacts in wild populations.

Our finding of high Hg concentrations has raised the question about its toxicity. Two hepatic Hg toxicity thresholds exist for marine mammals. One is 61 $\mu g\, g^{-1}$ WW for liver injuries and lymph cellular breakdown determined in dolphins (Rawson et al., 1993). The hepatic Hg concentrations found in false killer whales from our study exceeded the toxic threshold previously defined. As well, Wagemann and Muir (1984) established a range for hepatic damage between 100 and 400 $\mu g\, g^{-1}$ WW. Once again, liver samples of stranded individuals exceeded the minimum Hg tolerance level, and in average hepatic Hg concentration of false killer whale are close to surpass the maximum level of the range. However, no specific analyses on liver disease were carried out to establish a threat due to an excessive Hg accumulation. An evaluation of possible toxic impacts of chronic Hg exposure is also necessary. For instance, liver lesions consisting of lipofuscinosis (lysosomal digestive enzymes inhibition), central necrosis and lymphocytic infiltrations (Rawson et al., 1993) have been associated with chronic Hg accumulation 234 $\mu g\, g^{-1}$ DW in bottlenose dolphins (Rawson et al., 1993) and 248 $\mu g\, g^{-1}$ WW (~1000 $\mu g\, g^{-1}$ DW) in Blainville's beaked whale (*Mesoplodon densirostris*) (Law et al., 1997). Furthermore, higher Hg accumulation has been observed in diseased animals suggesting they lack ability to detoxify organic Hg efficiently as healthy animals (Siebert et al., 1999). Chronic low-level Hg exposure could suppress immune and endocrine system of animals, suggesting that Hg exposure has immune-modulatory effects accompanied by an increase in diseases, infections and health impairments (Kannan et al., 2006; Stavros et al., 2011).

Further integrative analysis of histopathology and immunohistochemistry datasets on stranded individuals will enable a better evaluation of potential interactions between Hg pollution and fitness parameters, with possible implications to mass-mortalities.

Of particular concern are the neurotoxic effects of MeHg. Mammals, especially those at high trophic position can accumulate MeHg in brain among other tissues, at levels associated with neurotoxicological damages (Basu and Head, 2010). Recently, it has been suggested that chronic Hg toxicity affects the brain of polar bears (*Ursus maritimus*), ringed seals (*Pusa hispida*), beluga whales (*Delphinapterus leucas*) (Krey et al., 2015; Ostertag et al., 2013) and pilot whales (Gajdosechova et al., 2016). Accordingly, threshold levels of Hg toxicity are important indicators of neurotoxic effects that can be expected in wild animals. Accumulation of Hg in brain leads to the onset of neurodegenerative diseases or dysfunction of central nervous system, followed by premature death (Clarkson and Magos, 2006). It is expected that structural or brain lesions, and functional effects (clinical outcomes) of Hg exposure are similar among mammals because its high affinity for sulphydryl group, since Hg interacts with proteins in a non-discriminate way (Basu and Head, 2010). Until now, some links of mass mortality events to higher Hg concentrations in different organs including brain tissues were developed (Gajdosechova et al., 2016; Siebert et al., 1999; Squadrone et al., 2015). While no overt toxicity may be apparent, it has been suggested that subtle biochemical and neurochemical changes may occur (Krey et al., 2015; Stavros et al., 2011) before affecting the structure or function of the nervous system (Manzo et al., 2001). Squadrone et al. (2015) found that in stranded sperm whales, Hg values were within the range that has been demonstrated to result in Hg-associated neurotoxic effects, such as altered orientation and space perception. Authors suggest that this fact could be one of the underlying causes which contribute to stranding episodes, and further indicate that more studies are required to better understand neurotoxicological effects of Hg together with Se in marine mammals.

Finally, it is important to mention that consumption of contaminated fish and cetaceans in some countries is the principal exposure pathway for Hg accumulation in humans, despite the health risk associated with high Hg levels (Endo et al., 2005; Sakamoto et al., 2015). The red meat, which mainly includes muscle and liver, and the blubber from cetaceans are the most popular whale and dolphin products marketed. They usually exceed the limit proposed for food consumption of 0.5 $\mu g\, g^{-1}$ WW (Endo et al., 2005). Furthermore, the EPA reference dose for Hg in fish tissue was set 0.40 $\mu g\, g^{-1}$ WW (US EPA, 2002) as well the FDA action limit for Hg of 1.0 $\mu g\, g^{-1}$ WW in fish, shellfish, crustaceans, and other aquatic animals (US FDA, 2000). Those values were used to compare with our results and the false killer whale tissues analyzed in this study fairly exceed the safety reference limits.

4. Conclusion

The study revealed a concerning amount of Hg and Se in stranded false killer whales at southern marine area of South America; displaying the first Se/Hg molar ratio as toxicity risk assessment in a large odontocete that will be useful as a baseline for future strandings. To our knowledge, our results are the first published Se assessment in kidneys, muscle, lung, spleen and reproductive tissues of the species. Liver systematically shows the highest concentration of Hg as other cetaceans with evident high potential for toxic elements accumulation. Also, higher concentrations of Hg and Se were found in spleen and lung tissues. The finding of high concentrations of Hg in lung has raised the question about its additional source of bioaccumulation aside of intestinal tract via diet. Muscle Hg concentrations represented high levels of Hg contamination loads. Overall, Hg and Se concentrations of false killer whales were higher to those observed in toothed cetaceans from other marine areas of South America, and concentration values varied within ranges measured in species worldwide (apart from the extremely high values in Mediterranean Sea). Concentrations of Hg in false killer whales indicate a possible toxicity risk to these cetacean populations, demanding additional study to further our understanding of effects by elevated toxic metal in cetaceans which strand in masse. Even when Hg concentrations reached worrying levels, Se may be play a role in counteracting Hg toxicity. We found that molar Se:Hg ratio varied among tissues, only in the case of kidney and testicle ratios were >1, suggesting that Se could protect false killer whales from Hg-associated toxicity. The Se/Hg molar ratio close to, or equal 1, suggest that individuals may be at toxicological risk for high concentrations of Hg or a deficiency of Se without a protective action against Hg toxicity. It must be emphasized that Hg concentrations were variable between tissues and specimens; but all of them fall into the threshold established for Hg hepatic damage. These concentrations warrant further histological studies for indications of health assessment as the opportunities arise. The long-term database of contaminants in tissues of stranded cetaceans along with pathological examination, are critical to determine biological effects on individuals and populations scale of vulnerable species.

Acknowledgements

Our special thanks to the officers of Armada de Chile, Tte. Claudio Zúñiga and Ramón Aguilar, and Chap. René Rojas and Jared Yáñez for their assistance during transportation, and to the manager of Estancia 5 de Enero (Mr. Ivelic), who facilitated access to the coastline. We are indebted to those who helped carry out the field labor and logistical support: Nathasja Haddow, Tamara Martínez, Constanza Cifuentes, Carolina Calabró, Benjamín Cáceres, Ricardo Matus, Susana Saez and Manuel Cáceres. Our appreciation to the Director of INACH and colleagues Jorge Gallardo, Geraldine Asencio and Diana Schofield of Laboratory “Jorge Berguño”, INACH. Field collection is under regulations established by SARNAPESCA and coordinated by Ministerio de Medio Ambiente, Chile. The study was approved by Ministerio de Ambiente y Desarrollo Sustentable de la Nación, Departamento de Biodiversidad y Dirección de Fauna Silvestre, Argentina (license N° 2680428/16). All samples were imported under CITES N° 16CL000003WS. We are grateful to the editor and anonymous reviewers for their remarks on the manuscript.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.chemosphere.2018.02.046.

References

Andre, J., Boudou, A., Ribeyre, F., Bernhard, M., 1991. Comparative study of mercury accumulation in dolphins (*Stenella coeruleoalba*) from French Atlantic and Mediterranean coasts. Sci. Total Environ. 104, 191–209.

Augier, H., Park, W.K., Ronneau, C., 1993. Mercury contamination of the striped dolphin *Stenella coeruleoalba* Meyen from the French Mediterranean coasts. Mar. Pollut. Bull. 26, 306–311.

Basu, N., Head, J., 2010. Mammalian wildlife as complementary models in environmental neurotoxicology. Neurotoxicol. Teratol. 32, 114–119.

Baird, R.W., Langelier, K.M., Stacey, P.J., 1989. First records of false killer whales, *Pseudorca crassidens*, in Canada. Can. Field Nat. 103 (3), 368–371.

Bellante, A., Sprovieri, M., Buscaino, G., Buffa, G., Di Stefano, V., Salvagio Manta, S., Barra, M., Filiciotto, F., Bonanno, A., Giacoma, C., Mazzola, S., 2012. Stranded cetaceans as indicators of mercury pollution in the Mediterranean Sea. Ital. J. Zool. 79 (1), 151–160.

Berry, M.J., Ralston, N.V.C., 2008. Mercury toxicity and the mitigating role of

selenium. EcoHealth 5, 456–459.
Bilandžić, N., Sedak, M., Đokić, M., Gomeržić, M.D., Gomeržić, T., Zadravec, M., Benić, M., Crnić, A.P., 2012. Toxic element concentrations in the bottlenose (*Tursiops truncatus*), striped (*Stenella coeruleoalba*) and Risso's (*Grampus griseus*) dolphins stranded in Eastern Adriatic Sea. Bull. Environ. Contam. Toxicol. 89, 467–473.
Bustamante, P., Garrigue, C., Breau, L., Caurant, F., Dabin, W., Greaves, J., Dodemont, R.Ç., 2003. Trace elements in two odontocete species (*Kogia breviceps* and *Globicephala macrorhynchus*) stranded in New Caledonia (South Pacific). Environ. Pollut. 124, 263–271.
Cáceres-Saez, I., Goodall, R.N.P., Dellabianca, N., Luis Cappozzo, L., Ribeiro Guevara, S., 2015. Commerson's dolphins (*Cephalorhynchus commersonii*) skin as a biomonitor of mercury and selenium in Subantartic waters. Chemosphere 138, 735–743.
Cardellicchio, N., Decataldo, A., Di, L.A., Misino, A., 2002. Accumulation and tissue distribution of mercury and selenium in striped dolphins (*Stenella coeruleoalba*) from the Mediterranean Sea (Southern Italy). Environ. Pollut. 116, 265–271.
Castellini, J., Rea, L.D., Lieske, C.L., Beckmen, K.B., Fadely, B.S., Maniscalco, J.M., O'Hara, T.M., 2012. Mercury concentrations in hair from neonatal and juvenile Steller sea lions (*Eumetopias jubatus*): implications based on age and region in this Northern Pacific Marine sentinel piscivore. EcoHealth 9, 267–277.
Caurant, F., Navarro, M., Amiard, J.C., 1996. Mercury in pilot whales: possible limits to the detoxification process. Sci. Total Environ. 186, 95–104.
Clarkson, T.W., Magos, L., 2006. The toxicology of mercury and its chemical compounds. Crit. Rev. Toxicol. 36, 609–662.
Correa, L., Rea, L.D., Bentzen, R., O'Hara, T.M., 2014. Assessment of mercury and selenium tissular concentrations and total mercury body burden in 6 Steller sea lion pups from the Aleutian Islands. Mar. Pollut. Bull 82, 175–182.
Cuvin-Aralar, A., Furness, R.W., 1991. Mercury and selenium interaction: a review. Ecotoxicol. Environ. Saf. 21, 283–294.
Daniels, L.A., 1996. Selenium metabolism and bioavailability. Biol. Trace Elem. Res. 54, 185–199.
Dietz, R., Sonne, C., Basu, N., Braune, B., O'Hara, T., Letcher, R.J., et al., 2013. What are the toxicological effects of mercury in Arctic biota? Sci. Total Environ. 443, 775–790.
Endo, T., Haraguchi, K., Hotta, Y., Hisamichi, Y., Lavery, S., Dalebout, M.L., Baker, C.S., 2005. Total mercury, methyl mercury and selenium levels in the red meat of small cetaceans sold for human consumption in Japan. Environ. Sci. Technol. 39, 5703–5708.
Endo, T., Kimura, O., Hisamichi, Y., Minoshima, Y., Haraguchi, K., Kakumoto, C., Kobayashi, M., 2006. Distribution of total mercury, methyl mercury and selenium in pod of killer whales (*Orcinus orca*) stranded in the northern area of Japan: comparison of mature females and calves. Environ. Pollut. 144, 145–150.
Endo, T., Hisamichi, Y., Kimura, O., Haraguchi, K., Scott Baker, C., 2008. Contamination levels of mercury and cadmium in melon-headed whales (*Peponocephala electra*) from a mass stranding on the Japanese coast. Sci. Total Environ. 401, 73–80.
Ferreira, I.M., Kasuya, T., Marsh, H., Best, P.B., 2014. False killer whales (*Pseudorca crassidens*) from Japan and South Africa: differences in growth and reproduction. Mar. Mamm. Sci. 30, 64–84.
Frodello, J.P., Roméo, M., Viale, D., 2000. Distribution of mercury in the organs and tissues of five toothed-whale species of the Mediterranean. Environ. Pollut. 108, 447–452.
Gajdosechova, Z., Brownlow, A., Cottin, N.T., Fernandes, M., Read, F.L., Urgast, D.S., Raab, A., Feldmann, J., Krupp, E.M., 2016. Possible link between Hg and Cd accumulation in the brain of long-finned pilot whales (*Globicephala melas*). Sci. Total Environ. 545–546, 407–413.
García-Alvarez, N., Fernandez, A., Boada, L.D., Zumbado, M., Zaccaroni, A., Arbelo, M., Sierra, E., Almunia, J., Luzardo, O.P., 2015. Mercury and selenium status of bottlenose dolphins (Tursiops truncatus): a study in stranded animals on the Canary Islands. Sci. Total Environ. 536, 489–498.
Geraci, J.R., Lounsbury, V.J., 2005. Marine Mammals Ashore: A Field Guide for Strandings. National Aquarium in Baltimore, Baltimore, MD.
Gui, D., Yu, R.-Q., Sun, Y., Chen, L., Tu, Q., Mo, H., Wu, Y., 2014. Mercury and selenium in stranded Indo-Pacific Humpback dolphins and implications for their trophic transfer in food chains. PLoS One 9 (10), e110336.
Hall, B.D., Bodaly, R.A., Fudge, R.J.P., Rudd, J.W.M., Rosenberg, D.M., 1997. Food as the dominant pthway of methylmercury uptake by fish. Water Air Soil Pollut. 100, 13–24.
Hansen, A.M.K., Bryan, C.E., West, K., Jensen, B.A., 2016. Trace element concentrations in liver of 16 species of cetaceans stranded on Pacific islands from 1997 through 2013. Arch. Environ. Contam. Toxicol. https://doi.org/10.1007/s00244-015-0204-1.
Haro, D., Aguayo-Lobo, A., Blank, O., Cifuentes, C., Dougnac, C., Arredondo, C., Pardo, C., Cáceres-Saez, I., 2015. A new mass stranding of false killer whale, *Pseudorca crassidens*, in the Strait of Magellan. Chile. Rev. Biol. Mar. Oceanog 50 (1), 149–155.
Itano, K., Kawai, S., Miyazaki, N., Tatsukawa, R., Fujiyama, T., 1984. Mercury and selenium levels in striped dolphins caught off the Pacific coast of Japan. Agric. Biol. Chem. 48 (5), 1109–1116.
Kannan, K., Perrotta, E., Thomas, N.J., 2006. Association between perfluorinated compounds and pathological conditions in Southern Sea otters. Environ. Sci. Technol. 40, 4943–4948.
Kasuya, T., 1986. False killer whale. In: Tamura, T., Ohsumi, S., Arai, S. (Eds.), Report of Investigation in Search of Solution for Dolphin Fishery Conflict in the Iki Island Area. Japan Fish. Agency, Tokyo, pp. 178–187.
Kemper, C., Gibbs, P., Obendorf, D., Marvanek, S., Lenghaus, C., 1994. A review of heavy metal and organochlorine levels in marine mammals in Australia. Sci. Total Environ. 154, 129–139.
Khan, M., Wang, F., 2009. Mercury–selenium compounds and their toxicological significance: toward a molecular understanding of the mercury-selenium antagonism. Environ. Toxicol. Chem. 28, 1567–1577.
Kehrig, H.A., Seixas, T.G., Di Beneditto, A.P.M., Malm, O., 2013. Selenium and mercury in widely consumed seafood from South Atlantic Ocean. Ecotoxicol. Environ. Saf. 93, 156–162.
Kehrig, H.A., Hauser-Davis, R.A., Seixas, T.G., Pinheiro, A.B., Di Beneditto, A.P.M., 2016. Mercury species, selenium, metallothioneins and glutathione in two dolphins from the southeastern Brazilian coast: mercury detoxification and physiological differences in diving capacity. Environ. Pollut. 213, 785–792.
Koen Alonso, M., Pedraza, S.N., Schiavini, A.C.M., Goodall, R.N.P., Crespo, E.A., 1999. Stomach contents of false killer whales (*Pseudorca crassidens*) stranded along the Strait of Magellan. Mar. Mamm. Sci. 15, 712–724.
Korbas, M., O'Donoghue, J.L., Watson, G.E., Pickering, I.J., Singh, S.P., Myers, G.J., Clarkson, T.W., George, G.N., 2010. The chemical nature of mercury in human brain following poisoning or environmental exposure. ACS Chem. Neurosci. 1 (12), 810–818.
Kunito, T., Nakamura, S., Ikemoto, T., Anan, Y., Kubota, R., et al., 2004. Concentration and subcellular distribution of trace elements in liver of small cetaceans incidentally caught along the Brazilian coast. Mar. Pollut. Bull. 49, 574–587.
Krey, A., Ostertag, S.K., Chan, H.M., 2015. Assessment of neurotoxic effects of mercury in beluga whales (*Delphinapterus leucas*), ringed seals (*Pusa hispida*), and polar bears (*Ursus maritimus*) from the Canadian Arctic. Sci. Total Environ. 509–510, 237–247.
Lahaye, V., Bustamante, P., Law, R.J., Learmonth, J.A., Santos, M.B., Boon, J.P., Rogan, E., Dabin, W., Addink, M.J., López, A., Zuur, A.F., Pierce, G.J., Caurant, F., 2007. Biological and ecological factors related to trace element levels in harbor porpoises (*Phocoena phocoena*) from European waters. Mar. Environ. Res. 64, 247–266.
Lailson-Brito, J., Cruz, R., Dorneles, P.R., Andrade, L., Azevedo Ade, F., Fragoso, A.B., Vidal, L.G., Costa, M.B., Bisi, T.L., Almeida, R., Carvalho, D.P., Bastos, W.R., Malm, O., 2012. Mercury-selenium relationships in liver of Guiana dolphin: the possible role of Kupffer cells in the detoxification process by tiemannite formation. PLoS One 7, e42162.
Langelier, K.M., Stacey, P.J., Baird, R.M., 1990. Stranded whale and dolphin program of BC–1989 report. Wildlife Vet Rep 3 (1), 10–11.
Law, R.J., Fileman, C.E., Hopkins, A.D., Baker, J.R., Harwood, J., Jackson, D.B., Kennedy, S., Martin, A.R., Morris, R.J., 1991. Concentrations of trace metals in the livers of marine mammals (seals, porpoises and dolphins) from waters around the British Isles. Mar. Pollut. Bull. 22 (4), 183–191.
Law, R.J., Allchin, C.R., Jones, B.R., Jepson, P.D., Baker, J.R., Spurrier, C.J.H., 1997. Metals and organochlories in tissues of a Blainville's Beaked Whale (*Mesoplodon densirostris*) and a killer whale (*Orcinus orca*) stranded in the United Kingdom. Mar. Pollut. Bull. 34, 208–212.
Lemos, L.S., de Moura, J.F., Hauser-Davis, R.A., de Campos, R.C., Siciliano, S., 2013. Small cetaceans found stranded or accidentally captured in southeastern Brazil: bioindicators of essential and non-essential trace elements in the environment. Ecotoxicol. Environ. Saf. 97, 166–175.
Leonzio, C., Focardi, S., Fossi, C., 1992. Heavy metals and selenium in stranded dolphins of the Northern Tyrrhenian (NW Mediterranean). Sci. Total Environ. 119, 77–84.
Mackey, E.A., Demiralp, R., Becker, P.R., Greenberg, R.R., Koster, B.J., Wise, S.A., 1995. Trace element concentrations in cetacean liver tissues archived in the National Marine Mammal Tissue Bank. Sci. Total Environ. 175, 25–41.
Mackey, E.A., Becker, P.R., Demiralp, R., Greenberg, R.R., Koster, B.J., Wise, S.A., 1996. Bioaccumulation of vanadium and other trace metals in livers of alaskan cetaceans and pinnipeds. Arch. Environ. Contain. Toxicol. 30, 503–512.
Manzo, L., Castoldi, A.F., Coccini, T., Prockop, L.D., 2001. Assessing effects of neurotoxic pollutants by biochemical markers. Environ. Res. 85, 31–36.
Mouton, M., Botha, A., Thornton, M., Mesjasz-Przybyłowicz, J., Przybyłowicz, W.J., 2015. Elemental distribution patterns in the skins of false killer whales (*Pseudorca crassidens*) from a mass stranding in South Africa, analyzed using micro-PIXE. Nucl. Instrum. Meth. Phys. Res. B. 363, 70–74.
Nigro, M., Leonzio, C., 1996. Intracellular storage of mercury and selenium in different marine vertebrates. Mar. Ecol. Prog. Ser. 135, 137–143.
Ostertag, S.K., Stern, G.A., Wang, F., Lemes, M., Chan, H.M., 2013. Mercury distribution and speciation in different brain regions of beluga whales *(Delphinapterus leucas)*. Sci. Total Environ. 456–457, 278–286.
Palmisano, F., Cardellicchio, N., Zambonin, P.G., 1995. Speciation of mercury in dolphin liver: a two-stage mechanism for the demethylation accumulation process and role of selenium. Mar. Environ. Res. 40, 109–121.
Rawson, A.J., Patton, G.W., Hofmann, S., Pietra, G.G., Johns, L., 1993. Liver abnormalities associated with chronic mercury accumulation in stranded Atlantic bottlenose dolphins. Ecotoxicol. Environ. Saf. 25, 41–47.
Rawson, A.J., Bradley, J.P., Teetsov, A., Rice, S.B., Haller, E.M., Patton, G.W., 1995. A role for airborne particulates in high mercury levels of some cetaceans. Ecotoxicol. Environ. Saf. 30, 309–314.
Roditi-Elasar, M., Kerem, D., Hornung, H., Kress, N., Shoham-Frider, E., Goffman, O., Spanier, E., 2003. Heavy metal levels in bottlenose and striped dolphins off the Mediterranean coast of Israel. Mar. Pollut. Bull. 46, 491–521.
Rojo-Nieto, E., Fernández-Maldonado, C., 2017. Assessing trace elements in striped

dolphins from the Strait of Gibraltar: clues to link the bioaccumulation in the westernmost Mediterranean Sea area and nearest Atlantic Ocean. Chemosphere 170, 41–50.
Riccialdelli, L., Goodall, R.N.P., 2015. Intra-specific trophic variation in false killer whales (*Pseudorca crassidens*) from the southwestern South Atlantic Ocean through stable isotopes analysis. Mamm. Biol. 80, 298–302.
Sakamoto, M., Itai, T., Yasutake, A., Iwasaki, T., Yasunaga, G., Fujise, Y., Nakamura, M., Murata, K., Chan, H.M., Domingo, J.L., Marumoto, M., 2015. Mercury speciation and selenium in toothed-whale muscles. Environ. Res. 145, 55–61.
Seixas, T.G., Kehrig, H.A., Costa, M., Fillmann, G., Di Beneditto, A.P.M., Secchi, E.R., Souza, C.M.M., Malm, O., Moreira, I., 2008. Total mercury, organic mercury and selenium in liver and kidney of a South American coastal dolphin. Environ. Pollut. 154, 98–106.
Siebert, U., Joiris, C., Holsbeek, L., Benke, H., Failing, K., Frese, K., Petzinger, E., 1999. Potential relation between mercury concentrations and necropsy findings in cetaceans from German waters of the North and Baltic Seas. Mar. Pollut. Bull. 38 (4), 285–295.
Sonne, C., Dam, M., Leifsson, P.S., Dietz, R., 2010. Liver and renal histopathology of North Atlantic long-finned pilot whales (*Globicephala melas*) contaminated with heavy metals and organochlorine compounds. Toxicol. Environ. Chem. 92 (5), 969–985.
Sonne, C., Leifsson, P.S., Dietz, R., 2013. Liver and renal lesions in mercury-contaminated narwhals (*Monodon monoceros*) from North West Greenland. Toxicol. Environ. Chem. 95 (3), 1–14.
Sørmo, E.G., Ciesielski, T.M., Øverjordet, I.B., Lierhagen, S., Eggen, G.S., Berg, T., Jenssen, B.M., 2011. Selenium moderates mercury toxicity in free-ranging freshwater fish. Environ. Sci. Technol. 45, 6561–6566.
Squadrone, S., Chiaravalle, E., Gavinelli, S., Monaco, G., Rizzi, M., Abete, M.C., 2015. Analysis of mercury and methylmercury concentrations, and selenium:mercury molar ratios for a toxicological assessment of sperm whales (*Physeter macrocephalus*) in the most recent stranding event along the Adriatic coast (Southern Italy, Mediterranean Sea). Chemosphere 138, 633–641.
Stacey, P.J., Baird, R.W., 1991. Status of the false killer whale, *Pseudorca crassidens*, in Canada. Can. Field Nat. 105, 189–197.
Stavros, H.C.W., Stolen, M., Durden, W.N., McFee, W., Bossart, G.D., Fair, P.A., 2011. Correlation and toxicological inference of trace elements in tissues from stranded and free ranging bottlenose dolphins (*Tursiops Truncatus*). Chemosphere 82 (11), 1649–1661.
Storelli, M.M., Giacominelli-Stuffler, R., Marcotrigiano, G., 2002. Mercury accumulation and speciation in muscle tissue of different species of sharks from Mediterranean Sea, Italy. Bull. Environ. Contam. Toxicol. 68, 201–210.
UNEP (United Nations Environment Programme), 2002. Chemicals. Global Mercury Assessment. Available at. http://www.chem.unep.ch/MERCURY/Report/Final%20report/final-assessment-report-25nov02.pdf.
US EPA (US Environment Protection Agency), 2002. Risk Assessment Forum. A Review of the Reference Dose and Reference Concentration Process (Washington, DC).
US FDA (US Food and Drug Administration), 2000. Guidance for Industry: Action Levels for Poisonous or Deleterious Substances in Human Food and Animal Feed (Washington, DC).
Vázquez-Medina, J.P., Zenteno-Savín, T., Elsner, R., 2007. Glutathione protection against dive-associated ischemia/reperfusion in ringed seal tissues. J. Exp. Mar. Biol. Ecol. 345, 110–118.
Wagemann, R., Snow, N.B., Lutz, A., Scott, D.P., 1983. Heavy metals in tissues and organs of the narwhal (*Monodon monoceros*). Can. J. Fish. Aquat. Sci. 40, 206–214.
Wagemann, R., Muir, D., 1984. Concentrations of heavy metals and organochlorines in marine mammals of Northern waters: overview and evaluation. Can. Tech. Rep. Fish. Aquat. Sci. 1279, 1–97.
Wagemann, R., Trebacz, E., Boila, G., Lockhart, W.L., 1998. Methylmercury and total mercury in tissues of arctic marine mammals. Sci. Total Environ. 218, 19–31.
Wagemann, R., Trebacz, E., Boila, G., Lockhart, W.L., 2000. Mercury species in the liver of ringed seals. Sci. Total Environ. 261, 21–32.
Watanabe, C., 2002. Modification of mercury toxicity by selenium: practical importance. Tohoku J. Exp. Med. 196, 71–77.
Wintle, N.J.P., Duffield, D.A., Barros Deceased, N.B., Jones, R.D., Rice, J.M., 2011. Total mercury in stranded marine mammals from the Oregon and southern Washington coasts. Mar. Mamm. Sci. 27, 268–278.