Chemosphere 138 (2015) 735–743

Contents lists available at ScienceDirect

Chemosphere

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

The skin of Commerson's dolphins (*Cephalorhynchus commersonii*) as a biomonitor of mercury and selenium in Subantarctic waters

Iris Cáceres-Saez [a,b], R. Natalie P. Goodall [b,c], Natalia A. Dellabianca [b,c], H. Luis Cappozzo [a,d], Sergio Ribeiro Guevara [e,*]

[a] *Laboratorio de Ecología, Comportamiento y Mamíferos Marinos (LECyMM), División Mastozoología, Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (MACN-CONICET), Av. Ángel Gallardo 470, C1405DJR Buenos Aires, Argentina*

[b] *Museo Acatushún de Aves y Mamíferos Marinos Australes, Sarmiento 44, 9410 Ushuaia, Tierra del Fuego, Argentina*

[c] *Centro Austral de Investigaciones Científicas (CADIC-CONICET), Houssay 200, V9410BFD Ushuaia, Tierra del Fuego, Argentina*

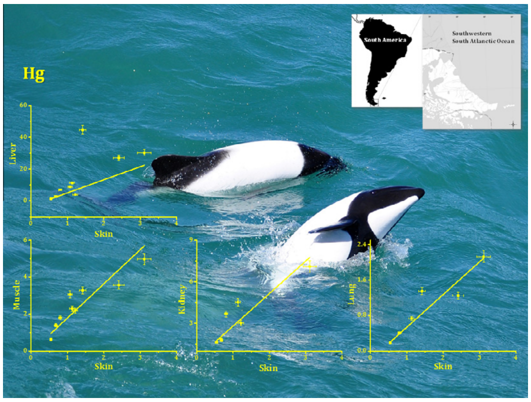
[d] *Centro de Estudios Biomédicos, Biotecnológicos, Ambientales y Diagnóstico (CEBBAD), Fundación de Historia Natural Félix de Azara, Departamento de Ciencias Naturales y Antropología, Universidad Maimónides, Hidalgo 775, C1405BCK Buenos Aires, Argentina*

[e] *Laboratorio de Análisis por Activación Neutrónica, Centro Atómico Bariloche-Comisión Nacional de Energía Atómica (CAB-CNEA), Av. E. Bustillo 9500, 8400 Bariloche, Argentina*

HIGHLIGHTS

- Skin of Subantarctic Commerson's dolphins was analyzed for Hg and Se bioindication.
- Liver, lung, kidney, muscle, and spleen tissues were correlated with skin contents.
- Skin Hg showed correlation with internal tissues allowing bioindication.
- Skin Se did not correlate with internal tissues due to biological regulation.
- Hg in muscle can be estimated from skin biopsies concentration by a factor of 1.85.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 3 May 2015
Received in revised form 28 June 2015
Accepted 8 July 2015

Keywords:
Mercury
Selenium
Skin biomonitoring
Marine environments
Small cetaceans
South Atlantic Ocean

ABSTRACT

The skin of bycaught Commerson's dolphins was tested for mercury (Hg) and selenium (Se) biomonitoring in Subantarctic environments. The correlation of levels detected in the skin with those found in internal tissues – lung, liver, kidney and muscle – was assessed to evaluate how skin represents internal Hg and Se distribution for monitoring purposes. Mercury in skin had a concentration range of 0.68–3.11 $\mu g\, g^{-1}$ dry weight (DW), while Se had a higher concentration range of 74.3–124.5 $\mu g\, g^{-1}$ DW. There was no significant correlation between selenium levels in any of the analyzed tissues. Thus, the skin selenium concentration did not reflect the tissular Se levels and did not provide information for biomonitoring. The lack of correlation is explained by the biological role of Se, provided that each tissue regulates Se levels according to physiological needs. However, the skin Hg level had significant positive correlation with the levels in internal tissues (ANOVA $p < 0.05$), particularly with that of muscle ($R^2 = 0.79$; ANOVA $p = 0.0008$). Thus, this correlation permits the estimation of Hg content in muscle based on the multiplication of skin biopsy levels by a factor of 1.85. Mercury bioindication using skin biopsies is a non-lethal approach that allows screening of a large number of specimens with little disturbance and makes possible an adequate sampling strategy that produces statistically valid results

---

* Corresponding author.
E-mail address: ribeiro@cab.cnea.gov.ar (S. Ribeiro Guevara).

http://dx.doi.org/10.1016/j.chemosphere.2015.07.026

in populations and study areas. The correlation between Hg levels in the skin and internal tissues supports the use of the epidermis of Commerson's dolphins for Hg biomonitoring in the waters of the Subantarctic, which is a poorly studied region regarding Hg levels, sources and processes.

1. Introduction

The evaluation of pollutant impact on marine ecosystems, both from natural and anthropic origins, is a subject of extensive research worldwide. This is the case for mercury (Hg), a heavy metal, which is highly toxic at very low concentrations. In its organic form (mostly monomethylmercury; MMHg), Hg is biomagnified in marine trophic webs, and it is a powerful neurotoxin for wildlife and for humans through fish consumption (Ullrich et al., 2001). Selenium (Se) is an essential element in biological systems but is toxic at high concentrations. Selenium has a high chemical affinity for Hg, and forms insoluble Hg–Se compounds. This sequesters Hg from biological processes and neutralizes its toxicity (Khan and Wang, 2009; Peterson et al., 2009; Sørmo et al., 2011). Therefore, the dual study of Se and Hg in aquatic food webs is important to ascertain the potential impact of Hg.

Cetacean skin biopsies are recommended as a non-invasive tool for assessing the eco-toxicological risk of populations and to conduct long-term environmental monitoring programs (Bryan et al., 2007; Fossi et al., 2000; Savery et al., 2013a; Stavros et al., 2007, 2011). Measuring Hg in the skin of piscivorous homeotherms is important to evaluate the exposure routes in marine environments and to assess the potential for toxicity to biota (Miller et al., 2011; Wöshner et al., 2008). Although highly mobile, their feeding habits provide clues on the sources and pathways. Additionally, the analysis of stable isotopes of Hg in skin biopsies, with regard to both the mass-dependent and the mass-independent isotopic ratios, provides key information on Hg sources and pathways (Jackson et al., 2008; Kwon et al., 2014). The odontocetes are long-lived, high-trophic-level mammals and therefore have a high potential for Hg bioaccumulation and biomagnification (Clayden et al., 2015). Furthermore, coastal dolphins and humans consume similar fish; thus, coastal dolphins can serve as a model for other cetaceans and humans and as sentinel species. Here, we study Hg and Se in the Commerson's dolphin (*C. c. commersonii*), an endemic odontocete in the southwestern South Atlantic Ocean with a distribution from 41°30′S to 55°S (Goodall et al., 1988, 1994).

The relationship between the skin Hg and Se content and that of other internal tissues is a key information required for the use of skin biopsies to monitor pollutants in marine environments. Although there are several reports on the elemental concentrations in the epidermis of cetaceans (Aubail et al., 2013; Dehn et al., 2006; Kunito et al., 2002; O'Hara et al., 2008; Savery et al., 2013b, 2014; Yang et al., 2002), there is little information on inter-tissue correlation or association between the heavy metal concentrations in such organs and those in the internal tissues. For Hg, several studies indicate that there is a correlation between the Hg concentration in skin and liver in odontocetes, such as Dall's porpoise (*Phocoenoides dalli*) (Yang et al., 2002), the harbor porpoise (*Phocoena phocoena*), the common dolphin (*Delphinus delphis*) (Aubail et al., 2013), the striped dolphin (*Stenella coeruleoalba*) (Aubail et al., 2013; Monaci et al., 1998; Borrell et al., 2015), the bottlenose dolphin (*Tursiops truncatus*) (Stavros et al., 2011; Aubail et al., 2013), and mysticetes including the bowhead whale (*Balaena mysticetus*) (O'Hara et al., 2008). Moreover, O'Hara et al. (2008) found that the epidermal Hg concentration was predictive of the blubber, hepatic and muscle tissue concentrations in bowhead whales. In addition, Aubail et al. (2013) showed a correlation between Hg in the skin and kidney in the common dolphin, the harbor porpoise, the bottlenose dolphin and the striped dolphin. More recently, Borrell et al. (2015) found a correlation between the Hg concentration in the skin and that found in the renal and muscle tissue of striped dolphins. The species considered in this study, the Commerson's dolphin, is classified as "Data Deficient" by the International Union for Conservation of Nature (IUCN) (IUCN, 2014). In Appendix II of the Convention on International Trade in Endangered Species (CITES), it is considered as one of the species that is not threatened but which may become so unless closely monitored. Previous research has shown that Commerson's dolphins are exposed to heavy metals and toxic elements (Cáceres-Saez et al., 2013a,b; Gil et al., 2006). This species consumes some prey species, which are targeted by the coastal fisheries. Although most fisheries are offshore, the artisanal captures are relevant for the local market. Therefore, monitoring the potentially toxic metals in their environment is useful to assess the impact on humans and for the development of management and conservation strategies.

The aim of this work is to study Hg and Se in the skin together with lung, spleen, liver, kidney and muscle tissues (which are the main tissues and organs in heavy metal dynamics, or which are physiologically relevant in marine mammals) using bycatch specimens of Commerson's dolphins from Tierra del Fuego to determine the correlation between the skin and internal tissues for monitoring purposes in Subantarctic marine ecosystems.

2. Materials and methods

2.1. Specimens studied and biological material analyzed

Nine bycaught specimens of Commerson's dolphins were recovered on the shores of Tierra del Fuego (52°30′–56°S; 65°–68°30′W; Fig. 1) in the austral summers from 2010 to 2012, as part of the field collection by the AMMA project (*Aves y Mamíferos Marinos Australes*) of the *Museo Acatushún*. Dolphin necropsies followed standard procedures (Norris, 1961). Whole organs and approximately 150 g of the epaxial muscle were excised from each specimen using a surgical blade. For the six specimens, samples from the central and posterior region of musculature were processed for the analysis. Once collected, each sample was placed in a small plastic bag and was kept frozen at −20 °C. The information recorded for each specimen included the sex (determined by direct observation of the genital patch or genital organs), the total length and the body weight. Three to four teeth were removed to estimate the age.

Samples were conditioned for analysis in the laboratory. After removal from the freezer, the tissue samples were lightly thawed, and the outer exposed tissue layer was trimmed to exclude any potential contamination during necropsy and storage. The tissue samples were handled using powder-free polyethylene gloves, and the samples were cut into pieces using the titanium-bladed knives and Teflon® tools. All tools and devices used for sample conditioning were previously washed in a 10% nitric acid solution and were double-rinsed with high-purity water (ASTM grade I). In total, 15–25 g was extracted and lyophilized from each organ and muscle tissue to achieve the same weight. The dried samples were ground to a fine powder with Teflon® tools. The skin samples were

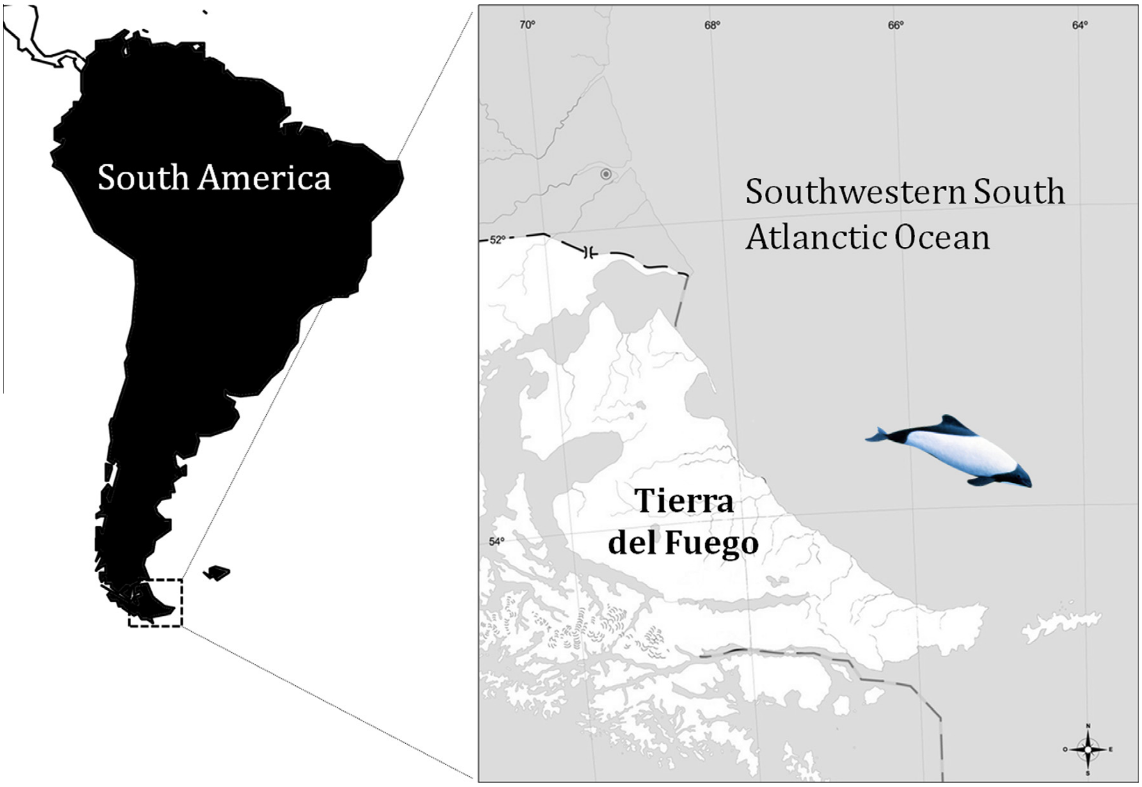


Fig. 1. Geographic location of the bycaught Commerson's dolphin (*C. commersonii*) specimens on the shores of Tierra del Fuego, Argentina.

shaved and excised from the underlying hypodermis (blubber) with a sterilized scalpel, and then lyophilized and sliced into small pieces. The aliquots, ranging from 100 to 150 mg, were placed in Suprasil AN® quartz ampoules in a laminar flow hood and were sealed for analysis.

The sex, age and body length of the specimens are reported in Table 1a. Age was determined using the Growth Layer Groups (GLGs) in dentine; each GLG was considered to be one year (Dellabianca et al., 2012; Lockyer et al., 1988). According to the age estimation, the specimens were classified into three groups: calves, suckling and still-nursing dolphins, with an age of less than one year; sexually immature juveniles between one and five years old; and sexually mature adults aged five years and older. In total, there were five GLGs (Goodall et al., 1988; Lockyer et al., 1988). This research uses Hg and Se content results from liver, kidney and muscle samples, as reported in previous work on this species (Cáceres-Saez et al., 2013b).

2.2. Mercury and selenium analysis

The concentration of Hg ([Hg]) and Se ([Se]) in a sample of each studied tissue was determined using Instrumental Neutron Activation Analysis (INAA). The samples were irradiated in the RA-6 nuclear reactor (MTR type, 1 MW thermal power), CAB – CNEA. The irradiation was performed in the reactor core (thermal, epithermal, and fast neutron fluxes of 2×10^{13}, 8×10^{11}, and 2×10^{12} n cm^{-2} s^{-1}, respectively) for 20 h. Two gamma-ray spectra were collected after decay times of 7 and 20 days. The gamma-ray spectra were collected using coaxial HPGe detectors (12% and 30% relative efficiency and 1.8 keV resolution at 1.33 MeV) and 4096-channel analyzers. The thermal and epithermal neutron fluxes were determined using the (n, γ) reactions of the Co–Au pair, using high purity Co and 0.1% Au–Al alloy wires. Mercury was determined by evaluating two activation products: ^{197}Hg and ^{203}Hg. The activation product ^{75}Se was analyzed to determine

Table 1a
Mercury and Se concentrations (μg g^{-1} DW) in the skin and internal tissues of Commerson's dolphins.

Specimen collection number	Sex	Age	Total length (cm)	Body weight (cm)	Skin			Lung			Spleen		
					Hg	Se	Se:Hg	Hg	Se	Se:Hg	Hg	Se	Se:Hg
RNP 2671	Male	0.5	109	18.1	1.21 ± 0.07	117.3 ± 7.1	245.5	n.a	n.a	n.a	n.a.	n.a.	n.a.
RNP 2701	Male	0.5	117.4	27	0.68 ± 0.04	96.5 ± 5.4	360.0	n.a	n.a	n.a	n.a.	n.a.	n.a.
RNP 2727	Female	0.5	99.3	25	0.54 ± 0.03	83.8 ± 5.0	392.1	0.19 ± 0.01	22.6 ± 1.70	303.8	0.29 ± 0.02	16.4 ± 1.30	141.7
RNP 2628	Male	1	118.9	27	1.07 ± 0.06	82.6 ± 4.7	196.1	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
RNP 2728	Male	1.5	116.6	28.5	0.80 ± 0.05	100.8 ± 5.9	319.3	0.41 ± 0.02	11.85 ± 0.88	73.78	0.57 ± 0.03	9.49 ± 0.71	41.93±
RNP 2669	Male	2	121	27.25	1.13 ± 0.07	75.5 ± 4.4	131.2	0.74 ± 0.04	13.7 ± 1.1	47.22	n.a.	n.a.	n.a.
RNP 2670	Female	7	139	31.2	3.11 ± 0.19	79.7 ± 4.7	65.1	2.13 ± 0.13	8.29 ± 0.62	9.89	n.a.	n.a.	n.a.
RNP 2724	Male	14	136.9	35	1.41 ± 0.08	74.3 ± 4.3	133.4	1.35 ± 0.08	10.08 ± 0.76	18.97	1.06 ± 0.06	7.41 ± 0.56	17.76
RNP 2725	Female	11	135.1	37.5	2.41 ± 0.14	124.5 ± 9.3	131.2	1.25 ± 0.07	13.32 ± 0.98	27.07	n.a.	n.a.	n.a.
Mean					1.38	92.78	219.32	1.01	13.3	80.1	0.64	11.1	67.1
SD					0.85	18.31	115.95	0.71	4.98	111.9	0.39	4.71	65.7
CV%					61.7	19.7	52.9	70.4	37.5	139.7	60.3	42.4	97.9

n.a. – not analyzed. The analytical uncertainty is reported after '±'. Se:Hg – Se to Hg molar ratio.

Table 1b
Mercury and Se concentrations ($\mu g\ g^{-1}$ DW) in internal tissues of Commerson's dolphins.

Specimen collection number	Liver			Kidney			Muscle		
	Hg	Se	Se:Hg	Hg	Se	Se:Hg	Hg	Se	Se:Hg
RNP 2671	3.89 ± 0.23	9.14 ± 0.69	5.97	3.03 ± 0.18	12.66 ± 0.97	10.61	2.19 ± 0.13	6.57 ± 0.48	7.62
RNP 2701	2.80 ± 0.17	10.58 ± 0.81	9.60	1.79 ± 0.11	13.5 ± 1.1	19.16	1.49 ± 0.09 1.29 ± 0.08	3.41 ± 0.27 3.02 ± 0.23	5.82 5.92
RNP 2727	1.30 ± 0.08	15.2 ± 1.2	29.66	1.64 ± 0.09	19.1 ± 1.5	29.59	0.58 ± 0.05 0.68 ± 0.06	8.62 ± 0.67 6.48 ± 0.48	37.50 24.32
RNP 2628	8.85 ± 0.53	12.8 ± 1.0	3.68	n.a.	n.a.	–	3.07 ± 0.18 3.06 ± 0.18	3.81 ± 0.29 2.89 ± 0.22	3.15 2.40
RNP 2728	7.06 ± 0.42	20.3 ± 1.6	7.30	3.68 ± 0.22	13.0 ± 1.0	8.97	1.85 ± 0.11 1.73 ± 0.10	2.57 ± 0.19 2.73 ± 0.21	3.53 4.01
RNP 2669	11.51 ± 0.69	13.4 ± 1.0	2.96	4.52 ± 0.27	12.55 ± 0.85	7.05	2.29 ± 0.14	3.2 ± 0.24	3.55
RNP 2670	30.4 ± 1.8	20.5 ± 1.6	1.71	7.1 ± 0.43	10.89 ± 0.79	3.90	4.99 ± 0.30	2.36 ± 0.19	1.20
RNP 2724	44.7 ± 2.7	36.7 ± 2.7	2.1	n.a.	n.a.	–	3.22 ± 0.19 3.35 ± 0.20	2.81 ± 0.22 2.70 ± 0.21	2.22 2.05
RNP 2725	27.2 ± 1.6	25.2 ± 1.9	2.4	n.a.	n.a.	–	3.77 ± 0.23 3.37 ± 0.20	3.66 ± 0.28 3.55 ± 0.30	2.47 2.68
Mean	15.30	18.20	7.26	3.63	13.6	13.2	2.46	3.9	7.2
SD	15.17	8.68	8.82	2.03	2.83	9.5	1.23	1.83	10.1
CV%	99.1	47.7	121.5	55.9	20.8	72.0	49.8	47.0	139.4

n.a. – not analyzed. The analytical uncertainty is reported after '±'. Se:Hg – Se to Hg molar ratio. Liver, kidney and muscle data of some specimens was published (Cáceres-Saez et al., 2013b). For six specimens, two samples of muscle were processed, extracted from the central and posterior body region, and the elemental concentrations measured were averaged for further correlations.

the Se concentrations. Corrections of the analytical interferences were performed, particularly that of ^{75}Se on ^{203}Hg. The Suprasil NA quartz impurity content was previously evaluated, and no Hg or Se content was detected. Analytical quality control was performed by analyzing Certified Reference Material NRCC TORT-2 (lobster hepatopancreas), and the results showed good agreement with the certificate values. The concentrations are expressed on a dry weight (DW) basis. Because [Hg] is reported frequently on a wet weight (WW) basis, the conversion factor from dry to wet weight was determined for each analyzed tissue to allow the comparison with all reported data in the literature. For the skin, a moisture content of 70% was assumed (Yang and Miyazaki, 2003).

2.3. Data analysis

Descriptive statistics were employed to evaluate [Hg] and [Se] in the analyzed skin and internal tissues. The concentrations are presented as the mean, standard deviation (in parenthesis) and coefficient of variation (CV%). Linear correlation analysis was performed to evaluate the relationship between [Hg] and [Se] levels in the skin and the corresponding levels in the lung, liver, kidney and muscle. This analysis was not performed for the spleen tissue due to the low sample size (three samples). For the specimens with two analyzed muscle samples, the two determinations were averaged for the correlation evaluation, provided that no significant difference was observed (Tables 1a and 1b). The data from all tissues and organs were analyzed to detect statistically significant differences using non-parametric (Kruskal–Wallis) tests. Due to the small sample sizes of calves, juveniles and adults, we only provide descriptive comparisons related to these ontogenetic classes. The threshold for statistical significance was set at $p < 0.05$. The analyses were conducted using OriginPro Version 8 (OriginLab Corporation, Northampton, MA 01060 USA, 2007).

3. Results and discussion

The occurrence of heavy metals and toxic elements in top predators, such as some cetaceans, is of global concern. Assessment of pollutants in these organisms, with the objective of species preservation, has been the subject of a large number of research studies (Kunito et al., 2002; Stavros et al., 2007; Wöshner et al., 2008). The study of tissues and organs in bycaught dolphins is a valuable source of chemico-toxicological information (Augier et al., 1993; Cardellicchio et al., 2002; Frodello et al., 2000). Elemental concentrations can be measured in a variety of internal tissue samples, specifically, hepatic, renal and muscular tissues. Among these biological materials, skin is the most accessible external organ and is easily sampled from the specimens in the field. Skin is one of the largest and most important organs in mammals. Its primary physiological function is that of an interface between the body and the external environment. The skin provides the foremost line of defense against mechanical damage, radiation, toxic compounds and micro-organisms (Martinez-Levasseur et al., 2010; Richelle et al., 2006; Sengupta et al., 2010). In cetaceans, this organ exhibits adaptive specializations, including an increase in lipid deposits and subdermal blubber reservoirs (Bryan et al., 2007; Yang et al., 2002). Additionally, the skin decreases drag due to absence of hair, sebaceous glands and other epidermal annexes (Pfeiffer and Jones, 1993). It has been suggested that lack of these features prevents the excretion and uptake of trace elements through openings in the skin and makes the cetaceans a closed system (Bryan et al., 2007). Moreover, it has been proposed that trace elements primarily accumulate in the multi-layered epidermis and dermis of the skin (Wagemann and Kozlowska, 2005).

3.1. Mercury and Se content

The concentration of Hg in samples of Commerson's dolphin skin was similar to that found in other tissues and ranged between 0.5 and 3 $\mu g\ g^{-1}$ DW (Fig. 2 and Tables 1a and 1b). No significant differences were observed between [Hg] in the skin, lung, muscle, kidney or spleen. Significant differences ($p < 0.01$) were found between [Hg] in the skin and in liver. However, higher values were observed in the kidney and lower in the spleen (Fig. 2). Age and body growth are important biotic parameters in the study of Hg, because Hg may accumulate in certain organs over the lifespan

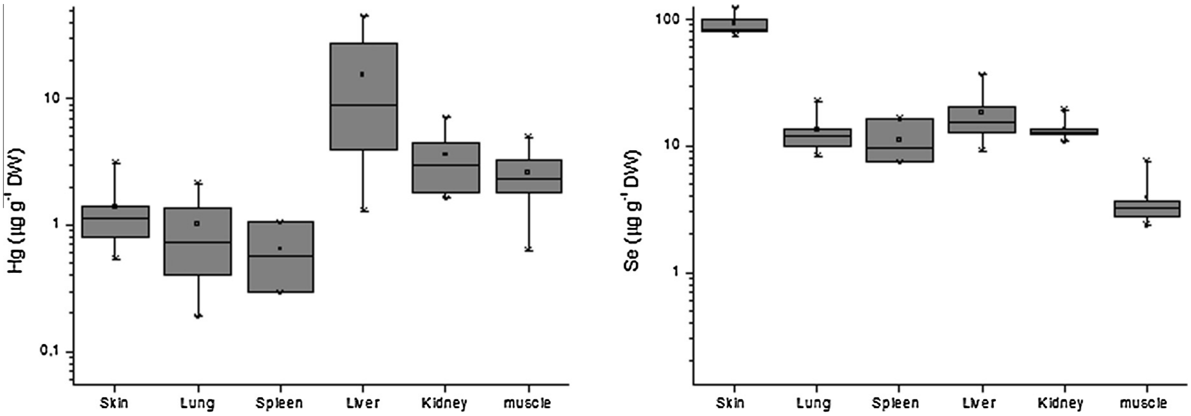


Fig. 2. Box plot indicating the Hg and Se concentrations in the skin and internal tissues of the Commerson's dolphins. The horizontal line inside the box represents the median, the bottom and top of the box constitute the interquartile range (25% and 75%) of the distribution, and the lines extending vertically from the boxes indicate the minimum and maximum values.

(Caurant et al., 1996; Lockhart et al., 2005; Stavros et al., 2011; Wagemann et al., 1996). Specifically, Hg tends to accumulate in the liver, an organ that metabolizes nutrients and essential elements and removes non-essential elements, compounds and toxins from the bloodstream in mammals (Augier et al., 1993; Frodello et al., 2000). Therefore, [Hg] and [Se] may increase with age and size and are linked to the formation and storage of SeHg in the liver (Caurant et al., 1996; O'Hara and O'Shea, 2001). A tendency of [Hg] to increase was observed in the liver and kidneys of the Commerson's dolphin specimens, as well as in the muscle tissue, where Hg is primarily in the MMHg form. Selenium increases with age only in the liver, which is consistent with the abovementioned formation and storage of SeHg. Furthermore, we observed [Hg] variation in the skin with growth. Specifically, [Hg] was lower in calves, 0.81(0.35) µg g^{-1} DW (average; standard deviation in parenthesis), than in adults, 2.31(0.81) µg g^{-1} DW, and was similar between the calf and juvenile classes, 1.0(0.18) µg g^{-1} DW. It is important to note that the epidermal molt eliminates Hg in odontocetes (Wagemann et al., 1996; Wagemann and Kozlowska, 2005), which is analogous to the elimination via hair or fur in other species. Moreover, the highest Hg concentration is found in the outer epidermal layer, and, during the skin molt, approximately 14% of the epidermal MMHg is eliminated (Wagemann et al., 1996).

Selenium concentrations were highest in the skin. The values for the kidney, liver, lung and spleen were similar. However, the muscle tissue showed the lowest concentrations (Tables 1a and 1b and Fig. 2). Regarding the variation of [Se] in skin with age, the calves (99.2(17) µg g^{-1} DW), juveniles (86.3(13) µg g^{-1} DW), and adults (92.8(27.5) µg g^{-1} DW) exhibited similar values. The epidermis was found to be the target tissue for Se deposition, as observed in previous studies (Borrell et al., 2015; Dehn et al., 2006; Stavros et al., 2011; Yang et al., 2002). This is attributed to the importance of Se in skin physiology. The low standard deviation (less than 20%; Tables 1a and 1b) indicates that [Se] is tightly regulated in this organ. In the studied Commerson's dolphin specimens, the skin [Se] ranged from 74 to 124 µg g^{-1} DW (22–37 µg g^{-1} WW), which is within the range of reported levels for the epidermis of dolphins and porpoises, 57–321 µg g^{-1} DW (17–96 µg g^{-1} WW; Augier et al., 1993; Monaci et al., 1998; Yang et al., 2002), and is in agreement with other studies (Dehn et al., 2006; Kunito et al., 2002; Lockhart et al., 2005; Savery et al., 2013a; Stavros et al., 2007).

Selenium uptake occurs via ingestion, primarily due to the Se-rich fish diet consumed by odontocetes (Caurant et al., 1996; Paludan-Müller et al., 1993; Wöshner et al., 2001). Selenium is a dietary micronutrient and is important for proper functioning of many organs, including the epidermis. Specifically, it has been indicated that Se in the form of glutathione peroxidase protects against UVB-induced skin damage and tumors in mammals by: (1) decreasing oxidative DNA damage, (2) preventing the production of cytokines, and (3) enhancing cellular and humoral immunity (Leccia et al., 1993; McKenzie, 2000; Richelle et al., 2006; Sengupta et al., 2010). Additionally, Se is known to be essential for keratinocyte function, and for the development of epidermal density and thickness (Richelle et al., 2006; Sengupta et al., 2010), taking into account that cetaceans seasonally slough epidermis (Savery et al., 2013a; St. Aubin et al., 1990; Wagemann and Kozlowska, 2005; Wöshner et al., 2001).

Regarding the potential of cetaceans to be used as biomonitors of Hg in marine systems, the situation in two geographical areas is briefly compared: the Mediterranean Sea and the Southern Ocean. The Mediterranean Sea is one of the most investigated marine systems in the world with a high Hg pollution level, which is affected by natural Hg inputs (Cossa et al., 2009; Heimbürger et al., 2010; Kotnik et al., 2015; and references therein). The Southern Ocean is free from direct industrial sources of contamination and is scarcely affected by local anthropogenic pollution. It exhibits lower Hg levels in water, although it is not well studied (Cossa et al., 2011; Mason and Sullivan, 1999). The mercury concentration in the liver of captured odontocetes in the Mediterranean Sea has a wide range, from 10 to 5000 µg g^{-1} DW (Augier et al., 1993; Cardellicchio et al., 2002; Frodello et al., 2000; Leonzio et al., 1992; Shoham-Frider et al., 2002). To date, little is known about the Hg exposure of small odontocetes within the Subantarctic food webs, but Hg levels in the liver of the Commerson's dolphin, which inhibits the waters around Tierra del Fuego, ranged from 1.3 to 45 µg g^{-1} DW. This is lower than for the Mediterranean Sea odontocetes and is among the lowest values for other dolphins from the South Atlantic Ocean (Cáceres-Saez et al., 2013a).

3.2. Interaction of Hg and Se

Marine mammals can be protected against Hg toxicity through a number of mechanisms, including demethylation, excretion/elimination (e.g., urine, feces, hair in pinnipeds), and interactions with other elements (O'Hara and O'Shea, 2001; Yang et al., 2008). Selenium counteracts negative effects of Hg through a number of direct-binding and antioxidant mechanisms (Koeman et al., 1973; Wagemann et al., 1996). Specifically, Se has a high chemical affinity for Hg in biological systems and forms insoluble Hg–Se complexes, thus sequestering Hg from biological processes and neutralizing its toxic effects (Khan and Wang, 2009; Peterson

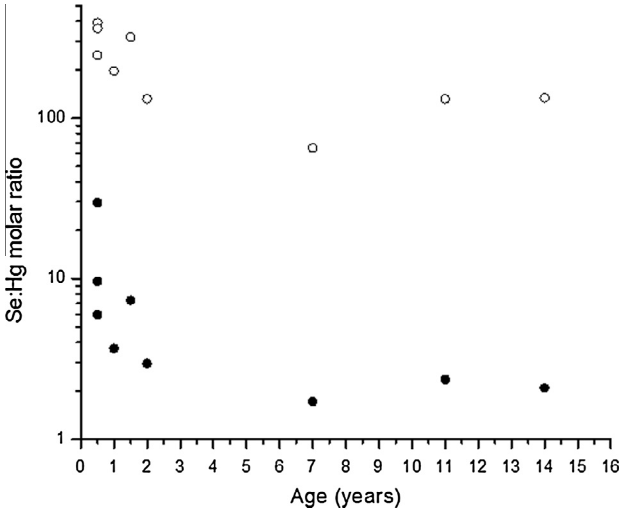


Fig. 3. The Se:Hg molar ratio in the skin (white dots) and the liver (black dots) vs. the age of the Commerson's dolphins (points at 0.5 years correspond to ages below 1 year).

et al., 2009; Sørmo et al., 2011). The availability of Se for binding Hg is indicated by the Se:Hg molar ratio in a tissue. Ratios greater than 1 indicate a molar excess of Se in the tissue, which implies the potential of Se to protect against Hg toxicity. However, ratios lower than 1 suggest limited Se protection (Sørmo et al., 2011). Measured skin Se:Hg molar ratios significantly exceed 1 (65–392; Tables 1a and 1b), which suggests high Se availability to combine with Hg in stable compounds.

The [Hg] was correlated with [Se] in the cetacean liver samples, together with low levels of MMHg. This is consistent with the formation and storage of Hg–Se stable compounds, provided that the liver is the organ that accumulates them (Augier et al., 1993; Cardellicchio et al., 2002; Koeman et al., 1973; Wagemann et al., 1996; Wöshner et al., 2001). This correlation was not observed in the epidermis (Augier et al., 1993; Dehn et al., 2006; Lockhart et al., 2005; Stavros et al., 2007). In this study, [Hg] and [Se] were not found to have a significant correlation in the skin ($p > 0.05$). Although there was a positive correlation of [Hg] with age due to Hg storage in inorganic forms, a [Hg]–[Se] correlation was not observed due to the much higher [Se] (Tables 1a and 1b). The trend of lower Se:Hg molar ratios with higher [Hg] for older individuals (Fig. 3) reveals an increasing [Hg] relative to [Se]. This could be attributed to the skin storage of Hg–Se stable compounds. Notably, the Se:Hg molar ratios in skin and liver, the organs which are known to accumulate Hg–Se stable compounds (Augier et al., 1993; Frodello et al., 2000), have a similar age-trend but with much higher values for the skin (Fig. 3). Keratin is the main structural protein found in the epidermis and contains multiple disulfide cross linkages as well as cysteine residues. Cysteine is an –SH amino acid and is likely the binding site for $MMHg^+$ in the keratinized tissues (Cernichiari et al., 1995). However, it is possible that Hg complexes occur in the skin of odontocetes, and Hg is deposited in the form of MMHg–SH complexes (Khan and Wang, 2009; Savery et al., 2013a).

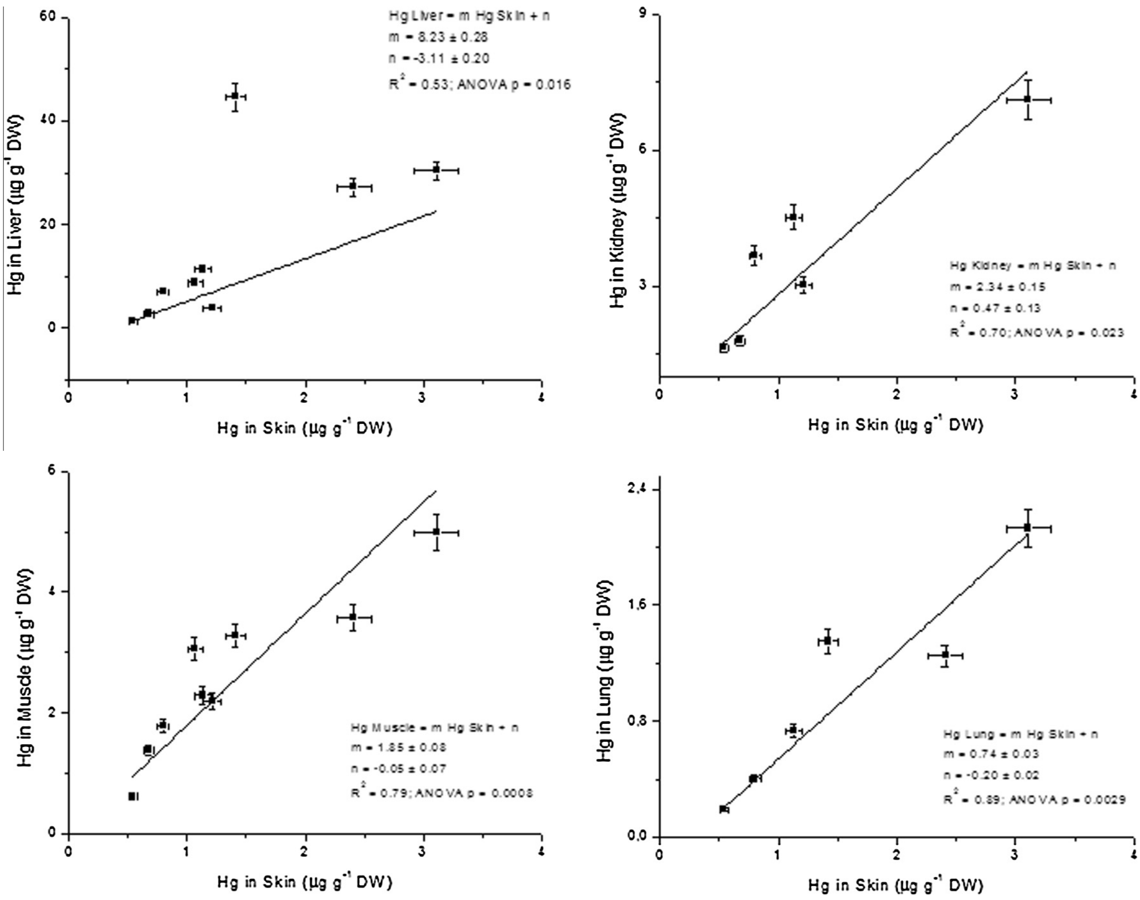


Fig. 4. Relationship between the Hg concentration in the skin and internal tissues of the Commerson's dolphins: liver, kidney, muscle and lung. The linear fitting parameters are reported for each case.

3.3. Skin-to-internal-tissue relationship

Significant linear positive correlations were found between [Hg] in the skin and in other studied tissues in Commerson's dolphins (Fig. 4). Specifically, the strong correlation ($R^2 = 0.79$; $p = 0.0008$) between the skin and muscle indicates that [Hg] in the skin is associated with that in the muscle. This outcome is important for monitoring purposes because Hg in the muscle is primarily in the MMHg form (Ullrich et al., 2001) and is associated with feeding habits and MMHg sources. Therefore, Hg in the epidermis indicates the level of Hg uptake by the whole organism but is strongly associated with MMHg accumulation in muscle and, hence, the MMHg uptake. Mercury in the skin of Commerson's dolphins is an accurate bioindicator of Hg accumulation in the whole organism, which allows us to predict [Hg] in the muscle. Under the study conditions, [Hg] in the muscle is estimated from the skin content using a factor of 1.85 (n parameter in linear fitting is 0; Fig. 4).

No significant correlation was found between the [Se] in the skin and that in internal tissues ($p > 0.05$; Fig. 5), which suggests that there was no direct association between Se accumulation in the skin and that in internal tissues. This is attributed to the biological role of Se, which is regulated by each tissue according to the physiological needs (Dehn et al., 2006; Kunito et al., 2002; Savery et al., 2013a). Therefore, the skin [Se] does not reflect the internal tissue content and cannot be used to monitor Se.

3.4. Skin Hg content of Commerson's dolphins as a proxy for monitoring the Subantarctic ecosystem

Bioindication has been widely used to evaluate the sources, distribution, processes or impact of different pollutants. In marine ecosystems, Hg is a pollutant of particular concern because it is harmful at very low concentrations to wildlife and humans through fish consumption. Transport, speciation and trophic transfer are key aspects in Hg cycling, which involves complex processes that are difficult to fully understand. Therefore, bioindication is of particular relevance in the study of Hg in aquatic systems and provides valuable information that is difficult to obtain by other means. The higher trophic levels of marine ecosystems are the target of Hg studies, given the ability of Hg to biomagnify. The higher trophic levels have the highest [Hg], primarily in the MMHg form, and provide information about the lower trophic levels, which indicates the exposure routes. The odontocetes fully fit this profile, particularly the Commerson's dolphin, which is a coastal cetacean. Regarding wildlife protection, exposure assessment in dolphins indicates marine areas where the species face risks from metal toxicity, and this type of assessment is therefore an important aspect of the management and conservation of cetacean wildlife.

Heavy metal monitoring using skin biopsies is a non-lethal approach that allows the screening of a large number of specimens with little disturbance to the animals. This makes it an adequate sampling strategy that produces statistically valid results in the study area or in the populations under study. Most studies report the elemental concentrations in the tissues of marine mammals as they relate to age and growth, tissue type, and geographic area, which varies among the individuals and species (Law, 1996). Nevertheless, for bioindication, the correlation of trace element concentrations between the skin and internal organs of cetaceans is of particular significance for the evaluation of the whole body content or concentrations in the key tissues. The linear correlation of [Hg] in the skin with the concentrations in other relevant internal organs and tissues, specifically in the muscle, supports the use

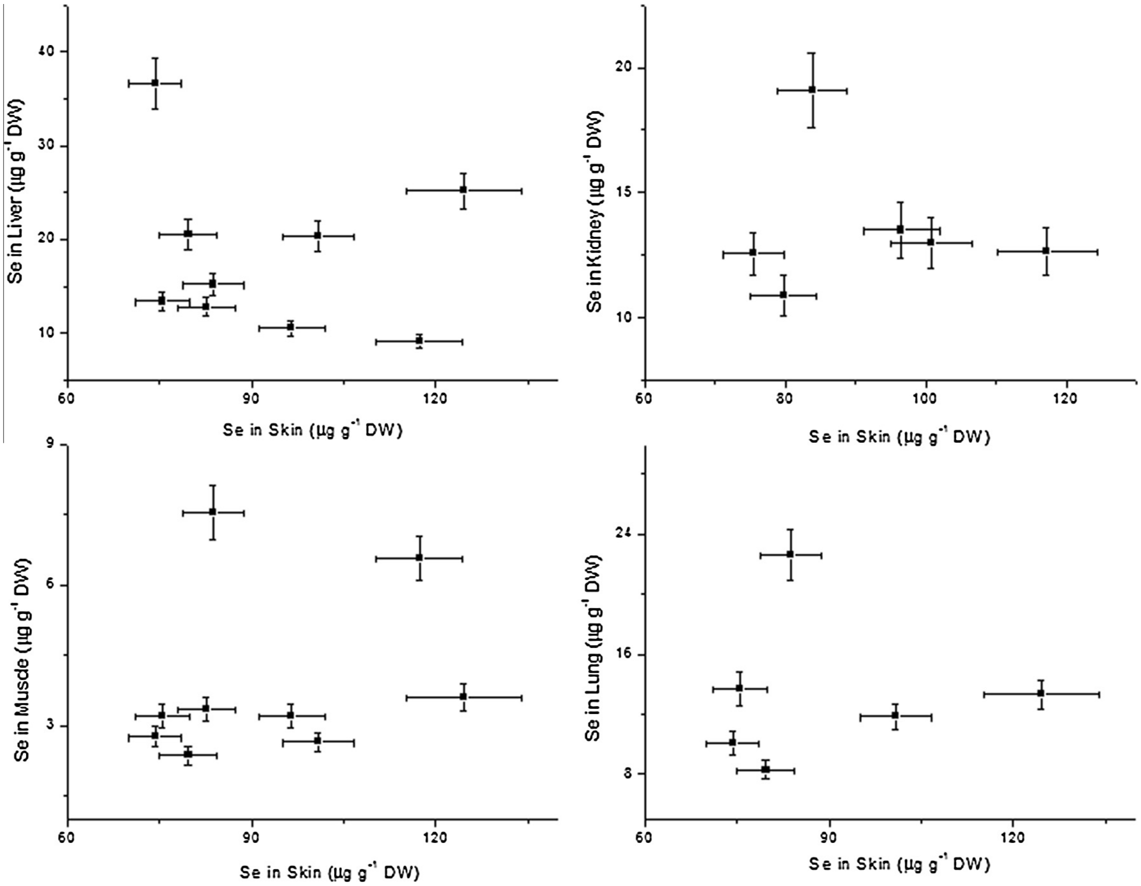


Fig. 5. Selenium concentrations in the skin and internal tissues of the Commerson's dolphins: liver, kidney, muscle and lung.

of skin biopsies from Commerson's dolphins for Hg bioindication in the water of the Subantarctic, which is a poorly studied region regarding Hg levels, sources and processes.

Acknowledgements

We sincerely thank the RA-6 reactor operation staff for irradiating the samples. We are grateful to A. Rizzo and M. Arcagni for laboratory support and to many volunteers who helped to collect and dissect the specimens at the Museo Acatushún de Aves y Mamíferos Marinos Australes, Ushuaia, Tierra del Fuego, Argentina. Additionally, we thank Lars-Eric Heimbürger and an unknown reviewer for their fruitful revision of the manuscript. The ICS deeply thanks M. Berzano for the photographs of Commerson's dolphins that were shared with us. The field work and research in the coastal areas of Tierra del Fuego is carried out under a permit from the local government. The biological material was transferred under licenses extended by the Secretaría de Ciencia y Tecnología, Secretaria de Desarrollo Sustentable y Ambiente, and SENASA, Tierra del Fuego, Argentina. ICS was supported by a postdoctoral fellowship from the Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina (CONICET) and was funded by Grants in Aid of Research from the Cetacean Society International and the Society for Marine Mammalogy. These results are part of the Ph.D. thesis of Iris Cáceres Saez.

References

Aubail, A., Méndez-Fernandez, P., Bustamante, P., Churlaud, C., Ferreira, M., Vingada, J.V., Caurant, F., 2013. Use of skin and blubber tissues of small cetaceans to assess the trace element content of internal organs. Mar. Pollut. Bull. 76, 158–169.

Augier, H., Benkoel, L., Chamlian, A., Park, W.K., Ronneau, C., 1993. Mercury, zinc and selenium bioaccumulation in tissues and organs of Mediterranean striped dolphins *Stenella coeruleoalba* Meyen. Toxicological result of their interaction. Cell. Mol. Biol. 39, 621–634.

Bryan, C.E., Christopher, S.J., Balmer, B.C., Wells, R.S., 2007. Establishing baseline levels of trace elements in blood and skin of bottlenose dolphins in Sarasota Bay, Florida: implications for non-invasive monitoring. Sci. Total. Environ. 388, 325–342.

Borrell, A., Clusa, M., Aguilar, A., Drago, M., 2015. Use of epidermis for the monitoring of tissular trace elements in Mediterranean striped dolphins (*Stenella coeruleoalba*). Chemosphere 122, 288–294.

Cáceres-Saez, I., Dellabianca, N., Goodall, R.N.P., Cappozzo, H.L., Ribeiro Guevara, S., 2013a. Mercury and selenium in Subantarctic Commerson's dolphins (*Cephalorhynchus c. commmersonii*). Biol. Trace Elem. Res. 151, 195–208.

Cáceres-Saez, I., Ribeiro Guevara, S., Dellabianca, N., Goodall, R.N.P., Cappozzo, H.L., 2013b. Heavy metals and essential elements in Commerson's dolphins (*Cephalorhynchus c. commmersonii*) from the southwestern South Atlantic Ocean. Environ. Monit. Assess. 185, 5375–5386.

Cardellicchio, N., Decataldo, A., Di Leo, A., Giandomenico, S., 2002. Trace elements in organs and tissues of striped dolphins (*Stenella coeruleoalba*) from the Mediterranean Sea (Southern Italy). Chemosphere 49, 85–90.

Caurant, F., Navarro, M., Amiard, J.C., 1996. Mercury in pilot whales: possible limits to the detoxification process. Sci. Total Environ. 186, 95–104.

Cernichiari, E., Toribara, T.Y., Lian, L., Marsh, D.O., Berlin, M.W., Myers, G.J., Cox, C., Shamlaye, C.F., Choisy, O., Davidson, P., Clarkson, T.W., 1995. The biological monitoring of mercury in the Seychelles study: methylmercury and human health. Neurotoxicology 16, 613–627.

Clayden, M.G., Arsenault, L.M., Kidd, K.A., O'Driscoll, N.J., Mallory, M.L., 2015. Mercury bioaccumulation and biomagnification in a small Arctic polynya ecosystem. Sci. Total Environ. 509–510, 206–215.

Cossa, D., Averty, B., Pirrone, N., 2009. The origin of methylmercury in open Mediterranean waters. Limnol. Oceanogr. 54 (3), 837–844.

Cossa, D., Heimbürger, L.E., Lannuzel, D., Rintoul, S.R., Butler, E.C.V., Bowie, A.R., Averty, B., Watson, R.J., Remenyi, T., 2011. Mercury in the Southern Ocean. Geochim. Cosmochim. Acta 75 (14), 4037–4052.

Dehn, L.A., Follmann, E.H., Rosa, C., Duffy, L.K., Thomas, D.L., Bratton, G.R., Taylor, R.T., O'Hara, T.M., 2006. Stable isotope and trace element status of subsistence-hunted bowhead and beluga whales in Alaska and gray whales in Chukotka. Mar. Pollut. Bull. 52, 301–319.

Dellabianca, N.A., Hohn, A.A., Goodall, R.N.P., 2012. Age estimation and growth layer patterns in teeth of Commerson's dolphins (*Cephalorhynchus c. commersonii*) in subantarctic waters. Mar. Mamm. Sci. 28, 378–388.

Fossi, M.C., Marsili, L., Neri, G., Casini, S., Bearzi, G., Politi, E., Zanardelli, M., Panigada, S., 2000. Skin biopsy of Mediterranean cetaceans for the investigation of interspecies susceptibility to xenobiotic contaminants. Mar. Environ. Res. 50, 517–521.

Frodello, J.P., Romeo, 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.

Gil, M.N., Torres, A., Harvey, M., Esteves, J.L., 2006. Metales pesados en organismos marinos de la zona costera de la Patagonia, Argentina continental. Rev. Biol. Mar. Oceanog. 41, 167–176.

Goodall, R.N.P., Galeazzi, A.R., Leatherwood, S., Miller, K.W., Cameron, I.S., Kastelein, R.K., Sobral, A.P., 1988. Studies of Commerson's dolphins *Cephalorhynchus commersonii*, off Tierra del Fuego, 1976–1984, with a review of information on the species in the South Atlantic. Rep. Int. Whal. Comm. 9, 3–70. Special Issue.

Goodall, R.N.P., Schiavini, A., Fermani, C., 1994. Net fisheries and net mortality of small cetaceans off Tierra del Fuego, Argentina. Rep. Int. Whal. Comm. 15, 295–304. Special Issue.

Heimbürger, L.E., Cossa, D., Marty, J.-C., Migon, C., Averty, B., Dufour, A., Ras, J., 2010. Methylmercury distributions in relation to the presence of nano- and picophytoplankton in an oceanic water column (Ligurian Sea, North-western Mediterranean). Geochim. Cosmochim. Acta 74 (19), 5549–5559.

IUCN, 2014. International Union for Conservation of Nature, Red List of Threatened Species. Version <www.iucnredlist.org>. Downloaded on 15 December 2014.

International Whaling Commission <http://iwc.int/scmain>.

Jackson, T.A., Whittle, M., Evans, M.S., Muir, D.C.G., 2008. Evidence for mass-independent and mass-dependent fractionation of the stable isotopes of mercury by natural processes in aquatic ecosystems. Appl. Geochem. 23, 547–571.

Khan, M.A.K., Wang, F., 2009. Mercury–selenium compounds and their toxicological significance toward a molecular understanding of the mercury–selenium antagonism. Environ. Toxicol. Chem. 28 (8), 1567–1577.

Koeman, J.H., Peeters, W.H., Koudstaal-Hol, C.H., Tjioe, P.S., de Goeij, J.J., 1973. Mercury–selenium correlations in marine mammals. Nature 245, 385–386.

Kotnik, J., Horvat, M., Ogrinc, N., Fajon, V., Zagar, D., Cossa, D., Spovieri, F., Pirrone, N., 2015. Mercury speciation in the Adriatic Sea. Mar. Pollut. Bull. http://dx.doi.org/10.1016/j.marpolbul.2015.05.037.

Kunito, T., Watanabe, I., Yasunaga, G., Fujise, Y., Tanabe, S., 2002. Using trace elements in skin to discriminate the populations of minke whales in southern hemisphere. Mar. Environ. Res. 53, 175–197.

Kwon, S.Y., Blum, J.D., Chen, C.Y., Meattey, D.E., Mason, R.P., 2014. Mercury isotope study of sources and exposure pathways of methylmercury in estuarine food webs in the northeastern U.S. Environ. Sci. Technol. 48, 10089–10097.

Law, R.J., 1996. Metals in marine mammals. In: Beyer, W.N., Heinz, G.H., Redmond-Norwood, A.W. (Eds.), Environmental Contaminants in Wildlife. Interpreting Tissues Concentrations SETAC Special Publication Series. Lewis Publishers, Boca Raton, pp. 357–376.

Leccia, M.T., Richard, M.J., Beani, J.C., Faure, H., Monjo, A.M., Cadet, J., Amblard, P., Favier, A., 1993. Protective effect of selenium and zinc on UV-A damage in human skin fibroblasts. Photochem. Photobiol. 58, 548–553.

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.

Lockhart, W.L., Stern, G.A., Wagemann, R., Hunt, R.V., Metner, D.A., DeLaronde, J., Dunn, B., Stewart, R.E.A., Hyatt, C.K., Harwood, L., Mount, K., 2005. Concentrations of mercury in tissues of beluga whales (*Delphinapterus leucas*) from several communities in the Canadian Arctic from 1981 to 2002. Sci. Total Environ. 351–352, 391–412.

Lockyer, C., Goodall, R.N.P., Galeazzi, A.R., 1988. Age and body length characteristics of *Cephalorhynchus commersonii* from incidentally-caught specimens of Tierra del Fuego. Rep. Int. Whal. Comm. 9, 103–118. Special Issue.

Martinez-Levasseur, L.M., Gendron, D., Knell, R.J., O'Toole, E.A., Singh, M., Acevedo-Whitehouse, K., 2010. Acute sun damage and photoprotective responses in whales. Proc. Roy. Soc. B. http://dx.doi.org/10.1098/rspb.2010.1903.

Mason, R.P., Sullivan, K.A., 1999. Mercury in the south and equatorial Atlantic. Deep Sea Res. Part II 46, 937–956.

McKenzie, R.C., 2000. Selenium, ultraviolet radiation and the skin. Clin. Exp. Dermatol. 25, 631–636.

Miller, D.L., Wöshner, V., Styer, E.L., Ferguson, S., Knott, K.K., Gray, M.J., Wells, R.S., O'Hara, T.M., 2011. Histologic findings in free-ranging Sarasota Bay bottlenose dolphin (*Tursiops truncatus*) skin: mercury, selenium, and seasonal factors. J. Wildl. Dis. 47, 1012–1020.

Monaci, F., Borrell, A., Leonzio, C., Marsili, L., Calzada, N., 1998. Trace elements in striped dolphin (*Stenella coeruleoalba*) from the western Mediterranean. Environ. Pollut. 99, 61–68.

Norris, K.S., 1961. Standardized methods for measuring and recording data on the smaller cetacea. J. Mammal. 42, 471–476.

O'Hara, T.M., O'Shea, T.J., 2001. Toxicology. In: Dierauf, L.A., Gulland, F.M.D. (Eds.), Handbook for Marine Mammal Medicine. CRC Press, Boca Roton, FL, pp. 471–520.

O'Hara, T.M., Hanns, C., Wöshner, V.M., Zeh, J., Bratton, G., Taylor, R., 2008. Essential and non-essential elements in the bowhead whale: epidermis-based predictions of blubber, kidney, liver and muscle tissue concentrations. J. Cetacean Res. Manage. 10, 107–117.

Paludan-Müller, P., Agger, C.T., Dietz, R., Kinze, C.C., 1993. Mercury, cadmium, zinc, copper and selenium in harbour porpoise (*Phocoena phocoena*) from West Greenland. Polar Biol. 13, 311–320.

Peterson, S.A., Ralston, N.V.C., Whanger, P.D., Oldfield, J.E., Mosher, W.D., 2009. Selenium and mercury interactions with emphasis on fish tissue. Environ. Bioindic. 4, 318–334.

Pfeiffer, C.J., Jones, F.M., 1993. Epidermal lipid in several cetacean species: ultrastructural observations. Anat. Embryol. 188, 209–218.

Richelle, M., Sabatier, M., Steiling, H., Williamson, G., 2006. Skin bioavailability of dietary vitamin E, carotenoids, polyphenols, vitamin C, zinc and selenium. Br. J. Nut. 96, 227–238.

Savery, L.C., Evers, D.C., Wise, S.S., Falank, C., Wise, J., Gianios Jr., C., Kerr, I., Payne, R., Thompson, W.D., Perkins, C., Zheng, T., Zhu, C., Benedict, L., Wise, J.P., 2013a. Global mercury and selenium concentrations in skin from free-ranging sperm whales (*Physeter macrocephalus*). Sci. Total Environ., 59–71

Savery, L.C., Wise, S.S., Falank, C., Wise, J., Gianios Jr., C., Thompson, W.D., Perkins, C., Mason, M.D., Payne, R., Kerr, I., Pierce Wise, J., 2013b. Global assessment of silver pollution using sperm whales (*Physeter macrocephalus*) as an indicator species. J. Environ. Anal. Toxicol.

Savery, L.C., Wise, J., Wise, S., Falank, C., Gianios Jr., C., Thompson, W.D., Perkins, C., Zheng, T., Zhu, C., Pierce Wise, J., 2014. Global assessment of arsenic pollution using sperm whales (*Physeter macrocephalus*) as an emerging aquatic model organism. Comp. Biochem. Physiol. Part C. http://dx.doi.org/10.1016/j.cbpc.2014.01.003.

Sengupta, A., Lichti, U.F., Carlson, B.A., Ryscavage, A.O., Gladyshev, V.N., Yuspa, S.H., Hatfield, D.L., 2010. Selenoproteins are essential for proper keratinocyte function and skin development. PLoS ONE 5 (8), e12249.

Shoham-Frider, E., Amiel, S., Roditi-Elasar, M., Kressa, N., 2002. Risso's dolphin (Grampus griseus) stranding on the coast of Israel (eastern Mediterranean). Autopsy results and trace metal concentrations. Sci. Total Environ. 295, 157–166.

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.

St. Aubin, D.J., Smith, T.G., Geraci, J.R., 1990. Seasonal epidermal molt in beluga whales, *Delphinapterus leucas*. Can. J. Zool. 69, 359–367.

Stavros, H.W., Bossart, G.D., Hulsey, T.C., Fair, P.A., 2007. Trace element concentrations in skin of free-ranging bottlenose dolphins (*Tursiops truncatus*) from the southeast Atlantic coast. Sci. Total Environ. 388, 300–315.

Stavros, H.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, 1649–1661.

Ullrich, S., Tanton, T., Abdrashitova, S.A., 2001. Mercury in the aquatic environment: a review of factors affecting methylation. Crit. Rev. Environ. Sci. Technol. 31, 241–293.

Wagemann, R., Innes, S., Richard, P.R., 1996. Overview and regional and temporal differences of heavy metals in Arctic whales and ringed seals in the Canadian Arctic. Sci. Total Environ. 186, 41–66.

Wagemann, R., Kozlowska, H., 2005. Mercury distribution in the skin of beluga (*Delphinapterus leucas*) and narwhal (*Monodon monoceros*) from the Canadian Arctic and mercury burdens and excretion by moulting. Sci. Total Environ. 351–352, 333–343.

Wöshner, V.W., O'Hara, T.M., Bratton, G.R., Suydam, R.S., Beasley, V.R., 2001. Concentrations and interactions of selected essential and non-essential elements in bowhead and beluga whales of Arctic Alaska. J. Wildl. Dis. 37, 693–710.

Wöshner, V., Knott, K., Wells, R., Willetto, C., Swor, R., O'Hara, T., 2008. Mercury and selenium in blood and epidermis of bottlenose dolphins (*Tursiops truncatus*) from Sarasota Bay, FL: interaction and relevance to life history and hematologic parameters. EcoHealth 5, 360–370.

Yang, J., Kunito, T., Tanabe, S., Amano, M., Miyazaki, N., 2002. Trace elements in skin of Dall's porpoises (*Phocoenoides dalli*) from the northern waters of Japan: an evaluation for utilization as non-lethal tracers. Mar. Pollut. Bull. 45, 230–236.

Yang, J., Miyazaki, N., 2003. Moisture content in Dall's porpoise (*Phocoenoides dalli*) tissues: a reference base for conversion factors between dry and wet weight trace element concentrations in cetaceans. Environ. Pollut. 121, 345–347.

Yang, D.Y., Chen, Y.W., Gunn, J.M., Belzile, N., 2008. Selenium and mercury in organisms: interactions and mechanisms. Environ. Rev. 16, 71–92.