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6 **Mercury and selenium in bottlenose dolphins (*Tursiops truncatus*): a**
7 **study in stranded animals from the Canary Islands**

8
9 *Natalia García-Alvarez¹, Antonio Fernández¹, Luis. D. Boada², Manuel Zumbado²,*
10 *Annalisa Zaccaroni³, Manuel Arbelo¹, Eva Sierra¹, Javier Almunia⁴, Octavio P. Luzardo².*

Formattato: Italiano (Italia)

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12
13 ¹Unit of Histology and Pathology, Institute of Animal Health (IUSA), Veterinary School, University of Las
14 Palmas de Gran Canaria, 35413 Arucas, Las Palmas, Spain.

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15 ²Toxicology Unit, Research Institute of Biomedical and Health Sciences (IUIBS), University of Las Palmas
16 de Gran Canaria, 35016, Las Palmas de Gran Canaria, Spain.

17 ³Department of Veterinary Medical Sciences, University of Bologna, Research Group on Large Pelagic
18 Vertebrates, Viale Vespucci 2, 47042 Cesenatico, FC, Italy.

19 ⁴Loro Parque Foundation. Camino Burgado, 38400 Puerto de la Cruz (Tenerife), Santa Cruz de Tenerife,
20 Spain.

Formattato: Italiano (Italia)

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23
24 * Corresponding author: Natalia García Álvarez, Unit of Histology and Pathology, Institute of Animal
25 Health (IUSA), Veterinary School, University of Las Palmas, 35413 Arucas, Las Palmas de Gran Canaria,
26 Spain. Tel: (+34) 928 45 97 11; Fax: (+34) 928 45 74 33; E-mail address: natalia.garcia117@alu.ulpgc.es

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ABSTRACT:

The mercury (Hg) level in the marine environment has tripled in recent decades, becoming a great concern because of its high toxic potential. This study is the first report of inorganic pollutants Hg and selenium (Se) status, and the first Se/Hg molar ratio assessment in bottlenose dolphins (*Tursiops truncatus*) inhabiting the waters of the Canary Islands. Total Hg and selenium (Se) concentrations were determined in the blubber and liver collected from 30 specimens stranded along the coasts of the archipelago from 1997 to 2013. The median values for total Hg in the blubber and liver were 80.83 and 223.77 $\mu\text{g g}^{-1}$ dry weight (dw), and the median levels for Se in both tissues were 7.29 and 68.63 $\mu\text{g g}^{-1}$ dw, respectively. Hg concentrations in the liver were similar to those obtained in bottlenose dolphins from the North Sea, the Western Atlantic Ocean and several locations in the Pacific Ocean. The Mediterranean Sea and South of Australia are the most contaminated areas for both elements in this cetacean species. However, it must be stressed that the hepatic contents levels of Hg and Se in the liver showed an increasing trend with the age of the animals. Furthermore, as expected, a strong positive correlation between Hg and Se was observed ($r_s=0.960$). Surprisingly, both younger and older specimens had a Se/Hg molar ratio different from 1, suggesting that these individuals may be at greater toxicological risk for high concentrations of both elements or a deficiency of Se without a protective action against Hg toxicity.

Keywords: bottlenose dolphins, *Tursiops truncatus*, Canary Islands, mercury, selenium, Se/Hg molar ratio, Mediterranean Sea, North Atlantic Ocean, South of Australia

52

53 1. INTRODUCTION

54

Mercury (Hg) is a natural element that is a ubiquitous environmental contaminant. It is distributed around the world by atmospheric transportation. The sources of Hg contamination can be both natural (e.g., degassing of the earth's crust, volcanic activities and forest fires) and anthropogenic (e.g., mining, chlorine industry, coal-burning power plants, cement and metallurgical industries, paper mills, agricultural pesticides, or medical waste incineration) (van de Merwe et al., 2010). Natural inputs might be highly relevant in certain areas (Andre et al., 1991), but industrial activities might increase the exposure to this toxic element (AMAP, 2011; Magos and Clarkson, 2006), and recently published data even suggested that the amount of Hg in water has almost tripled compared to the pre-industrial period (Lamborg et al., 2014). Hg in its inorganic form is moderately toxic, but once in the aquatic environment, it is quickly transformed into methylmercury (MeHg), a highly toxic form of Hg of most concern to the health of humans and biota. MeHg is strongly neurotoxic (Clarkson and Magos, 2006), harmful to the kidneys, lungs, the thyroid gland, and the immune system (De Guise et al., 1995); it is also teratogenic (Crespo-Lopez et al., 2009) and carcinogenic (Vos et al., 2003; Vos et al., 2000). In the marine environment, MeHg accumulates and biomagnifies along the food chain (Seixas et al., 2014) representing a serious threat, especially to top predators such as humans (Visnjevec et al., 2014) or cetaceans, which are exposed to this metal mainly via the diet (Storelli et al., 2005).

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75 It is well known that the toxic potential of Hg is suppressed in the presence of
76 sufficient amounts of selenium (Se) (Parizek and Ostadalova, 1967). This effect has
77 been shown in studies in a variety of species, including marine mammals (Cuvin-Aralar
78 and Furness, 1991; Frodello et al., 2000; Gui et al., 2014; Sakamoto et al., 2013)
79 exposed to these elements for a long time, even before the industrial period (Holsbeek
80 et al., 1998). Thus, several mechanisms for resistance to the adverse effects of Hg have
81 been proposed. On the one hand, Se can easily combined with various forms of Hg to
82 yield complexes with lower toxicity, such as methylmercury selenide (MeHg-Se),
83 methylmercury selenocysteinat (MeHg-Sec), or mercury selenide, tiemannite (HgSe),
84 which is considered the last step in Hg detoxification (Palmisano et al., 1995). These
85 compounds also contribute to the mobilization of mercury from the most vulnerable
86 targets (such as kidney or nervous system tissues) to other less sensitive bodily
87 regions, such as muscle. Furthermore, Se competes with Hg for its various biological
88 targets, which also contributes to lowering the potential toxicity of Hg (Khan and
89 Wang, 2009). Therefore, the Se/Hg molar ratio has been widely used (McHuron et al.,
90 2014; Mendez-Fernandez et al., 2014; Squadrone et al., 2015; Vos et al., 2003), and
91 many authors have established that Se, in a molar ratio of 1:1 or above with Hg,
92 protects against the toxic effects of this latter metal (Ganter et al., 1972; Ralston et
93 al., 2007; Ralston and Raymond, 2010; Sormo et al., 2011; Squadrone et al., 2015).
94 However, paradoxically, this protective action can be harmful to the body because
95 complex formation also results in the sequestration of both elements, causing them to
96 become biologically unavailable (Martoja and Berry, 1980). Se is a well-known
97 essential element with multiple biological functions, such as its critical participation in
98 reproduction, the metabolism of thyroid hormones or DNA synthesis, in addition to its

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99 important antioxidant role (anticarcinogenic activity), among other functions (Schwarz
100 and Foltz, 1957; Taylor et al., 2009; Zhang et al., 2014). Therefore, the presence of high
101 levels of Hg could lead to Se deficiency, which could even cause the death in extreme
102 cases (Chen, 2012; Sunde, 2006). Thus, the toxicological effects might be due to both
103 MeHg toxicity and the induced Se deficiency (Zhang et al., 2014). However, Se levels
104 have increased dramatically in many marine areas, presenting an environmental
105 toxicity problem (Lavery et al., 2008). Se pollution probably occurs as a result of
106 anthropogenic activities such as coal burning, smelting, ceramic and glass
107 manufacturing, or copper refining (van de Merwe et al., 2010). Therefore, to evaluate
108 the health status of the ecosystems, the simultaneous study of Hg and Se and the
109 relationship between them is of great interest, particularly in those species usually
110 considered as sentinels for environmental pollution.

111
112 Because of its large size, longevity, and high position within the food chain, many
113 authors have proposed the cetaceans as good sentinels for ocean health. Species with
114 a worldwide distribution such as the bottlenose dolphin (*Tursiops truncatus*), are
115 usually employed to assess global pollution and regional variations (Wilson et al.,
116 2012). Therefore, this species has been selected for the present study because
117 previous reports indicate that bottlenose dolphins clearly reflect the contamination of
118 the waters of the Canary Islands (Eastern Atlantic Ocean) due to their proximity to
119 likely anthropogenic sources (Garcia-Alvarez et al., 2014a; Garcia-Alvarez et al.,
120 2014b). Moreover, these cetaceans have been extensively studied, allowing
121 comparison of the results ~~of the results~~ of this research with other marine areas
122 around the world, to obtain more comprehensive approach to pollution observations.

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Bottlenose dolphins inhabit the Canary Islands as local resident populations that shows inter-island movements within the archipelago (Tobeña et al., 2014). This species faces a high exposure to organic pollutants (Garcia-Alvarez et al., 2014b) and is considered a valuable biomarker of the health status of the marine ecosystems. A high concentration of contaminants has also been reported in humans from the Archipelago (Luzardo et al., 2012; Luzardo et al., 2009) and in other marine animals from the Canary Islands waters (Camacho et al., 2014) and other nearby areas (Camacho et al., 2013). Although there is a previous research concerning a few inorganic pollutants in 12 bottlenose dolphins stranded on the canary coasts (Carballo et al., 2004), there is a need of more recent and comprehensive data from this marine region.

The major goals of this study were as follows: 1) adding to recently published information on chemical pollution in bottlenose dolphins from the Canary Islands (Garcia-Alvarez et al., 2014a; Garcia-Alvarez et al., 2014b), focusing on the Hg and Se concentrations in the blubber and liver of stranded animals, and studying their relationships and toxicity, and 2) reviewing published studies to date on both elements in bottlenose dolphins worldwide to assess the global impact of these elements on this species.

2. MATERIALS AND METHODS

2.1. Study area

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148 The Canary Archipelago is located in the Eastern North Atlantic Ocean near Europe and
149 North Africa. These islands are a protected territory with 12 marine Special Areas of
150 Conservation (SACs) because of the presence of bottlenose dolphins, species listed in
151 Annex II and IV in the European Habitats Directive (EC, 1992).

152

153 Bottlenose dolphins inhabit the Canary Islands as a local resident populations that
154 shows inter-island movements within the archipelago (Tobeña et al., 2014). This
155 species faces a high exposure to organic pollutants (Garcia-Alvarez et al., 2014b) and is
156 considered a valuable biomarker of the health status of the marine ecosystems. A high
157 concentration of contaminants has also been reported in humans from the
158 Archipelago (Luzardo et al., 2012; Luzardo et al., 2009) and in other marine
159 animals (Camacho et al., 2014) in nearby areas (Camacho et al., 2013). However, a lack
160 of data exists concerning heavy metals and other inorganic pollutants from this marine
161 region.

162

163 2.2. Sample collection

164

165 Over a period from 1997 to 2013, 29 each of blubber and liver samples were collected
166 from 30 bottlenose dolphins stranded on the Canary Islands coasts. According to the
167 literature, Hg and Se were found to accumulate in both tissues, reaching the highest
168 levels in the liver (Beck et al., 1997). Besides, these tissues have been selected to be in
169 accordance with previous studies of contaminants in stranded dolphins from this
170 Archipelago (Garcia-Alvarez et al., 2014a). The blubber is considered as a main target

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for pollutant assessment, in order to possible future comparisons with biopsy samples from free ranging cetacean. On the other hand, the liver tissue was also selected because pattern distribution of metals is tissue specific, being the mercury mostly concentrated in the liver (Krishna et al., 2003).

Tissue sampling and the state of decomposition of the stranded specimens were determined by adapting the Geraci and Lounsbury (2005) protocol. Thirteen males and 17 females (including 2 pregnant females) were ~~grouped-divided~~ into age categories i.e., newborn (1), calf (1), juveniles (5), subadults (11), adult (11) and old (1), based on body length and gonadal appearance. The bodily condition of the specimens was classified from a good to a very poor state according to morphological characteristics features. All of the characteristics of the animals studied are summarized in Table 1. Samples were stored in plastic bags at -80C in the Cetacean Tissue Bank of the University of Las Palmas de Gran Canaria (ULPGC) until analysis.

2.3. Sample preparation and analysis of trace elements

All samples were first lyophilized (freeze-dried) for a subsequent microwave digestion method using a Milestone ETHOS ONE oven. The fresh weight of each sample was recorded such that the results could be expressed both on a dry (dw) and a wet weight (ww) basis. In different vessels, 0.5 g aliquots of freeze-dried samples were mineralized with 6 ml of nitric acid plus 50 µl of Itrio (Y) as an internal standard. Each vessel was placed into the microwave oven to obtain solutions, which were then diluted to a final volume of 50 ml with distilled water. After digestion, the analysis of the elements was

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performed with an Inductively Coupled Plasma-Optic Emission Spectrometry method (ICP-OES) using a Perkin Elmer Optima 2100 DV instrument. Two blanks were run during each analysis to check chemical purity, and the accuracy of the method was verified with reference materials (lyophilized mussel; CRM 278, Community Bureau of Reference, BCR, Brussels). All the values of the reference materials were within certified limits. ~~The instrumental detection limits were 0.061 ng ml⁻¹ ww for Hg and 0.1 ng ml⁻¹ ww for Se.~~ The recovery values for Hg and Se were 120 ± 8% and 115 ± 11%, respectively. ~~The instrumental detection limits were 0.061 ng ml⁻¹ ww for Hg and 0.1 ng ml⁻¹ ww for Se.~~

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2.4. Data analysis

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~~2.4.~~ Calculation of the Se/Hg molar ratio

The molar ratio of Se to Hg was calculated as:

$$\text{Se/Hg} = (\text{Se}/78.96) / (\text{Hg}/200.59)$$

where 200.59 and 78.96 g mol⁻¹ are the molar masses of Hg and Se, respectively.

~~2.5.~~ Statistical analysis

Statistical analysis was performed with IBM SPSS Statistics v ~~22~~19.0. ~~Because trace elements did not follow~~ When conditions of normality (Kolmogorov-Smirnov and Shapiro-Wilk tests) ~~and-or~~ homogeneity of variances in all variable groups ~~were not satisfied~~, non-parametric tests were used. ~~Thus, the significant differences between~~

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~~group medians and distributions statistical significance between different categories~~
~~were was~~ assessed using the Mann–Whitney U-test and the Kruskal–Wallis test ~~for~~
~~differences between two or more independent groups, respectively~~. Spearman's

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correlation test was performed to determine a possible relationship between both
trace elements. As usual, the level of statistical significance was set at $p\text{-value} = 0.05$.

3. RESULTS AND DISCUSSION

Individual and descriptive statistics of Hg and Se concentrations in the blubber and
liver of bottlenose dolphins (mean \pm SD, median and range) are shown in ~~Table~~ 2. The
data are expressed as $\mu\text{g g}^{-1}$ (ppm) on a dry weight (dw) basis. However, to allow
comparison with other reports, the wet weight (ww) results were also determined
using conversion factors calculated for each sample based on their respective
percentages of dry residue (~~Table~~ 2). The mean correction factor for blubber and liver
tissue (0.48 and 0.28 respectively) are comparable with values reported in the
literature (Becker et al., 1995; Mackey et al., 1995). Mean and median Hg values of
83.36 and 80.83 $\mu\text{g g}^{-1}$ dw were found in the blubber, which were lower than the mean
and median Hg results in the liver (261.56 and 223.77 $\mu\text{g g}^{-1}$ dw, respectively). For Se,
the mean and median levels of 8.96 and 7.29 $\mu\text{g g}^{-1}$ dw in the blubber were much
lower than the Se concentration in the liver, in which the mean value of 211.20 $\mu\text{g g}^{-1}$
dw was quite far from the median of 68.63 $\mu\text{g g}^{-1}$ dw because of the data dispersion.

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Bioaccumulation of contaminants in marine mammals has been reported to be highly
dependent on both biotic and abiotic factors, such as sex, age, diet and pollution

gradients in the aquatic environment (Storelli et al., 2005). Thus, an analysis of Hg and Se concentrations against different variables (Table 1) is essential to fully understand the effects of these elements on the specimens studied.

3.1. Influence of sex and age on mercury and selenium levels

Age is the most important biotic factor for Hg and Se accumulation. Age increasing hepatic concentration of both trace elements was observed, the small and unequal sample sizes of age categories discouraged any statistical test assessment. This enables us to use the body length as a surrogate for age class. Testing despite a lack of statistical significance in their levels between consecutive age groups ($p>0.05$). Because of the small sample size of the age categories, it was interesting to analyze the correlations between the pollutant levels with and the length of the animal. Thus, the hepatic Hg was found to be positively correlated against this variable with a Spearman coefficient (r_s) of 0.769 (Fig. 1A). In accordance with previous authors (Bellante et al., 2012), an increasing trend throughout the life of cetaceans was observed (Fig. 1), probably due to bioaccumulation from the continuous uptake of Hg in the diet and the decreasing ability to excrete this metal and storage in stable forms such as HgSe (Aguilar et al., 1999; Mackey et al., 1995; Wagemann et al., 2000). Other authors also found an upward trend of hepatic Hg in sharks and rays with age (Gutierrez-Mejia et al., 2009; Storelli and Marcotrigiano, 2002), suggesting a higher rate of assimilation than excretion of Hg and a lower efficiency of detoxification. Moreover, the largest specimens may capture bigger prey, which are more likely to contain higher levels of Hg. Although essential trace elements are regulated via

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homeostasis in marine mammals (Mendez-Fernandez et al., 2014), Se levels in liver samples were notably higher in adults than in the youngest individuals (Fig. 1) and were also correlated with animal length ($r_s=0.764$) in accordance with other studies (Woshner et al., 2001). The high level of Se may result from its accumulation with Hg during the detoxification process or from a highly concentrated diet because most ocean fish are Se-rich, as was reported in a study in the Faroe Islands (Budtz-Jorgensen et al., 2007).

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Fig. 1B also shows tissue distribution of Hg and Se throughout the lifetime of the animals, highlighting the concentration of both elements mostly in the liver, with a statistically significant difference between tissues ($p=0.000$). Therefore, the liver appeared to be the preferential tissue, as indicated in previous studies (Bellante et al., 2012; Frodello et al., 2000).- However, it is also interesting to stress that the newborn and calf specimens of this research accumulated greater levels of Hg in the blubber than the liver, especially for the calf. Concerning the Se, the newborn showed equal levels in both tissues and the calf individual doubled the concentration in the blubber. These results were in contrast to the following sampling ages where the liver showed higher levels of both trace elements with an evident increasing trend. One could hypothesize that this variation on the tissue distribution is due to the different pollutant sources. Thus, the Hg and Se were initially transferred through placental barrier entering the fetal circulation to be transported to the liver for metabolism, and then distributed to the blubber for accumulation. The calf showed the highest concentrations of both elements in the blubber among all the animals studied, likely as a result of exposure through lactation. On the contrary, the juvenile group had the

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lowest level of Hg and Se in the blubber compared to the rest of the age categories. This may be due to the release of Hg and Se from the blubber into the circulation at weaning stage, which could be considered a period of negative energy balance (Louis et al., 2015). However, little is known concerning the factors that affect mobilization of pollutants from adipose tissue (Louis et al., 2014). Therefore, this finding should be interpreted with caution also because only one newborn and one calf of bottlenose dolphins were available for this study.

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In this study no influence of sex on Hg or Se accumulation was observed, as has been found in other marine areas of the North Atlantic Ocean (Mendez-Fernandez et al., 2014).

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3.2. Influence of stranding location on mercury and selenium levels

Clear differences for Hg and Se concentrations in the liver between the geographical areas, in which animals stranded, were found (with ~~p-value~~~~p-values~~ of 0.060 and 0.046 respectively). The results for the eastern Canary Islands of Lanzarote (LZ), Fuerteventura (FV) and Gran Canaria (GC) showed the highest Se and Hg levels as compared to the western islands of Tenerife (TF) and La Gomera (LG). Thus, median hepatic Hg molar concentration from animals stranded in the eastern islands (1824.21 nmol g⁻¹ dw) was 4 times greater than that from specimens stranded in the western islands (427.97 nmol g⁻¹ dw). This difference between both Canary regions was even more prominent for Se, which reached a 22-fold hepatic molar concentration in the eastern region of the Archipelago. In fact, there is a decreasing trend from the nearest

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to the furthest island from the African continent (Supp. Fig. 1). This finding could be related to geographical differences of natural and/or anthropogenic sources, but is more likely affected by the age of the animals at the various locations. The youngest individuals were found stranded in the western Canary Islands, so these results should be cautiously considered.

3.3. Temporal trends of mercury and selenium concentrations

Figure 2 illustrates the total Hg and Se concentrations in the blubber of individuals grouped according to the year of stranding (between 1999 and 2013). For this context, it is preferable to [analyze the blubber samples because no influence of age or length on Hg or Se levels in this tissue was obtained in the present study \(see Fig. 1B\)](#). Despite the low sample size of the groups (1999, n=2; 2000, n=1; 2002, n=1; 2003, n=2; 2004, n=1; 2005, n=5; 2007, n=1; 2008, n=4; 2009, n=2; 2010, n=2; 2011, n=5; 2012, n=2; 2013, n=1) [required careful interpretation](#), an increasing temporal trend of Hg in the blubber can be seen throughout the study period. [In addition, the Mann-Whitney U-test revealed a significant difference \(\$p=0.016\$ \) between 2005 and 2011, each with 5 specimens available.](#) ~~Excluding the samples from years with only one value, the Kruskal-Wallis test revealed a significant difference ($p=0.029$).~~ From the individual stranded in 1999 to the last one in 2013, the Hg level has tripled in the blubber, consistent with a recently published report (Lamborg et al., 2014). Lamborg's group found that deep and intermediate North Atlantic waters are abnormally enriched in Hg, probably because of anthropogenic activities such as mining and fossil fuel

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338 combustion. Furthermore, no temporal differences were observed in the Se burden in
339 blubber (Fig. 2) or the hepatic levels of either trace element (data not shown).

340

341 3.4. Study of the relationship between mercury and selenium

342

343 As previously mentioned, a common approach to assess the risk of exposure to Hg is to
344 determine the molar ratio of Hg and Se in the body (Se/Hg). A high positive correlation
345 between Hg and Se with an equimolar ratio in the liver as well as the protective effect
346 of Se against Hg toxicity is well documented (Civin-Aralar and Furness, 1991; Geraci,
347 1989; Koeman et al., 1973; Yang et al., 2007).

348

349 In the present study, the results showed that increasing Hg levels were associated with
350 increasing Se concentrations, as described for other dolphin populations (Palmisano et
351 al., 1995). Spearman's correlation coefficient (r_s), calculated between molar
352 concentrations of hepatic Hg and Se, showed a strong positive relationship (Fig. 3).

353 Excluding the outlier data for Se (CET 407), the correlation slightly decreased from
354 0.960 to 0.955, although the coefficient of determination (R^2) for linear regression
355 increased from 0.592 to 0.807. It is remarkable that the strongest linear association
356 ($R^2=0.973$) between these two elements occurred below 1500 nmol g^{-1} ($300 \mu\text{g g}^{-1} \text{ dw}$)
357 of Hg in the liver, comparable to a total Hg threshold of $100 \mu\text{g g}^{-1} \text{ ww}$, as obtained by
358 other authors (Palmisano et al., 1995). Above this concentration, the level of hepatic

359 Se significantly exceeded the Hg concentration, so the Se/Hg molar ratio was higher
360 than 1 (Fig. 3B and 4). Se was in molar excess of Hg in 11 of 29 livers evaluated (37.9%).
361 Other publications reported a similar levels of Se compared to Hg in both pelagic fish

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(Kaneko and Ralston, 2007) and cetaceans (Mendez-Fernandez et al., 2014), and the authors stated that this excess reflects the good health status of individuals or a high proportion of young animals. In the present study, individuals with a Se/Hg molar ratio above 1 were all included in older categories (Fig. 4), contrary to such statement and other results obtained for several cetaceans species (Caceres-Saez et al., 2013; Palmisano et al., 1995; Yang et al., 2007). Regarding the place of stranding, LZ and FV showed a Se/Hg molar ratio over 1; by contrast, GC, TF and LG had a median ratio below 1 (Supp. Fig. 1, insert panel). However, the limited sample size per group undercut any conclusion.

~~Recently, different criteria have been developed to determine whether the consumption of certain foods by humans presents a health risk, so dietary recommendation can be issued. The positive values correspond to a Se/Hg molar ratio above 1, but are also associated with a hidden risk for Se deficiency and poisoning. Zhang and colleagues attempted to develop a new criterion for an exposure assessment to Se/Hg that they called the benefit risk value (BRV), taking into account the amount of Se required for normal biological functions and the threshold intake value for Se poisoning. Unfortunately, such a calculation could not be performed in this study, due to a lack of specific information for marine mammals essential for the development of the equation, which might not be applicable to an assessment of health risk in bottlenose dolphins.~~

3.5. Assessment of the health risk of mercury and selenium

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386 Wagemann and Muir (1984), established a threshold for hepatic damage in marine
387 mammals in a range of 100-400 $\mu\text{g g}^{-1}$ ww Hg burden (Wagemann and Muir, 1984). In
388 supplementary figure 2, the total hepatic Hg concentration is individually compared to
389 this threshold. The results indicate that 10 of 29 livers of stranded individuals (34.5%)
390 exceeded the minimum Hg tolerance level, and 4 had values just below 105 $\mu\text{g g}^{-1}$ ww.
391 All these samples were from subadult and adult specimens, corresponding to 45.5% of
392 the total of subadults and adults in this study. Other authors obtained comparable
393 results for stranded bottlenose dolphins in Australian and Floridian waters (Lavery et
394 al., 2008; Stavros et al., 2011). These results coincided with animals with a Se/Hg molar
395 ratio greater than 1.

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396
397 ~~Experimental studies suggest that Hg intoxication could cause weight loss, toxic~~
398 ~~hepatitis, renal failure and death in marine mammals. Additionally, high Hg levels have~~
399 ~~been associated with parasitic infection and pneumonia, resulting in a lower~~
400 ~~resistance to infectious diseases. After an experimental intoxication of seals, the~~
401 ~~hepatic Hg level reached more than 500 $\mu\text{g g}^{-1}$ dw after death. Other reports~~
402 ~~associated chronic Hg accumulation with liver abnormalities observed in stranded~~
403 ~~bottlenose dolphins from the Atlantic Ocean. Rawson's group found a correlation~~
404 ~~between the lipofuscin pigment with the hepatic Hg concentration. Large deposits~~
405 ~~were observed when the Hg level exceeds 60 $\mu\text{g g}^{-1}$ ww.~~

406
407 ~~The organic form of Hg (MeHg) appears to be the form of Hg most toxic to animals.~~
408 ~~However, after the results of *in vitro* studies carried out by Betti and Nigro, 1996, an~~
409 ~~adaptation acquired by dolphins to counteract the toxic effects of MeHg was~~

~~suggested. As mentioned earlier, the formation of insoluble tiemannite granules provides the ability to endure high Hg exposures to odontocetes. Thus, Hg and Se levels above 2000 $\mu\text{g g}^{-1}$ dw were reported in animals with no signs of poisoning because of the protection provided by the combined presence of both trace elements; however, the energy cost of the detoxification is difficult to assess.~~

The results discussed above indicate that the youngest and oldest bottlenose dolphins may be of greater toxicological concern (Fig. 4). Although the newborn and the calf among the animals studied had the lowest Hg content, they were deficient in Se which could lead to Hg toxicity and they also had a Se/Hg molar ratio less than 1, indicating a limited protection by Se. This result is consistent with human studies in which authors argue that prenatal and postnatal Hg exposure negatively affects central nervous system functions (Rasmussen et al., 2005). Additionally, a molar ratio of 1 or lower may indicate that all of the available Se is bound to Hg, conferring a possible oxidative stress risk (Caceres-Saez et al., 2013). However, these results must be carefully considered because there was only one specimen available from each, newborn and calf categories. By contrast, the older animals had the highest concentrations of both Hg and Se in the liver and Se/Hg ratios greater than 1 suggesting a Se molar excess which could become toxic at high levels (O'Hara et al., 2003). Nevertheless, the inter-relationships between the Hg and Se concentrations, age, nutritional status and disease are complex (Law et al., 2012) and the limits of deficiency, essentiality, and poisoning is quite difficult to assess and not well studied.

3.6. Mercury and selenium in bottlenose dolphins from different marine areas

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435 A comparison of Hg and Se levels in bottlenose dolphins from the Canary Islands (this
436 study) and from different marine areas worldwide was made (Table 3). All these
437 published results, compiled as ranges of Hg concentrations in the liver, were plotted
438 on a map (Fig. 5), as well as others previously performed using Hg content in the hair
439 of pinnipeds (McHuron et al., 2014) and organic pollutants in cetaceans (Aguilar et al.,
440 2002). Bottlenose dolphins from the Mediterranean Sea had greater Hg concentrations
441 than published values elsewhere, as was previously reported for striped dolphins
442 (Andre et al., 1991). Even within the same marine area some differences in the Hg
443 content were observed. Thus, the Ligurian and Tyrrhenian Sea, showed the maximum
444 measured of hepatic Hg ($13150 \mu\text{g g}^{-1} \text{dw}$) ever reported before, followed by the
445 Adriatic Sea which appears to be significantly more polluted by Hg than the less-
446 contaminated Eastern Mediterranean coast. The Hg levels from the North Sea and the
447 Northeast Atlantic Ocean, including the Canary Islands (results from this study), were
448 similar to mean concentrations in bottlenose dolphins from the Western Atlantic
449 Ocean and from several locations in the Pacific Ocean (Hong Kong and east coast of
450 Australia), but not to results from the south coast of Australia where a higher Hg
451 contamination occurred that nearly matched the Adriatic Sea values (see Table 3 for
452 references). Thus, this last sea displayed 6 times higher Hg burden compared with the
453 results from bottlenose dolphins from the Canary Islands (present study).
454 Furthermore, the Tyrrhenian Sea showed the highest Hg value obtained in the
455 literature, more than 50 times greater than the values obtained in this study. On the
456 other hand, *T. truncatus* and *T. aduncus* from South of Australia had 3 to 7 times
457 higher Hg levels respectively, than the specimens from the Canary Archipelago.

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459 It has been reported that Mediterranean prey had higher Hg levels than Atlantic prey
460 [\(Lahaye et al., 2006\)](#), which explains the Hg enrichment in the Mediterranean food
461 webs, and also in the liver of bottlenose dolphins. The authors suggest that this might
462 be due to natural Hg sources in the Mediterranean Sea [\(Andre et al., 1991\)](#) and high
463 anthropogenic Hg emissions especially from France [\(Bellante et al., 2012\)](#).

464

465 There are not many studies on Se levels in the liver of bottlenose dolphins ([Table 3](#)).
466 The mean hepatic concentration of Se was below 50 $\mu\text{g g}^{-1}$ ww in most marine areas
467 worldwide, but two locations far exceeded this value, which also corresponded to
468 places that had the highest Hg burdens, the Ligurian Sea [\(Capelli et al., 2008\)](#) and the
469 liver of *Tursiops aduncus* in the south of Australia [\(Lavery et al., 2008\)](#). The bottlenose
470 dolphins from both regions showed 8 and 3-fold greater Se levels, respectively, than
471 the results obtained in this study. These geographical differences are difficult to
472 explain because Se is an essential element and many factors, such as dietary intake or
473 natural sources, but also differences in physiologic needs or the retention of Se for
474 detoxification processes, might influence its concentrations [\(McHuron et al., 2014\)](#).

475

476 4. Conclusions

477

478 The present study contains the first reported evidence of Hg and Se concentrations in
479 the blubber [of bottlenose dolphins stranded along the coasts of the Canary Islands](#) and
480 [broadens the data previously available in liver tissue of bottlenose dolphins from the](#)

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Canary Islands. In addition, it represents the first Se/Hg molar ratio assessment in cetaceans from this marine area.

There is an increasing temporal trend of Hg concentration during the period of the study (1997-2013) and is consistent with recently published results for Hg in Atlantic waters (Lamborg et al., 2014).

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Hg and Se accumulate in the liver of dolphins during their lifetime and are strongly positively correlated with each other. Hg increases with age-body length probably because of continual dietary uptake and Se due to detoxification processes or from eating Se-rich fish. Individuals with Se/Hg molar ratios over 1 are all subadults and adults. Conversely, young animals have lower Hg burdens and are also deficient in Se. Thus, according to our results, the youngest and oldest animals seem to be of greater toxicological concern. In addition, variation on these two elements in the blubber between the earliest stages of life (newborn and calf) and the following ages, likely indicates the influence of lactation and weaning on the lipophilic pollutant accumulation. Nevertheless, this finding must be carefully discussed considering the limited data available per age group.

A comparison of the present study with literature values from other worldwide marine areas indicates that hepatic Hg results from this part of the Northeast Atlantic Ocean are similar to those obtained in bottlenose dolphins from the North Sea, the Western Atlantic Ocean and several locations in the Pacific Ocean. The Mediterranean Sea and the South of Australia are hot spot contaminated areas for both elements; by contrast,

the median results of this study show that the bottlenose dolphin population from the Canary Islands is not especially threatened by Hg or Se. However, it must be emphasized that the concentrations of the elements were highly variable between specimens; some fall into the Hg threshold established for hepatic damage, and others are Se deficient. In light of these results, further work is required to assess the individual effects of high loads of Hg and either large amounts or a deficiency of Se. In addition, an evaluation of the possible toxic impact of chronic exposure is also necessary.

Acknowledgements

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FIGURE CAPTIONS

Figure 1. (A) Hepatic mercury (above) and selenium (below) concentrations in bottlenose dolphins correlated with the total length of the specimens with Spearman correlations (r_s) of 0.769 and 0.764 respectively. (B) Mean concentrations ($\mu\text{g g}^{-1} \text{ dw}$) of mercury (above) and selenium (below) in the blubber and liver of bottlenose dolphins comparing age groups. Sample size (n) is in brackets. Note that there is only one animal in the newborn, calf and old age categories.

529

530 Figure 2. Temporal distribution of total mercury and selenium concentrations ($\mu\text{g g}^{-1}$
531 dw) in blubber samples of bottlenose dolphins stranded from 1999 to 2013 [on the](#)
532 [Canary Islands coasts](#). The plot represents the mean [level](#) with standard deviation (SD).
533 [Sample size in each group: 1999 \(n=2\); 2000 \(n=1\); 2002 \(n=1\); 2003 \(n=2\); 2004 \(n=1\);](#)
534 [2005 \(n=5\); 2007 \(n=1\); 2008 \(n=4\); 2009 \(n=2\); 2010 \(n=2\); 2011 \(n=5\); 2012 \(n=2\);](#)
535 [2013 \(n=1\). Inset: temporal trends of both trace elements \(\$\mu\text{g g}^{-1}\$ dw, individual or](#)
536 [mean values\) in the liver of the animals studied. Sample size in each group: 1997 \(n=1\);](#)
537 [1999 \(n=2\); 2000 \(n=1\); 2002 \(n=1\); 2003 \(n=2\); 2005 \(n=5\); 2007 \(n=1\); 2008 \(n=4\);](#)
538 [2009 \(n=2\); 2010 \(n=2\); 2011 \(n=5\); 2012 \(n=2\); 2013 \(n=1\).](#)

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539

540 Figure 3. (A) Correlation between mercury and selenium molar concentrations (nmol g^{-1}
541 dw) in the liver of bottlenose dolphins from the Canary Islands. Spearman correlation
542 ($r_s=0.955$) excluding the outlier data (CET 407) with a graphic representation of linear
543 ($R^2=0.807$) and potential cubic regression ($R^2=0.880$). Linear regression of Hg molar
544 concentration below 1500 ($R^2=0.973$). (B) Dependence of the Se/Hg molar ratio on the
545 total mercury ($\mu\text{g g}^{-1}$ dw) in liver samples of bottlenose dolphins. Spearman correlation
546 ($r_s=0.943$) considering all samples ($n=29$) and its potential cubic regression ($R^2=0.937$).
547 Spearman correlation excluding the outlier value ($r_s=0.937$) and its linear regression
548 ($R^2=0.789$).

549

550 Figure 4. Trends for hepatic mercury and selenium levels with the age of bottlenose
551 dolphins (median of molar concentrations, nmol g^{-1} dw); the selenium outlier data (CET
552 407) is excluded. Sample size (n) is in brackets.

553

554 Figure 5. Relative mercury concentration in the liver of bottlenose dolphins from the
555 Canary Islands (the present study) and other marine areas worldwide (see Table 3 for
556 references). 1, < 50 ($\mu\text{g g}^{-1}$ ww); 2, 50-100 ($\mu\text{g g}^{-1}$ ww); 3, 100-500 ($\mu\text{g g}^{-1}$ ww); 4, > 500
557 ($\mu\text{g g}^{-1}$ ww).

558

559 Supplementary Figure 1. Median molar concentrations (nmol g^{-1} dw) of mercury and
560 selenium in the liver of bottlenose dolphins stranded on several islands of the Canary
561 Archipelago. The graphic inserted at the top right represents plot boxes of hepatic
562 Se/Hg molar ratios comparing location groups. LZ, Lanzarote (n=5); FV, Fuerteventura
563 (n=2); GC, Gran Canaria (n=8); TF, Tenerife (n=13); LG, La Gomera (n=1).

564

565 Supplementary Figure 2. Total mercury and selenium concentrations ($\mu\text{g g}^{-1}$ ww) in 29
566 livers of stranded bottlenose dolphins compared to the 100-400 $\mu\text{g g}^{-1}$ ww range
567 threshold proposed by Wagemann and Muir, 1984.

568

569 References

- 570 Aguilar A, Borrell A, Pastor T. Biological factors affecting variability of persistent pollutant
571 levels in cetaceans. *The Journal of Cetacean Research and Management* 1999; 83-116.
- 572 Aguilar A, Borrell A, Reijnders PJ. Geographical and temporal variation in levels of
573 organochlorine contaminants in marine mammals. *Marine environmental research*
574 2002; 53: 425-52.
- 575 AMAP. AMAP Assessment 2011: Mercury in the Arctic. Arctic Monitoring and Assessment
576 Programme (AMAP), Oslo, Norway, 2011.
- 577 Andre J, Boudou A, Ribeyre F, Bernhard M. Comparative study of mercury accumulation in
578 dolphins (*Stenella coeruleoalba*) from French Atlantic and Mediterranean coasts. *The*
579 *Science of the total environment* 1991; 104: 191-209.
- 580 Beck KM, Fair PA, McFee W, Wolf D. Heavy metals in livers of bottlenose dolphins stranded
581 along the South Carolina coast. *Marine Pollution Bulletin* 1997; 34: 734-739.

Formattato: Italiano (Italia)

582 Becker PR, Mackey EA, Suydam R, Early GA, Koster BJ, Wise SA. Relationship of silver with
583 selenium and mercury in the liver of two species of toothed whales (odontocetes).
584 [Marine pollution bulletin](#) 1995; 30: 262-271.

585 Bellante A, Sprovieri M, Buscaino G, Buffa G, Di Stefano V, Salvagio Manta D, et al. Stranded
586 cetaceans as indicators of mercury pollution in the Mediterranean Sea [Italian Journal](#)
587 [of Zoology](#) 2012; 79: 151-160.

588 Budtz-Jorgensen E, Grandjean P, Weihe P. Separation of risks and benefits of seafood intake.
589 [Environmental health perspectives](#) 2007; 115: 323-7.

590 Caceres-Saez I, Dellabianca NA, Goodall RN, Cappozzo HL, Guevara SR. Mercury and selenium
591 in subantarctic Commerson's dolphins (*Cephalorhynchus c. commersonii*). [Biological](#)
592 [trace element research](#) 2013; 151: 195-208.

593 Camacho M, Calabuig P, Luzardo OP, Boada LD, Zumbado M, Oros J. Crude Oil as a Stranding
594 Cause among Loggerhead Sea Turtles (*Caretta caretta*) in the Canary Islands, Spain
595 (1998-2011). [Journal of wildlife diseases](#) 2013; 49: 637-40.

596 Camacho M, Oros J, Henriquez-Hernandez LA, Valeron PF, Boada LD, Zaccaroni A, et al.
597 Influence of the rehabilitation of injured loggerhead turtles (*Caretta caretta*) on their
598 blood levels of environmental organic pollutants and elements. [The Science of the](#)
599 [total environment](#) 2014; 487: 436-42.

600 Capelli R, Das K, Pellegrini RD, Drava G, Lepoint G, Miglio C, et al. Distribution of trace elements
601 in organs of six species of cetaceans from the Ligurian Sea (Mediterranean), and the
602 relationship with stable carbon and nitrogen ratios. [The Science of the total](#)
603 [environment](#) 2008; 390: 569-78.

604 Carballo M, Aguayo S, Esperón F, Fernández A, De la Torre A, De la Peña E, et al. Exposición de
605 cetáceos a contaminantes ambientales con actividad hormonal en el Atlántico.
606 [Ecosistemas](#) 2004; 13: 39-44.

607 Clarkson TW, Magos L. The toxicology of mercury and its chemical compounds. [Critical reviews](#)
608 [in toxicology](#) 2006; 36: 609-62.

609 Crespo-Lopez ME, Macedo GL, Pereira SI, Arrifano GP, Picanco-Diniz DL, do Nascimento JL, et
610 al. Mercury and human genotoxicity: critical considerations and possible molecular
611 mechanisms. [Pharmacol Res](#) 2009; 60: 212-20.

612 Cuvín-Aralar ML, Furness RW. Mercury and selenium interaction: a review. [Ecotoxicology and](#)
613 [environmental safety](#) 1991; 21: 348-64.

614 Chen J. An original discovery: selenium deficiency and Keshan disease (an endemic heart
615 disease). [Asia Pacific journal of clinical nutrition](#) 2012; 21: 320-6.

616 De Guise S, Martineau D, Beland P, Fournier M. Possible mechanisms of action of
617 environmental contaminants on St. Lawrence beluga whales (*Delphinapterus leucas*).
618 [Environ Health Perspect](#) 1995; 103 Suppl 4: 73-7.

619 EC. Council Directive No. 92/43/EEC of 21 May 1992 on the conservation of natural habitats
620 and of wild fauna and flora, 1992.

621 Frodello JP, Romeo M, Viale D. Distribution of mercury in the organs and tissues of five
622 toothed-whale species of the Mediterranean. [Environmental pollution](#) 2000; 108: 447-
623 52.

624 Ganther HE, Goudie C, Sunde ML, Kopecky MJ, Wagner P. Selenium: relation to decreased
625 toxicity of methylmercury added to diets containing tuna. [Science](#) 1972; 175: 1122-4.

626 Garcia-Alvarez N, Boada LD, Fernandez A, Zumbado M, Arbelo M, Sierra E, et al. Assessment of
627 the levels of polycyclic aromatic hydrocarbons and organochlorine contaminants in
628 bottlenose dolphins (*Tursiops truncatus*) from the Eastern Atlantic Ocean. [Marine](#)
629 [environmental research](#) 2014a; 100: 48-56.

630 Garcia-Alvarez N, Martin V, Fernandez A, Almunia J, Xuriach A, Arbelo M, et al. Levels and
631 profiles of POPs (organochlorine pesticides, PCBs, and PAHs) in free-ranging common
632 bottlenose dolphins of the Canary Islands, Spain. [The Science of the total environment](#)
633 2014b; 493: 22-31.

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Formattato: Spagnolo (Spagna)

Formattato: Spagnolo (Spagna)

Formattato: Italiano (Italia)

Formattato: Portoghese (Brasile)

Formattato: Italiano (Italia)

634 Geraci JR. Clinical investigation of the 1987-88 mass mortality of bottlenose dolphins along the
 635 U.S. central and south atlantic coast. In: Final report to National Marine Fisheries
 636 Service and U.S. Navy, editor, 1989.
 637 Gui D, Yu RQ, Sun Y, Chen L, Tu Q, Mo H, et al. Mercury and selenium in stranded Indo-Pacific
 638 humpback dolphins and implications for their trophic transfer in food chains. *PloS one*
 639 2014; 9: e110336.
 640 Gutierrez-Mejia E, Lares ML, Sosa-Nishizaki O. Mercury and arsenic in muscle and liver of the
 641 golden cownose ray, *Rhinoptera steindachneri*, Evermann and Jenkins, 1891, from the
 642 upper Gulf of California, Mexico. *Bulletin of environmental contamination and*
 643 *toxicology* 2009; 83: 230-4.
 644 Holsbeek L, Siebert U, Joiris CR. Heavy metals in dolphins stranded on the French Atlantic
 645 coast. *The Science of the total environment* 1998; 217: 241-9.
 646 Kaneko JJ, Ralston NV. Selenium and mercury in pelagic fish in the central north pacific near
 647 Hawaii. *Biological trace element research* 2007; 119: 242-54.
 648 Khan MA, Wang F. Mercury-selenium compounds and their toxicological significance: toward a
 649 molecular understanding of the mercury-selenium antagonism. *Environmental*
 650 *toxicology and chemistry / SETAC* 2009; 28: 1567-77.
 651 Koeman JH, Peeters WH, Koudstaal-Hol CH, Tjioe PS, de Goeij JJ. Mercury-selenium
 652 correlations in marine mammals. *Nature* 1973; 245: 385-6.
 653 Krishna D, Debacker V, Pillet S, Bouqueneau JM. Heavy metals in marine mammals. In: Vos JG,
 654 Bossart G, Fournier M, O'Shea TJ, editors. *Toxicology of Marine Mammals*, 2003, pp.
 655 135-167.
 656 Lahaye V, Bustamante P, Dabin W, Van Canneyt O, Dhermain F, Cesarini C, et al. New insights
 657 from age determination on toxic element accumulation in striped and bottlenose
 658 dolphins from Atlantic and Mediterranean waters. *Marine pollution bulletin* 2006; 52:
 659 1219-30.
 660 Lamborg CH, Hammerschmidt CR, Bowman KL, Swarr GJ, Munson KM, Ohnemus DC, et al. A
 661 global ocean inventory of anthropogenic mercury based on water column
 662 measurements. *Nature* 2014; 512: 65-8.
 663 Lavery TJ, Butterfield N, Kemper CM, Reid RJ, Sanderson K. Metals and selenium in the liver
 664 and bone of three dolphin species from South Australia, 1988-2004. *The Science of the*
 665 *total environment* 2008; 390: 77-85.
 666 Law RJ, Barry J, Barber JL, Bersuder P, Deaville R, Reid RJ, et al. Contaminants in cetaceans
 667 from UK waters: status as assessed within the Cetacean Strandings Investigation
 668 Programme from 1990 to 2008. *Marine pollution bulletin* 2012; 64: 1485-94.
 669 Louis C, Dirtu AC, Stas M, Guiot Y, Malarvannan G, Das K, et al. Mobilisation of lipophilic
 670 pollutants from blubber in northern elephant seal pups (*Mirounga angustirostris*)
 671 during the post-weaning fast. *Environmental research* 2014; 132: 438-48.
 672 Louis C, Perdaens L, Suciu S, Tavoni SK, Crocker DE, Debier C. Mobilisation of blubber fatty
 673 acids of northern elephant seal pups (*Mirounga angustirostris*) during the post-
 674 weaning fast. *Comparative biochemistry and physiology. Part A, Molecular &*
 675 *integrative physiology* 2015; 183: 78-86.
 676 Luzardo OP, Henriquez-Hernandez LA, Valeron PF, Lara PC, Almeida-Gonzalez M, Losada A, et
 677 al. The relationship between dioxin-like polychlorobiphenyls and IGF-I serum levels in
 678 healthy adults: evidence from a cross-sectional study. *PloS one* 2012; 7: e38213.
 679 Luzardo OP, Mahtani V, Troyano JM, Alvarez de la Rosa M, Padilla-Perez AI, Zumbado M, et al.
 680 Determinants of organochlorine levels detectable in the amniotic fluid of women from
 681 Tenerife Island (Canary Islands, Spain). *Environmental research* 2009; 109: 607-13.
 682 Mackey EA, Demiralp R, Becker PR, Greenberg RR, Koster BJ, Wise SA. Trace element
 683 concentrations in cetacean liver tissues archived in the National Marine Mammal
 684 Tissue Bank. *The Science of the total environment* 1995; 175: 25-41.

Formattato: Spagnolo (Spagna)

Formattato: Italiano (Italia)

685 Magos L, Clarkson TW. Overview of the clinical toxicity of mercury. *Annals of clinical*
686 *biochemistry* 2006; 43: 257-68.

687 Martoja R, Berry JP. Identification of tiemannite as a probable product of demethylation of
688 mercury by selenium in cetaceans. A complement to the scheme of the biological cycle
689 of mercury. *Vie Milieu* 1980; 30: 7-10.

690 McHuron EA, Harvey JT, Castellini JM, Stricker CA, O'Hara TM. Selenium and mercury
691 concentrations in harbor seals (*Phoca vitulina*) from central California: health
692 implications in an urbanized estuary. *Marine pollution bulletin* 2014; 83: 48-57.

693 Mendez-Fernandez P, Webster L, Chouvelon T, Bustamante P, Ferreira M, Gonzalez AF, et al.
694 An assessment of contaminant concentrations in toothed whale species of the NW
695 Iberian Peninsula: part II. Trace element concentrations. *The Science of the total*
696 *environment* 2014; 484: 206-17.

697 O'Hara TM, Woshner V, Bratton G. Inorganic pollutants in Arctic marine mammals. In: Vos JG,
698 Bossart G, Fournier M, O'Shea TJ, editors. *Toxicology of Marine Mammals*, 2003, pp.
699 206-246.

700 Palmisano F, Cardellicchio N, Zambonin PG. Speciation of Mercury in Dolphin Liver: A Two-
701 Stage Mechanism for the Demethylation Accumulation Process and Role of Selenium.
702 *Marine environmental research* 1995; 40: 109-121.

703 Parizek J, Ostadalova I. The protective effect of small amounts of selenite in sublimate
704 intoxication. *Experientia* 1967; 23: 142-3.

705 Ralston NV, Blackwell JL, 3rd, Raymond LJ. Importance of molar ratios in selenium-dependent
706 protection against methylmercury toxicity. *Biological trace element research* 2007;
707 119: 255-68.

708 Ralston NV, Raymond LJ. Dietary selenium's protective effects against methylmercury toxicity.
709 *Toxicology* 2010; 278: 112-23.

710 Rasmussen RS, Nettleton J, Morrissey MT. A review of mercury in seafood: special focus on
711 tuna. *Journal of Aquatic Food Product Technology* 2005; 14: 71-100.

712 Sakamoto M, Yasutake A, Kakita A, Ryufuku M, Chan HM, Yamamoto M, et al.
713 Selenomethionine protects against neuronal degeneration by methylmercury in the
714 developing rat cerebrum. *Environmental science & technology* 2013; 47: 2862-8.

715 Schwarz K, Foltz CM. Selenium as an integral part of factor 3 against dietary necrotic liver
716 degeneration. *Journal of the American Chemical Society* 1957; 79: 3292.

717 Seixas TG, Moreira I, Siciliano S, Malm O, Kehrig HA. Differences in methylmercury and
718 inorganic mercury biomagnification in a tropical marine food web. *Bulletin of*
719 *environmental contamination and toxicology* 2014; 92: 274-8.

720 Sormo EG, Ciesielski TM, Overjordet IB, Lierhagen S, Eggen GS, Berg T, et al. Selenium
721 moderates mercury toxicity in free-ranging freshwater fish. *Environmental science &*
722 *technology* 2011; 45: 6561-6.

723 Squadrone S, Benedetto A, Brizio P, Prearo M, Abete MC. Mercury and selenium in European
724 catfish (*Silurus glanis*) from Northern Italian Rivers: can molar ratio be a predictive
725 factor for mercury toxicity in a top predator? *Chemosphere* 2015; 119: 24-30.

726 Stavros HC, Stolen M, Durden WN, McFee W, Bossart GD, Fair PA. Correlation and toxicological
727 inference of trace elements in tissues from stranded and free-ranging bottlenose
728 dolphins (*Tursiops truncatus*). *Chemosphere* 2011; 82: 1649-61.

729 Storelli MM, Giacomini-Stuffler R, Storelli A, Marcotrigiano GO. Accumulation of mercury,
730 cadmium, lead and arsenic in swordfish and bluefin tuna from the Mediterranean Sea:
731 a comparative study. *Marine pollution bulletin* 2005; 50: 1004-7.

732 Storelli MM, Marcotrigiano GO. Mercury speciation and relationship between mercury and
733 selenium in liver of *Galeus melastomus* from the Mediterranean sea. *Bulletin of*
734 *environmental contamination and toxicology* 2002; 69: 516-22.

735 Sunde RA. Selenium. In: Bowman BA, Russell RM, editors. *Present Knowledge in Nutrition*. ILSI
736 Press, Washington, D. C., 2006, pp. 480-197.

Formattato: Italiano (Italia)

737 Taylor D, Dalton C, Hall A, Woodroffe MN, Gardiner PH. Recent developments in selenium
 738 research. *British journal of biomedical science* 2009; 66: 107-16; quiz 129.
 739 Tobeña M, Escáñez A, Rodríguez Y, López C, Ritter F, Aguilar N. Inter-island movements of
 740 common bottlenose dolphins *Tursiops truncatus* among the Canary Islands: online
 741 catalogues and implications for conservation and management. *African Journal of*
 742 *Marine Science* 2014; 36: 137-141.
 743 van de Merwe JP, Hodge M, Olszowy HA, Whittier JM, Lee SY. Using blood samples to estimate
 744 persistent organic pollutants and metals in green sea turtles (*Chelonia mydas*). *Marine*
 745 *pollution bulletin* 2010; 60: 579-88.
 746 Visnjevec AM, Kocman D, Horvat M. Human mercury exposure and effects in Europe.
 747 *Environmental toxicology and chemistry / SETAC* 2014; 33: 1259-70.
 748 Vos JG, Bossart G, Fournier M, O'Shea TJ. *Toxicology of Marine Mammals*, 2003.
 749 Vos JG, Dybing E, Greim HA, Ladefoged O, Lambre C, Tarazona JV, et al. Health effects of
 750 endocrine-disrupting chemicals on wildlife, with special reference to the European
 751 situation. *Critical reviews in toxicology* 2000; 30: 71-133.
 752 Wagemann R, Muir D. Concentrations of heavy metals and organochlorines in marine
 753 mammals of northern waters: overview and evaluation *Can. Tech. Rep. Fish. Aquat. Sc.*
 754 *1984; 1279.*
 755 Wagemann R, Trebacz E, Boila G, Lockhart WL. Mercury species in the liver of ringed seals. *The*
 756 *Science of the total environment* 2000; 261: 21-32.
 757 Wilson RM, Kucklick JR, Balmer BC, Wells RS, Chanton JP, Nowacek DP. Spatial distribution of
 758 bottlenose dolphins (*Tursiops truncatus*) inferred from stable isotopes and priority
 759 organic pollutants. *Sci Total Environ* 2012; 425: 223-30.
 760 Woshner VM, O'Hara TM, Bratton GR, Suydam RS, Beasley VR. Concentrations and interactions
 761 of selected essential and non-essential elements in bowhead and beluga whales of
 762 arctic Alaska. *Journal of wildlife diseases* 2001; 37: 693-710.
 763 Yang J, Kunito T, Tanabe S, Miyazaki N. Mercury and its relation with selenium in the liver of
 764 Dall's porpoises (*Phocoenoides dalli*) off the Sanriku coast of Japan. *Environmental*
 765 *pollution* 2007; 148: 669-73.
 766 Zhang H, Feng X, Chan HM, Larssen T. New insights into traditional health risk assessments of
 767 mercury exposure: implications of selenium. *Environmental science & technology*
 768 *2014; 48: 1206-12.*
 769
 770