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Mercury and selenium status of bottlenose dolphins (Tursiops truncatus): A study in stranded animals on the Canary Islands

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6	Mercury and selenium in bottlenose dolphins (<i>Tursiops truncatus</i>): a		
7	study in stranded animals from the Canary Islands		
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28 ABSTRACT:

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30 The mercury (Hg) level in the marine environment has tripled in recent decades, 31 becoming a great concern because of its high toxic potential. This study is the first reports of inorganic pollutants Hg and 32 selenium (Se) status, and the first Se/Hg molar ratio assessment in bottlenose dolphins 33 (Tursiops truncatus) inhabiting the waters of the Canary Islands. Total Hg and selenium 34 (Se) concentrations were determined in the blubber and liver collected from 30 35 specimens stranded along the coasts of the archipelago from 1997 to 2013. The 36 median values for total Hg in the blubber and liver were 80.83 and 223.77 μ g g⁻¹ dry 37 weight (dw), and the median levels for Se in both tissues were 7.29 and 68.63 μ g g⁻¹ 38 dw, respectively. Hg concentrations in the liver were similar to those obtained in 39 bottlenose dolphins from the North Sea, the Western Atlantic Ocean and several 40 locations in the Pacific Ocean. The Mediterranean Sea and South of Australia are the 41 most contaminated areas for both elements in this cetacean species. However, it must 42 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 43 44 between Hg and Se was observed (rs=0.960). Surprisingly, both younger and older 45 specimens had a Se/Hg molar ratio different from 1, suggesting that these individuals 46 may be at greater toxicological risk for high concentrations of both elements or a 47 deficiency of Se without a protective action against Hg toxicity.

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53 **1. INTRODUCTION**

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55 Mercury (Hg) is a natural element that is a ubiquitous environmental contaminant. It is 56 distributed around the world by atmospheric transportation. The sources of Hg 57 contamination can be both natural (e.g., degassing of the earth's crust, volcanic 58 activities and forest fires) and anthropogenic (e.g., mining, chlorine industry, coal-59 burning power plants, cement and metallurgical industries, paper mills, agricultural 60 pesticides, or medical waste incineration) (van de Merwe et al., 2010). Natural inputs 61 might be highly relevant in certain areas (Andre et al., 1991), but industrial activities 62 might increase the exposure to this toxic element (AMAP, 2011; Magos and Clarkson, 63 2006), and recently published data even suggested that the amount of Hg in water has 64 almost tripled compared to the pre-industrial period (Lamborg et al., 2014). Hg in its 65 inorganic form is moderately toxic, but once in the aquatic environment, it is quickly 66 transformed into methylmercury (MeHg), a highly toxic form of Hg of most concern to 67 the health of humans and biota. MeHg is strongly neurotoxic (Clarkson and Magos, 68 2006), harmful to the kidneys, lungs, the thyroid gland, and the immune system (De 69 Guise et al., 1995); it is also teratogenic (Crespo-Lopez et al., 2009) and carcinogenic 70 (Vos et al., 2003; Vos et al., 2000). In the marine environment, MeHg accumulates and 71 biomagnifies along the food chain (Seixas et al., 2014) representing a serious threat, 72 especially to top predators such as humans (Visnjevec et al., 2014) or cetaceans, which 73 are exposed to this metal mainly via the diet (Storelli et al., 2005).

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It is well known that the toxic potential of Hg is suppressed in the presence of 75 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 selenocysteinate (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 regions, such as muscle. Furthermore, Se competes with Hg for its various biological 87 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 (Ganther 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.

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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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124	Bottlenose dolphins inhabit the Canary Islands as local resident populations that shows
125	inter-island movements within the archipelago (Tobeña et al., 2014). This species faces
126	a high exposure to organic pollutants (Garcia-Alvarez et al., 2014b) and is considered a
127	valuable biomarker of the health status of the marine ecosystems. A high
128	concentration of contaminants has also been reported in humans from the
129	Archipelago (Luzardo et al., 2012; Luzardo et al., 2009) and in other marine animals
130	from the Canary Islands waters (Camacho et al., 2014) and other nearby areas
131	(Camacho et al., 2013). Although there is a previous research concerning a few
132	inorganic pollutants in 12 bottlenose dolphins stranded on the canary coasts (Carballo
133	et al., 2004), there is a need of more recent and comprehensive data from this marine
134	region.
 135	
136	The major goals of this study were as follows: 1) adding to recently published
137	information on chemical pollution in bottlenose dolphins from the Canary Islands
138	(Garcia-Alvarez et al., 2014a; Garcia-Alvarez et al., 2014b), focusing on the Hg and Se
 139	concentrations in the blubber and liver of stranded animals, and studying their

140 relationships and toxicity, and 2) reviewing published studies to date on both elements

141 in bottlenose dolphins worldwide to assess the global impact of these elements on this

- 142 species.
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- 144 **2. MATERIALS AND METHODS**
- 145
- 146 2.1. Study area

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

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 considered a valuable biomarker of the health status of the marine ecosystems. A high 156 157 concentration of contaminants has also been reported in humans from the Archipelago (Luzardo et al., 2012; Luzardo et al., 2009) and in other marine 158 159 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 Over a period from 1997 to 2013, 29 each of blubber and liver samples were collected 165 166 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

levels in the liver (Beck et al., 1997). Besides, these tissues have been selected to be in

accordance with previous studies of contaminants in stranded dolphins from this

Archipelago (Garcia-Alvarez et al., 2014a). The blubber is considered as a main target

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171	for pollutant assessment, in order to possible future comparisons with biopsy samples	
172	from free ranging cetacean. On the other hand, the liver tissue was also selected	
173	because pattern distribution of metals is tissue specific, being the mercury mostly	
174	concentrated in the liver (Krishna et al., 2003).	
 175		
176	Tissue sampling and the state of decomposition of the stranded specimens were	
177	determined by adapting the Geraci and Lounsbury (2005) protocol. Thirteen males and	
178	17 females (including 2 pregnant females) were grouped_divided_into age categories	
179	i.e., newborn (1), calf (1), juveniles (5), subadults (11), adult (11) and old (1), based on	
180	body length and gonadal appearance. The bodily condition of the specimens was	
 181	classified from a good to a very poor state according to morphological	
182	characteristicsfeatures. All of the characteristics of the animals studied are	F
183	summarized in t able 1. Samples were stored in plastic bags at -80C in the Cetacean	
184	Tissue Bank of the University of Las Palmas de Gran Canaria (ULPGC) until analysis.	
185		
186	2.3. Sample preparation and analysis of trace elements	
187		
188	All samples were first lyophilized (freeze-dried) for a subsequent microwave digestion	
189	method using a Milestone ETHOS ONE oven. The fresh weight of each sample was	
190	recorded such that the results could be expressed both on a dry (dw) and a wet weight	
191	(ww) basis. In different vessels, 0.5 g aliquots of freeze-dried samples were mineralized	
192	with 6 ml of nitric acid plus 50 μl of Itrio (Y) as an internal standard. Each vessel was	
193	placed into the microwave oven to obtain solutions, which were then diluted to a final	
194	volume of 50 ml with distilled water. After digestion, the analysis of the elements was	
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195	performed with an Inductively Coupled Plasma-Optic Emission Spectrometry method	
196	(ICP-OES) using a Perkin Elmer Optima 2100 DV instrument. Two blanks were run	
197	during each analysis to check chemical purity, and the accuracy of the method was	
198	verified with reference materials (lyophilized mussel; CRM 278, Community Bureau of	
199	Reference, BCR, Brussels). All the values of the reference materials were within	
200	certified limits. The instrumental detection limits were 0.061 ng ml ⁻¹ ww for Hg and 0.1	
201	$\frac{1}{100}$ mg ml ⁴ ww for Se. The recovery values for Hg and Se were 120 ± 8% and 115 ± 11%.	Formattato: Inglese (Stati Uniti)
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202	respectively. The instrumental detection limits were 0.061 ng ml ⁻¹ ww for Hg and 0.1	
203	ng ml ⁻¹ ww for Se.	Formattato: Inglese (Stati Uniti)
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205	2.4. Data analysis	
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207	2.4. Calculation of the Se/Hg molar ratio	
208		
209	The molar ratio of Se to Hg was calculated as:	
210	Se/Hg = (Se/78.96) / (Hg/200.59)	
211	where 200.59 and 78.96 g mol ⁻¹ are the molar masses of Hg and Se, respectively.	
212		
213	2.5. Statistical analysis	
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215	Statistical analysis was performed with IBM SPSS Statistics v <u>2249.0. Because trace</u>	Formattato: Inglese (Regno Unito)
216	elements did not follow When conditions of normality (Kolmogorov-Smirnov and ,	Formattato: Inglese (Stati Uniti)
		Formattato: Inglese (Stati Uniti)
217	<u>Shapiro-Wilk tests</u>) and or homogeneity of variances in all variable groups, were not	Formattato: Inglese (Stati Uniti)
218	satisfied, non-parametric tests were used. Thus,	Formattato: Inglese (Stati Uniti)
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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 correlation test was performed to determine a possible relationship between both trace elements. As usual, the level of statistical significance was set at *p*-value = 0.05.

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225 3. RESULTS AND DISCUSSION

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227 Individual and descriptive statistics of Hg and Se concentrations in the blubber and 228 liver of bottlenose dolphins (mean ± SD, median and range) are shown in Itable 2. The 229 data are expressed as $\mu g \ g^{\text{-1}}$ (ppm) on a dry weight (dw) basis. However, to allow 230 comparison with other reports, the wet weight (ww) results were also determined 231 using conversion factors calculated for each sample based on their respective 232 percentages of dry residue (Ttable 2). The mean correction factor for blubber and liver 233 tissue (0.48 and 0.28 respectively) are comparable with values reported in the 234 literature (Becker et al., 1995; Mackey et al., 1995). Mean and median Hg values of 235 83.36 and 80.83 μ 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 μ g g⁻¹ dw, respectively). For Se, 236 the mean and median levels of 8.96 and 7.29 $\mu g \; g^{\text{-1}}$ dw in the blubber were much 237 238 lower than the Se concentration in the liver, in which the mean value of 211.20 μ g g⁻¹ 239 dw was quite far from the median of 68.63 μ g g⁻¹ dw because of the data dispersion. 240

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

Formattato: Inglese (Stati Uniti) Formattato: Inglese (Stati Uniti) 243 gradients in the aquatic environment (Storelli et al., 2005). Thus, an analysis of Hg and 244 Se concentrations against different variables (Table 1) is essential to fully understand 245 the effects of these elements on the specimens studied. 246 247 3.1. Influence of sex and age on mercury and selenium levels 248 249 Age is the most important biotic factor for Hg and Se accumulation. Albeit Aan 250 increasing hepatic concentration of both trace elements was observed, the small and 251 unequal sample sizes of age categories discouraged any statistical test assessment. 252 This enables us to use the body length as a surrogate for age class. Testingdespite a lack of statistical significance in their levels between consecutive age groups (p>0.05). 253 254 Because of the small sample size of the age categories, _it was interesting to analyze 255 the correlations between of 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 256 257 Spearman coefficient (rs) of 0.769 (Fig. 1A). In accordance with previous authors 258 (Bellante et al., 2012), an increasing trend throughout the life of cetaceans was 259 observed (Fig. 1), probably due to bioaccumulation from the continuous uptake of Hg 260 in the diet and the decreasing ability to excrete this metal and storage in stable forms 261 such as HgSe (Aguilar et al., 1999; Mackey et al., 1995; Wagemann et al., 2000). Other 262 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 263 rate of assimilation than excretion of Hg and a lower efficiency of detoxification. 264 265 Moreover, the largest specimens may capture bigger prey, which are more likely to 266 contain higher levels of Hg. Although essential trace elements are regulated via

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omeostasis in marine mammals (Mendez-Fernandez et al., 2014), Se levels in liver	Formattato: Inglese (Stati Uniti)
amples were notably higher in adults than in the youngest individuals (Fig. 1) and	Formattato: Inglese (Stati Uniti)
ere also correlated with animal length (r _s =0.764) in accordance with other studies	
Voshner et al., 2001). The high level of Se may result from its accumulation with Hg	Formattato: Inglese (Stati Uniti)
uring the detoxification process or from a highly concentrated diet because most	
cean fish are Se-rich, as was reported in a study in the Faroe Islands (Budtz-Jorgensen	Formattato: Inglese (Stati Uniti)
t al., 2007).	Formattato: Inglese (Stati Uniti)
g. 1B also shows tissue distribution of Hg and Se throughout the lifetime of the	
nimals, highlighting the concentration of both elements mostly in the liver, with a	Formattato: Inglese (Stati Uniti)
atistically significant difference between tissues ($p=0.000$). Therefore, the liver	
opeared to be the preferential tissue, as indicated in previous studies (Bellante et al.,	Formattato: Inglese (Stati Uniti)
012; Frodello et al., 2000) <u>However, it is also interesting to stress that the newborn</u>	
nd calf specimens of this research accumulated greater levels of Hg in the blubber	
nan the liver, especially for the calf. Concerning the Se, the newborn showed equal	
vels in both tissues and the calf individual doubled the concentration in the blubber.	
nese results were in contrast to the following sampling ages where the liver showed	
gher levels of both trace elements with an evident increasing trend. One could	
ypothesize that this variation on the tissue distribution is due to the different	
ollutant sources. Thus, the Hg and Se were initially transferred through placental	
arrier entering the fetal circulation to be transported to the liver for metabolism, and	
arrier entering the fetal circulation to be transported to the liver for metabolism, and nen distributed to the blubber for accumulation. The calf showed the highest	
arrier 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	
	amples were notably higher in adults than in the youngest individuals (Fig. 1) and amples were notably higher in adults than in the youngest individuals (Fig. 1) and there 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 uring the detoxification process or from a highly concentrated diet because most cean fish are Se-rich, as was reported in a study in the Faroe Islands (Budtz-Jorgensen t al., 2007). g. 1B also shows tissue distribution of Hg and Se throughout the lifetime of the nimals, highlighting the concentration of both elements mostly in the liver, with a satistically significant difference between tissues ($p=0.000$). Therefore, the liver oppeared to be the preferential tissue, as indicated in previous studies (Bellante et al., 012; 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 man the liver, especially for the calf. Concerning the Se, the newborn showed equal wels in both tissues and the calf individual doubled the concentration in the blubber. hese results were in contrast to the following sampling ages where the liver showed igher levels of both trace elements with an evident increasing trend. One could ypothesize that this variation on the tissue distribution is due to the different collutant sources. Thus, the Hg and Se were initially transferred through placental

291	lowest level of Hg and Se in the blubber compared to the rest of the age categories.	
292	This may be due to the release of Hg and Se from the blubber into the circulation at	
293	weaning stage, which could be considered a period of negative energy balance (Louis	
294	et al., 2015). However, little is known concerning the factors that affect mobilization of	
295	pollutants from adipose tissue (Louis et al., 2014). Therefore, this finding should be	Formattato: Inglese (Regno Unito)
296	interpreted with caution also because only one newborn and one calf of bottlenose	Formattato: Inglese (Regno Unito)
297	dolphins were available for this study.	
 298		
299	In this study no influence of sex on Hg or Se accumulation was observed, as has been	Formattato: Inglese (Stati Uniti)
300	found in other marine areas of the North Atlantic Ocean (Mendez-Fernandez et al.,	Formattato: Inglese (Stati Uniti)
301	2014).	Formattato: Inglese (Stati Uniti)
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303	3.2. Influence of stranding location on mercury and selenium levels	
304		
305	Clear differences for Hg and Se concentrations in the liver between the geographical	
306	areas, in which animals stranded, were found (with <i>p-values</i> of 0.060 and 0.046	Formattato: Inglese (Stati Uniti)
307	respectively). The results for the eastern Canary Islands of Lanzarote (LZ),	
308	Fuerteventura (FV) and Gran Canaria (GC) showed the highest Se and Hg levels as	
309	compared to the western islands of Tenerife (TF) and La Gomera (LG). Thus, median	
310	hepatic Hg molar concentration from animals stranded in the eastern islands (1824.21	
311	nmol g ⁻¹ dw) was 4 times greater than that from specimens stranded in the western	
312	islands (427.97 nmol g $^{-1}$ dw). This difference between both Canary regions was even	
313	more prominent for Se, which reached a 22-fold hepatic molar concentration in the	
314	eastern region of the Archipelago. In fact, there is a decreasing trend from the nearest	

315	to the furthest island from the African continent (Supp. Fig. 1). This finding could be	
316	related to geographical differences of natural and/or anthropogenic sources, but is	
317	more likely affected by the age of the animals at the various locations. The youngest	
318	individuals were found stranded in the western Canary Islands, so these results should	
319	be cautiously considered.	
320		
321	3.3. Temporal trends of mercury and selenium concentrations	
322		
323	Figure 2 illustrates the total Hg and Se concentrations in the blubber of individuals	
374	grouned according to the year of stranding (between 1999 and 2013) For this context	
121	grouped according to the year of stranding (between 1555 and 2015). For this context,	
325	it is preferable to analyze the blubber samples because no influence of age or length	Formattato: Inglese (Stati Uniti)
326	on Hg or Se levels in this tissue was obtained in the present study (see Fig. 1B). Despite	Formattato: Inglese (Stati Uniti)
 327	the low sample size of the groups (1999, n=2; 2000, n=1; 2002, n=1; 2003, n=2; 2004,	
328	n=1; 2005, n=5; 2007, n=1; 2008, n=4; 2009, n=2; 2010, n=2; 2011, n=5; 2012, n=2;	
329	2013, n=1) required careful interpretation, an increasing temporal trend of Hg in the	
330	blubber can be seen throughout the study period. In addition, the Mann-Whitney U-	
331	test revealed a significant difference (p=0.016) between 2005 and 2011, each with 5	Formattato: Tipo di carattere: Corsivo
332	specimens available. Excluding the samples from years with only one value, the	
333	Kruskal-Wallis test revealed a significant difference (p=0.029). From the individual	
 334	stranded in 1999 to the last one in 2013, the Hg level has tripled in the blubber,	
\$35	consistent with a recently published report (Lamborg et al., 2014). Lamborg's group	Formattato: Inglese (Stati Uniti)
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336	found that deep and intermediate North Atlantic waters are abnormally enriched in	Formattato: Inglese (Stati Uniti)
337	Hg, probably because of anthropogenic activities such as mining and fossil fuel	

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).

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341 3.4. Study of the relationship between mercury and selenium

342

As previously mentioned, a common approach to assess the risk of exposure to Hg is to
determine the molar ratio of Hg and Se in the body (Se/Hg). A high positive correlation
between Hg and Se with an equimolar ratio in the liver as well as the protective effect
of Se against Hg toxicity is well documented (Cuvin-Aralar and Furness, 1991; Geraci,
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 (rs), 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 (R²=0.973) between these two elements occurred below 1500 nmol g^{-1} (300 $\mu g g^{-1} dw$) 357 of Hg in the liver, comparable to a total Hg threshold of 100 μ g g⁻¹ ww, as obtained by other authors (Palmisano et al., 1995). Above this concentration, the level of hepatic 358 Se significantly exceeded the Hg concentration, so the Se/Hg molar ratio was higher 359 360 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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\$62 (Kaneko and Ralston, 2007) and cetaceans (Mendez-Fernandez et al., 2014), and the . 363 authors stated that this excess reflects the good health status of individuals or a high 364 proportion of young animals. In the present study, individuals with a Se/Hg molar ratio 365 above 1 were all included in older categories (Fig. 4), contrary to such statement and \$66 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 \$67 368 showed a Se/Hg molar ratio over 1; by contrast, GC, TF and LG had a median ratio 369 below 1 (Supp. Fig. 1, insert panel). However, the limited sample size per group 370 undercut any conclusion.

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372	Recently, different criteria have been developed to determine whether the
373	consumption of certain foods by humans presents a health risk, so dietary
374	recommendation can be issued . The positive values correspond to a Se/Hg molar ratio
375	above 1, but are also associated with a hidden risk for Se deficiency and poisoning.
376	Zhang and colleagues attempted to develop a new criterion for an exposure
377	assessment to Se/Hg that they called the benefit risk value (BRV), taking into account
378	the amount of Se required for normal biological functions and the threshold intake
379	value for Se poisoning. Unfortunately, such a calculation could not be performed in
380	this study, due to a lack of specific information for marine mammals essential for the
381	development of the equation, which might not be applicable to an assessment of
382	health risk in bottlenose dolphins.
ı 383	

384 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 \$87 mammals in a range of 100-400 μ 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 μ 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 al., 2008; Stavros et al., 2011). These results coincided with animals with a Se/Hg molar 394 395 ratio greater than 1. 396

\$97 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 hepatic Hg level reached more than 500 µg g⁻¹ dw after death . Other reports 401 402 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 μ g g⁻¹ ww. 406

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

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410	suggested . As mentioned earlier, the formation of insoluble tiemannite granules
411	provides the ability to endure high Hg exposures to odontocetes . Thus, Hg and Se
412	levels above 2000 µg g ⁻¹ dw were reported in animals with no signs of poisoning
413	because of the protection provided by the combined presence of both trace elements ;
414	however, the energy cost of the detoxification is difficult to assess.

416 The results discussed above indicate that the youngest and oldest bottlenose dolphins 417 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 418 419 could lead to Hg toxicity and they also had a Se/Hg molar ratio less than 1, indicating a 420 limited protection by Se. This result is consistent with human studies in which authors 421 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 422 423 may indicate that all of the available Se is bound to Hg, conferring a possible oxidative 424 stress risk (Caceres-Saez et al., 2013). However, these results must be carefully 425 considered because there was only one specimen available from each, newborn and 426 calf categories. By contrast, the older animals had the highest concentrations of both 427 Hg and Se in the liver and Se/Hg ratios greater than 1 suggesting a Se molar excess 428 which could become toxic at high levels (O'Hara et al., 2003). Nevertheless, the inter-429 relationships between the Hg and Se concentrations, age, nutritional status and 430 disease are complex (Law et al., 2012) and the limits of deficiency, essentiality, and 431 poisoning is quite difficult to assess and not well studied. 432

433 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 measured of hepatic Hg (13150 µg g⁻¹ dw) ever reported before, followed by the 444 445 Adriatic Sea which appears to be significantly more polluted by Hg than the lesscontaminated Eastern Mediterranean coast. The Hg levels from the North Sea and the 446 447 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 Ttable 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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It has been reported that Mediterranean prey had higher Hg levels than Atlantic prey
(Lahaye et al., 2006), which explains the Hg enrichment in the Mediterranean food
webs, and also in the liver of bottlenose dolphins. The authors suggest that this might
be due to natural Hg sources in the Mediterranean Sea (Andre et al., 1991) and high
anthropogenic Hg emissions especially from France (Bellante et al., 2012).

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458

465 There are not many studies on Se levels in the liver of bottlenose dolphins (Ttable 3). The mean hepatic concentration of Se was below 50 µg g⁻¹ ww in most marine areas 466 467 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 uence its concentrations, (McHuron et al., 2014).

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174	detoxification processes, might influ
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176	4. Conclusions
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The present study contains the first reported evidence of Hg and Se concentrations in
the blubber <u>of bottlenose dolphins stranded along the coasts of the Canary Islands</u> and
broadens the data previously available in liver tissue of bottlenose dolphins from the

481	Canary Islands. In addition, it represents the first Se/Hg molar ratio assessment in	
482	cetaceans from this marine area.	
 483		
484	There is an increasing temporal trend of Hg concentration during the period of the	
485	study (1997-2013) and is consistent with recently published results for Hg in Atlantic	
486	waters (Lamborg et al., 2014).	
 487		\langle
488	Hg and Se accumulate in the liver of dolphins during their lifetime and are strongly	
489	positively correlated with each other. Hg increases with age-body length probably	
 490	because of continual dietary uptake and Se due to detoxification processes or from	
491	eating Se-rich fish. Individuals with Se/Hg molar ratios over 1 are all subadults and	
492	adults. Conversely, young animals have lower Hg burdens and are also deficient in Se.	
493	Thus, according to our results, the youngest and oldest animals seem to be of greater	
494	toxicological concern. In addition, variation on these two elements in the blubber	
495	between the earliest stages of life (newborn and calf) and the following ages, likely	
496	indicates the influence of lactation and weaning on the lypophilic pollutant	
497	accumulation. Nevertheless, this finding must be carefully discussed considering the	
498	limited data available per age group.	
ı 499		
500	A comparison of the present study with literature values from other worldwide marine	

5 501 areas indicates that hepatic Hg results from this part of the Northeast Atlantic Ocean 502 are similar to those obtained in bottlenose dolphins from the North Sea, the Western 503 Atlantic Ocean and several locations in the Pacific Ocean. The Mediterranean Sea and 504 the South of Australia are hot spot contaminated areas for both elements; by contrast,

Formattato: Inglese (Stati Uniti) Formattato: Inglese (Stati Uniti) Formattato: Inglese (Stati Uniti) 505 the median results of this study show that the bottlenose dolphin population from the 506 Canary Islands is not especially threatened by Hg or Se. However, it must be 507 emphasized that the concentrations of the elements were highly variable between 508 specimens; some fall into the Hg threshold established for hepatic damage, and others 509 are Se deficient. In light of these results, further work is required to assess the 510 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 511 512 necessary.

513

514 Acknowledgements

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520

521 FIGURE CAPTIONS

522

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 ($\mu g g^{-1} dw$) 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 ($\mu g \ g^{\text{-}1}$	
531	dw) in blubber samples of bottlenose dolphins stranded from 1999 to 2013 on the	
532	<u>Canary Islands coasts</u> . The plot represents the mean <u>level</u> 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);	Formattato: Portoghese (Brasile)
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 (µg g ⁻¹ dw, individual or	
536	mean values) in the liver of the animals studied. Sample size in each group: 1997 (n=1);	Formattato: Portoghese (Brasile)
537	1999 (n=2); 2000 (n=1); 2002 (n=1); 2003 (n=2); 2005 (n=5); 2007 (n=1); 2008 (n=4);	
538	<u>2009 (n=2); 2010 (n=2); 2011 (n=5); 2012 (n=2); 2013 (n=1).</u>	
539		
 540	Figure 3. (A) Correlation between mercury and selenium molar concentrations (nmol g	
541	1 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 (µg 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	

555		
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 (μg g ⁻¹ ww); 2, 50-100 (μg g ⁻¹ ww); 3, 100-500 (μg g ⁻¹ ww); 4, > 500	
557	(μg 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	For
563	(n=2); GC, Gran Canaria (n=8); TF, Tenerife (n=13); LG, La Gomera (n=1).	
564		
l 565	Supplementary Figure 2. Total mercury and selenium concentrations (µg g ⁻¹ ww) in 29	
566	livers of stranded bottlenose dolphins compared to the 100-400 $\mu g \ g^{\text{-1}}$ ww range	
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