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

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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~~s 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 µg g^{-1} dry weight (dw), and the median levels for Se in both tissues were 7.29 and 68.63 µg 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: stranded bottlenose dolphins, *Tursiops truncatus*, Canary Islands, trace elements, heavy metals, trace elements, liver, blubber, molar ratio, Atlantic Ocean, cetacean.

1. INTRODUCTION

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).

It is well known that the toxic potential of Hg is suppressed in the presence of sufficient amounts of selenium (Se) (Parizek and Ostadalova, 1967). This effect has been shown in studies in a variety of species, including marine mammals (Cuvin-Aralar and Furness, 1991; Frodello et al., 2000; Gui et al., 2014; Sakamoto et al., 2013) exposed to these elements for a long time, even before the industrial period (Holsbeek et al., 1998). Thus, several mechanisms for resistance to the adverse effects of Hg have been proposed. On the one hand, Se can easily combined with various forms of Hg to yield complexes with lower toxicity, such as methylmercury selenide (MeHg-Se), methylmercury selenocysteinate (MeHg-Sec), or mercury selenide, tiemannite (HgSe), which is considered the last step in Hg detoxification (Palmisano et al., 1995). These compounds also contribute to the mobilization of mercury from the most vulnerable 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 targets, which also contributes to lowering the potential toxicity of Hg (Khan and Wang, 2009). Therefore, the Se/Hg molar ratio has been widely used (McHuron et al., 2014; Mendez-Fernandez et al., 2014; Squadrone et al., 2015; Vos et al., 2003), and many authors have established that Se, in a molar ratio of 1:1 or above with Hg, protects against the toxic effects of this ~~latter~~ metal (Ganther et al., 1972; Ralston et al., 2007; Ralston and Raymond, 2010; Sormo et al., 2011; Squadrone et al., 2015). However, paradoxically, this protective action can be harmful to the body because complex formation also results in the sequestration of both elements, causing them to become biologically unavailable (Martoja and Berry, 1980). Se is a well-known essential element with multiple biological functions, such as its critical participation in reproduction, the metabolism of thyroid hormones or DNA synthesis, in addition to its important antioxidant role (anticarcinogenic activity), among other functions (Schwarz and Foltz, 1957; Taylor et al., 2009; Zhang et al., 2014). Therefore, the presence of high levels of Hg could lead to Se deficiency, which could even cause the death in extreme cases (Chen, 2012; Sunde, 2006). Thus, the toxicological effects might be due to both MeHg toxicity and the induced Se deficiency (Zhang et al., 2014). However, Se levels have increased dramatically in many marine areas, presenting an environmental toxicity problem (Lavery et al., 2008). Se pollution probably occurs as a result of anthropogenic activities such as coal burning, smelting, ceramic and glass manufacturing, or copper refining (van de Merwe et al., 2010). Therefore, to evaluate the health status of the ecosystems, the simultaneous study of Hg and Se and the relationship between them is of great interest, particularly in those species usually considered as sentinels for environmental pollution.

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

Bottlenose dolphins inhabit the Canary Islands as a 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(Camacho et al., 2014) in nearby areas (Camacho et al., 2013). However, a lack of data exists concerning heavy metals and other inorganic pollutants from this marine region.

2.2. Sample collection

Over a period from 1997 to 2013, 29 each of blubber and liver samples were collected from 30 bottlenose dolphins stranded on the Canary Islands coasts. According to the 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 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 ~~t~~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.

2.4. Data analysis

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

The molar ratio of Se to Hg was calculated as:

Se/Hg = (Se/78.96) / (Hg/200.59)

where 200.59 and 78.96 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, ~~T~~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 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.

3. RESULTS AND DISCUSSION

Individual and descriptive statistics of Hg and Se concentrations in the blubber and liver of bottlenose dolphins (mean ± SD, median and range) are shown in T~~t~~able 2. The data are expressed as μg 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 (T~~t~~able 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 μg 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 μg g^{-1} dw, respectively). For Se, the mean and median levels of 8.96 and 7.29 μg g^{-1} dw in the blubber were much lower than the Se concentration in the liver, in which the mean value of 211.20 μg g^{-1} dw was quite far from the median of 68.63 μg g^{-1} dw because of the data dispersion.

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

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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. Albeit Aan 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. Testingdespite 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 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 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 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).

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 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.

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).

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

3.4. Study of the relationship between mercury and selenium

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).

In the present study, the results showed that increasing Hg levels were associated with increasing Se concentrations, as described for other dolphin populations (Palmisano et al., 1995). Spearman's correlation coefficient (r_s), calculated between molar concentrations of hepatic Hg and Se, showed a strong positive relationship (Fig. 3). Excluding the outlier data for Se (CET 407), the correlation slightly decreased from 0.960 to 0.955, although the coefficient of determination (R^2) for linear regression increased from 0.592 to 0.807. It is remarkable that the strongest linear association (R^2=0.973) between these two elements occurred below 1500 nmol g^{-1} (300 µg g^{-1} dw) of Hg in the liver, comparable to a total Hg threshold of 100 µg g^{-1} ww, as obtained by other authors (Palmisano et al., 1995). Above this concentration, the level of hepatic Se significantly exceeded the Hg concentration, so the Se/Hg molar ratio was higher than 1 (Fig. 3B and 4). Se was in molar excess of Hg in 11 of 29 livers evaluated (37.9%). Other publications reported a similar levels of Se compared to Hg in both pelagic fish

(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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Wagemann and Muir (1984), established a threshold for hepatic damage in marine mammals in a range of 100-400 µg g^{-1} ww Hg burden (Wagemann and Muir, 1984). In supplementary figure 2, the total hepatic Hg concentration is individually compared to this threshold. The results indicate that 10 of 29 livers of stranded individuals (34.5%) exceeded the minimum Hg tolerance level, and 4 had values just below 105 µg g^{-1} ww. All these samples were from subadult and adult specimens, corresponding to 45.5% of the total of subadults and adults in this study. Other authors obtained comparable 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 ratio greater than 1.

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~~Experimental studies suggest that Hg intoxication could cause weight loss, toxic hepatitis, renal failure and death in marine mammals . Additionally, high Hg levels have been associated with parasitic infection and pneumonia , resulting in a lower 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 associated chronic Hg accumulation with liver abnormalities observed in stranded bottlenose dolphins from the Atlantic Ocean . Rawson's group found a correlation between the lipofuscin pigment with the hepatic Hg concentration. Large deposits were observed when the Hg level exceeds 60 µg g^{-1} ww.~~

~~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~~

~~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 µg 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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A comparison of Hg and Se levels in bottlenose dolphins from the Canary Islands (this study) and from different marine areas worldwide was made (Table 3). All these published results, compiled as ranges of Hg concentrations in the liver, were plotted on a map (Fig. 5), as well as others previously performed using Hg content in the hair of pinnipeds (McHuron et al., 2014) and organic pollutants in cetaceans (Aguilar et al., 2002). Bottlenose dolphins from the Mediterranean Sea had greater Hg concentrations than published values elsewhere, as was previously reported for striped dolphins (Andre et al., 1991). Even within the same marine area some differences in the Hg 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 Adriatic Sea which appears to be significantly more polluted by Hg than the less-contaminated Eastern Mediterranean coast. The Hg levels from the North Sea and the Northeast Atlantic Ocean, including the Canary Islands (results from this study), were similar to mean concentrations in bottlenose dolphins from the Western Atlantic Ocean and from several locations in the Pacific Ocean (Hong Kong and east coast of Australia), but not to results from the south coast of Australia where a higher Hg contamination occurred that nearly matched the Adriatic Sea values (see Table 3 for references). Thus, this last sea displayed 6 times higher Hg burden compared with the results from bottlenose dolphins from the Canary Islands (present study). Furthermore, the Tyrrhenian Sea showed the highest Hg value obtained in the literature, more than 50 times greater than the values obtained in this study. On the other hand, *T. truncatus* and *T. aduncus* from South of Australia had 3 to 7 times 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).

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

4. Conclusions

The present study contains the first reported evidence of Hg and Se concentrations in the blubber of bottlenose dolphins stranded along the coasts of the Canary Islands and 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 lypophilic 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.

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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 (μg 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.

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

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

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

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

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

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

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