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Global mercury and selenium concentrations in skin from free-ranging sperm whales (*Physeter macrocephalus*)

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HIGHLIGHTS

- ► Mercury (Hg) is a priority pollutant for the oceans.
- ► Hg concentrations worldwide were surveyed in the skin of free-ranging sperm whales
- ► Global mean mercury concentrations in sperm whale skin were 2.5 μg g^{-1} ww.
- ► Mediterranean Sea had the highest mean mercury concentration of 6.1 μg g^{-1} ww
- ► Selenium concentrations suggest a protective effect against mercury toxicity.

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ABSTRACT

Pollution of the ocean by mercury (Hg) is a global concern. Hg persists, bioaccumulates and is toxic putting high trophic consumers at risk. The sperm whale (*Physeter macrocephalus*), is a sentinel of ocean health due to its wide distribution, longevity and high trophic level. Our aim was to survey Hg concentrations worldwide in the skin of free-ranging sperm whales considering region, gender and age. Samples were collected from 343 whales in 17 regions during the voyage of the research vessel, *Odyssey*, between 1999 and 2005. Skin was analyzed for total Hg and detected in all but three samples with a global mean of 2.5 ± 0.1 μg g^{-1} ranging from 0.1 to 16.0 μg g^{-1}. The Mediterranean Sea had the highest regional mean with 6.1 μg g^{-1} followed by Australia with 3.5 μg g^{-1}. Considering gender, females and males did not have significantly different global Hg concentrations. The variation among regions for females was significantly different with highest levels in the Mediterranean and lowest in Sri Lanka; however, males were not significantly different among regions. Considering age in males, adults and subadults did not have significantly different Hg concentrations, and were not significantly different among regions. The toxic effects of these Hg concentrations are uncertain. Selenium (Se), an essential element, antagonizes Hg at equimolar amounts. We measured total Se concentrations and found detectable levels in all samples with a global mean of 33.1 ± 1.1 μg g^{-1} ranging from 2.5 to 179 μg g^{-1}. Se concentrations were found to be several fold higher than Hg concentrations with the average Se:Hg molar ratio being 59:1 and no correlation between the two elements. It is possible Hg is being detoxified in the skin by another mechanism. These data provide the first global analysis of Hg and Se concentrations in a free-ranging cetacean.

1. Introduction

Mercury (Hg) pollution is widespread causing elevated concentrations in far reaches of the globe including the open ocean (Hylander and Goodsite, 2006); however, data on oceanic Hg distribution is

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limited (Sunderland et al., 2009). This heavy metal is a priority pollutant because of its toxicity (Vallee and Ulmer, 1972), stability in the atmosphere of ~1 year enabling global mobility and deposition (Selin et al., 2008; Temme et al., 2004), and ability to bioaccumulate and biomagnify in food webs (Mason and Benoit, 2003; Wiener et al., 2003; Mason and Sheu, 2002; Mason et al., 1995). The oceans are a net sink for an estimated 3.8 Mmol of Hg annually (Soerensen et al., 2010), and the global Hg cycle suggests that the deep ocean Hg concentration is increasing by a few percent per year (Mason and Sheu, 2002). Total Hg concentrations in surface ocean waters are typically in the low picomolar range from 0.5 to 4 pM (Wang et al., 2012; Sunderland and Mason, 2007; Cossa and Coquery, 2005; Laurier et al., 2004; Horvat et al., 2003; Mason and Sullivan, 1999; Mason et al., 1998; Cossa et al., 1997; Mason and Fitzgerald, 1993). Total Hg concentrations are more elevated in the ocean surface waters, unless there is ice cover, due to atmospheric deposition with lower concentrations in the upper ocean due to particle scavenging and higher concentrations at depth due to organic remineralization processes (Wang et al., 2012).

Mercury release to the atmosphere occurs directly from natural and anthropogenic sources. Once emitted it can be deposited and then emitted back into the atmosphere from ocean and land surfaces (Lindeberg et al., 2007). Atmospheric deposition of Hg is thought to be the primary source to open oceans (Mason and Sheu, 2002). Anthropogenic activities, primarily attributable to the combustion of fossil fuels (Pacyna et al., 2006), have increased the amount of Hg cycling between the land, atmosphere, and ocean by a factor of three to five (Selin, 2009). Temporal trends of Hg concentrations in hard tissues like hair, teeth and feathers show an order-of-magnitude increase of Hg concentrations in the Arctic that began in the mid- to late-19th century accelerating into the 20th century (Dietz et al., 2011, 2009); however, no consistent temporal trend can be generalized across species and tissues for the entire circumpolar Arctic during the last 30 years (Regit et al., 2011).

Of concern, is the elevated concentrations of Hg in fish and squid due bioaccumulation and biomagnification (Sunderland, 2007; Chen et al., 2008) raising a significant health concern for humans and piscivorous marine mammals like toothed whales (Hong et al., 2012). Methylmercury (MeHg) is the most toxicological relevant species of Hg due to the efficient bioaccumulation (Kraepiel et al., 2003) and biomagnification in the marine food chain (Cossa et al., 2009). Methylmercury (MeHg) can cause damage to nervous, excretory and reproductive systems (Wolfe et al., 1998). Marine mammals bioaccumulate varying concentrations of MeHg depending on species, diet, age, gender, reproductive status, geographic distribution and range of ocean habitat (Evers et al., 2008). MeHg concentrations are largely dependent on water chemistry, which controls MeHg speciation (Mason et al., 1995). The source of oceanic MeHg is currently debated; however, is thought to occur primarily by bacterial activity forming methyl and dimethyl mercury (Kraepiel et al., 2003) in deeper, minimal-oxygen, thermocline waters and brought to the surface through upwelling and diffusive transport (Mason and Sullivan, 1999; Mason and Fitzgerald, 1993). Additionally, a recent discovery found subsurface MeHg concentrations were associated with the nutrient maxima in the Pacific Ocean, Southern Ocean, and Mediterranean Sea suggesting that Hg methylation occurs within oxic ocean domains where organic matter is undergoing remineralization creating MeHg hotspots (Hammerschmidt and Bowman, 2012; Wang et al., 2012; Cossa et al., 2011; Heimbuerger et al., 2010; Cossa et al., 2009; Sunderland et al., 2009; Mason and Fitzgerald, 1990). Importantly, Hg concentrations in the upper 500 m of the water column has been found to vary seasonally with highest concentration in the summer months and lowest values in the winter (Laurier et al., 2004).

Methylmercury exposure to toothed whales is thought to occur primarily through the diet via the gastrointestinal tract, and secondly by absorption through the skin due MeHg's high lipid solubility (Mollenhauer et al., 2009) and the nature of toothed whale skin with its fragile superficial layers and dense subepidermic vascular system (Augier et al., 1993). Exposure can also occur through inhalation and placental and lactational transfer (Lailson-Brito et al., 2012). The diet of toothed whales includes larger and higher trophic level fish and squid. It has been found that fish with increased size and trophic level have higher Hg concentrations (Wiener et al., 2003; Watras et al., 1998). Once inside the body, MeHg crosses intercellular membranes distributing via the blood stream to all internal organs (Clarkson, 1994). In toothed whales, Hg concentrations have been found to be highest typically in liver > kidney > lung > muscle > skin (Lockhart et al., 2005; Frodello et al., 2000; Augier et al., 1993; Andre et al., 1990). Methylmercury can sequester into keratinized structures (Woshner et al., 2008; Wagemann and Kozlowska, 2005) and be eliminated by epidermal sloughing (Brookens et al., 2007). Mercury concentrations in cetacean skin have been found highest in the outermost layer (Wagemann and Kozlowska, 2005).

Marine mammals in general are known for their low susceptibility to Hg toxicity (Lockhart et al., 2005). Selenium (Se), an essential element, occurs at 1:1 molar ratios with Hg in several marine mammal tissues such as liver, kidney and brain (Ikemoto et al., 2004; Bustamante et al., 2003; Itano et al., 1984; Kosta et al., 1975; Koeman et al., 1973) binding strongly to Hg as mercury selenide (HgSe), an inert end product that subsequently bioaccumulates, having a protective effect against Hg toxicity (Khan and Wang, 2009; Yang et al., 2008; Arai et al., 2004; Venugopal and Luckey, 1978). Mercury selenide granules have commonly been found in the liver and kidney of cetaceans (Nigro and Leonzio, 1996; Rawson et al., 1995; Nigro, 1994; Martoja and Berry, 1980). This binding may reduce the bioavailability of Se impacting its biochemical roles (Falnoga and Tusek-Znidaric, 2007) that include antioxidant, antiviral, antimutagenic and anticarcinogenic properties (Schrauzer, 2009) that may make the marine mammal more susceptible to oxidative injury (Ralston and Raymond, 2010; Ralston et al., 2008; Burk et al., 2002).

Mercury has been found to have a clear positive linear correlation with Se in the liver and kidney in toothed whales (Hong et al., 2012; Seixas et al., 2008; Koeman et al., 1973) where there are low levels of MeHg measured, but this correlation is not found in the skin (Hong et al., 2012; Stavros et al., 2007; Lockhart et al., 2005; Augier et al., 1993) where the majority is MeHg (Stavros et al., 2007; Dehn et al., 2006; Wagemann et al., 1998, 1997). This may be because the skin does not have the ability to demethylate MeHg allowing for the formation of HgSe like in the liver and kidney; however, several MeHg–Se compounds have been proposed, but not yet confirmed to exist in vivo, including bis(methylmercuric)selenide, MeHg–selenocysteine (Khan and Wang, 2009) and methylmercury(II) seleno bis(S-glutathionyl) arsenic(III) (Korbas et al., 2008). It is possible these complexes may occur in the skin of toothed whales allowing for attenuation of MeHg toxicity. Additionally, MeHg can bind strongly to amino acids containing sulfur (-SR) such as cysteine forming MeHg-SR complexes (Khan and Wang, 2009; Kaur et al., 2006) and glutathione forming methylmercuric glutathionate (Lemmes et al., 2011).

Se concentrations in the skin of toothed whales are at much higher levels compared to Hg and typically at much higher levels compared to Se concentrations in other tissues (Stavros et al., 2007; Lockhart et al., 2005; Kunito et al., 2002; Yang et al., 2002; Dietz et al., 2000). This excess Se in the skin of cetaceans has been thought to be because they lack hair or glands for excretion (Yang et al., 2002) or because this excess Se is necessary in providing protection against solar ultraviolet radiation effects (Stavros et al., 2007). Even though Se is an essential element being identified in at least 25 seleno-proteins (Papp, 2007) and is known to be essential for proper keratinocyte function and skin development (Sengupta et al., 2010), it can be toxic.

It is unknown if the Se concentrations in the skin of toothed whales are toxic. Additionally, the precise mechanism of Se toxicity is unknown (USHHS, 2003), but is thought to be associated with the formation of free radicals causing DNA damage and its reactivity with thiols altering the function of DNA repair proteins (Letavayova et al., 2006). Se exposure to the marine ecosystem may occur by natural emissions such as volcanic tuff and black slate in some regions (Kunli et al., 2004) and by anthropogenic emissions from combustion of coal and oil, agricultural products and non-ferrous metal melting (Bosco et al., 2005). Approximately, 40% of total Se emissions result from anthropogenic activities (Wen and Carignan, 2007). In surface waters, Se is found mostly as selenate and selenite (Dungan and Frankenberger, 1999), which are both highly bioavailable species allowing for bioaccumulation and biomagnification posing a threat to aquatic wildlife (Lenz and Lens, 2009). Selenium concentrations in fish and lower invertebrate tissues have been found to be 2000 times the amount in the surrounding water (Wu, 2004).

The sperm whale, a toothed whale, is an integral species of the ocean ecosystem and can be utilized in monitoring ocean health. They have a wide distribution inhabiting all oceans providing a relatively unique opportunity for a broad geographic comparison of Hg concentrations (Whitehead, 2003). Sperm whales do not travel in predictable migration routes rather being influenced by prey distribution and suitable breeding habitats (Whitehead, 2003). Adult males journey pole to pole being found to travel 7400 km (Ivashin, 1967) while females stay together with their offspring in pods of 20 to 30 individuals between the 40 parallels being found to travel only 2200 km. Genetic data has found close matrilineal relationships between the members of the pods with some members being long-term companions for at least several years staying together in a certain region. It is believed each region of the world contains sub-populations each having their own genetic variability. Males stay with the pod until they are between 3 and 15 years of age when they break off from the group and form smaller “bachelor groups” with males of about the same age and travel to higher latitudes. As the males get older and larger, they are found solitary even near the poles in both hemispheres coming back to the pods to breed (Whitehead, 2003). For example, the Indian Ocean has sperm whales year round and is hypothesized to have three stocks with one off the southeast coast of Africa; an oceanic stock around Amsterdam and St. Paul Islands and an eastern stock off Western Australia (Gosho et al., 1984).

A sperm whale spends about 60% of its life in foraging dives with each dive lasting 30 to 60 min to depths of 300 to 3000 m (Whitehead, 2003). These dives give this cetacean exposure to not only the air at the water's surface but also a large and wide range in the ocean's water column. Sperm whales are toothed whales, rather than baleen whales, having a lower jaw of cone shaped teeth (Whitehead, 2003) and are high trophic consumers preying primarily on squid and fish (Gosho et al., 1984). Almost all of the Hg present in fish and squid is methylated (Endo et al., 2002). These whales are long-lived, surviving to 70 years or more (Whitehead, 2003). Thus, sperm whales have a high potential for biomagnification and bioaccumulation of Hg. Skin biopsies are an easy and relatively harmless way to determine Hg levels in a high trophic species and exploring its distribution in the oceans. The objectives of this study were to (1) establish a worldwide survey of Hg and Se concentrations in skin samples of free-ranging sperm whales from around the globe; (2) determine whether these element concentrations differ with region, age and gender, (3) evaluate Se to Hg molar ratios and (4) determine if there is a positive correlation of Hg to Se in the skin of sperm whales. It is essential to understand variations in Se:Hg molar ratios before identifying geographical patterns for Hg concentrations in this species (Burger et al., 2012).

2. Methods and materials

We measured total Hg and total Se concentrations in 343 free-ranging sperm whale skin biopsies from 17 regions around the world (Fig. 1) beginning in the Sea of Cortez traveling westward ultimately ending in Massachusetts, USA with 117 samples in the Pacific Ocean, 161 in the Indian Ocean, 30 in the Mediterranean Sea and 35 in the Atlantic Ocean. There were 228 adult female, 53 adult male and 62 subadult male samples.

2.1. Biopsies

Skin samples were collected between 1999 and 2005 using the research vessel, *Odyssey*, from healthy, free-ranging whales taken from the flank with a biopsy dart, as described in Wise et al. (2009). Sampling was carried out with simultaneous photo-identification of the whales minimizing duplication. Briefly, samples were divided into two pieces at the interface between the skin and blubber. The skin was used for metal analysis and genetic analysis. Classification of males as subadult or adult was estimated by the length of the whale. Corresponding classification of females could not be made reliably because of the overall smaller size compared to males.

2.2. Genotyping

Gender was determined by genotyping as published in Wise et al. (2009). DNA was extracted from skin and determined using PCR amplification reactions considering the SRY (male determining factor) gene. The keratin gene was used as an amplification control.

2.3. Inductively coupled plasma mass spectroscopy

We collected skin and blubber tissue in our biopsies; however, used the skin for the analysis of Hg concentrations because the blubber has been found to have very low and even undetectable levels of Hg in our samples (data not shown) and in other studies, which is thought to be due to the lack of thiol-containing proteins with which Hg strongly binds (Hong et al., 2012). Biopsy samples were analyzed for total Hg by EPA Method 1631 and total Se by EPA Method 6020. Due to the extremely small sample mass, averaging 0.023 g, the preparation methods were modified to conserve sample mass. Each sample was rinsed with deionized water, air dried in a laminar flow hood, weighed, placed in a digestion tube with nitric and sulfuric acids and refluxed for 4 h at 95 °C. The sample was cooled and split into two separate aliquots to complete the Hg and Se preparation procedure. The Hg aliquot was oxidized with bromine monochloride, trapped on a gold trap, and purged into a Brooks Rand cold vapor atomic fluorescence spectrometer (Seattle, WA) for analysis. For the Se aliquot, 2 ml deionized (DI) water and 3 ml of trace metal grade hydrogen peroxide were added, and the sample was again heated in the hot block until the effervescence subsided. Samples were cooled, and DI water was added until the final volume equaled 20 ml. The sample was analyzed using a Perkin Elmer/Sciex ELAN inductively coupled plasma/ mass spectrometer (Norwalk, CT) according to standard protocols.

Standard quality assurance procedures were employed. For the Hg analysis, the analysis of duplicate samples had a relative percent difference of 10 ± 8%; the method blanks measured below detection limit ($\mu g\ g^{-1}$); the spiked samples had a 98 ± 12% recovery; laboratory control samples (LCS) had an 89 ± 9% recovery; and the standard reference materials (SRM) (DOLT-3/DORM-2; NRC Canada) had an 85 ± 9% recovery. For the Se analysis, the analysis of duplicate samples had a relative percent difference of 16 ± 1%; the method blanks measured below detection limit; the spiked samples had a 100 ± 9% recovery; LCS had a 101 ± 7% recovery; and the SRM had a 117 ± 17% recovery. All quality control parameters, including all standard reference material data, were within method specifications. Data are presented in units of $\mu g\ g^{-1}$ wet weight (ww). The average analytical limit of detection (LOD) for Hg was 0.0004 $\mu g\ g^{-1}$ ww. For molar ratio analysis, wet weight data were recalculated into nmol/g by the formula: molar concentration ($nmol\ g^{-1}$ ww) = concentration ($\mu g\ g^{-1}$ ww) × 1000 / atomic

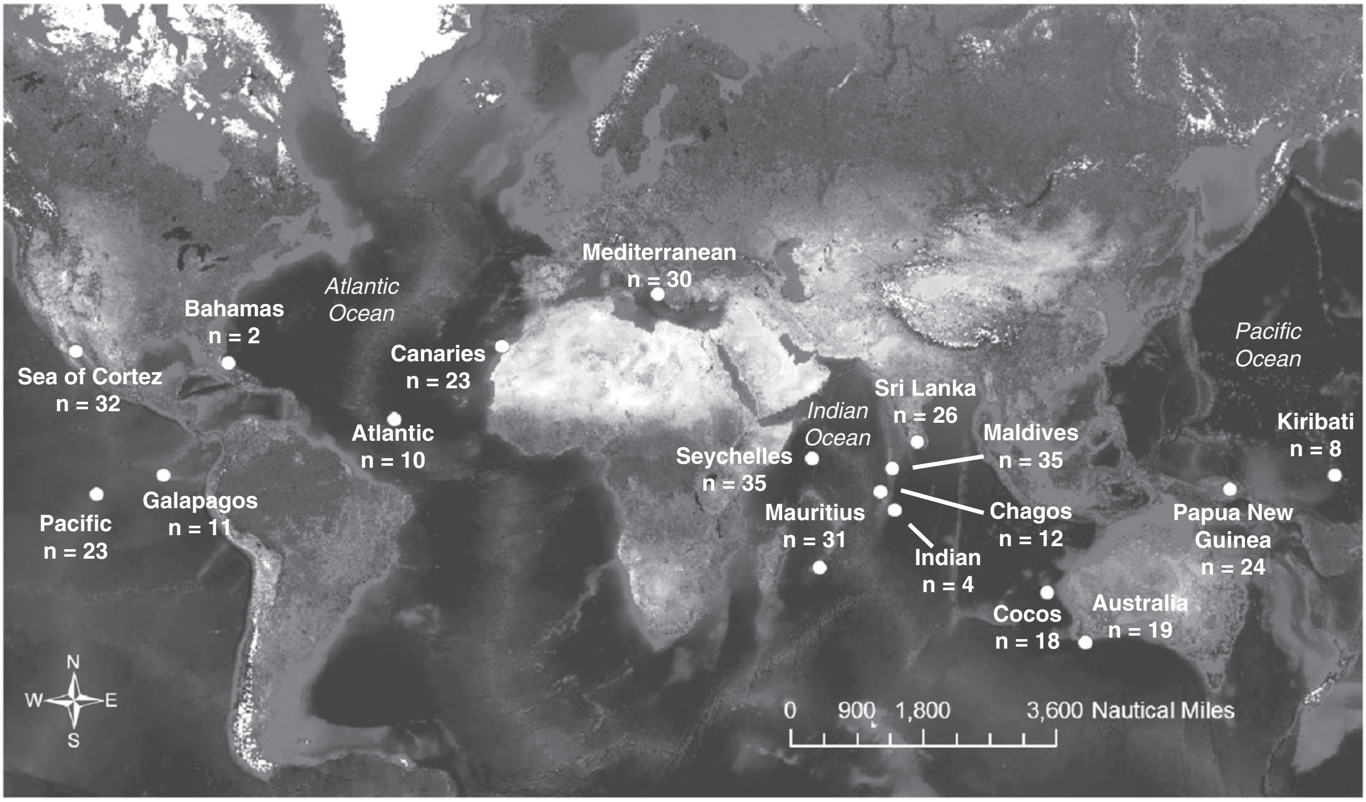

Fig. 1. Map showing the regional distribution of sperm whale samples during the voyage of the Odyssey organized into 17 regions with sample size denoted.

weight (g mol^{-1}). The atomic weights of Hg and Se are 200.59 and 78.96 g mol^{-1}, respectively. When comparing our Hg and Se concentrations to other studies, some data, which was originally presented on a dry weight basis, was converted to approximate wet weight basis by assuming a 75% moisture level.

2.4. Mapping

Whale locations were plotted using ArcGIS 9: ArcMap Version 9.3.1. These maps allowed us to group our samples into geographical regions.

2.5. Statistical analyses.

Mean values were compared using the analysis of variance, and differences for individual pairs of means were assessed for significance using t-tests ($p<0.05$) with Bonferroni correction for multiple comparisons according with our published methods (Wise et al., 2009). When the element was not detected in a specimen, a value of one-half LOD was used in the analysis. The distributions of values were skewed, so a normalizing logarithmic transformation was employed for the statistical analysis. The statistical testing was conducted using SAS 9.2 (SAS Institute, 2004).

3. Results and discussion

3.1. Mercury concentrations compared by region

At a global scale, Hg was present in all but three whales and detectable concentrations ranged from 0.1 to 16.0 μg g^{-1} with a global mean concentration of 2.5 ± 0.1 μg g^{-1} (Table 1). The mean Hg concentration from previous work was 2.9 μg g^{-1} and similar to our global mean (Table 3). The mean Hg concentrations for each of the four general regions of the world, Pacific, Indian, Mediterranean and Atlantic, were 2.3, 2.1, 6.1 and 2.5 μg g^{-1}, respectively. The sperm whale skin samples had mean Hg concentrations that were highest in the Mediterranean Sea > Atlantic Ocean > Pacific Ocean > Indian Ocean. Total and methylated Hg concentrations reported in water samples were highest in the Mediterranean Sea > Pacific Ocean > Atlantic Ocean (Sunderland et al., 2009; Cossa and Coquery, 2005; Horvat et al., 2003). Soerensen et al. (2012) recently found that there has been a 6% decline in surface water Hg concentrations since 1999 in the northern Atlantic Ocean. It is unknown why our samples did not reflect the lowering of Hg levels in seawater in the Atlantic Ocean.

It is well documented that deep-sea top predators of the Mediterranean have higher Hg concentrations compared to other oceans and seas (Saliot, 2005). This “mercury anomaly” may be attributed to the unique geochemical, biochemical and or ecological nature of this area (Cossa and Coquery, 2005; Saliot, 2005; Horvat et al., 2003). First, the most striking difference between Hg in the Mediterranean Sea to other oceans is the difference in speciation of Hg with higher MeHg levels to total Hg ratios in the Mediterranean (Cossa et al., 1997), which may be due to the formation and buildup of MeHg under the thermocline and near the sediment interface (Saliot, 2005). Secondly, Hg enriched minerals from cinnabar ore and hydrothermal activity is abundant in this area and may contribute to the area's high Hg level (Cossa and Coquery, 2005). A third possible explanation may be due to enhanced emissions of Hg from the sea surface. Re-emissions from water surfaces are partly governed by sunlight and temperature, and the warmer climate in the Mediterranean basin would enhance the fluxes from the water to the atmosphere (Wangbery et al., 2001). Fourthly, there may be more active atmospheric transformation processes. Photochemical processes in the marine boundary layer may lead to enhanced oxidation of elemental Hg vapor, which would lead to increased concentrations of reactive gaseous Hg and total particulate Hg via gas–particle interactions (Wangbery et al., 2001). Fifthly, it may be due to enhanced anthropogenic emissions with the Mediterranean Sea being affected not only by

Table 1
Mean and standard deviation of mercury concentrations in sperm whale skin (μg g^{-1}) for specific regions of the Pacific, Indian and Atlantic Oceans and the Mediterranean Sea.

Ocean/sea	Region	Sampling date (Mo/yr)	Sample size	Regional mean		Ocean/sea mean	
				Hg	Se	Hg	Se
Pacific	Sea of Cortez	Sept–Oct 1999	32	2.2 ± 0.4 (0.3–9.7)	42.0 ± 2.9 (8.8–85.1)	2.3	42.8
	Galapagos	April–June 2000	11	2.5 ± 0.5 (0.7–5.4)	27.6 ± 3.0 (17.1–48.9)		
	Pacific Crossing	July–Aug 2000	24	1.8 ± 0.2 (0.4–4.3)	32.7 ± 2.8 (4.1–68.1)		
	Kiribati	Dec 2000	8	2.1 ± 0.5 (0.2–4.9)	74.2 ± 16.6 (27.7–179.0)		
	Papua New Guinea	Feb–May 2001	24	1.7 ± 0.2 (0.1–4.1)	28.4 ± 2.9 (2.5–53.3)		
	Australia	Dec–Mar 2002	19	3.5 ± 0.3 (1.1–6.9)	52.1 ± 5.5 (20.3–124.5)		
Indian	Cocos	April–May 2002	18	2.1 ± 0.2 (0.2–4.7)	42.8 ± 4.8 (6.4–111.0)	2.1	29.7
	Indian Crossing	June–July 2002	4	2.2 ± 0.3 (1.5–3.0)	19.3 ± 3.4 (11.6–26.4)		
	Chagos	June–July 2002	12	2.2 ± 0.1 (1.4–3.0)	31.0 ± 3.4 (14.5–58.3)		
	Seychelles	Sept–Dec 2002	35 (34)[b]	2.0 ± 0.2 (0.1–4.9)	31.9 ± 3.0 (7.0–99.2)		
	Maldives	Jan–Mar 2003 & Feb–Mar 2004	35	2.3 ± 0.3 (0.5–11.9)	34.4 ± 4.9 (9.4–117.0)		
	Sri Lanka	April–May 2003	26	1.3 ± 0.1 (0.8–2.3)	15.7 ± 1.3 (5.9–32.6)		
	Mauritius	Nov–Dec 2003	31	2.5 ± 0.1 (1.3–3.8)	33.5 ± 1.7 (13.3–56.2)		
Med.	Mediterranean[a]	July–Nov 2004	30	6.1 ± 0.7 (0.3–16.0)	38.1 ± 3.4 (15.2–94.0)	6.1	38.1
Atlantic	Canaries	Jan–Feb 2005	23	1.7 ± 0.1 (0.7–3.8)	14.1 ± 1.3 (6.1–26.7)	2.5	17.7
	Atlantic Crossing	May 2005	10	2.0 ± 0.4 (0.4–4.4)	19.5 ± 2.9 (10.7–41.0)		
	Bahamas	July 2005	2	3.8 ± 0.7 (3.1–4.6)	19.5 ± 0.3 (19.2–19.7)		
	Global	Sept 1999–July 2005	343	2.5 ± 0.1 (0.1–16.0)	33.1 ± 1.1 (2.5-179.0)		

[a] Three whales had undetectable levels and one-half LOD, 0.0002 μg g^{-1} ww, was used in analysis.
( ) Sample size for Se data set.

Hg released in its vicinity but also from air masses enriched in Hg from regions of northern and northeastern Europe (Cossa and Coquery, 2005).

A map of the Mediterranean region is shown in Fig. 2 and denotes the 30 samples collected in three distinct areas: Southwestern Basin off the coast of Spain near the Alicante Canyon (Fig. 2A); Northwestern Basin in the Ligurian Sea (The Pelagos Sanctuary) off the coast of France in the area of the Provencal Escarpment (Fig. 2B); and the Ionian Sea off the coast of Greece near the Vavilov Hole (Fig. 2C). Of these whales, the 2 whales in the Southwestern Basin were both subadult males (biopsied November 2004); the 8 in the Northwestern Basin were all males with 5 being adult and 3 subadult (biopsied September 2004); and the 20 in the Ionian Sea were 11 females, 3 adult males and 6 subadult males (biopsied late July and August 2004) with 3 of these males having no detectable Hg. A strong seasonality for MeHg in the water column has been observed in the region with the highest concentrations occurring during the fall bloom when the phytoplankton biomass is dominated by nano- and picophytoplankton allowing for active organic matter remineralization and Hg methylation by the associated microbial communities (Heimbuerger et al., 2010). We biopsied these whales around the time of the fall bloom, so it is possible they were receiving a greater exposure to Hg during this time.

It is peculiar to find the 3 nondetects in this study to all be from males in the region with the highest Hg levels during a sampling time when Hg exposure is at its peak in the region, but not unusual seeing that Hg is not an essential element and finding very low to nondetectable Hg levels in cetacean skin would be expected. Sperm whales slough skin throughout the year; however, this cannot account for the nondetectable levels we found seeing that our biopsy includes a depth of skin all the way to the blubber interface and not just a superficial sampling from the top of the skin. All of the whales we sampled throughout the voyage would be actively sloughing skin no matter the location or season (Whitehead, 2003). Additionally, we sampled in the same area of each whale minimizing the possibility of differing Hg levels in skin being found in different locations on the body; however, research has found there is no significant difference in Hg concentration in respect to anatomical location in dolphins at least in the blubber or liver (Tilbury et al., 1997).

Genetic data suggests that the female and subadult sperm whales in the Mediterranean constitute a separate population from other regions (Engelhaupt et al., 2009) suggesting these whales likely received their Hg exposure within the Mediterranean Sea. However, it is difficult to determine the exact area within the Mediterranean Sea the females and subadult males received their Hg exposure seeing that there is no evidence of sperm whale population fragmentation in the Mediterranean (Engelhaupt et al., 2009), and they have been found to travel between the western and eastern Mediterranean basins through either the Strait of Sicily or the Strait of Messina (Frantzis et al., 2011). The adult sperm whales range over large distances (Whitehead, 2003) and genetic data supports that males have a long-range genetic dispersal probably on an oceanic scale (Engelhaupt et al, 2009), so it is uncertain if these adult males received their exposure from the Mediterranean or elsewhere.

Table 2
Mercury and selenium concentrations (μg g^{-1}) measured in skin by gender, female (F) and male (M) sperm whales, and by age, adult (A) and subadult (SM) male sperm whales. Regions are named for the closest land body or region; data are presented as mean ± standard error with minimum and maximum values in parentheses. ( ) indicates there was one less sample for the Se data set. One-half the detection concentration (0.0002 μg g^{-1}) was used in the analysis.

Region	Sample size				Mercury				Selenium			
	F	M	A	SM	F	M	A	SM	F	M	A	SM
Sea of Cortez	20	12	12	0	2.7 ± 0.5 (0.6–9.7)	1.0 ± 0.2 (0.3–2.4)	1.0 ± 0.2 (0.3–2.4)	–	41.7 ± 4.0 (8.8–85.1)	42.5 ± 4.3 (18.3–69.5)	42.5 ± 4.3 (18.3–69.5)	–
Galapagos	0	11	3	8	–	2.5 ± 0.5 (0.7–5.4)	3.4 ± 1.0 (2.2–5.4)	2.2 ± 0.6 (0.7–5.1)	–	27.6 ± 3.0 (17.1–48.9)	30.8 ± 9.4 (17.1–48.9)	26.3 ± 2.7 (17.8–39.0)
Pacific Crossing	17	6	6	0	1.6 ± 0.2 (0.4–4.2)	2.4 ± 0.5 (0.9–4.3)	2.4 ± 0.5 (0.9–4.3)	–	32.8 ± 3.5 (4.1–68.1)	32.6 ± 5.0 (15.0–46.1)	32.6 ± 5.0 (15.0–46.1)	–
Kiribati	8	0	0	0	2.1 ± 0.5 (0.2–4.9)	–	–	–	74.2 ± 16.6 (27.7–179.0)	–	–	–
Papua New Guinea	14	10	5	5	1.8 ± 0.3 (0.6–4.1)	1.5 ± 0.2 (0.1–2.0)	1.4 ± 0.3 (0.1–2.0)	1.6 ± 0.2 (1.0–2.0)	29.2 ± 3.6 (2.5–53.3)	27.2 ± 5.0 (9.6–48.8)	26.9 ± 7.4 (13.2–48.8)	27.6 ± 0.1 (9.6–47.4)
Australia	10	9	0	9	3.9 ± 0.5 (2.6–6.9)	3.0 ± 0.5 (1.1–5.1)	–	3.0 ± 0.5 (1.1–5.1)	63.3 ± 7.6 (37.2–124.5)	39.6 ± 5.9 (20.3–78.9)	–	39.6 ± 5.9 (20.3–78.9)
Cocos	18	0	0	0	2.1 ± 0.2 (0.2–4.7)	–	–	–	42.8 ± 4.8 (6.4–111.0)	–	–	–
Indian Crossing	0	4	4	0	–	2.1 ± 0.3 (1.5–3.0)	2.1 ± 0.3 (1.5–3.0)	–	–	19.3 ± 3.4 (11.6–26.4)	19.3 ± 3.4 (11.6–26.4)	–
Chagos	0	12	0	12	–	2.2 ± 0.1 (1.4–3.0)	–	2.2 ± 0.1 (1.4–3.0)	–	31.0 ± 3.4 (14.5–58.3)	–	31.1 ± 3.5 (14.5–58.3)
Seychelles	25	12 (11)	6 (5)	4	2.0 ± 0.2 (0.1–4.9)	1.8 ± 0.2 (1.1–3.6)	2.0 ± 0.4 (1.3–3.6)	1.6 ± 0.2 (1.1–2.1)	35.3 ± 3.6 (17.2–99.2)	22.6 ± 3.3 (9.6–38.2)	23.3–4.5 (10.9–34.1)	21.8 ± 0.6 (9.6–38.2)
Maldives	26	9	4	5	2.3 ± 0.5 (0.5–11.9)	2.3 ± 0.2 (1.5–3.2)	2.7 ± 0.2 (2.3–3.2)	1.9 ± 0.2 (1.5–2.6)	36.2 ± 5.7 (10.6–117.1)	29.4 ± 10.1 (9.4–89.9)	12.9 ± 1.7 (9.4–16.9)	42.6 ± 16.4 (13.5–89.9)
Sri Lanka	25	1	1	0	1.3 ± 0.1 (0.8–2.3)	1.5	1.5	–	16.1 ± 1.3 (8.0–32.6)	5.9	5.9	–
Mauritius	28	3	2	1	2.5 ± 0.1 (1.3–3.8)	2.4 ± 0.2 (2.1–2.8)	2.2 ± 0.03 (2.1–2.2)	2.8	33.6 ± 1.7 (13.3–56.2)	32.8 ± 9.7 (23.1–52.2)	23.1 ± 0.02 (23.1–23.2)	52.2
Mediterranean	11	19	8	11	8.4 ± 0.9 (5.6–16.0)	4.7[a] ± 0.7 (0.3–11.6)	4.4[a] ± 1.3 (3.8–11.6)	4.9[a] ± 0.9 (0.3–9.0)	46.6 ± 5.4 (32.0–94.0)	33.2 ± 4.0 (15.2–74.7)	29.3 ± 5.0 (15.2–55.1)	36.1 ± 5.9 (17.5–74.7)
Canaries	6	17	2	4	1.7 ± 0.2 (0.7–3.8)	1.5 ± 0.3 (0.9–2.7)	1.9 ± 0.8 (1.1–2.7)	1.3 ± 0.2 (0.9–1.7)	14.3 ± 1.6 (6.1–26.7)	13.7 ± 2.3 (7.1–22.1)	8.6 ± 1.5 (7.1–10.1)	16.2 ± 2.6 (11.3–22.1)
Atlantic Crossing	8	2	0	2	1.9 ± 0.5 (0.4–4.4)	2.3 ± 1.7 (0.6–4.0)	–	2.3 ± 1.7 (0.6–4.0)	21.0 ± 3.5 (10.7–41.0)	13.7 ± 1.8 (11.9–15.5)	–	13.7 ± 1.8 (11.9–15.5)
Bahamas	1	1	0	1	4.6	3.1	–	3.1	19.7	19.2	–	19.2
Global	**228**	**115**	**53**	**62**	2.4 ± 0.1 (0.1 − −11.6)	2.5 ± 0.2 (0.1 − −11.6)	**2.3 ± 0.3** **(0.1–11.6)**	**2.7 ± 0.2** **(0.3–9.0)**	**34.7 ± 1.5** **(2.5–179.0)**	**30.0 ± 1.6** **(5.9–89.9)**	**28.5 ± 2.1** **(5.9–69.5)**	**31.3 ± 2.3** **(9.6–89.9)**

[a] One or more whales had undetectable Hg concentrations.

Between regions, Hg concentrations were significantly different from each other ($F(16, 326) = 8.03$; $p < 0.0001$) with the Mediterranean having the highest mean Hg concentration at 6.1 ± 0.7 μg g^{-1}, which is similar to the mean for other Mediterranean studies of 5.7 μg g^{-1} (Table 3). Pairwise t-tests showed that whales in the Mediterranean Sea had significantly different Hg levels than those in the Atlantic, Canaries, Chagos, Cocos, Galapagos, Kiribati, Maldives, Mauritius, Pacific, Papua New Guinea, Sea of Cortez and Seychelles. The lowest Hg mean concentration was in Sri Lanka at 1.3 ± 0.1 μg g^{-1}.

Possible reasons for regional differences in Hg concentrations may first be that the region justifiably has higher Hg levels and is a 'hot spot.' However, other uncontrollable factors may influence Hg levels between regions, particularly diet (Bowles, 1999). Deep-water squid are the major food source for sperm whales, and differences in squid species between regions or even a change to consuming more fish in the diet than squid may cause changes in Hg exposure (Frodello et al., 2002). There is limited literature on Hg levels in squid; however, cephalopods in the North Atlantic contained Hg levels between 0.01 to 0.03 μg g^{-1} ww (Law et al., 1997) and in the north eastern Atlantic waters contained 0.01 to 0.9 μg g^{-1} ww (Bustamante et al., 2006), whereas marine fish typically contain higher levels that range from 0.05 to 1 μg g^{-1} ww with most species having < 0.2 μg g^{-1} ww (Carvalho et al., 2002). Secondly, differences between regions may be due to genetic variability causing differences in absorption, distribution, metabolism and or excretion of Hg. Thirdly, some regions may have a higher percentage of the toxicologically relevant form of Hg, MeHg, which biomagnifies up the food chain. MeHg concentrations are largely dependent on water chemistry, which controls MeHg speciation (Mason et al., 1995). Fourthly, we sampled sperm whales along an equatorial radius around the globe throughout the year for 5 years. It is possible there are seasonal differences that may contribute to the differences in Hg levels (Bowles, 1999), and the time of sampling is indicated in Table 1.

3.2. Mercury concentrations compared by gender

We considered Hg concentrations in skin by gender (Table 2). Detectable Hg concentrations in female whales were globally on average 2.4 ± 0.1 μg g^{-1} ranging from 0.1 to 16.0 μg g^{-1} (all females had detectable concentrations) and in males were 2.5 ± 0.2 μg g^{-1} ranging from 0.1 to 11.6 μg g^{-1} (3 male whales had undetectable concentrations of Hg). Overall, the mean Hg concentration for females and males were not significantly different ($F(1,341) = 2.02$; $p = 0.16$).

Although Hg accumulation may be influenced by gender (Stavros et al., 2011), Hg concentrations are expected to be less in females due to the ability to pass their body burden of MeHg to their offspring through the placenta (Storelli and Macrotrigiano, 2000) or lactation (Frodello et al., 2000). Additionally, gender differences may be due to different hormone metabolisms causing a difference in accumulation (Caurant et al., 1996); males having a wider distribution than females with males feeding at the poles and at deeper depths; males being in contact with colder water temperatures where Hg speciation may differ; and males having a different diet preying on larger squid than females, which may have higher Hg concentrations (Whitehead, 2003). However, several studies including this one have found the skin of toothed whales do not have significant gender related

Table 3
Mercury concentrations ($\mu g\ g^{-1}$) reported in the skin of toothed whales.

Species	Date	Location	Condition	n	Total Hg ($\mu g\ g^{-1}$ ww)	Reference
Oceanic dolphin						
Bottlenose dolphin	1993–2001	Israel (Mediterranean)	Stranded	13	4.2	Roditi-Elasar et al. (2003)
Bottlenose dolphin	1998–2002	Portugal	Stranded	2	2.9	Carvalho et al. (2002)
Bottlenose dolphin	2000–2007	South Carolina	Stranded	12	0.5	Stavros et al. (2011)
Bottlenose dolphin	2003–2005	Sarasota Bay, FL	Free-ranging	54	2.2	Woshner et al. (2008)
Bottlenose dolphin	2004–2005	South Carolina	Free-ranging	74	0.4	Stavros et al. (2007)
Bottlenose dolphin	2003–2004	Sarasota Bay, FL	Free-ranging	35	2.2	Miller et al. (2011)
Common dolphin	1998–2002	Portugal	Stranded	15	0.7	Carvalho et al. (2002)
Long-finned pilot whale	1993	Corisca (Mediterranean)	Stranded	1	6.8	Frodello et al. (2000)
Risso's dolphin	1996	Corisca (Mediterranean)	Stranded	1	7.8	Frodello et al. (2000)
Short-beaked common dolphin	1994	Corisca (Mediterranean)	Stranded	1	3.8	Frodello et al. (2000)
Striped dolphin	1988–1990	France (Mediterranean)	Stranded	13	10.7	Augier et al. (1993)
Striped dolphin	1990–1993	Spain (Mediterranean)	Stranded	34	2.7	Monaci et al. (1998)
Striped dolphin	1990–1993	Italy (Mediterranean)	Stranded	55	2.5	Monaci et al. (1998)
Striped dolphin	1993–2001	Israel (Mediterranean)	Stranded	5	5.1	Roditi-Elasar et al. (2003)
Striped dolphin	1994	Corisca (Mediterranean)	Stranded	1	4.8	Frodello et al. (2000)
Narwhal and Beluga						
Beluga	1981–2002	Canadian Arctic	Hunted	176	0.7	Lockhart et al. (2005)
Beluga	1981–2002	Alaska	Hunted	11	0.6	Woshner et al. (2001)
Beluga	1993	Canadian Arctic	Hunted	27	1.4	Wagemann and Kozlowska (2005)
Beluga	1993–1994	Western Canadian Arctic	Hunted	65	0.8	Wagemann et al. (1998)
Beluga	1993–1994	Eastern Canadian Arctic	Hunted	45	0.6	Wagemann et al. (1998)
Narwhal	1992–1994	Eastern Canadian Arctic	Hunted	48	0.6	Wagemann et al. (1998)
Porpoise						
Dall's porpoise	2000	Japan	Harpoon Fishery	1	1.2	Yang et al. (2006)
Harbor porpoise	1988–1989	Greenland	Hunting	34	0.5	Paludan-Muller et al. (1993)
Sperm whale						
Sperm whale	2000–2005	Worldwide	Free-ranging	343	2.5	This study
Sperm whale	2000–2005	Mediterranean	Free-ranging	30	6.1	This study
Summary						
Toothed whales	1981–2007	Worldwide	Free-ranging, stranded or hunted	689	2.9	14 different studies
Toothed whales	1988–2001	Mediterranean	Stranded	124	5.4	4 different studies

differences in Hg concentrations (Woshner et al., 2008, 2001; Monaci et al., 1998; Paludan-Muller et al., 1993). It is possible that gender related differences just are not reflected in the skin of toothed whales. For our study, there is also the possibility that since we were unable to determine age of the females we sampled that they were overall older than the males and feeding at a higher trophic level, which may mask any gender differences in Hg concentrations (Woshner et al., 2008).

Females had significantly different skin Hg concentrations between regions ($F(13,214)=8.59$; $p<0.0001$) with the highest mean Hg concentration in the Mediterranean Sea with $8.4 \pm 0.9\ \mu g\ g^{-1}$ ranging from 5.6 to 16.0 $\mu g\ g^{-1}$. Pairwise t-tests found that females in the Mediterranean had significantly different Hg concentrations compared to all other regions that had female samples, except Australia ($p=0.30$) and the Bahamas ($p=1.00$). The region with the lowest mean Hg concentration for females was in Sri Lanka with a mean of $1.3 \pm 0.1\ \mu g\ g^{-1}$ ranging from 0.8 to 2.3 $\mu g\ g^{-1}$. Pairwise t-tests found that females in Sri Lanka had significantly different Hg concentrations than the Mediterranean, Mauritius and Australia ($p>0.05$). It is possible some of the differences in Hg concentrations between regions for females could be due to genetic variability (Whitehead, 2003) or that some females were lactating or pregnant, which may alter Hg concentrations (Caurant et al., 1996). Nielsen et al. (2000) found that pregnant pilot whales had less Hg in their serum compared to non-pregnant female pilot whales. This may be due to elimination of Hg through transplacental transfer of Hg to the fetus or a change in diet. In the case of pilot whales, lactating pilot whales ate a larger proportion of fish in their diet whereas squid is normally the major food source (Nielsen et al., 2000).

The variation among regions for males was not significantly different ($F(14,100)=0.42$; $p=0.97$). However, the region with the highest mean Hg concentration for males was in the Mediterranean with $4.7 \pm 0.7\ \mu g\ g^{-1}$ ranging from 0.3 to 11.6 $\mu g\ g^{-1}$. Males had the lowest mean Hg concentration in the Sea of Cortez with $1.0 \pm 0.2\ \mu g\ g^{-1}$ ranging from 0.3 to 2.4 $\mu g\ g^{-1}$.

Males may not have differences in Hg concentrations between regions because of their wide habitat range that increases with age. Considering the high mobility of male sperm whales, the levels of Hg concentrations in their skin may reflect the general contamination of the large and poorly defined area in which they live (Pompe-Gotal et al., 2009) and not our smaller defined regions.

3.3. Mercury concentrations compared by age

We examined the effect of age in male sperm whales considering adults ($n=53$) and subadults ($n=62$) (Table 2). The global mean Hg concentration for adult males was $2.3 \pm 0.3\ \mu g\ g^{-1}$ ranging from 0.1 to 11.6 $\mu g\ g^{-1}$ and for subadult males was $2.7 \pm 0.2\ \mu g\ g^{-1}$ ranging from 0.3 to 9.0 $\mu g\ g^{-1}$. Adult males and subadult males did not have significantly different Hg concentrations ($F(1,113)=1.63$; $p=0.20$). When controlling for region, adult males were still not significantly different between region ($F(10,42)=0.50$; $p=0.88$) and subadult males were also not significantly different between regions ($F(10,51)=0.14$; $p=1.00$).

Studies have considered the most important biotic parameter to be considered is the age of marine mammals, since Hg potentially accumulates throughout life (Pompe-Gotal et al., 2009; Caurant et al., 1996). Several studies have found a positive correlation between Hg

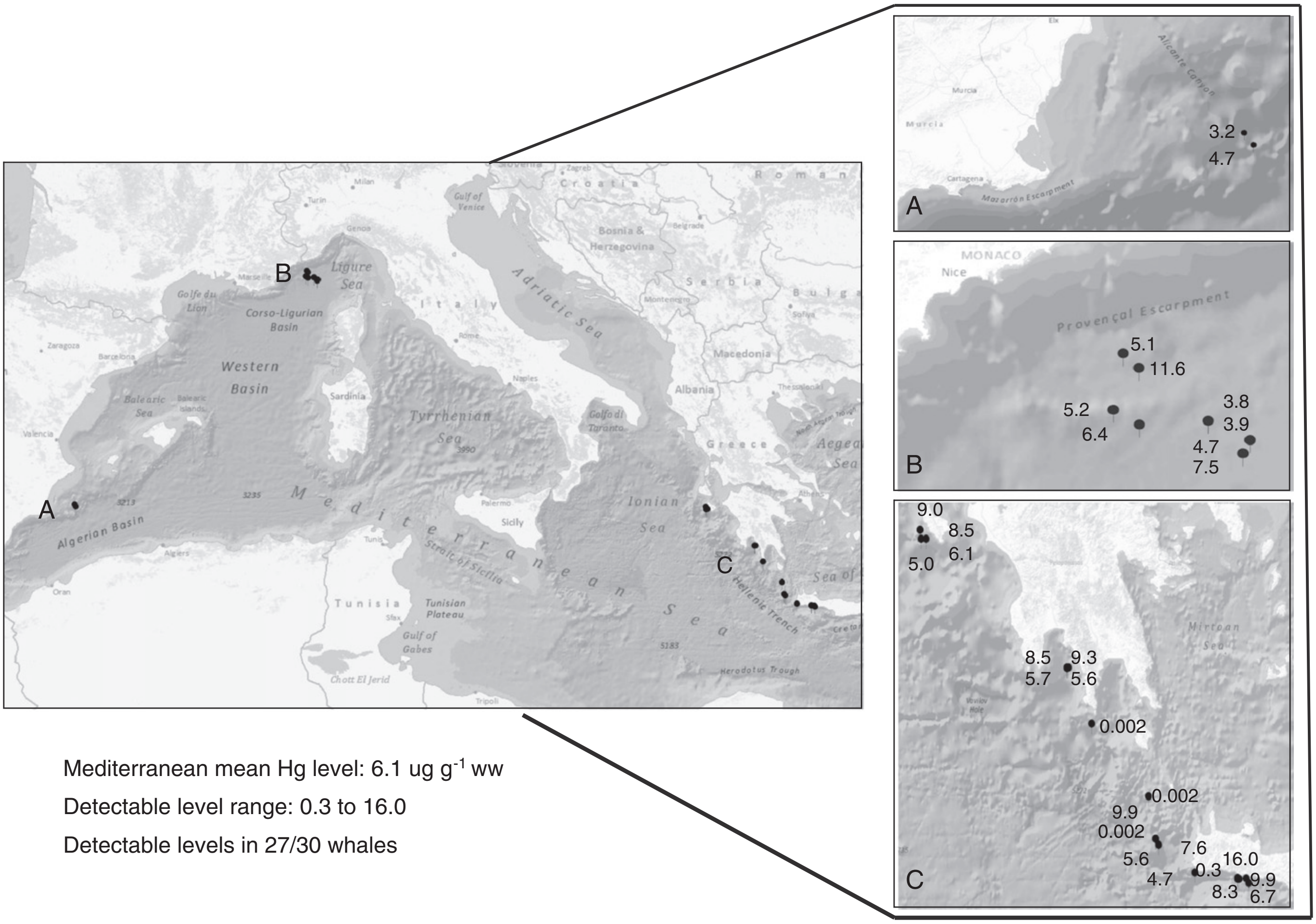

Fig 2. Map showing the distribution of sampling locations in the Mediterranean Sea, and the mercury concentrations in skin (μg g^{-1}) measured for sperm whales collected near the coast of A) Spain (n = 2), B) France (n = 8) and C) Greece (n = 20).

concentrations with age in the skin of dolphins, belugas and narwhals, which are all toothed whales (Miller et al., 2011; Stavros et al., 2011; Woshner et al., 2008; Lockhart et al., 2005; Wagemann and Kozlowska, 2005; Roditi-Elasar et al., 2003; Woshner et al., 2001; Monaci et al., 1998; Wagemann et al., 1998), and this was thought to be due to the bioaccumulation of Hg from birth and the fact that larger whales eat bigger and higher-trophic prey that contain greater amounts of Hg (Miller et al., 2011). It is unclear why our study did not find a correlation of Hg with age. Stavros et al. (2007) also found that a population of dolphins did not have a positive relationship between Hg in skin and age and suggested the extensive ecosystem where they sampled dolphins may create a difference in dietary exposure, which could obscure finding similar correlations between total Hg and dolphin age. This may be the similar case with our study. We sampled male sperm whales in an equatorial radius around the globe. This extensive range may have caused differences in dietary exposure obscuring a positive correlation between Hg and age. It is also possible that there is not a positive age correlation of Hg levels in sperm whale skin.

Between regions, adult males were not significantly different from one another (F(10,42) = 0.50; p = 2.1), and subadult males were also not significantly different from one another (F(10,51) = 0.14; p = 1.00) (Table 2). The highest mean Hg concentration by region for male adults was in the Mediterranean at 4.4 ± 1.3 μg g^{-1} ranging from 3.8 to 11.6 μg g^{-1} and for subadults in the Mediterranean at 4.9 ± 0.9 μg g^{-1} ranging from 0.3 to 9.0 μg g^{-1}. The lowest mean Hg concentration for male adults was in the Sea of Cortez at 1.0 ± 0.2 μg g^{-1} ranging from 0.3–2.4 μg g^{-1} and for subadults in the Canaries at 1.3 ± 0.2 μg g^{-1} ranging from 0.9 to 1.7 μg g^{-1}. We also considered simultaneously gender, age and region in a linear regression model. Consistent with the early analysis we found a significant effect for region (F(16, 324): 8.62; p<0.001) but not for age (F(1,324): 0.13; p = 0.71). However, in this model, gender (F(1,324): 5.21; p = 0.02) was found to be significant.

3.4. Selenium concentrations in the skin

Selenium was present in all whales (Table 1). The bioavailability of Se to these whales occurs most likely through ingestion (Caurant et al., 1996) where absorption is unregulated leading to 50–90% of dietary Se being utilized. Homeostasis of Se is maintained by excretion (Burk et al., 2002). Detectable concentrations of Se ranged from 2.5 to 179.0 μg g^{-1}, and the global mean concentrations was 33.1 ± 1.1 μg g^{-1} for Se. Our global mean for Se concentrations in sperm whale skin was higher than the mean Se concentration calculated from 15 other studies (Table 4). Sperm whales appear to accumulate higher levels of Se in their skin compared to other toothed whales. The concentrations for the four general regions of the world, Pacific, Indian, Mediterranean and were 42.8, 29.7, 38.1 and 17.7 μg g^{-1}, respectively. Se concentrations were significantly different in some regions compared to others (Table 1; F(16, 325) = 11.09; p<0.0001). The highest mean concentration of Se was in Kiribati at 74.2 ± 16.6 μg g^{-1} and Australia at 52.1 ± 5.5 μg g^{-1}. The lowest mean Se concentrations were found in the Canaries at 14.1 ± 1.3 μg g^{-1} and Sri Lanka at 15.7 ± 1.3 μg g^{-1}.

Table 4
Selenium concentrations (μg g^{-1}) of Se in the skin of toothed whales.

Species	Date	Location	Condition	n	Total Se (μg g^{-1} ww)	Reference
Oceanic dolphin						
Bottlenose dolphin	1998–2002	Portugal	Stranded	2	15.0	Carvalho et al. (2002)
Bottlenose dolphin	2000–2007	South Carolina	Stranded	12	6.6	Stavros et al. (2011)
Bottlenose dolphin	2002–2004	Sarasota Bay, FL	Free-ranging	40	5.5	Bryan et al. (2007)
Bottlenose dolphin	2003–2005	Sarasota Bay, FL	Free-ranging	47	5.5	Woshner et al. (2008)
Bottlenose dolphin	2004–2005	South Carolina	Free-ranging	74	6.0	Stavros et al. (2007)
Bottlenose dolphin	2003–2004	Sarasota Bay, FL	Free-ranging	32	5.4	Miller et al. (2011)
Common dolphin	1998–2002	Portugal	Stranded	15	16.3	Carvalho et al. (2002)
Striped dolphin	1988–1990	Mediterranean	Stranded	13	14.4	Augier et al. (1993)
Striped dolphin	1990–1993	Mediterranean (Spain)	Stranded	34	20.3	Monaci et al. (1998)
Striped dolphin	1990–1993	Mediterranean (Italy)	Stranded	15	26.9	Monaci et al. (1998)
Narwhal and Beluga						
Beluga	1981–2002	Canadian Arctic	Hunted	176	4.9	Lockhart et al. (2005)
Beluga	1983–1997	Alaska	Hunted	17	9.6	Woshner et al. (2001)
Beluga	1984–1994	Eastern Arctic	Hunted	45	4.8	Wagemann et al. (1996)
Beluga	1993–1994	Western Arctic	Hunted	55	4.0	Wagemann et al. (1996)
Porpoise						
Dall's porpoise	2000	Japan	Harpoon Fishery	1	78.1	Yang et al. (2006)
Sperm whale						
Sperm whale	2000–2005	Worldwide	Free-ranging	342	33.1	This study
Global mean					**14.9**	**All studies**

Female whales had a global mean Se concentration of 34.7 ± 1.5 μg g^{-1} ranging from 2.5 to 179.0 μg g^{-1}, and males had a global mean of 30.0 ± 1.6 μg g^{-1} ranging from 5.9 to 89.9 μg g^{-1} (Table 2). Se levels in female and male whales were not significantly different ($F(1,340) = 1.94$; $p = 0.17$) even when females were compared to adult males ($F(1, 278) = 2.58$; $p = 0.11$) and to subadult males ($F(1, 288) = 0.31$; $p = 0.58$). The variation among regions for females was significantly different ($F(13,214) = 11.52$; $p < 0.0001$). The region with the highest mean Se concentration for females was in Kiribati at 74.2 ± 16.6 μg g^{-1} ranging from 27.7 to 179.0 μg g^{-1}. Pairwise t-tests found that females near Kiribati had significantly different Se concentrations than females in the Atlantic, Canaries, Maldives, Pacific, Papua New Guinea and Sri Lanka ($p < 0.05$). The region with the lowest Se concentration for females was in the Canaries with 14.3 ± 1.6 μg g^{-1} ranging from 6.1 to 26.7 μg g^{-1}. Pairwise t-tests found that females in the Canaries had significantly different Se concentrations compared to females in the Australia, Cocos, Kiribati, Maldives, Mauritius, Mediterranean, Pacific, Sea of Cortez, Seychelles ($p < 0.05$). The variation among regions for males was significantly different ($F(14,99) = 3.52$; $p < 0.0001$). Males had the highest regional mean Se concentration in the Sea of Cortez with 42.5 ± 4.3 μg g^{-1} ranging from 18.3 to 69.5 μg g^{-1}. Pairwise t-tests found that males in the Sea of Cortez had significantly different Se concentrations than males in the Canaries ($p = 0.001$). Males had the lowest regional mean Se concentration in Sri Lanka at 5.9 μg g^{-1} ($n = 1$). All the other regions had a mix of males and females in our study. When controlling for region in the Se data set, females versus males ($F(1,323) = 2.83$; $p = 0.09$) and females versus adult males ($F(1,263) = 4.17$; $p = 0.04$) were still not significantly different.

Global mean Se concentration for adult males was 28.5 ± 2.1 μg g^{-1} ranging from 5.9 to 69.5 μg g^{-1} for adults and for subadults is 31.3 ± 2.3 μg g^{-1} ranging from 9.6 to 89.9 μg g^{-1} (Table 2). Adult males and subadult males did not have significantly different Se concentrations ($F(1,111) = 1.15$; $p = 0.29$). Between regions, adult males were significantly different ($F(10,41) = 4.98$; $p = 0.0001$). The highest mean regional Se concentration for adult males was in the Sea of Cortez at 42.5 ± 4.3 μg g^{-1} ranging from 18.3 to 69.5 μg g^{-1} and for subadults was in Mauritius at 52.2 μg g^{-1} ($n = 1$). The lowest mean Se concentration for male adults was in Sri Lanka at 5.9 μg g^{-1} ($n = 1$) and for subadults in the Atlantic at 13.7 ± 1.8 μg g^{-1} ranging from 11.9 to 15.5 μg g^{-1}. Pairwise t-tests found that the Sea of Cortez adult males had significantly different Se concentrations than the Canaries, Maldives and Sri Lanka adult males (p value < 0.05). Between regions, subadult males were not significantly different ($F(9,51) = 2.00$; $p = 0.06$). We then considered simultaneously gender, age and region in a linear regression model for Se levels. Consistent with the early analysis we found a significant effect for gender ($F(1,323)$: 8.87; $p = 0.003$) and for region ($F(16, 324)$: 11.86; $p < 0.0001$) but not for age ($F(1,324)$: 0.56; $p = 0.46$).

3.5. Selenium:mercury molar ratios

The exact Se:Hg ratio that protects against toxicity is a controversial issue; however, it is important for risk assessment (Burger et al., 2012). Our data show ratios of Se to Hg well above 1:1 suggesting a protective effect (Fig. 3). The global mean Se:Hg ratio was 59:1 having no molar ratios below 1:1. The skin biopsy with the ratio closest to 1:1 was in the Pacific with 3:1, and the furthermost was in Papua New Guinea with 1719:1. The region with the closest Se:Hg ratio to 1:1 was the Bahamas with 3:1 and the Mediterranean Sea with 14:1.

There was a great deal of variation of Se:Hg among individuals, which may be a result of differences in prey, the proportion of different prey items in the diet, and foraging location (Burger et al., 2012). The actual Se:Hg ratio that is protective is unknown (Ralston et al., 2008; Burger and Gochfeld, 2012), but it has been suggested that Se:Hg molar ratios above 1 are protective against adverse mercury effects (Ralston et al., 2008; Peterson et al., 2009). However, there is no biological basis for determining an exact Se:Hg ratio that is protective; indeed Se binds several other cations as mentioned, and any protection seen would be relative (Lemire et al., 2010). Future work will investigate the possible antagonistic effects of Se on Hg-induced toxicity in sperm whale skin cells.

3.6. Selenium and mercury in sperm whale skin

The positive correlation between Se and Hg in cetaceans was first documented by Koeman et al. (1973) in adult dolphins, porpoises and seals (Augier et al., 1993); however has not been found in the skin as

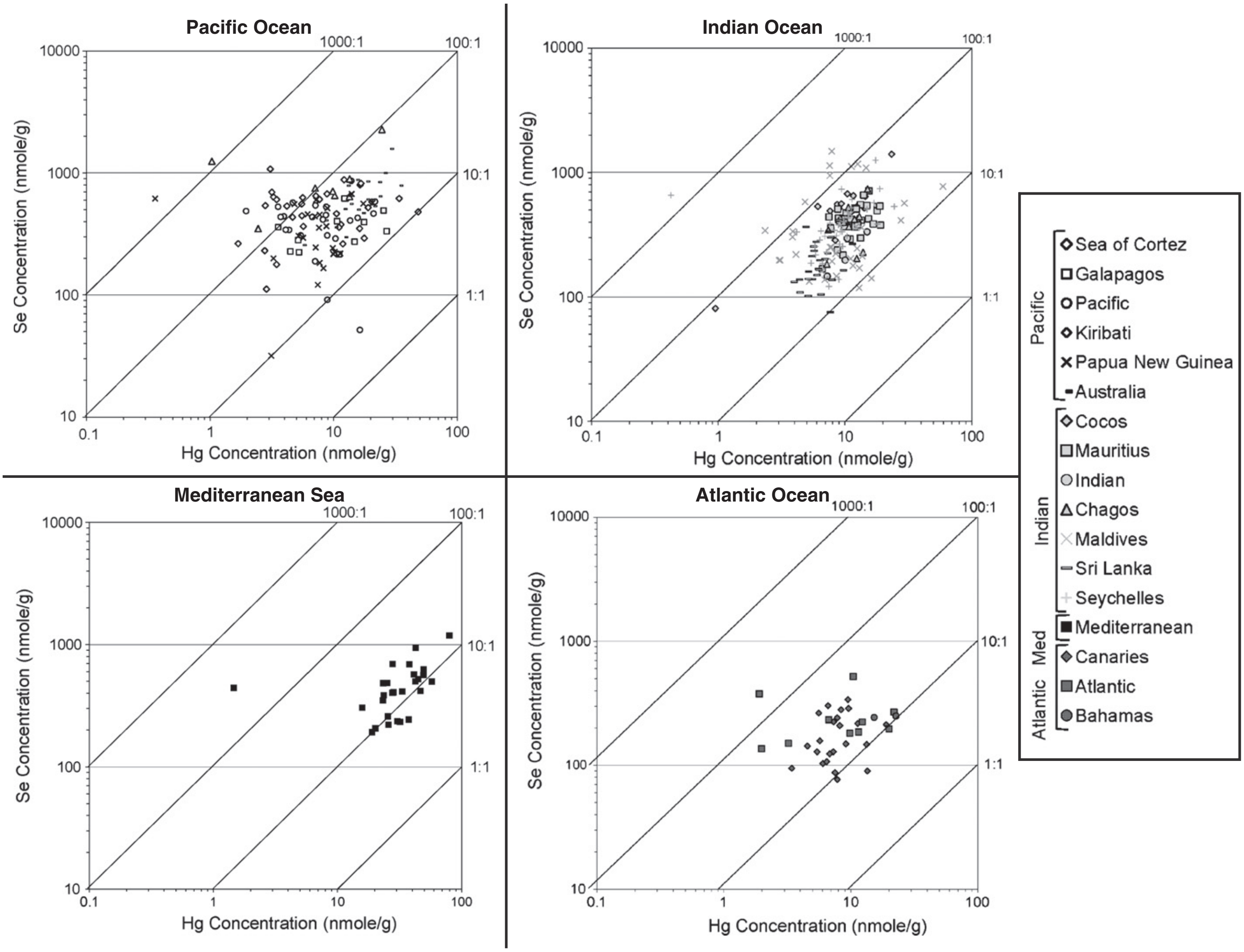

Fig. 3. Molar ratios of Se:Hg per sample by region: Pacific, Indian, Mediterranean and Atlantic Ocean/Sea. Diagonal lines represent varying molar ratios from 1:1 to 1000:1.

mentioned. In our study, we also did not find a positive correlation between Se and Hg concentrations in our samples, so that with increasing Hg concentration we did not necessarily find an increase in Se concentrations.

3.7. Comparison of selenium in skin samples

The sperm whales in this study had an enrichment of Se in their skin, which is in agreement with other studies of Se levels in the skin of cetaceans (Stavros et al., 2007; Lockhart et al., 2005; Kunito et al., 2002; Yang et al., 2002). Additionally, Dietz et al. (2000) found toothed whale skin had the most enriched concentration of Se in the skin compared to 48 other Greenland marine species.

In cetaceans, skin is reported to accumulate Se because they lack hair or glands for excretion (Yang et al., 2002) and because Se may provide protection for dolphins against solar ultraviolet A (UVA) radiation effects (Stavros et al., 2007) and other toxic metals such as arsenic (As) (Zwolak and Zaporowska, 2012; Korbas et al., 2008), cadmium (Cd) (Zwolak and Zaporowska, 2012), silver (Ag) (Nakazawa et al., 2011; Jonas et al., 2007), chromium (Cr) (Soudani et al., 2011a,b; Schrauzer, 2006), iron, vanadium (Zwolak and Zaporowska, 2012), bismuth, cobalt, copper, iron, lead, manganese, molybdenum, nickel, tin and zinc (Schrauzer, 2009). The excess Se may also be just for normal functioning of the skin and to increase skin density and thickness along with improving surface parameters like scaling and roughness (Sengupta et al., 2010) especially seeing that sperm whale slough skin continuously throughout the year (Whitehead, 2003; Richard et al., 1996).

3.8. Skin Hg concentrations as a proxy for predicting concentrations in other organs

We used a nonlethal sampling method to measure body burdens of Hg by biopsying skin. Previous work has shown that skin does not typically have the highest Hg concentrations in tissues of toothed whales with values being highest in liver and kidney (Hong et al., 2012; Lockhart et al., 2005; Frodello et al., 2000; Augier et al., 1993; Andre et al., 1990). This suggests that the Hg concentrations in the liver and kidney of the whales we biopsied may have higher Hg values than reported for skin. In Dall's porpoises, a positive correlation of Hg concentration between skin and liver suggests that skin could be used as a tool for monitoring liver Hg concentrations (Yang et al., 2002). The observed effects level of Hg in whale liver is about 65 μg g^{-1} ww, and toothed whales have Hg levels that are approaching or exceed this toxicological threshold (AMAP, 2011). It is unclear if our sperm whale samples had liver concentrations approaching this threshold.

4. Conclusions

Oceanic Hg pollution is a widespread concern due to its global distribution and potential for toxicity, bioaccumulation and biomagnification; however, data is limited on Hg distribution in the oceans. From 17 regions analyzed, the highest Hg concentrations were found in the Mediterranean, and these were similar to other studies of deep-sea top predators in the region. The females and sub-adult male sperm whales from this region are thought to stay within the Mediterranean Sea suggesting they received the Hg exposure within the region; however, their wide distribution within the area makes it difficult to determine the exact location of the exposure. The adult male sperm whales travel widely between pole to pole with no exact migration route making it very difficult to determine the location of where they received exposure. The Mediterranean is thought to have the highest Hg concentrations due to geochemical, biochemical or ecological origin indicative of the area. Our skin biopsies did not find significant gender differences in Hg levels in agreement with several other studies examining concentrations in toothed whale skin. We also did not find a significant difference in Hg concentration between ages for males, which was similar to findings in one other study of toothed whales. It is possible the extensive habitat of the adult male sperm whales may obscure the positive correlation between Hg concentrations with age in the skin of sperm whales. Further genetic analysis of the whales in our data set may give insight into the possible location of Hg exposure for these whales.

The Se concentrations found in these samples suggest a protective effect of Hg toxicity with Se:Hg molar ratios being well over 1:1; however, there is no biological basis for determining an exact Se:Hg ratio that is protective especially seeing that Se binds to several other cations. This enrichment of Se in the skin is in agreement with other studies in cetacean skin. There was not a positive correlation found between Hg and Se concentrations, and it is possible Hg in the skin of sperm whales binds also to sulfur containing amino acids allowing for attenuation of Hg toxicity. It is unclear the possible toxicity these Hg levels may induce on these whales; however, marine mammals are known to have an apparent tolerance to Hg. Further, cell culture analysis may shed light on the possible toxicity of Hg at these concentrations in sperm whale skin cells cultured during this voyage and if Se provides a protective effect. Hg concentrations in the skin are known to be less than levels found in the liver and kidney of toothed whales suggesting the concentrations of Hg in the liver and kidney of these sperm whales we sampled are higher than what we report in the skin.

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