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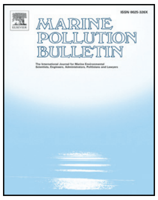

# A survey of trace element distribution in tissues of the dwarf sperm whale (*Kogia sima*) stranded along the South Carolina coast from 1990–2011

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## ABSTRACT

Few studies report trace elements in dwarf sperm whale (*Kogia sima*). As high trophic level predators, marine mammals are exposed through diet to environmental contaminants including metals from anthropogenic sources. Inputs of Hg, Pb, and Cd are of particular concern due to toxicity and potential for atmospheric dispersion and subsequent biomagnification. Liver and kidney tissues of stranded *K. sima* from coastal South Carolina, USA, were analyzed for 22 trace elements. Age-related correlations with tissue concentrations were found for some metals. Mean molar ratio of Hg:Se varied with age with higher ratios found in adult males. Maximum concentrations of Cd and Hg in both tissues exceeded historical FDA levels of concern, but none exceeded the minimum 100 μg/g Hg threshold for hepatic damage. Tissue concentrations of some metals associated with contamination were low, suggesting that anthropogenic input may not be a significant source of some metals for these pelagic marine mammals.

The dwarf sperm whale (*Kogia sima*) is generally found living over the continental shelf and slope adjacent to tropical and temperate ocean coasts (Rice, 1998 and McAlpine, 2009). They appear to feed primarily on deep-water cephalopods, as well as other prey types, including fish and crustaceans (Aguiar dos Santos and Haimovici, 2001; Jefferson et al., 1993; McAlpine, 2009). Adult male dwarf sperm whales generally weigh between 135 and 272 kg and may reach lengths of up to 270 cm. Adult females may be slightly smaller than males (Plön, 2004).

Dwarf sperm whales (*K. sima*) are the third most frequently stranded marine mammal in South Carolina, USA and usually strand as either single animals or mother–calf/juvenile pairs (Coastal Carolina University, 2014). Between 1990 and 2011, twelve dwarf sperm whales stranded along the South Carolina coast. Because of difficulty in positively distinguishing the dwarf sperm whale at sea, much of current knowledge about *K. sima* is based on stranded individuals or those taken by fisheries (Caldwell and Caldwell, 1989). The International Union for Conservation of Nature (IUCN) classifies *K. sima* as a “data deficient species” on its Red List of Threatened Species; there are insufficient data to make an assessment of its risk of extinction (Taylor et al., 2012) .

In addition to uncertainty in regard to the status of dwarf sperm whale populations, there is concern for their health (Taylor et al., 2007 and Ross, 2006). Due to their nearshore distribution, *K. sima* may be susceptible to human activities and pollution (Culik, 2011), including the ingestion of marine debris, potential for ship strikes, and fish net entanglement. Unexplained strandings in the Gulf of Mexico and the Atlantic coast of Florida have warranted further concern about the population (Waring et al., 2006). Exposure to pollutants can result in oxidative stress which has been linked to the development of disease. Stranded Kogiids have shown degenerative heart disease, immune system deficiencies, and heavy parasite infestations (Culik, 2011; Bossart et al., 2007; Bossart, 2011).

Increased human activity in recent decades has accelerated inputs of contaminants, including metals, to the marine environment. As a long-lived apex predator, *K. sima* may act as an indicator of marine pollution with the potential to accumulate significant quantities of contaminants including metals. Heavy metals are well known environmental pollutants that accumulate in the bodies of odontocetes and have potential to constitute a toxicological risk for the species (Caurant et al., 1996; Endo et al., 2002; Haraguchi et al., 2000; Simmonds et al., 2002; Stavros et al., 2011). Seawater enrichment with heavy metals from natural occurrence or from anthropogenic impact may lead to an increase of metal burdens entering marine food chains (Fernandez et al., 2007). The impact of metal contamination is not limited to coastal waters; elevated concentrations of heavy metals (Co, Cr, and Ni) have been reported in a cephalopod (*Nautilus macromphalus*) that inhabits depths of several hundred meters (Bustamante et al., 2000).

Although metals generally occur at low concentrations in the oceans, marine organisms bioaccumulate trace elements with significant increase of metal burdens through the food web, especially toxic elements such as Hg or Cd (Gerpe et al., 2006; Meador et al., 1999). In

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odontocentes, Cd has been known to accumulate in the internal organs (Honda et al., 1983; Endo et al., 2002; Gerpe et al., 2006; Holsbeek et al., 1998) and bind to metallothioneins (Das et al., 2000). Although Hg has a high affinity for metallothioneins, only a small amount of total Hg has been shown to be bound to metallothioneins in marine mammals (Caurant et al., 1996; Wagemann et al., 1998; Holsbeek et al., 1998).

Marine mammals are principally exposed to methylHg through diet. The Hg present in fish and squid is methylated (Caurant et al., 1996; Das et al., 2000; Gerpe et al., 2006), but the majority of Hg accumulated in marine mammal internal organs is inorganic mercury (Wagemann et al., 1998 and Cardellicchio et al., 2002). Demethylation of methylHg, followed by the formation of a less toxic Hg-Se complex, is thought to occur in cetacean livers as a protective physiological mechanism (Das et al., 2000 and Endo et al., 2004). An imbalance of Hg and Se can lead to oxidative stress that can impair protein function, damage DNA, and damage membrane lipids leading to onset of disease (Arteel and Sies, 2001). Increasing molar ratios of Hg:Se with increasing Hg and increasing age have been documented in dolphins and correlated with the progression of heart disease in adult *Kogia breviceps* males (Itano et al., 1984; Bryan et al., 2012).

To date, few studies have reported metal concentrations in *Kogiid* tissues. The aim of the present study was to examine the distribution of major and minor trace elements in the liver and kidney of *K. sima*. Results are contrasted among organ type, age class, size, and gender to provide information on heavy metal concentrations in a top marine predator. Because Se is recognized to decrease toxicity of Hg, molar ratios of Hg:Se are compared for gender and age in liver samples.

Stranded dwarf sperm whales, *K. sima*, were collected along the South Carolina coast from 1990 – 2011 by staff from the National Oceanic and Atmospheric Administration's (NOAA) Center for Coastal Environmental Health and Biomolecular Research laboratory in Charleston, SC, using established marine mammal necropsy protocols (Geraci and Lounsbury, 2005). Animals were either euthanized (code 1) or freshly dead (code 2) to reduce post-mortem tissue degradation; they were either stranded alone or in cow-calf pairs (Fig. 1).

For the present study, neonates are considered to be < 105 cm; with juveniles representing the next size class up to the median length at attainment of sexual maturity by gender. Based on the study of dwarf sperm whales off southern Africa, which found the age at maturity to be five years of age (Plön, 2004); median adult lengths are considered to be 197 cm for males and 215 cm for females. In this study, a female stranded with its presumed calf had a length of 212 cm, so the lower limit for adult females was adjusted to 212 cm. For age class comparisons, neonates were grouped with juveniles. In the laboratory, necropsies were performed, life history data were collected (Table 1), and the kidney and liver were removed and frozen at −80 °C until subsampling for trace element analysis.

Subsamples (~10 g) of liver or kidney tissue were homogenized and stored in pre-cleaned polypropylene containers at −80 °C until processing for analysis. Homogenized tissue samples were prepared for inductively coupled plasma mass spectrometry (ICP-MS) analysis by microwave-assisted digestion in PTFE pressurized vessels with ultrapure nitric acid followed by ultrapure 30% $H_2O_2$. Resulting solutions were diluted with Millipore deionized water prior to trace element analysis (EPA Method 3052, 1996). For total Hg determination, tissue samples were weighed directly into nickel boats for analysis.

An additional portion (~2.5 g) of each homogenate was dried to estimate the dry fraction. The dry fraction of each tissue sample was determined gravimetrically with an analytical balance after drying for 48 h in an 80 °C oven. Average moisture content of tissues was 78.1 ± 1.6% for the kidney (range = 76.2–80.9%) and 70.0 ± 5.2% for liver (range = 61.3–79.0%).

A Perkin Elmer Sciex ELAN® 6100 Inductively Coupled Plasma Mass Spectrometer with an AS-91 autosampler (Perkin Elmer, Inc., Waltham, MA) was used to measure the following 39 isotopes of 22 elements: $^{107}$Ag, $^{109}$Ag, $^{27}$Al, $^{75}$As, $^{136}$Ba, $^{137}$Ba, $^{9}$Be, $^{111}$Cd, $^{112}$Cd, $^{114}$Cd, $^{59}$Co, $^{52}$Cr, $^{53}$Cr, $^{63}$Cu, $^{65}$Cu, $^{54}$Fe, $^{57}$Fe, $^{6}$Li, $^{7}$Li, $^{55}$Mn, $^{60}$Ni, $^{206}$Pb, $^{207}$Pb, $^{208}$Pb, $^{121}$Sb, $^{123}$Sb, $^{77}$Se, $^{82}$Se, $^{117}$Sn, $^{119}$Sn, $^{120}$Sn, $^{203}$Tl, $^{205}$Tl, $^{235}$U, $^{238}$U, $^{51}$V, $^{64}$Zn, $^{66}$Zn, and $^{68}$Zn in all samples. Reported values are the average of listed isotopes for an element. A multiple element internal standard ($^{45}$Sc, $^{72}$Ge, $^{103}$Rh, $^{175}$Lu) was added to each sample and calibration standard. Standard solutions were purchased from High Purity Standards (Charleston, SC). Samples were diluted as necessary for bracketing by calibration curves. Quality assurance for ICP-MS data included analysis of duplicate samples, reagent blanks, standard reference material, NIST SRM 1566b (NIST, Gaithersburg, MD), and Trace Metals in Drinking

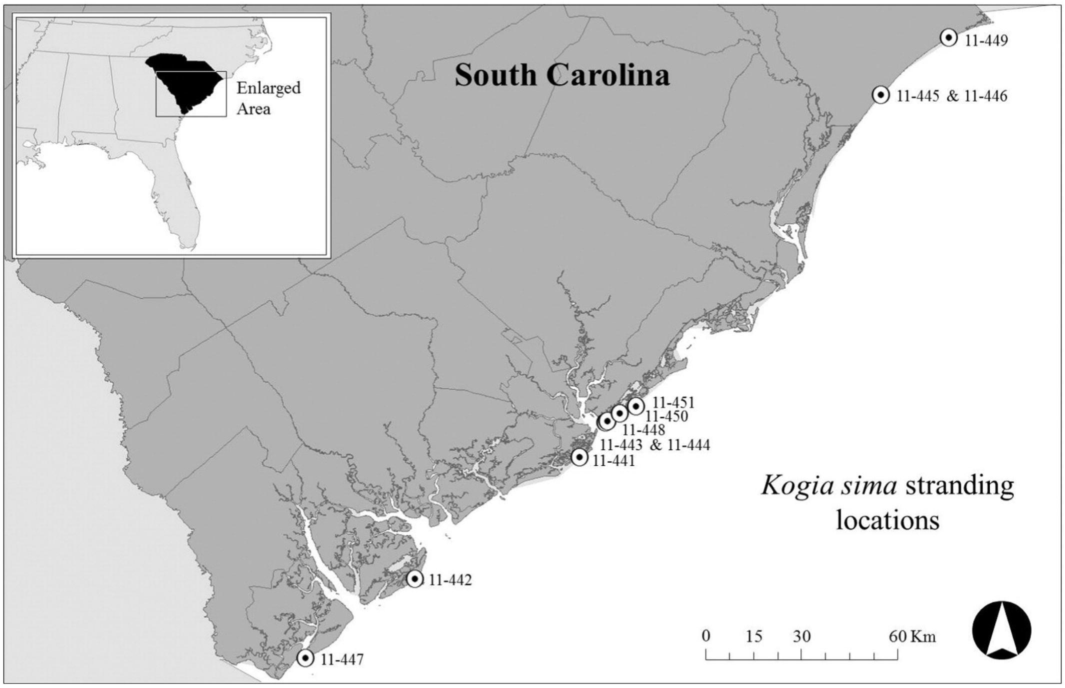

**Fig. 1.** Dwarf sperm whale stranding locations along the South Carolina coast from 1990–2011 labeled with NOAA sample number.

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