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Global Assessment of Silver Pollution using Sperm Whales (*Physeter macrocephalus*) as an Indicator Species

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Abstract

Silver pollution in the marine environment is of concern, particularly, with the rapid increasing use of silver nanoparticles in consumer products providing additional sources of silver emissions. Silver is highly toxic and known to bioaccumulate in aquatic organisms; however, the risk silver poses to the marine ecosystem is poorly understood. The sperm whale (*Physeter macrocephalus*), a toothed whale having a wide global distribution and high trophic level, is a sentinel of ocean health. The aim of this study was to provide a global baseline for silver as a marine pollutant using the sperm whale as an indicator species. Skin biopsies were collected in free-ranging sperm whales around the globe during the voyage of the research vessel, *Odyssey*, during 2000 and 2005. Total silver levels were measured in 298 sperm whales from 16 regions. Detectable levels were found in 176 whales and ranged from 0.1 to 4,179.0 µg/g ww with a global mean level of 16.9 ± 14.1 µg/g ww. The highest mean level was found in whales sampled in waters near Seychelles with 123.3 µg/g ww, and the lowest mean in whales near Chagos with 0.1 µg/g ww. These data provide an important global baseline for silver pollution that precedes the recent emergence of silver nanoparticles.

Keywords: Silver; Sperm whale; Skin biopsies; Atlantic Ocean; Indian Ocean; Pacific Ocean; Mediterranean Sea

Abbreviations: Ag: Silver; AgNPs: Silver Nanoparticles; ww: wet weight; dw: dry weight

Introduction

Silver (Ag) is a pollutant of concern in marine waters due to its toxicity, chemistry and bioavailability [1]. Ag is one of the most toxic trace metals known [2] secondary to only mercury [3]. Ag contamination has been a marine concern for some time, largely due to its toxicity in marine invertebrates and fish [1,4].

Ag can enter the marine environment from mining, photographic industry, combustion of wastes (fossil fuels, municipal and industrial), electronic applications and cloud seeding [3,5,6]. Natural sources include weathering of rock and soil erosion [5]. The natural concentration of Ag in marine waters is very low, 0.1 to 0.3 ng/L, and in oxidized sediments is also low, 0.1 µg/g dry weight (dw), making any amount of anthropogenic input a substantial enrichment of this metal into the environment [1]. Currently, Ag is regulated at 1.9 µg/L in saltwater [7].

Once in seawater, Ag binds with chloride keeping a substantial portion in solution allowing for enhanced bioavailability to marine life and increased distribution by currents [2,8]. At lower salinity, Ag exists as AgCl and is bioavailable, and at higher salinity Ag exists as AgCl⁻, AgCl²⁻ and AgCl³⁻ and is less bioavailable [9]. Ag concentration in seawater increases with depth so that surface waters contain <1-25 pM and deep waters contain 7-80 pM depending on region [9,10]. Additionally, Ag distributions appear to be dependent on silicate concentration in vertical profiles of the water column with a positive correlation of Ag to silicate with increasing depth [11].

Recently, Ag has become commonly used as a nanoparticle material in a wide range of consumer products including washing

machines, detergents, clothing, food packaging, containers, dietary supplements, medical devices, plastics and refrigerators due to its antibacterial properties [12-15]. The antimicrobial properties of silver nanoparticles (AgNPs) are of particular concern for if, inadvertently, they enter the marine ecosystem they may inhibit the natural microbial activity important for decomposition, nutrient cycling and removal [15]. AgNPs have the potential to be more toxic than dissolved-Ag [16]; however, there is debate as to whether the toxicity is due to the nanoparticle or the dissolved Ag released from the nanoparticle in the presence of water [14]. AgNPs have not had clear guidelines for the regulation of their release into the environment established [17].

The extent of Ag pollution prior to the dawn of the nanoparticle age is poorly understood. Measurements of Ag in marine waters are not widespread and assessments of Ag levels in indicator marine species have only been done sporadically. Studies in cetaceans show that Ag bioaccumulates [18-21], but so far a baseline for Ag has not been determined. Such a baseline would be useful in future assessments of the potential effects of AgNPs on Ag levels in the marine environment. Additionally, most studies on Ag compounds investigate acute time points. However, chronic low levels of Ag may lead to toxic body

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burden in long-lived organisms, such as the sperm whale, and need to be investigated further especially with the rise of silver nanotechnology.

Between 2000 and 2005, the research vessel, *Odyssey*, collected skin biopsies from free-ranging sperm whales from the Pacific, Indian and Atlantic Oceans and the Mediterranean Sea. Sperm whales have a global distribution and feed high on oceanic food chains and may be at risk for accumulation of Ag in their tissues. Since these samples were collected prior to the extensive use of AgNPs in consumer products, these biopsies provide a rare opportunity to establish a global baseline prior to the introduction of this new class of chemicals.

Materials and Methods

We measured total Ag concentrations in 298 free-ranging sperm whale skin samples collected from 16 regions around the globe (Figure 1) with 94 samples in the Pacific, 156 in the Indian, 23 in the Mediterranean and 25 in the Atlantic. We considered 194 female and 104 male sperm whales including 51 adult and 53 subadult males.

Biopsies

During the voyage of the research vessel, *Odyssey*, between 2000 and 2005, skin biopsies were collected from free-ranging sperm whales as described previously [22]. Sampling was carried out simultaneously with photo-identifications of individual whales to minimize duplication. The behavior of all whales sampled appeared to be healthy. Samples were taken from the whale's flank, a location that has been shown to elicit the fewest reactions [23]. We used a 50 mm stainless steel cylindrical biopsy dart. Samples were removed from the biopsy dart

and divided into two pieces at the interface between skin and blubber. These two pieces were stored separately for later genetic and metal analysis. All tissue samples were frozen at -20°C within a few minutes of collection. The samples were shipped frozen to the Wise Laboratory. Age classification of males as subadult or adult was estimated by the length of the whale; however, classification of females could not be made reliably because of their overall smaller size compared to males. Sloughed skin samples were additionally collected with a net and were also classified for gender and analyzed for Ag concentrations.

Genotyping

Gender was determined by genotyping as published in our methods [23]. DNA was extracted from a piece of whale skin, and gender was determined by PCR amplification reactions in which the SRY (male determining factor) gene was amplified. The keratin gene was used as an amplification control for all samples. Male samples showed both the keratin band (~311bp) and SRY (male) band at ~152bp. Female samples showed only the keratin band at ~311bp. Primer sequences were the following:

SryPMF: 5'CATTGTGTGTGGTCTCGTGATC

SryPMR: 5'AGTCTCTGTGCCTCCTCGAA

KF: 5'AGATCAGGGGTTCATGTTTCTTTGCG

KR: 5'TTTACAGAGGTACCCAAGCCTAAG

Inductively coupled plasma mass spectroscopy

Whale skin samples were analyzed for total Ag using a Perkin-

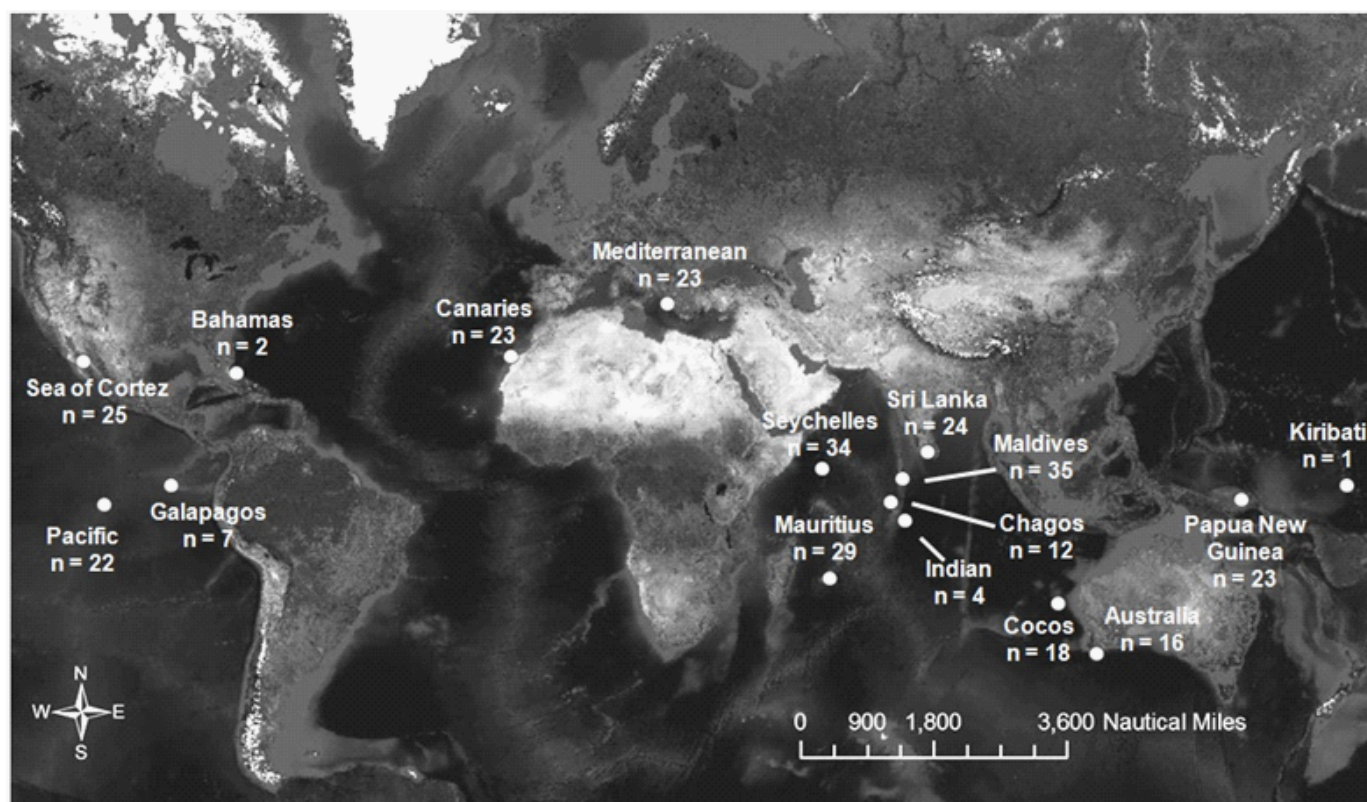


Figure 1: Regional distribution of sperm whale samples during the Voyage of the *Odyssey*. Samples are organized into 16 regions with sample size (n) denoted. The voyage began in the Sea of Cortez traveling westward ultimately ending in Massachusetts, USA.

Elmer/Sciex ELAN inductively coupled plasma-mass spectrometer (ICP-MS) according to our published methods [23]. Samples were rinsed with deionized water and allowed to air dry in a laminar flow hood to minimize contamination. Approximately 0.1 g of tissue was placed in a digestion vessel, 2 ml of Optima grade nitric acid (Fisher Scientific, Pittsburg, PA) was added, the vessel placed in a hot block, and refluxed at 95°C for 4 h. The sample was cooled, 2 ml Optima grade hydrogen peroxide (Fisher Scientific, Pittsburg, PA) and deionized water (3:2 v/v) was added, heated until the effervescence subsided, cooled, and brought up to a final volume of 20 ml. Standard quality assurance procedures were employed (Table 1) and include the analysis of standard reference materials, a duplicate sample and a pre-digestion spike. Instrument response was evaluated initially and after the 10 samples, using commercially available calibration verification standards (Claritas PPT multi-element solution 2- Spex CertiPrep, Metuchen, NJ; individual elemental standards – SCP Science, Baie-D'Urfe, Quebec, Canada) and a blank. All calibration verifications (n=5) were within the acceptance criterion of 85-115% recovery and all the preparation blank values were below 3× the limit of detection. Standard reference material (DOLT-4 - NIST, Charleston, SC) was used to assess method performance, where applicable. Interference check solutions (ICS A and ICS A+B- High Purity Standards, Charleston, SC) were analyzed with all sample runs checking for matrix effects that might interfere with sample analysis. The mean limit of detection (LOD) and project quantitation limit (PQL) values are presented in Table 1, and the PQL is 10 times higher than the LOD due to slight variability between samples due to differences in sample mass. The LOD was the lowest analyte concentration likely to be reliably distinguished from the blank and at which detection is feasible. The LOD was previously determined by utilizing both the measured blank and test replicates of a matrix matched sample known to contain a low concentration of analyte. Additionally, a series of PQL samples was run for each element to assess low concentration analytical performance, with an acceptance criterion of 50-150%. All samples were diluted 2× for analysis by ICP-MS.

Results are reported in units of µg/g wet weight of tissue (µg/g ww). Levels in the literature are mostly reported as per dry weight (dw) of tissue and do not include information on water content. Hence in order to compare our data with literature, we transformed literature values to wet weight values by multiplying them by a factor of 0.25, which corresponds to the dry/wet weight ratio of skin and blubber.

Mapping

Whale locations were plotted using ArcGIS 9: ArcMap Version 9.3.1. These maps allowed us to infer geographical areas in which exposure may have occurred.

Statistics

Mean values were compared using analysis of variance. Differences for individual pairs of means were assessed via t-tests with the Bonferroni correction for multiple comparisons also according to our

published methods [23]. When Ag was not detected in a specimen, a value of one-half the detection limit was used in the analysis. Statistical testing was performed on log-transformed data due to the skew of the distributions of the untransformed data. The statistical analyses were all conducted in SAS 9.2 (SAS Institute, 2004).

Results

Silver levels by region

Ag was detected in 178 of the 298 sperm whales sampled with a global mean of 16.9 ± 14.1 µg/g ww and detectable levels ranging from 0.04 to 4,179 µg/g ww (Table 2). There are two samples in the dataset with high Ag concentrations that should be noted, a subadult male in Seychelles with a Ag concentration of 4,179 µg/g ww and a female in Sri Lanka with 297.3 µg/g ww. Mean Ag concentrations for the four general regions of the world, Pacific, Indian, Mediterranean and Atlantic, were 4.2, 23.1, 1.5 and 5.9 µg/g ww, respectively. By region, the highest mean Ag concentration was measured in waters near Seychelles (n=34) with 123.3 ± 122.9 µg/g ww ranging from 0.1 to 4,179 µg/g ww and Sri Lanka (n=24) with 12.5 ± 12.4 µg/g ww ranging from 0.1 to 297.3 µg/g ww. The lowest regional Ag mean concentration was in whales sampled during the Indian Ocean crossing with all four samples undetectable (one-half LOD = 0.04 µg/g ww) and in whales near the Chagos Archipelago in the Indian Ocean with 0.1 ± 0.1 µg/g ww ranging from 0.1 to 0.7 µg/g ww. The variation among regions was statistically significant ($F(15,281)=5.40$; $p<0.0001$). Pair-wise t-tests showed regions that differed ($p<0.05$) included: Mauritius to Papua New Guinea; Sri Lanka to Papua New Guinea and Sea of Cortez; and Papua New Guinea to Chagos, Cocos, Indian Ocean, Maldives, Mauritius, Seychelles and Sri Lanka.

Silver levels by gender

We considered Ag levels by gender (Table 3). Detectable Ag levels were found in 107 of the 194 females sampled with a global mean of 3.5 ± 1.7 µg/g ww ranging from 0.04 to 297.3 µg/g ww. Detectable Ag levels were found in 71 of the 104 males sampled with a global mean of 42.0 ± 40.2 µg/g ww ranging from 0.1 to 4,179 µg/g ww. The global mean Ag concentration was not statistically different in males compared to females ($F(1,295)=3.40$; $p=0.07$) even when females were compared to adult males ($F(1,242)=2.35$; $p=0.13$) and to subadult males ($F(1,244)=1.87$; $p=0.17$). With removal of the male from Seychelles with a Ag concentration of 4,179 µg/g ww, the global mean for males was 1.9 ± 0.8 µg/g ww ranging from 0.1 to 75.6 µg/g ww, the global mean Ag concentration still was not statistically different when comparing males to females ($F(1,294)=2.25$; $p=0.13$).

Whales were not evenly distributed by region as females were not found to biopsy in the Galapagos, Indian Ocean crossing or Chagos. Similarly, males were not found to biopsy in the waters around Kiribati and Cocos Islands. All other regions had a mix of males and females at the time the *Odyssey* was present and collecting samples. Controlling

Table 1: Quality assurance and quality control data for Ag analysis.

Metal	LOD ^a (µg/g ww, mean)	PQL ^b (ppm, mean)	Calibration Blank (ppm, mean) n=5	Duplicate (RPD) ^c (%) n=1	LCS recovery (%) n=1	Spike recovery (%) n=1	SRM ^d recovery (%) DORM-3/ DOLT-4 n=1
Ag	0.08	0.80	BDL ^e	10.7	95.3	87.2	103.2

^aLOD=Limit of detection

^bPQL=Project quantitation limit

^cRPD=Relative percent difference

^dSRM=Standard reference material

^eBDL=Below detection limit

Table 2: Global distribution of sperm whale skin Ag concentrations.

Ocean/Sea	Region ^a	n	# of ND ^c	Regional Mean ^b	Ocean/Sea Mean ^b
Pacific	Sea of Cortez	25	6	1.4 ± 0.4 (0.2-6.5)	4.2
	Galapagos	7	3	2.2 ± 1.5 (0.1-10.7)	
	Pacific	22	5	2.0 ± 0.9 (0.1-15.7)	
	Kiribati	1	0	4.2	
	Papua New Guinea	23	4	10.3 ± 5.1 (0.2-94.4)	
	Australia	16	8	4.8 ± 4.3 (0.1-68.7)	
Indian	Cocos	18	6	0.2 ± 0.1 (0.04-1.3)	23.1
	Indian	4	4	ND	
	Chagos	12	6	0.1 ± 0.1 (0.1-0.7)	
	Seychelles	34	12	123.3 ± 122.9 (0.1-4179.0)	
	Maldives	35	24	0.3 ± 0.1 (0.3-2.1)	
	Sri Lanka	24	20	12.5 ± 12.4 (0.1-297.3)	
	Mauritius	29	13	1.9 ± 1.7 (0.1-49.7)	
Mediterranean	Mediterranean	23	4	1.5 ± 0.5 (0.2-9.5)	1.5
Atlantic	Canaries	23	5	0.5 ± 0.1 (0.1-1.5)	5.9
	Bahamas	2	0	11.2 ± 10.5 (0.6-21.7)	
Global		298	120	16.9 ± 14.1 (0.04-4179.0)	

^aRegions are named for the closest land body or ocean region

^bAll data are presented in µg/g ww ± standard error

^cWhales with nondetectable Ag levels had one-half LOD (0.04 µg/g ww) used in analysis

n: Sample size

() Minimum and maximum values.

ND: Nondetect

Table 3: Sperm whale skin Ag concentration by gender across regions.

Ocean/Sea	Region ^a	n		Ag ^b	
		M	F	M	F
Pacific	Sea of Cortez	12 ^c	13 ^c	1.1 ± 0.4 (0.2-1.7)	1.8 ± 0.6 (0.2-6.5)
	Galapagos	7 ^c	0	2.2 ± 1.5 (0.1-10.7)	-
	Pacific	6 ^c	16 ^c	2.9 ± 2.6 (0.1-15.7)	1.6 ± 0.8 (0.1-13.0)
	Kiribati	0	1	-	4.2
	Papua New Guinea	10 ^c	13 ^c	11.1 ± 7.5 (0.2-75.6)	9.8 ± 7.1 (0.4-94.4)
	Australia	8 ^c	8 ^c	0.4 ± 0.2 (0.1-1.3)	9.2 ± 8.5 (4.3-68.7)
Indian	Cocos	0	18 ^c	-	0.2 ± 0.1 (0.04-1.3)
	Indian	4 ^c	0	ND	-
	Chagos	12 ^c	0	0.1 ± 0.1 (0.1-0.7)	-
	Seychelles	9 ^c	25 ^c	464.9 ± 464.3 (0.1-4,179.0)	0.3 ± 0.1 (0.1-1.8)
	Maldives	9 ^c	26 ^c	0.4 ± 0.2 (0.3-2.1)	0.2 ± 0.05 (0.3-1.0)
	Sri Lanka	1 ^c	23 ^c	ND	13.0 ± 12.9 (0.1-297.3)
	Mauritius	3	26 ^c	0.1 ± 0.01 (0.06-0.08)	2.1 ± 1.9 (0.1-49.7)
Mediterranean	Mediterranean	16 ^c	7	1.0 ± 0.6 (0.2-9.5)	2.6 ± 1.0 (0.4-6.1)
Atlantic	Canaries	6	17 ^c	0.7 ± 0.2 (0.1-1.4)	0.5 ± 0.1 (0.1-1.5)
	Bahamas	1	1	0.6	21.7
Total	Global	104	194	42.0 ± 40.2 (0.1-4179.0)	3.5 ± 1.7 (0.04-297.3)

M: Male; F: Female

n: Sample size

- None sampled

^aRegions are named for the closest land body or ocean region

^bAll data are mean total Ag concentration presented in µg/g ww ± standard error with minimum and maximum values in ()

^cOne or more whale had undetectable Ag concentrations. One-half the detection concentration (0.01 µg/g ww) was used in the analysis

for region, Ag levels in males and females were statistically different from one another ($F(16,280)=5.06$; $p<0.0001$) as well as Ag levels in adult males being statistically different than females ($F(15,228)=5.41$; $p<0.0001$).

Considering gender by region, the region with the highest Ag concentration for females was in the waters near the Bahamas ($n=1$) with $21.7 \mu\text{g/g ww}$ and in Sri Lanka ($n=23$) with $13.0 \pm 12.9 \mu\text{g/g ww}$ ranging from 0.1 to $297.3 \mu\text{g/g ww}$. The lowest regional mean concentration for females was near the Cocos Islands ($n=18$) with $0.2 \pm 0.1 \mu\text{g/g ww}$ ranging from 0.04 to $1.3 \mu\text{g/g ww}$ and in the Maldives ($n=26$) with $0.2 \pm 0.05 \mu\text{g/g ww}$ ranging from 0.3 to $1.0 \mu\text{g/g ww}$. The variation among regions was statistically significant ($F(11,180)=5.91$; $p<0.001$). Pair-wise t-tests comparing females by region found significant differences ($p<0.05$) in Ag levels of females between the regions of the Cocos compared to the Mediterranean, Papua New Guinea, and Sea of Cortez; Maldives to the Mediterranean, Papua New Guinea, and Sea of Cortez; Mauritius to Mediterranean, Papua New Guinea, and Sea of Cortez; Mediterranean to Seychelles and Sri Lanka; Papua New Guinea to Seychelles and Sri Lanka; and Sea of Cortez to Seychelles and Sri Lanka.

The highest regional mean Ag concentration in males was in Seychelles ($n=9$) with a mean of $464.9 \pm 464.3 \mu\text{g/g ww}$ ranging from 0.1 to $4,179 \mu\text{g/g ww}$ and in Papua New Guinea ($n=10$) with $11.1 \pm 7.5 \mu\text{g/g ww}$ ranging from 0.2 to $75.6 \mu\text{g/g ww}$. If the male sample from Seychelles with the high Ag concentration is removed from the analysis, then Papua New Guinea is the region with the highest mean Ag concentration for the males sampled. The lowest regional mean Ag concentration in males was in the Indian Ocean with all four samples

having undetectable levels (one-half LOD= $0.04 \mu\text{g/g ww}$). There were statistical differences among males between regions ($F(13,90)=2.00$; $p=0.03$) whether the high sample in Seychelles is removed or not ($F(13,89)=2.35$; $p=0.01$). Papua New Guinea was significantly different than Chagos and the Indian Ocean Crossing.

Silver concentration comparisons by age in males

We found no conclusive evidence that older and subsequently larger males had higher Ag burdens (Table 4). Adult males ($n=51$) had a mean Ag concentration of $1.5 \pm 0.6 \mu\text{g/g ww}$ ranging from 0.1 to $23.6 \mu\text{g/g ww}$ compared to subadult males ($n=53$) with $81.0 \pm 78.8 \mu\text{g/g ww}$ ranging from 0.1 to $4179 \mu\text{g/g ww}$, and this difference was not statistically significant ($F(1,102)=0.01$; $p=0.93$). When the subadult male in Seychelles with the high concentration of Ag was removed from the analysis, the mean Ag concentration for subadult males ($n=52$) is $2.2 \pm 1.5 \mu\text{g/g ww}$ ranging from 0.1 to $75.6 \mu\text{g/g ww}$ and again the Ag concentrations in subadult male whales were not statistically different than adult male whales ($F(1,101)=0.40$; $p=0.53$).

There was no statistically significant variation across regions in adult ($F(10,40)=1.23$; $p=0.30$) or subadult males ($F(9,43)=1.87$; $p=0.08$). The highest regional mean Ag concentration for adult male was in the Papua New Guinea with $5.0 \pm 4.7 \mu\text{g/g ww}$ ranging from 0.2 to $23.6 \mu\text{g/g ww}$ and then in Mauritius with $0.07 \pm 0.01 \mu\text{g/g ww}$ ranging from 0.06-0.07 $\mu\text{g/g ww}$. The highest regional mean for subadults was in the Seychelles with $1,045 \pm 1,045 \mu\text{g/g ww}$ ranging from 0.4 to $4179 \mu\text{g/g ww}$, and the lowest in Chagos with $0.1 \pm 0.7 \mu\text{g/g ww}$ ranging from 0.1 to $0.7 \mu\text{g/g ww}$. If the subadult male from Seychelles with the high Ag concentration is removed from the analysis, there is a significant difference across regions for subadult males ($F(9,42)=2.31$; $p=0.03$).

Table 4: Sperm whale skin Ag concentration by age in males across regions.

Ocean/Sea	Region ^a	n		Ag ^b	
		SM	A	SM	A
Pacific	Sea of Cortez	0	12 ^c	-	1.1 ± 0.4 (0.2-1.7)
	Galapagos	5 ^c	2 ^c	2.8 ± 2.0 (0.1-10.7)	ND
	Pacific	0	6 ^c	-	0.2 ± 23.6 (0.3-2.4)
	Kiribati	0	0	-	-
	Papua New Guinea	5 ^c	5	17.2 ± 14.6 (1.9-75.6)	5.0 ± 4.7 (0.2-23.6)
	Australia	8 ^c	0	0.4 ± 0.2 (0.1-1.3)	-
Indian	Cocos	0	0	-	-
	Indian	0	4 ^c	-	ND
	Chagos	12 ^c	0	0.1 ± 0.7 (0.1-0.7)	-
	Seychelles	4 ^c	5 ^c	$1,045.0 \pm 1,045.0$ (0.4-4179.0)	0.9 ± 0.4 (0.1-2.4)
	Maldives	5 ^c	4 ^c	0.2 ± 0.1 (0.26-0.32)	0.8 ± 0.5 (0.9-2.1)
	Sri Lanka	0	1 ^c	-	0.1
	Mauritius	1	2	0.08	0.07 ± 0.01 (0.06-0.07)
Mediterranean	Mediterranean	8 ^c	8 ^c	0.5 ± 0.2 (0.4-1.4)	1.5 ± 1.1 (0.2-9.5)
Atlantic	Canaries	4	2	0.7 ± 0.3 (0.1-1.4)	0.6 ± 0.5 (0.1-1.1)
	Bahamas	1	0	0.6	-
Total	Global	53	51	81.0 ± 78.8 (0.1-4179.0)	1.5 ± 0.6 (0.1-23.6)

A: Adult male; SM: Subadult male

n: Sample size

-None sampled

^aRegions are named for the closest land body or ocean region

^bAll data are mean total Ag concentration presented in $\mu\text{g/g ww} \pm$ standard error with minimum and maximum values in ()

^cThis region had whales with nondetectable Ag concentrations. One-half the detection concentration ($0.04 \mu\text{g/g ww}$) was used in the analysis

with the highest levels in Papua New Guinea (n=5) with 17.2 ± 14.6 $\mu\text{g/g ww}$ ranging from 1.9 to 75.6 $\mu\text{g/g ww}$.

Silver levels in sloughed skin

Ag levels were measured in two sloughed skin samples found in Seychelles that were from males (data not shown). The Ag levels of the two samples were 0.6 and 21.7 $\mu\text{g/g ww}$. In comparison to the skin biopsy samples of males from Seychelles that had a mean Ag concentration of 464.9 ± 464.3 $\mu\text{g/g ww}$ ranging from 0.1 to 4179 $\mu\text{g/g ww}$, the sloughed skin levels were lower than the skin biopsy mean; however, still within the minimum and maximum Ag levels found. If the high sample found in Seychelles is removed, then the mean Ag concentration for this region for males is 0.7 ± 0.3 $\mu\text{g/g ww}$, which would put one of the sloughed skin samples on the higher end of the range found in the biopsies. Taken together, Ag can be eliminated from the skin by epidermal sloughing.

Discussion

Global distribution of Ag

To our knowledge, this is the first global study of the distribution of Ag, a contaminant of concern due to its toxicity and ability to bioaccumulate in the marine environment. We found detectable Ag levels in every region we tested except the Indian Ocean crossing where the four biopsies had undetectable levels. The wide distribution of Ag may be explained by the fact that once this metal enters marine waters it binds strongly to chloro complexes causing Ag to be highly bioavailable and remain in solution enabling wide dispersal [1] with a residence time <1 year [24].

One example of its wide dispersal is from wastewater fallouts off of Los Angeles and San Diego where the Ag gradient extends over 250 km [25]. Ag is shown to be bioavailable in marine sediments where it undergoes high bioaccumulation [1]; however, Ag is predominantly bound to sulfur in sediment, which is a less toxic form of Ag [26]. Sperm whales feed at deep ocean depths (up to 5000 m) including the ocean floor on squid, fish, rays and sharks [27] that may bioaccumulate Ag levels from these marine sediments.

Silver levels in cetacean skin

Our data are the first to report Ag levels in sperm whales. We sampled free-ranging sperm whales limiting the option of available tissue to skin and blubber. Metals accumulate at higher levels in the skin, so we focused on skin levels. Only one other study considers Ag levels in cetacean skin and reports 0.003 $\mu\text{g/g ww}$ in a Dall's porpoise found on the Sanriku coast of Japan [28]. This concentration is consistent with the low end of our range; however, it is only one animal.

Our limited data in sloughed skin from Seychelles show Ag concentrations within the range of levels found in the skin biopsies showing that epidermal sloughing can be a route for elimination of Ag, although likely a minor one.

Possible routes of exposure to silver for the sperm whale

The source of Ag exposure for the sperm whales sampled cannot be determined from a skin biopsy; however, may occur through ingestion, inhalation and skin absorption. Ingestion of Ag occurs through consumption of contaminated prey since Ag bioaccumulates in marine organisms and also through swallowing seawater. Dolphins have been reported to swallow about 1.5 l/day [29], but the amount is unknown in sperm whales. Once ingested the exact mechanism of Ag uptake into a

cell is unclear; however, it is believed to enter through active transport [2].

Inhalation is another possible route of Ag exposure to sperm whales because whales inhale large volumes of air and hold their breath for long periods of time allowing for more absorption. But, inhalation is rarely considered in the marine environment. Recently, we calculated a theoretical amount of chromium (Cr) inhalation in whales based on marine air levels. Although these air Cr levels were low, the calculation shows that the whales would likely inhale an amount of Cr equivalent to occupational exposure workers [22]. Overall, the majority of Ag emissions into the environment binds to sediments or suspended particles near the release site and becomes immobile; however, a portion is transported long distances through air [2]. In general, atmospheric Ag concentrations have been found to be <1 ng/m³ [5], but could be much higher if near a silver-based industry.

Ag could also possibly enter the sperm whale through skin absorption; however, this is unlikely due to the fact that Ag has not been shown to enter a cell by passive diffusion [2].

Regional differences of silver levels in toothed whales

Between regions, Ag concentrations in our data set varied with levels being the highest in the Indian>Atlantic>Pacific>Mediterranean. The Pacific waters have been found to have higher Ag concentrations than the Atlantic [9], and it is unknown why the Ag concentrations in these skin biopsies did not reflect the seawater concentrations found in other studies.

Our data are the first to report Ag levels in whales from the Indian Ocean and Mediterranean Sea. Marine pollution is poorly understood in the Indian Ocean, despite the importance of this body of water. This report builds on our previous study of Cr levels, which were generally high in whales from these regions [22], and extends our knowledge to include Ag. More work is needed to understand pollution in the Indian Ocean.

In 1999, the *Indian Ocean Experiment* was conducted and found surprising high levels of air pollution over the Indian Ocean and was suggested to originate from fossil fuel combustion and biomass burning from south and Southeast Asia and India [30]. Ag emissions into the Indian Ocean from river input was discovered from the coast of Kenya where Ag sediment concentrations were found ranging from 1.3 to 18.5 $\mu\text{g/g dw}$ [31]. These two studies along with this study are revealing the impact of pollution and specifically Ag pollution to the Indian Ocean.

Our average concentration for the Atlantic Ocean was 5.9 $\mu\text{g/g ww}$. Ag concentrations in deep seawater from the Atlantic have been found ranging from 7 to 23 pM [9], which in comparison to our mean Ag concentration of 54.7 μM depicts the ability of Ag bioaccumulation in an oceanic species.

Our mean Ag concentration for the Atlantic was on the high end of other Ag concentration reported in this region and included samples from the Canaries (0.5 $\mu\text{g/g ww}$) and the Bahamas (11.2 $\mu\text{g/g ww}$). Pilot whales (*Globicephala melas*) from Cape Cod (n=8) have liver Ag concentrations of 0.2 $\mu\text{g/g ww}$ [18]. Law et al. [19] consider Ag concentration in liver tissue from several species of cetaceans including long-finned pilot whale (*Globicephala melas*), Sowerby's beaked whale (*Mesoplodon bidens*), fin whale (*Balaenoptera physalus*), minke whale (*Balaenoptera*), pygmy sperm whale (*Kogia breviceps*) and Blainville's beaked whale (*Mesoplodon densirostris*) that stranded off the east coast of England and Wales. The individual levels range from 0.05-5.5 $\mu\text{g/g ww}$ though the number of animals are small (one per species) [19].

In the Pacific Ocean, the mean Ag concentration was 4.2 µg/g ww. As concentrations in seawater from the deep waters of the Pacific range from 22 to 80 pM [10,24,32] in comparison to our mean Ag concentration of 38.9 µM again showing the ability of Ag to bioaccumulate in sperm whales.

Our mean Pacific Ag concentration was well above the range reported in other studies from this area. Ag levels in the liver and kidney from several species of toothed whales including the bottlenose dolphin (*Tursiops truncatus*), common dolphin (*Delphinus delphis*) and melon-headed whale (*Peponocephala electra*) that had stranded off the east coast of Australia were all less than 1.0 µg/g ww though the number of animals were small (three or less per species) [21].

Hotspots of silver levels in the oceanic environment

Our data and the published literature are consistent with the hypothesis that there are hotspots of Ag exposure concentrating to a moderate extent in marine mammals [5]. We found that Ag levels in sperm whale skin are generally low, but there are a few hotspots that had skin samples with Ag levels greater than 10 µg/g ww in the Pacific crossing, Australia, Mauritius, Seychelles, Sri Lanka and Papua New Guinea. Additionally, a whale in the waters near Seychelles had the highest Ag concentration sampled of 4,179 µg/g ww.

The Pacific is an interesting case as our data may suggest that there is an increase in Ag levels or perhaps there are regional hotspots within it. Previous marine Ag studies largely focus on opportunistically obtained samples in a few well-researched areas of the Northern hemisphere. Thus, it is unclear if Ag levels observed reflected regional problems or something of a more global concern.

Silver levels compared by gender

Data in beluga whales from Alaska show that females had higher (33.1 µg/g ww; n=6) Ag concentrations than males (21.2 µg/g ww; n=9); however, it is unknown if this difference is significant or not [18]. We did not find that females sperm whales had statistically significant higher Ag levels compared to males.

Silver levels by age

Becker et al. [18] reports that Ag levels in the liver of pilot and beluga whales appear to increase with age but not with length. Klaine et al. [33], finds in freshwater fish that silver nitrate toxicity is higher in 28 day old fish compared to 3 day old fish. However, we did not find that our subadult males differed in Ag concentrations compared to adults. This may be a consequence of not being able to determine a more exact age of the whales or may be due to the small sample size per region and the considerable range of Ag concentrations in many of the regions that may have obscured possible age-related differences.

Skin Ag levels in relation to levels in other organs

Ag is known to bioaccumulate in marine mammals [2]. Studies in rats and mice report Ag first accumulates in the liver and then kidney, spleen, skin, bones and muscle [2]. Klaassen [34] also shows that in rats, rabbits and dogs that ¹¹⁰Ag administered intravenously accumulates to highest concentrations in the liver 2 h after treatment. Therefore, it is probable that the Ag concentrations in the liver of our sampled sperm whales were higher than what was measured in the skin.

Other studies considering Ag in whale species were from stranded or subsistence-hunted animals so internal organs were available, particularly liver. In subsistence-hunted gray whales (*Eschrichtus*

robustus) (n=6-14) from the Arctic, mean Ag concentrations were 1, 11 and 310 µg/g ww for kidney, brain and liver, respectively [20]. Mean Ag levels in the liver of subsistence-hunted beluga whales from Alaska (*Delphinapterus leucas*) are 19.3 µg/g ww in Point Hope (n=4) and 31.0 µg/g ww in Point Lay (n=10) [18]. Thus, our global mean Ag concentration (16.9 µg/g ww) in skin of sperm whales lies at the low end of the average Ag levels found in livers of toothed whales in the Arctic (19-210 µg/g ww).

Possible effects of elevated Ag levels

The hotspots of elevated Ag levels in our data set may render marine organisms more sensitive to the toxicity of other chemicals and to diseases especially those known to induce lipid peroxidation themselves [35]. There is evidence that Ag is a potent inhibitor of selenium, an essential element that functions as an antioxidant for the cell particularly being an integral part of glutathione peroxidase [36]. Ag has also been shown to be a potent inhibitor of glutathione peroxidase [37,38] and to induce lipid peroxidation [18]. The effect of the higher Ag concentrations found in the skin of these whales is unknown; however, this organ may act as a reservoir for Ag where it is slowly removed by eternal sloughing.

Conclusions

The data shown here represent a global baseline for marine Ag, which was established using the skin of sperm whales as an indicator species. These data indicate that sperm whales are indeed exposed to Ag, but Ag concentrations were generally low in these animals prior to 2005, thus providing an important and useful baseline to eventually determine if AgNPs are impacting the marine environment. These data suggest that Ag concentrations may be increasing in the Pacific though more work is needed to assess whether these observations reflect a general trend in the Pacific or it is due to more localized regional events. Additional work is needed to understand the toxicity of Ag to whales and how it transports through the environment to reach remote regions.

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