A Whale of a Tale: A One Environmental Health Approach to Study Metal Pollution in the Sea of Cortez.

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Highlights

- We measured 23 metals and Se in skin of 7 whale species from the Sea of Cortez.
- Metal levels appear to be decreasing in these whales over time, except Al, Cr, Fe and Ni are remaining the same or increasing.
- Our data indicate a similar exposure route across species for Al, Cr, Fe and Ni, likely not dietary.
- Al, Cr and Ni are known to be toxic to mammals and may pose a threat to the health of whale populations in the Sea of Cortez.

Abstract

Marine metal pollution is an emerging concern for human, animal, and ecosystem health. We considered metal pollution in the Sea of Cortez, which is a relatively isolated sea rich in biodiversity. Here there are potentially significant anthropogenic inputs of pollution from agriculture and metal mining. We considered the levels of 23 heavy metals and selenium in seven distinct cetacean species found in the area. Our efforts considered two different periods of time: 1999 and 2016/17. We considered the metal levels in relation to (1) all species together across years, (2) differences between suborders *Odontoceti* and *Mysticeti*, (3) each species individually across years, and (4) gender differences for each of these comparisons. We further compared metal levels found in sperm whale skin samples collected during these voyages to a previous voyage in 1999, to assess changes in metal levels over a longer timescale. The metals Mg, Fe, Al, and Zn were found at the highest concentrations across all species and all years. For sperm whales, we observed decreased metal levels from 1999 to 2016/2017, except for iron (Fe), nickel (Ni), and chromium (Cr), which either increased or did not change during this time period. These results indicate a recent change in the metal input to the Sea of Cortez, which may indicate a decreased concern for human, animal, and ecosystem health for some metals, but raises concern for the genotoxic metals Cr and Ni. This work was supported by NIEHS grant ES016893 (J.P.W.) and numerous donors to the Wise Laboratory.

Keywords

One Environmental Health; Sea of Cortez; metals; whales; Gulf of California

1. Introduction

The ocean serves as a terminal sink for chemicals released into the environment from either anthropogenic or natural sources. Oceans are an essential but finite resource and we are only starting to understand the extent of pollution impacts on marine ecosystems. Many government agencies have banned the use of persistent chemical contaminants (e.g. DDT, PCBs) due to their detrimental effects on the environment or possible links to human disease, yet these contaminants continue to be a problem for environmental health (Breivik et al., 2007; Loganathan et al., 1994). Metals are frequently overlooked as a class of environmental contaminants due to their natural occurrences. The majority of heavy metal environmental pollution is due to anthropogenic activities (Tchounwou et al., 2012). Recent investigations have shown that metals are global marine pollutants (Bjerregaard et al., 2015; Jarup, 2003; Wise, Sr., et al., 2009). Both the environmental impacts and toxic potential of a number of metals are well established (e.g. chromium, lead, mercury, etc.) (ATSDR, 1999; 2007; 2012), yet it remains difficult to limit toxic metals in food, water, and living spaces. As metals continue to spread and accumulate in our environment, it is imperative we understand how they will affect the health of wildlife, humans, and ecosystems alike, a concept known as One Environmental Health (Perez and Wise, 2018).

The Sea of Cortez (aka "Vermillion Sea" or "Gulf of California") is a relatively isolated marine body of water between the Baja California peninsula and mainland Mexico with a surface area of approximately 160,000 square kilometers. It is considered one of the richest seas in biodiversity and includes several UNESCO World Heritage Sites. Captain Jacques Cousteau famously referred to this body of water as “the world’s aquarium.” The Sea of Cortez and its islands are home to 695 vascular plant species, 891 fish species (90 of which are endemic), 493 bird species, and 32 marine mammal species, including the rarest cetacean on the planet, the vaquita. In addition, almost all major oceanographic processes occur in this area. Thus, the Sea of Cortez serves as a natural experiment for speciation research. Due to this rich biodiversity, its relative isolation, and high rates of primary productivity, it is considered an ecosystem with high priority for conservation. Most human marine activities in this area are centered on tourism and fishing, and are limited to a few small cities along the coast. However, there are potentially significant inputs of pollution from agriculture, fishing practices, and metal mining that occur along the coasts.

There are several studies that have considered metal levels in smaller, benthic organisms (e.g. oysters and mussels), some marine plants, and sediments along the Sea of Cortez coastlines, and only one other study has considered metals in a cetacean species (Villa et al., 1993; Gardner et al., 2006; Cadena-Cardenas et al., 2009; Jara-Marini et al., 2009; Jimenez et al., 2005; Ruelas-Inzunza and Páez-Osuna, 2000; Szteren and Aurioles-Gamboa, 2013; Roldan-Wong et al., 2018). Three other papers have considered metal levels in Sea of Cortez cetaceans that were found stranded, and measured internal organ metal levels (Bernardo Villa et al., 1993; Méndez et al., 2002; Ruelas-Inzunza and Páez-Osuna, 2002). Importantly, Sea of Cortez whales have been reported to be less exposed to anthropogenic activities than other regions when considering persistent organic pollutants (Nino-Torres et al., 2009; Fossi et al., 2014; Fossi et al., 2016). However, much remains to be evaluated for metal exposure and health risks in Sea of Cortez whales. We have previously reported an assessment of metal levels in sperm whales sampled around the world which included the Sea of Cortez and which we report again here as reference values for our more recent voyages from 2016 and 2017 spring seasons. Importantly, these data are the first to assess how metal levels are changing in whales from the Sea of Cortez over a long period of time (17-18 years) and a short period of time (1 year). Here, the levels of 23 metals and selenium (Se) in skin samples collected from seven different species of Sea of Cortez whales during 1999, 2016, and 2017 are reported.

2. Materials and Methods

2.1 Sample Collection

Skin biopsies were collected from free-ranging adult or subadult whales in the Sea of Cortez in the springs of 2016 and 2017. Our platform was the research vessel *Martin Sheen*, an 80-foot motor-sailer. Visual efforts were taken in 1-2 hour shifts from the crow's nest, weather permitting. Upon encountering a whale, two whale biopsiers would take positions in the bowsprit. As much detail about the whale and the biopsy was recorded as possible, including suspected age (adult or subadult), bodily location from where the biopsy was collected, whale's reaction (e.g. tail flick), any identifying markings (e.g. scars and flukes), GPS coordinates of the encounter, and number of individuals present.

2.2 Biopsies

Biopsy collection was the same between 1999, 2016, and 2017 voyages. Biopsies were consistently collected as previously described (Wise *et al.*, 2009) from the flank of the whale's back, a few feet caudal to the dorsal fin, in order to avoid hitting any critical body parts (e.g. blowhole or eyes). The biopsy dart was a modified crossbow bolt constructed of a hydrostatic buoy behind a stainless steel tip approximately 20 mm in length and 6 mm in diameter. The hydrostatic buoy doubled as a means to keep the arrow afloat and to prevent the arrow from penetrating the whale beyond the 20 mm tip or getting stuck in the whale's flank. After the biopsy arrow was retrieved, the sample was removed from the tip and processed on a sterile plate. Processing of the biopsy sample consisted of separating the skin and blubber, dividing each into two pieces for different types of analyses, storing the samples in a -20 ºC freezer temporarily (i.e. in the field), then storing in -80 ºC until analyses.

2.3 Genotyping

Gender was determined by genotyping based on published methods (Yang and Miyazaki, 2003). Analyses were performed in duplicate to validate results. DNA was extracted from a piece of whale skin using standard methods (Carvalho *et al.*, 2002). Gender was determined by PCR amplification reactions by amplifying the SRY (male determining factor) according to published methods (Yang and Miyazaki, 2003). The keratin gene was used as an amplification control for all samples; hence, male samples showed both the keratin band (~311 bp) and SRY band (~152 bp) whereas females only showed the keratin band. Primer sequences were the following:

- SryPMF: 5’CATTGTGTGTGGTCTCGTGATC
- SryPMR: 5’AGTCTCTGTGCCTCCTCGAA
- KF: 5’ AGATCAGGGGTTCATGTTTCTTTGC
- KR: 5’ TTTACAGAGGTACCCAAGCCTAAG

2.4 Inductively Coupled Plasma Mass Spectrometry

Samples were analyzed for total metal level using inductively coupled plasma mass spectrometry (ICPMS) according to our published methods using a Perkin-Elmer/Sciex ELAM ICPMS (Wise *et al.*, 2009). Interference check solutions were analyzed with all sample runs to compensate for any matrix effects which might interfere with sample analysis. Standard quality assurance procedures were employed (Tables 1 and 2). Instrument response was evaluated initially, after every 10 samples, as well as at the end of each analytical run using calibration verification standard and blank. All data are presented as ppm. Whale skin samples were measured as ug metal per g tissue wet weight.

Table 1. Mean quality assurance and quality control data for analysis, for 2016/17 samples.

Element	LOD[a] (ppm)	Blank	Duplicate RPD[b] (%)	LCS[c] % Recovery	Spike % Recovery	SRM[d] % Recovery
Ag	0.04	BDL[e]	8.5	102.5	103.3	96.3
Al	4.80	BDL	7.6	97.4	100.2	N/A
As	0.04	BDL	11.3	99.0	98.8	98.8
Au	0.12	BDL	*	98.3	107.5	N/A
Ba	0.04	BDL	*	94.1	94.2	N/A
Be	0.04	BDL	*	99.2	103.9	N/A
Cd	0.07	BDL	8.4	97.7	100.1	103.9
Co	0.04	BDL	8.0	108.2	105.2	N/A
Cr	0.27	BDL	5.2	103.9	94.3	110.7
Cu	0.20	BDL	13.0	104.2	106.4	96.3
Fe	7.24	BDL	8.2	108.8	110.0	107.2
Li	0.07	BDL	*	100.3	102.1	N/A
Mg	6.32	BDL	5.1	100.7	105.8	N/A
Mn	0.07	BDL	9.7	101.5	99.2	N/A
Mo	0.07	BDL	13.7	102.9	101.7	N/A
Ni	0.07	BDL	9.1	102.3	95.2	76.6
Pb	0.07	BDL	*	97.3	106.1	96.1
Sb	0.12	BDL	*	104.1	107.2	N/A
Se	0.07	BDL	8.3	96.2	95.8	105.0
Sr	0.04	BDL	4.5	96.6	88.9	N/A
Ti	0.07	BDL	4.2	106.8	101.0	N/A
V	0.04	BDL	*	102.2	105.7	N/A
Zn	0.32	BDL	11.3	105.9	116.6	109.6
Hg	0.03	BDL	3.9	100.7	103.7	98.9

[a] LOD= Limit of detection; [b] RPD= Relative percent difference; [c] LCS= Laboratory control sample;

[d] SRM= Standard reference material (DOLT-4; DORM-3); [e] BDL= Below detection limit

* All duplicate measurements were below the Project Quantitation Limit

Table 2. Mean quality assurance and quality control data for analysis, for 1999 samples.

Element	LOD[a] (ppm)	Blank	Duplicate RPD[b] (%)	LCS[c] % Recovery	Spike % Recovery	SRM[d] % Recovery
Ag	0.01	BDL[e]	9.2	101.2	100.7	103.7
Al	0.40	BDL	*	97.0	104.1	N/A
As	0.02	BDL	7.6	103.1	95.2	108.9
Au	0.01	BDL	*	85.7	106.9	N/A
Ba	0.01	BDL	*	97.7	94.2	N/A
Be	0.01	BDL	*	100.1	106.1	N/A
Cd	0.09	BDL	6.0	99.4	103.5	101.1
Co	0.01	BDL	*	103.6	107.0	N/A
Cr	0.04	BDL	9.5	103.3	99.1	N/A
Cu	0.04	BDL	9.4	97.8	110.8	102.7
Fe	0.40	BDL	10.7	102.7	111.4	101.4
Mg	1.10	BDL	4.6	100.0	102.1	N/A
Mn	0.01	BDL	10.2	92.3	97.8	N/A
Ni	0.01	BDL	10.8	101.5	92.4	90.3
Pb	0.01	BDL	*	102.8	103.3	104.1
Se	0.01	BDL	6.2	103.6	106.0	114.3
Sn	0.02	BDL	*	95.5	90.3	N/A
Sr	0.01	BDL	10.1	95.1	92.6	N/A
Ti	0.09	BDL	8.7	105.9	101.5	N/A
Zn	0.45	BDL	10.4	103.4	111.2	108.3
Hg	0.002	BDL	2.9	96.8	100.1	85.8

[a]LOD= Limit of detection; [b]RPD= Relative percent difference; [c]LCS= Laboratory control sample;

[d]SRM= Standard reference material (DOLT-3; DORM-2); [e]BDL= Below detection limit

* All duplicate measurements were below the Project Quantitation Limit

2.5 Statistics

Since the original data are non-negative and right skewed, the log transformation was applied, so the log-transformed data follows a normal or at least approximately normal distribution. The one-way ANOVA and multiple comparisons tests were used to determine whether there was evidence of differences among groups. The independent two sample t-test was used to test the significant differences between groups. The criterion for statistical significance was $p < 0.05$. All analyses were conducted using the R software package (R 3.5.2).

3. Results

The levels of 23 metals and Se were measured in skin biopsies from seven species of free-ranging adult whales in the Sea of Cortez in 1999, 2016, and 2017: sperm whales (*Physeter macrocephalus*), humpback whales (*Megaptera novaeangliae*), fin whales (*Balaenoptera physalus*), pilot whales (*Globicephala macrorhyncus*), blue whales (*Balaenoptera musculus*), Bryde's whales (*Balaenoptera brydei*), and a minke whale (*Balaenoptera acutorostrata*). See Table 3 for sample numbers across species and years.

Table 3. Whale Skin Biopsies Collected

	1999		**2016**		**2017**		
Whale	*Female*	*Male*	*Female*	*Male*	*Female*	*Male*	***Total***
Sperm	21	12	5	0	0	1	***39***
Pilot			3	0	2	3	***8***
Humpback			1	4	5	11	***21***
Fin			2	11	9	23	***45***
Blue			1	3	0	4	***8***
Bryde's			0	3	2	3	***8***
Minke			0	1	0	0	***1***
Total	***33*** ***(21 F, 12 M)***		***34*** ***(12 F, 22 M)***		***63*** ***(18 F, 45 M)***		

3.1 Cumulative Whale Metal Levels over Time

Metal levels in all skin samples collected were analyzed, including 39 sperm whales, 8 pilot whales, 21 humpback whales, 45 fin whales, 8 blue whales, 8 Bryde's whales and 1 minke whale (Table 3), and how metal levels changed over time was assessed (Figures 1 and 2). In general, the data showed decreasing levels of metals (Mg, Zn, Se, Mn, Cu, Ti, As, Hg, Sr, and Pb) in whale skin between 1999, 2016, and 2017; however, skin metal levels for Fe, Al, and Ni increased with time, while Cr levels did not change (Figure 1). As expected, the highest levels observed were for the essential metals: Fe, Mg, and Zn. With regards to metals of public health concern, the highest levels were for Al, Ni, and Cr. The next highest metal levels were for Cu, Mn, Ti, and Se. Levels were low for other metals of public health concern, including arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg). Some metals were not detected or detected infrequently in the sample set and were left out of further discussion: cobalt (Co), lithium (Li), beryllium (Be), antimony (Sb), gold (Au), vanadium (V), silver (Ag), and barium (Ba). For the metals discussed here, any individual samples that were non-detects were assigned half of the limit of detection and averaged with the other samples, as is standard practice.

Considering differences by gender (Figure 2), levels of Mg, Zn, Se, Cu, Ti, As, Hg, Sr, and Pb significantly decreased in females over time (i.e. 1999 vs 2016 or 2017), while for males Mg, Fe, Zn, Se, Cu, Ti, As, Hg, and Sr levels significantly decreased over time. In males, there was a significant decrease in Pb levels from 1999 to 2016 (2.3-fold), but a significant increase from 2016 to 2017 (2.1-fold), essentially returning back to 1999 levels. Levels for both Al (2.3 and 2.8-fold for 2016 and 2017, respectively) and Ni (3.5 and 2.5-fold for 2016 and 2017, respectively) significantly increased in males over time, while in females mean Al levels increased with time in a similar pattern to males but was not statistically significant.

3.2 Whale Metal Levels Considered by Suborders over Time

Whales (cetaceans) are organized into the suborders mysticetes (baleen whales) and odontocetes (toothed whales) to reflect their trophic level in the food web. We considered changes in whales by these suborders, though our 1999 data set did not include mysticete whales. This approach also allowed for a more refined assessment of changing metal levels over time (1999 odontocetes vs 2016/17 odontocetes) and for an assessment of differences between trophic levels over the same time period (2016/17 odontocetes vs mysticetes) (see Figures 3 and 4). Due to low odontocete sample numbers for 2016 and 2017, we combined these years to assess long-term changes (i.e. vs. 1999) only. Long term changes in odontocetes showed statistically significant decreased mean levels for Mg, Zn, Se, Cu, Ti, As, Sr, and Pb and significantly increased mean levels for Fe (2.3-fold) and Ni (3.2-fold). A 1.4-fold increase in mean Al levels was not significant (Figure 3). When differences in metals by trophic level were considered (Figure 3), mysticetes exhibited lower mean levels of Zn, Se, and especially Hg (138-fold lower) than odontocete levels, but higher mean levels of Al and Mg (1.5 and 1.7-fold), respectively. No significant differences were observed for Fe, Mn, Cr, Ti, Sr, and Pb levels.

Gender differences within odontocetes and mysticetes were considered (Figure 4). Significantly higher levels of Mg (2.1-fold), Se (1.3-fold), Al (5.8-fold), and As (5.5-fold) in 2016/17 were observed in odontocete females compared to males; however, no metal levels were higher in odontocete males compared to females. For mysticetes, significantly higher mean levels of Fe (2.2-fold), Mn (1.5-fold), Ni (3.1-fold), Ti (1.6-fold), Hg (6-fold), and Sr (2.2-fold) were observed in males compared to females; however, no metals were observed higher in mysticete females compared to males.

3.3 Essential Metal Levels Considered by Species over Time

Differences in essential metals (Mg, Fe, Zn, Se, Cu, Mn, and Mo) within species over time and between species in the same years were assessed (Figure 5). There were no significant differences between odontocete species (pilot and sperm whales) sampled within the same year, while there were significantly higher Zn (2.3-fold) and Se (2.1-fold) levels in pilot whales compared to sperm whales in 2016. For pilot whales, there were no significant changes over time. For sperm whales there were significant decreases from 1999 to 2016 in mean metal levels for Mg (1.7-fold), Zn (3-fold), and Se (9.8-fold); while there was a 2-fold significant increase in mean Fe levels.

Gender differences between odontocetes species were considered (Figure 6). Due to low numbers of male sperm whales in 2016/17, gender differences in sperm whales were not statistically addressed. For pilot whales, significantly higher Se (1.1-fold) levels were observed in females compared to males, but no differences were shown in any other essential metals.

For mysticetes, significant differences between species (blue, Bryde's, humpback, and fin whales) sampled within the same year were observed (the sample number for minke whales was too small for statistical comparison). Overall, Bryde's whales had the highest metal levels in 2016 and 2017, while fin whales typically had the lowest metal levels. Statistically significant differences between species within the same year are summarized in Tables 4 and 5. For blue whales and humpback whales, there were no significant changes over time for essential metals (Figure 5). For fin whales, there were significant increases from 2016 to 2017 in mean metal levels of Cu (3.2-fold) and Mn (1.5-fold), but no observed increases for essential metals. For Bryde's whales there were significantly decreased levels for Fe (5.6-fold) and Cu (1.9-fold) from 2016 to 2017, while mean levels of Mg, Zn, Se, and Mn were lower in 2017 than 2016, but were not significant.

Differences in essential metal levels between genders within mysticete species were considered (Figure 6). Due to low sample numbers between genders, 2016 and 2017 samples were combined and gender differences were assessed for humpback and fin whales only. Fin whales did not exhibit any differences between males and females. Humpback whales exhibited significantly higher Fe (2.9-fold) and Mn (2.2-fold) levels in males than females. Mg (1.8-fold) and Cu (3.9-fold) levels were also higher in males than females but were not statistically significant.

When odontocete species and mysticete species were compared, significantly higher Zn levels were observed for both pilot and sperm whales compared to any individual mysticete species in 2016 and 2017. Pilot whale Mg levels (411.17 ± 116.5 ug/g) were significantly lower than Bryde's whales (1589 ± 512.2 ug/g) in 2016 and significantly lower than all mysticete species in 2017.

3.4 Non-essential Metal Levels Considered by Species over Time

Change in non-essential metals (Al, Cr, Ni, Ti, As, Hg, Sr, and Pb) within species over time and between species in the same years were assessed (Figure 6). No significant differences between odontocete species for non-essential metals (Al, Cr, Ni, Ti, As, Hg, and Pb) sampled within the same year were observed. For pilot whales, a significant decrease of mean Al (8.5-fold), As (9.5-fold), and Ti (4.5-fold) levels were observed from 2016 to 2017. For sperm whales, a trend of increasing Al (2.4-fold higher in 2016 vs 1999) and Cr (1.2-fold higher in 2016 vs 1999) levels was observed, and significantly decreased mean levels for Ti (6.2-fold), Sr (24-fold) and Pb (19.9-fold) from 1999 to 2016 were observed.

Differences between genders for odontocetes species were considered (Figure 6). Due to low numbers of male sperm whales in 2016/17 gender differences in sperm whales were not statistically assessed. In pilot whales, higher mean levels were observed in males compared to females for Al (1.5-fold), As (1.6-fold), Hg (1.6-fold), and Sr (1.6-fold), while females exhibited higher Pb levels (1.8-fold) than males, but these data were not statistically significant.

Differences between mysticete species (blue, Bryde's, humpback, and fin whales) over time were assessed (Figure 5). Unlike the essential metals, no individual whale species exhibited overall higher non-essential metal levels. Statistically significant differences between species within the same year are summarized in Tables 4 and 5. For the non-essential metals, Bryde's whales did not exhibit statistically significant differences between 2016 and 2017. For blue whales, a 3.6-fold decrease in Ti levels from 2016 to 2017 ($p = 0.002$) was observed. In addition, a 1.7-fold decrease in mean Cr and a 2.4x increase in mean Ni levels was observed but these changes were not statistically significant. For fin whales, mean levels for Cr (1.9-fold), Ni (5-fold), and Pb (6.9-fold) significantly increased while levels of Ti (2-fold) significantly decreased from 2016 to 2017. For humpback whales, there was a 2.3-fold significant decrease in Cr level from 2016 to 2017 (15.17 ± 4.66 vs. 6.68 ± 2.47 ug/g, respectively).

Differences between genders for mysticete species were considered (Figure 6). Due to low numbers of female Bryde's and blue whales in 2016/17 gender differences in these species were not statistically assessed. No differences between male and female fin whales were observed. For humpback whales, higher mean levels of Cr (4.5-fold), Ni (7.5-fold), and Pb (3.4-fold, not significant) were observed in males compared with females, while females exhibited higher Al (2-fold) compared to males.

Statistical differences between species within the same year were assessed (data are summarized in Tables 4 and 5). Most notably, significantly higher Hg levels were observed for both pilot and sperm whales (odontocetes) compared with any individual mysticete species in 2016 and 2017.

Table 4. 2016 Statistically Different Skin Metal Levels Between Species (p<0.05)

	Sperm Whales	*Pilot Whales*	*Blue Whales*	*Bryde's Whales*	*Fin Whales*	*Humpback Whales*	*Minke Whales*
Sperm Whales		Se, Zn, Sr, Ag, Cd, V	Zn, Hg	Fe, Cu, Zn, Hg, Cd, Co	Cu, As, Se, Zn, Hg, Cd	Cu, Se, Zn, Hg, Cd	-
Pilot Whales			Se, Zn, Hg, Sr	Mg, Fe, Cu, Zn, Hg, Cd, Co	Cu, Se, Zn, Hg, Sr, Ag	Se, Zn, Hg, Ti, Sr	-
Blue Whales				Cu, Se, Co	Pb, Co, Ba		-
Bryde's Whales					Fe, Cu, Se, Pb, Zn, Co	Fe, Cu, Se, Co	-
Fin Whales						Cr	-
Humpback Whales							-
Minke Whales							

Table 5. 2017 Statistically Different Skin Metal Levels Between Species ($p<0.05$)

	Pilot Whales	*Blue Whales*	*Bryde's Whales*	*Fin Whales*	*Humpback Whales*
Pilot Whales		Se, Zn, Hg	Mg, Cu, As, Zn, Hg	Mg, Al, As, Zn, Hg	Mg, Se, Zn, Hg
Blue Whales			Cu, Se	Al	Al
Bryde's Whales				Cu, Se	Cu, Se
Fin Whales					As
Humpback Whales					

4. Discussion

Whales are key apex species for ocean systems, and as such can provide a useful snapshot of an ecosystem's health. The Sea of Cortez is one of the most biodiverse marine ecosystems on the planet, and is home to 37 cetacean species, including 8 mysticetes (blue, Bryde's, humpback, fin, minke, sei, and North Pacific right whales), 16 delphinids, 2 phocoenidae (including the vaquita, the most endangered cetacean on the planet), 9 ziphiids (beaked whales), both Kogia species (dwarf and pygmy sperm whales) and sperm whales (Páez-Osuna et al., 2017). Five of these species are listed as endangered or critically endangered (blue, fin, sei, and North Pacific right whales and the vaquita) and the sperm whale is listed as threatened. Anthropogenic impacts on these marine mammals greatly diversified starting in the 1970s, bringing pollution, noise, tourism, vessel collisions, fisheries interactions, habitat modification, and climate change effects (Arellano-Peralta et al., 2015). Importantly, adverse health effects in marine mammals are commonly related to the level of anthropogenic pollution in their environments (Mori et al., 2008; Mos et al., 2006). Here, the focus is on the current status of metals pollution in whales encountered in the Sea of Cortez.

4.1 Previously Reported Metal Levels in the Sea of Cortez

Metal pollution is of particular concern in the Sea of Cortez due to the high incidence of mining that occurs along the coast. In the Mexican state, Baja California Sur, mining makes up approximately 25% of the commercial industry (plaster, limestone, phosphorus, copper, gold, silver, manganese, and chrome). Metal mining activities are well-documented to have detrimental effects on local ecosystems by leaching many toxic chemicals that can cause serious risks for human and wildlife health (Espinosa-Reyes et al., 2014; Armendariz-Villegas et al., 2015). Many other studies have documented levels of metals in the Sea of Cortez via sampling environmental substrates (sediment, seawater), flora (sea grasses, mangroves), and fauna (zooplankton, fish, bivalves, birds, sea turtles and marine mammals) (Villa et al., 1993; Gardner et al., 2006; Cadena-Cardenas et al., 2009; Jara-Marini et al., 2009; Jimenez et al., 2005; Ruelas-Inzunza and Páez-Osuna, 2000; Szteren and Aurioles-Gamboa, 2013; Roldan-Wong et al., 2018). The metals most often reported were Cd, Zn, Pb, Cu, Ni, Fe, Mn, and Co, and the highest levels reported were for Zn, Fe, Mn, Cd, and Al.

Four other studies have considered marine mammal species. Bernardo Villa et al. (1993) reported metal levels in heart, kidney, and liver from vaquita (*P. sinus*). Méndez et al. (2002) reported metal concentrations in liver, lung, heart, muscle, blubber, and kidney from gray whales that beached in Sinaloa and Baja California Sur, Ruelas-Inzunza. Páez-Osuna (2002) reported metals in gray and sperm whales that beached in Sinaloa and Sonora Szteren and Aurioles-Gamboa (2013) reported bone metal levels in California sea lions (*Z. californianus*). Overall, the values reported for vaquita (collected in 1988) were lower in Ni and Mg, and higher in Fe than our reported values for cetaceans (or odontocetes) in 1999, 2016, and 2017. The higher Fe is likely due to the enriched hemoglobin content in heart, kidney and liver relative to skin.

In 1999, four gray whales (*Eschrichtius robustus*) and one sperm whale were found stranded along the coast of Mexican states Sonora and Sinaloa. Metal levels (Fe, Zn, Mn, Cu, Cd, and Pb) were assessed in kidney, liver and muscle. These whales exhibited much higher Fe, Cu, and Cd levels compared to our mean reported sperm whale levels from the same year. Also in 1999, gray whales that were found stranded on the coast of Baja California Sur and Sinaloa were assessed for metal loads (Méndez et al., 2002). It was observed that these whales all died of various infections, which may have been influenced by metal loads in the whales. For example, Cd levels were all higher than the reported threshold (0.21 ppm) for Cd immunotoxicity (Desforges et al., 2016). As with Ruelas-Inzunza's study, metal levels for Fe, Cu, and Cd were much higher in this study than our reported levels for 1999 sperm whales. Bone metal levels for California sea lion collected between 1974-94 reported overall higher levels of Cd, Pb, Ni, Co, and As, and lower levels of Zn, Fe, and Hg (Szteren and Aurioles-Gamboa, 2013).

Importantly, a report by Roldán-Wong et al (2018) assessed bioaccumulation and biomagnification of metals in octopus tissues collected in the same area and roughly the same time as this study. They reported indications of biomagnification occurring for Cd, Co, Cu, Fe, Mn, Pb, and Zn from the Baja chocolate clam (*M. suqlida*) to Hubb's octopus (*Octopus hubbsorum*). They considered the tissue levels of metals in mantle/muscle, digestive glands, and branchial hearts relative to the international standards for human consumption and found Cd, Cu, Ni, and Pb levels in digestive glands and/or branchial hearts exceeded these standards but mantle/muscle levels were safe for consumption. Given that odontocete whales also eat octopi, and do not distinguish mantle/muscle from other organs, these values may also indicate levels of concern for these species. According to their results, Cu and Ni exhibited high biomagnification factors, which may contribute to some of the levels observed in the whale samples from this study. This study is the first Sea of Cortez-focused investigation to consider large whales (mysticetes and odontocetes), multiple species of whales (seven species) and a broad suite of metals (23 metals) reported to date. This study is also the first whale study to consider how metal levels are changing in whales from a specific region over a long period of time (1999 vs 2016 vs 2017).

4.2 Changes in Metal Levels with Time

This study initially considered a broad view to evaluate how metal levels in whale tissues are changing over time. For this analysis all whale samples were pooled by year and differences were only considered by year (1999 n = 33; 2016 n = 34; 2017 n = 63). From this broad point of view, a trend for decreasing metal levels in whale skin with time, over both long periods (17-18 years) and short periods (1 year) of time were observed. Three metals were exceptions and showed increasing levels with time: Al, Fe, and Ni. Of these metals, Fe is the only essential metal whereas both Ni and Al have well documented toxic effects known or suspected to induce cancers, neurological dysfunction, reproductive failure, and developmental effects (ATSDR – Al, Ni). When data by gender (Figure 2) were considered we found significantly decreased levels of Ti, As, Hg, Sr, Cu, Se, Zn, and Mg with time for both males and females, whereas Al, Fe, and Ni were significantly increased in males but not females.

When metal level changes in odontocetes were considered from 1999 to 2016/17 we observed significantly lower levels for Mg, Zn, Se, Cu, Ti, As, Hg, Sr, and Pb; whereas we observed significantly increased levels for Fe and Ni (Figure 3). Both Ni and Fe were reported to have biomagnification factors of 233 and 11, respectively, by Roldán-Wong et al. (2018). Hence, these elevated metals levels may indicate rising metal levels in the food chain from metal mining in the area.

When metal level changes in species (Figure 5) were considered, sperm whales exhibited lower mean levels of Mg, Mn, Zn, Se, Cu, Ti, Sr, and Pb and higher mean levels for

Fe, Al, and Ni from 1999 to 2016. Sperm whale levels for As, Cr, and Hg did not change. Between 2016 and 2017, pilot whales only exhibited significantly lower Ti, As, and Sr. Blue whales exhibited significantly lower levels of Ti only (2.18 ± 0.51 vs 0.61 ± 0.11 ug/g, respectively). Bryde's whales exhibited significantly lower levels of Fe and Cu only. Fin whales exhibited higher Cu, Mn, Cr, Ni, and Pb, whereas Ti was the only metal significantly lower (1.39 ± 0.23 vs 0.71 ± 0.09 ug/g, respectively). Humpback whales exhibited significantly lower Cr only (15.17 ± 4.66 vs 5.97 ± 0.80 ug/g, respectively).

4.3 Considerations between Trophic Levels

When differences between odontocetes and mysticetes within the same years (2016/17 combined) were considered, (Figure 3) we observed higher Mg levels in mysticetes compared to odontocetes (704.02 ± 52.58 vs 403.67 ± 61.34 ug/mg, respectively), whereas there were significantly higher Zn, Se, and Hg levels in odontocetes compared to mysticetes. It's likely the differences in essential metals (Mg, Zn, and Se) are due to differences in physiological levels between suborders, and perhaps due to difference in physiological function. We previously observed similar differences between mysticete and odontocete species in the Gulf of Mexico (Wise, Jr. et al., 2018).

No differences between odontocetes and mysticetes for Al, Cr, Ni, Ti, As, Sr, and Pb were observed. The lack of differences for these metals likely indicates an exposure route other than dietary as they feed on different trophic levels of the food chain. In addition, several of these metals are known to have poor gastrointestinal uptake in other mammalian species, which is likely consistent in whales (ATSDR 1999, 2007, 2008, 2012). It is possible that whales' exposures to metals like Cr and Ni are instead via inhalation, though there are no recent studies that report ambient air metals.

We expect the significantly higher Hg levels in odontocetes (1.86 ± 0.41 ug/mg vs 0.05 ± 0.02 ug/mg for odontocetes and mysticetes, respectively; $p = 2.2e-16$) likely reflects biomagnification of Hg, though Hg biomagnification has yet to be demonstrated in the Sea of Cortez. In this study, 2016/17 odontocete Hg levels were 8.5-fold higher than the highest level observed in octopuses sampled near Santa Rosalia during 2015/16, which suggests evidence for biomagnification of Hg (Roldán-Wong et al. 2018). So far only one other study has considered Hg levels in Sea of Cortez marine mammals, which reported a much lower range of 0.02-0.16 ug/g dry weight in California sea lion skulls collected throughout the area from 1978-1994 (Szteren and Aurioles-Gamboa, 2013). The Hg levels reported here are also much higher than levels we observed in mysticetes from the Gulf of Maine (<0.10 ug/g mean Hg levels for fin, humpback, and minke whales), but lower than levels we observed in Bryde's whales following the Deepwater Horizon oil spill (6-8 ug/g mean Hg) (Wise, Jr. et al., 2017, 2019). When these data are considered by gender, significantly higher Hg, Ni, Ti, Sr, Mn, and Fe levels in male mysticetes were observed compared to females. This distinction is important because metals can be transferred from mothers to offspring during pregnancy and lactation (Maunder et al., 2013; Chen et al., 2014; Noel et al., 2016). Hence, future studies should also consider metal levels in whale calves from the Sea of Cortez.

4.4 Considerations between Species

Differences between odontocete species were considered (pilot and sperm whales). We were able to biopsy only one of two sperm whales we encountered in 2017, and so were limited to statistical comparisons with sperm whales for 2016 only. Despite the differences in size, prey species, and ecological impact between pilot and sperm whales, we observed few statistically significant differences between pilot and sperm whales. Significantly higher levels of Zn (2.3-fold), Se (2.1-fold), and Sr (18.4-fold) were observed in pilot whales compared to sperm whales. We previously observed a similar difference for Zn between pilot and sperm whales in the Gulf of Mexico (Wise, Jr. et al., 2018). Intriguingly, vaquita Zn levels in heart, kidney, and liver were in a similar range (71-107 ug/g dry weight, or 96-1.9 ug/g wet weight) to levels in odontocete species from our study (Bernardo Villa et al., 1993). When compared to mysticete species, both pilot and sperm whales exhibited significantly higher levels of Zn and Hg, likely due to biomagnification of these metals through the food web. Notably, we observed similar metal levels across species for most metals of environmental concern: Al, Cr, Ni, Ti, As. This suggests (1) that these whale species are all similarly exposed to these metals, and (2) these are likely recent exposures since both migratory (blue, humpback and sperm whales) and resident (fin, Bryde's, and likely pilot and minke whales) species have similar metal levels. Further in support of metal exposure being recent, whales are known to slough skin in warmer waters and blue whales were demonstrated to incorporate environmental nitrogen and carbon isotopes into their skin every 193 ± 61 days (i.e. within the last 6 months). Given the 2016/17 samples from this study were collected at the end of the winter season, it is likely the skin metal levels were from exposures in the Sea of Cortez (Busquets-Vass, et al., 2017).

There are some significant differences between species for these metals. Most notably, 2017 Al levels were significantly higher in fin whales compared with humpback or blue whales from the same year. Significantly higher Cr levels were observed in 2016 humpback whales vs. fin whales (4.9-fold) and significantly higher arsenic levels were observed in 2017 fin whales vs. humpback whales (1.5-fold). Pb levels were significantly higher in 2016 blue and Bryde's whales vs. fin whales (4-fold and 14.4-fold, respectively). Levels reported here for Al are similar to levels previously reported in California sea lion skull bone, but Ni and Pb levels are much lower in our data (Szteren and Aurioles-Gamboa, 2013). When compared to levels reported in vaquita (heart, kidney, and liver) our reported Ni levels were higher whereas Pb levels were lower, especially when comparing between odontocete species (Bernardo Villa et al., 1993). Two studies reported metal levels for internal organs in gray whales and a sperm whale that stranded in the Sea of Cortez. While stranded animals are typically unhealthy and may exhibit higher metal loads as a result, these studies provide some reference values. Gray whale Ni levels were similar to our reported 2016 blue, fin, and minke whales, but were consistently lower than all other data. Gray whale Pb levels were higher than all species except for 1999 sperm whales and 2016 Bryde's whales which exhibited similar Pb levels(Méndez et al., 2002; Ruelas-Inzunza and Páez-Osuna, 2002). Gray whale Cd levels were consistently higher than our reported levels (Méndez et al., 2002; Ruelas-Inzunza and Páez-Osuna, 2002).

4.5 Considerations between Genders

Significant differences in metal loads can occur between males and females due to maternal off-loading of metals to calves, differences in habitat use that lead to different environmental exposures, differences in physiology between genders, or differences in behavior (e.g. mothers raising calves in shallow coastal waters) (Ersts and Rosenbaum, 2003; Smultea, 1994). Since these differences in whale populations are unable to be evaluated, we utilized our metals data to understand how males and females are impacted differently.

Differences between genders in all whales across years (Figure 2) were considered first. In 1999, females exhibited significantly higher Ti levels compared with males (3.7-fold), whereas Ti levels were the same between genders in 2016. In 2017 Ti levels in males were significantly higher than females. Similarly, in 1999, males exhibited significantly higher arsenic levels than females (1.5-fold) whereas in 2016 this pattern is reversed (2.3x higher in females) and in 2017 we observed no differences between genders. For Hg, higher levels were observed in females compared with males for both 1999 and 2016 (2.7-fold and 52.7-fold, respectively). However, 2017 data showed higher levels in males than females (7.3-fold). These Hg observations appear peculiar, until gender differences were considered by suborders (odontocetes vs mysticetes).

As mentioned earlier, higher levels of Hg were observed in odontocetes vs mysticetes, and in 2016 all odontocete samples were females. As shown in Figure 4, Hg levels between male and female odontocetes were similar when 2016 and 2017 were combined whereas 2016/17 mysticetes exhibited significantly higher Hg levels in males compared with females

(4.3-fold). Differences between males and females of the same species during the 2016/17 sampling seasons were not detected (see Figure 6). When gender differences for Ti levels were considered by suborders, no significant differences for 2016/17 odontocetes were observed and slightly but significantly higher Ti levels were found in male mysticetes compared with females (1.6-fold). However, gender differences for any individual species were not statistically different. For arsenic, significantly higher levels in 2016/17 female odontocetes were observed when compared with males (5.5-fold) but no differences between genders in mysticetes. Again, gender differences for any individual species were not detected. Significant differences between male and female mysticetes for Fe, Mn, Ni, and Sr levels (see Figure 4) were observed, and were most notable for Ni (3.1-fold higher in males). For odontocetes, significant differences between genders for Mg, Se, and Al were observed and in each case females exhibited higher levels than males (2.1-fold, 3.5-fold, and 5.8-fold for Mg, Se, and Al, respectively) The only gender differences detected at the species level were in humpback whales and were for Fe, Mn, Al, Cr, and Ni. Both Cr and Ni were significantly higher in females than males (4.5-fold and 7.6-fold, respectively), whereas Fe, Mn, and Al levels were significantly higher in males compared to females (2.9-fold, 2.2-fold, and 2-fold, respectively).

4.6 Comparisons Metals in Cetaceans from Other Regions

We have previously reported metal levels for whales in the Gulf of Mexico and the Gulf of Maine from 2010-2012 – two areas with high anthropogenic input of metals pollution (Wise, Jr. et al., 2018, 2019). When we consider mean metal levels for all whales sampled in each of these regions, higher levels of Mg, Zn, and Al were observed in the Sea of Cortez compared with either the Gulfs of Maine or Mexico whereas higher levels of Cr and Ni were observed in the latter. For all species assessed, mean metal levels for Mg and Al were much higher in the Sea of Cortez compared with either Gulf regions. Mysticete species (humpback, fin, and minke whales) in the Sea of Cortez consistently exhibited lower Ti levels compared to the same species in the Gulf of Maine. Other intra-species differences across regions included: higher Fe, Mn, Cu, Cr, and Ni levels in Gulf of Maine fin whales; higher Cr, Ni, and Cu levels in Gulf of Mexico sperm whales; higher Zn, Cu, Hg, and Pb levels in Gulf of Mexico pilot whales; and lower Zn levels in Gulf of Maine humpback whales.

5. Conclusions

Overall metal levels were decreasing with time (1999 vs 2016/17) in whale skin samples from the Sea of Cortez, though there were a few exceptions (Al, Cr, Fe, and Ni) which appear to be increasing or not changing relative to 1999 levels. Levels for Mg and Al were higher in the Sea of Cortez compared with our previous reports in similar species from the Gulf of Maine and Gulf of Mexico. Unfortunately, Al, Cr, and Mg are rarely looked at in Sea of Cortez biota and are difficult to compare for the region. Interestingly, Cd and Pb are frequently reported elevated in biota from this region but were low in whale skin samples. Perhaps this is in part due to these metals having low affinity for skin accumulation as the levels were observed much higher for internal organs of whales from two previous studies (Bernardo Villa et al., 1993; Páez-Osuna et al., 2002, 2017; Méndez et al., 2002). Future work should aim to include Al, Cr, Fe, and Ni in studies assessing metal levels in Sea of Cortez biota. Considerations for metal pollution from fossil fuel combustion associated with marine activities (e.g. shipping, fishing, tourism) should also be considered for marine mammal protections, as this has been demonstrated as a point source for these metals.

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Figure Legends

Figure 1. Metal Levels in All Whales Sampled over Time. The levels of essential metals in whale skin samples from the Sea of Cortez in 1999 (n = 33), 2016 (n = 34), and 2017 (n = 64) were measured. The mean (±SEM) ug metal/g tissue (w/w) for essential metals (**A, B**) and non-essential metals (**C, D**) in all species sampled is reported to determine overall status of whales as apex species for the Sea of Cortez; *p<0.05 vs 1999, $^{\#}$p<0.05 vs 2016.

Figure 2. Metal Levels in All Whales Sampled, by Gender, over Time. The levels of essential metals in whale skin samples from the Sea of Cortez in 1999 (n = 33), 2016 (n = 34), and 2017 (n = 64) were measured. The mean (±SEM) ug metal/g tissue (w/w) for essential metals (**A, B**) and non-essential metals (**C, D, E**) in males vs. females for all species sampled is reported to determine overall gender differences in whales as apex species for the Sea of Cortez; *p<0.05 between genders; $^{\alpha}$p<0.05 vs 1999 females; $^{\beta}$p<0.05 vs 2016 females; $^{@}$p<0.05 vs 1999 males; $^{\$}$p<0.05 vs 2016 males.

Figure 3. Metal Levels in Odontocetes vs Mysticetes. The mean (±SEM) ug metal/g tissue (w/w) for essential metals (**A, B**) and non-essential metals (**C, D**) in all odontocetes and all mysticetes sampled is reported to determine trophic effects on metal levels in whales from the Sea of Cortez; *p<0.05.

Figure 4. Metal Levels in Odontocetes vs Mysticetes, by Gender. The mean (±SEM) ug metal/g tissue (w/w) for essential metals (**A, B**) and non-essential metals (**C, D, E**) in all odontocetes and all mysticetes sampled, separated by gender is reported to determine differences in males and females within trophic levels in whales from the Sea of Cortez; *p<0.05.

Figure 5. Metal Levels in Individual Whale Species. The mean (±SEM) ug metal/g tissue (w/w) for essential metals in odontocete species (**A, C**) and mysticete species (**B, D**), and non-essential metals in odontocete (**E, F**) and mysticetes (**E, G**) species sampled is reported to determine metal level differences between species in whales from the Sea of Cortez; *$p<0.05$ vs 1999; $^{\#}p<0.05$ vs 2016; $^{@}p<0.05$.

Figure 6. Metal Levels in Individual Whale Species, by Gender. The mean (±SEM) ug metal/g tissue (w/w) for essential metals in odontocete species (**A, C**) and mysticete species (**B, D**), and non-essential metals in odontocete (**E, F, H**) and mysticetes (**E, G, I**) species sampled are reported to determine metal level differences between species in whales from the Sea of Cortez; *$p<0.05$.

Figure 1

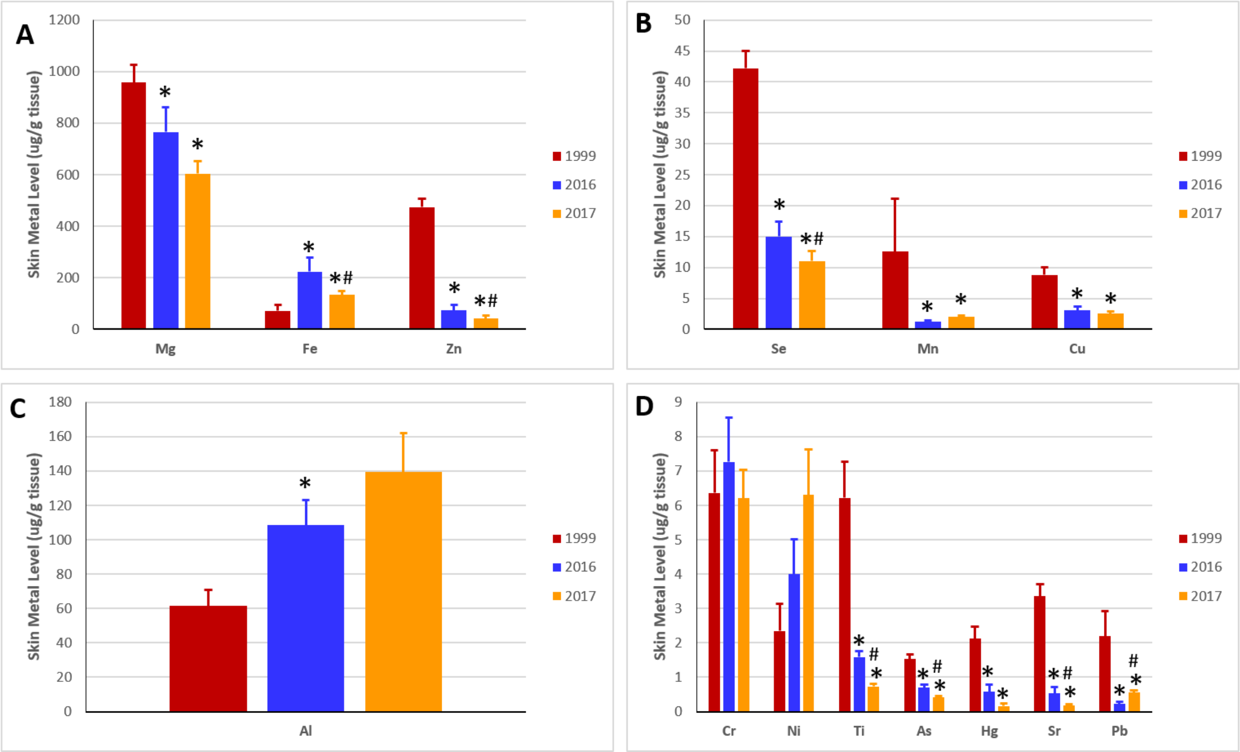

A
Skin Metal Level (ug/g tissue)
0
200
400
600
800
1000
1200
Mg
Fe
Zn
1999
2016
2017
*
*
*
*#
*
*#
B
Skin Metal Level (ug/g tissue)
0
5
10
15
20
25
30
35
40
45
50
Se
Mn
Cu
*
*#
*
*
*
*
C
Skin Metal Level (ug/g tissue)
0
20
40
60
80
100
120
140
160
180
Al
*
D
Skin Metal Level (ug/g tissue)
0
1
2
3
4
5
6
7
8
9
Cr
Ni
Ti
As
Hg
Sr
Pb
*
#
*
*
#
*
*
*
*
#
*
*
#
*

Figure 2

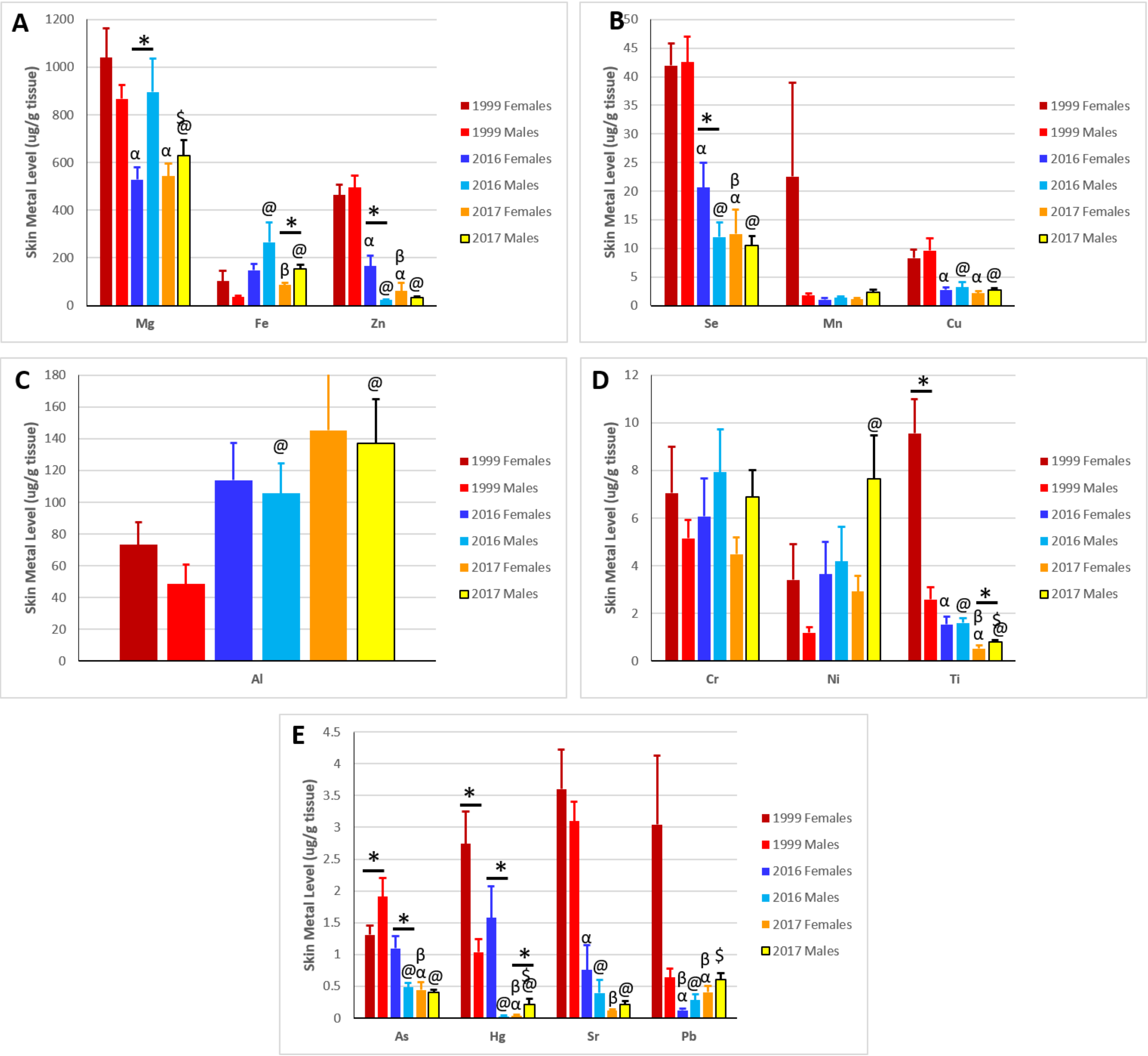

A
Skin Metal Level (ug/g tissue)
0
200
400
600
800
1000
1200
Mg
Fe
Zn
B
Se
Mn
Cu
0
5
10
15
20
25
30
35
40
45
50
C
Al
0
20
40
60
80
100
120
140
160
180
D
Cr
Ni
Ti
0
2
4
6
8
10
12
E
As
Hg
Sr
Pb
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
*
α
β
@
$
1999 Females
1999 Males
2016 Females
2016 Males
2017 Females
2017 Males

Figure 3

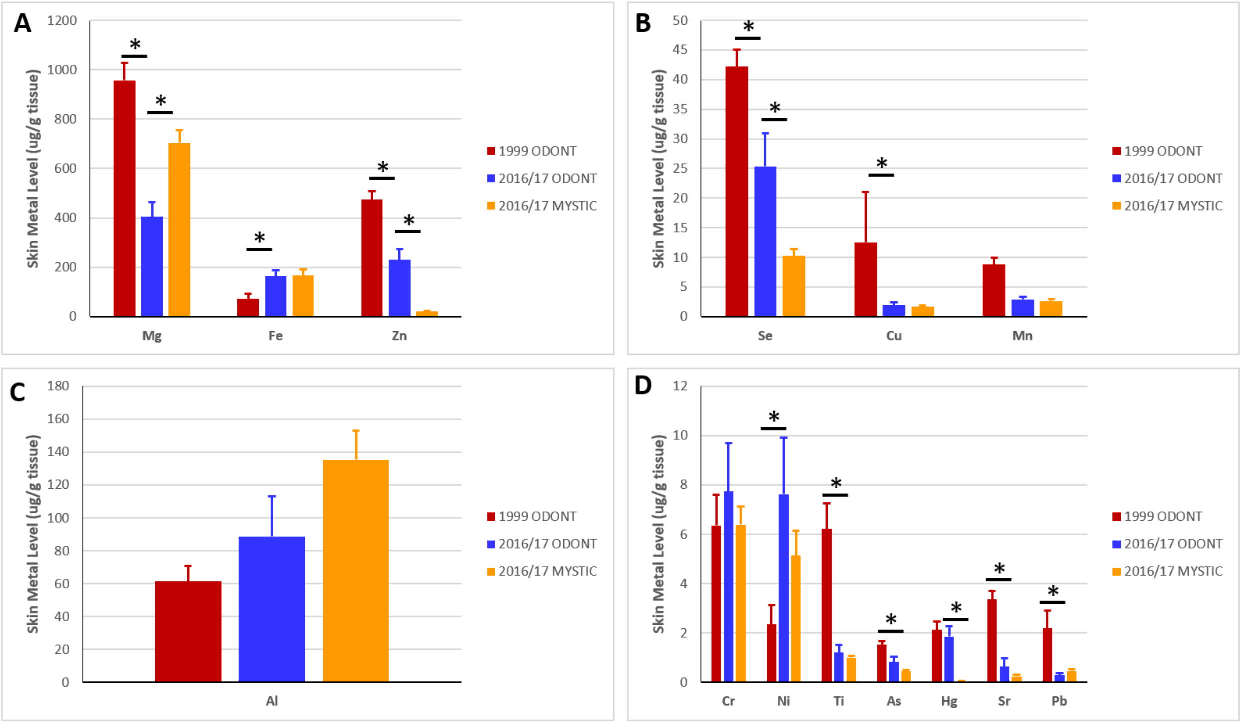

A
Skin Metal Level (ug/g tissue)
0
200
400
600
800
1000
1200
Mg
Fe
Zn
*
1999 ODONT
2016/17 ODONT
2016/17 MYSTIC
B
Skin Metal Level (ug/g tissue)
0
5
10
15
20
25
30
35
40
45
50
Se
Cu
Mn
*
1999 ODONT
2016/17 ODONT
2016/17 MYSTIC
C
Skin Metal Level (ug/g tissue)
0
20
40
60
80
100
120
140
160
180
Al
1999 ODONT
2016/17 ODONT
2016/17 MYSTIC
D
Skin Metal Level (ug/g tissue)
0
2
4
6
8
10
12
Cr
Ni
Ti
As
Hg
Sr
Pb
*
1999 ODONT
2016/17 ODONT
2016/17 MYSTIC

Figure 4

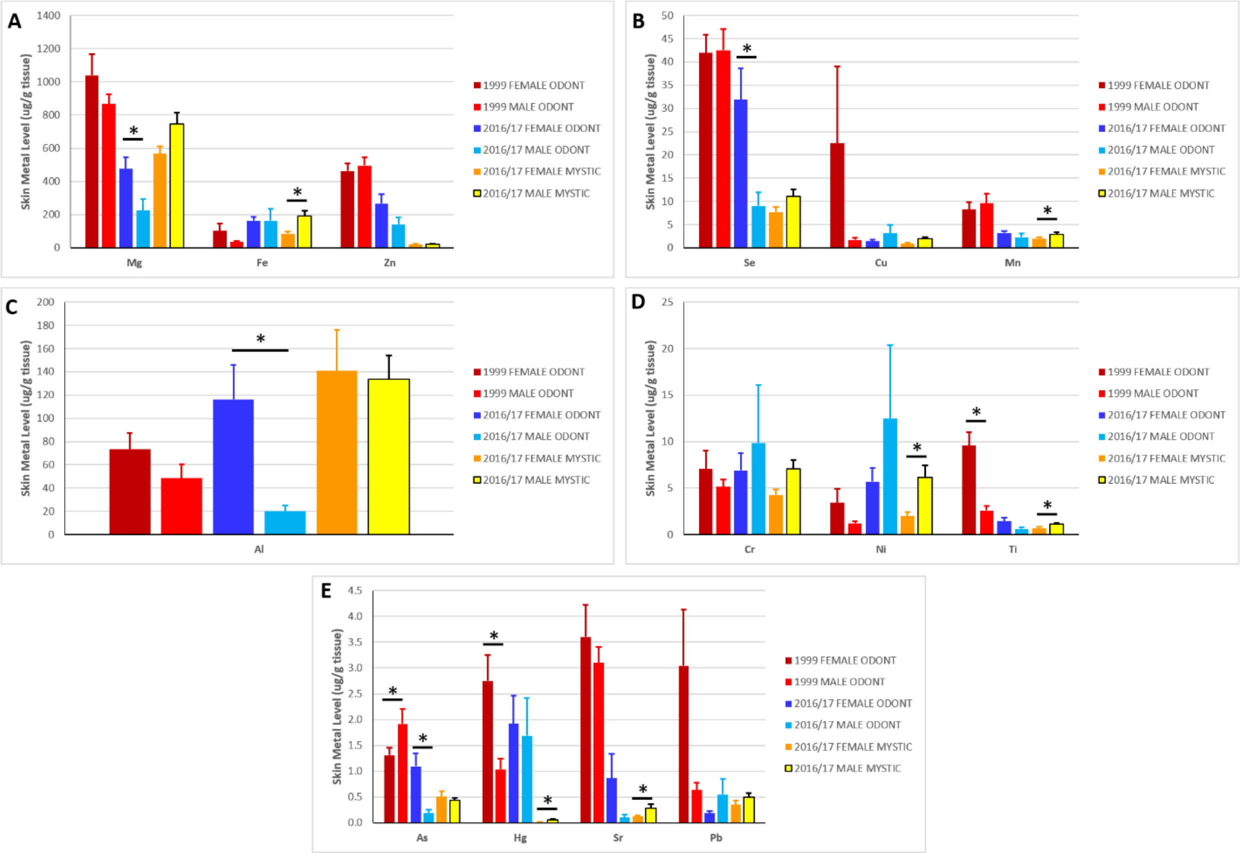

A
B
C
D
E
Skin Metal Level (ug/g tissue)
Mg
Fe
Zn
Se
Cu
Mn
Al
Cr
Ni
Ti
As
Hg
Sr
Pb
*
1999 FEMALE ODONT
1999 MALE ODONT
2016/17 FEMALE ODONT
2016/17 MALE ODONT
2016/17 FEMALE MYSTIC
2016/17 MALE MYSTIC

Figure 5.

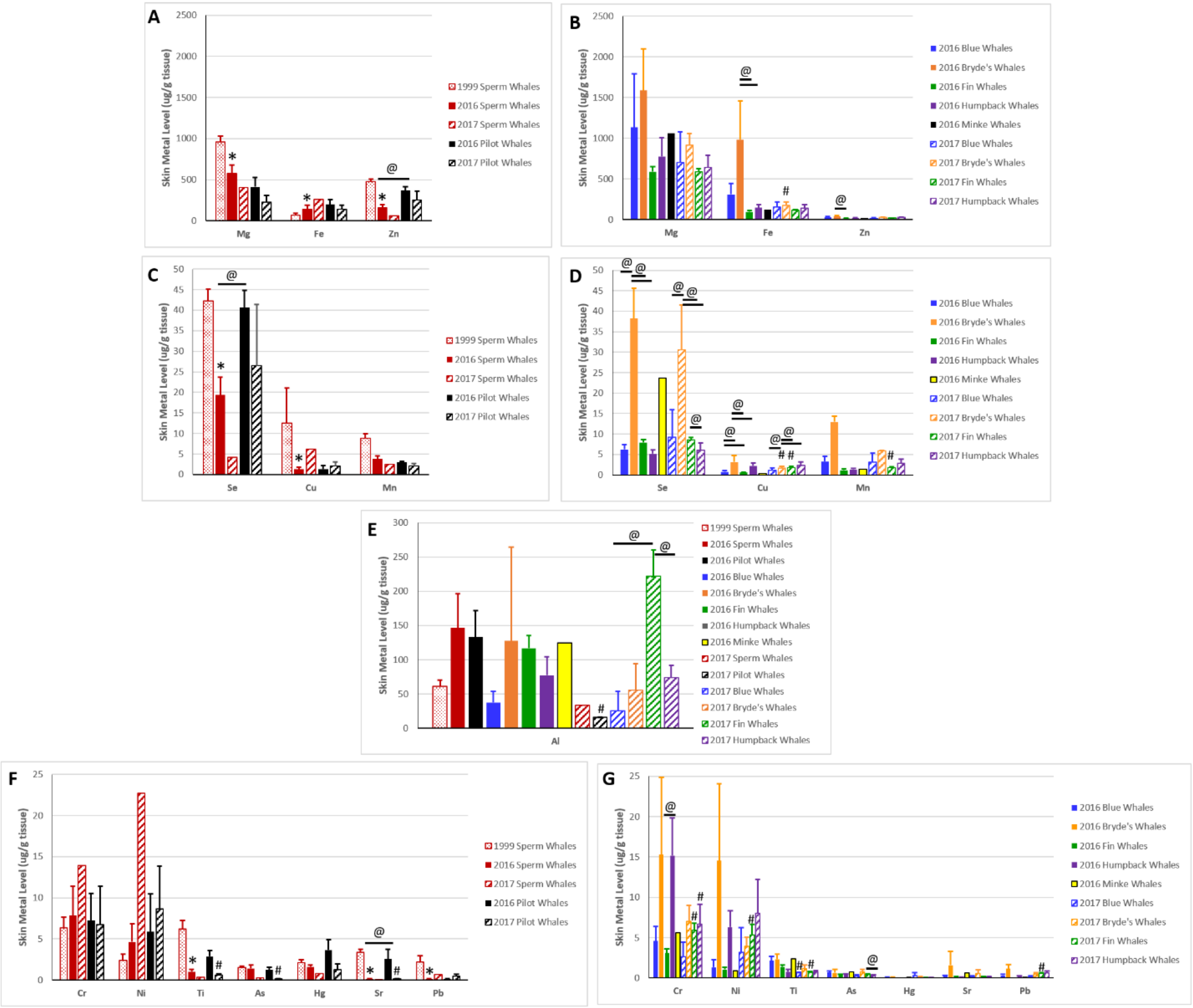
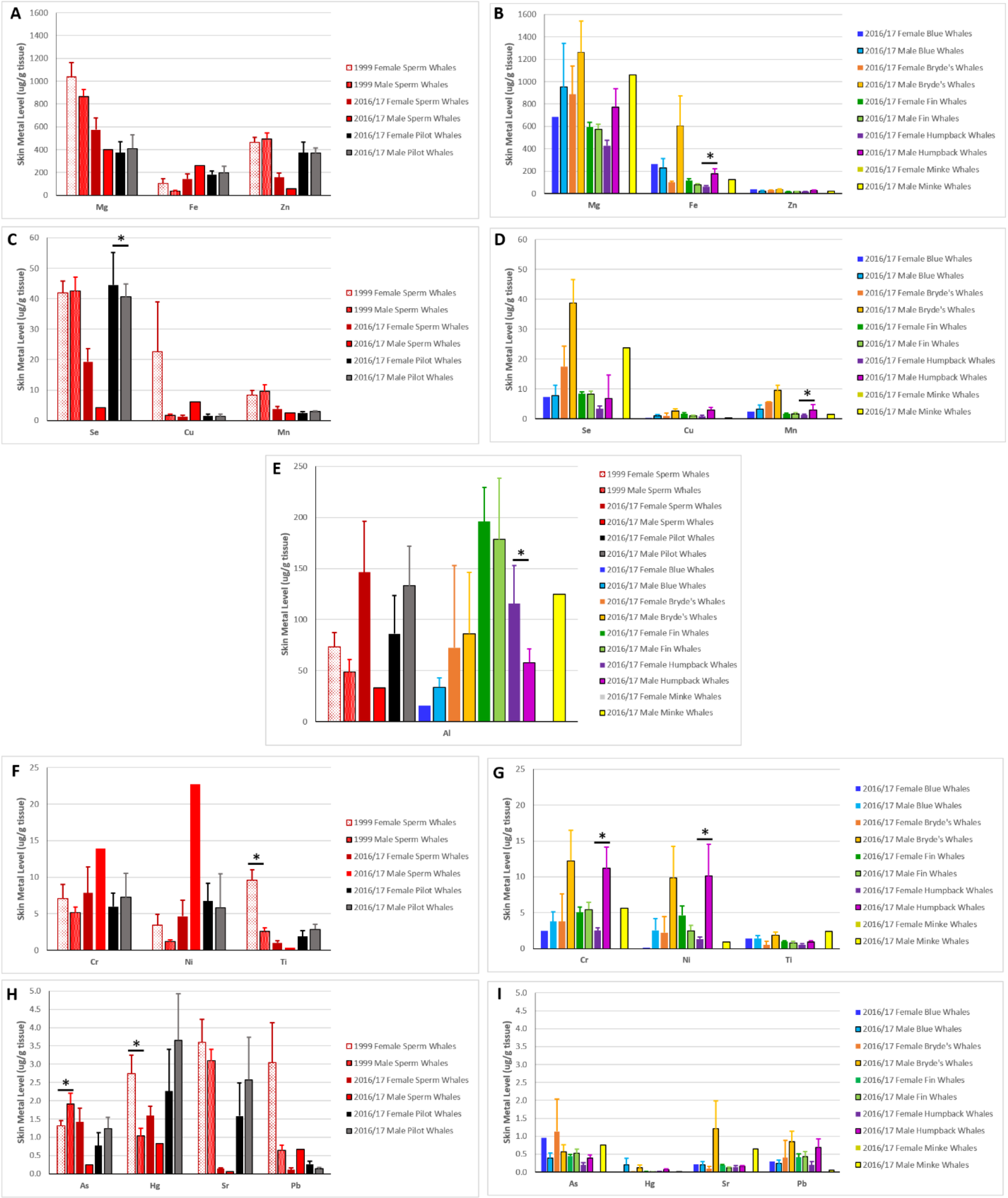

A
B
C
D
E
F
G
Skin Metal Level (ug/g tissue)
Mg
Fe
Zn
Se
Cu
Mn
Al
Cr
Ni
Ti
As
Hg
Sr
Pb
*
@
#
1999 Sperm Whales
2016 Sperm Whales
2017 Sperm Whales
2016 Pilot Whales
2017 Pilot Whales
2016 Blue Whales
2016 Bryde's Whales
2016 Fin Whales
2016 Humpback Whales
2016 Minke Whales
2017 Blue Whales
2017 Bryde's Whales
2017 Fin Whales
2017 Humpback Whales

Figure 6


A
B
C
D
E
F
G
H
I
Skin Metal Level (ug/g tissue)
0
200
400
600
800
1000
1200
1400
1600
Mg
Fe
Zn
Se
Cu
Mn
Al
Cr
Ni
Ti
As
Hg
Sr
Pb
*
1999 Female Sperm Whales
1999 Male Sperm Whales
2016/17 Female Sperm Whales
2016/17 Male Sperm Whales
2016/17 Female Pilot Whales
2016/17 Male Pilot Whales
2016/17 Female Blue Whales
2016/17 Male Blue Whales
2016/17 Female Bryde's Whales
2016/17 Male Bryde's Whales
2016/17 Female Fin Whales
2016/17 Male Fin Whales
2016/17 Female Humpback Whales
2016/17 Male Humpback Whales
2016/17 Female Minke Whales
2016/17 Male Minke Whales
0
10
20
30
40
50
60
0
50
100
150
200
250
0
5
10
15
20
25
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0