



Metal Levels in Whales from the Gulf of Maine: A One Environmental Health approach

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H I G H L I G H T S

- Gulf of Maine whales exhibited metal levels in their skin tissues.
- Chromium levels in the whales were similar to levels reported in exposed workers.
- Nickel levels in the whales were similar to levels reported in exposed workers.

A R T I C L E I N F O

Article history:

Received 3 May 2018

Received in revised form

8 October 2018

Accepted 16 October 2018

Available online 18 October 2018

Handling Editor: Martine Leermakers

Keywords:

One health

Gulf of Maine

Metals

Chromium

Nickel

Humpback whale

Fin whale

Minke whale

A B S T R A C T

One Environmental Health has emerged as an important area of research that considers the interconnectedness of human, animal and ecosystem health with a focus on toxicology. The great whales in the Gulf of Maine are important species for ecosystem health, for the economies of the Eastern seaboard of the United States, and as sentinels for human health. The Gulf of Maine is an area with heavy coastal development, industry, and marine traffic, all of which contribute chronic exposures to environmental chemicals that can bioaccumulate in tissues and may gradually diminish an individual whale's or a population's fitness. We biopsied whales for three seasons (2010–2012) and measured the levels of 25 metals and selenium in skin biopsies collected from three species: humpback whales (*Megaptera novaeangliae*), fin whales (*Balaenoptera physalus*), and a minke whale (*Balaenoptera acutorostrata*). We established baseline levels for humpback and fin whales. Comparisons with similar species from other regions indicate humpback whales have elevated levels of aluminum, chromium, iron, magnesium, nickel and zinc. Contextualizing the data with a One Environmental Health approach finds these levels to be of potential concern for whale health. While much remains to understand what threats these metal levels may pose to the fitness and survival of these whale populations, these data serve as a useful and pertinent start to understanding the threat of pollution.

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1. Introduction

The ocean serves as the final sink for most chemicals released into the environment by natural or anthropogenic sources. The ocean is an essential, but finite, resource and we are only beginning

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to understand the impacts of pollution on the global marine ecosystem. Society banned or phased out the use of many chemical contaminants (e.g. DDT, PCBs) due to their toxic effects on the environment or links to human disease; however they continue to be a problem for environmental health (Breivik et al., 2007; Loganathan and Kannan, 1994). Metals are often overlooked as a class of environmental contaminants because they are naturally occurring. However, the majority of modern heavy metal pollution is anthropogenic (Tchounwou et al., 2012). Data now show metals are global marine pollutants (Bjerregaard et al., 2015; Jarup, 2003; Wise et al., 2009). The toxic potential and environmental impacts of a number of metals is well established (e.g. lead, mercury, chromium, etc.), yet it remains a challenge to keep toxic metals out of 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.

The Gulf of Maine is an important waterway both for its economic value and for its high biodiversity. A number of species in the Gulf of Maine are intensely studied including the North Atlantic right whale (*Eubalaena glacialis*), fin whale and humpback whale. However, very little is known about pollutants in these whales. One study reported data indicating Gulf of Maine humpback whales had some of the highest levels of persistent organic pollutants (e.g. Σ PCBs, Σ DDTs, Σ chlordanes, and Σ PBDEs) when compared to other populations found in US coastal waters (Elfes et al., 2010). Two studies reported high levels of chromium in right whales and fin whales (Wise et al., 2008, 2009, 2015). There has also been no consideration of metal levels over time.

To understand the health of these whales better, we conducted three research voyages in 2010, 2011, and 2012. An important initial step in understanding the threats to the health of these whales is determining what chemicals they are encountering. We collected skin biopsies from individual whales and analyzed their skin metal levels. In this study, we report our findings regarding the levels of 25 heavy metals and selenium (Se) in Gulf of Maine whale skin over this three-year period.

2. Materials and methods

2.1. Sample collection

Skin biopsies were collected from free-ranging adult humpback, fin, and minke whales in the Gulf of Maine in the summer of 2010, and the autumn of 2011 and 2012. Our platforms were the research vessel *Odyssey*, a 93-foot motor-sailer ketch, and the research vessel *Caribana*, an 80-ft motor-sailer. Whales were located visually from various observation platforms above the deck from sunrise to sunset. These platforms are on top of the pilothouse (approximately 10 feet above the *Odyssey* deck), halfway up the main mast (approximately 30 feet above the *Odyssey* or *Caribana* decks), and the crow's nest near the top of the main mast (approximately 50 feet off the *Odyssey* deck). Whale spotters worked in 1–2 h shifts from one of the platforms, weather permitting. The biopsies were taken from the bow of the boat and details about the whale and biopsy were recorded, including estimated age (adult or subadult), biopsy location, any reaction by the whale, any identifying markings, GPS coordinates of the biopsy and number of individual whales in the group.

2.2. Biopsies

Biopsies were collected as previously described (Wise et al., 2018). Briefly, biopsies were collected from the flank of the

whale's back using a stainless steel tip approximately 20 mm in length and 6 mm in diameter. After retrieving the biopsy arrow, the sample was removed from the tip, samples were processed on-board to separate the skin and blubber using a ceramic knife and glass petri dish and frozen for storage. For our purposes, skin refers to all physiological layers above the blubber. The skin samples were further divided into approximately half with one piece used for metal analysis and one for genotyping analysis to determine gender. Previously, we demonstrated that metals are not released from the biopsy darts into the samples (Wise et al., 2009).

2.3. Gender determination

DNA was extracted from whale skin using standard methods (Wise et al., 2014; Carvalho et al., 2002). Gender was determined by PCR amplification of the SRY and keratin genes based on our published methods (Wise et al., 2018). Male samples have two bands (keratin and SRY) while females have one (keratin).

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 ELAN 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 (Supplemental Table 1). 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. Whale skin samples were analyzed for 25 metals and Se, and measured as ug metal per g tissue wet weight.

2.5. Statistics

Means, standard deviations, standard error, Geomeans, and standard deviations of Geomean were calculated for all groups and subgroups. Because the distributions of values were skewed, a normalizing logarithmic transformation was used for statistical testing. Generalized linear model (GLM) was used to explore the impacts of gender and year among the whales. P-values less than 0.05 were regarded as statistically significant, and no adjustment was made for multiple comparisons. The statistical analyses were all conducted in SAS 9.3 (SAS Institute, Cary, NC).

3. Results

We measured the levels of 25 metals and Se in skin biopsies from three species of free-ranging adult whales in the Gulf of Maine in 2010, 2011, and 2012: humpback whales (*Megaptera novaeangliae*), fin whales (*Balaenoptera physalus*), and a minke whale (*Balaenoptera acutorostrata*). We biopsied 33 humpback whales (6 in 2010, 8 in 2011, and 19 in 2012), 9 fin whales (2 in 2010, 4 in 2011, and 3 in 2012), and 1 minke whale (2012).

3.1. Cumulative whale metal levels

We analyzed metal levels in all whale skin samples we collected including 33 different humpback whales, 9 fin whales, and 1 minke whale (Table 1). In general, the pattern of metal accumulation we observed were consistent across species (Figs. 1–5). The highest levels observed were for the essential metals: Iron (Fe), magnesium (Mg) and zinc (Zn) and the metals of public health concern: Aluminum (Al), nickel (Ni) and chromium (Cr). There were also

Table 1
Whale skin biopsies collected.

| Whale | 2010 | | 2011 | | 2012 | |
|----------|--------|------|--------|------|--------|------|
| | Female | Male | Female | Male | Female | Male |
| Humpback | 3 | 3 | 2 | 6 | 9 | 10 |
| Fin | 2 | 0 | 0 | 4 | 1 | 2 |
| Minke | 0 | 0 | 0 | 0 | 1 | 0 |

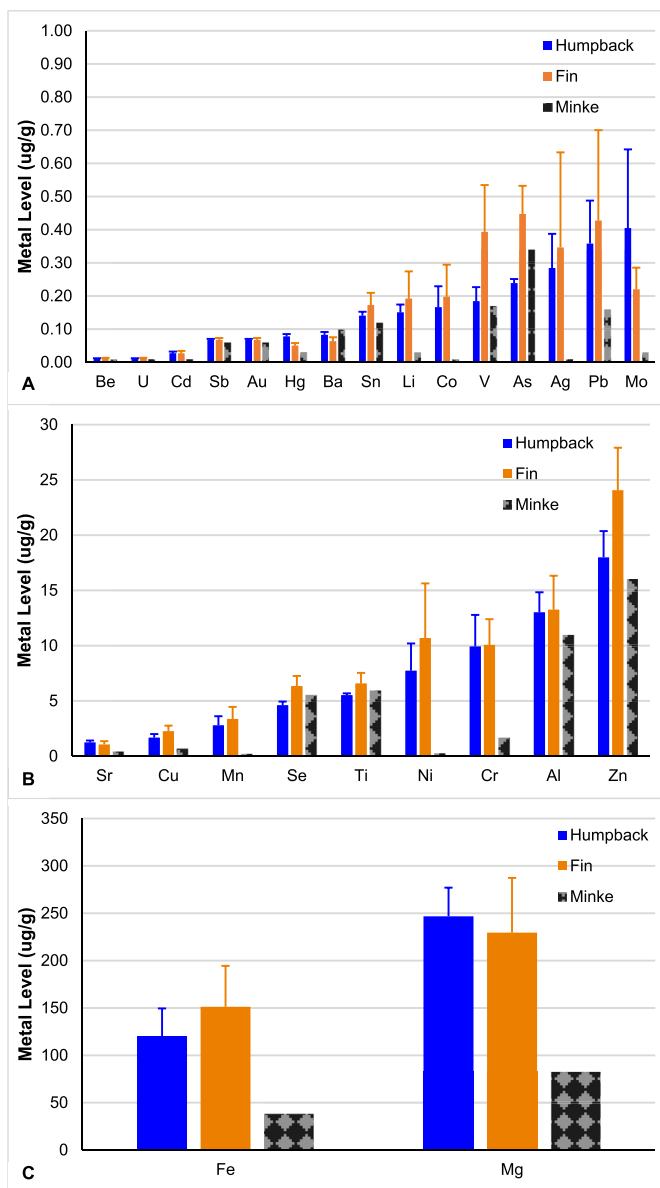


Fig. 1. Metal Levels in Humpback, Fin and Minke Whales Biopsied in the Gulf of Maine. We measured 25 metals and Se in all whale skin biopsies. Sample sizes were 33, 9, and 1 for humpback, fin and minke whales, respectively. Data represented as mean ± standard error in units of µg/g tissue. Data are divided into panels solely for optimal visualization. (A) Metals with mean skin levels <1 µg/g; (B) Metals with mean skin levels between 1 and 50 µg/g; (C) Metals with mean skin levels >50 µg/g.

comparatively high levels of titanium (Ti) and selenium (Se). Levels were low for other metals of public health concern: Arsenic (As), cadmium (Cd), cobalt (Co), lead (Pb), mercury (Hg) and uranium (U) (Figs. 1–5).

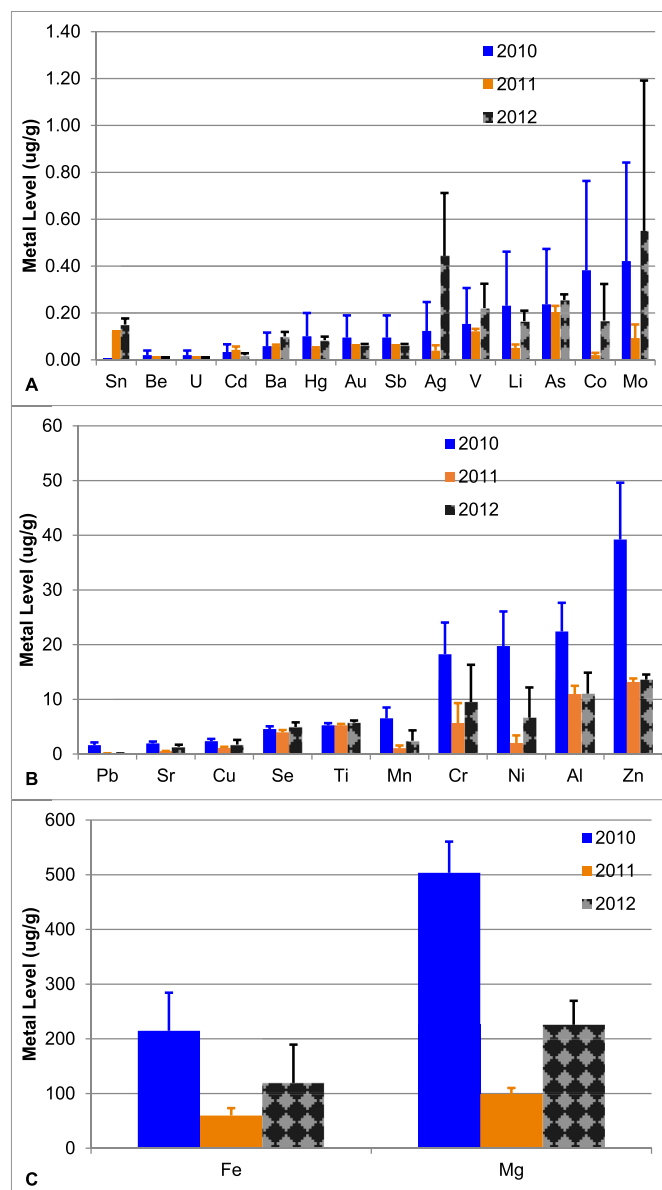


Fig. 2. Metal Levels in Humpback Whales Biopsied in the Gulf of Maine over Time. We measured 25 metals and Se in the humpback whale skin biopsies. Sample sizes were 6, 8, and 19 for 2010, 2011, and 2012 respectively. Data represented as mean ± standard error in units of µg/g tissue. (A) Metals with mean skin levels <1 µg/g; (B) Metals with mean skin levels between 1 and 50 µg/g; (C) Metals with mean skin levels >50 µg/g.

3.2. Whale metal levels considered by gender and over time

For the humpback whales, we had sufficient numbers to conduct GLM analyses for all metals to assess the impacts of gender and time (Supplementary Table 2). We found no interaction between gender and year, and no significant differences between genders after adjusting for year (Supplementary Table 3). We also did not find any statistical differences between genders when we investigated differences in metal levels across years by gender (Fig. 2).

In humpback whales, we did find significant decreasing metal levels over time ($p < 0.05$) for metals with high levels: Cr, Mg, manganese (Mn), Ni and Zn, and with low levels: antimony (Sb), beryllium (Be), gold (Au), lead (Pb), strontium (Sr) and uranium (U)

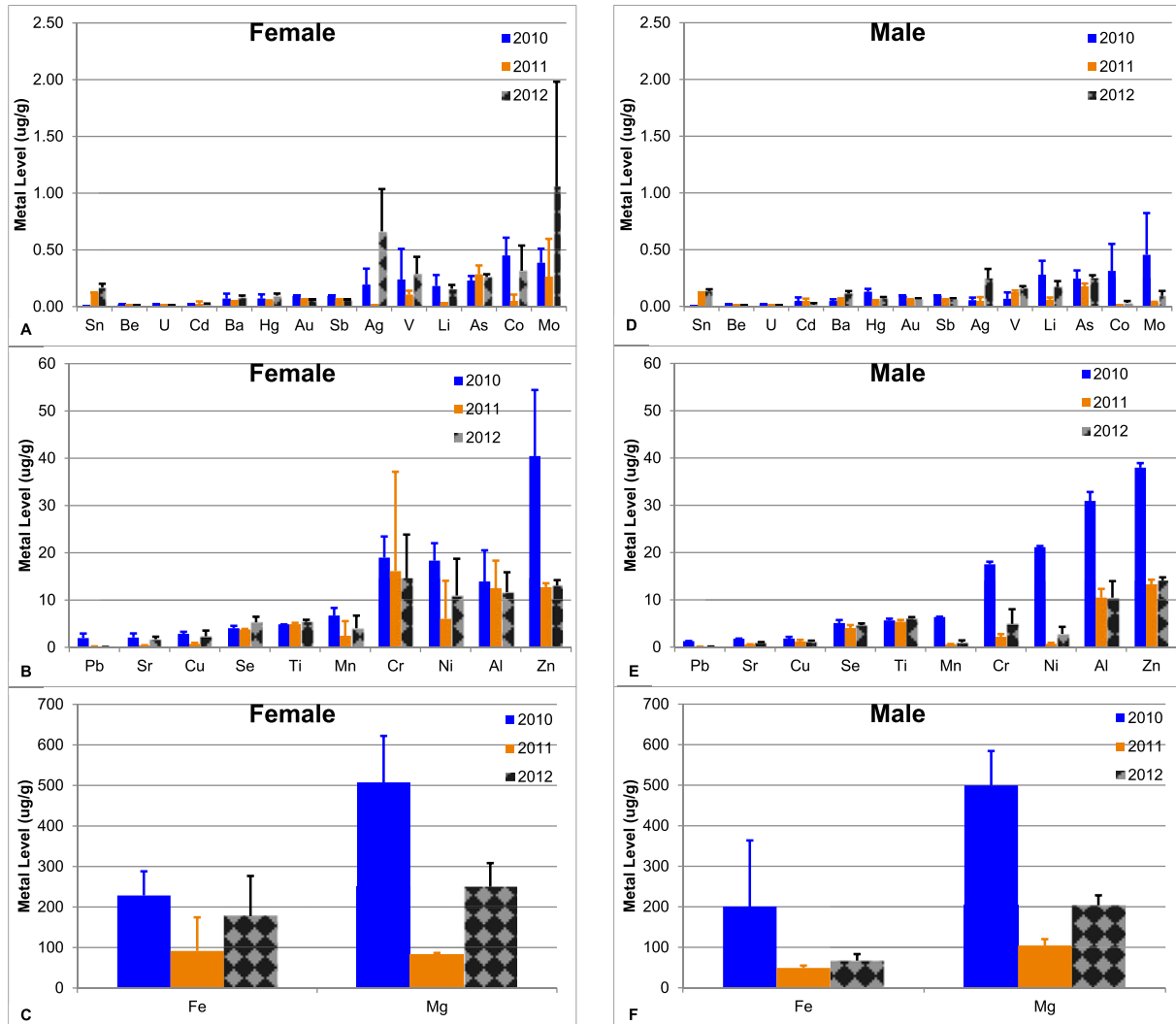


Fig. 3. Metal Levels in Female vs Male Humpback Whales Biopsied in the Gulf of Maine over Time. Sample sizes were—for 2010: 3 males and 3 females; for 2011: 6 males and 2 females; for 2012: 10 males and 9 females. Data represented as mean \pm standard error in units of $\mu\text{g/g}$ tissue. Data are separated into panels solely for optimal visualization. (A,D) Metals with mean skin levels $<1 \mu\text{g/g}$; (B,E) Metals with mean skin levels between 1 and $50 \mu\text{g/g}$; (C,F) Metals with mean skin levels $>50 \mu\text{g/g}$.

(Figs. 2 and 3). For the fin whales, the interaction term between year and gender was not included in the GLM model because there were only female samples in 2010 and only male samples in 2011 (Supplementary Table 4). We did find significant decreasing metal levels over time ($p < 0.05$) for metals with high levels: copper (Cu), Fe, Mg and Zn, and for metals with low levels Sb, Be, Au, and U (Fig. 4 & Supplementary Table 5). Though the levels were overall low, we observed increasing metal levels over time for Hg, Ba and Sn (Fig. 4 & Supplementary Table 5).

4. Discussion

Whales are key species in the ocean ecosystem. Many whale species are endangered or threatened either as a species or as specific populations within the species, and even though some species are not listed, many suffer low reproductive rates (Lockyer, 1984; Kraus et al., 2001). Some species, like the North Atlantic right whale in the Gulf of Maine are severely endangered. These right whales have a very small population of only about 400–500 individuals and have a very low reproductive rate compared to their close cousins the Southern right whale (*Eubalaena australis*) (Fujiwara and Caswell, 2001). Anthropogenic impacts including

boats striking whales and whales becoming entangled in fishing gear are well-defined threats with active efforts seeking to prevent them (Panigada et al., 2006; Reeves et al., 2012). However, the causes of the whale's low reproduction rates are poorly understood, and likely involve other stressors such as chemical and noise pollution.

The Gulf of Maine whales, including right, humpback, fin and minke whales are vital marine species and a valuable indicators for human and ecosystem health. Previous reports have described exposure to metals as a possible health concern for the whales. For example, Cr levels were found to be high in biopsies from North Atlantic right whales (7.1 ppm Cr) and fin whales (10.07 ppm Cr) from the Gulf of Maine, which was 11–16-fold higher than Cr levels found in biopsies from Southern right whales (0.64 ppm Cr) (Wise et al., 2008, 2015; Martino et al., 2013).

This study is the first to report a broad suite of metal levels in Gulf of Maine humpback, fin and minke whales. Right whales were unavailable to sample due to their low population numbers. Thus, in this study we establish a broader baseline of metals in the Gulf of Maine whales. We found measurable levels of toxic and essential metals in whales from the Gulf of Maine. In general, these levels declined over time from 2010 to 2012, although we cannot yet

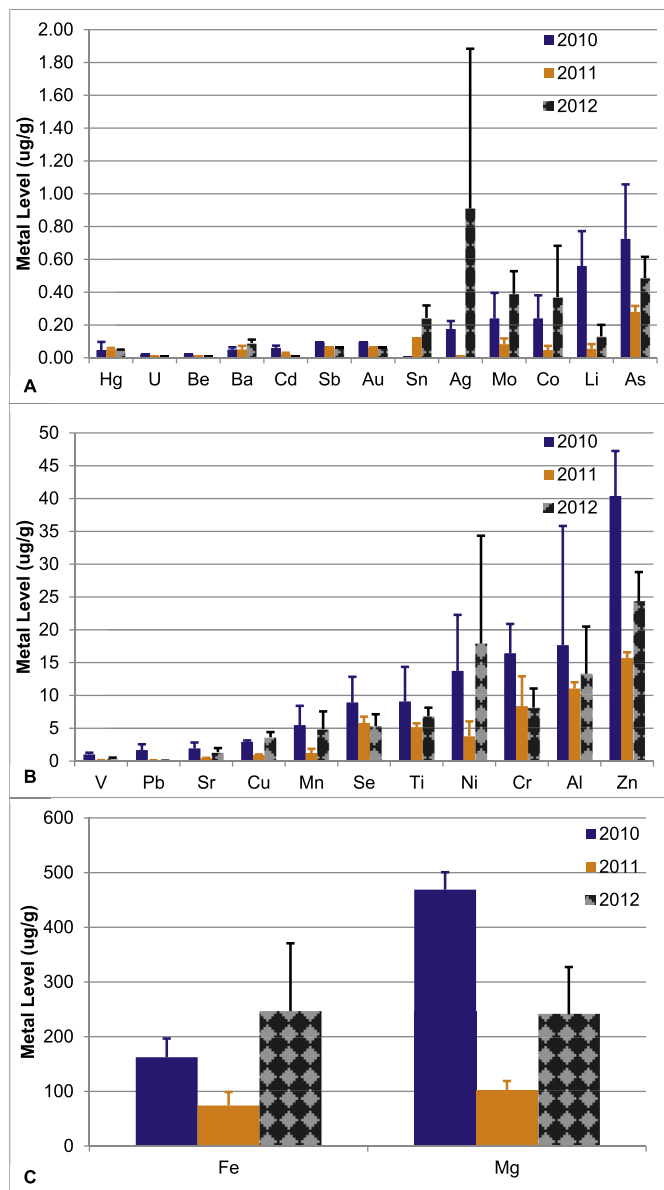


Fig. 4. Metal Levels in Fin Whales Biopsied in the Gulf of Maine over Time. We measured 25 metals and Se in the fin whale skin biopsies. Sample sizes were 2, 4, and 3 for 2010, 2011, and 2012 respectively. Data represented as mean \pm standard error in units of ug/g tissue. (A) Metals with mean skin levels <1 ug/g; (B) Metals with mean skin levels between 1 and 50 ug/g; (C) Metals with mean skin levels >50 ug/g.

determine if this change over time reflects possibly improving conditions or possibly due to seasonal variations at the start of the season versus the end of the season (NB: 2010 whales were biopsied in July 2011 and 2012 whales were biopsied in autumn).

Notably, these baseline levels are among the first for these three species anywhere. We could not locate any other published reports of metal levels in humpback whales in any organ, or fin whale skin, aside from our previous report focused on cell culture studies (Wise et al., 2015). We did find two studies of metals in minke whale skin (Kunito et al., 2002; Shoham-Frider et al., 2014). Our data from one Gulf of Maine minke whale skin biopsy were higher for vanadium (V), manganese (Mn) and barium (Ba), lower for Sr, and similar for Cu and Hg; however all of these levels were below 0.70 ppm and most were below 0.22 ppm. Our observed Cr (1.71 ppm) and Zn (16.03 ppm) levels were similar to those studies and consistent

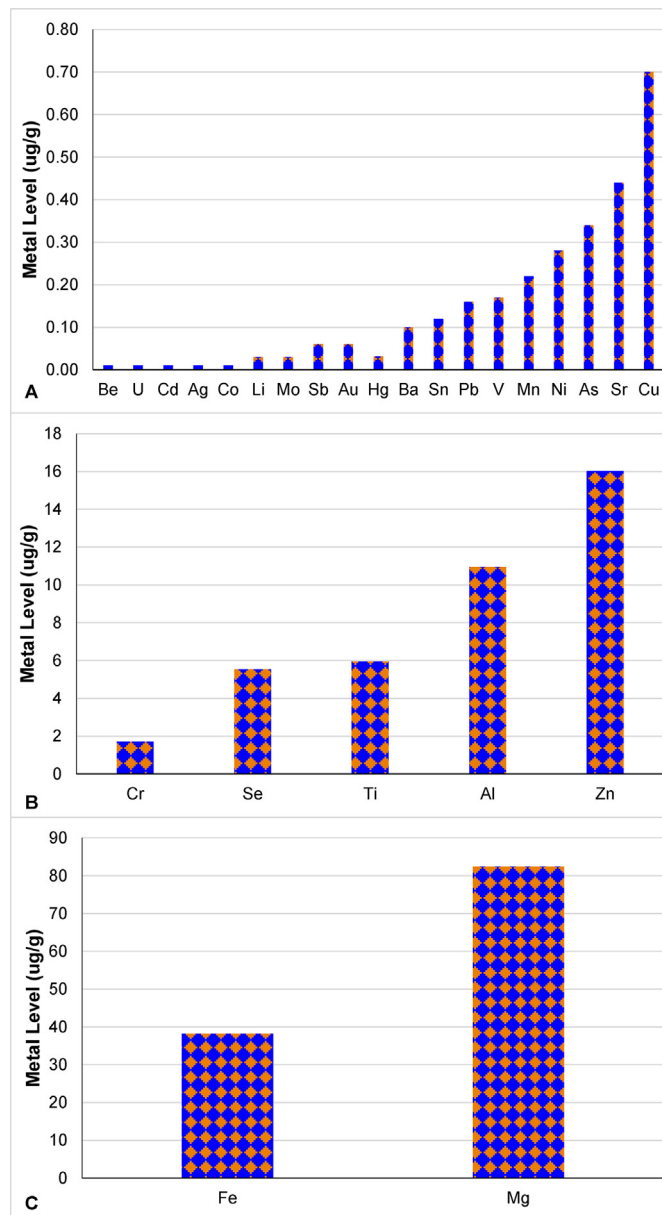


Fig. 5. Metal Levels in a Minke Whale Biopsied in the Gulf of Maine in 2012. We measured 25 metals and Se in a single minke whale skin biopsy. Data represented as mean in units of ug/g tissue. (A) Metals with mean skin levels <1 ug/g; (B) Metals with mean skin levels between 1 and 50 ug/g; (C) Metals with mean skin levels >50 ug/g.

with observations that marine mammals typically have Cr levels below 2 ppm (Law et al., 1996; Macke et al., 1995), while our reported level for Se (5.53 ppm) was at the low end of their range of levels for female minke whales.

While skin biopsies are limited in scope to evaluate burden of metal levels in internal organs, several studies have found positive correlations between metal levels observed in skin to those observed in internal organs such as liver and kidney for Cr, Hg, Mn, Cu, and Zn (Kunito et al., 2002; Shoham-Frider et al., 2014; Aubail et al., 2013; Monaci et al., 1998; Stavros et al., 2011; Yang and Miyazaki, 2003). Fin whale levels were reported in organs other than skin, although Al, Cr or Ni, the highest toxic metal levels in these Gulf of Maine whales were not considered in these studies. For example, one study reported Cd, Cu, Hg and Zn in muscle, liver and kidney tissue in fin whales from Spain (31 whales) and Iceland

(5 whales), captured during whaling activities between 1983 and 1984 (Sanpera et al., 1996). The levels we observed in fin whale skin were much lower for Cd (0.01–0.07 ppm) than levels observed in livers (Cd 0.16–5.23 ppm) or kidney (Cd 0.91–58.75 ppm), but were similar for Zn (13.80–45.25 ppm in our study; 16.60–58.75 ppm in liver, 9.48–48.58 ppm in kidney) from Spanish or Icelandic fin whales, respectively. Cu levels in our samples had a higher range (0.70–4.85 ppm) than levels observed in muscle (0.26–1.48 ppm) from these fin whales, but again were much lower than levels observed in livers or kidneys. Skin Hg levels were relatively low (0.01–0.08 ppm) in our samples when compared to muscle (0.08–0.36 ppm), liver (0.16–1.41 ppm), or kidney (0.15–0.75 ppm). These differences could be due to the organs considered or due to different exposures in different regions.

Al, Cr and Ni were the highest toxic metal levels in these Gulf of Maine whales. These three metals are not often considered in baleen whale studies, and even less frequently in baleen whale skin. One study considered skin biopsies in Southern right whales, allowing us to consider comparisons with whales that feed at the same trophic level, but live in a different region. This comparison shows Gulf of Maine humpback and fin whales have much higher levels of Cr and Ni. For example, comparisons of southern right whales with the humpback whales showed 16-fold higher levels of Cr and 41-fold higher levels of Ni, while comparisons with the fin whales showed 16-fold higher levels of Cr and 56-fold higher levels of Ni. By contrast, Al levels were much closer (1.4-fold elevated in the Gulf of Maine whales), and other toxic metals (e.g. Hg, Sn, Au, and Ag (silver) were similarly low (<0.5 ppm) in both populations. Thus, comparisons with other whales with a similar lifestyle, but living in a different region indicate levels in the Gulf of Maine whales are much higher for Cr and Ni, raising concern because these two metals can cause significant toxicity. The only available toxicity data of these metals in a whale-specific model are whale cell culture studies (including fin whale and right whales) that show Cr is cytotoxic and genotoxic to whale cells (Wise et al., 2008, 2015; Browning et al., 2017; Li Chen et al., 2009a, 2009b, 2012; Pabuwat et al., 2013). Such outcomes are consistent with the major toxic outcomes of concern for Cr, reproductive and developmental toxicity and cancer. Ni and Cr are both reproductive and developmental toxicants, and human carcinogens (ATSDR, 2012; ATSDR, 2000; ATSDR, 2005; Hayes, 1997).

Because of the lack of toxicology studies in whales, it is difficult to interpret the risk these levels pose to the health of these whale populations. One way to provide insight into these levels is to consider a One Environmental Health approach, which is a subspecialty within the One Health approach. Whereas, the One Health paradigm broadly considers the interconnectedness of human, animal and ecosystem health, One Environmental Health focuses on toxic chemicals in that paradigm. Cr and Ni are known to cause these disease outcomes in humans; thus, we took advantage of this approach to inform the interpretation of the whale data.

First, considering humans with no occupational exposure to metals, the data confirm the baleen whale comparisons indicating the Gulf of Maine whale Ni and Cr levels are much higher. For example, Schroeder et al. (1970) reported a mean level of 0.31 ppm Cr in human skin for people with no known Cr exposure. By comparison, the Cr levels we observed in whales were 32-fold (humpback whales), 33-fold (fin whales), and 5.5-fold (minke whale) higher than the 0.31 ppm level reported for humans. We were unable to locate skin levels of Ni from unexposed people, but mean nickel levels in lung tissue ranged from 0.016 to 2.1 ppm. Because metal levels in skin are generally found to be much lower than in internal organs, the human skin levels are probably much

lower than these lung levels. Nevertheless, while the minke level was consistent with this human tissue level, the Ni levels we observed in humpback and fin whales were 3.7-fold and 5.1-fold higher, respectively. This comparison shows the Ni and Cr levels are also much higher in humpback and fin whales than humans without occupational exposure.

Next, we compared the Gulf to Maine whales to workers with metal-induced disease. The only available levels were from workers who died of Cr- or Ni-induced lung cancer. For example, mean Cr lung levels in workers were 20.4 ppm, while mean levels ranged from 10 to 280 ppm for Ni workers (Edelman and Roggli, 1989; Tsuneta et al., 1980). For both metals the workers had decades of exposure. The levels we observed for skin in both humpback (9.93 ppm Cr; 7.73 ppm Ni) and fin whales (10.07 ppm Cr; 10.68 ppm Ni) are in this range (e.g. 10.68 ppm Ni in fin whales) or approach them. We again note that skin levels are usually lower compared with internal organs, and thus, one would anticipate higher lung levels in these whales. The Cr workers illustrate this potential outcome as well. Lung cancer autopsies rarely measure skin metal levels; however, we did find two such cases. One case reported a worker with lung levels ranging from 33 to 45.6 ppm Cr who had a skin level of 0.05 ppm Cr; 66–912x lower in the skin (Mancuso, 1997). The other case reported a worker with lung levels ranging from 3 to 12 ppm who had a skin level of 0.16 ppm (although this case stated the skin sample included the subcutaneous tissue around it); 19–75x lower in the skin (Mancuso, 1997). We do not know how much higher Cr levels in whale lung are compared to whale skin, but if one used a mere 2-fold difference from whale skin to whale lung as a possible difference, the projected Cr and Ni levels in whale lung would be well within the levels of workers with occupational disease. Thus, comparisons with Cr and Ni workers show the Gulf of Maine whale levels from environmental exposure are similar to occupational levels resulting from decades of exposure.

These comparisons to other whale and human outcomes raises questions about the source of the metals to the whales, which is uncertain. There are three possible routes of exposure: ingestion, dermal absorption and inhalation. Ingestion and dermal exposure are unlikely to be the major routes of exposure for whales. Both Ni and Cr are poorly absorbed by the digestive system, and both do not biomagnify in food webs like Hg does (ATSDR, 2012; ATSDR, 2000; ATSDR, 2005). Humpback and fin whales are baleen whales and consequently, feed low on the food chain. They are not top predators, which is evident from their low mercury levels, which are typically high in marine species that feed higher on the food chain, such as sperm whales (Savery et al., 2013). Ni and Cr are poorly absorbed by oral and dermal routes of exposure in mammals and do not typically remain in the marine water column to allow for dermal exposures and instead typically settle into sediments (ATSDR, 2012; ATSDR, 2000; ATSDR, 2005). Thus, there is no *a priori* reason to expect these two metals would achieve such levels as we observed from these two routes of exposure.

The remaining exposure route is inhalation, although inhalation of metals has never been measured in whales. Whales have a large lung capacity and hold their breaths for long periods of time, which may enhance absorption of inhaled metal particles. For example, a fin whale's lung volume is estimated to be 2 m³ with a respiration rate of 24 breaths/h (Brodie, 1975). The migration range of our study whales includes the entire Eastern US seaboard, where abundant metal manufacturing and industrial releases have occurred. Metal levels in the air are higher in areas near industrial sources. For example, the annual average Cr atmospheric concentration ranged from 0.4 to 5.5 µg/m³ near chromate manufacturing

Table 2
Whale vs Human Hypothetical Comparison of Ambient Air Cr Exposure.

| Species | Chromium Concentration in Air ($\mu\text{g}/\text{m}^3$) | Lung Volume (m^3/breath) | Breathing Rate (breath/h) | Exposure Time (h/day) | Daily Cr(VI) Exposure ($\mu\text{g}/\text{day}$) |
|---------------------|--|--|---------------------------|-----------------------|--|
| Fin Whale | 5.5 | 2 | 24 | 24 | 2217.6 |
| Human Cr(VI) Worker | 5 | 0.0005 | 1200 | 8 | 24 |

in Corpus Christi, Texas (ATSDR, 2000), while for most towns and cities Cr levels range from range from 1 to $100 \text{ ng}/\text{cm}^3$ (Singh et al., 1999). Marine atmospheric Cr and Ni levels are infrequently measured, but a report of air in Baltimore harbor reported a level Cr of $0.226 \mu\text{g}/\text{m}^3$ (ATSDR, 2000). Using the physiological parameters for fin whales exposed to this level of Cr in their air, the whales would have inhaled $260 \mu\text{g}$ Cr per day. Estimates indicate that 35% of Cr in the air is hexavalent [Cr(VI)], thus, a whale could possibly inhale $91 \mu\text{g}$ Cr(VI)/day. If levels in marine air were to reach the higher levels of Corpus Christi, a whale could inhale $2217.6 \mu\text{g}$ Cr(VI)/day.

Of course, there are no data to indicate whether inhaling $91\text{--}2217 \mu\text{g}$ Cr(VI) per 24 h is toxic to a whale, as such studies cannot be done. Data from cell culture studies indicate $0.7 \mu\text{g}$ Cr(VI)/24 h is cytotoxic and genotoxic to whale cells (Wise et al., 2008, 2015; Browning et al., 2017; Li Chen et al., 2009a, 2009b, 2012; Pabuwal et al., 2013). This level is 130-fold to 3169-fold lower than hypothetical amounts a whale might inhale as discussed above.

Another way to consider whether the levels are concerning, is to consider how the amount a whale inhales compares to an occupationally exposed Cr(VI) worker. Currently, the U.S. Occupational Safety and Health Administration (OSHA) states an allowable limit for workers exposed to Cr(VI) to be $5 \mu\text{g}/\text{m}^3$, measured as an 8-hr time-weighted average (OSHA, 2006; OSHA, 2018). Given that people breathe 0.0005 m^3 of air per breath and take about 20 breaths/min a worker would breathe $72 \mu\text{g}/\text{day}$ (sample calculation: $5 \mu\text{g}/\text{m}^3 \times 0.0005 \text{ m}^3/\text{breath} \times 20 \text{ breath}/\text{min} \times 60 \text{ min}/\text{h} \times 8 \text{ h}/\text{day} = 24 \mu\text{g}/\text{day}$). It should be noted that this occupational Cr(VI) exposure level is not a safe level per se, as it still carries a substantial cancer risk (estimated 10–45 cancers/1000 people) (OSHA, 2006; OSHA, 2018). Thus, a whale might inhale 4 to 92-times as much Cr(VI) as a Cr(VI) worker per day ($2215 \mu\text{g}$ or $91 \mu\text{g}$ versus $24 \mu\text{g}$). For simplicity, this hypothetical comparison is summarized in Table 2.

One can do the same type of comparison for Ni. Ambient air levels for Ni range from 0.002 to $6.1 \mu\text{g}/\text{m}^3$ (ATSDR, 2005). Thus, at these levels our hypothetical whale would inhale $2.3\text{--}7027 \mu\text{g}$ Ni. The OSHA standard for Ni is $1 \text{ mg}/\text{m}^3$ measured as an 8-hr time-weighted average (OSHA, 2018). Thus, a worker would inhale $4.8 \mu\text{g}/\text{day}$, well within the low range for our hypothetical whale.

Of course, whale lungs are much bigger than human lungs and cell cultures are not a complete lung. Thus, the direct comparison is imprecise. However, it is important to remember that Cr(VI) does not typically distribute throughout the lung. Thus, correct comparison is not whale lung volume to human lung volume. Instead, Cr accumulates at lung bifurcation sites, which concentrate the particles in a much smaller surface area of comparison (Holmes et al., 2008). The surface area of whale and human lung bifurcation sites are both unknown so we cannot compare them. However, the whale cell culture doses that induced toxicity were 130–3169 fold lower than the hypothetical amount a whale might inhale considering ambient air. Simply comparing full lung volume, a whale lung is only about 330-times bigger than a human lung, which is at the low end of this cell culture comparison. Thus, these comparisons also suggest inhalation may be a very important route of exposure and support a conclusion that the whale levels are of concern.

5. Conclusions

In conclusion, many of the threats to whale populations are immediate concerns that may threaten the lives of individuals when they encounter them (e.g. ship strikes, entanglements, whaling); metals, however, are a much more sinister threat that can gradually impair the health of a population. Our data show baleen whales in the Gulf of Maine are exposed to metal levels much higher than baleen whales in other regions. Comparisons with outcomes in whale cell cultures and humans indicate the levels are of a health concern. Such a conclusion is consistent with a previous report indicating the Gulf of Maine humpback whales have highest levels of persistent organic pollutants (ΣPCBs , ΣDDTs , $\Sigma\text{chlordanes}$, and ΣPBDEs) in the world (based on ten distinct populations), indicating a heavier exposure to anthropogenic pollutants in this region (Elfes et al., 2010) and extend this observation to Cr and Ni. Current environmental changes, such as ocean acidification, are altering ocean chemistry and increasing release of some metals from their environmental sinks back into the water column (Noyes et al., 2009). Thus, it is imperative to continue to evaluate contaminant loads in important marine organisms such as whales, so we can better understand and predict how our changing marine environments will affect health for whales, humans and the ecosystem.

Funding

This work was supported by the National Institute of Environmental Health Sciences [ES016893 (J. Wise Sr., PI)]; Army Research Office [W911NF-09-1-0296 (J. Wise Sr., PI)]; University of Southern Maine, the Maine Center for Toxicology and Environmental Health; the Campbell Foundation; the Ocean Foundation; Ocean Alliance; and the many individual and anonymous Wise Laboratory donors.

Acknowledgments

The authors declare no competing financial interests. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Environmental Health Sciences, the National Institutes of Health, the Army Research Office. Work was conducted under National Marine Fisheries Service permit #1008-1637-03 (J. Wise Sr., PI) and permit #751-1614 (Iain Kerr, PI). We would like to thank Amie Holmes, Shouping Huang, Selma Botman, Timothy Stevens, W. Douglas Thompson, and all the Wise Laboratory members and volunteers for their help with technical support, whale spotting and sample collection. We thank Iain Kerr, Rick Walker, and all of the *Caribana* boat crew for their help with sample collection and logistics. Finally, we thank all the volunteers who support us in this project.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.chemosphere.2018.10.120>.

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