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A three year study of metal levels in skin biopsies of whales in the Gulf of Mexico after the Deepwater Horizon oil crisis

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ABSTRACT

In response to the explosion of the *Deepwater Horizon* and the massive release of oil that followed, we conducted three annual research voyages to investigate how the oil spill would impact the marine offshore environment. Most investigations into the ecological and toxicological impacts of the Deepwater Horizon Oil crisis have mainly focused on the fate of the oil and dispersants, but few have considered the release of metals into the environment. From studies of previous oil spills, other marine oil industries, and analyses of oil compositions, it is evident that metals are frequently encountered. Several metals have been reported in the MC252 oil from the Deepwater Horizon oil spill, including the nonessential metals aluminum, arsenic, chromium, nickel, and lead; genotoxic metals, such as these are able to damage DNA and can bioaccumulate in organisms resulting in persistent exposure. In the Gulf of Mexico, whales are the apex species; hence we collected skin biopsies from sperm whales (*Physeter macrocephalus*), short-finned pilot whales (*Globicephala macrorhynchus*), and Bryde's whales (*Balaenoptera edeni*). The results from our three-year study of monitoring metal levels in whale skin show (1) genotoxic metals at concentrations higher than global averages previously reported and (2) patterns for MC252-relevant metal concentrations decreasing with time from the oil spill.

1. Introduction

After the *Deepwater Horizon* exploded, over 4.9 million barrels (> 779 million liters) of MC252 light sweet crude oil were spilled into the Gulf of Mexico and over 200 million liters of dispersants were applied on the surface and at the well head (McNutt et al., 2012). The size of such a spill in an offshore marine environment is unprecedented; as are the volume and methods of dispersant application. In response to this incident, a large proportion of the literature has focused on figuring out the final size of the spill, the environmental fate of the oil, or approximating the environmental impact – while few have assessed the health impacts on the environment.

Metals are known to be present in crude oil, elevated in the environments surrounding oil industries, and elevated after oil spills (Adedara et al., 2013; Benson and Etesin, 2008; Efe, 2010; Fowler et al., 1993; Gondal et al., 2006; Kuhuhawar et al., 2012; Osuji and Onojake, 2004). However, for this crisis, only a few reports assessing the environmental and health effects of the oil have considered metals; which reported detection of aluminum (Al), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), vanadium (V), and zinc (Zn) (Carmichael et al., 2012; Liu et al., 2012; Joung and Shiller, 2013; Steffy et al., 2013; Fitzgerald and Gohlke, 2014; Wise Jr. et al., 2014; Botello et al., 2015; Granneman et al., 2017). In particular, Mg, Al, Mn, Fe, Ni, and Pb were observed to increase in concentration as the sea mousse became more weathered (Liu et al., 2012). Here, we present data on metal levels in three whale species from the Gulf of Mexico: Bryde's, pilot, and sperm whales.

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Bryde's whales (*Balaenoptera edeni*) are mysticetes that primarily feed on very small species of fish (e.g. sardines or herring) and generally live solitary lives except to mate and raise calves (Tershy, 1992; Tershy et al., 1993; Waring et al., 2013a). While Bryde's whales live in all oceans across the globe, the specific population in the Gulf of Mexico is isolated and was recently identified as a distinct subspecies (Rosel and Wilcox, 2014). Furthermore, this population is the only residential population of mysticetes in the Gulf of Mexico (Wursig, 2017). With a population size of approximately 33 individuals, this subspecies is one of the rarest of the great whales (Rosel and Wilcox, 2014).

Sperm whales (*Physeter macrocephalus*) are odontocetes that primarily feed on squids and fishes found in the deep abyss; they also have a cosmopolitan distribution, but the population in the Gulf of Mexico is a residential population with few individuals (primarily males) leaving or entering the area each year (O'Hern and Biggs, 2009; Waring et al., 2013c; Wursig, 2017). Their population size is currently estimated at 763 individuals; the global population of sperm whales is currently classified as vulnerable and has an unknown status as to whether it is declining or improving (Waring et al., 2013c). A recent report showed the matriarchal sperm whale pods remained in waters about 200–3500 m deep, south and southwest of the Mississippi/Atchafalaya river mouths (Ortega-Ortiz et al., 2012). Sperm whales have a relatively large residential population in the northern Gulf of Mexico, are apex predators at the highest trophic level, and are known to use echolocation to communicate and hunt their prey (Waring et al., 2013c). This population of whales is critical to the ecological health and stability of the Gulf of Mexico, and thus they are an important species to study.

Short-finned pilot whales (*Globicephala macrorhynchus*) are also odontocetes, but prey on mesopelagic fishes and squids not nearly at the same depths as sperm whales (Waring et al., 2013b; Wursig, 2017). It is currently unknown if the population in the Gulf of Mexico is distinct from the Atlantic stock, but it is currently classified as distinct for management purposes (Waring et al., 2013b). This northern Gulf of Mexico stock is estimated to be 2415 individuals (Wursig, 2017). These are an important species to study, as they share a similar trophic level and habitat to sperm whales. Despite the vast size of these animals and the critical ecological role they play in the ocean ecosystem, (both at the surface and the ocean floor), our knowledge of their lives and health is very limited. It is clear that both Bryde's and sperm whale populations reside in areas of the Gulf that were most heavily affected by the oil.

To better understand the health of these whales and the potential toxic effects of the oil spill, our group conducted three research voyages in the summers of 2010, 2011, and 2012 to collect biopsies of skin and blubber from these whales to analyze their contaminant loads. Our initial report observed high concentrations of nickel and chromium in these Gulf whales in the immediate aftermath of the spill (August–November) following capping of the riser; with a mean Ni concentration of 15.9 ± 3.5 ppm (range 1.7–94.6 ppm wet weight) and a mean Cr concentrations of 12.8 ± 2.6 ppm (range 2.0–73.6 ppm wet weight), which were significantly elevated when compared to global means (2.4 ± 0.4 ppm Ni, 9.3 ± 1.0 ppm Cr) (Wise Sr. et al., 2009; Wise Jr. et al., 2014). In this study, we report our findings regarding the concentrations of 26 metals in Gulf whale skin over a three-year period after the spill, with a focus on metals also found in the MC252 oil. The precise route and duration of exposure cannot be determined, because the oil was burned, metal exposure from the spill could have occurred through oral, dermal or inhalation routes and may have lasted for a short time to several months. Since metals accumulate, the exposure may last for years inside the animal.

2. Materials and methods

2.1. Sample collection

Skin biopsies were collected from free-ranging adult Bryde's, pilot, and sperm whales in the northern Gulf of Mexico in the summers of 2010, 2011, and 2012 (Wise Jr. et al., 2014) (see also Table 2). Our platform was the research vessel *Odyssey*, a 93-ft motor-sailer ketch. The *Odyssey* was specially equipped to acoustically track echolocating whales, using an underwater hydrophone array and RainbowClick software. This equipment was used 24 h per day while we were in open sea in conjunction with visual efforts from various observation platforms above the deck from sunrise to sunset. These platforms are on top of the pilot house (approximately 10 ft above the deck), halfway up the main mast (approximately 30 ft above the deck), and the crow's nest near the top of the main mast (approximately 50 ft off the deck); visual efforts were taken in 1–2 h shifts from one of the platforms, weather permitting. Upon encountering a whale, one whale biopsier would take a position on the *Odyssey*'s “whale boom”, a 30-ft pole on the starboard bow with a deer stand attached to the end; a second, backup biopsier would be positioned in the bowsprit. This “whale boom” allowed the primary biopsier to get closer to the whale while keeping the research vessel at a respectful distance. The backup biopsier was positioned to only release an arrow if the primary biopsier missed, did not make an attempt, or was incapable of making an attempt (e.g. if the whale moved too close). As much detail about the whale and the biopsy was recorded as possible, including suspected age (adult or subadult), location 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

Biopsies were consistently collected from the left flank of the whale's back, approximately 1 m caudal to the dorsal, 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 in a sterile biosafety cabinet (generously donated by the Baker Company). Processing of the biopsy sample consisted of separating the skin and blubber using a ceramic knife and glass petri dish and finally isolating a section between the skin and blubber (where living, dividing skin cells reside) for tissue culture. Hence, for our purposes, skin refers to all physiological layers above the blubber. The skin samples were then further divided each into two pieces; one for metals analysis and one for genotyping analysis. Previously, we demonstrated that metals are not released from the biopsy darts into the samples (Wise Sr. et al., 2009). All animal procedures complied with approved institutional animal care protocols.

2.3. Genotyping

Gender was determined by genotyping based on published methods (Yang and Miyazaki, 2003). DNA was extracted from a piece of whale skin using standard methods (Carvalho et al., 2002; Wise Jr. et al., 2014). Gender was determined by PCR amplification reactions by amplifying the SRY (male determining factor) gene (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 as follows:

- SryPMF: 5′-CATTGTGTGTGGTCTCGTGATC-3′
- SryPMR: 5′-AGTCTCTGTGCCTCCTCGAA-3′
- KF: 5′-AGATCAGGGGTTCATGTTTCTTTGC-3′
- KR: 5′-TTTACAGAGGTACCCAAGCCTAAG-3′