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Associations between metal exposure and lesion formation in offshore Gulf of Mexico fishes collected after the Deepwater Horizon oil spill

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ABSTRACT

The objectives of this study were to: (1) examine patterns of short- and long-term metal exposure within the otoliths of six offshore fish species in varying states of health, as indicated by the presence of external skin lesions, and (2) determine if there was a change in otolith metal concentrations concurrent with the Deepwater Horizon (DWH) oil spill. Otoliths collected from 2011 to 2013 in the Gulf of Mexico (GOM) were analyzed for a suite of trace metals known to be associated with DWH oil. We found that lesioned fish often had elevated levels of otolith ^{60}Ni and ^{64}Zn before, during, and after the DWH oil spill. In addition, metal exposure varied according to species-specific life history patterns. These findings indicate that lesioned individuals were exposed to a persistent source of trace-metals in the GoM prior to the oil spill.

1. Introduction

After the *Deepwater Horizon* drilling platform exploded on April 20, 2010, approximately 4.9 million barrels of crude oil were released into the Gulf of Mexico (GoM) at a depth of 1500 m before the Macondo well could be capped (Camilli et al., 2010). The *Deepwater Horizon* (DWH) oil spill was not only the largest oil spill in US history, it was also the first to release oil at the deep seafloor and to involve extensive use of chemical dispersants at depth (Lubchenco et al., 2012). Due to the depth of the Macondo wellhead and its distance offshore, subsurface and benthic oil from the DWH oil spill may have directly impacted marine fishes over an unprecedented depth range (0–1500 m) and spatial distribution (3200 km^2) (Valentine et al., 2014). Several studies have documented substantial overlap of the known extent of surface oil with the distributions of adult and larval fishes, suggesting the potential for oil exposure in many fish species (Chakrabarty et al., 2012; Muhling et al., 2012; Rooker et al., 2013). Physiological biomarkers of sub-lethal oil exposure have been detected in several fish species since the spill occurred; the prevalence of external skin lesions on fish and concentrations of biliary polycyclic aromatic hydrocarbons (PAHs) were higher in the vicinity of the spill site compared to the West Florida Shelf, and have decreased since the oil spill occurred, suggesting exposure to PAH pollution from an episodic event (Murawski et al., 2014). Alterations of genome expression in liver and gill tissue have been observed in resident fish from Louisiana marshes that were likely exposed to weathered crude oil from the spill (Dubansky et al., 2013). Yet, establishing a causal link between the spill and any of the sub-lethal effects of oil exposure (e.g., disease prevalence) measured in fishes is problematic due to a lack of baseline data and the rapid deterioration of oil-exposure biomarkers as a result of the efficient metabolization of PAHs by the fishes themselves (Varanasi et al., 1989).

The unique, metal signatures recorded within fish otoliths could potentially serve as oil-exposure biomarkers that would not degrade over time. Fish otoliths are metabolically inert stones (primarily consisting of aragonitic $CaCO_3$) located within teleost ears that incorporate trace elements from the surrounding water and diet (Campana et al., 1995). Previous studies have demonstrated that trace metals, particularly Ni and V, can be highly enriched in crude oil and are characteristic of the geographic origin of the oil (Fingas, 2011). In a review of oil-spill literature, Gohlke et al. (2011) concluded that trace metals in oil can accumulate in marine organisms at levels above the baseline. For instance, concentrations of Ni and V in mollusks have been used as tracers of exposure to past oil spills (Amiard et al., 2004). Thus, the detection of crude-oil-associated metals in fish otoliths could indicate exposure to spilled oil. A laboratory-based study by Morales-Nin et al. (2007) demonstrated that some of the trace metals in fuel oil (e.g., Na, Mg, Sr, Cr, Ni, and Cu) were incorporated through the diet into the otoliths of juvenile Turbot (*Scophthalmus maximus*).

The metal content of the DWH crude oil, as identified by Liu et al. (2012), includes Mg, Al, V, Cr, Fe, Ni, Co, Cu, Zn, and Pb. Additionally,

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the 1.84 million gallons of chemical dispersants (Corexit 9500A) applied by BP to dissipate the oil also contain trace amounts of As, Cr, and Cu (USEPA, 1995). Steffy et al. (2013) identified a significant increase from 2009 to 2010 in the concentrations of Ni, Cr, and Pb in northern GoM (NGoM) sediments, suggesting the spilled oil and dispersants contributed a new source of trace metals to the GoM. Elevated concentrations of Co identified within a subsurface intrusion of natural oil and gas (1000–1400 m depth) (Joye et al., 2011) were also attributed to the DWH oil (Joung and Shiller, 2013).

Studies of coastal marine organisms in the GoM have thus far not suggested a significant change in trace-metal tissue concentrations due to the DWH oil spill (Carmichael et al., 2012; Apeti et al., 2013). In coastal saltmarshes of the northern GoM, researchers collected Gulf Killifish (*Fundulus grandis*) after the oil spill at oiled and non-oiled reference sites to determine if oil exposure could be detected in Gulf Killifish otoliths (Nelson et al., 2014). The authors found no consistent differences in the metal signatures of otoliths from fish collected at oiled versus non-oiled sites. However, this result may be misleading because most of the fish analyzed by Nelson et al. (2014) were spawned after the DWH oil spill occurred. Additionally, these authors did not use Gulf Killifish otoliths to establish baseline conditions prior to exposure, but instead relied on comparisons between presumptive oiled versus non-oiled sites.

In the present study, we took advantage of the chronological deposition of metals in otoliths in order to examine the metal-exposure histories and establish baseline conditions for offshore fish species collected after the DWH oil spill occurred. Otoliths grow continually by the concentric deposition of calcium carbonate layers, resulting in identifiable daily and annual increments (Pannella, 1971) that can be used to determine fish age and growth rates (Campana and Neilson, 1985). Additionally, otoliths are useful because no resorption or alteration of an otolith occurs under stressful conditions (Campana and Neilson, 1985), although stress has been observed to affect the incorporation of some metals into fish otoliths (Kalish, 1992). Thus, the dual function of an otolith as a recorder of both fish age and trace elements provides a history of the annual element exposure through the environment and the diet of an individual fish throughout its lifetime.

In the aftermath of the DWH oil spill, federal and state agencies conducted post-spill sampling of nearshore fish more frequently than deepwater fish (Fitzgerald and Gohlke, 2014), despite the substantial overlap of oil with the distributions of offshore fish species (Chakrabarty et al., 2012). The only studies of metal exposure in marine organisms from the offshore environment of the GoM alternately suggested an increase in metal exposure (Lu et al., 2012; Wise et al., 2014) or no change (Fitzgerald and Gohlke, 2014) due to the DWH oil spill. In either case, these studies were confounded by an important lack of baseline data. The ability to establish baseline metal concentration in fish immediately prior to the DWH oil spill is invaluable, considering the only comprehensive dataset for comparison in the GoM is from fish tissue collected in 1978 (Hall et al., 1978).

The objectives of the present study were to (1) examine patterns of short- and long-term metal exposure within the otoliths of several offshore fish species in varying states of health, as indicated by the presence of external skin lesions, and (2) determine if there was a change in otolith metal concentrations concurrent with the DWH oil spill. These objectives were addressed by analyzing the lifetime otolith chemistries of six offshore fish species collected in the GoM following the DWH oil spill. This approach is unique because it allowed us to establish baseline conditions immediately preceding the DWH oil spill for individuals that were collected after the spill. Unlike previous approaches, which relied on collection of individuals prior to the oil spill in order to establish baseline conditions, this novel approach does not require any a priori information on fish location during the time period of the oil spill or the extent of the oil spill, and can be applied to a wide array of species.

2. Methods

2.1. Study site and specimen collection

Longline surveys were conducted during the summer months (June–August) of the three years that followed the DWH oil spill (2011—2013). Fish were collected at stations along transects in the region of the DWH oil spill and off the West Florida Shelf (Fig. 1). Each station was fished for 2 h using baited demersal long lines in an 8 km string at a depth of approximately 10–150 m. For additional details on field collection methods refer to Snyder et al. (2015). Total length, weight, and sex were recorded for the target fish species. Both sagittal otoliths were extracted from a subsample of the target species at the time of capture and stored for later processing.

Otoliths from the following species collected in the field were selected for analysis using a stratified-random design across transects: Red Grouper, *Epinephelus morio*; Red Porgy, *Pagrus pagrus*; Red Snapper, *Lutjanus campechanus*; Southern Hake, *Urophycis floridana*; Tilefish, *Lopholatilus chamaeleonticeps*; and Yellowedge Grouper, *Hyporthodus flavolimbatus*. These species were selected for analysis because there is the potential for overlap of the oil spill with the known distributions of these fishes (Chakrabarty et al., 2012). Additionally, these species exhibit a variety of life history patterns (Table S1) that could provide insight into different pathways of exposure to DWH oil.

2.2. Sample preparation

Most otolith preparation procedures took place in a Class 100 laminar flow bench to avoid contamination of otoliths with trace metals. To clean residual tissue off of otoliths, they were scrubbed using a soft-bristled brush, immersed in 36% ultrapure hydrogen peroxide for 3 min, rinsed three times in ultrapure water, and allowed to dry in clean vials for 24 h. Otoliths were embedded in Epoxies Etc. 20-3068 epoxy adhesive and mounted on trace-metal-clean petrographic slides using Crystal Bond™ 509 adhesive. Thin transverse sections (1 mm) were cut through the primordium region with a Buehler IsoMet® low speed saw. Otolith sections were polished until the core was reached using a sequence of 68, 13, and 3 μm grit waterproof silicon carbide papers wetted with ultrapure water. Polished sections were mounted on clean petrographic slides using Crystal Bond™ 509 adhesive, rinsed with ultrapure water, sonicated for 5 min, and air-dried for 24 h.

2.3. Data collection and processing

Otoliths were ablated in a sealed chamber using a PhotonMachines Analyte.193 excimer UV laser ablation (LA) system. An Agilent 7500CX Quadrupole Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) was used to assay the ablated otolith material. For each sample, a suite of 9 isotopes was measured: ^{24}Mg, ^{51}V, ^{53}Cr, ^{57}Fe, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{64}Zn, and ^{208}Pb. ^{43}Ca was also measured for use as an internal standard. These isotopes were selected for analysis because they are less likely to have inter-element isobaric interferences during analysis and they have been measured in the Macondo crude oil from the DWH oil spill (Liu et al., 2012).

The LA-ICP-MS instrumentation was tuned prior to data collection while ablating a National Institute of Standards (NIST) 612 glass wafer to maximize analytical sensitivity and minimize interferences. Background element counts were quantified prior to otolith ablation by collecting a gas blank for 60 s. A NIST 612 glass wafer was ablated two times for 60 s before and after each sample transect run to use as an external calibration reference material. Ablation of otoliths occurred as a line scan along a transect with a width of 64.1 μm extending from the primordium to the edge of the otolith along the sulcal groove. The pulse frequency of the laser was set at 10 Hz, while the laser travel speed was set to 10 μm/s. In most cases, 1–2 pre-ablation transects preceded the sampling transect to remove potential contaminants,

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