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Preliminary Analysis of the Effects of the Macondo Oil Spill on Coastal Diving Ducks in the Northern Gulf of Mexico

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Authors' contributions

This work was carried out in collaboration among all authors. Authors CWM, JFV, SBS and JJD designed the study. Authors CWM, JFV, SBS, TCK and JJD managed the literature searches, collected and analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The explosion and sinking of the Macondo drilling platform resulted in one of the largest marine oil spills in history but with largely uncertain ecological consequences. Among the lesser studied but potentially greater concerns are population and toxicological effects of the spill on migratory birds, including many economically-important waterfowl that overwinter in the area. Here, we present a preliminary analysis of oil effects to coastal diving ducks.
Study Design: Oiled areas of coastal Alabama and reference areas on the Florida Gulf of Mexico coast were used to assess oil impacts in waterfowl.
Place and Duration of Study: Waterfowl were collected in oiled and unoiled areas along the northern Gulf of Mexico during the winter following the 2010 Deepwater Horizon oil spill.

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Methodology: Specimens of scaup (*Aythya* spp), buffleheads (*Bucephala albeola*), and redheads (*Aythya americana*) were collected from local hunters to make comparisons of isotopic carbon signatures (n = 31, n = 6, and n = 12 total for scaup, bufflehead and redheads, respectively), a measure previously used to indicate oil hydrocarbon incorporation into tissues, using elemental analysis-isotope ratio mass spectrometry (EA-IRMS). A subset of these samples was analyzed for liver hydrocarbon concentrations using Gas Chromatography / Mass Spectrometry (GC / MS).
Results: Although based on a small sample size, we found little evidence of assimilation of hydrocarbons in waterfowl was detected based on isotopic signatures or liver concentrations with the exception of one redhead that had liver concentrations of 46 µg/kg Benzo[k]fluoranthene.
Conclusion: We speculate on possible explanations for the lack of oil indicators in waterfowl tissues including the rapid incorporation of oil into alternate food web pathways, degradation of oil prior to arrival, patchy oil distributions, low sample size, and/or insensitive metrics.

Keywords: Aythya; Bucephala; scaup; redhead; bufflehead; PAH; Macondo; deepwater horizon.

1. INTRODUCTION

Waterfowl (i.e., ducks, swans, and geese) have been deemed "the most prominent and economically important group of migratory birds in America" [1]. The degradation of coastal ecosystems due to the cumulative effects of numerous, persistent anthropogenic disturbances such as habitat loss, invasive species, and overharvesting [2,3] has resulted in the increased vulnerability of waterfowl populations. Among those of highest concern are the "diving" ducks, once commonly found in coastal areas [4].

The April 20, 2010 explosion of the Deepwater Horizon (DwH) oil rig off the coast of Louisiana represented a large-scale disturbance [5] that may have negatively impacted coastal populations of migratory waterfowl overwintering in the area. The capping of the Macondo wellhead occurred on July 10, 2010 after an estimated 4.9 million barrels of Sweet Louisiana Crude [6] had entered the waters of the northern Gulf of Mexico. Many areas of the region were impacted by the spill [7], with oil washing ashore into a number of wetland habitats [8] and entering some components of the planktonic base of the region's nearshore food web [9]. Marine birds are known to be vulnerable to effects of oil spills, as exemplified by previous oil spills, including the Exxon-Valdez [10]. Oiling of birds can result in direct mortality by smothering or toxicological effects, or indirectly by initiating hypothermia from the fouling of insulating plumage.

In North America, waterfowl typically move from their summer breeding grounds, located in the north-central United States and south-central Canada between October and January to their wintering grounds where they remain until early spring [11-12]. While many wintering areas exist, among the most important in the southeastern United States are the estuaries of the northern Gulf of Mexico. While these birds are dispersed over a large area during summer, many species aggregate in waters of the Gulf of Mexico during winter [13], with some species exhibiting high site fidelity [14] and others actively occupying a large geographic area. Recent estimates indicate that some 92% of the continental population of redheads alone overwinters in the Gulf of Mexico, particularly in the Laguna Madre area [15]. These waterfowl feed almost exclusively on coastal seagrasses, submerged aquatic vegetation, and emergent marsh plants and the shelter-seeking fauna these vegetated areas support [16-18], many of which were inundated by oil in the months following the explosion of the DwH platform.

Here, we present the results of several physiological assessments used previously to assess oil incorporation into tissues [9,16]. Specifically, we compare carbon isotopic analyses to detect potential shifts in signatures due to the assimilation of oil in birds collected from impacted areas in Alabama and unimpacted reference areas in Florida. Previous studies [9,19] have used carbon isotopes to trace oil due to the depleting nature of oil ($-27.3^0/_0$, [9]). While carbon signatures can change for reasons other than oil exposure, this method provides an inexpensive measure of oil accumulation. Additionally, polycyclic aromatic hydrocarbon (PAH) concentrations in liver tissues were also analyzed in a subset of collected specimens to allow direct comparison with previous studies [16].

2. MATERIALS AND METHODS

2.1 Physiological Metrics

Redhead, scaup, and bufflehead were collected in oiled areas of coastal Alabama, as well as outside the oiled area in Florida (Fig. 1) during the winter of 2011 immediately following the oil spill. Numerous instances of oil washing ashore were observed throughout the summer in the impacted area [7] and documented by the National Oceanic and Atmospheric Administration's Shoreline Assessment and Cleanup Teams and their mapping efforts (SCAT maps) [20]. Likewise, the reference area, chosen because of its geographic proximity and similarity (i.e. available habitats, food resources), lacked any report of oil washing ashore, sheens, or tarballs on SCAT maps.

To collect birds, decoys mimicking the targeted species were placed on the water to lure them within the range of firearms, where they were quickly dispatched and returned to Dauphin Island Sea Lab for processing. Additionally, supplemental samples of liver tissues were collected from hunters in the reference region, along with information on specific collection locations. All specimens were collected during January 2011 during the legal harvesting season, with the exception of one bufflehead taken on December 20, 2010. Birds were taken at the end of the harvesting season to increase exposure time to potential oil contaminants and increase the likelihood of finding any potential hydrocarbons in bird tissues. Although the specific ages were not measured, all birds were adults and taken from separate flocks.

To analyze carbon isotopic signatures of collected birds, leg muscle tissue was excised, rinsed with distilled water, freeze-dried, and ground to a fine powder using a mortar and pestle. All samples were shipped to Washington State University Isotope Facility (http://www.isotopes.wsu.edu/) where they were packed into tin capsules and analyzed using elemental analysis-isotope ratio mass spectrometry (EA-IRMS). A total of 31 scaup (n = 17 from impact, n = 14 from reference area), 6 bufflehead (n = 3 in each area), and 12 redhead (n = 12 in impact area, no data from reference area) were used in isotopic comparisons. Although no redheads were collected from the reference area, we have included this data in hopes of providing baseline information for future studies.

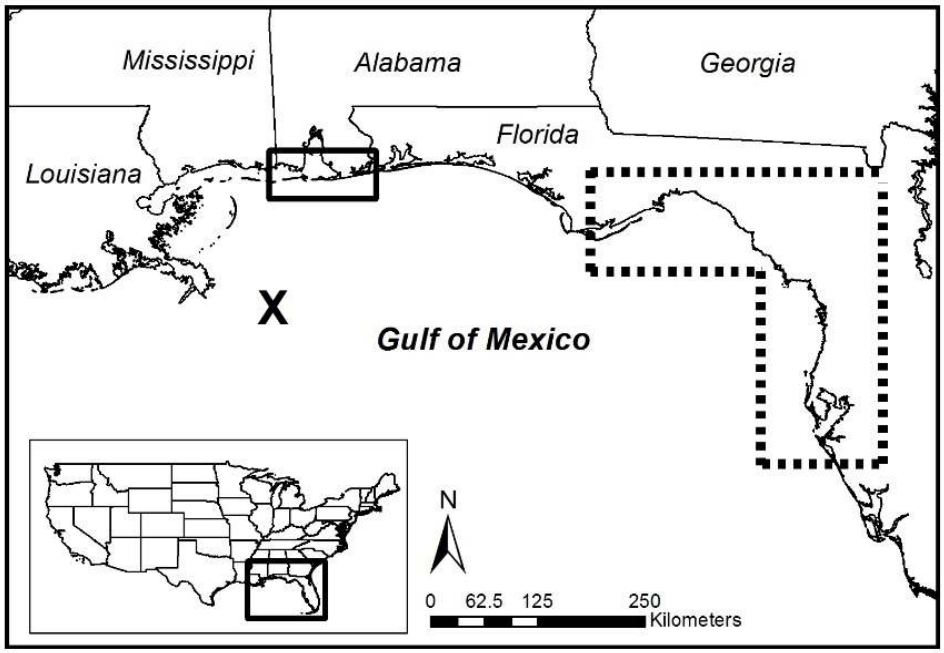

Fig. 1. Study sites in the Gulf of Mexico indicating origin of oil at the Deepwater Horizon drilling site (denoted by the "X"), as well as sites waterfowl collection locations in impact (solid box) and reference (dashed box) areas

Proportions of isotopes were calculated using the formula [18]:

$$\delta^{13}C\ (^{0}/_{00}) = [(R_{sample}/R_{standard})-1] \times 1000$$

Where $\delta^{13}C$ is the ratio of $^{13}C/\ ^{12}C$ in the sample divided by the ratio in Pee Dee Belemnite standard.

A subset of samples was also used to analyze polycyclic aromatic hydrocarbon concentrations in liver tissues. Percent (%) lipids were documented for each sample as a measure of potential hydrocarbon accumulation. A known mass of liver tissue was homogenized and combined with anhydrous sodium sulfate to create an effusive mixture. Methylene chloride solvent was added to the dried sample and extracted using an ultrasonic disruptor for three minutes and solvent decanted. This step was repeated three times. The decanted solvent was then filtered and concentrated, allowed to dry overnight, and weighed to calculate the % lipid concentration according to the following formula:

$$\% \text{ Lipids} = \frac{Wr}{1 \text{ mL}(Ws/FV)} \times 100$$

Where Wr = weight of dried residue (g), Ws = weight of extracted sample (g), and FV = final volume (g). Quality control was performed using cod liver oil containing a known amount of lipids.

We tested for the assimilation of hydrocarbons in waterfowl following the extraction procedure described above, concentrating the dried extract to a volume of 1.0 mL. This extract was analyzed using Gas Chromatography / Mass Spectrometry (GC/MS) after an initial calibration verification was passed. Additional quality control was achieved by analyzing method blanks, laboratory control sample, matrix spike, matrix duplicate once for every 20 samples, and surrogates at a frequency of 1 per sample. Calibration curves were generated by analyzing known standards prior to analysis. A more detailed analysis of redheads than buffleheads/scaup was performed for comparison with previous published estimates [16]. All hydrocarbon analyses were performed by TestAmerica, a commercial analytical lab in Mobile, AL.

2.2 Statistical Analyses

In all analyses, assumptions of normality (Shapiro-Wilk test) and homoscedasity (Levene's test) were tested prior to analysis and transformations of the data were performed if necessary. Comparisons of % lipids and $\delta^{13}C$ were made using Mann Whitney tests. All results were considered significant at $p \leq 0.05$ and analyses were performed using SigmaStat v3.5.

3. RESULTS AND DISCUSSION

No significant difference in $\delta^{13}C$ was detected between impact and reference areas for bufflehead ($W = 7.0$, $P = 0.19$) or scaup ($W = 298.0$, $P = 0.3114$) (Fig. 2). Both bufflehead and scaup exhibited $\delta^{13}C$ values of approximately $-20^{0}/_{00}$, while redheads had values of approximately $-16^{0}/_{00}$.

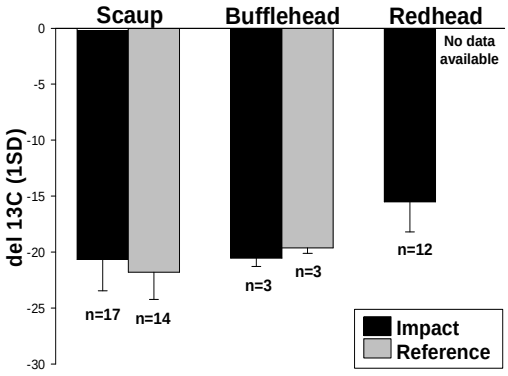

Fig. 2. $\delta^{13}C$ comparisons of scaup and bufflehead taken at reference (gray) and impact (black) areas

Percent lipids in buffleheads did not vary significantly between impact (mean = 2.2%) and reference sites (mean = 2.8%) ($W = 9.0$; $P = 0.66$). In a similar fashion, site differences (impact mean = 2.2% and reference mean = 2.3%) in lipid concentrations scaup were not found ($W = 31.5$; $P = 0.80$). Redheads were found to contain 1.75% ± 0.57 (95CI) lipid liver concentrations.

In all hydrocarbon tests, PAH concentrations were below the instrument's minimum detectable levels for redheads (Table 1) and buffleheads/ scaup (Table 2), with the exception of 1 redhead which contained 46 µg/kg Benzo[k]fluoranthene.

Results presented here failed to identify short-term effects of the DwH spill on diving ducks; however, we caution that additional studies over larger spatial and temporal scales, and at greater replication, are needed to assess the generality

of these results. It is our intent that these preliminary results join a growing list of studies examining the effects of oil on environmental concerns and the publication of such results aid future DwH studies to more efficiently allocate time, effort, and financial investments when investigating potential impacts throughout the larger impacted area, especially for highly migratory species that cover large geographic areas. Here, we demonstrate no significant depletion of the carbon isotopic signature and, with the exception of one redhead, all liver PAH values were below detectable limits (Tables 1 and 2). Although this conclusion was based on a low number of samples and additional research is needed, these findings appear to diverge from initial reports on pelagic plankton [9] and microbes [21], but are in agreement with some population-level studies of fishes [22-23], insects [24], and macrophytes [8,25] that fail to find oil effects or demonstrate rapid recovery.

The lack of hydrocarbons measured in this study could be due to a number of reasons. Oil present in the impacted area may have been rapidly incorporated into alternate microbial pathways of the food web and therefore unavailable to the birds in the fall/winter months. Using isotopic signatures as a proxy for oil assimilation, Graham et al. [9] described a rapid shift in the δ^{13} carbon signature in planktonic organisms concurrent with presence of oil in coastal Alabama. More recent literature, however, has suggested that isotopes may be a poor indicator of hydrocarbon accumulation, even in filter feeders such as barnacles and mussels [26].

Table 1. Hydrocarbon concentrations in redheads taken at the impact site

Compound	Concentration (µg/kg)	Method detection Limit (µg/kg) + 1 SD
Naphthalene	*Below detectable limits*	15.3 + 2.1
1-Methylnaphthalene	*Below detectable limits*	5.3 + 0.8
2-Methylnaphthalene	*Below detectable limits*	7.2 + 1.0
C1-Naphthalenes	*Below detectable limits*	15.3 + 2.1
C2-Naphthalenes	*Below detectable limits*	15.3 + 2.1
C3-Naphthalenes	*Below detectable limits*	15.3 + 2.1
C4-Naphthalenes	*Below detectable limits*	15.3 + 2.1
Acenaphthene	*Below detectable limits*	6.1 + 0.8
Acenaphthylene	*Below detectable limits*	9.1 + 1.2
Fluorene	*Below detectable limits*	5.7 + 0.8
C1-Fluorenes	*Below detectable limits*	5.7 + 0.9
C2-Fluorenes	*Below detectable limits*	5.7 + 0.10
C3-Fluorenes	*Below detectable limits*	5.7 + 0.11
Anthracene	*Below detectable limits*	6.4 + 0.9
Phenanthrene	*Below detectable limits*	7.5 + 1.1
C1-Phenanthrenes/Anthracenes	*Below detectable limits*	7.5 + 1.2
C2-Phenanthrenes/Anthracenes	*Below detectable limits*	7.5 + 1.3
C3-Phenanthrenes/Anthracenes	*Below detectable limits*	7.5 + 1.4
C4-Phenanthrenes/Anthracenes	*Below detectable limits*	7.5 + 1.5
Fluoranthene	*Below detectable limits*	6.5 + 0.9
Pyrene	*Below detectable limits*	10.3 + 1.6
C1-Fluoranthenes/pyrene	*Below detectable limits*	10.3 + 1.7
C2-Fluoranthenes/Pyrene	*Below detectable limits*	10.3 + 1.8
C3-Fluoranthenes/Pyrene	*Below detectable limits*	10.3 + 1.9
Benzo[a]anthracene	*Below detectable limits*	15.8 + 2.4
Chrysene	*Below detectable limits*	8.1 + 1.1
C1-Chrysenes	*Below detectable limits*	8.1 + 1.2
C2-Chrysenes	*Below detectable limits*	8.1 + 1.3
C3-Chrysenes	*Below detectable limits*	8.1 + 1.4
C4-Chrysenes	*Below detectable limits*	8.1 + 1.5
Benzo[b]fluoranthene	*Below detectable limits*	8.9 + 1.2
Benzo[k]fluoranthene	*Below detectable limits* - 46	8.5 + 1.4
Benzo[a]pyrene	*Below detectable limits*	8.5 + 1.5
Perylene	*Below detectable limits*	11.3 + 1.6
Indeno[1,2,3-cd]pyrene	*Below detectable limits*	16.8 + 2.4
Dibenz(a,h)anthracene	*Below detectable limits*	9.7 + 1.4
Benzo[g,h,i]perylene	*Below detectable limits*	9.8 + 1.3
Dibenzofuran	*Below detectable limits*	8.1 + 1.1

Table 2. Hydrocarbon concentrations in scaup and bufflehead from impact and reference areas

Compound	Impact site*	Reference site*	Impact site*	Reference site*	Limit + 1 SD*
1-Methylnaphthalene	*Below detectable limits*	*Below detectable limits*	*Below detectable limits*	*Below detectable limits*	20.8 + 5.2
2-Methylnaphthalene	*Below detectable limits*	*Below detectable limits*	*Below detectable limits*	*Below detectable limits*	23.5 + 5.8
Acenaphthene	*Below detectable limits*	*Below detectable limits*	*Below detectable limits*	*Below detectable limits*	38.2 + 9.4
Acenaphthylene	*Below detectable limits*	*Below detectable limits*	*Below detectable limits*	*Below detectable limits*	22.3 + 5.5
Anthracene	*Below detectable limits*	*Below detectable limits*	*Below detectable limits*	*Below detectable limits*	54.3 + 13.4
Benzo[a]anthracene	*Below detectable limits*	*Below detectable limits*	*Below detectable limits*	*Below detectable limits*	25.9 + 6.5
Benzo[a]pyrene	*Below detectable limits*	*Below detectable limits*	*Below detectable limits*	*Below detectable limits*	39.4 + 9.8
Benzo[b]fluoranthene	*Below detectable limits*	*Below detectable limits*	*Below detectable limits*	*Below detectable limits*	34.5 + 8.5
Benzo[g,h,i]perylene	*Below detectable limits*	*Below detectable limits*	*Below detectable limits*	*Below detectable limits*	30.9 + 7.7
Benzo[k]fluoranthene	*Below detectable limits*	*Below detectable limits*	*Below detectable limits*	*Below detectable limits*	32.1 + 8.0
Chrysene	*Below detectable limits*	*Below detectable limits*	*Below detectable limits*	*Below detectable limits*	23.5 + 5.8
Dibenz(a,h)anthracene	*Below detectable limits*	*Below detectable limits*	*Below detectable limits*	*Below detectable limits*	50.5 + 12.6
Fluoranthene	*Below detectable limits*	*Below detectable limits*	*Below detectable limits*	*Below detectable limits*	22.3 + 5.5
Fluorene	*Below detectable limits*	*Below detectable limits*	*Below detectable limits*	*Below detectable limits*	34.5 + 8.5
Indeno[1,2,3-cd]pyrene	*Below detectable limits*	*Below detectable limits*	*Below detectable limits*	*Below detectable limits*	37.0 + 9.1
Naphthalene	*Below detectable limits*	*Below detectable limits*	*Below detectable limits*	*Below detectable limits*	41.9 + 10.4
Phenanthrene	*Below detectable limits*	*Below detectable limits*	*Below detectable limits*	*Below detectable limits*	27.1 + 6.8
Pyrene	*Below detectable limits*	*Below detectable limits*	*Below detectable limits*	*Below detectable limits*	20.8 + 5.2

**Note: all concentrations in ug/kg*

Moreover, the turnover time needed for isotopic shifts due to oil in muscle tissue to be observable may occur over longer time frames than that measured here. Alternatively, a recent investigation into the microbial uptake of oil has demonstrated that microbial communities demonstrated an increase in species more efficient in hydrocarbon assimilation when oil slicks are present [21,27]. Should hydrocarbons have been diverted into this pathway, it is possible oil could have been degraded in the offshore waters of the Gulf of Mexico [27]. Finally, it has been suggested that food webs are often comprised of multiple "compartments" or "pathways," some of which may not be linked [28]. This is thought to be true in species-rich regions such as the northern Gulf of Mexico, and has been suggested to be the reason for the lack of clear trophic cascades in tropical ecosystems [29]. Another explanation is that many of the species studied here frequently utilize open water and, when they arrived in the winter following the spill, oil may have already washed ashore and deposited onto higher elevation habitats.

Our study is among the first ecological assessments focused on impacts of the Macondo oil spill on waterfowl. However, we acknowledge potential limitations in the current study that highlight the need for further investigations to make a more comprehensive assessment of waterfowl impacts. While the results presented here show negligible PAH uptake by waterfowl in coastal Alabama, we are not suggesting these results to be representative of the larger impact area and it is uncertain at what levels PAH assimilation becomes ecologically important in waterfowl. Birds taken at our impact sites in coastal Alabama may not have been exposed to high levels as birds in coastal Louisiana, an area closer and more heavily impacted by the Deepwater Horizon event. It is also important to note that we measured direct concentration of oil in livers and carbon isotopes, and birds may have converted hydrocarbons to other unmeasured compounds. Additional study taking this into account, for example measuring CYP1A activity [10], needs to be performed to confirm the conclusions presented here. The sublethal impact of oil is also worthy of consideration, as only direct assimilation was measured here.

4. CONCLUSION

Although based on a low sample size, we found little evidence of oil incorporation into waterfowl tissues using stable isotope and PAH analyses. We note numerous mechanisms that may obscure identification of oil impacts and discuss potential limitations of the current study. It is our hope that this data can be utilized by researchers with aspirations of building a larger data set to gain further insight into oil spill impacts.

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CONSENT

It is not applicable.

ETHICAL APPROVAL

No approval was necessary as donations of specimens were taken from local hunters in each region and thus no collection was necessary.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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