Marine Mammals and Respiration: ***Evidence of Poor Pulmonary Health in Bottlenose Dolphins Following the Deepwater Horizon Oil Spill***

Cynthia R. Smith[1*], Teresa K. Rowles[2], Forrest M. Gomez[1], Kathleen M. Colegrove[3], Ryan Takeshita[1], Eric S. Zolman[1], Brian C. Balmer[1], Randall S. Wells[4], Forrest I. Townsend,[5] Lori H. Schwacke[1]

[1]National Marine Mammal Foundation, 2240 Shelter Island Drive, Suite 200, San Diego, California 92106, USA

[2]National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Office of Protected Resources, 1315 East West Highway, Silver Spring, Maryland 20910, USA

[3]Zoological Pathology Program, University of Illinois College of Veterinary Medicine, 3300 Golf Road, Brookfield, Illinois 60513, USA

[4]Chicago Zoological Society, c/o Mote Marine Laboratory, 1600 Ken Thompson Parkway, Sarasota, Florida 34236, USA

[5]Bayside Hospital for Animals, 251 Racetrack Road NE, Fort Walton Beach, Florida 32547, USA

ABSTRACT

[Abstract#688234] The *Deepwater Horizon* (DWH) disaster resulted in large-scale contamination of bays, sounds, and estuaries in the northern Gulf of Mexico (GoM), home to multiple stocks of bottlenose dolphins. Inhalation, direct aspiration, ingestion with subsequent aspiration, and dermal absorption of oil and its toxic components were all considered possible routes of exposure for dolphins living within the oil spill footprint. To determine if dolphins were adversely impacted, capture-release health assessments were performed in heavily-oiled Barataria Bay (BB), Louisiana, and in Sarasota Bay (SB), Florida, a comparison site with no DWH oil contamination. Initial studies were conducted as part of a Natural Resource Damage Assessment (2011-2014), with follow-on studies supported by the Gulf of Mexico Research Initiative (2016-2018). To specifically evaluate pulmonary health, transthoracic ultrasound techniques previously developed for managed dolphins were applied to wild dolphins. Results showed that BB dolphins were ~5 times more likely to have moderate to severe lung disease than SB dolphins in 2011, the year following the spill. Concurrent pathology investigations of dead dolphins in the northern GoM reported similar pulmonary findings. In 2013 and 2014, moderate to severe lung disease persisted among BB dolphins, and remained elevated relative to the prevalence at the SB comparison site. More recent live animal health assessments (2016-2018) showed long-term persistence and potential worsening of moderate to severe lung disease in BB dolphins, specifically in animals alive during the oil spill (prevalence of 0.20, 0.35, and 0.55 in 2016, 2017, and 2018, respectively). Long-term monitoring of dolphin populations is critical to fully understand the potential for and timeline of individual and population recovery from the impacts of a large-scale oil spill event, as well as the cost-benefit trade-offs for restoration activities. In particular, BB dolphins provide valuable insight into the long-lasting effects of oil and oil-related contaminants on animal, human, and ecosystem health.

INTRODUCTION

Deepwater Horizon Oil Spill

In April 2010, the *Deepwater Horizon* (DWH) offshore drilling rig exploded and sank, resulting in large-scale contamination of bays, sounds, and estuaries in the northern Gulf of Mexico

(GoM) (Michel et al. 2013). More than 3 million barrels of oil (~900 million pounds) were released before the well was sealed (U.S. v. BP et al. 2015), resulting in an oil slick that spanned ~43,000 square miles of ocean and oiled over 1,000 miles of shoreline habitats (ERMA 2015, Michel et al. 2013). Although clean-up efforts removed ~600 million pounds of oil-contaminated waste from Gulf waters and nearshore and coastal environments of the northern GoM (EPA 2011), the oil spill caused substantial injury to marine life (DWH NRDA Trustees 2016).

Injury to Marine Mammals

As DWH oil spread throughout the northern GoM, marine mammals were documented swimming through the oil (Aichinger Dias et al. 2017). Response monitoring activities from April to September 2010 documented over 1,100 cetaceans from at least 10 species of dolphins and whales swimming through thick surface oil or surface oil sheen (Aichinger Dias et al. 2017, Wilkin et al. 2017). Marine mammals living within the oil spill footprint were likely exposed through multiple routes, including inhalation, direct aspiration, ingestion with or without subsequent aspiration, and dermal absorption of oil and its toxic components (Takeshita et al. 2017, Smith et al. 2017).

In the months to follow, one of the largest and longest marine mammal mortality events occurred in the northern GoM, lasting from 2010 through July 2014 (Litz et al. 2014). Marine mammal responders routinely collected biological data and samples from carcasses, and after a comprehensive investigation into all potential causes of the mortality event, the most likely cause was determined to be the DWH oil spill (Litz et al. 2014, Venn-Watson et al. 2015a). Some of the most consistent necropsy findings in non-perinate dolphins recovered within the oil spill footprint were bronchopneumonia and adrenal gland atrophy, and an increased prevalence of fetal distress and *in utero* pneumonia was found in dead perinates (Venn-Watson et al. 2015b, Colegrove et al. 2016).

Live dolphins were also examined to investigate the chronic health effects of the oil spill. The temporary capture of dolphins for comprehensive health examinations was performed in heavily-oiled Barataria Bay (BB), Louisiana, as well as oil-impacted Mississippi Sound (MS), Mississippi/Alabama. Dolphins were also examined in Sarasota Bay (SB), Florida, a comparison site with no DWH oil contamination. Initial studies were conducted from 2011-2014 as part of a Natural Resource Damage Assessment (NRDA), with follow-on studies funded by the Gulf of Mexico Research Initiative (2016-2018).

Results from the live dolphin health studies performed during the NRDA were consistent with findings from the concurrent dead dolphin investigations. Specifically, live dolphins were diagnosed with multiple health issues, including moderate to severe lung disease, poor body condition, an impaired stress response, and hematological/serum chemistry indicators of inflammation, hypoglycemia, and abnormal iron levels (Schwacke et al. 2014). From 2011-2013, nearly half of the dolphins evaluated in oil-impacted habitats (BB and MS) were considered unhealthy, indicated by a guarded or worse prognosis, and 17% percent of examined dolphins received a poor or grave prognosis, meaning they were not expected to survive (Schwacke et al. 2014).

The increased prevalence of dolphins with compromised health coincided with high mortality rates within the oil spill footprint. Follow-up studies of BB dolphins using mark-recapture survival models yielded estimated annual mortality rates of 13.2-19.6% in the years immediately following the spill (Lane et al. 2015, McDonald et al. 2017), which were much higher than mortality rates previously reported for bottlenose dolphins using similar techniques near

Charleston, South Carolina (4.9%), and Sarasota, Florida (3.8%) (Speakman et al. 2010, Wells et al. 1990). Alternative hypotheses were considered, including exposure to harmful algal blooms (DWH NRDA Trustees 2016), persistent organic pollutants (Balmer et al. 2015), and infectious disease outbreaks (Venn-Watson et al. 2015a). These factors were ruled out as likely contributors to the increased mortality rates documented within the oil spill footprint, leaving exposure to toxic oil components as the most likely cause of death.

Pulmonary Disease in Dolphins

To specifically evaluate pulmonary health in dolphins likely exposed to DWH oil and related byproducts, transthoracic ultrasound techniques previously developed for managed dolphins (Smith et al. 2012) were used on wild dolphins temporarily captured for health assessments. The dolphin body is well-suited for ultrasound, as their skin is smooth and hairless, so doesn't require any preparation before conducting the exam. Current ultrasound machines are powerful enough to penetrate dolphin blubber and rugged enough to be used in extreme field conditions (e.g. high air temperatures, saltwater environments, unstable operating platforms). Pulmonary abnormalities can be rapidly detected and are divided into previously defined categories (Smith et al. 2012): (1) pleural effusion, or fluid surrounding the lungs; (2) superficial pulmonary nodules, or <2cm round/ovoid foci of non-aerated lung; (3) pulmonary masses, or 2cm or greater well-defined areas of non-aerated lung; (4) alveolar-interstitial syndrome, or evidence of reduced air in the lung and replacement of air with cellular infiltrate, and (5) pulmonary consolidation, where fluid or cellular infiltrate is occupying the alveolar spaces in the lungs. Once examinations have been completed, each lung field (left and right) is given an overall score as follows: normal (no evidence of disease), mild, moderate, or severe lung disease (Schwacke et al. 2014, Smith et al. 2017).

In the year following the spill, dolphins examined within the oil spill footprint had a 5-fold higher prevalence of moderate to severe lung disease when compared to dolphins living outside the oiled region, based on ultrasound examination (0.34 in BB versus 0.7 in the comparison site) (Schwacke et al. 2014). During 2013 and 2014, the prevalence of moderate to severe lung disease among BB dolphins decreased slightly (0.23 and 0.25, respectively), but remained elevated relative to the prevalence at the SB non-oiled comparison site. Concurrent pathology investigations of dead dolphins recovered from the northern GoM in the years following the spill reported similar pulmonary findings (Colegrove et al. 2016). The prevalence of bacterial pneumonias, many of which were severe, in carcasses recovered within the oil spill footprint was significantly higher than in comparison populations (0.22 (oiled) vs 0.02 (non-oiled)). Pneumonias were caused by multiple bacterial pathogens indicating that lung disease was not due to infection with a single, highly pathogenic bacterium, but rather was due to secondary infection of damaged lung tissue (Venn-Watson et al. 2015b).

The increased prevalence of lung disease in dolphins living within the DWH oil spill footprint was consistent with respiratory findings from humans exposed to oil and its toxic components (Zock et al. 2007, Jung et al. 2013, Alexander et al. 2018, Gam et al. 2018a, Gam et al. 2018b). However, relatively few studies have focused on the chronicity of respiratory disease post-exposure. Therefore, to determine if pulmonary disease would persist in wild dolphins living within the oil spill footprint, BB dolphins were examined during capture-release health assessments in 2016, 2017, and 2018 as part of two Gulf of Mexico Research Initiative (GoMRI) supported investigations to better understand the chronic health effects of the DWH disaster.

METHODS

Dolphin capture-release health assessments

Dolphin health assessments were conducted via capture, temporary restraint, and release in oil-impacted BB during 2016, 2017, and 2018, as well as SB (non-oiled comparison site) in 2016 and 2018. For comparative analyses over time, we included previously reported pulmonary data from health assessments in BB (2011, 2013, 2014) and health assessments in SB (2011, 2013) (Schwacke et al. 2014, Smith et al. 2017). We also analyzed additional ultrasound images that had been collected in SB in 2012, 2015, and 2017. Capture-release methodologies and diagnostic sampling techniques are described elsewhere and were similar during all study years and across locations (Wells et al. 2004, Schwacke et al. 2014, Barratclough et al. 2019). Briefly, dolphins were encircled with a seine net and supported by experienced handlers for examination. We avoided small calves with a known or suspected age of less than two years. The amount of time each animal spent out of the water and overall time restrained was minimized, and veterinary staff continuously monitored animals throughout their health assessments.

Pulmonary ultrasound evaluation

Pulmonary ultrasound exams were performed by experienced marine mammal sonographers (CS, FG) using the dorsal-ventral slide technique as previously described (Smith et al. 2012). Pulmonary abnormalities were detected following standardized methods (Smith et al. 2012, Schwacke et al. 2014), to include pleural effusion, alveolar-interstitial syndrome, pulmonary masses, pulmonary nodules, and pulmonary consolidation. Alveolar-interstitial syndrome (AIS) was graded as mild, moderate, or severe as follows: mild – occasional clusters of ring-down artifacts; moderate – frequent clusters of ring-down artifacts, distributed throughout the dorsal, ventral, or entire lung field; severe – contiguous ring-down artifacts that created a ‘white-out’ effect and loss of reverberation artifact, detected in multiple areas. When present, distribution of

AIS throughout the lung field was noted. Pulmonary nodules (<2cm) and masses (≥2cm) were measured and described.

Photo-identification

We compared dorsal fin images of the dolphins that we sampled to images of known individuals collected from prior photo-identification studies (McDonald et al. 2017, Wells et al. 1990). Our team has conducted photo-identification studies in BB since 2010, and in SB since 1970; therefore, a majority of the dolphins were known. Based on photographic sighting history, we categorized each individual dolphin as being alive in 2010 during the DWH spill, or being born after the spill. This allowed us to compare the prevalence of lung disease in BB dolphins presumably exposed to DWH oil in 2010 to those born after the spill, and to compare similar age cohorts between the two study sites.

Statistical analysis

To examine potential time trend in the prevalence of lung disease for dolphins that were alive in 2010 when the DWH spill occurred, we applied a generalized additive model (GAM) with smoothing splines. We created a binary outcome variable to indicate lung scores that were normal or showed mild lung disease (0), versus those that showed moderate to severe lung disease (1). We used a binomial distribution and logit link for the GAM, and included site (BB/SB) as an additional binary covariate. Because of the short time span, we had limited samples of dolphins born after 2010, therefore we pooled samples across years. To examine potential differences in lung disease prevalence between the two sites for the younger cohort, we applied a similar GAM, but without the smoothing spline term for time. We generated heatmaps stratified by site and age cohort to visually examine prevalence of the various lung abnormalities. For AIS, the heatmap was based on mean score with moderate scores assigned 0.5 and severe scores assigned 1.0, inferring that severe scores were twice as bad as moderate scores; for all other (binary) variables, the heat maps display prevalence of cases. We conducted an additional GAM, again with smoothing splines, to evaluate temporal changes in worsening AIS dorsal to ventral (yes/no) for dolphins alive at the time of the spill. All analyses were conducted in R (ref); we used the mgcv package for GAM analyses.

RESULTS

Sample demographics

Between 2011 and 2018, we evaluated the pulmonary health of 171 BB dolphins; 132 of which were alive at the time of the spill and 18 born after the oil spill. We could not definitively classify 21 BB dolphins to an age cohort. Over the same time period, we assessed the pulmonary health of 103 dolphins from the unoiled comparison site (SB), 84 which were alive in 2010 and 19 born after 2010.

Dolphins alive in 2010 during the DWH spill

Our GAM analysis indicated that sampling site strongly influenced the prevalence of moderate to severe lung disease ($p<0.001$), with dolphins sampled in BB, which was heavily oiled by the DWH disaster, having the higher prevalence. Many of the lung scores for BB dolphins were severe (prevalence of 0.23 in 2017 and 0.22 in 2018 in oiled BB), while none of the SB dolphins alive in 2010 were diagnosed with severe lung disease post-DWH (2011-2018).

There was some evidence that prevalence of moderate to severe lung disease changed over the years (GAM smooth term $p=0.06$ for BB, $p=0.07$ for SB); however, the increases were primarily confined to one or two years: 2018 in BB and 2016-2017 in SB. The prevalence of moderate to severe lung disease in BB was 0.20, 0.35, and 0.55 in 2016, 2017, and 2018, respectively. During the period of highest prevalence of moderate lung disease in SB (no SB animals alive at the time of the spill were diagnosed with severe disease), there was also significant uncertainty in the estimates due to limited sample sizes.

AIS was a significant factor in the BB lung disease cases, and in some cases worsened dorsal to ventral within the lung field. While dorsal to ventral worsening was not seen at all in SB dolphins, the prevalence increased over the years for BB dolphins that were alive at the time of the DWH spill ($p<0.001$) and was highest in 2018, the final year of sampling. We documented other lung abnormalities including pleural effusion, pulmonary nodules, consolidation, and masses in BB. Some of these abnormalities, including pulmonary nodules, were also seen in SB, albeit to a much lesser extent.

Dolphins born after the DWH spill in 2010

Prevalence of moderate or severe lung scores did not differ between BB and SB for dolphins born in 2010 after the DWH spill ($p=0.67$), and was relatively low. Nodules were the most commonly observed abnormality for both BB and SB cohorts.

DISCUSSION

In the aftermath of the DWH disaster and subsequent oiling of coastal habitats, we found a high prevalence of moderate to severe lung disease in bottlenose dolphins living within one of the heavily-oiled estuaries (BB). BB dolphins have high site fidelity, meaning that animals tend to live in the same region for multiple years and are unlikely to leave (Lane et al. 2015; Wells et al. 2017). Our follow-on studies proved critical in determining that dolphin pulmonary health was not improving. The high prevalence of moderate to severe lung disease suggests a chronic, progressive lung damage has occurred following oil exposure, which should be considered when investigating potential pathways and mechanisms of respiratory compromise. Other possible contributing factors include an increased susceptibility to lung infections due to oil-induced immune system aberrations, cardiac damage with secondary pulmonary compromise, and age-related health changes.

In bottlenose dolphins and other cetaceans (porpoises, dolphins, and whales), the lungs serve a dual purpose of both respiration and buoyancy control (Ridgway et al. 1969). Investigations of dolphin pulmonary anatomy have shown that terminal airways are reinforced with cartilage, and myoelastic sphincters are found surrounding terminal bronchioles and alveolar entrances (Simpson & Gardner 1972). The alveoli can completely collapse at depth, forcing air into the reinforced air spaces, presumably for prevention of decompression sickness (Ridgway et al. 1969). The presence of moderate to severe pulmonary disease would be expected to impair these physiologic mechanisms, negatively impact buoyancy, and increase energetic demands. This is supported by the authors' (CRS, FMG, FIT) personal observations of managed dolphins diagnosed with moderate to severe pulmonary disease that have altered swim and dive patterns, likely due to a decrease in functional lung capacity and concomitant impaired buoyancy.

Pulmonary damage and subsequent respiratory compromise would not be unusual for mammals several years following exposure to an oil spill. Respiratory symptoms were reported in humans following inhalation exposure from multiple spills, including the DWH disaster (Alexander et al. 2018, Rusiecki et al. 2018). In addition to dolphins and humans, studies involving fish and mice experimentally exposed to DWH oil and/or byproducts reported respiratory injury (Brown-Peterson et al. 2015, Jaligama et al. 2015, Pan et al. 2018). Potential mechanisms of primary injury have been compared across taxa, including oxidative damage, cellular damage, and cellular necrosis. Secondary pathways have also been investigated, including immunotoxicity, cardiotoxicity, and chronic stress.

Based on comprehensive examination of all available data collected to date, chronic pulmonary disease was likely a significant factor in the overall poor health of dolphins living within the oil spill footprint, and there were additional consequences to dolphins that had sustained this injury. To help define clinical significance to individual animals, oxygenation and blood gas analyses were conducted (2016 and 2017), which identified evidence of compensatory acid-base imbalances in dolphins with lung disease (Sharp et al. 2017). Poor pulmonary health and acid-base imbalances could help explain the sustained high rates of reproductive failure in BB (Lane et al. 2015; Kellar et al. 2016; Smith et al. 2020), as maternal illness and related adverse health outcomes could put pregnancies at risk and impact a female's ability to adequately care for her young. Additionally, overall health scores have been determined over time (2011-2018) in BB dolphins and showed that dolphin population health has not improved over time, but instead worsened in 2017 and 20178 (Schwacke et al. 2020).

A limitation of this study was the small number of SB dolphins (comparison site) sampled during 2016 and 2017 that were alive during the DWH oil spill (5 in 2016; 8 in 2017), and caution must be taken when interpreting the increasing trend of moderate lung disease during those two years. Although the prevalence of moderate lung disease increases, the prevalence of pulmonary abnormalities remains low. There are no cases of pulmonary masses, pulmonary consolidation, or dorsal-to-ventral worsening of AIS in SB during 2017 or 2018. Additionally, there are no cases of severe pulmonary disease diagnosed in SB during the entire post-DWH disaster study period, compared to a prevalence of 0.23 in 2017 and 0.22 in 2018 in oiled BB.

This study shows strong evidence of a chronic and potentially progressive respiratory injury in bottlenose dolphins living within the oil spill footprint. Other species of cetaceans living further off-shore were exposed, but their health was difficult to evaluate and few data exists to determine the potential adverse health impacts. However, the nearshore bottlenose dolphin data suggests that any cetacean with a similar exposure to DWH oil or its byproducts could sustain a similar injury. Long-term monitoring of dolphin populations living within the oil spill footprint is critical for fully understanding the potential for and timeline of individual and population recovery from the impacts of a such a large-scale oil spill event, to include extrapolation of impacts to other cetacean populations in the northern GoM. BB dolphins in particular provide valuable insight into the long-lasting effects of oil and associated contaminants on animal, human, and ecosystem health.

ACKNOWLEDGMENTS

This research was made possible in part by a grant from The Gulf of Mexico Research Initiative, and as part of the Deepwater Horizon Natural Resource Damage Assessment. Data are publicly available through the Gulf of Mexico Research Initiative Information & Data Cooperative (GRIIDC) at https://data.gulfresearchinitiative.org (doi:10.7266/N7H41PTV, 10.7266/n7-76aj-rp39, 10.7266/n7-sv57-1h12). Live dolphin health assessments in Barataria Bay were conducted under NMFS permit numbers 932-1905/MA-009526 18786 issued to the NMFS Marine Mammal Health and Stranding Response Program. Sarasota Bay health assessments were performed under National Marine Fisheries Service Scientific Research Permit No. 15543 and approved by Mote Marine Laboratory's Institutional Animal Care and Use Committee. We appreciate the efforts of numerous organizations and their researchers, veterinarians, and technicians who provided support for the health assessment projects, which include our home institutions.

REFERENCES

Aichinger Dias, L., Litz, J., Garrison, L., Martinez, A., Barry, K., & Speakman, T. (2017) Exposure of cetaceans to petroleum products following the *Deepwater Horizon* oil spill in the Gulf of Mexico. Endang Species Res 33:119–125.

Alexander, M., Engel, L.S., Olaiya, N., Wang, L., Barrett, J., Weems, L., Schwartz, E.G., & Rusieki, J.A. (2018) The *Deepwater Horizon* oil spill Coast Guard cohort study: A cross-sectional study of acute respiratory health symptoms. Environ Res 162:196-202. doi: 10.1016/j.envres.2017.11.044.

Balmer, B.C., Ylitalo, G.M., McGeorge, L.E., Baugh, K.L., Boyd, D., Mullin, K.D., Rosel, P.E., Sinclair, C., Wells, R.S., Zolman, E.S., & Schwacke, L.H. (2015) Persistent organic pollutants (POPs) in blubber of common bottlenose dolphins (*Tursiops truncatus*) along the northern Gulf of Mexico coast, USA. Sci Total Environ 527:306-312.

Barratclough, A., Wells, R.S, Schwacke, L.H, Rowles, T.K., Gomez, F.M., Fauquier, D., Sweeney, J., Townsend, F.I., Hansen, L., Zolman, E.S., Balmer, B.C., & Smith, CR. (2019) Health assessments of common bottlenose dolphins (*Tursiops truncatus*): past, present, and potential conservation applications. Front Vet Sci 6:444.

Brown-Peterson, N.J., Krasnec, M., Takeshita, R., Ryan, C.N., Griffitt, K.J., Lay, C., Mayer, G.D., Bayha, K.M., Hawkins, W.E., Lipton, I. & Morris, J. (2015) A multiple endpoint analysis of the effects of chronic exposure to sediment contaminated with *Deepwater Horizon* oil on juvenile Southern flounder and their associated microbiomes. Aquat Tox 165:197-209.

Colegrove, K.M., Venn-Watson, S., Litz, J., Kinsel, M.J., Terio, K.A., Fougeres, E., Ewing, R., Pabst, D.A., McLellan, W.A., Raverty, S., Saliki, J., Fire, S., Rappucci, G., Bowen-Stevens, S., Noble, L., Costidis, A., Barbieri, M., Field, C., Smith, S., Carmichael, R.H., Chevis, C., Hatchett, W., Shannon, D., Tumlin, M., Lovewell, G., McFee, W., & Rowles, T.K. (2016) Fetal distress and *in utero* pneumonia in perinatal dolphins during the Northern Gulf of Mexico unusual mortality event. Dis Aquat Org 119:1-16.

Deepwater Horizon Natural Resource Damage Assessment (NRDA) Trustees. (2016) *Deepwater Horizon* oil spill: Final Programmatic Damage Assessment and Restoration Plan and Final Programmatic Environmental Impact Statement. Retrieved from *http://www.gulfspillrestoration.noaa.gov/restoration-planning/gulf-plan*

ERMA (2015) Web application: Deepwater Gulf Response Environmental Response Management Application, National Oceanic and Atmospheric Administration. *http://gomex.erma.noaa.gov/*

EPA (2011) EPA should clarify and strengthen its waste management oversight role with respect to oil spills of national significance (11-P-0706). US Environmental Protection Agency, Office of Inspector General, Washington, DC. *http://www.epa.gov/oig/reports/2011/20110926-11-P-0706.pdf*

Gam, K.B., Kwok, R.K., Engel, L.S., Curry, M.D., Stewart, P.A., Stenzel, M.R., McGrath, J.A., Jackson, W.B. 2nd, Jensen, R.L., Keil, A.P., Lichtveld, M.Y., Miller, A.K., Sandler, D.P. (2018a) Lung function in oil spill response workers 1-3 years after the Deepwater Horizon disaster. Epidemiology 29(3):315-322. doi: 10.1097/EDE.0000000000000808.

Gam, K.B., Engel, L.S., Kwok, R.K., Curry, M.D., Stewart, P.A., Stenzel, M.R., McGrath, J.A., Jackson, W.B. 2nd, Lichtveld, M.Y., Sandler, D.P. (2018b) Association between Deepwater Horizon oil spill response and cleanup work experiences and lung function. Environ Int 121(Pt

1):695-702. doi: 10.1016/j.envint.2018.09.058.

Jaligama, S., Chen, Z., Saravia, J., Yadav, N., Lomnicki, S.M., Dugas, T.R., & Cormier, S.A. (2015) Exposure to Deepwater Horizon crude oil burnoff particulate matter induces pulmonary inflammation and alters adaptive immune response. Environ Sci Technol 21;49(14):8769-76. doi: 10.1021/acs.est.5b01439.

Jung, S.C., Kim, K.M., Lee, K.S., Roh, S., Jeong, W.C., Kwak, S.J., Lee, I.J., Choi, Y.H., Noh, S.R., Hur, J.I., Jee, Y.K. (2013) Respiratory effects of the Hebei Spirit oil spill on children in taean, Korea. Allergy Asthma Immunol Res 5(6):365-70. doi: 10.4168/aair.2013.5.6.365.

Kellar, N.M., Lane, S.M., Smith, C.R., Speakman, T.R., Balmer, B.C., Trego, M.L., Catelani, K.N., Robbins, M.N., Allen, C.D., Wells, R.S., Zolman, E.S., Rowles, T.K., & Schwacke, L.H. (2016) Low reproductive success rates of common bottlenose dolphins (*Tursiops truncatus*) in the northern Gulf of Mexico following the *Deepwater Horizon* disaster (2010-2015). Endang Species Res 33:143-158. doi.org/10.3354/esr00775.

Lane, S.M., Smith, C.R., Mitchell, J., Balmer, B.C., Barry, K.P., McDonald, T., Mori, C.S., Rosel, P.E., Rowles, T.K., Speakman, T.R., Townsend, F.I., Tumlin, M.C., Wells, R.S., Zolman, E.S., & Schwacke, L.H. (2015) Reproductive outcome and survival of common bottlenose dolphins sampled in Barataria Bay, Louisiana, USA, following the *Deepwater Horizon* oil spill. Proc R Soc B 282:20151944.

Litz, J., Baran, M.A., Bowen-Stevens, S.R., Carmichael, R.H., Colegrove, K.M., Garrison, L.P., Fire, S.E., Fougeres, E.M., Hardy, R., Holmes, S., Jones, W., Mase-Guthrie, B.E., Odell, D.K., Rosel, P.E., Saliki, J.T., Shannon, D.K., Shippee, S.F., Smith, S.M., Stratton, E.M., Tumlin, M.C., Whitehead, H.R., Worthy, G.A., & Rowles, T.K. (2014) Review of historical unusual mortality events (UMEs) in the Gulf of Mexico (1990–2009): Providing context for the multiyear Northern Gulf of Mexico cetacean UME declared in 2010. Dis Aquat Org 112: 161-175. doi:10.3354/dao02807.

McDonald, T.L., Hornsby, F., Speakman, T., Zolman, E., Mullin, K.D., Sinclair, C., Rosel, P., Thomas, L., & Schwacke, L. (2017) Survival, density, and abundance of common bottlenose dolphins in Barataria Bay following the *Deepwater Horizon* oil spill. Endang Species Res 33:193-209. doi.org/10.3354/esr00806.

Michel, J., Owens, E.H., Zengel, S., Graham, A., Nixon, Z., Allard, T., Holton, W., Reimer, P.D., Lamarche, A., White, M., Rutherford, N., Childs, C., Mauseth, G., Challenger, G., & Taylor, E. (2013) Extent and degree of shoreline oiling: *Deepwater Horizon* oil spill, Gulf of Mexico, USA. PLoS One 8(6):e65087.

Pan, Y.K., Khursigara, A.J., Johansen, J.L., & Esbaugh, A.J. (2018) The effects of oil induced respiratory impairment on two indicies of hypoxia tolerance in Atlantic croaker (*Micropogonias undulatus*). Chemosphere 200:143-150.

Ridgway, S.H., Scronce, B.L., Kanwisher J. (1969) Respiration and deep diving in the bottlenose porpoise. Science 166(913):1651-4.

Rusieki, J., Alexander, M., Schwartz, E.G., Wang, L., Weems, L., Barrett, J., Christenbury, K., Johndrow, D., Funk, R.H., & Engel, L.S. (2018) The *Deepwater Horizon* oil spill Coast Guard study. Occup Environ Med 75(3):165-175. doi: 10.1136/oemed-2017-104343.

Schwacke, L.H., Smith, C.R., Townsend, F.I., Wells, R.S., Hart, L.B., Balmer, B.C., Collier, T.K., De Guise, S., Fry, M.M., Guillette, L.J., Lamb, S.V., Lane, S.M., McFee, W.E., Place, N.J., Tumlin, M.C., Ylitalo, G.M., Zolman, E.S., & Rowles, T.K. (2014) Health of common bottlenose dolphins (*Tursiops truncatus*) in Barataria Bay, Louisiana, following the *Deepwater Horizon* oil spill. Environ Sci Technol 48(1), 93-103. doi:10.1021/es403610f.

Schwacke, L.H., Smith, C.R., Gomez, F.M, Rowles, T.K., Wells, R.S., Zolman, E.S., Speakman, T., Herrman, J., Takeshita, R., and Thomas, L. (2020) Health trends for bottlenose dolphins in the 8 years following the *Deepwater Horizon* oil spill: Evidence for lack of resilience. Proceedings of the Gulf of Mexico Oil Spill & Ecosystem Science Conference.

Sharp, S.M., Baird, M., Cendejas, V., Gomez, F.G., Meegan, J.M., Townsend, F.I., Jensen, E.D., Schwacke, M., Venn-Watson, S.K., Rowles, T.K., Schwacke, L.H., and Smith, C.R. (2017) A pilot study to evaluate the utility of blood gas analysis and capnography in determining maternal oxygenation status in bottlenose dolphins *(Tursiops truncatus*) following the Deepwater Horizons oil spill. Proceedings of the International Association of Aquatic Animal Medicine Annual Meeting.

Simpson, J.G., Gardner, M.B. (1972) Comparative microscopic anatomy of selected marine mammals. In: Ridgway SH (ed) Mammals of the sea: biology and medicine. Charles C. Thomas Publishers, Springfield, IL, p.298-418.

Smith, C.R., Solano, M., Lutmerding, B.A., Johnson, S.P., Meegan, J.M., Le-Bert, C.R., Emory-Gomez, F., Cassle, S., Carlin, K., & Jensen, E.D. (2012) Pulmonary ultrasound findings in a bottlenose dolphin *Tursiops truncatus* population. Dis Aquat Org 101:243-255.

Smith, C.R., Rowles, T.K., Hart, L.B., Townsend, F.I., Wells, R.S., Zolman, E.S., Balmer, B.C., Quigley, B., Ivančić, M., McKercher, W., Tumlin, M.C., Mullin, K.D., Adams, J.D., Wu, Q., McFee, W., Collier, T.K., & Schwacke, L.H. (2017) Slow recovery of Barataria Bay dolphin health following the *Deepwater Horizon* oil spill (2013-2014), with evidence of persistent lung disease and impaired stress response. Endang Species Res 33:127-142. doi.org/10.3354/esr00778.

Smith, C.R., Gomez, F.G., Colegrove, K.M., Rowles, T.K., Zolman, E.S., Speakman, T.R., Musser, W.B., Barratclough, A., Balmer, B.C., Takeshita, R., & Schwacke, L.H. (2020) Sustained maternal illness and low reproductive success rate in Barataria Bay bottlenose dolphins following the *Deepwater Horizon* oil spill. Proceedings of the Gulf of Mexico Oil Spill & Ecosystem Science Conference.

Speakman, T.R., Lane, S.M., Schwacke, L.H., Fair, P.A., & Zolman, E.S. (2010) Mark-recapture estimates of seasonal abundance and survivorship for bottlenose dolphins (*Tursiops truncatus*) near Charleston, South Carolina, USA. J Cetacean Res Manag11(2):153-162.

Takeshita, R., Sullivan, L., Smith, C., Collier, T., Hall, A., Brosnan, T., Rowles, T., & Schwacke, L. (2017). The *Deepwater Horizon* oil spill marine mammal injury assessment. Endang Species Res 33:95-106.

U.S. v. BP et al. (United States of America v. BP Exploration & Production, Inc., et al) (2015) Findings of fact and conclusions of law: Phase two trial. In re: Oil spill by the oil rig *Deepwater Horizon* in the Gulf of Mexico, on April 20, 2010, No. MDL 2179, 2015 WL 225421. LA. E.D. Jan. 15, 2015. Doc. 14021. U.S. District Court for the Eastern District of Louisiana. *http://www.laed.uscourts.gov/OilSpill/Orders/1152015FindingsPhaseTwo.pdf* .

Venn-Watson, S., Garrison, L., Litz, J., Fougeres, E.M., Mase, B., Rappucci, G., Stratton, E.M., Carmichael, R.H., Odell, D.K., Shannon, D.K., Shippee, S.F., Smith, S.M., Staggs, L., Tumlin, M.C., Whitehead, H., & Rowles, T. (2015a) Demographic clusters identified within the Northern Gulf of Mexico common bottlenose dolphin (*Tursiops truncatus*) unusual mortality event: January 2010-June 2013. PLoS One 10(2). doi:e0117248.

Venn-Watson, S., Colegrove, K.M., Litz, J., Kinsel, M., Terio, K., Saliki, J., Fire, S., Carmichael, R., Chevis, C., Hatchett, W., Pitchford, J., Tumlin, M., Field, C., Smith, S., Ewing, R., Fauquier, D., Lovewell, G., Whitehead, H., Rotstein, D., McFee, W., Fougeres, E., & Rowles,

T. (2015b). Adrenal gland and lung lesions in Gulf of Mexico common bottlenose dolphins (*Tursiops truncatus*) found dead following the *Deepwater Horizon* oil spill. PLoS One 10(5). doi:10.1371/journal.pone.0126538.

Wells, R.S., Scott, M.D. (1990) Estimating bottlenose dolphin population parameters from individual identification and capture-release techniques. Report of the International Whaling Commission, Special Issue 12:407-415.

Wells, R.S., Rhinehart, H.L., Hansen, L.J., Sweeney, J.C., Townsend, F.I., Stone, R., Casper, D., Scott, M.D., Hohn, A.A., & Rowles, T.K. (2004) Bottlenose dolphins as marine ecosystem sentinels: developing a health monitoring system. EcoHealth 1:246−254.

Wells, R. S., L. H. Schwacke, T. K. Rowles, B. C. Balmer, E. Zolman, T. Speakman, F. I. Townsend, M. C. Tumlin, A. Barleycorn and K. A. Wilkinson. (2017) Ranging patterns of common bottlenose dolphins (*Tursiops truncatus*) in Barataria Bay, Louisiana, following the *Deepwater Horizon* Oil Spill. Endang Species Res 33:159-180. doi:10.3354/esr00732.

Wilkin, S.M., Rowles, T.K., Stratton, E., Adimey, N., Field, C.L., Wissmann, S., Shigenaka, G., Fougères, E., Mase, B., Southeast Region Stranding Network, & Ziccardi, M.H. (2017) Marine mammal response operations during the *Deepwater Horizon* oil spill. Endang Species Res 33: 107−118.

Zock, J.P., Rodríguez-Trigo, G., Pozo-Rodríguez, F., Barberà, J.A., Bouso, L., Torralba, Y., Antó, J.M., Gómez, F.P., Fuster, C., Verea, H.; SEPAR-Prestige Study Group. (2007) Prolonged respiratory symptoms in clean-up workers of the prestige oil spill. Am J Respir Crit Care Med 15;176(6):610-6. doi: 10.1164/rccm.200701-016OC.