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Contribution to the Theme Section 'Effects of the Deepwater Horizon oil spill on protected marine species'

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

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ABSTRACT: The 2010 *Deepwater Horizon* (DWH) disaster resulted in large-scale oil contamination of the northern Gulf of Mexico. As part of the Natural Resource Damage Assessment designed to investigate the potential impacts of the DWH oil spill, comprehensive health assessments were conducted on bottlenose dolphins *Tursiops truncatus* living in oiled bays (Barataria Bay [BB], Louisiana, and Mississippi Sound [MS], Mississippi/Alabama) and a reference bay with no evidence of DWH oil contamination (Sarasota Bay [SB], Florida). As previously reported, multiple health issues were detected in BB dolphins during 2011. In the present study, follow-on capture-release health assessments of BB dolphins were performed (2013, 2014) and indicated an overall improvement in population health, but demonstrated that pulmonary abnormalities and impaired stress response persisted for at least 4 yr after the DWH disaster. Specifically, moderate to severe lung disease remained elevated, and BB dolphins continued to release low levels of cortisol in the face of capture stress. The proportion of guarded or worse prognoses in BB improved over time, but 4 yr post-spill, they were still above the proportion seen in SB. Health assessments performed in MS in 2013 showed similar findings to BB, characterized by an elevated prevalence of low serum cortisol and moderate to severe lung disease. Prognosis scores for dolphins examined in MS in 2013 were similar to BB in 2013. Data from these follow-on studies confirmed that dolphins living in areas affected by the DWH spill were more likely to be ill; however, some improvement in population health has occurred over time.

KEY WORDS: Dolphin · Health · Oil · Toxicology · Pulmonary · Stress · Cortisol · Prognosis

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INTRODUCTION

The explosion and subsequent collapse of the *Deepwater Horizon* (DWH) drilling platform in April 2010 resulted in large-scale contamination of bays, sounds, and estuaries in the northern Gulf of Mexico (GoM) (Michel et al. 2013). An estimated 3.19 million barrels (~507 000 m^3) of Mississippi Canyon Block 252 (MC252) oil were released before the well was sealed (US v. BP et al. 2015). MC252 is a sweet crude oil containing thousands of chemicals, including polycyclic aromatic hydrocarbons (PAHs) and volatile organic compounds (VOCs) such as benzene, toluene, and xylene (Dietrich et al. 2014, DWH NRDA Trustees 2016). The oil slick spanned ~43 000 square miles (~111 000 km^2) of ocean and oiled over 1000 miles (1600 km) of shoreline habitats (Michel et al. 2013, ERMA 2015). Cleanup efforts have removed 600 million pounds (270 million kg) of oil-contaminated waste from Gulf waters and the nearshore and coastal environments of the northern GoM (EPA 2011).

The northern GoM is home to numerous stocks of common bottlenose dolphins *Tursiops truncatus* that rely on healthy ecosystems to support successful foraging, growth, and reproduction (Waring et al. 2015). One of the most heavily oiled bays in the northern GoM was Barataria Bay (BB), Louisiana, where persistent contamination of shoreline was documented during the most recent ground observations in September 2014 (ERMA 2014). Inhalation, direct aspiration, ingestion with subsequent aspiration, and dermal absorption of MC252 oil and its toxic components were all considered possible routes of exposure to dolphins in BB and other contaminated habitats. A vast body of published literature has shown that exposure to toxic oil components can lead to both acute and chronic adverse health effects in humans and other animals.

As part of the Natural Resource Damage Assessment (NRDA) designed to determine the potential health impacts of the DWH disaster on bottlenose dolphins living in the northern GoM, capture-release health assessments were performed during the summer of 2011 in heavily oiled BB and in Sarasota Bay (SB), Florida, a control site with no evidence of DWH oil contamination. Multiple health issues were detected in BB dolphins, including poor body condition, an impaired stress response, moderate to severe lung disease, and hematological/serum chemistry indicators of inflammation, hypoglycemia, and abnormal iron levels (Schwacke et al. 2014). Nearly half of the dolphins evaluated were considered unhealthy, indicated by a guarded or worse prognosis, and 17% of dolphins were given a poor or grave prognosis, meaning they were not expected to survive. Similarly, follow-up studies of BB dolphins utilizing mark-recapture survival models yielded estimated annual mortality rates ranging from 13.2 to 19.6% in the years immediately following the spill (Lane et al. 2015, McDonald et al. 2017, this Theme section), which are much higher than mortality rates previously reported for bottlenose dolphins using similar techniques in Charleston, South Carolina (4.9%), and Sarasota, Florida (3.8%) (Wells & Scott 1990, Speakman et al. 2010). Alternative factors such as accumulation of persistent organic pollutants (Balmer et al. 2015), exposure to harmful algal blooms (DWH NRDA Trustees 2016), and infectious disease outbreaks (Venn-Watson et al. 2015a) were ruled out as likely contributors to disease, leaving exposure to toxic oil components as the most likely cause of the increased morbidity and mortality.

Concurrent studies focused on dead dolphin retrieval, necropsy, and histopathology in the northern GoM have reported similar findings in the aftermath of the DWH oil spill (Venn-Watson et al. 2015a,b). As part of an unusual mortality event (UME) investigation following the spill, dolphins recovered within the oil spill footprint had potentially lethal changes to their adrenal gland (33%) and primary bacterial pneumonia (22%), consistent with the previous findings of impaired stress responses and moderate to severe lung disease in live-sampled dolphins. The prevalence of abnormal adrenal glands and lung disease in the dead, stranded dolphins were significantly higher than the prevalences among reference populations, and were not attributable to common causes of cetacean mortality events such as morbillivirus outbreaks and brevetoxicosis (Litz et al. 2014, Venn-Watson et al. 2015b). Further, an unusually high number of dead dolphins were documented in BB beginning in August 2010 and lasting until December 2011 (Venn-Watson et al. 2015a), overlapping in time with the periods of heaviest oiling in the bay (Michel et al. 2013). The weight of evidence from both the live and dead dolphin studies following the DWH disaster supports that exposure to oil components resulted in increased morbidity and mortality of bottlenose dolphins in BB.

In the present study, follow-up capture-release health assessments were performed in BB (in 2013 and 2014) to document disease outcome in individual dolphins and to examine the recovery process of a population previously documented with a high prevalence of disease. To address potential differences

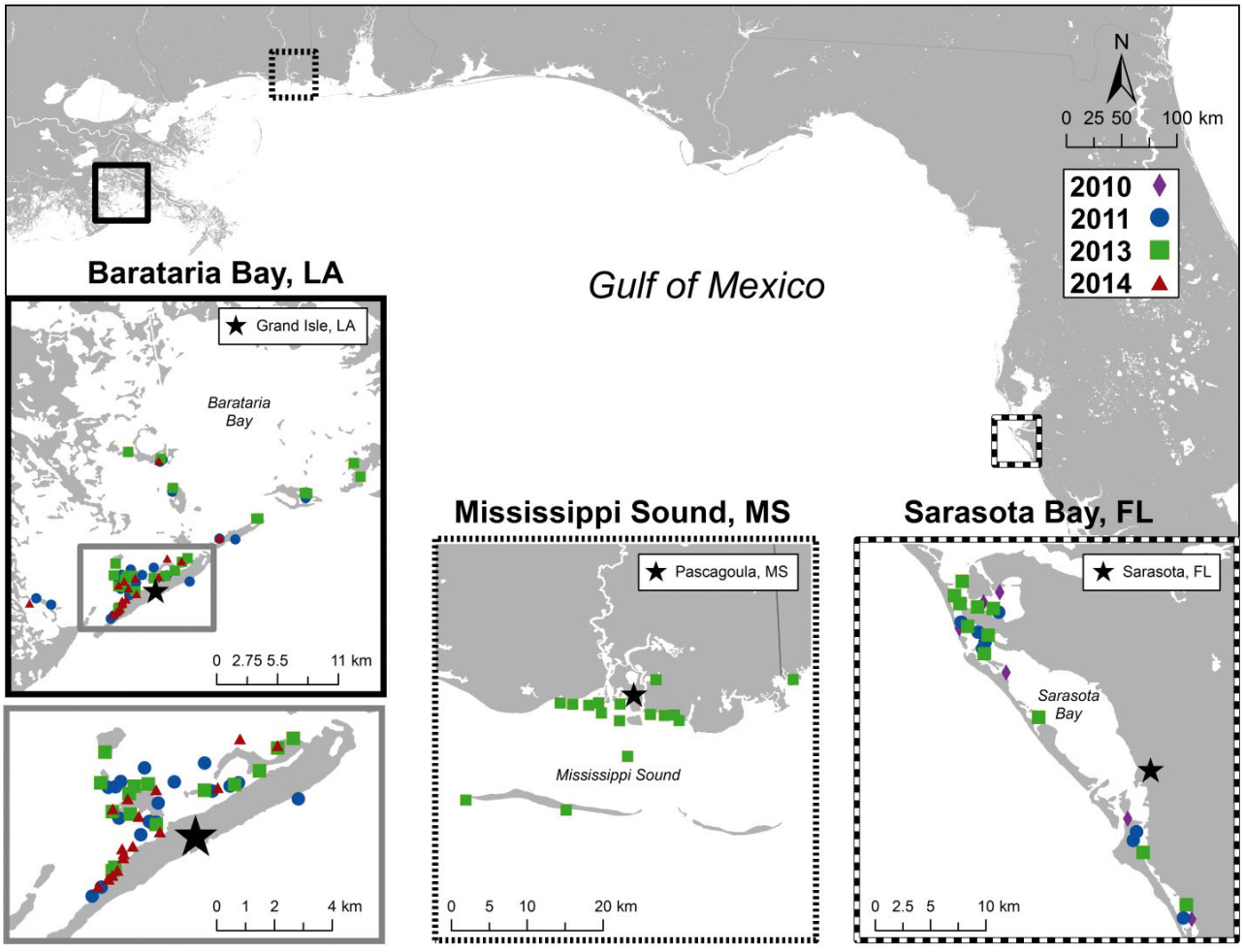

Fig. 1. Capture-release health assessment locations for bottlenose dolphins *Tursiops truncatus* sampled in Barataria Bay (2011, 2013, 2014), Mississippi Sound (2013), and Sarasota Bay (2010, 2011, 2013)

in geographic locations within the northern GoM, dolphins from another oiled area, Mississippi Sound (MS), Mississippi/Alabama, were also evaluated in 2013. Both were compared to additional data from the unoiled SB reference site, evaluated again in 2013. The same methodologies were employed to provide consistency between sites and years, allowing for accurate interpretation of results with the goal of determining progression, persistence, and/or resolution of previously identified health issues in the years following the DWH oil spill.

MATERIALS AND METHODS

Dolphin capture-release

Dolphin (*Tursiops truncatus*) health assessments, via temporary capture, restraint, and release, were conducted in BB from June 17–28, 2013 and June 9–20, 2014, and in MS during July 22–August 2, 2013 (Fig. 1). Health assessments were also conducted in SB from May 6–10, 2013 to build upon preceding reference data from an area without significant DWH oiling. Previously reported data from 2011 health assessments in BB and 2010–2011 health assessments in SB were included for comparative analyses over time (Schwacke et al. 2014). Capture-release methodologies and diagnostic sampling for the NRDA have been described elsewhere (Wells et al. 2004, Schwacke et al. 2014). Briefly, dolphins were encircled with a seine net and restrained by experienced handlers for examination. Small calves with a known or suspected age of <2 yr were avoided. The amount of time each animal spent out of the water was minimized, and veterinary staff continuously monitored animals throughout their health assessments.

Health diagnostics

For 2013 and 2014 assessments, blood for diagnostic testing was collected via venipuncture of the ventral fluke vasculature before the dolphin was brought on-board the research vessel (pre-sampling) and just prior to the dolphin being released (post-sampling), aiming for a minimum 30 min interval between collection times. Whole blood, serum, and plasma samples were sent to the Animal Health Diagnostic Center at Cornell University's College of Veterinary Medicine for hematological, serum chemistry, and hormone analyses. Sample collection and analysis methods for the endocrine assays followed methods described previously by Schwacke et al. (2014).

Physical examinations, including morphologic measurements, physical observations, and the extraction of a tooth for age determination, were conducted as previously described (Schwacke et al. 2014). Morbillivirus testing via PCR analysis was performed on blowhole swab samples when available, using techniques previously described (Sierra et al. 2014). *Brucella* sp. PCR testing was performed on blowhole swabs and genital slit swab samples when available, using techniques previously described (Wu et al. 2014). PCR test results were reported as either positive or negative.

Ultrasound evaluation

Reproductive ultrasound exams, pregnancy confirmation, and due date estimations were performed as previously described (Smith et al. 2013, Schwacke et al. 2014, Wells et al. 2014), utilizing M-mode and color Doppler interrogation if needed to determine fetal viability. Pulmonary ultrasound exams were performed utilizing the dorsal-ventral slide technique as previously described (Smith et al. 2012, Schwacke et al. 2014). Pulmonary abnormalities were described as previously reported for dolphins (Smith et al. 2012, Schwacke et al. 2014), including pleural effusion, alveolar-interstitial syndrome (AIS), pulmonary masses, pulmonary nodules, and pulmonary consolidation. 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; or severe: contiguous ring-down artifacts that created a 'white-out' effect and loss of reverberation artifact, detected in multiple areas (Fig. 2). Pulmonary nodules (<2 cm) and masses (≥2 cm) were measured and described.

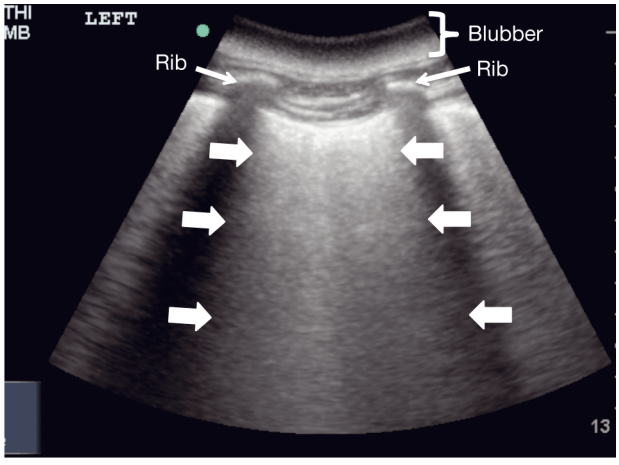

Fig. 2. Representative dorsal plane ultrasound image of an area of dolphin (*Tursiops truncatus*) lung affected with severe alveolar-interstitial syndrome (AIS). Contiguous ring-down artifacts create a 'white-out' appearance (white block arrows). Green dot designates that cranial is to the left and caudal is the right

Prognosis determination

Prognosis determinations were assigned for each dolphin as previously described (Schwacke et al. 2014). Animals that received a good or fair prognosis were within what was considered expected limits for wild dolphins, as these animals would likely survive the health conditions documented. Animals that fell into the guarded prognosis category had conditions that were of concern and would have warranted follow-up care in a clinical setting; therefore it was unknown if these animals could overcome the documented conditions without intervention or treatment. Animals receiving poor or grave prognoses were considered to be in ill health and unlikely to overcome the health issues without medical intervention.

Data and statistical analysis

Four sets of reference intervals were used to evaluate the health of BB, SB, and MS dolphins: (1) hematology (Schwacke et al. 2009), (2) serum chemistry (Schwacke et al. 2009), (3) body condition (Hart et al. 2013), and (4) cortisol (Hart et al. 2015). Schwacke et al. (2014) reported hematological and serum biochemical abnormalities grouped by health panels (i.e. inflammation, hypoglycemia, abnormal iron, hepatobiliary abnormalities, anemia, electrolyte/mineral imbalance, and impaired renal function). These health panels were based on single or com-

bined abnormalities determined by hematological and serum biochemical reference intervals, and methods to identify health panel cases (Schwacke et al. 2014) were replicated for this study (e.g. abnormal iron was defined as an elevation of 2 or more of the following: serum iron, total iron binding capacity, % transferrin saturation). A dolphin was considered a case if a parameter value was below or above the respective reference threshold, and the clinical importance of a high or low value varied depending on the analyte under consideration (e.g. low hemoglobin for anemia vs. high neutrophil count for inflammation). Similarly, body condition for each animal was determined based on reference intervals for mass:length ratio as described in Hart et al. (2013) and Schwacke et al. (2014), where non-pregnant individuals with a mass:length ratio below the lower reference threshold were considered to be in poor body condition. Subsequent to the Schwacke et al. (2014) study, 95th percentile reference intervals have been constructed for bottlenose dolphin cortisol concentrations in serum collected during the pre- and post-sampling bleeds (Hart et al. 2015). These reference intervals were used to re-evaluate the serum cortisol concentrations of SB and BB dolphins sampled in 2010–2011, as well as detect cases among the SB 2013, BB 2013, BB 2014, and MS 2013 cohorts. Only samples collected in <30 min elapsed time of net deployment were used for pre-sampling reference interval evaluations (Hart et al. 2015). Post-sampling cortisol was unavailable for SB dolphins sampled in 2010–2011 or BB 2011 dolphins, as post-sampling blood was not collected. Aldosterone concentrations were compared to the assay detection limit, as outlined in Hart et al. (2015).

The prevalence of multiple health measures including body condition, low cortisol, and health panel cases were calculated for each site and year, along with exact binomial confidence intervals (CIs). There was no difference in the prevalence of cases for any of the health measures between the 2 SB sampling years ($p > 0.05$ for all comparisons); therefore the 2 years were combined to increase sample size and statistical power. If the 95% CIs for the prevalence estimate of low cortisol and poor body condition did not contain expected proportions (0.025 for cortisol and poor body condition; Hart et al. 2013, 2015), the prevalence of observed cases was considered statistically significant. For the health panels, the prevalence of cases in each BB year and MS was compared to SB using a 1-tailed Fisher's exact test. Comparisons to SB were re-calculated for BB 2011 because of the additional SB samples since Schwacke et al. (2014). R 2.15.3 (R Foundation for Statistical Computing, 2010) and SAS 9.3 (SAS Institute, 2003) were used for statistical analyses. For statistical comparisons that included recaptured animals between years, the earliest capture-release event was used for analyses.

The prevalence of pulmonary abnormalities in MS and each BB year was compared to SB using a 1-tailed Fisher's exact test. Comparisons to SB were re-calculated for BB 2011 because of the additional SB samples since the Schwacke et al. (2014) paper. The same methodology was applied to the prevalence of normal to mild versus moderate to severe AIS, as well as normal to mild versus moderate to severe overall lung scores. Similarly, the proportion of guarded or worse prognoses for MS and all years in BB were compared with the proportion in SB using a series of 1-tailed Fisher's exact tests. Comparisons to SB were re-calculated for BB 2011, due to the additional animals assessed and assigned a prognosis in SB 2013.

RESULTS

Sample demographics

In 2013 and 2014, respectively, 30 (15 female, 15 male) and 32 (24 female, 8 male) dolphins were captured, sampled, and released in BB (Table 1, Fig. 1). Of the 30 dolphins sampled in 2013, 3 had been previously sampled in 2011; and of the 32 dolphins sampled in 2014, 7 dolphins had been previously sampled in 2011 and 3 in 2013 (Table 2, Fig. 1). In MS, a total of 20 dolphins were captured and sampled, including 9 females and 11 males (Table 1, Fig. 1). Fifteen dolphins were sampled during 2013 SB health assessments (Table 1, Fig. 1), including 4 animals from the original 26 unique individuals sampled in 2010–2011, bringing the total number of unique individuals in the reference sample over the 3 years to 37. Sex and distribution of age classes was similar between the reference site and the other 2 sites (Table 1), the exception being that the MS sample had a lower proportion of adults (Table 1, $p = 0.04$).

Physical examination

The prevalence of poor body condition, or underweight dolphins, in BB was significantly elevated in 2011 (25%; Schwacke et al. 2014); however, the percentage decreased to be within expected levels in 2013 (9%) and 2014 (6%) (Fig. 3). Among the 4 re-

Table 1. Demographic data for bottlenose dolphins *Tursiops truncatus* captured and released from Sarasota Bay (SB; data combined from all sampling years), Barataria Bay (BB), and Mississippi Sound (MS). Confirmed and probable pregnancies are indicated as 'pregnant'. Criteria for assigning age/sex class were adopted from Schwacke et al. (2009, 2010). Calf criteria: age <2 yr; length <200 cm. Subadult criteria: age ≥2 yr and <10 yr; length ≥200 cm and <240 cm. Adult criteria: age ≥10 yr; length ≥240 cm

Site	Dates	Calf		Juvenile/subadult		Adult		Pregnant	Total
		Male	Female	Male	Female	Male	Female		
SB[a]	2010–2011[b] and May 6–10, 2013	0	0	11	7	7	12	4	37
BB	2011[b]	0	0	3	13	9	7	11[c]	32
	Jun 17–28, 2013	0	1	10	10	5	4	5	30
	Jun 9–20, 2014	0	0	3	10	5	14	16[c]	32
MS	Jul 22–Aug 2, 2013	0	2	6	7	5	0	7	20

[a]Excludes recaptured dolphins
[b]Data from Schwacke et al. (2014)
[c]Includes probable early pregnant; specifically, 1 of 11 in BB 2011 and 5 of 16 in BB 2014

Table 2. Health panel and other abnormalities among Barataria Bay bottlenose dolphins *Tursiops truncatus* recaptured in subsequent years of health assessment sampling. 1: abnormality observed, 0: abnormality not observed, na: data not available for a given parameter. Green cells: improvement; orange cells: decline; yellow cells: no change between recapture years. For blood-based assessments, only pre-sampling measurements were utilized

Animal ID	Year	Inflammation	Hypoglycemia	Abnormal iron	Hepatobiliary abnormalities	Anemia	Electrolytes and mineral abnormalities	Body condition	Low cortisol	Overall lung score	Prognosis
Y00	2011	0	0	0	0	0	0	1	1	Mild	Good
	2013	0	0	0	0	0	0	0	0	Mild	Fair
Y03	2011	1	0	0	0	0	0	0	0	Moderate	Guarded
	2014	0	0	0	1	0	0	0	0	Mild	Fair
Y06	2011	0	0	0	0	0	0	0	0	Normal	Fair
	2013	0	0	0	0	0	0	0	0	Normal	Good
Y08	2011	0	0	0	0	0	0	1	1	na	na
	2014	0	0	0	0	0	0	1	1	Mild	Fair
Y15	2011	0	0	0	1	0	0	0	0	Normal	Guarded
	2014	0	0	0	0	0	0	0	0	Mild	Fair
Y17	2011	1	0	0	0	0	0	0	0	Mild	Guarded
	2014	0	0	0	0	0	0	0	0	Mild	Good
Y19	2011	0	0	0	0	0	0	0	0	Normal	Good
	2014	0	0	0	0	0	0	1	0	Mild	Fair
Y21	2011	0	1	0	0	0	0	na	0	Severe	Poor
	2014	0	0	0	0	0	0	na	0	Severe	Poor
Y23	2011	0	0	0	0	0	0	0	1	Mild	Good
	2013	0	0	0	0	0	0	0	0	Mild	Good
Y26	2013	1	1	0	1	1	0	0	0	Moderate	Poor
	2014	0	0	0	0	0	0	0	na	Mild	Fair
Y34	2013	1	1	0	0	0	0	0	0	Mild	Fair
	2014	1	0	0	0	0	0	0	na	Mild	Fair
Y39	2011	1	0	1	0	0	0	na	1	Moderate	Guarded
	2014	0	0	1	0	0	0	na	1	Mild	Guarded
Y48	2013	0	0	0	0	0	0	1	0	Mild	Guarded
	2014	0	0	0	0	0	0	0	0	Mild	Fair

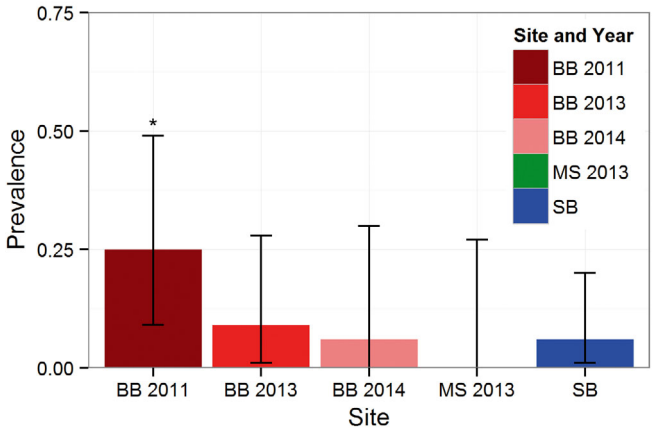

Fig. 3. Prevalence and 95% binomial CIs for bottlenose dolphins *Tursiops truncatus* captured in Sarasota Bay (SB; data combined from all sampling years, 2010–2011, 2013), Barataria Bay (BB), and Mississippi Sound (MS) with low mass: length ratios compared to 95th percentile reference intervals. *Significantly higher than expected prevalence (0.025); $\alpha = 0.05$

captured BB dolphins that were underweight, 2 (Y00, Y48) had gained weight and were within normal limits. Additionally, 1 dolphin (Y08) remained significantly underweight, and another (Y19) moved from normal to underweight (Table 2). The prevalence of underweight dolphins was within expected levels at 6% for the combined SB dataset. None of the dolphins sampled in MS during 2013 were underweight (Fig. 3).

Twenty-one percent (6/29) of the dolphins examined in BB during 2011 had extensive (>50%) or complete tooth loss as diagnosed by oral examination (Schwacke et al. 2014). In subsequent BB captures (2013, 2014), an additional 3 dolphins (of 49 newly examined animals) had extensive or complete tooth loss. Tooth loss abnormalities were not limited to older animals, as the age range spanned 30 yr and included a dolphin as young as 12 yr old. SB dolphins previously sampled did not have extensive tooth loss (Schwacke et al. 2014), and in 2013, only 1 older dolphin (43 yr old) was diagnosed with extensive tooth loss accompanied by extensive tooth wear. None of the MS dolphins were observed with extensive or complete tooth loss.

Clinical pathology and health panels

Clinicopathologic abnormalities previously detected in BB dolphins (2011) included a high prevalence of inflammation, hypoglycemia, and abnormal iron cases when compared to SB dolphins (Schwacke et al. 2014). During subsequent sampling of BB dolphins (2013, 2014), these clinicopathologic abnormalities resolved, with no significant differences detected in inflammation, hypoglycemia, or iron panels as compared to the SB reference site (Table 3). In MS dolphins (2013), none of the health panel prevalence estimates were significantly different from the SB reference data. It should be noted that when comparisons were repeated between BB dolphins (2011) and SB dolphins using the combined data from all sampling years as the reference (2010–2011, 2013), all differences were still significant at $p = 0.05$, with the exception of the hepatobiliary panel ($p = 0.09$). Additionally, no significant differences in hepatobiliary, anemia, or electrolyte/mineral imbalance panels were detected in either BB (2013, 2014) or MS dolphins (2013) when compared to the newly combined SB reference dataset (Table 3). Among BB dolphins that were resampled during 2013–2014, 4 of the 5 inflammation cases improved upon recapture, and all of the dolphins that were hypoglycemic during their first capture resolved by their second capture (Table 2). Only 1 of the recaptured dolphins had an abnormal iron panel in 2011, and it remained abnormal when recaptured in 2014.

Endocrine assessment

In 2011, serum cortisol concentrations were evaluated relative to previously reported minimum values from dolphins sampled at other southeastern US sites (Schwacke et al. 2014). In the present study, pre-sampling cortisol concentrations were re-evaluated using newly available reference intervals (Hart et al. 2015). For pre-sampling cortisol, 12 (41%), 4 (14%), and 8 (27%) of the 2011, 2013, and 2014 BB dolphins, respectively, had low pre-sampling concentrations (i.e. below the 95% reference threshold). Based on an expected prevalence of 2.5% or less, the number of observed pre-sampling low-cortisol cases was significantly elevated for all sampling years in BB as well as MS (3; 19%), whereas the number of SB animals with low pre-sampling cortisol (1; 3%) was within expected intervals (Table 4). Post-sampling blood was not collected in 2011; therefore, we could not evaluate cortisol response following the capture-release sampling period for dolphins during that year. Of the BB 2013–2014 and MS 2013 dolphins sampled, cortisol for only 1 BB dolphin remained low at the post-sampling bleed (Table 4). Of the 4 dolphins with low pre-sampling cortisol that were resampled in subsequent captures, 2 remained low, and 2 were within expected limits upon recapture (Table 2). Similar to findings in

Table 3. Observed counts, case prevalence, and 95% binomial CIs (in parentheses) for hematological and serum biochemical panel abnormalities among bottlenose dolphins *Tursiops truncatus* sampled in Sarasota Bay (SB; data combined from all sampling years), Barataria Bay (BB), and Mississippi Sound (MS). p_{SB}: p-value from a 1-tailed Fisher's exact test comparing prevalence of abnormality to reference site SB. (–): Not applicable. *Significant difference from SB ($\alpha = 0.05$)

Panel	SB	BB						MS	
	2010, 2011, 2013	2011	p_{SB}	2013	p_{SB}	2014	p_{SB}	2013	p_{SB}
Sample size	**37**	**32**		29, 30[a]		**32**		**20**	
Inflammation									
Observed cases	5	11	0.04*	8	0.13	6	0.39	5	0.23
Prevalence	0.14	0.34		0.28		0.19		0.25	
95% CI	(0.05–0.29)	(0.19–0.53)		(0.13–0.47)		(0.07–0.36)		(0.09–0.49)	
Hypoglycemia									
Observed cases	0	7	0.003*	2	0.19	0	–	2	0.12
Prevalence	0	0.22		0.07		0		0.1	
95% CI	(0.00–0.10)	(0.09–0.40)		(0.01–0.23)		(0.00–0.11)		(0.01–0.32)	
Abnormal iron									
Observed cases	1	7	0.02*	1	0.69	3	0.25	0	1
Prevalence	0.03	0.22		0.03		0.09		0	
95% CI	(0.00–0.14)	(0.09–0.40)		(0.00–0.18)		(0.02–0.25)		(0.00–0.12)	
Hepatobiliary abnormalities									
Observed cases	2	6	0.09	3	0.38	4	0.27	2	0.44
Prevalence	0.05	0.19		0.1		0.13		0.1	
95% CI	(0.01–0.18)	(0.07–0.36)		(0.02–0.27)		(0.04–0.29)		(0.01–0.32)	
Anemia									
Observed cases	1	4	0.14	2[a]	0.42	2	0.44	3	0.12
Prevalence	0.03	0.13		0.07		0.06		0.15	
95% CI	(0.00–0.14)	(0.04–0.29)		(0.01–0.22)		(0.01–0.21)		(0.03–0.38)	
Electrolytes and minerals									
Observed cases	2	1	0.85	3	0.38	0	1	2	0.44
Prevalence	0.05	0.03		0.1		0		0.1	
95% CI	(0.01–0.18)	(0.00–0.16)		(0.02–0.27)		(0.00–0.11)		(0.01–0.32)	
Renal function									
Observed cases	0	0	–	0	–	0	–	0	–
Prevalence	0	0		0		0		0	
95% CI	(0.00–0.10)	(0.00–0.11)		(0.00–0.12)		(0.00–0.11)		(0.00–0.12)	

[a]The full suite of samples was not collected for 1 animal (1 animal was only evaluated for anemia)

2011 (Schwacke et al. 2014), BB dolphins determined as having low cortisol concentrations also had low aldosterone concentrations; in fact, 92% (11/12) of low-cortisol cases in 2013 and 2014 had aldosterone measures below the assay limit of detection. Of the 3 MS dolphins with abnormally low cortisol, 2 also had aldosterone below the limit of detection.

Infectious disease diagnostics

Only 1 animal (Y31, BB 2011) tested positive for dolphin morbillivirus by PCR analyses of blowhole swab samples. All remaining BB animals tested for morbillivirus were negative: 12 dolphins in 2011, 27 in 2013, and 31 in 2014 (included 4 recaptured dolphins that remained negative). Additionally, all MS and SB dolphins tested for morbillivirus were negative (17 and 12 dolphins, respectively). Similarly, the vast majority of dolphins tested for *Brucella* sp. were negative by PCR analyses of blowhole and genital swab samples, when available. One dolphin in BB 2014 (Y89, blowhole swab) and another in MS 2013 (675, blowhole swab) tested positive for *Brucella* sp. The remaining BB, MS, and SB dolphins tested negative: 32 dolphins in BB 2011, 27 in BB 2013, 30 in BB 2014, 14 in MS 2013, and 11 in SB 2013.

Reproductive ultrasound

Pregnancy data from all health assessments is summarized in Table 1, as determined by reproductive ultrasound examination. Criteria for probable preg-

Table 4. Observed counts, prevalence, and 95% binomial CIs (in parentheses) for bottlenose dolphins *Tursiops truncatus* captured and released in Sarasota Bay (SB; data combined from all sampling years), Barataria Bay (BB), and Mississippi Sound (MS) with low cortisol. 'Expected' is the number of cases or fewer that would be expected in any of the populations, assuming a prevalence of 0.025 for observations below the cortisol 95th percentile reference interval. (–): No data. *Significantly higher than the expected prevalence of 0.025 ($\alpha = 0.05$)

	SB	BB			MS
	2010, 2011, 2013	2011	2013	2014	2013
Pre-sampling low cortisol					
Sample size	31	29	29	30	16
Expected cases	3 or fewer	3 or fewer	3 or fewer	3 or fewer	2 or fewer
Observed cases	1	12	4	8	3
Prevalence	0.03	0.41*	0.14*	0.27*	0.19*
95% CI	(0.00–0.17)	(0.24–0.61)	(0.04–0.32)	(0.12–0.46)	(0.04–0.46)
Post-sampling low cortisol					
Sample size	12	–	28	31	17
Expected cases	2 or fewer	–	3 or fewer	3 or fewer	2 or fewer
Observed cases	0	–	0	1	0
Prevalence	0	–	0	0.03	0
95% CI	(0.00–0.26)	–	(0.00–0.12)	(0.00–0.17)	(0.00–0.20)

nancies included a serum progesterone >5 ng ml^{-1}, and the detection of a corpus luteum on either ovary, with or without the presence of uterine fluid. Criteria for confirmed pregnancies were the same as probable, plus the presence of a fetus and uterine fluid. Additional detail regarding reproductive outcome in study animals is outside the scope of this paper; however, data, analyses, and discussion are included in Kellar et al. (2017, this Theme Section).

Pulmonary ultrasound

In 2011, pulmonary ultrasound determined that BB dolphins were approximately 5 times more likely to have moderate to severe lung disease when compared to SB dolphins (Schwacke et al. 2014). The higher prevalence of moderate to severe lung disease resulted from cases of moderate to severe AIS in BB dolphins (14 cases), pulmonary masses which were only diagnosed in BB dolphins (3 cases), and cases of pulmonary consolidation (6 cases). For the present study, data from SB 2011 and 2013 exams were combined as the reference, since no significant differences in the prevalence of lung abnormalities or lung scores were observed between SB years ($p > 0.05$). For SB dolphins examined in both 2011 and 2013 (3 dolphins), only 2011 data were included in analyses. Prevalence of moderate to severe lung scores, pulmonary abnormalities, and AIS severity were calculated for each BB and MS sampling year, and compared to the expanded SB reference data.

The prevalence of moderate to severe lung disease remained elevated in BB dolphins during 2013 (23%) and 2014 (25%) when compared to SB dolphins (7%); however, the difference was no longer statistically significant at the $\alpha = 0.05$ threshold (2013: $p = 0.10$; 2014: $p = 0.07$) (Table 5). Of the 10 dolphins that were examined in BB 2011 and then reexamined in 2013 or 2014, lung scores remained stable (5 cases), improved (2 cases), worsened (2 cases), or could not be determined due to insufficient data (1 case).

Evidence of AIS in BB increased from 62% in 2011 to a significantly elevated prevalence of 90–88% in subsequent years, specifically as compared to the SB reference of 52% (Table 6). When severity was considered, moderate to severe AIS was considered significantly elevated in all 3 BB years (34–48%) when compared to SB (7%) (Table 7). Additionally, the prevalence of pulmonary nodules was significantly elevated in BB 2013 (70%), but not in other years.

Results in MS (2013) were similar to BB, with 40% of dolphins examined having moderate to severe lung disease, a significant elevation over SB (Table 5). Additionally, MS dolphins had a high prevalence of AIS (85%), moderate to severe AIS (50%), and pulmonary consolidation (35%) when compared to SB (Tables 6 & 7).

Severe lung disease cohort

Fourteen dolphins were diagnosed with severe lung disease, exclusively in the oiled sites: 11 in BB (5 in 2011, 2 in 2013, and 4 in 2014) and 3 in MS

Table 5. Number of cases, prevalence and 95% binomial CIs (in parentheses) for overall lung scores for *Tursiops truncatus* in Sarasota Bay (SB; data combined from all sampling years), Barataria Bay (BB), and Mississippi Sound (MS). p_{SB}: p-value from a 1-tailed Fisher's exact test comparing prevalence of moderate to severe lung score to reference site SB. *Significant difference from SB ($\alpha = 0.05$)

Lung score	SB	BB						MS	
	2011, 2013[a]	2011	p_{SB}	2013	p_{SB}	2014	p_{SB}	2013	p_{SB}
Sample size (total)	27	29		30		32		20	
Normal to mild									
Observed cases	25	19		23		24		12	
Prevalence	0.93	0.66		0.77		0.75		0.6	
95% CI	(0.76–0.99)	(0.46–0.82)		(0.58–0.91)		(0.57–0.89)		(0.36–0.81)	
Moderate to severe									
Observed cases	2	10*	0.01	7	0.1	8	0.07	8*	0.01
Prevalence	0.07	0.34		0.23		0.25		0.4	
95% CI	(0.01–0.24)	(0.18–0.54)		(0.10–0.42)		(0.11–0.43)		(0.19–0.64)	

[a]Recaptures excluded

Table 6. Pulmonary abnormalities in *Tursiops truncatus* in Sarasota Bay (SB; data combined from all sampling years), Barataria Bay (BB), and Mississippi Sound (MS), with number of cases, prevalence, and 95% binomial CIs (in parentheses). p_{SB}: p-value from a 1-tailed Fisher's exact test comparing prevalence of each pulmonary abnormality to reference site SB. (–): Not applicable. *Significant difference from SB ($\alpha = 0.05$)

	SB	BB						MS	
	2011, 2013[a]	2011	p_{SB}	2013	p_{SB}	2014	p_{SB}	2013	p_{SB}
Sample size	27	29		30		32		20	
Pleural effusion									
Observed cases	1	3	0.33	2	0.54	2	0.56	0	1
Prevalence	0.04	0.1		0.07		0.06		0	
95% CI	(0.00–0.19)	(0.02–0.27)		(0.01–0.22)		(0.01–0.21)		(0.00–0.17)	
Pulmonary nodules									
Observed cases	9	10	0.58	21*	0.006	17	0.1	11	0.12
Prevalence	0.33	0.34		0.7		0.53		0.55	
95% CI	(0.17–0.54)	(0.18–0.54)		(0.51–0.85)		(0.35–0.71)		(0.32–0.77)	
Pulmonary masses									
Observed cases	0	3	0.13	0	–	0	–	0	–
Prevalence	0	0.1		0		0		0	
95% CI	(0.00–0.13)	(0.02–0.27)		(0.00–0.12)		(0.00–0.11)		(0.00–0.17)	
Pulmonary consolidation									
Observed cases	2	6	0.15	5	0.26	7	0.12	7*	0.02
Prevalence	0.07	0.21		0.17		0.22		0.35	
95% CI	(0.01–0.24)	(0.08–0.40)		(0.06–0.35)		(0.09–0.40)		(0.15–0.59)	
Alveolar-interstitial syndrome									
Observed cases	14	18	0.31^	27*	0.002	28*	0.003	17*	0.02
Prevalence	0.52	0.62		0.9		0.88		0.85	
95% CI	(0.32–0.71)	(0.42–0.79)		(0.73–0.98)		(0.71–0.96)		(0.62–0.97)	

[a]Recaptures excluded

(2013). One dolphin (Y21) with severe lung disease was examined twice (BB 2011 and BB 2014), and was only included in the cohort during her original capture event. All animals with severe lung disease had evidence of moderate to severe AIS and pulmonary consolidation. Additional findings within this cohort included pleural effusion (29%; 4/14), pulmonary nodules (57%; 8/14), and pulmonary masses (21%; 3/14). Prognosis scores for animals with severe lung disease were guarded or worse, with the majority having a score of poor or grave (71%; 10/14).

Table 7. Prevalence and 95% binomial CI (in parentheses) for alveolar-interstitial syndrome (AIS) severity in *Tursiops truncatus* in Sarasota Bay (SB; data combined from all sampling years), Barataria Bay (BB), and Mississippi Sound (MS). p_{SB}: p-value from a 1-tailed Fisher's exact test comparing prevalence of moderate to severe AIS to reference site SB. *Significant difference from SB (α = 0.05)

	SB	BB						MS	
	2011, 2013[a]	2011	p_{SB}	2013	p_{SB}	2014	p_{SB}	2013	p_{SB}
None or mild									
Observed cases	25	15		16		21		10	
Prevalence	0.93	0.52		0.53		0.66		0.5	
95% CI	(0.76–0.99)	(0.33–0.71)		(0.34–0.72)		(0.47–0.81)		(0.27–0.73)	
Moderate or severe									
Observed cases	2	14*	0.0007	14*	0.001	11*	0.01	10*	0.001
Prevalence	0.07	0.48		0.47		0.34		0.5	
95% CI	(0.01–0.24)	(0.29–0.67)		(0.28–0.66)		(0.19–0.53)		(0.27–0.73)	

[a]Recaptures excluded

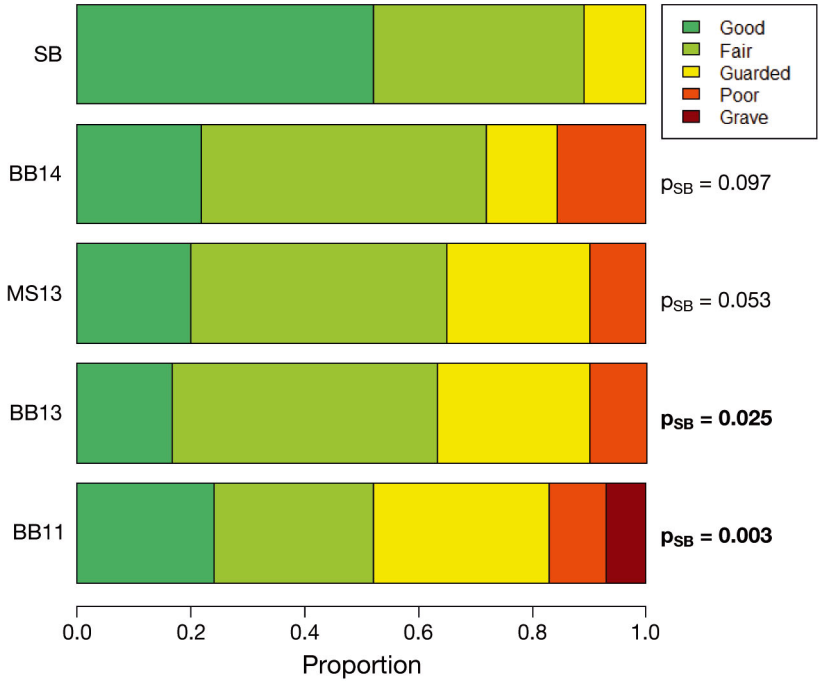

Fig. 4. Overall prognosis scores for bottlenose dolphins *Tursiops truncatus* captured and released in Barataria Bay (BB; 2011, 2013–2014), Mississippi Sound (MS; 2013), and Sarasota Bay (SB; data combined from all sampling years, 2010–2011, 2013). p_{SB}: p-value from a 1-tailed Fisher's exact test comparing prevalence of guarded or worse prognosis to reference site SB. **Bold**: significantly different from SB (α = 0.05)

Overall prognosis

The proportion of guarded or worse prognoses in BB improved over time when compared to SB. Specifically, the BB prevalence of dolphins scored as guarded or worse decreased from 48% in 2011 (14/29), to 37% in 2013 (11/30), and 28% in 2014 (9/32), when compared to SB at 11% (3/27) (Fig. 4). The differences in proportion were statistically significant when comparing SB and BB 2011 (p = 0.003) and SB versus BB 2013 (p = 0.025), and no longer significant when comparing SB to BB 2014 (p = 0.097) (Fig. 4). MS animals examined in 2013 had similar scores to BB 2013, with 35% of animals scoring as guarded or worse (7/20). Among the 12 BB dolphins recaptured in subsequent sampling years and assigned prognosis scores for both capture events, 6 improved, 4 were stable, and 2 degraded (Table 2).

DISCUSSION

Follow-up assessments of dolphins living in heavily oiled BB indicated an overall improvement in population health, but demonstrated that pulmonary abnormalities and impaired stress response persisted for at least 4 yr after the DWH disaster. Specifically, moderate to severe lung disease remained elevated and dolphins in 2013–2014 continued to have low levels of cortisol in the face of capture stress. Prevalence of moderate to severe lung disease among BB dolphins decreased slightly with time since the spill (34% in 2011, and 23–25% in 2013–2014), but was still elevated relative to expected prevalence (7%) based on the SB reference site. Health assessments performed at another

contaminated site (MS) showed similar findings to BB. MS dolphins had low serum cortisol levels and a high prevalence of moderate to severe lung disease, based primarily on findings of moderate to severe AIS and pulmonary consolidation.

In contrast to the slowly improving prevalence of moderate to severe lung disease was the increased proportion of AIS among BB dolphins in 2013–2014 and MS dolphins in 2013. Smith et al. (2012) reported ultrasound evidence of AIS as consistent with pneumonia, pulmonary edema, and pulmonary fibrosis in dolphins. These findings could be secondary to an oil-induced injury. Pulmonary damage and subsequent respiratory compromise would not be unusual for mammals several years following exposure to an oil spill. In fact, acute and chronic respiratory symptoms were reported in humans following inhalation exposure from multiple spills, including the *Prestige*, *Hebei Spirit*, *Tasman Spirit*, and DWH oil spills (Suárez et al. 2005, Carrasco et al. 2006, Janjua et al. 2006, Zock et al. 2007, 2012, Sim et al. 2010, Jung et al. 2013, Sandler et al. 2014).

During the 2011 assessments of BB dolphins, pneumonia was considered a likely cause of AIS based on associated ultrasound findings and blood-based evidence of inflammation, anemia, and possible septicemia (Schwacke et al. 2014). One of the study dolphins (Y12) stranded dead 5.5 mo after examination, and necropsy findings confirmed severe pneumonia (Lane et al. 2015). A concurrent study of stranded, dead dolphins recovered from BB during 2010–2011 reported a high prevalence of primary bacterial pneumonia (Venn-Watson et al. 2015b). Results from these live and dead animal investigations, conducted within 18 mo of the DWH disaster, indicated that exposure to toxic oil components was the likely cause of lung disease based on the high level of oil contamination in BB (Michel et al. 2013), high levels of PAHs documented in BB (Allan et al. 2012), and a lack of evidence for alternative causes (Schwacke et al. 2014, Venn-Watson et al. 2015a,b). Inhalation or aspiration of oil components could have resulted in direct pulmonary damage with subsequent pneumonia, or a damaged immune system with an overall increased susceptibility to infectious disease.

Although the persistence of AIS in live dolphins examined in BB 2013–2014 and MS 2013 could indicate continued susceptibility to pneumonia, improvement in other relevant health parameters (e.g. inflammation, anemia, hypoglycemia) suggests development of chronic conditions such as pulmonary fibrosis that wouldn't necessarily be accompanied by clinicopathologic abnormalities. AIS has also been associated with pulmonary fibrosis in dolphins (Smith et al. 2012) and chronic interstitial diseases in humans (Smargiassi et al. 2013); therefore, the development of a chronic and potentially progressive disease following oil-induced pulmonary injury should be fully explored. This outcome would not be unprecedented, as humans and animals exposed to oil mist and/or vapor have developed pulmonary fibrosis (Lykke et al. 1979, Skyberg et al. 1992). Histopathologic assessments of stranded, dead dolphins recovered from the oil spill footprint beginning in 2013 and later are needed to elucidate whether chronic, fibrotic diseases are increasing in prevalence among oil-impacted dolphins.

Identifying 14 cases of severe lung disease among 99 unique animals receiving pulmonary ultrasound exams within the DWH oil spill footprint was a remarkable finding that should not be overlooked for its importance in understanding the potential long-term impacts of oil exposure on pulmonary health. All severe cases of lung disease had ultrasound evidence of both pulmonary consolidation and moderate to severe AIS, with the majority of animals having severe AIS (86%). Severe AIS, previously considered uncommon in dolphins, and characterized by contiguous ring-down artifacts that create a 'white-out' effect, has been correlated with severe pneumonia and pulmonary edema (Smith et al. 2012). Although difficult to confirm without histopathologic examination of tissues, severe AIS could also be consistent with severe fibrotic changes. Prognosis scores for animals with severe lung disease were guarded or worse, with the majority having a score of poor or grave (71%), underscoring the impact of pulmonary health on animal survival. This severe lung disease cohort provided further evidence that exposure to DWH oil could result in pulmonary damage with secondary pneumonia, edema, and/or fibrosis in dolphins, and emphasizes the importance of continued studies of stranded, dead animals recovered within the oil spill footprint to help determine the pathologic processes underlying the development of severe, debilitating lung disease.

Infectious disease diagnostic results did not support either morbillivirus or *Brucella* as significant contributing factors to the prevalence of moderate to severe overall lung disease or moderate to severe AIS. morbillivirus was important to rule out since it has been previously reported to cause unusual mortality events in bottlenose dolphins (Litz et al. 2014). Only 1 BB dolphin (2011) tested positive for morbillivirus by PCR analysis of a blowhole swab sample, which is consistent with results from Fauquier et al.

(2017, this Theme Section) demonstrating sporadic cases of dolphin morbillivirus in the northern GoM between 2010 and 2014 as confirmed by PCR analysis of samples from stranded dolphins. Although *Brucella* would not be expected to cause a cluster of lung disease cases in free-ranging dolphins, the organism has been detected in dead, stranded dolphins from the GoM (Venn-Watson et al. 2015b). Therefore, PCR testing was performed to rule out an epizootic of brucellosis. Only 1 MS animal (2013) and 1 BB animal (2014) had blowhole swab samples test positive for *Brucella*, confirming that *Brucella* was not a driver for the increased prevalence of moderate to severe lung disease in BB or MS dolphins. Similarly, Venn-Watson et al. (2015b) did not identify any known or novel pathogens that could explain the increased morbidity and mortality reported in dolphins affected by the DWH disaster, further supporting that DWH oil exposure was the most likely cause of advanced lung disease.

Evidence of adrenal compromise and an impaired stress response also persisted beyond 2011. BB dolphins had a high prevalence of low cortisol with associated low aldosterone values in the face of capture and restraint, a technique previously proven to elicit a stress response in dolphins (Thomson & Geraci 1986, St. Aubin et al. 1996). Although the prevalence of animals with low cortisol decreased in 2013 and 2014 (Table 4), the difference from expected values remained statistically significant. MS dolphins had similar findings, with 19% of dolphins having low serum cortisol values. To better characterize adrenal impairment in 2013 and 2014, additional blood samples were collected when possible at least 30 min after initial sampling and following health assessment procedures (referred to as post-sampling). The majority of post-sampling cortisol values were within expected bounds; therefore, the animal's stress response in 2013–2014 was consistent with a delayed response rather than complete suppression. Unfortunately, post-sampling blood samples were not collected in 2011, so the ability of those animals to mount a delayed stress response remains unknown.

An animal's ability to secrete appropriate and timely amounts of cortisol during a stressful situation relies on a properly functioning adrenal cortex and hypothalamic-pituitary-adrenal axis (Rosol et al. 2001). In a study of dead stranded dolphins in the northern GoM following the DWH oil spill, 50% of carcasses recovered from BB had unusually thin adrenal cortices (N = 9) (Venn-Watson et al. 2015b). Based on histologic and gross pathologic examinations of these carcasses, Venn-Watson et al. (2015b) ruled out autoimmune disease, metastatic neoplasia, chronic illness, fungal infection, miliary tuberculosis, stress, and poor nutrition as likely causes of adrenal cortical injury. However, contaminant exposure could not be ruled out, and that study concluded that exposure to oil contaminants from the DWH disaster led to injury of the adrenal cortex in GoM dolphins. These data are consistent with the present findings and further support that exposure to toxic oil components caused a persistent, impaired stress response in BB and MS dolphins.

Although evidence of adrenal compromise and pulmonary abnormalities persisted, other health trends showed signs of improvement. Body condition improved with time in BB, incrementally decreasing from 25% in poor body condition in 2011 to 9% and 6% in 2013 and 2014, respectively. Poor body condition has been positively correlated with poor survival in common dolphins *Delphinus delphis* (Sharp et al. 2014); therefore, the decreasing prevalence of BB dolphins diagnosed with poor body condition could be partially attributable to the death of underweight dolphins between 2011 and 2013–2014. In fact, 2 of the 5 BB dolphins diagnosed as underweight in 2011 were either confirmed or presumed dead within a year after their evaluation (Lane et al. 2015).

Similar results were observed for Prince William Sound river otter (*Lutra canadensis*) populations assessed for injury following the 1989 *Exxon Valdez* oil spill (EVOS). Immediately and up to 2 yr post-EVOS, river otters sampled from oiled sites had a significantly lower mass:length ratio than otters sampled from non-oiled sites (Duffy et al. 1993, Bowyer et al. 2003). However, a difference in mass:length ratio was not detected for otters from oiled habitats sampled 3, 7, and 9 yr post-EVOS as compared to otters from non-oiled areas, and body mass significantly increased over time (Duffy et al. 1994, Bowyer et al. 2003).

BB dolphins also showed improvement in previously observed clinicopathologic abnormalities, including inflammation, hypoglycemia, and altered iron metabolism. In subsequent sampling years, 28% (2013) and 19% (2014) of BB dolphins still had indications of inflammation, but these proportions were not significantly greater when compared to SB dolphins. There were no hypoglycemia cases observed in BB in 2014, and the number of cases with abnormal iron and anemia reduced by nearly half. Among MS dolphins sampled in 2013, the prevalence of health panel cases was very similar to findings from BB in 2013; since sampling did not occur in 2011, we cannot determine if this was an improvement over time.

As reported by Schwacke et al. (2014), nearly half of the dolphins examined in BB 1 yr following the DWH oil spill were deemed unhealthy, as evidenced by the prevalence of guarded or worse prognosis scores (48%). In 2013 and 2014, the prevalence of unhealthy animals improved (37 and 28% respectively), but still remained elevated in comparison to the SB reference site (11%), although the difference between BB 2014 and the SB reference site was no longer statistically significant at the $\alpha = 0.05$ threshold ($p = 0.097$). Dolphins examined in MS in 2013 had a prevalence of unhealthy animals that closely paralleled BB 2013 (MS: 35% vs. BB: 37%). These data confirmed that animals living in areas affected by the DWH oil spill were more likely to be ill, and demonstrated that some improvement in prevalence has occurred over time. Long-term health monitoring is needed to determine if the BB prognosis scores will continue to improve with time, and if a similar trend will occur in MS.

It can be difficult to discern whether the improvements in population health observed over time in BB resulted from individuals recovering from health issues, the death and loss of sick animals from the population, or a combination of recovery and loss. However, dolphins initially sampled in BB 2011 and then resampled in BB 2013–2014 did provide some insight into the health of survivors. Improvement was seen in many, but not all, of the clinicopathologic, endocrine, and body condition abnormalities previously identified in resampled BB dolphins (Table 2). Of the 12 BB dolphins sampled in subsequent years and assigned a prognosis score, none of the animals with a good/fair prognosis score degraded into the guarded or worse prognosis category, and half of the animals improved with time. Additional data from previously sampled BB dolphins would be needed to determine the relevance of these findings to overall population recovery.

Although evidence of lung disease and an impaired stress response persisted in heavily oiled BB, signs of gradual improvement have emerged. From BB 2011 to 2014, incremental improvements were detected in clinicopathologic findings, body condition scores, and overall prognosis scores. Similar findings in MS, another DWH contaminated site, strengthened the supposition that exposure to toxic oil components likely caused and/or contributed to the adverse health conditions detected in the aftermath of the DWH disaster. Long-term monitoring of these impacted dolphin populations is critical for fully understanding the potential for and timeline of individual and population recovery from a large-scale oil spill event. Special emphasis should be placed on the well-studied BB population, as these dolphins will continue to provide valuable insight into the long-lasting effects of oil on marine animal and ecosystem health.

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LITERATURE CITED

Allan SE, Smith BW, Anderson KA (2012) Impact of the *Deepwater Horizon* oil spill on bioavailable polycyclic aromatic hydrocarbons in the Gulf of Mexico coastal waters. Environ Sci Technol 46:2033–2039

Balmer BC, Ylitalo GM, McGeorge LE, Baugh KL and others (2015) Persistent organic pollutants (POPs) in blubber of common bottlenose dolphins (*Tursiops truncatus*) along the northern Gulf of Mexico coast, USA. Sci Total Environ 527–528:306–312

Bowyer RT, Blundell GM, Ben-David M, Jewett SC, Dean TA, Duffy LK (2003) Effects of the *Exxon Valdez* oil spill on river otters: injury and recovery of a sentinel species. Wildl Monogr 153:1–53

Carrasco JM, Lope V, Pérez-Gómez B, Aragonés N and others (2006) Association between health information, use of protective devices and occurrence of acute health problems in the *Prestige* oil spill clean-up in Asturias and Cantabria (Spain): a cross-sectional study. BMC Public Health 6:1

DWH NRDA (*Deepwater Horizon* Natural Resource Damage Assessment) Trustees (2016) *Deepwater Horizon* oil spill: final programmatic damage assessment and restoration plan and final programmatic environmental impact statement. NOAA, Silver Spring, MD. www.gulfspillrestoration.noaa.gov/restoration-planning/gulf-plan (accessed 1 May 2016)

Dietrich DE, Bowman MJ, Korotenko KA, Bowman HE (2014) Oil spill risk management: modeling Gulf of Mexico circulation and oil dispersal. John Wiley & Sons, Hoboken, NJ and Scrivener Publishing, Salem, MA

Duffy LK, Bowyer RT, Testa JW, Faro JB (1993) Differences in blood haptoglobin and length-mass relationships in river otters (*Lutra canadensis*) from oiled and nonoiled areas of Prince William Sound, Alaska. J Wildl Dis 29: 353–359

Duffy LK, Bowyer RT, Testa JW, Faro JB (1994) Evidence of recovery of body mass and haptoglobin values of river otters following the *Exxon Valdez* oil spill. J Wildl Dis 30: 421–425

EPA (US Environmental Protection Agency) (2011) EPA should clarify and strengthen its waste management oversight role with respect to oil spills of national significance (11-P-0706). EPA, Office of Inspector General, Washington, DC. www.epa.gov/office-inspector-general/report-epa-should-clarify-and-strengthen-its-waste-management-oversight (accessed 12 Nov 2015)

ERMA (Environmental Response Management Application) (2014) Web application: Deepwater Gulf response environmental response management application. NOAA, Washington, DC. http://gomex.erma.noaa.gov/ (accessed 1 Oct 2015)

ERMA (Environmental Response Management Application) (2015) Web application: Deepwater Gulf response environmental response management application. NOAA, Washington, DC. http://gomex.erma.noaa.gov/ (accessed 2 Nov 2015)

Fauquier DA, Litz J, Sanchez S, Colegrove K and others (2017) Evaluation of morbillivirus exposure in cetaceans from the northern Gulf of Mexico 2010–2014. Endang Species Res 33:211–220

Hart LB, Wells RS, Schwacke LH (2013) Reference ranges for body condition in wild bottlenose dolphins *Tursiops truncatus.* Aquat Biol 18:63–68

Hart LB, Wells RS, Kellar N, Balmer BC and others (2015) Adrenal hormones in common bottlenose dolphins (*Tursiops truncatus*): influential factors and reference intervals. PLOS ONE 10:e0127432

Janjua NZ, Kasi PM, Nawaz H, Farooqui SZ and others (2006) Acute health effects of the *Tasman Spirit* oil spill on residents of Karachi, Pakistan. BMC Public Health 6:84

Jung SC, Kim KM, Lee KS, Roh S and others (2013) Respiratory effects of the *Hebei Spirit* oil spill on children in Taean, Korea. Allergy Asthma Immunol Res 5:365–370

Kellar NM, Speakman TR, Smith CR, Lane SM and others (2017) 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

Lane SM, Smith CR, Mitchell J, Balmer BC and others (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 JA, Baran MA, Bowen-Stevens SR, Carmichael RH and others (2014) Review of historical unusual mortality events (UMEs) in the Gulf of Mexico (1990-2009): providing context for the multi-year northern Gulf of Mexico cetacean UME declared in 2010. Dis Aquat Org 112: 161–175

Lykke AW, Stewart BW, O'Connell PJ, Le Mesurier SM (1979) Pulmonary responses to atmospheric pollutants I: an ultrastructural study of fibrosing alveolitis evoked by petrol vapour. Pathology 11:71–80

McDonald TL, Hornsby FE, Speakman TR, Zolman ES and others (2017) Survival, density, and abundance of common bottlenose dolphins in Barataria Bay (USA) following the *Deepwater Horizon* oil spill. Endang Species Res 33:193–209

Michel J, Owens EH, Zengel S, Graham A and others (2013) Extent and degree of shoreline oiling: *Deepwater Horizon* oil spill, Gulf of Mexico, USA. PLOS ONE 8:e65087

Rosol TJ, Yarrington JT, Latendresse J, Capen CC (2001) Adrenal gland: structure, function, and mechanisms of toxicity. Toxicol Pathol 29:41–48

Sandler D, Engel L, McGrath J, Payne J and others (2014) Respiratory symptoms in oil spill clean-up workers participating in the Gulf STUDY. In: Abstr 2014 Conf Int Soc Environ Epidemiol (ISEE), Seattle, WA, 24–28 Aug 2014. National Institute of Environmental Health Sciences, Research Triangle Park, NC. Environ Health Perspect: abstr 2278

Schwacke LH, Hall AJ, Townsend FI, Wells RS and others (2009) Hematologic and serum biochemical reference intervals for free-ranging common bottlenose dolphins (*Tursiops truncatus*) and variation in the distributions of clinicopathologic values related to geographic sampling site. Am J Vet Res 70:973–985

Schwacke LH, Twiner MJ, DeGuise S, Balmer BC and others (2010) Eosinophilia and biotoxin exposure in bottlenose dolphins (*Tursiops truncatus*) from a coastal area impacted by repeated mortality events. Environ Res 110: 548–555

Schwacke LH, Smith CR, Townsend FI, Wells RS and others (2014) Health of common bottlenose dolphins (*Tursiops truncatus*) in Barataria Bay, Louisiana, following the *Deepwater Horizon* oil spill. Environ Sci Technol 48: 93–103

Sharp SM, Knoll JS, Moore MJ, Moore KM and others (2014) Hematological, biochemical, and morphological parameters as prognostic indicators for stranded common dolphins (*Delphinus delphis*) from Cape Cod, Massachusetts, U.S.A. Mar Mamm Sci 30:864–887

Sierra E, Sánchez S, Saliki JT, Blas-Machado U, Arbelo M, Zucca D, Fernández A (2014) Retrospective study of etiologic agents associated with non-suppurative meningoencephalitis in stranded cetaceans: a retrospective study in the Canary Islands. J Clin Microbiol 52:2390–2397

Sim MS, Jo IJ, Song HG (2010) Acute health problems related to the operation mounted to clean the *Hebei*

Spirit oil spill in Taean, Korea. Mar Pollut Bull 60:51–57
Skyberg K, Rønneberg A, Christensen CC, Naess-Andresen CF, Borgersen A, Refsum HE (1992) Lung function and radiographic signs of pulmonary fibrosis in oil exposed workers in a cable manufacturing company: a follow up study. Br J Ind Med 49:309–315
Smargiassi A, Inchingolo R, Soldati G, Copetti R and others (2013) The role of chest ultrasonography in the management of respiratory diseases: document II. Multidiscip Respir Med 8:55
Smith CR, Solano M, Lutmerding BA, Johnson SP and others (2012) Pulmonary ultrasound findings in a bottlenose dolphin *Tursiops truncatus* population. Dis Aquat Org 101:243–255
Smith CR, Jensen ED, Blankenship BA, Greenberg M and others (2013) Fetal omphalocele in a common bottlenose dolphin (*Tursiops truncatus*). J Zoo Wildl Med 44:87–92
Speakman TR, Lane SM, Schwacke LH, Fair PA, Zolman ES (2010) Mark-recapture estimates of seasonal abundance and survivorship for bottlenose dolphins (*Tursiops truncatus*) near Charleston, South Carolina, USA. J Cetacean Res Manag 11:153–162
St. Aubin DJ, Ridgway SH, Wells RS, Rhinehart HL (1996) Dolphin thyroid and adrenal hormones: circulating levels in wild and semidomesticated *Tursiops truncatus*, and influence of sex, age, and season. Mar Mamm Sci 12: 1–13
Suárez B, Lope V, Pérez-Gómez B, Aragonés N and others (2005) Acute health problems among subjects involved in the cleanup operation following the *Prestige* oil spill in Asturias and Cantabria (Spain). Environ Res 99:413–424
Thomson CA, Geraci JR (1986) Cortisol, aldosterone, and leucocytes in the stress response of bottlenose dolphins, *Tursiops truncatus*. Can J Fish Aquat Sci 43:1010–1016
US 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. US District Court for the Eastern District of Louisiana, New Orleans, LA. www.laed.uscourts.gov/sites/default/files/OilSpill/Orders/1152015FindingsPhaseTwo.pdf (accessed 12 Nov 2015)
Venn-Watson S, Garrison LP, Litz J, Fougeres E and others (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:e0117248
Venn-Watson S, Colegrove KM, Litz J, Kinsel M and others (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:e0126538
Waring G, Josephson E, Maze-Foley K, Rosel P (2015) U.S. Atlantic and Gulf of Mexico marine mammal stock assessments — 2014. NOAA Tech Memo NMFS-NE-231. NOAA, National Marine Fisheries Service, Northeast Fisheries Science Center, Gloucester, MA
Wells RS, Scott MD (1990) Estimating bottlenose dolphin population parameters from individual identification and capture-release techniques. Rep Int Whal Comm Spec Issue 12:407–415
Wells RS, Rhinehart HL, Hansen LJ, Sweeney JC and others (2004) Bottlenose dolphins as marine ecosystem sentinels: developing a health monitoring system. EcoHealth 1:246–254
Wells R, Smith CR, Sweeney J, Townsend FI and others (2014) Fetal survival of common bottlenose dolphins (*Tursiops truncatus*) in Sarasota Bay, Florida. Aquat Mamm 40:252–259
Wu Q, McFee W, Goldstein T, Tiller RV, Schwacke L (2014) Real-time PCR assays for detection of *Brucella* spp. and the identification of genotype ST27 in bottlenose dolphins (*Tursiops truncatus*). J Microbiol Methods 100: 99–104
Zock JP, Rodríguez-Trigo G, Pozo-Rodríguez F, Barberà JA and others, for the SEPAR-Prestige Study Group (2007) Prolonged respiratory symptoms in clean-up workers of the *Prestige* oil spill. Am J Respir Crit Care Med 176: 610–616
Zock JP, Rodríguez-Trigo G, Rodríguez-Rodríguez E, Espinosa A and others (2012) Persistent respiratory symptoms in clean-up workers 5 years after the *Prestige* oil spill. Occup Environ Med 69:508–513

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