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Health of Common Bottlenose Dolphins (*Tursiops truncatus*) in Barataria Bay, Louisiana, Following the *Deepwater Horizon* Oil Spill

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Supporting Information

ABSTRACT: The oil spill resulting from the explosion of the *Deepwater Horizon* drilling platform initiated immediate concern for marine wildlife, including common bottlenose dolphins in sensitive coastal habitats. To evaluate potential sublethal effects on dolphins, health assessments were conducted in Barataria Bay, Louisiana, an area that received heavy and prolonged oiling, and in a reference site, Sarasota Bay, Florida, where oil was not observed. Dolphins were temporarily captured, received a veterinary examination, and were then released. Dolphins sampled in Barataria Bay showed evidence of hypoadrenocorticism, consistent with adrenal toxicity as previously reported for laboratory mammals exposed to oil. Barataria Bay dolphins were 5 times more likely to have moderate−severe lung disease, generally characterized by significant alveolar interstitial syndrome, lung masses, and pulmonary consolidation. Of 29 dolphins evaluated from Barataria Bay, 48% were given a guarded or worse prognosis, and 17% were considered poor or grave, indicating that they were not expected to survive. Disease conditions in Barataria Bay dolphins were significantly greater in prevalence and severity than those in Sarasota Bay dolphins, as well as those previously reported in other wild dolphin populations. Many disease conditions observed in Barataria Bay dolphins are uncommon but consistent with petroleum hydrocarbon exposure and toxicity.



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INTRODUCTION

The explosion on the *Deepwater Horizon* (DWH) drilling platform in April 2010, the collapse of the rig, and subsequent flow of oil until the well was capped in mid-July 2010 resulted in the release of an estimated 4.9 million barrels of oil into the northern Gulf of Mexico. The spill drew immediate concern for cetaceans such as sperm, Bryde's, pygmy sperm, and beaked whales, as well as multiple delphinid species that reside in pelagic and continental shelf waters near the DWH wellhead. Oil then spread to more than 1000 miles of the Gulf coast from western Louisiana to the Florida panhandle,[1] prompting concern also for coastal stocks as well as many of the 32 bay, sound, and estuary (BSE) stocks of the common bottlenose dolphin (*Tursiops truncatus*) in the northern Gulf of Mexico.[2] Dolphins, as well as other cetaceans, appear to be able to detect the presence of oil but do not necessarily avoid it.[3] In the months following the DWH spill, dolphins were observed in oiled waters, including BSE waters, at times swimming through surface oil and with oil adhering to their skin. Dolphins therefore had potential for exposure to oil through direct contact at the surface and in the water column, through incidental ingestion from water or sediments while feeding, and through ingestion of contaminated prey. In addition, dolphins breathe immediately above the air–water interface and thus can be exposed to volatile and aerosolized petroleum-associated compounds through inhalation. Resultant health effects from oil via any of these exposure routes are largely unknown for cetaceans. While cetacean mortalities have been suggested in association with prior oil spill events,[4] data to assess specific pathologies that could be linked to oil exposure have been lacking due to the difficulties associated with recovering fresh carcasses during a spill event.

In an attempt to better understand the potential sublethal and/or chronic health effects of exposure to DWH oil for cetaceans, dolphin capture–release health assessments were conducted. Capture–release studies to evaluate the health of wild dolphins have been conducted previously in a number of sites along the southeast U.S. coast.[5−7] On the basis of these prior studies, reference intervals have been established for many health measures, including hematologic and serum biochemical parameters.[8,9] In addition, patterns of abnormalities have been identified for some populations under stress.[10,11]

Here, we describe results from capture–release health assessments conducted in two Gulf of Mexico sites following the DWH oil spill: Barataria Bay, Louisiana, an area that received prolonged and heavy oiling,[12] and Sarasota Bay, Florida, where no oil was observed following the DWH spill (Figure S1). Some areas of Barataria Bay were still closed to fishing due to residual oil[13] at the time that the assessments were conducted (August 2011). The two study sites provide an opportunity to compare the health status of two populations representing extremes of DWH oil exposure for Gulf of Mexico BSE dolphins. Along with standard health assessment methods including physical examination and clinicopathologic measures which have been previously applied and reported for other dolphin populations,[5,8,9] ultrasound examinations of lungs were conducted to assess respiratory health.

MATERIALS AND METHODS

Dolphin Capture–Release. Bottlenose dolphins were temporarily captured and released on site in Sarasota Bay, Florida (SB) during May 16–20, 2011 and in Barataria Bay, Louisiana (BB) during August 3–16, 2011 as part of the *Deepwater Horizon* Natural Resource Damage Assessment (NRDA). Additional data from a prior capture–release health assessment conducted by the Chicago Zoological Society in SB, May 17–21, 2010 were also included in analyses.

Methods followed those described for prior dolphin capture–release studies.[5] Dolphins were caught by deploying a seine net around a group of 1–4 dolphins, forming a "compass." If a dolphin did not entangle itself quickly in the net, the size of the compass was reduced to limit the amount of open water available to the dolphin, thereby forcing it to eventually become entangled or enabling it to be manually restrained by handlers. Once a dolphin was in-hand, it was restrained by handlers and, if necessary, disentangled from the net.

Health Diagnostics. Samples were obtained for a suite of diagnostics (Table 1). The majority of the diagnostic assays have been conducted routinely in previous dolphin health assessment studies.[5,10,11,14] Pulmonary ultrasound was added utilizing techniques recently described by Smith et al.[15] for managed-care dolphins.

Blood for diagnostics was drawn from the ventral fluke vasculature while the dolphin was held in the water, and females of sufficient size to be reproductively mature were examined with ultrasound to determine pregnancy status. Most dolphins were brought onboard a processing vessel for weighing and morphometric measurements, physical examination, and additional diagnostic sampling; later-stage pregnant females were given abbreviated health evaluations in the water. One tooth was extracted under local anesthesia and sectioned according to established protocols to determine age.[16,17] Once processing was completed, each dolphin was released.

Blood samples were collected and sent to the Animal Health Diagnostic Center at Cornell University College of Veterinary Medicine for hematology, serum chemistry, and evaluation of endocrine function using standard methods.

Contaminant Analysis. Surgical biopsies of skin and blubber were taken for chemical analysis as previously described.[18] Blubber samples were analyzed for a suite of persistent organic pollutants (POPs) using gas chromatography/mass spectrometry[19,20] (see the SI for detailed methods). POP concentrations have been shown to significantly decline following parturition and lactation[18] and are therefore difficult to interpret in reproductively mature females. For this reason, only samples from males were submitted for POP analysis.

Data and Statistical Analysis. The relationship of mass/length for each individual was compared to 95th percentile reference intervals as established by Hart et al.,[9] and the prevalence of individuals considered significantly underweight (i.e., with mass below the lower threshold for their given length) was calculated. The established reference intervals were based on nonpregnant dolphins[9] and would be expected to underestimate the lower threshold for mass of a pregnant dolphin due to increases in body size that occur from carrying a fetus. Therefore, pregnant dolphins were not included in low body mass prevalence calculations.

Prevalence of pulmonary abnormalities including pleural effusion, alveolar-interstitial syndrome (AIS), pulmonary nodules, masses, and consolidation[21−27] (described in Table 2) were calculated. AIS, defined as an increase of fluid or cellular infiltrate in the interstitium, was further classified as normal (no AIS), mild, moderate, or severe. Lung scores were assigned to each animal based on overall lung findings and the presence/absence and severity of abnormalities. Overall lung scores were assigned, ranging from normal (no disease detected) to severe disease, by

Table 1. Primary Diagnostic Procedures Performed As Part of Dolphin Health Assessments

diagnostic	principal end points of interest	objective/potential links to oil exposure
physical examination	body condition, heart rate, respiratory rate and character, examination for skin lesions, oral assessment	part of routine health assessment
pulmonary assessment	pleural effusion, pulmonary nodules, pulmonary masses, consolidation, alveolar interstitial syndrome (AIS)	to evaluate lung health and detect disease: respiratory abnormalities include those reported in humans or other mammals exposed via ingestion, inhalation, or aspiration to petroleum-associated compounds[44−48]
hematology	differential white blood cell count (WBC), red blood cell indices	part of routine health assessment: anemia and both ↑ and ↓ WBC counts noted in other mammals following oil exposure;[34,35] ↓ platelet, ↑ hemoglobin and hematocrit reported for DWH cleanup participants[49]
serum chemistry	glucose, hepatobiliary enzymes, electrolytes, minerals	part of routine health assessment: elevated hepatobiliary enzymes noted in other mammal species and DWH cleanup participants following oil exposure[33−35,49]
adrenal hormones	cortisol, aldosterone	part of routine health assessment: decreased cortisol response to stress challenge noted in other mammal species following oral oil exposure;[36,37] adrenal hypertrophy reported following inhalation of toluene in rats[71]
thyroid hormones	total thyroxine (TT4), free thyroxine (fT4), total triiodothyronine (T3)	part of routine health assessment: thyroid effects associated with induction of cytochrome P450 enzymes, providing a potenital pathway for PAH toxicity[72]
reproductive hormones	progesterone, testosterone, estradiol	part of routine health assessment: progesterone measures along with ultrasound are used to assess reproductive status, particularly pregnancy
chemical analysis of blubber	persistent organochlorine pollutants (POPs)	part of routine health assessment: understanding prior exposure to POPs important because some classes may induce toxic effects similar to petroleum hydrocarbons

Table 2. Pulmonary Abnormalities Observed in Sarasota Bay and Barataria Bay Dolphins[a]

		number of cases		prevalence (95% CI)		
abnormality	description	Sarasota Bay (N = 15)	Barataria Bay (N = 29)	Sarasota Bay	Barataria Bay	p value
pleural effusion	excessive fluid between visceral and parietal pleura	1	3	0.07 (0.00−0.32)	0.10 (0.02−0.27)	0.58
pulmonary nodules	round to ovoid foci of nonaerated peripheral lung, typically measuring <2 cm	5	10	0.33 (0.12−0.62)	0.35 (0.18−0.54)	0.61
pulmonary masses	well-defined rounded areas of nonaerated peripheral lung, measuring >2 cm diameter	0	3	0.00 (0.00−0.22)	0.10 (0.02−0.27)	0.28
consolidation	fluid or infiltrate accumulation in the alveoli	1	6	0.07 (0.00−0.32)	0.21 (0.08−0.40)	0.23

[a] p values calculated using a one-tailed Fisher's exact test.

the experienced marine mammal veterinarian (C.R.S.) who conducted the ultrasound exam, as well as a board-certified veterinary radiologist. The prevalence of each pulmonary abnormality and prevalence of moderate−severe AIS and moderate−severe overall lung score were compared between the two sites using one-tailed Fisher's Exact Tests (package fisher.test, R Version 2.11.1, The R Foundation for Statistical Computing). Relative risk for moderate−severe lung disease was computed as the ratio of the probability occurring in the BB versus SB group.

Hematology and serum chemistry values for each dolphin were compared with age-class specific 95th percentile reference intervals previously established for wild dolphin populations.[8] Analytes were organized into panels representing pathologic processes (e.g., inflammation) or organ systems (e.g., hepatobiliary). The prevalence of cases with 95% exact binomial confidence intervals was computed for each panel and compared between BB and SB using a one-tailed Fisher's Exact Test.

Robust reference intervals have not been established for serum endocrine hormone concentrations in wild dolphins; therefore mean endocrine hormone concentrations were compared between BB and SB using standard multivariate models. A multivariate analysis of variance (MANOVA) was conducted for thyroid hormones (thyroxine, free thyroxine, triiodothyronine), and a multivariate analysis of covariance (MANCOVA) was conducted for adrenal hormones (cortisol, aldosterone).

Sex and age/length class were included as covariates for comparison of serum thyroid hormone concentrations, as these parameters have previously been reported to be influential factors.[11,28]

The capture and restraint required for sampling of a dolphin would be expected to elicit a stress response involving elevation of serum cortisol and potentially aldosterone.[29] Therefore, elapsed time, defined as the time from when the net was initially set to the time that the blood sample was collected was included as a covariate for analysis of cortisol and aldosterone. An interaction term for site*elapsed time was also included.

Two experienced marine mammal veterinarians (F.I.T., C.R.S.) reviewed findings from physical examination, ultrasound, hematology, and serum chemistry to determine an overall prognosis for each dolphin. The prognosis categories were good (favorable outcome expected), fair (favorable outcome possible), guarded (outcome uncertain), poor (unfavorable outcome expected), and grave (death considered imminent).

Concentrations of POPs in blubber were compared between the two sites using a MANCOVA. POP concentrations in males have been shown to increase with age.[18] Age was not available for 2 of the sampled dolphins; therefore length as an indicator of age was included as a covariate in the model.

RESULTS

Animals. Thirty-two dolphins (20 ♀, 12 ♂) from BB were temporarily captured and given health evaluations during August, 2011. Twenty-seven dolphins (14 ♀, 13 ♂) were captured and evaluated from SB in May 2010 (N = 12) and May 2011 (N = 15) (Figure S1). One female dolphin captured in May 2011 in SB had also been sampled in May 2010; only the 2011 sample was included for statistical analyses. Dolphins were categorized into age classes as described by Schwacke et al.[10] There was not a significant difference at the α = 0.05 critical threshold for sex ratio

(Fisher Exact Test, $p = 0.135$) or age-class distribution (Fisher Exact Test, $p = 0.200$) of dolphins sampled between the two sites (Table S1).

Physical Examination. A high proportion of BB dolphins was determined to be in poor body condition based on mass/length relationship as compared to established reference intervals[9] (Figure 1). Five of 20 dolphins (0.25, 95% CI = 0.09−0.49) in BB were classified as significantly underweight, as compared to 1 of 24 SB dolphins (0.04, 95% CI = 0.00−0.21).

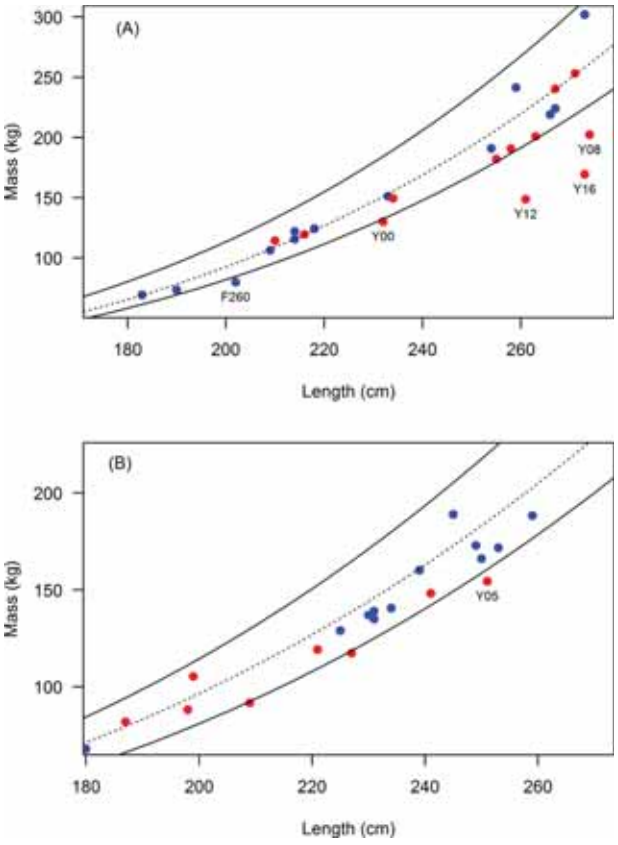


Figure 1. Total mass versus total length for (A) male and (B) female dolphins in Sarasota Bay, FL (blue circles) and Barataria Bay, LA (red circles). Lines represent 95th percentile body condition reference ranges.[9] Labeled points are freeze-brand identifiers for individuals with a mass−length relationship below the 2.5th percentile.

Ten and two dolphins sampled in BB and SB, respectively, were pregnant (fetus detected via ultrasound); an additional dolphin in BB was considered a probable early pregnancy based on the presence of a corpus luteum, and elevated serum progesterone. One of the BB pregnancies was determined to be nonviable. Ultrasound examination of that fetus detected no heart beat or movement; estimated gestational age was 5 months based on the skull biparietal diameter of 4.5 cm.

Three BB dolphins presented with complete or near complete tooth loss. Two females, 42 and 16 years of age, had only 8 and 2 remaining teeth, respectively, and one adult male (undetermined age, 258 cm length) had no teeth (Figure S2). Bottlenose dolphins normally have between 76 and 108 teeth.[30] Three additional dolphins (ages 16, 22, and 21 years) presented with incomplete but extensive tooth loss (more than half of teeth missing). All six of the dolphins presenting with extensive tooth loss also presented with mild−moderate gingival hyperplasia. Extensive tooth loss was not observed in any SB dolphins.

Pulmonary Assessment. Prevalence of pleural effusion and pulmonary nodules were very similar between BB and SB dolphins (Table 2). While pulmonary masses were only found in BB, and BB dolphins had a higher prevalence of consolidation, differences were not statistically significant at the $\alpha = 0.05$ critical threshold for either of these end points (Table 2). The severity and demographics of AIS differed between the sites (Figure 2A).

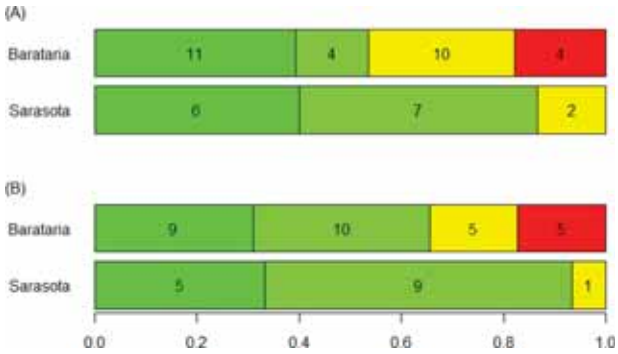


Figure 2. Proportion of dolphins categorized as having normal (green), mild (yellow-green), moderate (yellow), and severe (red) (A) alveoloar interstitial syndrome and (B) overall lung disease. Numbers inside bars represent number of cases in each category.

The prevalence of moderate−severe AIS was greater for BB dolphins ($p = 0.023$). Most (77%) of the SB dolphins with AIS were juvenile/subadult, while only 39% of BB dolphins with AIS were juvenile/subadult. Similarly, all SB dolphins with pulmonary nodules were juvenile/subadult as compared to 40% of BB dolphins.

Considering all pulmonary abnormalities for each individual to determine an overall classification of lung disease, BB dolphins presented with a higher prevalence of moderate−severe lung disease (Figure 2B) and were 5 times more likely to have moderate−severe lung disease as compared to SB dolphins (34% vs 7%, $p = 0.044$). Representative ultrasound images are presented in the SI (Figure S3).

Clinical Pathology. Multiple clinicopathologic abnormalities were identified in BB dolphins (Table 3, Table S2) with the most prevalent abnormalities involving markers of inflammation (41%), hypoglycemia (22%), altered iron metabolism (22%), and hepatobiliary disease (19%). In contrast, the prevalence for any indicator of inflammation in SB dolphins was 8%, and none of the SB dolphins presented with hypoglycemia or abnormalities in iron or hepatobiliary panels (Table 3).

No significant renal panel abnormalities were present in either dolphin population, and only one BB dolphin showed multiple electrolyte/mineral abnormalities. High potassium was noted in four BB dolphins (Table S2), but none of these dolphins had sodium or chloride concentration abnormalities. Low sodium was noted in three BB dolphins. Additional details for panel abnormalities are provided in the Supporting Information.

Endocrine Assessment. Dolphins sampled from BB exhibited low serum concentrations of adrenal hormones when compared to dolphins sampled from SB (Figure 3). Serum cortisol was significantly lower for BB dolphins ($p < 0.001$) even when considering elapsed time to sample collection that mildly influenced cortisol ($p = 0.024$). When considered separately,

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Table 3. Hematological and Serum Biochemical Parameter Panels and Prevalence of Abnormalities in Sarasota Bay and Barataria Bay Dolphins[a]

		number of cases		prevalence (95% CI)		
panel	criteria	Sarasota Bay (N = 26)	Barataria Bay (N = 32)	Sarasota Bay	Barataria Bay	p value
inflammation	elevation of one or more: neutrophils, lymphocytes, eosinophils, monocytes, basophils; and/or increased serum globulin or decreased albumin	2	13	0.08 (0.01−0.25)	0.41 (0.24−0.59)	0.004[b]
hypoglycemia	glucose below lower reference limit	0	7	0.00 (0.00−0.13)	0.22 (0.09−0.40)	0.011[b]
iron panel	elevation of 2 or more: serum iron, total iron binding capacity, % saturation of transferrin	0	7	0.00 (0.00−0.13)	0.22 (0.09−0.40)	0.011[b]
hepatobiliary	abnormal value for 2 or more liver enzymes (ALT, AST, GGT, or LDH)	0	6	0.00 (0.00−0.13)	0.19 (0.07−0.36)	0.022[b]
anemia	hemoglobin below lower reference limit	0	4	0.00 (0.00−0.13)	0.13 (0.04−0.29)	0.085
electrolytes and minerals	abnormal value for 2 or more: potassium, sodium, chloride, calcium, phosphate or magnesium	0	1	0.00 (0.00−0.13)	0.03 (0.00−0.16)	0.552
renal function	elevation of both blood urea nitrogen and creatinine	0	0	0.00 (0.00−0.13)	0.0 (0.00−0.11)	1.000

[a] p values were calculated using a one-tailed Fisher's exact test. [b] Indicates significant p values at the α = 0.05 threshold.

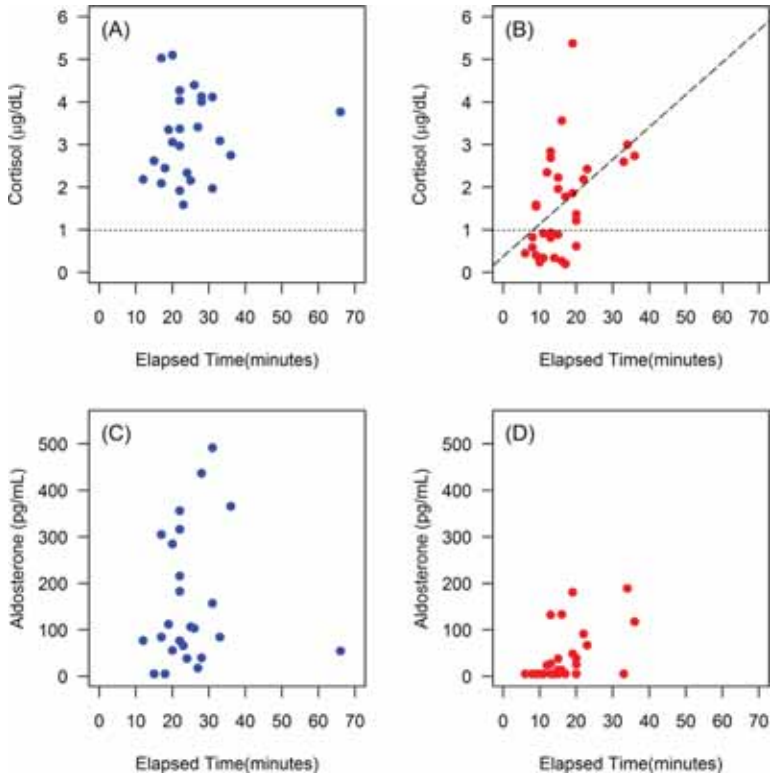


Figure 3. Serum cortisol concentrations versus elapsed time to sampling for (A) Sarasota (N = 26) and (B) Barataria (N = 32) dolphins and serum aldosterone concentrations versus elapsed time for (C) Sarasota (N = 26) and (D) Barataria (N = 32) dolphins. The dotted line represents minimum cortisol measured in dolphins sampled during prior studies in St. Joseph Bay, FL and Beaufort, NC. The dashed line in B is a regression line for elapsed time versus cortisol (p = 0.006, r^2 = 0.222).

elapsed time was found to significantly affect cortisol for BB dolphins (p = 0.006), but not for SB dolphins (p = 0.517).

Aside from comparing sample means, cortisol measures were compared to minimum values from dolphins sampled from prior studies in St. Joseph Bay, Florida (n = 30, Table S3) and Beaufort, North Carolina[31] (Figure 3A,B). Forty-four percent of BB dolphins had cortisol measures below the minimum values previously measured for St. Joseph Bay and Beaufort dolphins (0.99 μg/dL and 1.0 μg/dL, respectively); none of the SB cortisol values fell below these minima.

Aldosterone concentrations also differed between BB and SB (p < 0.001). Seventeen (53%) dolphins sampled in BB exhibited aldosterone concentrations below the assay detection limit (DL), whereas only two dolphins (8%) sampled from SB showed a value below the DL (Figure 3C,D).

There were no significant differences in serum thyroid hormone concentrations between BB and SB (p = 0.586); only age class was determined to be a significant factor for total thyroxine (p < 0.001), free thyroxine (p < 0.001), and triiodothyronine (p = 0.040).

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Overall Prognosis. On the basis of a review of clinical findings, 14 of 29 (48%) dolphins evaluated from BB were given a guarded or worse prognosis. The proportion of adults versus juveniles/subadults given a guarded or worse prognosis did not differ (Table S4; $p = 0.715$) and neither did the proportion of males versus females ($p = 0.450$). In contrast to BB findings, 1 out of 15 dolphins (7%) sampled in 2011 from SB was given a guarded prognosis and all others were given good or fair prognoses. Ultrasound examinations were not completed for three of the BB dolphins and were not conducted at all for SB dolphins sampled in 2010. This limited the data available for evaluation; therefore prognoses were not assigned for these animals.

Chemical Analysis. Polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane related compounds (DDTs), chlordane compounds, and polybrominated diphenyl ethers (PBDE) were the POP classes found in greatest concentration in blubber samples. Concentrations for all four of these contaminant classes were greater in SB dolphins as compared to BB dolphins (Figure 4), although only the differences in DDT

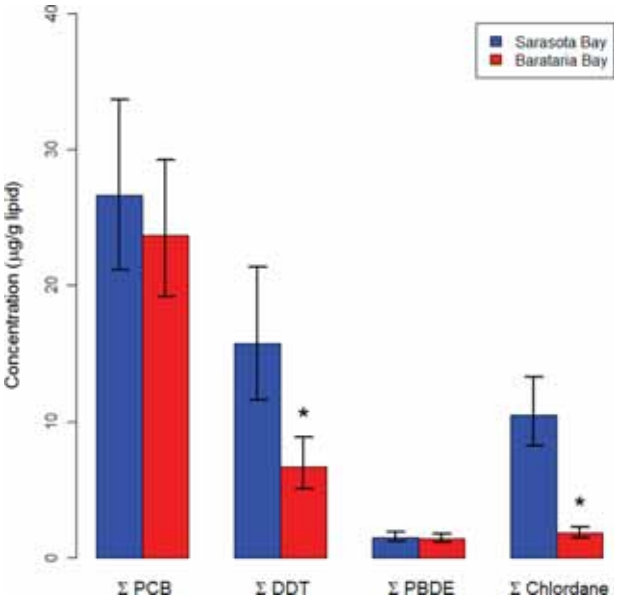


Figure 4. Concentration of POPs in blubber of male bottlenose dolphins from Sarasota Bay (blue) and Barataria Bay (red). Bars represent geometric mean concentration computed at the mean dolphin body length (239.4 cm) and whiskers represent 95% confidence intervals around the mean. Asterisks represent significant differences between the two sites with $\alpha = 0.05$.

and chlordane compounds were statistically significant at the $\alpha = 0.05$ threshold ($p = 0.001$ and $p < 0.001$, respectively). Dieldrin, mirex, and hexochlorobenzene (HCB) were found in the next highest concentrations (Table S5) and were also significantly higher in SB as compared to BB ($p = 0.004$, $p < 0.001$, $p = 0.022$, respectively). Concentrations of hexachlorocyclohexanes (HCHs), aldrin, and endosulfan were found at the lowest concentrations. Only HCHs were higher in BB as compared to SB ($p = 0.017$).

DISCUSSION

BB dolphins demonstrated a number of relatively uncommon disease conditions consistent with effects noted previously in marine wildlife following oil spills[32,33] and in experimental studies of petroleum hydrocarbon toxicity in mammals.[34−37] We present a conceptual model (Figure 5) to illustrate the potential connections among the observed conditions.

Most notably, BB dolphins had abnormally low measures of adrenal hormones consistent with hypoadrenocorticism. In domestic animals and humans, clinical diagnosis of hypoadrenocorticism often relies on the adrenocorticotropic hormone (ACTH) stimulation test, which involves administration of injectable ACTH, followed by timed measurement of serum cortisol levels to assess adrenal response.[38,39] ACTH is a pituitary hormone that stimulates the adrenal glands to release cortisol, the primary glucocorticoid for dolphins. While it was not feasible to perform the ACTH stimulation test in wild dolphins, the capture process, including a brief chase, encirclement with a net, and restraint by a team of human handlers, itself would be expected to cause adrenostimulation. In fact, an earlier study using captive dolphins compared the increase in serum cortisol concentration following injection of synthetic ACTH versus a simulated chase−capture where the dolphins were repeatedly chased and corralled and determined that injection of synthetic ACTH did not increase cortisol beyond that naturally stimulated by handling.[29] Yet in nearly half of the dolphins sampled in BB, serum cortisol was below the minimum value previously measured from dolphins sampled using the same capture method[10,31] (Figure 3B). A majority of the dolphins with low serum cortisol also exhibited low to nondetectable concentrations of aldosterone, a mineralcorticoid also produced by the adrenal gland. Cortisol and aldosterone concentrations were correlated (Spearman's rank-order correlation $r_s = 0.73$, $p < 0.001$), and 79% of BB dolphins with abnormally low cortisol (below 0.99 μg/dL) also demonstrated aldosterone measures below DL. The reduced concentrations of both of these hormones produced in the adrenal cortex, and the consistency of the reduced concentrations within individuals, suggests compromised adrenal response.

Other observed serum chemistry abnormalities in BB dolphins, including hypoglycemia and hyperkalemia, provide supporting evidence for underlying hypoadrenocorticism. Cortisol has a role in gluconeogenesis and hypoglycemia is a characteristic feature of hypoadrenocortism.[40] We examined the relationship between serum glucose and cortisol concentrations in the dolphins and found a significant association, and 5 of 7 hypoglycemic cases from BB had cortisol measures below the minimum concentration measured in SB dolphins (Figure S4).

Aldosterone plays an important role in regulating the retention of sodium and excretion of potassium, and hyperkalemia and hyponatremia occurring together or independently are also classic clinicopathologic findings associated with hypoadrenocorticism in dogs and people.[38,39] These abnormalities were present in some BB dolphins (13% and 9%, respectively) but not in any SB dolphins. The abnormalities occurred in BB dolphins that also had relatively low concentrations of aldosterone; 3 of the 4 hyperkalemia cases had aldosterone concentrations below the limit of detection (Figure S5).

Similar adrenal profiles were seen in mink fed either bunker C or artificially weathered fuel oil as part of an experimental study.[37] Mink exposed to the oil showed lower serum cortisol response following injection of ACTH, and adrenal hypertrophy was documented on necropsy. Mohr et al.[36,37] suggest that the observed adrenal effects could be due to inhibition of steroidogenesis by components in the fuel oil, which is consistent

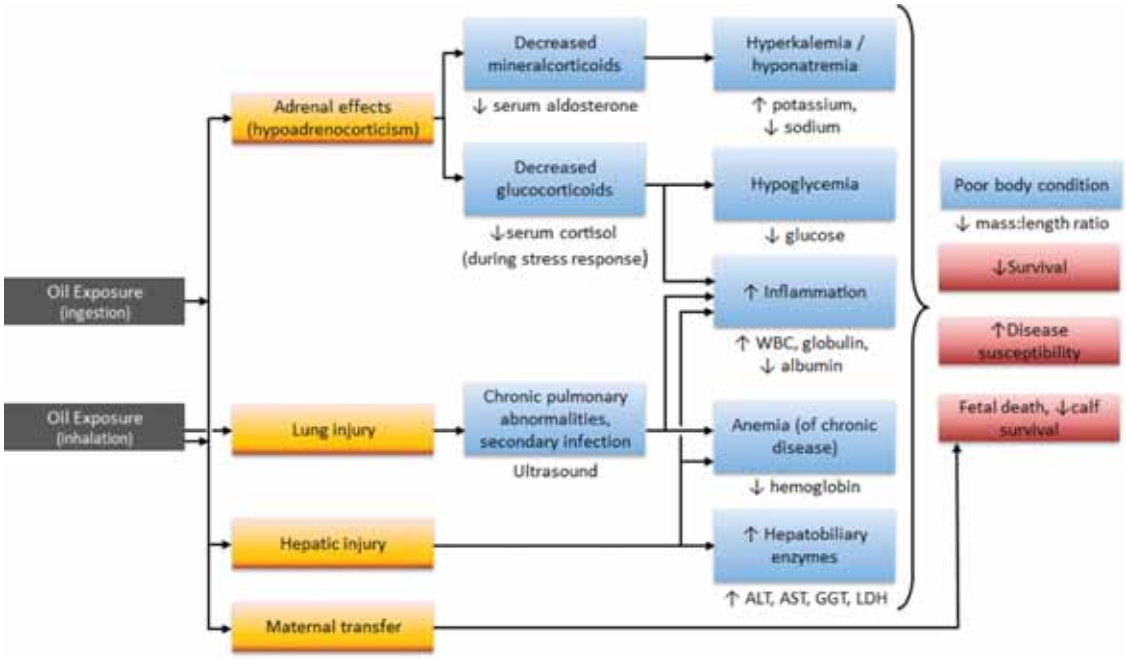


Figure 5. Conceptual model for health effects previously reported for mammals in association with oil exposure (yellow boxes) that could lead to the abnormalities observed in Barataria Bay dolphins (blue boxes), and ultimately to end points such as decreased survival and fecundity. Text below boxes represents indicators that were measured in this study, and up/down arrows indicate the direction of the change in those indicators that would be consistent with abnormalities observed.

with the general toxic pathway for chemical inhibition of adrenal response described by Harvey and Sutcliffe.[41]

The long-term outcome of dolphins with hypoadrenocortism, particularly wild dolphins which cannot be treated, is uncertain. Although rare, hypoadrenocortism has been well-studied in some species such as domestic canines and humans,[38,39,42] but we were unable to find published reports of hypoadrenocortism in dolphins. If left untreated in humans and companion animals, hypoadrenocorticism can be life-threatening, particularly during episodes of illness, stress, or pregnancy. Clearly if the dolphins' HPA axis is compromised and they are unable to mount a sufficient stress response, this would tax critical physiological processes such as metabolism and cardiovascular function and increase the risk of adrenal crisis or other potentially fatal complications.

BB dolphins were 5.0 times more likely to have moderate−severe lung scores as compared to SB dolphins. AIS was the most common pulmonary finding in both dolphin populations, but the prevalence of moderate−severe AIS was much higher in BB (48%) as compared to SB (13%). In the SB dolphins with AIS, most had mild AIS and were juveniles/subadults. Pulmonary nodules were also relatively common, with 33% of SB dolphins detected to have small lung nodules, and all of these animals were juveniles/subadults. These findings are consistent with low-grade lungworm infection and mild verminous pneumonia, which would not be unusual in this age class.[43] In contrast, only 39% of dolphins with AIS in BB were juvenile/subadult, and the majority of AIS was categorized as moderate−severe. These findings are not consistent with mild lungworm infections, and based on additional findings of pulmonary masses and consolidation, other causes of pneumonia were considered more likely in the BB dolphins, including bacterial, fungal, and viral infections, which could be primary or secondary to underlying lung injury.

The lung disease observed in BB dolphins is consistent with laboratory studies and clinical reports of humans and animals exposed via ingestion, inhalation, or aspiration to petroleum hydrocarbons.[44−48] Pneumonitis, airway inflammation, and delayed pulmonary edema are associated with inhalation exposure, and even at low occupational exposure levels, petroleum-derived hydrocarbons have been linked to decreased pulmonary function, chronic bronchitis, and airway inflammation.[45,46] Furthermore, ingestion of petroleum hydrocarbons in humans and other animals has led to aspiration pneumonia.[47,48] Therefore, the moderate−severe lung disease findings observed in BB dolphins are consistent with exposure to oil resulting in either primary lung injury and secondary pneumonia or primary aspiration pneumonia. Alternatively, primary pneumonia from a common infectious agent should also be considered. Although we consider this unlikely, the investigation of pathologies in stranded dolphins from the BB area could help to determine if a common infectious agent is associated with the high prevalence of lung disease.

The higher prevalence of serum hepatobiliary abnormalities in the BB population raises the possibility of oil spill-related hepatotoxicity or altered enzyme production, or both. Increased activities of hepatocellular transaminases were reported in sea otters following the EVOS and were also a consistent finding in the experimental oil exposure studies that followed.[33−35] In addition, higher levels were noted in human subjects that participated in the DWH oil spill cleanup as compared to a geographically similar but presumably unexposed cohort.[49] Hepatic enzyme induction is often observed following xenobiotic exposure and could be associated with other chemicals such as POPs. POPs and polycyclic aromatic hydrocarbons (PAHs), which are considered the most toxic constituents of oil,[50] can mediate toxic effects similarly through the aryl hydrocarbon receptor (AhR).[51] Liver transaminase abnormalities in association

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with high concentrations of PCBs were reported for dolphins near Brunswick, Georgia (USA).[11] In addition, environmental exposure to DDT, a legacy organochorine pesticide that has been banned in the U.S. for decades but is persistent in the environment, should be considered as an alternative causal factor for both hepatotoxicity and hypoadrenocorticism. A DDT derivative, *o,p′*-DDD (mitotane), destroys adrenal cortices and in fact has been used as a treatment for *hyper*adrenocorticism[38] and has also been associated with hepatotoxicity.[52]

However, concentrations were actually lower in BB dolphins as compared to SB dolphins for the broad range of contaminants tested (Figure 4, Table S5), including Σ DDT, Σ chlordane, dieldrin, mirex, and HCB. Only Σ HCH was found to be higher in BB dolphins, and although the difference was statistically significant, the measured concentrations were extremely low, 3−4 orders of magnitude less than the Σ PCB, Σ DDT, or even newer compounds such as Σ PBDE. The POPs concentrations measured in BB dolphins were also low relative to dolphins from other locations of the U.S. coast. For example, Σ DDT in male dolphins sampled from 14 locations in the western North Atlantic and northern Gulf of Mexico ranged from 8.03 to 51.0 μg/g lipid,[53] while Σ DDT measured in the BB dolphins was only 6.70 μg/g lipid. Similarly, Σ chlordane in BB dolphins was lower than reported for all of the other sites, and Σ PBDE in BB dolphins was lower than 12 of the 14 sites. Therefore, an association between the observed hepatic abnormalities or the compromised adrenal function in BB dolphins with background POPs seems highly unlikely.

High serum iron concentration and concurrent high transferrin saturation was noted in seven BB dolphins, suggesting potential iron overload. We are unaware of any direct association between oil exposure and iron overload. However, the observed iron levels could be a characteristic of the population and of genetic origin. Hemochromatosis has been reported in captive dolphins which originated from a wild dolphin stock in the same geographic region,[54] and in fact, when compared with reference intervals derived from that captive population,[55] the iron levels observed in the BB dolphins are well within the normal range. Given the potential for genetic influence, the clinical relevance of the observed elevated iron measures is unknown.

The excessive tooth loss in some BB dolphins was surprising and to our knowledge has not been reported in other dolphin populations. It is not uncommon for older dolphins to experience tooth wear and moderate tooth loss as part of the aging process[30,56] However, the extensive loss of teeth in their entirety and often without evidence of wear in remaining teeth in both young and old dolphins was unexpected. Tooth loss, both with and without observable periodontitis, has previously been reported in beluga whales (*Delphinapterus leucus*) exposed to a number of toxic compounds including PAHs, metals, and POPs in the St. Lawrence estuary, Quebec, Canada.[57] Similarly, alveolar bone depletion and associated tooth loss was reported in pinnipeds from heavily contaminated waters of the Baltic, and the bone lesions were observed in young animals as well as older animals.[58] A number of experimental studies have shown that some PAHs can induce tumors as well as increased cell proliferation, hyperplasia, and inflammation in the oral cavity,[59−61] which could lead to tooth loss. In addition, experimental studies of mink exposed to contaminants which act through the AhR have found alveolar bone loss and proliferation of squamous cells in the alveolar cavity, leading to extreme tooth loss and jaw lesions.[62,63] However, without additional diagnostics and an understanding of the period of time over which the tooth loss occurred, it is impossible to determine etiology. We cannot rule out infectious, behavioral, or nutritional factors as potential causes of the gingival hyperplasia and extensive tooth loss observed in BB dolphins.

BB dolphins were generally in poor body condition, and 25% were classified as being underweight. Two of the underweight dolphins (Y05, Y12) had multiple disease issues, including severe lung disease, and were given a grave prognosis; therefore, the low mass/length ratio is consistent with their overall condition. For the other three underweight animals (Y00, Y08, Y16), the poor condition could relate to adrenal issues, as all three of these dolphins had very low cortisol measures and weight loss can be associated with hypoadrenocortism.[38]

A nonviable fetus was detected in a female dolphin in BB and gestational age of the fetus was estimated at 5 months. Dolphin midgestational abortion has not been documented in the literature. To our knowledge, the only dolphin abortions currently reported in the literature are late-term abortions due to *Brucella* infection.[64] One author (C.R.S.) has detected early embryonic loss and early fetal loss with ultrasound in managed dolphins, but losses during the second trimester of pregnancy are either underdiagnosed or uncommon. The mother in BB was determined to have moderate lung disease and a poor prognosis overall, and this could have contributed to her inability to sustain a pregnancy.

While many of the health effects observed in BB dolphins are suggestive of a toxic insult and consistent with effects associated with petroleum hydrocarbon exposure, we do not have prespill health data for BB dolphins and cannot dismiss the possibility that other pre-existing environmental stressors made this population particularly vulnerable to effects from the oil spill. While there have been some smaller oil spills along the Louisiana coast[65] that could potentially have provided a chronic low-level exposure to PAHs or other petroleum-associated contaminants, a study that used passive sampling devices to monitor bioavailable PAHs before and after DWH oil reached the shoreline reported that preoiling concentrations of 33 measured PAHs in surface waters adjacent to Grand Isle, LA (3.8 ng/L) were lower than concentrations at other sites in Mississippi (7.3 ng/L), Alabama (9.1 ng/L), and Florida (3.9 ng/L).[66] These measurements of bioavailable PAHs at a single preoiling time point do not preclude the possibility that prior events might have led to transient increases in PAHs or other petroleum-associated compounds in BB, but such increases would surely be much lower than the 45-fold increase in bioavailable concentration of PAHs reported after the DWH spill for Grand Isle (maximum concentration reached 170 ng/L).[66] Nevertheless, we cannot rule out the possibility that other environmental stressors exist in BB, and this suggests a need to continue monitoring of BB dolphins over time to determine if the health of this population will improve.

Forty-eight percent of the dolphins sampled from BB were given a guarded or worse prognosis, and 17% were graded as poor or grave, indicating that they were not expected to survive. Some level of disease is expected for any wild population. Populations are exposed to a plethora of environmental pathogens and temporal fluctuation in available prey resources can lead to variation both within and among populations with regard to health state.[5,14,67,68] However, the severity of disease, poor body condition, and high prevalence of abnormalities seen in BB dolphins is in stark contrast with the overall health status of dolphins from the SB reference site, as well as with health

conditions previously documented in bottlenose dolphins from other U.S. coastal sites (e.g., refs 5, 6, 10).

Given these findings, it would be surprising not to see a concurrent elevation in dolphin mortality for BB. In fact, dolphin mortalities in the BB area have been elevated and are considered part of an ongoing Unusual Mortality Event (UME) declared by NOAA Fisheries[69] that covers the broader northern Gulf of Mexico region. The UME period was declared to have begun February, 2010 two months prior to the DWH oil spill, and since that time the frequency of strandings has fluctuated both spatially and temporally. The timing and underlying pathologies for the strandings are being examined as part of the UME investigation to understand the potential differing causal factors, including the DWH oil spill, which may be contributing to the variable intensity and pattern of mortalities. The UME is ongoing and as of November 3, 2013 over 1051 cetacean strandings have been reported.[70]

The severe disease documented by this study and the continued elevation of mortalities raise significant concerns regarding both short-term and long-term impacts on the Barataria Bay dolphin population. Continued photographic monitoring studies, also being conducted as part of the *Deepwater Horizon* NRDA, will help to elucidate potential impacts on dolphin reproduction and long-term survival.

■ ASSOCIATED CONTENT

Supporting Information

Additional details for methods and results, supplemental figures, and summary tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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