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

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Details for Materials and Methods – Field Methods

Dolphin Capture-Release

Females ≥ 220 cm were considered sufficient size to potentially be reproductively mature. Therefore, in Barataria Bay (BB) female dolphins ≥ 220 cm were held in the water until an ultrasound examination was conducted to assess pregnancy status. In Sarasota Bay (SB), known mature females (known from studies initiated in 1970) and females of unknown maturity status were held in the water until an ultrasound examination was conducted to assess pregnancy status. Dolphins determined to be pregnant were evaluated by the veterinary team to determine whether the animal could be taken onboard the processing boat. The decision as to whether or not to bring the dolphin onboard was based on stage of pregnancy, a brief physical examination, and assessment of behavioral cues indicative of stress. Some pregnant females were examined in-water and sampled as deemed appropriate by the lead veterinarian. Non-pregnant females and males were brought aboard a processing vessel for physical examination, ultrasound evaluation to assess lung condition and screen for organ abnormalities, body weight and morphometrics, diagnostic sampling, freeze-brand marking, VHF and/or satellite-linked tag attachment (BB only) and photographic documentation. The condition of a captured dolphin was continuously monitored for signs of distress (1, 2); efforts were made to minimize the amount of time each sampled dolphin spent out-of-water. If the lead veterinarian felt that a dolphin was becoming overly stressed, as evidenced by abnormal respirations, arching, or abnormal heart rate, the dolphin was returned to the water and held until the veterinarian felt that it was safe to continue processing or required immediate release. For this reason, some samples (*e.g.*, tooth) were not collected from every dolphin.

Sample Processing

Blood samples were collected for hematology, serum chemistry and protein electrophoresis, and evaluation of endocrine function using standard methods as previously described (3-5). For hematology, blood tubes were gently rocked to ensure mixing with the anti-coagulant and to prevent clot formation, followed by cooling. Blood smears were made for microscopic examination to determine differential leukocyte counts. For serum chemistry and hormone analysis, samples were allowed to sit approximately 30 minutes to allow for clot formation. Samples were then centrifuged onboard the processing vessel, transferred to vials and kept cool. At the end of each day whole blood for CBC and serum for biochemistry were wrapped and shipped on ice packs for overnight delivery to Animal Health

Diagnostic Center (AHDC) at Cornell University College of Veterinary Medicine. Serum samples for hormone analysis were frozen at -80ºC and shipped to AHDC at the end of the field period.

Additional samples of blood, urine, feces, gastric fluid, milk, and blowhole swabs were collected for analyses to include functional immune assays, biotoxin analyses, chemical contaminant analysis and/or serology that are beyond the scope of this paper and will be described elsewhere.

Diagnostic Ultrasound

Equipment

A portable GE Voluson i (GE Healthcare, Waukesha, WI, USA) ultrasound unit with a 2 to 5 MHz 3D/4D or curvilinear transducer was utilized to perform dolphin ultrasound exams. The ultrasound unit was fitted with a personal video heads-up display (Z800, eMagin, Bellevue, WA, USA) to improve the ability of the sonographer to see the ultrasound image in bright sunlight.

Technique

Ultrasound exams were performed with either the animal resting on a foam pad in the processing vessel or with the animal lightly restrained in the water. Onboard exams were typically performed with the animals in right lateral recumbency. Occasionally, exams were performed in sternal recumbency if other procedures or the animal's condition required them to be in this position.

All ultrasound exams were performed in real-time B-mode. Image settings were optimized for each animal and organ evaluated. Tissue harmonic imaging and speckle reduction imaging (low) were utilized. When appropriate, color Doppler was used to study blood flow patterns. Due to the time constraints of working with animals in the field, the left side of the animal was consistently scanned, with a special focus on pulmonary and reproductive health evaluation. The left lung, left ovary and both uterine horns in females, and left lung and left testicle in males were carefully evaluated. Occasionally, the right lung or right ovary was evaluated if time allowed. In cases where a fetus was detected, sonographic evaluation of the fetus was also performed.

Reproductive ultrasound

Female reproductive tracts were examined and evaluations were consistent with those previously described (6, 7). Any evidence of pregnancy, including the presence of a corpus luteum or uterine fluid, warranted close inspection of the horns for an embryonic vesicle or fetus. If a fetus was detected, the biparietal skull diameter was measured and the fetus staged according to size. The fetus was further evaluated for viability based on detection of a heartbeat, fetal movement, and basic organ definition.

Due to time constraints, M mode was not utilized to measure fetal heart rate. In one case, color Doppler was used to interrogate the fetal and umbilical cord vessels to document presence/absence of blood flow.

Image acquisition and interpretation

All ultrasound examinations were performed by an experienced marine mammal veterinarian. For each animal, representative still images were captured of all organs examined and occasionally cine loops recorded as time allowed. Data sheets were completed by the veterinarian immediately following the exam while on the boat, representing the veterinarian's most significant findings. Upon return to land, the images were reviewed in a dark room to facilitate more thorough evaluation of the sonograms and measurement of significant structures when appropriate. Additional review was performed by the veterinarian with a board-certified marine mammal veterinary radiologist. During this review, final ultrasound interpretations were made and abnormalities recorded, as well as overall lung scores confirmed ranging from normal (no disease detected) to severe disease.

Details for Materials and Methods - Laboratory Analyses

Endocrine Analyses

Serum concentrations of aldosterone, total thyroxine (TT4), and total triiodothyronine (TT3) were measured by solid-phase ^{125}I radioimmunoassays (RIA) using commercially available kits (Siemens Healthcare Diagnostic Inc., Los Angeles, CA). Serum cortisol concentration was measured by an automated chemiluminescent enzyme immunoassay (Immulite, Siemens Healthcare Diagnostic Inc., Los Angeles, CA). Serum free thyroxine (FT4) concentration was measured by RIA following equilibrium dialysis to separate bound from free T4 (Antech Diagnostics, Irvine, CA). For each of the hormones measured, pooled samples were used to validate each assay. Three or more samples contributed to each of the pools used for the validations. Each validation included an assessment of the intra-assay coefficient of variation (CV), whereby a dolphin pool was analyzed 10 times in a single assay run. The intra-assay CVs for all hormones were less than 10%. Each sample pool was also serially diluted by four progressive dilutions and spiked with four known concentrations of the relevant hormone. For most of the hormones analyzed, parallelism was demonstrated by the serial dilution of a pooled dolphin sample. However, the concentration of the pooled dolphin samples for cortisol was relatively low (3.8 µg/dL) and serial dilution resulted in concentrations that were below the lower limit of the standard curve. Therefore, for validation of the cortisol assay, spike/recovery evaluation was used to demonstrate

parallelism. In general, all dolphin samples were run in a single assay for each hormone. One aldosterone and two cortisol samples were analyzed in a follow-up assay, because the CV's of the duplicates for those samples were greater than 25% within the original assay. Every assay met all quality assurance criteria, and internal controls (canine, equine, feline, bovine) were run in each assay.

Contaminant Analyses

Blubber samples of bottlenose dolphins were extracted and analyzed for persistent organic pollutants (POPs) using the gas chromatography/mass spectrometry (GC/MS) method of Sloan *et al*.(8). This method involves: (1) extraction of tissues using dichloromethane in an accelerated solvent extraction procedure, (2) clean-up of the dichloromethane sample extract on a single stacked silica gel/alumina column, (3) separation of POPs from lipid or other biogenic material by high-performance size exclusion liquid chromatography, and (4) analysis on a low-resolution quadrupole GC/MS system equipped with a 60-meter DB-5 GC capillary column and an electron impact mass spectrometer in selected ion monitoring mode. The instrument was calibrated using sets of up to ten multi-level calibration standards of known concentrations. Percent lipid was determined gravimetrically as described in (8).

Sum PCBs is the sum of congeners 17, 18, 28, 31, 33, 44, 49, 52, 66, 70, 74, 82, 87, 95, 99, 101/90, 105, 110, 118, 128, 138/163/164, 149, 151, 153/132, 156, 158, 170, 171, 177, 180, 183, 187/159/182, 191, 194, 195, 196, 199, 200, 201, 202, 205, 206, 207, 208, 209. Sum DDTs is the sum of *o,p*'-DDD, *p,p'*-DDD, *o,p*'-DDE, *p,p*'-DDE, *o,p*'-DDT and *p,p*'-DDT. Sum chlordanes is the sum of oxychlordane, *gamma*-chlordane, nona-III-chlordane, heptachlor, heptachlor epoxide, *alpha*-chlordane, *trans*-nonachlor and *cis*-nonachlor. Sum hexachlorocyclohexanes (HCHs) is the sum of *alpha*-, *beta*-, and *gamma*-HCH isomers, and sum PBDEs is the sum of congeners 28, 47, 49, 66, 85, 99, 100, 153, 154, 155, 183. Additional POPs determined in the dolphin blubber samples include hexachlorobenzene (HCB), aldrin, dieldrin, mirex and endosulfan I.

A method blank and a National Institute of Standards and Technology (NIST) blubber Standard Reference Material (SRM 1945) were analyzed with each dolphin blubber set as part of a performance-based quality assurance program (9). Concentrations of individual analytes measured in SRM 1945 were in excellent agreement with the certified or reference values published by NIST. Other quality control samples met established laboratory criteria.

Details for Materials and Methods – Data and Statistical Analyses

Unless otherwise noted, statistical tests were conducted in Statistica (Version 9.0, StatSoft Inc), graphs were made using R (Version 2.11.1, The R Foundation for Statistical Computing). Kernel density estimation was done in R using the *density* function. Exact binomial confidence intervals were computed using Mathcad (2001 Professional MathSoft, Inc.).

Hematology and Serum Chemistry Data

Hematology and serum chemistry values for each dolphin were compared with 95th percentile reference intervals for the appropriate age class, previously established for wild dolphin populations (10, 11). The prevalence of individuals with values above the upper reference threshold and below the lower reference threshold was computed for each parameter along with exact binomial 95% confidence interval (CI). The computed prevalence and 95% CI was compared with expected prevalence (0.025); if 95% CI did not include 0.025, the prevalence was considered to be significantly different than the expected (Table S2). Hematology and serum chemistry parameters were organized into health panels representing pathologic processes or organ systems as described by Hall *et al*. (12); health panel criteria were developed in consultation with a clinical pathologist (M Fry). Prevalence of cases for each panel was computed and compared between BB and the reference site using a Fisher Exact Test (package fisher.test, R Version 2.11.1, The R Foundation for Statistical Computing). For the inflammation panel (Table 1, S2), dolphins with an abnormal value for any one of the seven indicators were classified as a case. Hypoglycemia (low glucose) and anemia (low hemoglobin) were each considered as their own panel. For all other panels, there is strong correlation among parameters and abnormal measures of multiple indicators would be expected. Therefore, the prevalence of cases with more than one abnormal indicator was computed and was used for comparison between the two sites.

Endocrine Data

Serum aldosterone concentrations that were below the limit of detection were substituted with a value of one half of the detection limit.

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). Wilks' lambda was computed to assess multivariate significance, and when significant, a univariate analysis was conducted for each

dependent variable. Model assumptions were evaluated using normal probability plots and a Levene's test for homogeneity of variance. When model assumptions were not met, a non-parametric method (Mann-Whitney U test) was employed.

Sex and age/length class (subadult, adult) were included as covariates for comparison of serum thyroid hormone concentrations as these parameters have previously been reported to be influential factors (5, 13).

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 (14). Therefore, elapsed time from when the net was initially set to the time that the blood sample was collected was calculated for each dolphin and included as a covariate for analysis of cortisol and aldosterone. An interaction term for site*elapsed time was also included.

Details for Results

Ultrasound

One of the Barataria Bay pregnancies was determined to be nonviable, based on the lack of fetal heartbeat, fetal movement, and organ definition as well as an overall increased echogenicity consistent with fetal maceration. There was no blood flow in the fetal or umbilical cord when interrogated with Doppler. Estimated due date based on fetal skull biparietal diameter would have been 27MAR2012 +/- 2 weeks, although fetus may have been dead for several days, so actual due date would have been slightly earlier. The subject female was diagnosed with moderate lung disease and an overall poor prognosis, therefore poor maternal health was a likely contributor to fetal death.

Panel abnormalities

The most common abnormalities associated with inflammation were neutrophilia (present in 6 dolphins from BB and 1 from SB) and hypoalbuminemia (present in 5 and none, respectively). Three of the BB dolphins with neutrophilia had other laboratory evidence of inflammation (hyperglobulemia, hypoalbuminemia, or monocytosis), and also had ultrasonographic evidence of lung disease.

Increased lactate dehydrogenase (LDH) activity was the most prevalent abnormality in the hepatobiliary panel for both SB (19%) and BB (50%) populations (Table S2). None of the SB cases with increased LDH had increased activity of other hepatobiliary enzymes suggesting hepatocellular damage, and in 3 of the 5 cases, LDH was only slightly increased over the upper reference limit (LDH = 532, 547,

569 U/L; upper reference limit [URL] = 530 U/L); in the remaining 2 cases (LDH = 601, 903 U/L) creatine kinase activity was also increased, and the increased activities of both enzymes was considered likely attributable to minor muscle trauma related to capture and restraint. In contrast, 5 of 16 BB dolphins with increased LDH also had increased activity of at least one other hepatobiliary enzyme and an additional 5 BB cases had LDH values > 600 U/L (2 of them increased more than 2-fold above the URL).

Erythrogram data from some dolphins were also consistent with anemia of inflammation. Decreased hemoglobin concentration occurred in 4 dolphins from BB and none from the reference population; none of the anemic animals had a reticulocyte concentration exceeding the maximum value in dolphins from SB.

Endocrine Data

Multivariate tests of significance indicated that site and elapsed time were associated with adrenal hormones ($p=0.003$ and 0.032, respectively) but the site*elapsed time term was not significant ($p=0.175$). A subsequent univariate F-test with cortisol as the dependent variable indicated significant effects for site ($p<0.001$) and elapsed time ($p=0.009$). Although the site*elapsed time effect was not statistically significant at the $\alpha=0.05$ level, it was very close to significance ($p=0.061$). Therefore, regression analyses for elapsed time versus serum cortisol concentration were conducted separately for SB and BB samples.

A subsequent univariate F-test for aldosterone concentrations showed deviation from model assumptions. A Levene's test indicated non-homogeneity of variances ($p<0.001$); therefore, a non-parametric Mann-Whitney U test was also conducted. The parametric F-test indicated that site had a significant effect on aldosterone ($p=0.001$), but elapsed time did not ($p=0.197$). The Mann-Whitney U test supported the conclusion of a significant difference between the two sites ($p<0.001$).

Chemical Analysis Data

Concentrations of multiple POPs (Σ PCBs, Σ DDTs, Σ PBDEs, Σ Chlordanes, Σ HCHs, aldrin, dieldrin, HCB, endosulfan) were compared between SB and BB using a MANCOVA with length as a continuous covariate. The multivariate F values (Wilks λ) were significant for both length ($p=0.003$) and site ($p<0.001$); therefore, a univariate test for each POP class was conducted.

Residual plots and a Levene's test for homogeneity of variances were used to assess potential violation of model assumptions. Data for most contaminant groups were found to non-normal (left-skewed) and/or the assumption for homogeneity of variances was violated. Therefore, data were log-

transformed and analyses were repeated. Following transformation, the hypothesis of homogeneous variances was rejected only for Σ HCHs (Levene's test, p=0.011).

Due to the potential violation of model assumptions for the Σ HCHs, nonparametric analyses were used to further examine this contaminant class. Length and Σ HCHs were not significantly correlated (Spearman rank order correlation, p=0.284), therefore a Mann-Whitney U test was used to compare SB versus BB concentrations without considering length.

Table S1. Breakdown of sampled dolphins from Sarasota Bay and Barataria Bay by age and sex class. Criteria for assigning age/sex-class were adopted from (10, 11). There was not a significant difference at the α=0.05 threshold in sex ratio (Fisher exact test, p=0.135) or age-class distribution (Fisher exact test, p=0.200) for dolphins sampled between the two sites.

Age/Sex-Class	Criteria[a]	Sarasota Bay	Barataria Bay
Calf	< 2 years; < 200 cm	0	0
Juvenile/Subadult	≥ 2 years and < 10 years; ≥ 200 cm and < 240 cm		
Male		8	3
Female		4	13
Adult	≥ 10 years; ≥ 240 cm		
Male		5	9
Female		9	7
Total		**26**	**32**

[a] Age was used for assigning class; if age was not available, assignment was based on length.

Table S2. Hematology and serum biochemical parameter abnormalities observed in dolphins sampled in Sarasota Bay and Barataria Bay, indicating number of cases above/below the reference interval. Assuming prevalence of 0.025 for cases below and above 95th percentile reference thresholds, the expected number of cases in either population would be 3 or fewer. Red font indicates that number of cases is higher than expected.

	Hematological/Biochemical Parameter	Number of Cases Sarasota Bay (N=26)	Barataria Bay (N=32)
Inflammation			
	↑neutrophil	1	6
	↑lymphocyte	1	3
	↑eosinophil	0	1
	↑monocyte	0	1
	↑basophil	0	0
	↑globulin	0	3
	↓albumin	0	5
Iron panel			
	Iron (↓ /↑)	0 / 1	1 / 9
	Total Iron Binding Capacity (↓ /↑)	0 / 0	1 / 0
	% Saturation (↓ /↑)	1 / 2	2 / 9
Hepatobiliary			
	Alanine aminotransferase (↓ /↑)	0 / 0	1 / 2
	Aspartate transaminase (↓ /↑)	0 / 0	2 / 3
	Gamma-glutamyl transferase (↓ /↑)	1 / 0	1 / 3
	Lactate dehydrogenase (↓ /↑)	0 / 5	0 / 16
Anemia			
	↓Hgb	0	4
	↓Hemoglobin, ↓MCV	0	1
	↓Hgb, ↑MCV	0	1
	↓Hgb, normal MCV	0	2
Electrolytes & Minerals			
	Potassium (↓ /↑)	0 / 1	0 / 4
	Sodium (↓ /↑)	0 / 0	3 / 1
	Chloride (↓ /↑)	0 / 0	0 / 0
	Calcium (↓ /↑)	0 / 1	1 / 0
	Phosphate (↓ /↑)	0 / 1	0 / 0
	Magnesium (↓ /↑)	0 / 0	0 / 0
Renal function			
	Blood urea nitrogen	1 / 0	1 / 0
	Creatinine	0 / 1	1 / 1
Other (non-specific)			
	AP (↓ /↑)	1 / 1	1 / 0
	CK (↓ /↑)	2 / 5	11 / 1

Table S3. Serum cortisol concentrations measured from dolphins sampled during health assessments in St. Joseph Bay, Florida in April 2005 and July 2006 (11). Elapsed time from net deployment to blood collection ranged from 6 - 56 minutes.

Date	Cortisol (µg/dL)
4/18/2005	3.18
4/18/2005	4.14
4/18/2005	6.78
4/19/2005	2.32
4/20/2005	3.18
4/20/2005	4.46
4/20/2005	2.82
4/21/2005	2.48
4/25/2005	4.93
4/25/2005	2.97
4/28/2005	2.50
4/28/2005	3.87
7/17/2006	3.06
7/19/2006	3.24
7/19/2006	2.37
7/19/2006	1.92
7/19/2006	2.67
7/20/2006	1.91
7/20/2006	1.08
7/20/2006	1.28
7/20/2006	3.20
7/21/2006	0.99
7/21/2006	2.12
7/21/2006	1.55
7/25/2006	2.52
7/25/2006	2.02
7/27/2006	2.14
7/28/2006	2.44
7/28/2006	1.61
7/28/2006	3.92

Table S4. Breakdown of prognosis by age- and sex-class for Sarasota Bay and Barataria Bay. The proportion of adults versus juveniles/subadults given a guarded or worse prognosis did not differ significantly at the α=0.05 level (Table S4; p=0.715), and neither did the proportion of males versus females (p=0.450).

	Prognosis				
	Good	**Fair**	**Guarded**	**Poor**	**Grave**
Sarasota Bay					
Juvenile/Subadult (combined sexes)	4	3	1	0	0
Male	4	2	1	0	0
Female	0	1	0	0	0
Adult (combined sexes)	3	4	0	0	0
Male	1	2	0	0	0
Female	2	2	0	0	0
Total	**7**	**7**	**1**	**0**	**0**
Barataria Bay					
Juvenile/Subadult (combined sexes)	5	3	5	1	0
Male	1	2	0	0	0
Female	4	1	5	1	0
Adult (combined sexes)	2	5	4	2	2
Male	0	4	2	1	1
Female	2	1	2	1	1
Total	**7**	**8**	**9**	**3**	**2**

Table S5. Geometric means (µg/g lipid) and p-values from MANCOVA examining concentrations of chemical contaminants measured in blubber samples of male dolphins from Sarasota Bay versus Barataria Bay with length as a covariate. Red text indicates significant p-value at the α=0.05 level. Marginal (least squares) means are shown for classes where length covariate is significant and are computed for length at mean (239.4 cm).

		Sarasota Bay (N=10)	Barataria Bay (N=12)	Length	Site
Σ PCB	Mean	26.7	23.7	**< 0.001**	0.461
	L95%	21.1	19.2		
	U95%	33.6	29.2		
Σ DDT	Mean	15.7	6.70	**< 0.001**	**0.001**
	L95%	11.6	5.08		
	U95%	21.3	8.82		
Aldrin	Mean	0.001	0.002	0.524	0.202
	L95%	0.001	0.001		
	U95%	0.002	0.002		
Dieldrin	Mean	0.672	0.321	0.932	**0.004**
	L95%	0.494	0.242		
	U95%	0.915	0.425		
Endosulfan	Mean	0.001	0.002	0.517	0.205
	L95%	0.001	0.001		
	U95%	0.002	0.002		
Σ PBDE	Mean	1.50	1.41	**0.009**	0.710
	L95%	1.17	1.13		
	U95%	1.90	1.75		
Σ Chlordane	Mean	10.4	1.81	**<0.001**	**<0.001**
	L95%	8.21	1.46		
	U95%	13.3	2.25		
HCB	Mean	0.065	0.039	0.096	**0.022**
	L95%	0.052	0.032		
	U95%	0.080	0.048		
Mirex	Mean	0.690	0.067	**<0.001**	**<0.001**
	L95%	0.505	0.050		
	U95%	0.943	0.089		
Σ HCH	Median*	0.004	0.008	0.284*	**0.017***

*Non-parametric methods were used for Σ HCHs, therefore median (µg/g lipid) is shown rather than LS mean. Length p-value for Σ HCHs is from Spearman rank order correlation and site p-value is from Mann-Whitney U test.

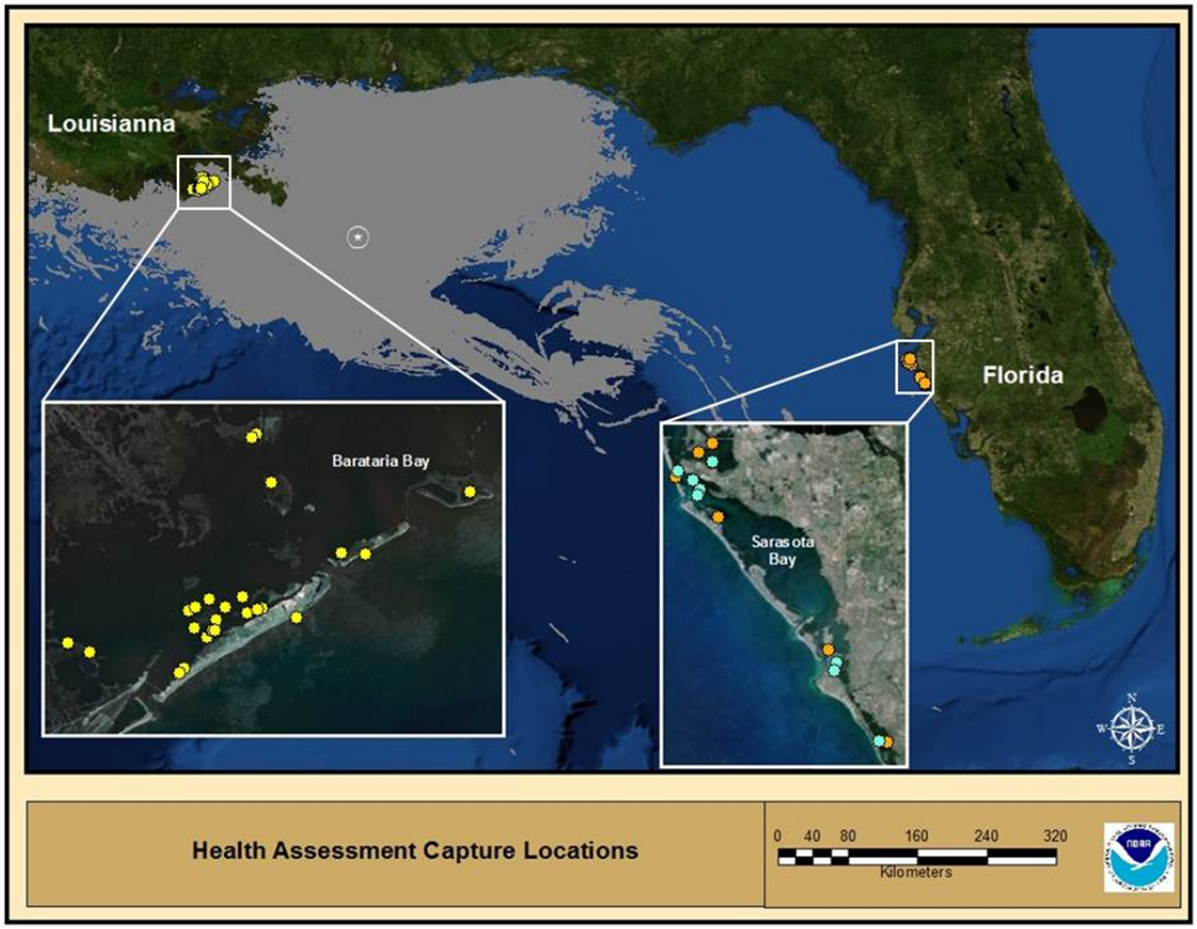

Figure S1. Locations of dolphins sampled during 2010 Sarasota Bay (orange circles), 2011 Sarasota Bay (blue circles) and 2011 Barataria Bay dolphin capture-release health assessments. Gray shaded area on base map indicates the cumulative National Environmental Satellite, Data and Information Service (NESDIS) Anomaly Analysis April-August 2010. The white star/circle indicates the wellhead location.

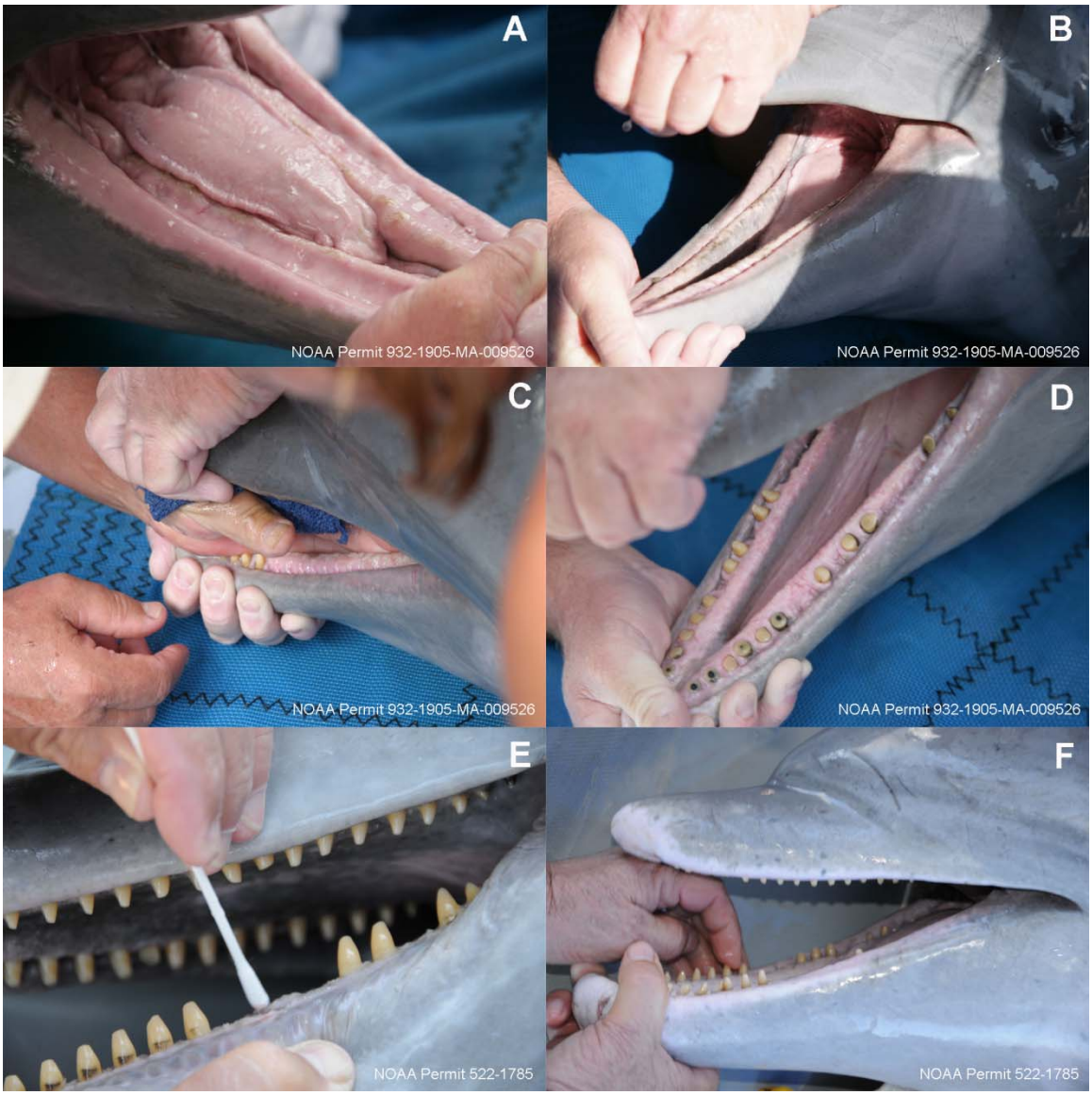

Figure S2. Tooth loss and wear among Barataria Bay dolphins (A=Y10; B=Y01; C=Y09; D=Y05) and Sarasota Bay dolphins (E=FB193, F=FB90). Complete (A), near complete (B-C), and extensive tooth loss (D) were only seen in Barataria Bay dolphins. Tooth loss in Sarasota dolphins (E-F) was much less severe.

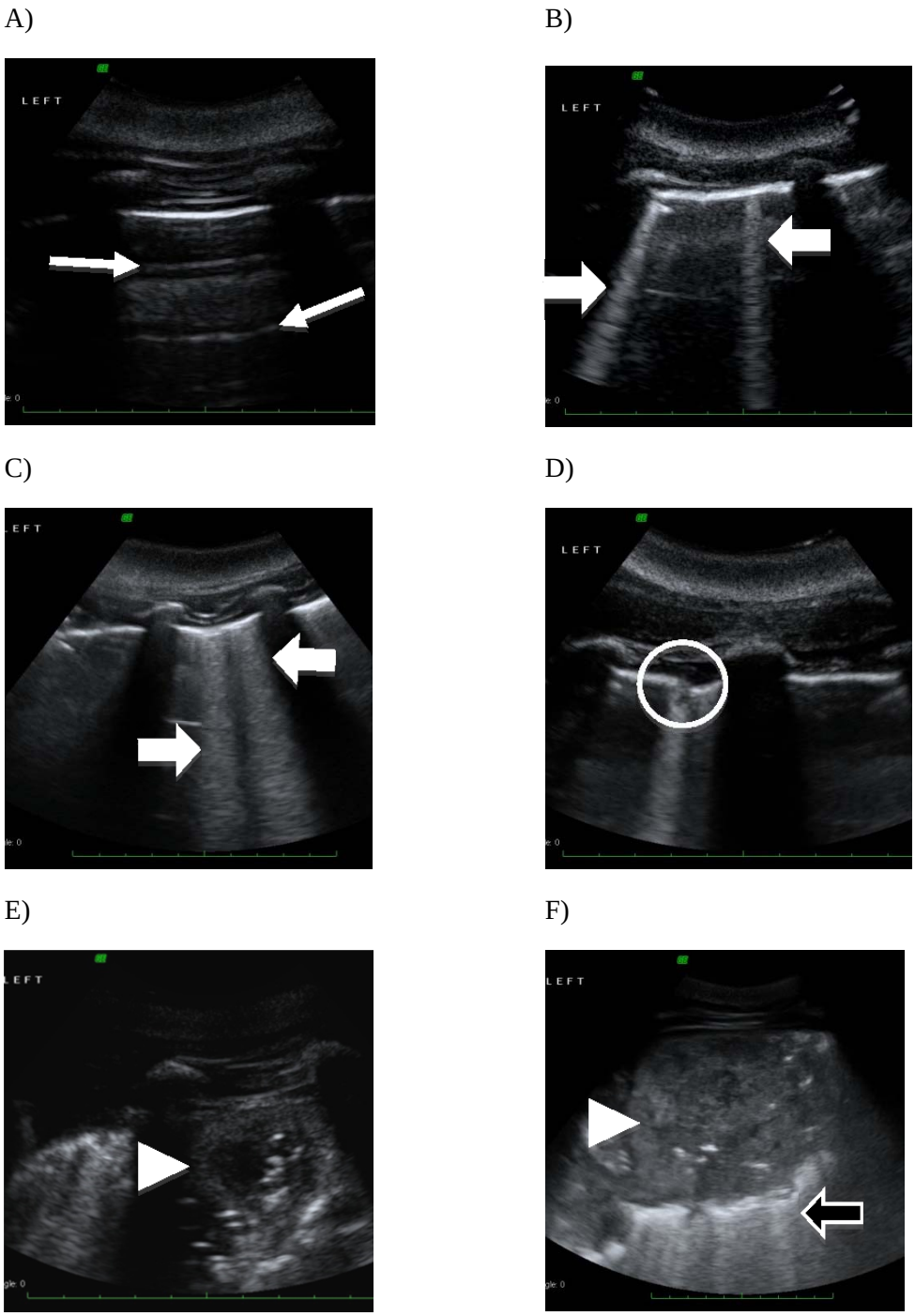

Figure S3. Representative dorsal plane sonograms of the left lungs of dolphins examined in Barataria Bay. Sonograms represent: A) normal lung; B) mild lung disease; C,D) moderate lung disease; and E,F) severe lung disease. Normal lung was characterized by the presence of reverberation artifact (S3A, line arrows), which is created by air-filled lung. Mild lung disease findings included ring-down artifacts (3B, block arrows) found throughout the lung, consistent with alveolar-interstitial syndrome. Moderate lung disease findings included coalescing ring-down artifacts (S3C, block arrows) consistent with significant alveolar-interstitial syndrome (AIS) and pulmonary nodules/lesions (S3D, circle). Severe lung disease findings included cavitated pulmonary masses with hypoechoic centers as seen in 3E (arrow head), and a large pulmonary mass as seen in S3F (arrow head) that measured greater than 16cm, with consolidation and AIS deep to the mass (black arrows).

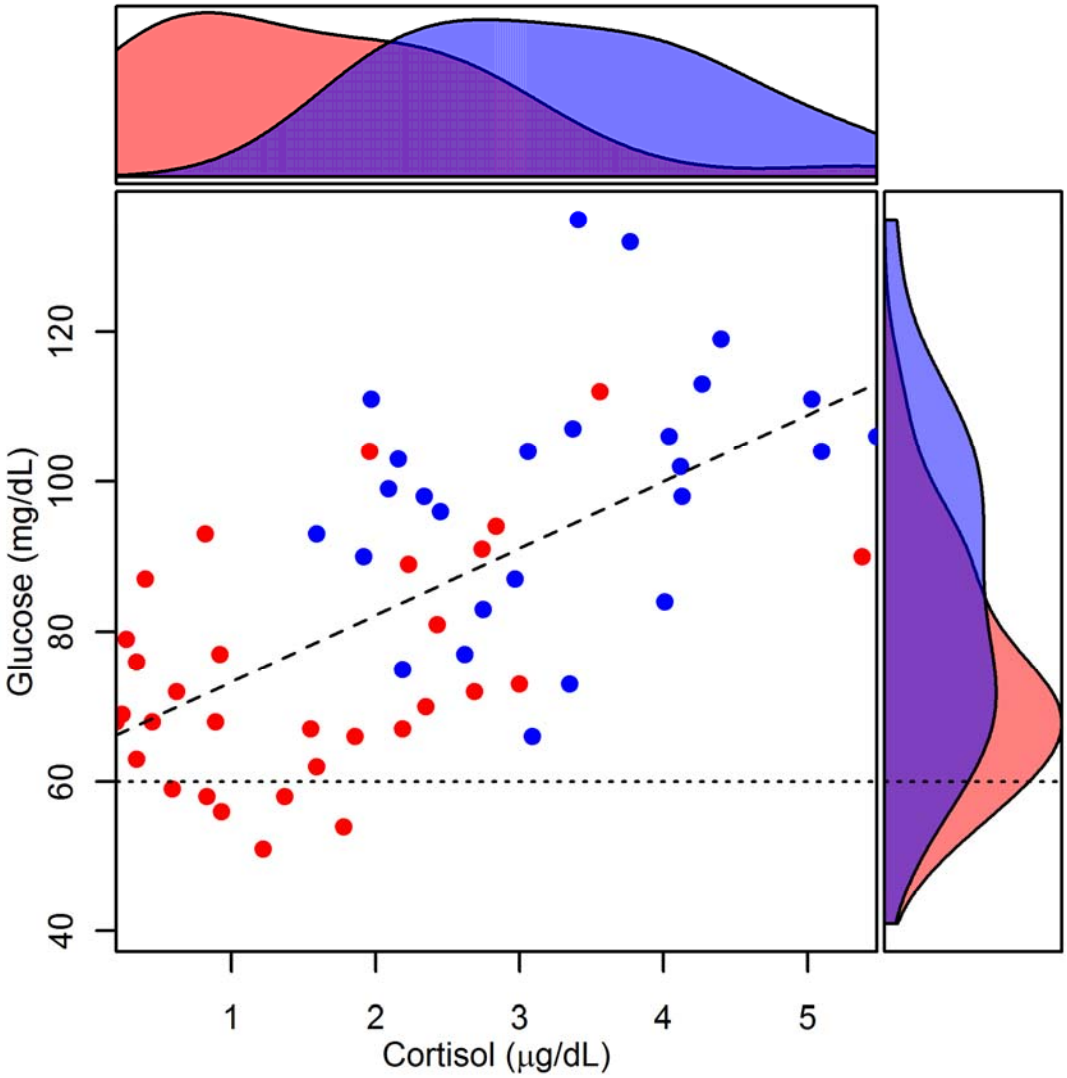

Figure S4. Serum cortisol versus glucose concentrations (N=57) for dolphins sampled in Barataria Bay (red circles) and Sarasota Bay (blue circles). Simple linear regression (dashed line) showed a significant relationship between cortisol and glucose ($p<0.001$, $r=0.62$, $y=64.0813+8.8768*x$). Dotted line represents lower reference limit (2.5th percentile) for glucose in adult dolphins [21]. Densities at top and right for cortisol and glucose, respectively, were computed using kernel density estimation. Red and blue densities represent Barataria and Sarasota, respectively. One dolphin (Y05) was excluded from regression analysis because its measured glucose value (10 mg/dL) was considered to be an outlier, determined by a Grubb's test.

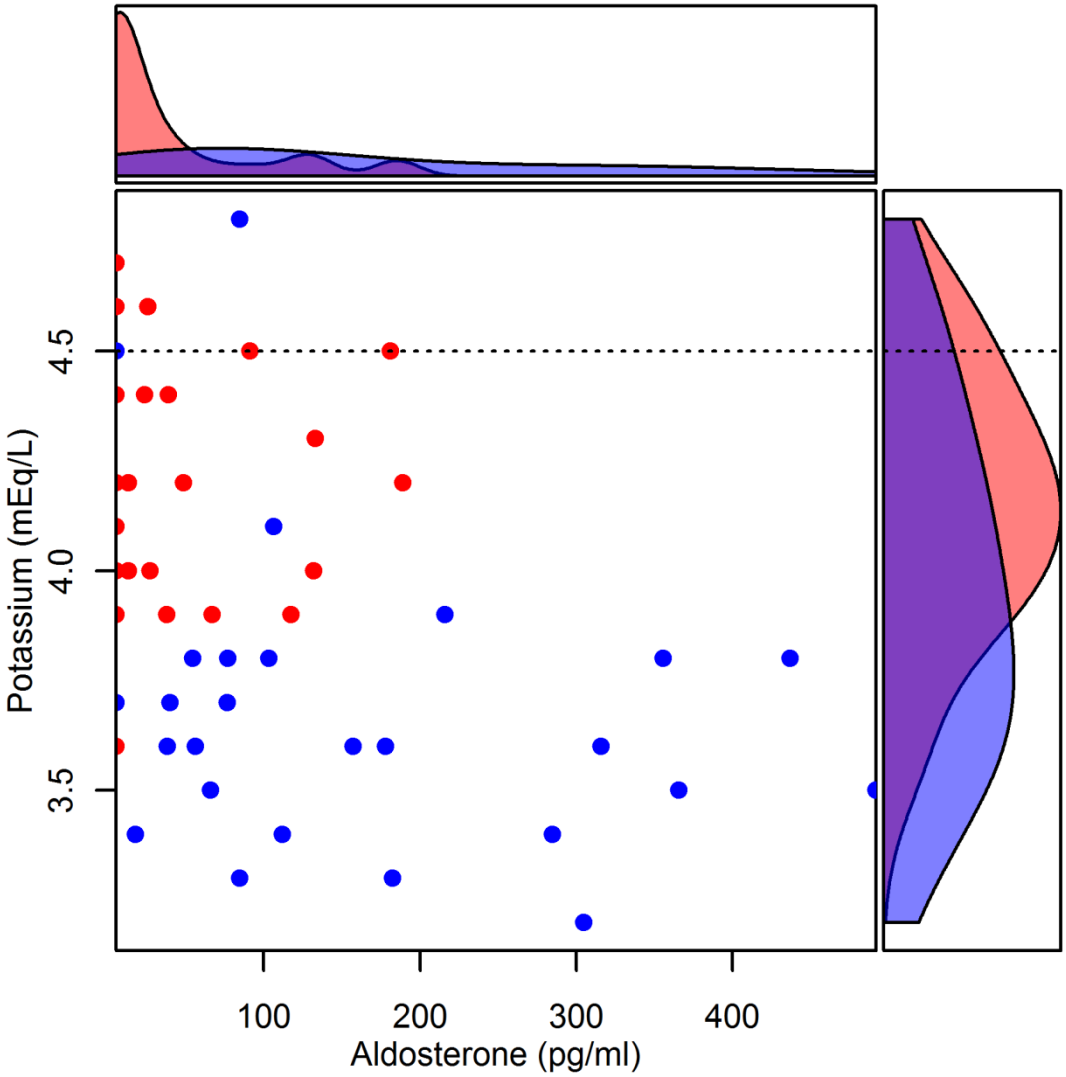

Figure S5. Serum aldosterone concentration versus serum potassium for dolphins sampled in Barataria Bay (red circles) and Sarasota Bay (blue circles). Spearman's rank-order correlation r_s = -0.40, p=0.002. Dotted line represents upper reference threshold for potassium (10). Densities at top and right for aldosterone and potassium, respectively, were computed using kernel density estimation. Red and blue densities represent Barataria and Sarasota, respectively. Of the four Barataria Bay dolphins classified as hyperkalemic (red circles above potassium upper reference threshold, two values for potassium=4.7 mEq/L are overlaid), 3 had serum aldosterone concentrations below the limit of detection.

References

1. Norman SA, Hobbs RC, Foster J, Schroeder JP, & Townsend FI (2004) A review of animal and human health concerns during capture-release, handling and tagging of odontocetes. *Journal of Cetacean Research and Management* 6(1):53-62.
2. Loughlin T, Cunningham L, Gales N, Wells R, & Boyd I (2010) Marking and capturing. *Marine Mammal Ecology and Conservation: A Handbook of Techniques*, eds Boyd I, Bowen D, & Iverson S (Oxford University Press, New York, NY), pp 16-41.
3. Schwacke LH*, et al.* (2010) Eosinophilia and biotoxin exposure in bottlenose dolphins (Tursiops truncatus) from a coastal area impacted by repeated mortality events. *Environ Res* 110(6):548-555.
4. Wells RS*, et al.* (2004) Bottlenose dolphins as marine ecosystem sentinels: Developing a health monitoring system. *EcoHealth* 1:246-254.
5. Schwacke LH*, et al.* (2012) Anaemia, hypothyroidism and immune suppression associated with polychlorinated biphenyl exposure in bottlenose dolphins (Tursiops truncatus). *Proceedings of the Royal Society B-Biological Sciences* 279(1726):48-57.
6. Brook FM (2001) Ultrasonographic imaging of the reproductive organs of the female bottlenose dolphin, *Tursiops truncatus aduncas*. *Reproduction* 121(3):419-428.
7. Smith CR*, et al.* (*In Press*) Fetal omphalocele in an Atlantic bottlenose dolphin (*Tursiops truncatus*). *Zoo and Wildlife Medicine*.
8. Sloan CA*, et al.* (2004) Northwest Fisheries Science Center Procedures for Extraction, Cleanup and Gas Chromatography/Mass Spectrometry Analysis of Sediments and Tissues for Organic Contaminants. in *NOAA Tech Memo* (NMFS, US Department of Commerce, Seattle, WA).
9. Sloan CA*, et al.* (2006) Quality assurance plan for analyses of environmental samples for polycyclic aromatic compounds, persistent organic pollutants, fatty acids, stable isotope ratios, lipid classes, and metabolites of polycyclic aromatic compounds. in *NOAA Tech Memo* (NMFS, US Department of Commerce, Seattle, WA).
10. Schwacke L*, et al.* (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(8):973-985.
11. Schwacke LH*, et al.* (2010) Eosinophilia and biotoxin exposure in bottlenose dolphins (Tursiops truncatus) from a coastal area impacted by repeated mortality events. *Environ. Res.* 110(6):548-555.
12. Hall A, Gulland F, Hammond J, & Schwacke L (2010) Epidemiology, disease and health assessment. *Marine Mammal Ecology and Conservation*, ed IL Boyd WB, SJ Iverson (Oxford University Press Inc., New York), pp 144-164.
13. Aubin DS, Ridgway S, Wells R, & Rhinehart H (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):1-113.
14. Thomson C & Geraci J (1986) Cortisol, aldosterone and leucocytes in the stress response of bottlenose dolphins, *Tursiops truncatus*. *Can J Fish Aquat Sci* 43:7.