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# Hematological, biochemical, and morphological parameters as prognostic indicators for stranded common dolphins (*Delphinus delphis)* from Cape Cod, Massachusetts, U.S.A.

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## Abstract

The current paucity of published blood values and other clinically relevant data for short-beaked common dolphins, *Delphinus delphis,* hinders the ability of veterinarians and responders to make well-informed diagnoses and disposition decisions regarding live strandings of this species. This study examined hematologic, clinical chemistry, and physical parameters from 26 stranded common dolphins on Cape Cod, Massachusetts, in light of their postrelease survival data to evaluate each parameter's efficacy as a prognostic indicator. Statistically and clinically significant differences were found between failed and survived dolphins, including lower hematocrit, hemoglobin, $TCO_2$, and bicarbonate and higher blood urea nitrogen, uric acid, and length-to-girth ratios in animals that failed. In general when compared to survivors, failed dolphins exhibited acidosis, dehydration, lower PCVs, and decreased body condition. Additionally, failed dolphins had the highest ALT, AST, CK, LDH, GGT, and lactate values. These blood values combined with necropsy findings indicate that there are likely a variety of factors affecting postrelease survival, including both preexisting illness and stranding-induced conditions such as capture myopathy. Closer evaluation of these parameters for stranded common dolphins on point of care

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analyzers in the field may allow stranding personnel to make better disposition decisions in the future.

Key words: dolphin, *Delphinus*, stranding, release, satellite tag, hematology, clinical chemistry, morphometrics, capture myopathy, disease.

Common dolphins are an abundant species of small delphinid found in the Atlantic, Pacific, and Indian Oceans, as well as in the Mediterranean and Black Seas (Natoli 2006). Primarily a pelagic animal, the common dolphin is found from near-shore waters to thousands of kilometers offshore, and associates with conspecifics in small groups, large groups, and super-pods numbering in the thousands (Evans 1994). There are two main subspecies of the common dolphin: long-beaked (*Delphinus capensis)* and short-beaked *(Delphinus delphis).* In the western North Atlantic, only a single population of short-beaked common dolphins is found (Westgate and Read 2007). Common dolphin strandings have been documented throughout their range, especially in the eastern North Atlantic (MacLeod *et al*. 2005), Mediterranean (Bearzi *et al.* 2003), on the west coast of the United States (Cowan *et al.* 1986, Seagars and Jozwiak 1991), and along the northeastern coast of North America from Nova Scotia to Florida (Waring *et al.* 2009).

Cape Cod, Massachusetts, experiences one of the highest and most consistent rates of cetacean stranding in the world (Walsh *et al.* 2001, Geraci and Lounsbury 2005, Bogomolni *et al.* 2010). Over the 10 yr period from 2002 through 2011, 1,320 cetaceans stranded on Cape Cod shores during both individual and mass stranding events. *Delphinus delphis* comprised over one-third of these cases ($n = 474$), 172 of which were found alive and in need of rapid response and humane care. The International Fund for Animal Welfare's Marine Mammal Rescue and Research Team (IFAW) responds to all marine mammal strandings on Cape Cod and in southeastern Massachusetts. IFAW staff biologists and veterinarians conduct standardized health evaluations, including on-site blood analysis for each live dolphin that strands in order to determine the best course of action. Due to a dearth of suitable rehabilitation space in the region, disposition options are usually limited to euthanasia, immediate release, or relocation and release from shore. In this case, relocation and release involves overland transport of the dolphins in enclosed trailers from the stranding site (usually a gently sloping, shallow beach or estuary in Cape Cod Bay) to a beach within one hour transport distance with a steep near-shore bathymetric slope. In order to provide optimal triage and aid in animal disposition decision making, stranding response teams use the best available clinical information, which is often not species- or region-specific.

Despite their global abundance and prevalence in the stranding record, little has been documented regarding the hematology, clinical chemistry, or other diagnostically relevant data for common dolphins. Until recently, the CRC Marine Mammal Medicine Handbook published the only available *Delphinus delphis* blood value ranges, derived from only two captive common dolphins that were rehabilitated at SeaWorld following stranding (Bossart *et al.* 2001). Common dolphins are not a predominant species in captivity (Corkeron 2002), thus it is not likely that existing *D. delphis* blood value ranges will be expanded upon from the captive population. In addition, normal blood values from wild dolphins have been shown to differ from captive dolphins due to physiologic adaptations to captivity (Asper *et al.* 1990, Bossart *et al.* 2001). While there are limitations to utilizing captive animal reference ranges to evaluate wild stranded dolphins, often there are no available alternatives due to the logistical challenges of collecting blood from free-ranging delphinids.

Furthermore, stranded dolphin blood values differ from those of both wild and captive dolphins due to stranding-induced stresses (Koopman *et al.* 1999, Walsh *et al.* 2001), indicating that a separate set of acceptable blood parameter ranges is most appropriate for stranded animals. In 2012, Sampson *et al.* published blood data from three mass stranded common dolphins that were satellite tagged and released on Cape Cod between 2006 and 2009. Therein, the authors called for further work to establish which blood parameters provide the most useful prognostic information in stranding events.

Since 2010, IFAW has been operating a satellite tagging program to better evaluate postrelease success of stranded dolphins. The current study employs IFAW's postrelease satellite tag data to test the hypothesis that certain health assessment parameters are predictive of stranded common dolphin survival. Here we report on the relative prognostic value of 24 hematology, 32 clinical chemistry, and four physical examination parameters from 26 common dolphins stranded on Cape Cod, Massachusetts.

## Methods

In this study, data were analyzed from common dolphins that stranded on Cape Cod between January 2010 and June 2012, the time within which standardized protocols for blood collection and documentation were established and IFAW's satellite tagging program was ongoing. Live dolphins that stranded and were deemed releasable were transported across land in an enclosed trailer to a suitable release site that optimized access to deep water from the beach. Animals were always released the same day as they stranded. In order to determine which animals were releasable, IFAW staff biologists and veterinarians conducted standardized health assessments on all live-stranded dolphins. The health evaluations consisted of the following: basic data collection (location, date, mass or single stranding, sex), morphometric measurements (straight length, weight, axillary girth), physical examination (respiration rate, heart rate, reflexes, external examination), stress and behavioral evaluations (noting flatulence, foamy feces, belching, arching, thrashing, tail fluttering, vocalizations), ultrasound exam (to evaluate blubber thickness and pregnancy, when possible), and in-field blood analyses.

### *Environmental Parameters*

For each day that an animal in this study stranded, air and sea temperature data were obtained from the Northeastern Regional Association of Coastal and Ocean Observing Systems (http://NERACOOS.org). The daily average air and sea temperature at 1 m depth from Massachusetts Bay Buoy AO1 were used. Data regarding total time that the animals were stranded on land were not consistently collected for the animals in this study, and thus, could not be analyzed.

### *Morphometrics*

Standard straight lengths were collected for every dolphin according to accepted methods (Norris 1961). To minimize the stress of physical manipulation on live dolphins, axillary girths were measured as half-girths from dorsal midline to ventral midline and then doubled. Weights were obtained in the field by placing the dolphin

on IFAW's dolphin cart and rolling the cart such that the four wheels rested on four separate industrial grade postal scales. Weights from the four scales were summed then the weight of the cart, foam padding, and stretcher were subtracted to calculate the weight of the animal.

*Blood Collection*

For each dolphin, blood was collected from the dorsal fluke periarterial venous rete. The venipuncture site was prepped with a betadine scrub and blood was collected directly into vacutainer tubes using a 21 g, 3/4 in. winged infusion set with a BD Vacutainer adapter and holder. One to three 7 mL red top tubes (no additive) and one 4 mL green top tube (lithium heparin) were collected for serum and plasma determinations and one 3 mL lavender top tube ($K_2$EDTA) was collected for Complete Blood Count (CBC). If more than 1 h would pass between collection and analysis, blood was stored with ice packs in an insulated cooler.

*Blood Analysis*

EDTA-preserved whole blood samples were analyzed in-house on the VetScan HM2 Hematology System (Abaxis, Union City, CA) either during the stranding response in the temperature-controlled rescue trailer or immediately after return from the field and within 8 h of drawing blood. The HM2 analyzer provided an 18-parameter CBC, using impedance methodology to count red blood cells (RBCs), white blood cells (WBCs), and platelets (PLTs), measure their respective cell sizes, and to provide a 3-part WBC differential, including absolute granulocyte (Gra), lymphocyte (Lym), and monocyte (Mon) counts. Hemoglobin concentration (Hgb) was measured spectrophotometrically. Calculated parameters included percentages of lymphocytes, monocytes, granulocytes, hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDWc%), platelet hematocrit (PCT%), mean platelet volume (MPV), and platelet distribution width (PDWc%). Per manufacturer instructions, the equine species profile was selected for sample analysis and the machine was operated according to manufacturer's specifications.

Heparinized blood samples were run on an i-STAT 1 System handheld analyzer (Abbott Laboratories, Abbott Park, IL) within 1 h of blood draw for clinical chemistry and blood gas analyses. Three different i-STAT test cartridges were used, each with chemically sensitive biosensors on a silicon chip that were configured to perform specific tests, including anion gap, base excess (BEecf), bicarbonate ($HCO_3$), chloride ($Cl^-$), creatinine (Crea), glucose (Glu), hematocrit (Hct), hemoglobin (Hgb), lactate, ionized calcium (iCa), carbon dioxide partial pressure ($PCO_2$), potassium ($K^+$), sodium ($Na^+$), pH, urea nitrogen (BUN)/urea, oxygen partial pressure ($PO_2$), oxygen saturation ($sO_2$), and total carbon dioxide ($TCO_2$). Since comparable assays in different cartridges use the same methodology, their results were combined for statistical analysis. The i-STAT 1 analyzer uses a conductivity-based method to measure blood hematocrit. After correction for electrolyte concentration, the measured conductivity is inversely related to the blood hematocrit, and blood hemoglobin concentration is calculated from this value. The i-STAT 1 analyzer was maintained and run according to manufacturer's specifications.

Following stranding response, red top tubes were centrifuged at 3,200 RPM for 10 min and at least 1 mL of serum was collected into 1.5 mL bullet tubes for serum

chemistry analysis. Serum samples and whole blood collected into EDTA were stored at 4°C until they could be transported to a reference laboratory (IDEXX Laboratories, Inc., North Grafton, MA) for additional analyses. At the reference lab, serum samples were processed using an AU 5421 chemistry analyzer (Olympus, Center Valley, PA), which measured the following parameters: albumin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), BUN, calcium, chloride, cholesterol, creatine kinase (CK), creatinine, gamma-glutamyl transpeptidase (GGT), globulin, glucose, lactate dehydrogenase (LDH), phosphorus, potassium, sodium, $TCO_2$, total bilirubin, total protein, and uric acid. The following calculated parameters were also provided: albumin:globulin ratio, anion gap, BUN:creatinine ratio, and sodium:potassium ratio.

EDTA anticoagulated blood was analyzed at the reference laboratory using an XT-V Automated Hematology Analyzer (Sysmex, Kobe, Japan), an instrument that uses fluorescent flow cytometry technology. Samples were processed using the instrument thresholds established for *monkey* and the following parameters were reported: WBC, RBC, Hgb, Hct, MCV, MCH, MCHC, absolute and percentage segmented neutrophils, lymphocytes, monocytes, eosinophils, and basophils. Blood that was assayed within 72 h of venipuncture was included in this study, since most parameters are stable for this period of time (Geraci and Medway 1974, Varela 2006).

Blood from each animal was not always run on all available analyzers (HM2, i-STAT 1, AU5421, and XT-V), resulting in a varied number of samples for the different components of this study. For animals that stranded multiple times, only blood from their first stranding was used for analysis.

### *Satellite Tagging*

Select stranded common dolphins were affixed with single-pin satellite tags to the proximal third of the dorsal fin, approximately 3.0 cm cranial to the trailing edge (fin thickness at this site ranged from approximately 6–12 mm), according to the methods in Balmer *et al.* (2011). A standard plastic identification tag (AllFlex bullet sheep tag) was also attached to the distal one-third of the trailing edge of each animal's dorsal fin to facilitate resightings at sea. Two models of satellite tags were used in the study: (1) Kiwisat 202 Cetacean Fin Tags (Model K2F161) from Sirtrack Ltd. (Havelock North, NZ) with integrated VHF transmitters, a 12 h on/12 h off duty cycle and an expected battery life of 45 d; and (2) time- and depth-recording satellite tags (MK-10A model) from Wildlife Computers (Redmond, WA) with varying duty cycles and an expected battery life of at least 60 d. Dive data from the MK-10A tags will not be reported here. Location data were obtained from both tag types using Argos tracking services (CLS America) and the data were subsequently filtered with R ArgosFilter algorithm (Freitas *et al.* 2008). The only data from the satellite tags that were used in this study were the total number of days transmitted.

### *Necropsy and Sample Analysis*

All fresh dead animals included in the study were necropsied and sampled according to standard IFAW protocols. Histological slides were prepared by Histology Consultation Services (Everson, WA) and sent to Dr. Dave Rotstein, Consulting Veterinary Pathologist (Olney, MD), for review. Necropsied animals with a suspected *Brucella* infection had frozen tissue samples sent for *Brucella* culture to

USDA APHIS National Veterinary Services Laboratory (Ames, IA). Cultured tissue samples included: ovary, prescapular lymph node, tracheobronchial lymph node, kidney, spleen, brain, and testis. Whole blood and tissue samples (uterus, ovary, amniotic fluid, testes, kidney, fibrotic mass, brain, and spleen) were also sent to the University of Illinois College of Veterinary Medicine's Veterinary Diagnostic Laboratory (Urbana, IL) for RT-PCR analysis. Tissues with inconclusive RT-PCR results were genetically sequenced by the same lab. Additionally, frozen serum and whole blood samples were sent to Mystic Aquarium (Mystic, CT) for *Brucella* cELISA assays.

A subset of necropsied dolphins had samples submitted for biotoxin analysis to the Marine Biotoxins Program Lab of NOAA National Ocean Service (Charleston, SC), including liver, kidney, gastric contents, and/or feces. Samples were analyzed for the presence of saxitoxin using a receptor binding assay (Van Dolah *et al.* 2012). Samples were also analyzed for domoic acid and okadaic acid and its related congeners (dinophysistoxin and pectenotoxin) using tandem mass spectrometry coupled with liquid chromatographic separation (Wang *et al.* 2012).

### *Data and Statistical Analyses*

All stranded common dolphin cases between January 2010 and June 2012 were reviewed and placed into two groups for analysis: "failed" and "survived." Animals with satellite tag transmission durations of 3 wk or more were categorized as "survived." The 3 wk postrelease survival period was based on previously published interpretations of survival from satellite tagged delphinids (Wells *et al.* 2008, 2009; Sampson *et al.* 2012). A more recently suggested 6 wk cut off period for determining postrelease survival in delphinids (Wells *et al.* 2013) was not used in our study due to the unique characteristics of common dolphin ecology, behavior, and fin morphology, as well as differences in tag attachment and programming. The "failed" group included live-stranded common dolphins that met either of the following criteria: (1) died during the initial response effort and had blood drawn prior to death, or (2) satellite or ID tagged and initially released but later restranded and either died or were euthanized by IFAW staff due to poor health. Stranded common dolphins that did not fit into either the "survived" or "failed" category were not included in this study.

Receiving Operator Characteristic (ROC) curve analysis was performed using MedCalc statistical software (Mariakerke, Belgium) to evaluate the difference between "failed" and "survived" groups for each parameter; each instrument was analyzed as an isolated data set since results are not reliably comparable across labs/analyzers (Hall *et al.* 2007). For each parameter, the ROC curve provided test sensitivity (the proportion of failed dolphins that the test correctly identified) and specificity (the proportion of survivors that the test correctly identified), a suggested clinical cut-off value, and *P* value for significance. Due to the small sample size, the nonparametric Mann-Whitney-Wilcoxon (MWW) test was additionally conducted on the significant parameters from the ROC curve analysis to further evaluate their relevance. Significance for all tests was determined at $\alpha < 0.05$.

Several physical exam parameters including heart rate, respiratory rate, body mass index [mass (kg)/length $(m)^2$], length-to-girth ratio and length-to-weight ratio were also assessed for their value as predictors of survival using the same methods. Differences in air and water temperatures from the day of stranding for survivors *vs.* failures were evaluated for statistical significance using a MWW test for nonparametric data.

The data set was also separately analyzed based on sex and stranding type (single or mass stranding) to evaluate the effect of these factors on the above tests. This was accomplished with a MWW test on the ROC curve-determined significant parameters for the 26 dolphin sample set.

## Results

### *Overview*

Between January 2010 and June 2012, IFAW responded to 330 reports of stranded common dolphins on Cape Cod and in southeastern Massachusetts, 143 of which were found alive (43%). During this time frame, a large and extended stranding event of common dolphins occurred on Cape Cod from 12 January through 3 April 2012. This event involved a total of 215 stranded animals, 98 of which stranded live (45%), thus comprising 69% of all the live animals during the study period.

Ten of the 143 live stranded animals fit the definition of "failed" [three females (30%), seven males (70%); three single stranded (30%), seven mass stranded (70%)]. Two of these animals were satellite tagged, released, restranded and either died the next day (IFAW11-026Dd) or were found dead and moderately decomposed 9 d after the initial release (IFAW11-003Dd). The remaining eight animals were ID-tagged only. One of these animals restranded live 3 d after release and was euthanized due to poor condition. Two animals died during the initial stranding response, but had blood drawn prior to death. The rest of the animals were found dead between 1 and 9 d after they were released, in varying states of decomposition. See Table 1 for a summary of the individual animals included in this study.

Sixteen satellite tagged dolphins posted transmission durations of 3 wk or more to comprise the "survived" group for this study [5 females (31%), 11 males (69%); 4 single stranded (25%), 12 mass stranded (75%)], see Table 1. One of the satellite tagged animals included in this group (IFAW12-194Dd) restranded with a group of dolphins two days after the initial stranding, however this animal was deemed healthy and was relocated and released with the rest of the group. His satellite tag continued to transmit for 21 d following the second release.

Two known pregnant animals (IFAW12-004Dd and IFAW12-201Dd) were included in this study, both of which failed to survive. One of the two was found to be pregnant upon necropsy and the other was diagnosed in the field by ultrasound. The latter was the only female in the study for which a complete ultrasound exam was conducted. It is reasonable to assume that a proportion of the females that did not receive either a complete ultrasound examination or a necropsy were likely pregnant as well; thus, to exclude the two known pregnant animals would not be appropriate.

### *Environmental Parameters*

No difference was found in the average daily air or water temperature on the day of stranding between the survivors ($n_S = 16$) and the failures ($n_F = 10$) in this study.

### *Physical Parameters*

Failed dolphins presented with significantly larger length-to-girth ratios ($n_F = 6$, $\bar{x} = 2.08$, $R = 1.73–2.50$) than those that survived ($n_S = 8$, $\bar{x} = 1.77$, $R = 1.68–2.04$;

*Table 1.* Animal disposition summary.

| Animal ID | Stranding date | Sex | Stranding type | Initial disposition | Restrand | Final disposition | Satellite tag | # Days transmitted | Category |
|---|---|---|---|---|---|---|---|---|---|
| IFAW10-060Dd | 26 February 2010 | F | Mass | Released | N/A | - | Kiwisat202 | 22 | Survived |
| IFAW10-063Dd | 26 February2010 | M | Mass | Released | N/A | - | Kiwisat202 | 44 | Survived |
| IFAW10-230Dd | 29 November 2010 | M | Single | Released | N/A | - | MK-10A | 21 | Survived |
| IFAW10-238Dd | 3 December 2010 | F | Single | Released | N/A | - | MK-10A | 21 | Survived |
| IFAW11-022Dd | 14 February 2011 | M | Mass | Released | N/A | - | Kiwisat202 | 39 | Survived |
| IFAW11-026Dd | 19 February 2011 | M | Single | Released | 20 February 2011 | Died | Kiwisat202 | <1 | Failed |
| IFAW11-249Dd | 31 August 2011 | M | Mass | Released | 9 September 2011 | Found dead | - | N/A | Failed |
| IFAW11-252Dd | 31 August2011 | M | Mass | Released | 3 September 2011 | Euthanized | - | N/A | Failed |
| IFAW11-284Dd | 21 November 2011 | M | Single | Released | N/A | - | Kiwisat202 | 25 | Survived |
| IFAW12-003Dd | 12 January 2012 | F | Single | Released | 21 January 2012 | Found dead | Kiwisat202 | 8 | Failed |
| IFAW12-004Dd | 13 January 2012 | F | Mass | Died | N/A | - | - | N/A | Failed |
| IFAW12-008Dd | 14 January 2012 | F | Mass | Released | N/A | - | Kiwisat202 | 29 | Survived |
| IFAW12-012Dd | 14 January 2012 | M | Mass | Died | N/A | - | - | N/A | Failed |
| IFAW12-016Dd | 14 January 2012 | M | Mass | Released | 15 January 2012 | Found dead | - | N/A | Failed |
| IFAW12-032Dd | 16 January 2012 | M | Mass | Released | N/A | - | Kiwisat202 | 31 | Survived |
| IFAW12-033Dd | 16 January 2012 | M | Mass | Released | 22 January 2012 | Found dead | - | N/A | Failed |
| IFAW12-078Dd | 19 January 2012 | M | Mass | Released | N/A | - | Kiwisat202 | 41 | Survived |
| IFAW12-112Dd | 1 February 2012 | M | Mass | Released | N/A | - | Kiwisat202 | 67 | Survived |
| IFAW12-124Dd | 4 February 2012 | M | Mass | Released | N/A | - | Kiwisat202 | 44 | Survived |
| IFAW12-160Dd | 9 February 2012 | M | Mass | Released | N/A | - | Kiwisat202 | 30 | Survived |
| IFAW12-194Dd | 1 March 2012 | M | Mass | Released | 3 March 2012 | Rereleased | Kiwisat202 | 24 | Survived |
| IFAW12-197Dd | 6 March 2012 | M | Mass | Released | N/A | - | Kiwisat202 | 21 | Survived |
| IFAW12-201Dd | 8 March 2012 | F | Single | Released | 9 March 2012 | Found dead | - | N/A | Failed |
| IFAW12-202Dd | 9 March 2012 | F | Single | Released | N/A | - | Kiwisat202 | 22 | Survived |
| IFAW12-204Dd | 11 March 2012 | F | Mass | Released | N/A | - | Kiwisat202 | 41 | Survived |
| IFAW12-228Dd | 3 April 2012 | M | Mass | Released | 5 April 2012 | Found dead | - | N/A | Failed |

ROC $P < 0.0001$; MWW $P = 0.0117$), meaning that their girth was small relative to their length. Length-to-weight ratio, BMI ($n_F = 10$, $n_S = 12$), respiration rates ($n_F = 13$, $n_S = 17$), and heart rates ($n_F = 6$, $n_S = 14$) were not found to be statistically significant prognostic indicators of failure.

*Hematology*

Whole blood from eight failed dolphins ($n_F = 8$) and 11 survivors ($n_S = 11$) was analyzed on the in-house HM2 and from five failures ($n_F = 5$) and 14 survivors ($n_S = 14$) on the reference lab XT-V hematology analyzer. With ROC curve analysis, the following hematological parameters were found to be significantly lower in failed animals than in those that survived, across all applicable analyzers: red blood cells (HM2, XT-V), hemoglobin (HM2, XT-V, i-STAT), hematocrit (HM2, XT-V, i-STAT), and red cell distribution width (HM2). The results shown in Tables 2 and 3 include sensitivity, specificity, and cut-off values for the significant hematology and serum chemistry parameters, respectively. Nonparametric testing supported these findings for all the above parameters, except for RBC count from the HM2 (MWW $P = 0.0524$). All other parameters not listed in Tables 2 and 3 had nonsignificant findings.

Absolute monocyte counts from the XT-V were higher in failed animals (ROC curve $P < 0.0001$), but this difference was not found to be significant with nonparametric testing (MWW $P = 0.126$). Additionally, the HM2 monocyte values showed no difference between groups. The absolute difference between the failed ($F$) and survived ($S$) groups from XT-V was so small that it was not likely clinically relevant ($\bar{x}_S = 102.5$/uL, $\bar{x}_F = 112$/uL). All other hematologic values were not found to differ significantly between groups.

*Clinical Chemistry*

Reference lab AU5421 chemistry results were obtained from five failure and 15 survivor dolphins ($n_F = 5$, $n_S = 15$). Field i-STAT clinical chemistry results varied in the number of samples for each parameter as follows: $n_F = 5$ and $n_S = 8$ ($Na^+$, $K^+$, $Cl^-$, Glu, BUN, Hct, and Hgb); $n_F = 4$ and $n_S = 8$ (anGap and creatinine); $n_F = 6$ and $n_S = 6$ (BEecf, $HCO_3$, lactate, $PCO_2$, pH, $PO_2$, and $sO_2$); $n_F = 6$ and $n_S = 8$ ($TCO_2$); and $n_F = 4$ and $n_S = 6$ (iCa). Across all applicable analyzers, BUN (i-STAT, AU5421) and uric acid (AU5421) concentrations were higher in animals that failed than those that survived (Table 3). All dolphins that failed had BUN concentrations greater than 45 mg/dL as analyzed by the i-STAT (100% sensitivity), but the test specificity was only 62.5%, indicating that nearly 40% of animals with elevated BUN concentrations actually survived (ROC $P < 0.0001$, MWW $P = 0.0232$). Failed dolphins also presented with lower albumin concentrations (ROC $P = 0.00601$, MWW $P = 0.0324$) and lower albumin-to-globulin ratios (ROC $P < 0.0001$, MWW $P = 0.003$) than dolphins that survived.

Chloride concentrations as analyzed by the i-STAT were higher in dolphins that failed (ROC $P < 0.0001$, MWW $P = 0.0128$), but AU5421 chloride values did not differ between groups. i-STAT ionized calcium was found to be lower in animals that failed than animals that survived (ROC $P < 0.0001$), but nonparametric testing found the sample size too small for analysis ($n_F = 4$, $n_S = 6$). AU5421 total calcium values did not differ between groups.

Total $CO_2$ from the i-STAT differed between groups, but the AU5421 values for this parameter did not, which was likely a result of the increased time between blood

*Table 2.* Significant hematology parameters by survival type from the ROC curve analysis. Published ranges from Bossart *et al.* (2001; $n = 2$) provided when available. NS = not significant.

| Parameter | Analyzer | Category | *n* | Median (range) | ROC curve sensitivity | ROC curve specificity | ROC curve criterion | Area under ROC curve | ROC curve analysis *P*-value | Mann-Whitney-Wilcoxin *P* -value |
|---|---|---|---|---|---|---|---|---|---|---|
| Hematocrit (%) published range: 46–55 | Abaxis HM2 | Failed | 8 | 37.93 (31.41–46.16) | 100.0 | 72.7 | ≤46.16 | 0.852 | 0.0003 | 0.0117 |
| | | Survived | 11 | 48.13 (23.8–55.13) | | | | | | |
| | i-STAT | Failed | 5 | 36 (32–46) | 80.0 | 100.0 | ≤40 | 0.950 | <0.0001 | 0.0105 |
| | | Survived | 8 | 51.5 (41–59) | | | | | | |
| | IDEXX | Failed | 5 | 42.4 (38.5–54.4) | 80.0 | 100.0 | ≤45 | 0.900 | 0.0001 | 0.0108 |
| | | Survived | 14 | 55.1 (47.7–62.0) | | | | | | |
| Hemoglobin (g/dL) published range: 16.1–19.4 | Abaxis HM2 | Failed | 8 | 15.45 (13.5–18.30) | 87.5 | 90.9 | ≤16.8 | 0.920 | <0.0001 | 0.0026 |
| | | Survived | 11 | 18 (16.7–19.60) | | | | | | |
| | i-STAT | Failed | 5 | 12.2 (10.9–15.6) | 80.0 | 100.0 | ≤13.6 | 0.950 | <0.0001 | 0.0105 |
| | | Survived | 8 | 17.5 (13.9–20.1) | | | | | | |
| | IDEXX | Failed | 5 | 14.6 (13.2–17.3) | 100.0 | 85.7 | ≤17.3 | 0.957 | <0.0001 | 0.0035 |
| | | Survived | 14 | 19 (15.7–21.6) | | | | | | |
| Absolute monocytes (#/μL) published range: 120–350 | IDEXX | Failed | 5 | 122 (61–147) | 100.0 | 57.1 | >51 | 0.743 | 0.0337 | 0.126 (NS) |
| | | Survived | 14 | 48 (0–540) | | | | | | |
| Red blood cell count ($\times 10^{12}$/L) published range: 4.6–4.9 | Abaxis HM2 | Failed | 8 | 3.89 (3.08–5.40) | 7.5 | 72.7 | ≤4.62 | 0.773 | 0.0274 | 0.0524 (NS) |
| | | Survived | 11 | 5.01 (2.05–5.80) | | | | | | |
| Red blood cell count (million/μL) published range: 4.6-4.9 | IDEXX | Failed | 5 | 4.42 (3.82–5.89) | 60.0 | 100.0 | ≤4.42 | 0.821 | 0.0194 | 0.0414 |
| | | Survived | 14 | 5.75 (5.01–6.42) | | | | | | |
| Red blood cell distribution width (%) | Abaxis HM2 | Failed | 8 | 14.6 (13.3–20.4) | 100.0 | 54.5 | >13.2 | 0.801 | 0.0042 | 0.0316 |
| | | Survived | 11 | 13.2 (12.3–14.7) | | | | | | |

*Table 3.* Significant serum chemistry parameters by survival type from the ROC curve analysis. Published ranges from Bossart *et al.* (2001; $n = 2$) provided when available.

| Parameter | Analyzer | Category | *n* | Median (range) | ROC curve sensitivity | ROC curve specificity | ROC curve citerion | Area under ROC curve | ROC curve analysis *P*-value | Mann-Whitney-Wilcoxin *P*-value |
|---|---|---|---|---|---|---|---|---|---|---|
| Albumin (mmol/L) published range: 3.9–4.7 | IDEXX | Failed | 5 | 3.4 (2.8–4.0) | 80.0 | 86.7 | ≤ 3.5 | 0.833 | 0.0060 | 0.0324 |
| | | Survived | 15 | 3.8 (3.1–4.3) | | | | | | |
| Albumin: Globulin ratio | IDEXX | Failed | 5 | 0.8 (0.6–1.0) | 80.0 | 100.0 | ≤ 0.8 | 0.960 | <0.0001 | 0.003 |
| | | Survived | 15 | 1.1 (0.9–1.4) | | | | | | |
| Base excess (Beecf) (mmol/L) | i-STAT | Failed | 6 | 7.5 (4.0–13.0) | 66.7 | 100.0 | ≤ 8 | 0.806 | 0.0291 | 0.093 (NS) |
| | | Survived | 6 | 12 (9.0–15.0) | | | | | | |
| Blood urea nitrogen (mg/dL) | i-STAT | Failed | 5 | 58 (51–83) | 100.0 | 62.5 | >45 | 0.900 | <0.0001 | 0.0232 |
| | | Survived | 8 | 42 (28–55) | | | | | | |
| published range: 22–46 | IDEXX | Failed | 5 | 58 (50–74) | 100.0 | 73.3 | >48 | 0.920 | <0.0001 | 0.0067 |
| | | Survived | 15 | 42 (30–58) | | | | | | |
| Ionized calcium (mmol/L) | i-STAT | Failed | 4 | 0.99 (0.93–1.04) | 100.0 | 83.3 | ≤ 1.04 | 0.917 | <0.0001 | insufficient sample size |
| | | Survived | 6 | 1.08 (1.01–1.12) | | | | | | |
| Chloride (mmol/L) published range: 120–121 | i-STAT | Failed | 5 | 116 (110–117) | 80.0 | 100.0 | >112 | 0.937 | <0.0001 | 0.0128 |
| | | Survived | 8 | 108 (102–112) | | | | | | |
| $HCO_3$ (mmol/L) | i-STAT CG4 | Failed | 6 | 31.55 (29.3–37.0) | 83.3 | 100.0 | ≤ 33 | 0.917 | <0.0001 | 0.0203 |
| | | Survived | 6 | 37.4 (35.0–39.300) | | | | | | |
| $PCO_2$ (mmHg) | i-STAT | Failed | 6 | 47.1 (26.8–75.3) | 66.7 | 100.0 | ≤ 48.8 | 0.806 | 0.0390 | 0.093 (NS) |
| | | Survived | 6 | 59.75 (53.8–77.5) | | | | | | |
| Total $CO_2$ (mmol/L) | i-STAT | Failed | 6 | 33 (31–39) | 83.3 | 87.5 | ≤ 35 | 0.865 | 0.0011 | 0.0285 |
| | | Survived | 8 | 39 (34–41) | | | | | | |
| Uric Acid (mg/dL) | IDEXX | Failed | 5 | 0.5 (0.2–1.7) | 60.0 | 100.0 | > 0.3 | 0.860 | 0.0015 | 0.0209 |
| | | Survived | 15 | 0.2 (0.1–0.3) | | | | | | |

draw and analysis for reference laboratory (AU5421) samples. $PCO_2$ and base excess (i-STAT) were both found to be lower in animals that failed with ROC analysis, but not with nonparametric methods ($PCO_2$: ROC $P = 0.0390$, MWW $P = 0.093$; base excess: ROC $P = 0.0291$, MWW $P = 0.093$). Bicarbonate concentration analyzed by the i-STAT, was found to be lower in failed dolphins (ROC $P < 0.0001$, MWW $P = 0.0203$).

Based on the i-STAT parameters that were significant with both parametric and nonparametric analyses, failed dolphins presented with lower $TCO_2$ and $HCO_3$ than animals that survived. ROC analysis determined that a $TCO_2$ of less than or equal to 35 mmol/L indicated failure with 87.5% specificity and 83.3% sensitivity, and no survivors had a $TCO_2$ of less than 34 mmol/L, but three failures did (Table 4). Additionally, a blood $HCO_3$ concentration of less than or equal to 33 mmol/L indicated failure with 100% specificity (no surviving animals had an $HCO_3$ concentration that low), however, 16.7% of the animals that failed had an $HCO_3$ value higher than this cut-off (sensitivity of 83.3%). Higher overall lactate concentrations were seen in dolphins that died ($n_F = 6$, $\bar{x}_{\text{lactate}} = 3.05$ mmol/L, $R = 0.72$–$6.34$) compared to survivors ($n_S = 6$, $\bar{x}_{\text{lactate}} = 2.12$ mmol/L, $R = 0.79$–$4.19$). However, these differences were not found to be statistically significant (ROC Curve AUC = 0.583, $P = 0.6525$).

No other examined chemistry values were found to significantly differ between groups. Some failed animals had elevated liver (ALT, AST, GGT, LDH) and/or muscle (AST, CK, LDH) enzymes, though the differences were not statistically significant. The high outlier LDH and AST values in failed animals did not correlate with high MCHC, indicating that hemolysis was not the driving factor for these parameters. No surviving dolphins, but some failed dolphins, had CK > 397 U/L ($n_F = 2/5$), ALT > 432 U/L ($n_F = 1/5$), AST > 1,059 U/L ($n_F = 1/5$), LDH > 1,197 IU/L ($n_F = 2/5$), GGT > 138 U/L ($n_F = 1/5$), or lactate > 4.19 mmol/L ($n_F = 1/6$).

Of the parameters that were found to differ significantly by survival group, none were found to differ by sex or stranding type when nonparametric tests were applied to the rebatched data set. See Tables S1 and S2 for hematological and biochemical parameters by sex and by stranding type. In general, failed animals presented with a wider range of values than survivors in nearly every parameter.

*Table. 4.* Suggested indicators of poor prognosis. Ranges with 100% specificity for failures, *i.e.*, no survivors had values in these ranges.

| Parameter (analyzer) | Total tested | | Poor prognosis range | Test sensitivity ($n_F$ in range) |
|---|---|---|---|---|
| | $n_F$ | $n_S$ | | |
| Hemoglobin (i-STAT) | 5 | 8 | <13.9 g/dL | 40% (2) |
| Hemoglobin (HM2) | 8 | 11 | <16.7 g/dL | 75% (6) |
| Hematocrit (i-STAT) | 5 | 8 | <41% | 80% (4) |
| $HCO_3$ (i-STAT) | 6 | 6 | <35 mmol/L | 83% (5) |
| $TCO_2$ (i-STAT) | 6 | 8 | <34 mmol/L | 50% (3) |
| BUN (i-STAT) | 5 | 8 | >55 mg/dL | 60% (3) |
| Uric Acid (IDEXX) | 5 | 15 | >0.3 mg/dL | 60% (3) |
| Length:Girth Ratio (N/A) | 6 | 8 | >2.05 | 33% (2) |

*Necropsy and Sample Analysis*

Necropsies were performed on 9 of the 10 failed dolphins; IFAW11-249Dd was not necropsied due to its advanced state of decomposition at the time of report. Histological analyses were completed on eight of nine necropsied animals (not IFAW12-012Dd), encompassing one partial (IFAW12-004Dd) and seven complete tissue sample sets. Lesions were found in multiple body systems in all but three dolphins (IFAW12-004Dd, IFAW12-012Dd, and IFAW12-016Dd) grossly and all but the dolphin with a partial sample set, histologically. Table S3 presents a summary of all histological lesions observed in the sampled failed dolphins. Only lesions of suspected clinical significance are noted here.

Observations in the respiratory tract that may have contributed to the stranding in 4/9 necropsied dolphins included chronic interstitial pneumonia ($n = 3$), verminous pneumonia (nematodiasis $n = 1$; tematodiasis $n = 1$), pulmonary congestion ($n = 1$), edema ($n = 2$), fibrosis ($n = 1$), suspected aspiration pneumonia ($n = 1$), and gross pleural adhesions with a diptheritic membrane covering the cranial pleura ($n = 1$). Pertinent lesions in the hepatobiliary system in 4/9 dolphins included the following: acute centrilobular hepatocellular necrosis ($n = 1$) and chronic periportal hepatitis and biliary hyperplasia ($n = 3$), with verminous hepatitis (trematodiasis: *Campulla sp.*) in one case.

Two necropsied dolphins had significant gross abnormalities in the cardiovascular system. One dolphin had a fluid-filled pericardial sac (35 mL) and widespread congestion of the peripheral blood vessels; another had pericardial adhesions (suspect restrictive pericarditis) and pale myocardial tissue. Only mild, multifocal myocardial fibrosis was found in these two animals histologically. Musculoskeletal lesions of significance were found in four dolphins, all of which were thin or emaciated on necropsy and two of which had flexure of the caudal spine (peduncle). One animal with spinal curvature also had muscular atrophy, a fibrotic peduncular mass, chronic peduncular tendonitis, and dermatitis at the site of flexure (a restranded animal). The other dolphin with spinal flexure also had occipital joint fibrosis. The only significant gastrointestinal abnormality observed in the nine necropsied animals was acute pancreatic hemorrhage in one dolphin. No significant urinary tract findings were observed.

Two dolphins were pregnant, one with a 37 cm male fetus and the other with a 54 cm female fetus. The amniotic fluid of the larger fetus was green-tinged, but the fetuses were otherwise normal. Reproductive tract lesions were observed in the two dolphins with spinal flexure, including testicular interstitial fibrosis and interstitial orchitis in the male and oophoritis and granulomatous perimetritis in the female. Additional gross lesions of potential significance included one dolphin with gross verminous otitis media and another with icteric blubber that corresponded to observed hepatic lesions and clinically elevated liver values. An incidental finding in one dolphin was lymph node angiomatosis, which has primarily been reported in Atlantic bottlenose dolphins, *Tursiops truncatus* (Rawson *et al.* 1992, Turnbull and Cowan 1999). Pulmonary angiomatosis has been reported in common dolphins in association with verminous pneumonia (Diaz-Delgado *et al.* 2012), but was not observed in the animals examined.

Five of the nine necropsied dolphins, all from 2012, had samples sent for *Brucella* analysis. The two dolphins with musculoskeletal and reproductive tract lesions tested positive for *Brucella* sp., one on serology, tissue culture (ovary) and PCR/genetic sequencing (fibrotic mass) and the other on tissue culture (spleen and testis) and

PCR/genetic sequencing (spleen). The remaining three cases, including the two pregnant animals, tested negative for *Brucella* sp. through the following tests: whole blood PCR and cELISA and tissue PCR ($n = 1$); serology and tissue PCR and culture ($n = 1$); or tissue PCR and culture ($n = 1$). Five of the 16 survivors had serum ($n = 3$) or whole blood ($n = 2$) sent for *Brucella* cELISA analyses. Two dolphins were positive on serum cELISA (IFAW12-194Dd, IFAW12-204Dd) and the remaining three were negative.

Three of the necropsied dolphins had biotoxin samples analyzed. Two dolphins (IFAW12-003Dd, IFAW12-016Dd) were negative for all toxins (saxitoxin, domoic acid, okadaic acid and its congeners). The only fecal sample submitted (IFAW12-033Dd) had trace amounts of pectenotoxin-2 present. All other samples from this animal (gastric contents and liver) were negative for all tested biotoxins.

Based on the combined set of diagnostics, necropsy and histological examinations for each necropsied animal, the following is a list of the most likely cause of stranding and/or death for each case: parasitism (pulmonary nematodiasis in IFAW11-026Dd and trematodiasis in IFAW12-033Dd), infectious pneumonia, likely aspiration pneumonia (IFAW11-252Dd), brucellosis and chronic liver disease (IFAW12-003Dd, IFAW12-228Dd), terminal hypoxia possibly exacerbated by chronic pulmonary fibrosis (IFAW12-016Dd), and undetermined/spontaneous (including both pregnant dolphins: IFAW12-004Dd, IFAW12-012Dd, IFAW12-201Dd).

## Discussion

In summary, differences in certain blood parameters and length-to-girth ratios were found between dolphins that survived and dolphins that failed, signifying that these may be valuable prognostic indicators for stranded common dolphins. No differences were found in any parameter when examined by sex or stranding type. Previous studies have shown sex-specific differences in some blood parameters for bottlenose dolphins (Goldstein *et al.* 2006, Venn-Watson *et al.* 2007, Schwacke *et al.* 2009), and further work may help elucidate whether the observed similarity here is in fact a new finding for common dolphins. Likewise, the comparable values among single and mass stranded dolphins is interesting, since it is commonly believed that singly stranded dolphins are more often ill or injured than mass stranded dolphins (Geraci and Lounsbury 2005, Bogomolni *et al.* 2010). However, it is beyond the scope of this study to determine whether mass and single stranded dolphins are equally healthy or equally compromised. Additionally, it is important not to use the survived *vs*. failed ratio in this paper as an indicator of the prevalence of animals that actually lived following release, in that by default we cannot evaulate the number of nonsatellite tagged animals that survived. Thus, this study simply compares the survivor *vs*. failure subsets for the utility of different triage parameters in terms of prognostic value.

While statistically significant differences were found between survivors and failures for a number of parameters, their level of clinical significance must also be evaluated. Failed dolphins had lower serum albumin concentrations, albumin:globulin ratios, and red blood cell counts, as well as higher red cell distribution width compared to survivors, but the statistical significance of these differences does not translate into clinical significance (see Table 2, 3). Additionally, the discrepancy across analyzers regarding the significance of the chloride comparison between groups was likely due to a previously reported i-STAT error. Varela *et al.* (2006) found that an increased

BUN concentration (as seen in the failed dolphins) caused a falsely elevated chloride reading on the i-STAT analyzer (also as seen in the failed dolphins), and thus the observed difference on the i-STAT in the present study is likely a result of this analyzer artifact.

The significant parameters that warrant clinical consideration and further discussion include length:girth ratio, Hct, Hgb, $TCO_2$, $HCO_3$, BUN, and uric acid (especially when the latter two are combined). Given the small sample size and considerable overlap in the ranges of most of the parameters for survivors and failures from this study, disposition decisions based on statistical or even clinical significance should be made with caution. Grouping animals with a potentially wide variety of ailments together for analysis may moderate extreme values and dilute prognostic efficacy. Furthermore, there are inherent pitfalls in using assumed survivors as the control for comparisons with known failures. Since no normal reference ranges are available in this species for evaluation, this approach was warranted, but the importance of taking a conservative approach when interpreting these data must be underscored.

When deciding whether or not to euthanize a stranded dolphin, it seems best to focus on more specific tests that are most likely to exclude surviving dolphins. With this in mind, we examined the data with regards to tests that have 100% specificity for failure in the clinically significant parameters, the summary of which is presented in Table 4. For physical parameters, no survivors had length:girth ratios >2.05, while two failures fell within this range. Regarding hematological parameters, 2/5 failed dolphins had hemoglobin <13.9 g/dL on the i-STAT, 6/8 failed dolphins had hemoglobin <16.7 g/dL on the HM2 and 4/5 failed dolphins had hematocrit <41% on the i-STAT, while no survivor dolphins had values that fell within these ranges. From chemistry and blood gas analyses on the i-STAT analyzer, 5/6 failures had $HCO_3$ <35 mmol/L, 3/6 failures had $TCO_2$ <34 mmol/L, 3/5 failures had BUN >55 mg/dL, and the same 3/5 animals had uric acid >0.3 mg/dL from IDEXX, while no survivors had values that fell within these ranges. Of all the failed dolphins, 7/10 had parameters that fell within the 100% specific ranges shown in Table 4. Six of these animals had more than one parameter within these ranges: two failed animals had 6/7 and one each had 5/7, 4/7, 3/7, 2/7, and 1/7 poor prognosis parameters. We could have improved our prognostic efficacy for the failed animals in this study by 60% by using the criterion for poor prognosis as an animal with more than one parameter within the ranges on Table 4. While more data are needed to support the establishment of more stringent release criteria, this set of poor prognosis ranges may be a useful tool for responders in the field in combination with a complete physical and behavioral exam and any additional available diagnostics.

*Anemia*

One of the clearest patterns that developed between failures and survivors from this study was that failed dolphins presented more anemic than survivors, as indicated by clinically significant decreases in hematocrit percentage and hemoglobin concentration. Many factors could cause anemia in these animals, including inflammation/chronic disease, liver disease, poor nutritional status, pregnancy, or low-grade blood loss. The lack of difference in MCV and MCHC among the groups complicates clinical discrimination between these causes. The MCV and MCHC of the seven failed animals with poor prognosis Hgb values (four of which also had poor prognosis Hct values) were examined individually and compared to the ranges presented in Bossart

*et al.* (2001) for common dolphins (MCV 100–114 fL; MCHC 35–40 g/dL) and Schwacke *et al.* (2009) for capture-release bottlenose dolphins (MCV 103–131 fL; MCHC 32–37 g/dL). Based on these comparisons, the best description of these dolphins' anemia is micro/normocytic (MCV: 88–106 fL) and normochromic (MCHC: 32.1–42.9 g/dL), suggesting a potentially nonregenerative process, which is uncommon in marine mammals (Bossart *et al.* 2001). However, the reference lab blood smear analysis reported slight polychromasia and anisocytosis from two animals with increased RDWc% on the in-house HM2 analyzer, signifying that the anemia in some animals may be regenerative in nature.

For the dolphins with nonregenerative anemia, chronic disease/inflammation, liver disease, pregnancy, or poor nutritional status are possible differentials. Anemia of chronic disease in dolphins has been associated with a decrease in albumin (observed, but not likely clinically significant here), alkaline phosphatase (not observed), and serum iron (not examined), as well as an increase in plasma fibrinogen, erythrocyte sediment rate (not examined), and white blood cell count (not observed) (Bossart *et al.* 2001). In light of the chronic disease processes observed in 5/9 necropsied dolphins, this etiology of anemia cannot be ruled out. One of the microcytic dolphins (IFAW12-003Dd) had evidence of substantial liver disease, which may have caused a nonregenerative anemia in this animal and is a reported cause of microcytic anemia in dogs and cats. Both liver disease and inflammation/chronic disease can cause anemia, in part, through abnormalities in iron metabolism. If the anemia was due to pregnancy, Hgb and Hct values would likely have differed between the male and female groups, which was not the case. Nutritional deficiencies, although rare in animals, may result in a nonregenerative anemia. Iron deficiencies caused by poor nutrition could potentially lead to the observed microcytosis of some of the anemic dolphins. The theory of nutritional deficiency causing anemia is additionally supported by the poorer body condition (larger length:girth ratio) and dehydration (increased BUN and uric acid) of failed animals.

For the animals with evidence of regeneration, their anemia may have been caused by low grade blood loss or iron deficiency. It is likely that these animals may have been stranding with or because of a preexisting condition, especially given the 3–5 d lag for regeneration to become evident in peripheral blood. However, no origins of chronic blood loss were identified in postmortem examinations of failed dolphins, nor were their total serum protein concentrations decreased. Another potential cause for the anemia is parasitic infection, given that the death of one anemic dolphin was attributed, at least partially, to pulmonary trematodiasis. However, the second animal with significant parasitism was not anemic, and neither the trematode nor nematode genera identified in these two animals traditionally cause anemias in marine mammals. Further research into the etiology of the anemia is needed, but it appears to be due to preexisting conditions and should be considered poorly prognostic when making disposition decisions.

### *Acid-Base Balance*

Failed animals were found to have lower $HCO_3$ and $TCO_2$ than animals that survived, indicative of a metabolic acidosis. This condition could be anticipated for stranded dolphins based on their muscular exertion when attempting to swim while beached, and has been suggested but not demonstrated in the literature (St. Aubin 2002, Sampson *et al.* 2012). There was no significant difference in lactate levels between failures and survivors, but one failure presented with the most

extremely elevated lactate value. Metabolic acidosis may also be indicative of anaerobic cellular respiration due to tissue ischemia that results when aquatically adapted animals lie recumbent while stranded (Herráez *et al.* 2007). The absolute values of the above parameters however, were not low enough to be indicative of a classic terrestrial animal metabolic acidosis ($\bar{x}_F$ $HCO_3$ = 32.18 mmol/L; $\bar{x}_S$ $HCO_3$ = 37.15 mmol/L). In the current study, the mean time from blood draw to analysis for blood gas samples was 34.8 min for failed dolphins and 24.3 min for survivors, which may have affected the results. Varela *et al.* (2006) found that dolphin blood gas samples were best when run within 10 min of sample collection. Future work should specifically focus on reducing the time between sample collection and analysis.

*BUN and Uric Acid*

Increased BUN and uric acid concentrations for the failed animals indicate that they may have been more dehydrated that those that survived (Bossart *et al.* 2001). The three failed dolphins with poor prognosis BUN values were the same three that had poor prognosis uric acid values (Table 4), potentially suggesting a higher level of prognostic value when both parameters are elevated and within these ranges. In addition to dehydration, increased BUN can also be caused by other processes that decrease the glomerular filtration rate, such as heart failure or vascular collapse (shock), both of which have been known to have stranding-related etiologies (Turnbull and Cowan 1998, Geraci and Lounsbury 2005, Herráez *et al.* 2007). Since creatinine levels were normal, either a process that does not markedly elevate creatinine (GI hemorrhage or high protein diet) or an early stage prerenal azotemia that had not yet caused an elevation in creatinine values may have been involved. The latter explanation is more likely than the former since no significant GI hemorrhage was observed on necropsy and a high protein diet in failures is not likely. In dolphins, elevated uric acid concentration is indicative of dehydration, but is not generally useful in evaluating liver or kidney function (Bossart *et al.* 2001). Importantly, if failed animals were suffering from dehydration as suspected, their concurrent anemia was likely more severe than detected by the analyzers.

Failed animal dehydration was likely caused by malnutrition, either from decreased food intake or decreased absorptive capacity, since dolphins derive all their water from food. No significant chronic gastrointestinal lesions were observed to indicate that malabsorption was the cause, and as such, decreased food consumption is more likely. In addition, the larger length-to-girth ratios of failures indicate that these animals were less robust in body condition than their survivor counterparts. This decreased fitness suggests that a preexisting disease process may have contributed to the occurrence of the initial stranding event and eventually to the animal's demise. The lack of a difference in length-to-weight ratios and BMI between the groups is perplexing, as it would be logical that weight would increase in proportion to girth. Seasonal differences may play a part in creating this discrepancy in terms of natural variations in nutritional condition and/or gestation/lactation stages of females, but there was not enough seasonal variability in the data set to examine this hypothesis fully. It should be noted that none of the dolphins in either group were classified as emaciated during the subjective staff health assessment at the time of initial stranding, but a higher proportion of failed animals were noted as thin or slightly thin (33%) than survivors (19%) and four of the failures were classified as thin or emaciated at necropsy. Recent ingesta are rarely if ever

found on necropsy in the upper gastrointestinal tract of stranded dolphins in this locale, whether or not evidence of disease or emaciation is present, and thus this metric is not useful for evaluating recent feeding activity. Further work is needed to properly assess the relationships between morphological parameters (girth, BMI), feeding behavior, energy and fluid balance, and overall health status in stranded dolphins.

*Capture Myopathy*

Findings from a few of the failed animals in this study may be suggestive of stranding-induced capture myopathy (CM), a metabolic muscle disease found primarily in wildlife (Spraker 1993). Cetaceans have been suggested to suffer from CM during stranding and transport events (Colgrove 1978; Herráez *et al.* 2007, 2013), during which the animal's system is flooded with endogenous catecholamines that help meet the body's metabolic requirements by altering blood flow and increasing metabolism to the most vital systems. A byproduct of this process is a decrease in perfusion of other tissues and resulting ischemia, acidosis, and potential necrosis.

The centrilobular zone hepatocellular necrosis found in one of the failed dolphins was likely caused by stranding-induced ischemia, either from capture myopathy or physical vessel obstruction. Animals with CM or any decrease in tissue perfusion will be more likely to be susceptible to reperfusion injuries postrelease (usually within 24–48 h), and would presumably be more likely to fail. Four of the ten failed dolphins died or were found dead within two days of their initial release, supporting the timeline of this disease process. Clinical detection of the degree of tissue perfusion present in stranded dolphins will likely improve the selection process for release candidates and result in the release of a higher percentage of fit animals that are more likely to survive postrelease.

Capture myopathy is difficult to diagnose clinically (Herráez 2007), but certain serum chemistry values may aid in clinical screening, including elevated AST, BUN, CK, and LDH levels (Spraker 1993), such as those seen in some of the failed animals in this study. Elevations in AST and CK were observed in mass stranded striped dolphins from Western Australia, while LDH and BUN levels were not examined (Gales 1992). In our study, failed dolphins had higher BUN concentrations than survivors and while AST, CK, and LDH did not significantly differ between groups, all the exceptionally high outliers observed for AST, CK, and LDH were noted in the failed group. These findings indicate that at least some failed dolphins may have experienced capture myopathy, which may in turn have affected their chance of postrelease survival. Interpreting some of these CM-related values must be done conservatively though, as AST and LDH are nontissue specific and both are potentially increased by sample hemolysis (not in this study) and other preexisting conditions, such as liver disease (a condition observed in two failed dolphins). Capture myopathy should also be considered as a differential diagnosis in animals with metabolic acidosis, a disorder also observed in failed animals of this study. As such, having access to LDH, CK, AST, BUN, and blood gas values in the field may prove beneficial for future prognostication. However, more research and an increased sample size are needed to further establish the effectiveness of these parameters as prognostic indicators.

Other CM-associated lesions previously documented in stranded dolphins from other locales, such as skeletal or cardiac rhabdomyolysis or renal lesions were not observed in the failed dolphins in this study. It is unclear whether that is due to a true

lack of lesions or a sampling or processing bias. In the future, a more intensive and targeted sampling method, such as in Turnbull and Cowan (1998) combined with immunostaining as in Herráez *et al.* (2007, 2013) would be a more sensitive approach to detect the prevalence of CM in failed stranded dolphins on Cape Cod.

*Comparative Hematology and Clinical Chemistry*

Compared to the ranges published in Bossart *et al.* (2001) for common dolphins, this data set represented wider ranges in all but one parameter (total bilirubin) for both survivors and failures. This is not surprising, considering the inherent individual variation in normal blood values (Hall *et al.* 2007) and the fact that the number of individuals in this study was thirteen times greater than those included in Bossart *et al.* (2001). This wider range may also reflect that these animals are not in optimal health during the stranding event, and it should be emphasized that the ranges for survivors presented here are not intended to be interpreted as "normal" common dolphin values.

Blood values from stranded *D. delphis* in this study predominantly overlapped with ranges found for capture-release bottlenose dolphins, *Tursiops truncatus* (Schwacke *et al.* 2009), though some differences of potential clinical significance were observed. Compared to wild-caught *T. truncatus*, stranded *D. delphis* often had lower WBC, Neut, Eos, Na, Cl, Ca, P, total protein, Alb, A:G, MCV, MCH, MCHC, BUN, and creatinine. Additionally, stranded dolphins often had higher lymph, Glu, ALP, ALT, AST, LDH, GGT, glob, uric acid, CK, RBC, Hgb, Hct, and BUN:creatinine than the *T. truncatus* in that study. Total bilirubin levels of stranded *D. delphis* were entirely higher (0.2–0.90 mg/dL) than the range found for *T. truncatus* (0–0.2 mg/dL), though this may not be a clinically significant difference. It is worth noting, however, that the total bilirubin concentrations from *D. delphis* in this study agreed with the range from *D. delphis* in Bossart *et al.* (2001). Potassium levels in stranded dolphins had a wider range (2.4–6.4 mEq/L) than capture-release bottlenose dolphins (3.2–4.5 mEq/L), which may have clinical significance since potassium is tightly maintained within a narrow physiological range. The overall differences between these two data sets were relatively small in magnitude except for the values of ALP, ALT, AST, LDH, GGT, and CK, which were considerably higher in stranded dolphins. These differences may suggest a few things: that there may be dissimilar stress processes involved in the stress of stranding *vs.* the stress of capture and release, that the stress of capture-release is more acute than that of stranding, that stranded animals are less healthy than capture-release dolphins, or that different species of dolphins have different physiological ranges for these parameters. Given the magnitude of these particular differences, it seems more likely to be a pathological difference rather than a species-driven one.

*Survival Metric*

This study was conducted under the assumption that satellite tag transmission durations of 3 wk are sufficient to indicate postrelease success. This duration was deduced to be a reasonable predictor of survival based on previous publications on small delphinid satellite tagging that used transmission durations between 9 d and 1 mo as indicators of survival (Wells *et al.* 2008, 2009; Sampson *et al.* 2012). A more recent retrospective review that was published after this study was conducted indicated that a minimum six week transmission period is a better estimate of postrelease

survival (Wells *et al.* 2013). However, certain conditions of our study made it more likely that the satellite tags would have a shorter life span than those in this recent review, including the following: the satellite tags were all single-pin (not three-pin), common dolphins have smaller and thinner dorsal fins for attachment and faster average swim speeds (Rohr *et al.* 2002) than bottlenose dolphins, animals were tagged in the western North Atlantic during the winter months when offshore sea conditions were known to be rough, and the tags were programmed with a shorter expected battery life of only 45–60 d. The survivors' transmitted locations from this study were documented within known common dolphin habitat, further suggesting postrelease success. Given all of these factors, the authors believe that the 3 wk tag transmission duration was an acceptable metric for common dolphin survival in the western North Atlantic and is likely a sufficient indication of postrelease survival for this group of animals.

*2012 Stranding Event*

Since 65% (17/26) of the animals in this study stranded during one year (2012), the data set may be biased by the circumstances of that particular year. Extensive samples were collected from the 2012 dolphins and some are still pending further analyses, but thus far, no single common factor has been implicated in causing this unusually large and extended stranding. Four of the 10 dolphins tested for *Brucella sp.* from this study, all from 2012 were found to be positive (two survivors and two failures). Bogomolni *et al.* (2008) found that 3/5 common dolphins sampled from a single mass stranding event were positive for *Brucella* on brain and uterus PCR, indicating that the proportion of animals in this study with *Brucella* infection is not a novel finding. The clinical significance of the trace biotoxin levels that were detected in one of the three analyzed dolphins from this study can be difficult to interpret, given the lack of data regarding background levels in this population, the small quantity detected, and the lack of other fecal samples for comparison. No corresponding histological lesions in the GI tract were observed in the pectenotoxin-2-positive dolphin, though this is not unexpected even in clinical cases. Overall, the affected body systems from the histologically analyzed samples from this study were similar to those documented from other stranding events (Hohn *et al.* 2006, Bogomolni *et al.* 2010). The most common disease processes found in stranded cetaceans from Bogomolni *et al.* (2010) were bacterial pneumonia and verminous pneumonia, both of which were determined to be causes of death in failed animals in this study as well.

*Environmental Parameters*

The lack of difference between air and water temperature on the day of stranding among survivors and failures may be a result of the lack of wide temperature variation in this data set, since all but two of the stranded animals came ashore between late November and early April. The total time stranded was not available for the dolphins in this study, but this parameter may play a role in determining postrelease success, especially with regard to the dolphin in this study with acute tissue hypoxia. Although frequently difficult to deduce, stranding responders should make efforts to document or estimate total time stranded to better answer questions regarding the importance of this parameter as a prognostic indicator for stranded cetaceans.

*Conclusions and Future Work*

In summary, this study has demonstrated that length-to-girth ratios, hematocrit, hemoglobin, $TCO_2$, $HCO_3$, and the combination of BUN and uric acid, may be useful in predicting the survival of stranded common dolphins. Hct, Hgb, $TCO_2$, and $HCO_3$ were all lower and BUN and uric acid were all higher in animals that failed. This study found that no surviving dolphins had these parameters falling within the ranges in Table 4, and those with multiple poor prognostic indicators may be at greater risk for failure postrelease. Dolphins whose stranding and/or death was partially attributed to chronic liver disease demonstrated clinically elevated serum liver values. Having access to these parameters in the field as well as those involved with capture myopathy may be useful diagnostic tools for stranding personnel. While this study represents the largest collection of standardized blood samples from stranded common dolphins to the authors' knowledge, our results should be interpreted cautiously with an understanding of their limitations given the small sample size and difficult field conditions. Future work should focus on increasing the sample size to more robustly evaluate the efficacy of these parameters as prognostic indicators. Particular attention should be paid to improving the evaluation of preexisting illnesses (especially liver disease, brucellosis, and pneumonia), stranding-related acid-base disturbances and capture myopathy in stranded dolphins through thorough clinical and postmortem analyses.

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## Supporting Information

The following supporting information is available for this article online at http://onlinelibrary.wiley.com/doi/10.1111/mms.12093/suppinfo.

*Table S1*. Nonparametric analysis by sex and stranding type of the significant hematological parameters from the 26 *Delphinus delphis*.

*Table S2*. Nonparametric analysis by sex and stranding type of the significant serum chemistry parameters from the 26 *Delphinus delphis*.

*Table S3*. Histology summary from subset of failed dolphins.