

BLOOD CHEMISTRY AND HEMATOLOGY VALUES IN HEALTHY AND REHABILITATED ROUGH-TOOTHED DOLPHINS (*STENO BREDANENSIS*)

Charles A. Manire,^{1,5} C. Melanie Reiber,^{2,6} Cécile Gaspar,³ Howard L. Rhinehart,^{1,7} Lynne Byrd,¹ Jay Sweeney,⁴ and Kristi L. West^{2,8,9}

¹ Dolphin and Whale Hospital, Mote Marine Laboratory and Aquarium, 1600 Ken Thompson Parkway, Sarasota, Florida 34236, USA

² College of Natural and Computational Sciences, Hawaii Pacific University, 45-045 Kamehameha Hwy., Kaneohe, Hawaii 96744, USA

³ Dolphin Quest French Polynesia, BP 1021, Papetoai, 98729, French Polynesia

⁴ Dolphin Quest, 4467 Saratoga Ave., San Diego, California 92107, USA

⁵ Current address: Loggerhead Marinelife Center, 14200 US Highway One, Juno Beach, Florida 33408, USA

⁶ Current address: Espenmoosstrasse 4, 9008 St. Gallen, Switzerland

⁷ Current address: PO Box 11283, Hilo, Hawaii 96721, USA

⁸ Current address: Hawaii Institute of Marine Biology, University of Hawaii at Manoa, PO Box 1346, Kaneohe, Hawaii 96744, USA

⁹ Corresponding author (email: kristiw@hawaii.edu)

ABSTRACT: Rehabilitation efforts for live stranded marine mammals are guided by diagnostic measures of blood chemistry and hematology parameters obtained from each individual undergoing treatment. Despite the widespread use of blood parameters, reference values are not available in the literature from healthy rough-toothed dolphins (*Steno bredanensis*) with which to infer the health status of an animal. We examined serum or plasma chemistry and hematology data from 17 rough-toothed dolphins either housed at Dolphin Quest French Polynesia or during their rehabilitation at the Dolphin and Whale Hospital in Sarasota, Florida, US between 1994 and 2005. Blood parameters were compared among healthy animals, rehabilitation animals that were eventually released, and rehabilitation animals that died. This study indicated significant differences in many blood parameters for the poorly known rough-toothed dolphin that are likely to vary between healthy and sick animals. These included aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, bicarbonate, and globulins, which were greater in sick dolphins, and alkaline phosphatase and total protein which were greater in healthy individuals. Total white blood cell counts were lower in healthy animals as were the absolute numbers of neutrophils, monocytes, and eosinophils. Analysis of first blood sample levels for glucose, sodium, and erythrocyte sedimentation rate may have value for triage and prognostic evaluation.

Key words: Cetacean, clinical pathology, hematology, rehabilitation, rough-toothed dolphin, *Steno bredanensis*.

INTRODUCTION

The rough-toothed dolphin, *Steno bredanensis*, inhabits warm pelagic waters throughout the world's oceans (West et al. 2011). In the southeastern US, rough-toothed dolphins are commonly sighted in the Gulf of Mexico and along the Atlantic coast (Davis et al. 1998) and have mass-stranded during several isolated events in Florida waters between 1997 and 2005 (Wells et al. 1999; Waring et al. 2009).

Postmortem investigations of stranded dolphins of various species indicate the majority of stranded animals are affected with serious disease such as bronchopneumonia, fibrosis,

gastroenteritis, pancreatitis, hepatitis, and encephalitis at the time of death (Bogomolni et al. 2010; Arbelo et al. 2013; Bossart et al. 2013). The state of health of individual dolphins is evaluated, in part, by clinical pathology where substances in serum or plasma are measured. Because these are closely related to the physical state of the individual animal independent of species, these parameters are routinely used to help identify acute, chronic, and pathologic conditions in stranded animals antemortem (Lutz and Dunbar-Cooper 1987; Walsh et al. 2001). In some cases, blood samples are taken and hematologic data obtained to aid in determin-

ing the most-likely candidates for rehabilitation, to help establish a prognosis, and to implement and monitor a treatment regimen (Geraci and Lounsbury 2005; Sharp et al. 2014).

Hematology and blood chemistry data are available for several species of delphinids, but in the case of rough-toothed dolphins the only reference blood values available in the published literature are from two animals from Florida sampled over a 2-wk period just prior to release following rehabilitation (Manire and Rhinehart 2000) and a single blood sample from a stranded newborn calf from Brazil (Bastos et al. 2004).

Our objectives were to: 1) compare blood chemistry and hematology data between healthy and unhealthy rough-toothed dolphins to establish “normal” and “abnormal” values for this species; and 2) evaluate the accuracy of initial blood samples in predicting outcome during rehabilitation of rough-toothed dolphins.

MATERIALS AND METHODS

Animals

Fourteen rough-toothed dolphins that were part of three mass strandings in Florida and had undergone rehabilitation between 1997 and 2005 at the Mote Marine Laboratory, Dolphin and Whale Hospital (DWH) in Sarasota, Florida were included in this study (see Supplementary Material Table S1). Ten of these dolphins, two females and eight males, were cared for until they either died ($n=5$) or were successfully rehabilitated and released ($n=5$). Four others died soon after arrival and were included only in the initial blood sample analysis. Also included were three rough-toothed dolphins that were housed in Moorea, French Polynesia, at Dolphin Quest French Polynesia (DQFP), each for a minimum of 3 yr between 1994 and 2001 (Supplementary Material Table S1). In French Polynesia, Dolphin 16, a juvenile male, and Dolphin 17, a juvenile female, were wild-caught in May 1994. Both were healthy until their deaths (2001 and 1997, respectively); causes of death were unknown. Dolphin 15, a stranded juvenile female, was considered healthy after 150 d of care following her stranding in 1997 until her death from drowning in 2000 (Supplementary Material Table S1).

Medication

All animals were treated with various drugs during their rehabilitation until they reached healthy status or died. Because administered medication was changed frequently (in response to its effect on each individual), the influence of medication was not considered in this study. However, at both DQFP and the DWH, the dolphins rarely received any medication that would directly influence blood values. At DQFP, the animals were only treated with multivitamins and antibiotics for short periods of time. At DWH, all dolphins received a variety of antibiotics, probiotics, ulcer treatment (sucralfate and proton pump inhibitors), and multivitamins. Occasionally, and only in particular cases, the dolphins would receive antiparasitic, analgesic, aspirin, or anti-fungal (the latter known to affect liver enzyme levels occasionally) drugs and rarely corticosteroids (known to increase glucose levels).

Blood sampling and hematologic measurements

On a regular basis (usually every other day at the start of care and once or twice a week prior to release), blood samples were collected from each rehabilitation dolphin at DWH. Approximately 10 mL of blood were taken by venipuncture from the central tail vein in the fluke while the dolphin was restrained (necessary for wild, untrained animals). In most cases at DQFP, blood was collected as a trained voluntary procedure where dolphins were sampled approximately bimonthly for 3 yr. Dolphin P was initially sampled daily and transitioned to bimonthly sampling during the first 60 d of care. Hematologic parameters were obtained within 24 h of sampling using standard protocols and an electronic cell counter (Sysmex XT-2000i for DQFP and Sysmex XE 2100 for DWH; Sysmex America Inc., Mundelein, Illinois, USA) and manual cell differentials established counts of white blood cell parameters. The serum or plasma parameters were also obtained within 24 h on automated equipment (Hitachi 912 Roche for DQFP and Hitachi Modular Chemistry System for DWH; MedTechTrade AG, Uster, Switzerland). Electrolytes considered in this study included potassium, sodium, calcium, chloride, and bicarbonate (total CO_2). Measured liver parameters included total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (AP) and protein parameters included total protein and albumin. Globulin values were calculated. Measured or calculated red blood cell parameters included total red blood cell count (RBC), hemoglobin, hematocrit, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume

(MCV), and erythrocyte sedimentation rate (ESR). White blood cell parameters included total white blood cell count (WBC) and absolute counts of neutrophils, lymphocytes, monocytes, and eosinophils. Not all tests were run on every sample, hence the n value for each test varied.

Sample grouping

Each blood sample was categorized as representing one of three groups. Healthy samples were defined as those collected from captive individuals in French Polynesia (except for the first 150 d of rehabilitation for Dolphin P) or within 30 d prior to release for the surviving animals at DWH. Surviving-sick samples were categorized as those from compromised individuals that ultimately survived, and those samples were collected for the first 150 d of care for Dolphin P at DQFP and up until 30 d prior to release during rehabilitation at DWH. Fatally-sick samples represented collection during the entire rehabilitation of individuals that eventually died. To compare methodologies used by the two facilities, the healthy samples from each facility were compared.

Reference ranges for rough-toothed dolphins were determined by using bootstrapping procedures to calculate 95% reference intervals around the median for each hematologic parameter in the healthy dolphin group. Although data were normally distributed for the parameters of calcium, globulin, RBC, hemoglobin, and platelets, bootstrapping procedures were consistently applied to all parameters to establish reference intervals. To determine the validity of using a single blood sample to make decisions regarding rehabilitation following mass strandings, the first blood draws from 14 rough-toothed dolphins admitted at DWH were considered separately. Of these, nine died or were euthanized within 78 d of admission and five were rehabilitated and released. From the first blood sample upon admission, irrespective of survival, individual parameters were identified for further examination if 40% or greater of the values represented outliers when compared to the raw data distribution of the healthy group. The first blood sample values for sodium, AST, ALT, ESR, glucose, and lactate dehydrogenase (LDH) were in the outlier range for 40% or greater of the samples. Values for these six parameters were then compared to survival outcome.

Statistics

All data were tested for normality and homogeneity of variance. When data failed the Levene's test for equality of variance, data were transformed using a reciprocal transformation for

calcium and an exponential transformation for total protein, albumin, and hemoglobin. A one-way analysis of variance with Tukey's honest significant difference post hoc test using SPSS version 14 (SPSS Inc., Chicago, Illinois, USA) was performed to determine if significant differences were present among the three groups. When comparing values between the two facilities, a t -test was used for calcium. Neutrophils were square root-transformed, MCHC was reciprocal transformed, and total protein, hemoglobin, ESR, WBC, and glucose were natural log transformed and then compared using a t -test. All other parameters were not normally distributed or did not meet the assumption of equal variances and were compared using a Mann-Whitney rank sum test. A Bonferroni correction for multiple comparisons was used, and accordingly the level of significance when comparing values between the two facilities was set to $\alpha=0.002$.

RESULTS

Electrolyte parameters

Calcium and chloride concentrations in the blood of rough-toothed dolphins did not vary significantly among the three groups. A significant difference was apparent in sodium concentrations between the surviving-sick and both the fatally-sick and healthy groups and in potassium concentrations between the fatally-sick and surviving-sick groups (Table 1). However, the healthy and fatally-sick groups were not significantly different for either electrolyte ($P_{\text{Na}}=0.727$, $P_{\text{K}}=0.386$). Mean bicarbonate (total CO_2) was found to be lowest in the healthy and highest in the fatally-sick groups ($P<0.001$; Fig. 1).

Liver parameters

Significant differences were present among the groups for all four of the liver parameters considered in the study. Mean AST concentration in the healthy dolphin group was significantly lower (68% and 73%) than in both the fatally-sick and surviving-sick groups, respectively (Table 1 and Fig. 1). For ALT, the means in the surviving-sick and fatally-sick groups were similar ($P=1.000$), but significantly lower mean concentrations ($\sim 63\%$) were present in the healthy group ($P=0.001$). Mean AP levels were much higher (72% and 80%) in

TABLE 1. Mean (SE) values for the biochemistry and hematology parameters and number of samples (*n*) for each of three groups of rough-toothed dolphins (*Steno bredanensis*). Groups were defined as fatally-sick, survived-sick, or healthy based on captive display animals or the rehabilitation outcome of 14 rough-toothed dolphins housed at the Dolphin and Whale Hospital in Sarasota, Florida, USA or at Dolphin Quest French Polynesia. Groups were compared using a one-way analysis of variance. Parameters in bold were significantly different between the groups indicated.

Parameter ^a	Significance (<i>P</i>) ^b	Fatally-sick			Survived-sick			Healthy		
		Mean	SE	<i>n</i>	Mean	SE	<i>n</i>	Mean	SE	<i>n</i>
Electrolytes										
Calcium* (mg/dL)	0.059 ^c	8.83	0.04	179	8.69	0.03	255	8.70	0.13	53
Chloride (mEq/L)	0.330	118.9	0.4	180	118.3	0.3	255	118.9	0.5	66
Potassium (mEq/L)	0.023 (FS/SS)	3.85	0.04	180	3.97	0.02	189	3.93	0.05	66
Sodium (mEq/L)	0.005 (FS/SS,SS/H)	153.0	0.3	180	152.1	0.2	189	153.4	0.3	68
Bicarbonate (mEq/L)	0.000 (FS/SS,FS/H)	28.54	0.27	162	26.69	0.25	247	24.49	0.84	34
Liver										
AST (U/L)	0.000 (FS/H,SS/H)	840	79	180	993	76	258	269	11	76
ALT (U/L)	0.000 (FS/H,SS/H)	135	16	179	135	8	255	50	4	65
AP (U/L)	0.000 (FS/H,SS/H)	124	14	180	172	14	258	615	43	106
Bilirubin (mg/dL)	0.000 (FS/SS,FS/H,SS/H)	0.31	0.02	176	0.41	0.01	256	0.54	0.07	54
Proteins										
Total protein* (g/dL)	0.040 (FS/H)	7.32	0.05	180	7.44	0.04	254	7.54	0.09	72
Albumin* (g/dL)	0.000 (FS/SS,FS/H,SS/H)	3.24	0.03	180	3.51	0.02	254	3.89	0.09	52
Globulin (g/dL)	0.013 (FS/SS)	4.08	0.04	180	3.93	0.04	254	3.86	0.09	52
Red blood cells										
RBC (10 ⁶ /mm ³)	0.000 (FS/SS,SS/H)	4.41	0.03	178	3.94	0.03	258	4.26	0.08	69
Hemoglobin (g/dL)	0.000 (FS/SS,FS/H,SS/H)	14.7	0.1	178	13.4	0.1	258	14.1	0.2	69
Hematocrit (%)	0.005 (FS/SS,SS/H)	46.8	0.3	178	43.2	0.0	280	47.8	2.3	170
MCH (pg)	0.000 (FS/SS,SS/H)	33.46	0.05	178	34.02	0.09	256	33.39	0.27	66
MCV (fL)	0.000 (FS/SS,FS/H)	106.4	0.4	178	110.3	0.4	257	109.2	1.5	68
MCHC (g/dL)	0.000 (FS/SS,FS/H)	31.5	0.1	176	30.9	0.1	257	30.7	0.3	66
ESR (at 60 min)	0.000 (FS/SS,FS/H,SS/H)	21.0	1.5	160	30.8	1.7	261	8.9	0.8	128
White blood cells										
WBC (10 ³ /mm ³)	0.000 (FS/SS,FS/H,SS/H)	11.34	0.39	178	10.00	0.35	262	7.57	0.22	157
Neutrophils (absolute)	0.000 (FS/SS,FS/H,SS/H)	8,626	364	178	7,083	284	286	4,879	174	157
Lymphocytes (absolute)	0.000 (FS/SS,FS/H,SS/H)	935	41	178	1,390	47	286	1,129	47	157
Monocytes (absolute)	0.000 (FS/H,SS/H)	503	37	178	581	45	285	271	13	157
Eosinophils (absolute)	0.000 (FS/SS,FS/H,SS/H)	1,209	53	178	1,420	57	286	824	53	157
Other										
Glucose (mg/dL)	0.000 (FS/SS,FS/H,SS/H)	139	3	180	126	2	254	103	2	49
BUN (mg/dL)	0.000 (FS/SS,SS/H)	50.4	0.6	180	49.6	0.5	257	52.4	1.5	92
Creatinine (mg/dL)	0.000 (FS/SS,SS/H)	1.13	0.06	180	0.86	0.02	257	1.09	0.07	65
LDH (U/L)	0.003 (FS/H,SS/H)	2,761	141	180	2,945	271	251	1,199	52	51

^a Data that were transformed are indicated with an asterisk (*). AST = aspartate aminotransferase; ALT = alanine aminotransferase; AP = alkaline phosphatase; RBC = red blood cell count; MCH = mean corpuscular hemoglobin; MCV = mean corpuscular volume; MCHC = mean corpuscular hemoglobin concentration; ESR = erythrocyte sedimentation rate; WBC = white blood cell count; BUN = blood urea nitrogen; LDH = lactate dehydrogenase.

^b Significant differences between groups indicated by the following: FS = fatally-sick; SS = survived-sick; H = healthy.

^c Not significant.

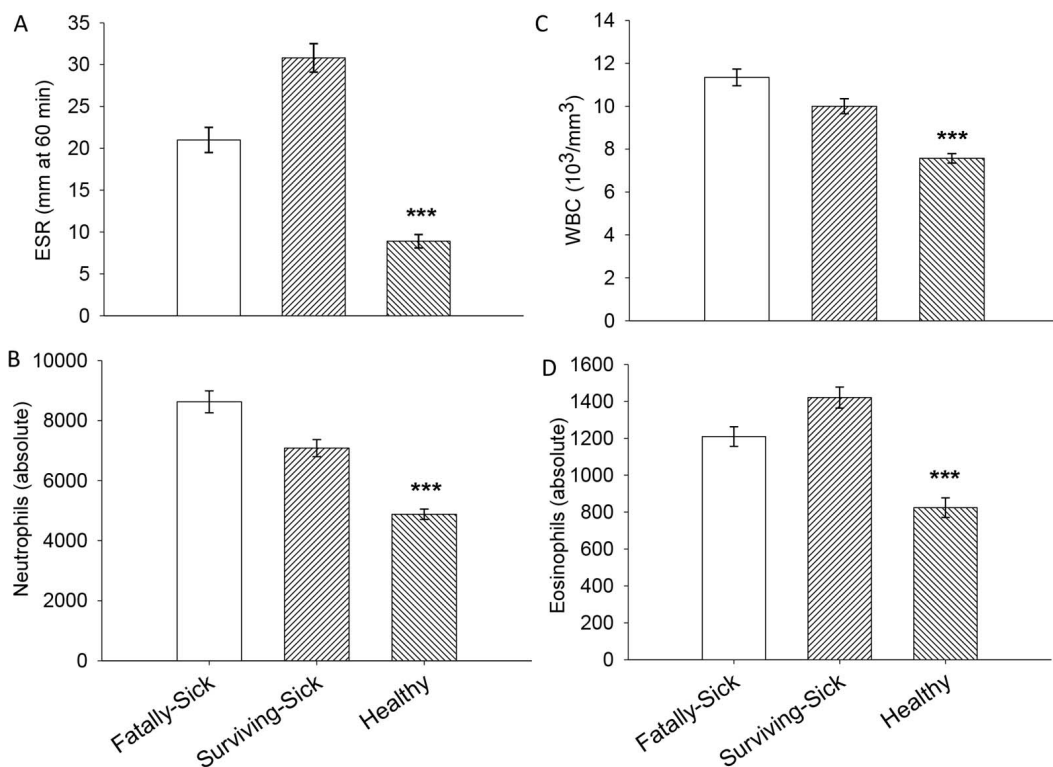


FIGURE 1. Comparisons of hematology parameters for three groups of rough-toothed dolphins (*Steno bredanensis*) that represent fatally-sick, surviving-sick, and healthy dolphins housed at the Dolphin and Whale Hospital in Sarasota, Florida, USA or at Dolphin Quest French Polynesia. Parameters include (A) erythrocyte sedimentation rate (ESR); (B) absolute neutrophil count; (C) total white count (WBC); and (D) absolute eosinophil count. *** indicates $P < 0.001$.

the healthy group than in the surviving-sick or fatally-sick groups, respectively ($P < 0.001$), regardless of survival outcome. Likewise, mean total bilirubin concentrations were significantly different between the healthy (lower value) and the surviving-sick and fatally-sick groups, independent of survival ($P < 0.001$; Table 1).

Protein parameters

Mean globulin levels were significantly lower in the healthy group ($P = 0.016$) and significantly higher in the fatally-sick group ($P = 0.033$) when compared to mean values of the surviving-sick group. Albumin was significantly different ($P < 0.001$) among all of the groups with the lowest mean concentrations in the fatally-sick group (Table 1). The surviving-sick group had 9% higher mean albumin and

the healthy group had 20% higher mean albumin than the fatally-sick group. Additionally, the total serum proteins differed significantly ($P = 0.040$) between the surviving-sick and fatally-sick groups, with the highest mean values in the healthy group when compared to the surviving-sick and fatally-sick groups (Table 1).

Red blood cell parameters

Several red blood cell parameters varied significantly, but there was no clear pattern among them (Table 1). The ESR was found to be significantly different among the groups ($P < 0.001$), with mean values significantly lower (58–71%) in the healthy group when compared to the fatally-sick or surviving-sick groups (Table 1 and Fig. 2).

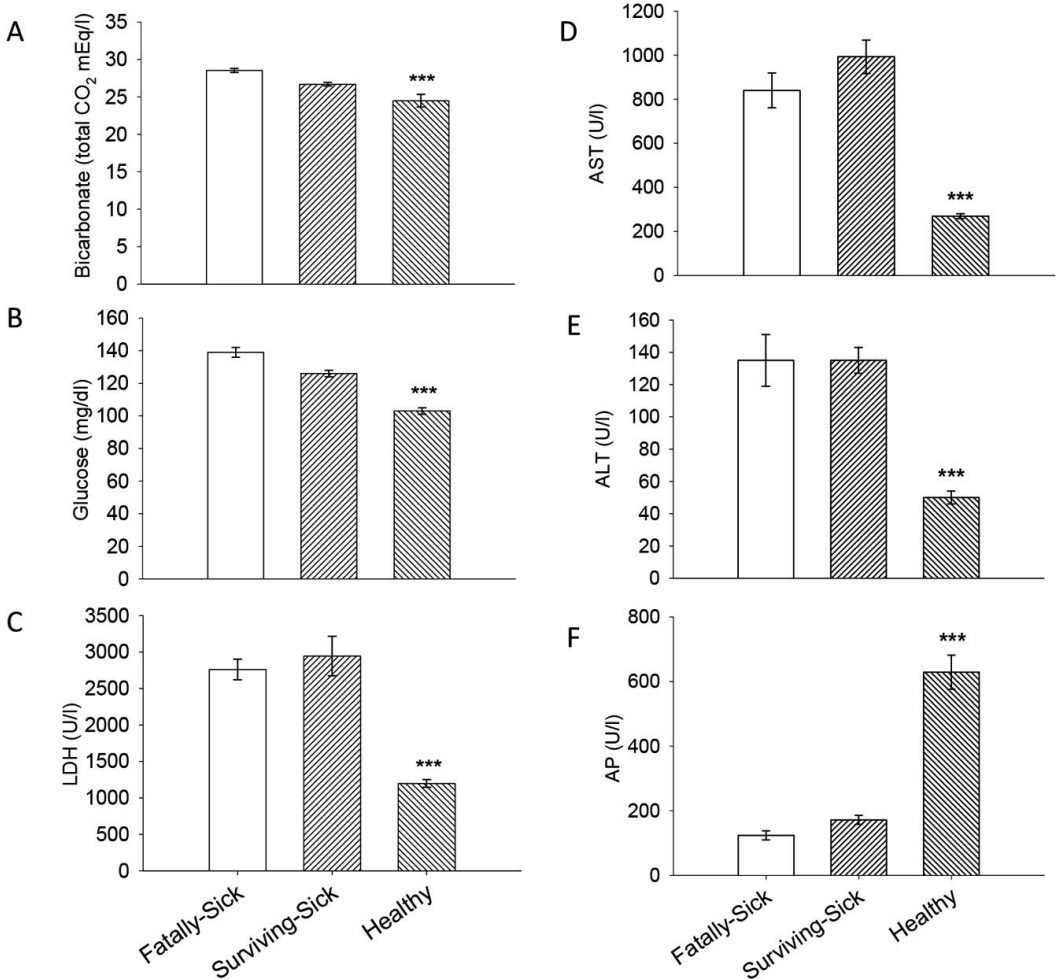


FIGURE 2. Comparisons of blood chemistry and liver parameters for three groups of rough-toothed dolphins (*Steno bredanensis*) that represent fatally-sick, surviving-sick, and healthy dolphins housed at the Dolphin and Whale Hospital in Sarasota, Florida, USA or at Dolphin Quest French Polynesia. Parameters include (A) bicarbonate (total CO₂); (B) glucose; (C) lactate dehydrogenase (LDH); (D) aspartate aminotransferase (AST); (E) alanine aminotransferase (ALT); and (F) alkaline phosphatase (AP). *** indicates $P < 0.003$.

White blood cell parameters

White blood cell counts were significantly different ($P < 0.001$) among the three groups of dolphins. The mean total WBC for the healthy group was 24–33% lower than the surviving-sick and fatally-sick groups (Table 1). Mean neutrophil counts were 43% and 31% lower in the healthy group than mean values in the fatally-sick and surviving-sick groups, respectively, which was statistically significant ($P < 0.001$). Significant differences ($P < 0.001$) were apparent among the three

groups in terms of the lymphocyte counts, with mean counts of the surviving-sick group being significantly higher (21–49%; $P < 0.001$) than those of the fatally-sick group. Mean monocyte counts were significantly higher ($P < 0.001$) in both the surviving-sick and fatally-sick groups compared to the healthy group. Similarly, the eosinophil counts differed ($P < 0.001$), with the mean counts of the healthy group being approximately 30–40% lower than mean values in the surviving-sick and fatally-sick groups (Table 1).

Other

A one-way analysis of variance detected a significant difference ($P < 0.001$; Fig. 1) in blood glucose among the three groups, with significantly lower mean values in the healthy group compared to the mean values of the surviving-sick and fatally-sick groups. Creatinine concentrations indicated significant differences ($P < 0.001$) among the three groups but mean values were similar between the healthy and fatally-sick groups. The mean value of blood urea nitrogen was significantly higher ($P = 0.001$) in the healthy group. The mean LDH was also significantly lower (57–59%) in the healthy group than mean LDH in either the surviving-sick or fatally-sick groups ($P < 0.001$; Table 1).

Comparison between the two facilities and healthy reference ranges

Comparison of the values from healthy dolphins at DWH and those at DQFP indicated a few significant differences (Table 2). The biochemistry values that were significantly different included only AP and total bilirubin. The red cell hematology values that were significantly different included RBC and hemoglobin, which led to differences in the calculated values for MCH, MCV, and MCHC. White cell values that were different included WBC and neutrophil and eosinophil counts. Reference ranges established using bootstrapping procedures from healthy animals at both facilities are based on 95% confidence intervals (Table 2).

First blood samples

When the first blood samples from the 14 individuals admitted to DWH were examined, sodium, AST, ALT, ESR, glucose, and LDH were represented by outlier values in 40% or greater of the samples when compared to the distribution of raw data from the healthy group. These six parameters were then examined according to survival outcome and indicated the following: sodium was lower than reference ranges in 5/9 animals that died and in 1/5 that survived; AST was higher in 6/9 animals that died and 5/5 animals that

survived; ALT was higher in 3/9 animals that died and 1/5 that survived; ESR was higher in 6/9 animals that died and in 3/5 that survived; glucose was higher in 9/9 animals that died and in 2/5 that survived; and LDH was higher in 6/9 animals that died and 4/5 that survived. Glucose, ESR, and sodium seemed to have some value as prognostic indicators when considered together, especially when two of the three were outliers. In all but one of the nine animals that died, at least two of these three parameters were represented by outliers when compared to the distribution of raw data from the healthy group (8/9). In the five survivors, 2/5 had at least two of these parameters that were outlier values. First blood sample values, survival outcome, and number of days until death in the animals that died is provided for all parameters in Supplementary Material Table S2.

DISCUSSION

Comparisons among groups and prognostic value of first blood samples

For each parameter evaluated in this study, findings from the comparison of healthy, surviving-sick, and fatally-sick groups were considered along with the prognostic value of the first blood sample upon admission. During live strandings, veterinarians and animal care response teams must often make disposition decisions based on only a single blood sample (Sharp et al. 2014). However, values of some of the parameters considered in this study may be influenced secondarily to a stress response associated with stranding and transport, and cortisol values obtained from some of the study dolphins during rehabilitation and from the healthy dolphins at DQFP suggest that endogenous cortisol release during stranding and transport is significant (West 2002). A more comprehensive and informative approach to evaluating the usefulness of the first blood sample as a prognostic indicator was to combine these results with our comparisons among the groups, where many more blood samples are considered over time, and blood values from the healthy dolphin

TABLE 2. Comparison of means from healthy rough-toothed dolphins (*Steno bredanensis*) housed at the Dolphin and Whale Hospital in Sarasota, Florida, USA (DWH) and at Dolphin Quest French Polynesia (DQFP). Reference ranges were generated using bootstrapping procedures that represent combined values from the eight healthy captive dolphins. Ranges represent 95% confidence intervals. Parameters that are significantly different using a Bonferroni correction ($\alpha < 0.002$) between the two facilities are shown in bold.

Parameter ^a	Significance (<i>P</i>)	Facility				Reference ranges
		DWH		DQFP		
		Mean	SEM	Mean	SEM	
Electrolytes						
Calcium (mg/dL)	0.145	8.8	0.044	8.6	0.240	8.8–9.0
Chloride (mEq/L)	0.838	119.5	0.35	118.4	1.56	118.2–120.4
Potassium (mEq/L)	0.074	4.0	0.069	3.7	0.066	3.6–4.0
Sodium (mEq/L)	0.198	153.9	0.266	152.5	0.878	153.4–154.6
Bicarbonate (mEq/L)	0.182	28.3	1.324	31.7	2.404	24.3–27.7
Liver						
AST (U/L)	0.978	266.2	15.83	280.9	38.23	230.0–258.3
ALT (U/L)	0.093	55.7	4.392	33.8	8.252	35.5–51.5
AP (U/L)	0.001	365.0	48.45	773.4	55.69	330.2–808.8
Bilirubin (mg/dL)	0.001	0.32	0.018	1.24	0.209	0.3–0.5
Proteins						
Total protein (g/dL)	0.092	7.7	0.099	7.45	0.158	7.5–7.7
Albumin (g/dL)	0.411	3.9	0.003	4.67	0.247	3.8–4.0
Globulin (g/dL)	ND ^b					3.6–4.2
Red blood cells						
RBC ($10^6/\text{mm}^3$)	0.001	4.05	0.087	4.91	0.074	4.2–4.8
Hemoglobin (g/dL)	0.001	13.70	0.194	15.52	0.242	13.9–14.9
Hematocrit (%)	0.040	45.5	0.748	45.6	0.617	43.7–45.1
MCH (pg)	0.001	34.0	0.332	34.8	0.308	32.1–33.6
MCV (fL)	0.001	115.5	1.118	90.9	0.346	106.0–112.8
MCHC (g/dL)	0.001	29.5	0.199	31.6	0.235	29.5–30.6
ESR (at 60 min)	0.667	8.7	1.003	5.8	0.353	4.9–7.1
White blood cells						
WBC ($10^3/\text{mm}^3$)	0.001	5.8	0.282	7.0	0.194	6.1–6.7
Neutrophils (absolute)	0.001	3,091	231.6	5,174	157.4	4,169–4,692
Lymphocytes (absolute)	0.955	1,158	87.37	1,111	36.54	953–1,135
Monocytes (absolute)	0.017	301	32.08	230	13.91	184–260
Eosinophils (absolute)	0.001	1,315	107.6	561	50.08	408–712
Other						
Glucose (mg/dL)	0.666	102.3	2.302	98.3	6.936	97–102
BUN (mg/dL)	0.441	51.0	3.216	49.3	2.342	49–52
Creatinine (mg/dL)	0.572	0.92	0.035	1.08	0.094	0.9–1.0
LDH (U/L)	ND ^b					985–1,505

^a AST = aspartate aminotransferase; ALT = alanine aminotransferase; AP = alkaline phosphatase; RBC = red blood cell count; MCH = mean corpuscular hemoglobin; MCV = mean corpuscular volume; MCHC = mean corpuscular hemoglobin concentration; ESR = erythrocyte sedimentation rate; WBC = white blood cell count; BUN = blood urea nitrogen; LDH = lactate dehydrogenase.

^b ND = not determined due to small sample size from one facility.

group are not influenced by the stress of stranding and transport.

Most routine blood chemistry and hematology parameters were considered in this study. Overall, electrolytes did not appear to be a good indicator of health status or survival in rough-toothed dolphins, although dramatic increases or decreases would likely lead to a grave prognosis. Sodium had some value as a prognostic indicator, as sodium concentrations in the first blood sample were represented by low outliers in 5/9 of the dolphins that died. Surviving-sick dolphins had slightly lower sodium than did healthy dolphins. Although bicarbonate, also reported as total carbon dioxide, has not been routinely reported in cetaceans, it is the only electrolyte in this study that showed highly significant differences and a consistent decrease from fatally-sick through surviving-sick to healthy dolphin groups. A study comparing stranded common dolphins (*Delphinus delphis*) that were categorized as either failed or survived found the opposite trend, with lower TCO_2 and HCO_3 in the failed dolphins (Sharp et al. 2014). These conflicting results warrant further investigation to determine how disturbances in acid-base balance may impact survival prognosis in dolphins.

Liver problems are a common finding in stranded dolphins that die (Di Guardo et al. 1995). Unlike electrolytes, all of the liver parameters considered in this study showed highly significant differences among the three groups. First blood sample AST and ALT values were often represented by outlier values when compared to healthy dolphins, regardless of survival outcome. Initial elevations in liver enzymes are likely related to transport and suggest that AST, ALT, and AP may not be especially valuable in predicting survival outcome from only a single blood sample collected soon after transport. However, the difference between healthy and sick groups was pronounced, with lower AST and ALT values and higher AP concentrations in the healthy group. Elevated AST may also increase secondary to corticosteroid release or skeletal muscle damage from overexertion (Sjogren 2007; Watson 2014), which could

occur during a live stranding, and heightened cortisol was observed in the first blood samples from some of the animals examined (West et al. 2002). Total AP activity in domestic animals is the product of two isoenzymes: one of hepatic origin and the other of osteoclast origin. The level of AP is typically elevated in young, growing animals with increased osteoblastic activity, or AP elevations can signify liver problems (Smith 2015). In contrast, AP has been shown to decrease with infection (bacterial or viral) in bottlenose (*Tursiops truncatus*) and Pacific white-sided (*Lagenorhynchus obliquidens*) dolphins (Fothergill et al. 1991), and low values are often applied as prognostic tools in cetacean medicine (Bossart et al. 2001). We observed significantly lower AP values in the surviving-sick and fatally-sick dolphins, which may be a reflection of the high proportion of repeat blood samples from young, growing dolphins that comprised our healthy group, of the ill health status and greater likelihood of infections within sick groups, or a combination of both factors in both groups. Although total bilirubin was higher in the healthy group, it is unlikely that the difference in total bilirubin is clinically significant because one healthy dolphin had a consistently elevated bilirubin level and methodology-related differences were observed.

In cetaceans, infectious processes can lead to increases in globulin levels and decreases in albumin levels (Ridgway 1972). The sick rough-toothed dolphins had lower total protein and albumin and higher globulin concentrations. Because the lowest albumin concentrations were found in the fatally-sick group and steadily increased through rehabilitation efforts, albumin may be a good indicator of health and survival in stranded rough-toothed dolphins, especially when trends are considered.

Red blood cell parameters are usually evaluated as a unit rather than individually. The RBC, hematocrit, and hemoglobin generally vary directly with one another in all mammals (Bossart and Dierauf 1990). In stranded common dolphins that died, their hematocrit and hemoglobin were lower than

in the animals that survived (Sharp et al. 2014), but in this study red blood cell parameters did not vary consistently when compared among the healthy and sick groups. Hemoglobin and RBC were highest in the fatally-sick rough-toothed dolphins but MCV was lowest in this group of dolphins and highest in the surviving dolphins. It is possible that this reflects a lack of erythrocytic response in the dolphins that died, where decreased red blood cell mass may have led to increased bone marrow production and the release of larger red blood cells in the surviving dolphins.

Erythrocyte sedimentation rate is a measure of the presence and intensity of inflammation in cetaceans and is often used as a dolphin prognostic indicator (Bossart et al. 2001; Fair et al. 2006). In this study, ESR correlated well with health status of rough-toothed dolphins; the lower the sedimentation rate the healthier the dolphin (Fig. 2). Additionally, ESR was often represented by a high outlier value when examining the first blood sample upon admission to DWH. First blood sample ESR was represented by high outlier values in the majority of the animals that died (6/9) but was also high in the 3/5 that survived. The use of ESR as a prognostic indicator of survival from a single blood sample was increased if glucose or sodium values were also represented by outliers. Although not specific, determining ESR upon admittance to a rehabilitation facility was included as a recommendation when reviewing postintervention survival in odontocetes (Wells et al. 2013).

White blood cell parameters are an indirect measurement of the effectiveness of the immune system. As an animal's immune system fights against parasites or infections, white blood cells may be elevated. This may be the most-commonly used hematology parameter for assessing general health (Brown and Hunter 1993). This study found that both the WBC and the absolute counts of most leucocytes (neutrophils, monocytes, eosinophils) were lower in the healthy group than in the surviving-sick and fatally-sick groups, with the exception of lymphocytes. Lower lympho-

cyte counts in the last two mentioned groups are likely due to illness, a suppressed immune system, or a heightened release of endogenous cortisol as part of the stress response (or a combination) in the sick dolphins. Transportation stress or treatment with glucocorticoids can cause lymphopenia and neutrophilia in bottlenose dolphins (Medway and Geraci 1964; Medway et al. 1970; Reidarson and McBain 1999). As expected, in this study neutrophil counts were lower in healthy dolphins as opposed to those that were sick and undergoing rehabilitative care (Fig. 2). Increased neutrophil levels are often reflective of infection (Brown and Hunter 1993) but can also increase during inflammation and stress (Harvey 2012; Kolaczowska and Kubes 2013). The observation of increased neutrophils would be consistent with both the greater probability of infections in the sick dolphins or with increased endogenous cortisol release. We would also expect the sick dolphins to have greater inflammation, as suggested by the high ESR values in the first blood samples. However, we did not examine neutrophil maturity or cytoplasmic toxic changes that would support inflammation. Monocyte counts were also lower in the healthy group, as these cells are precursors of macrophages which are increased in the presence of dead or dying cells (Brown and Hunter 1993). Despite the usefulness of WBCs in assessing health status, and the trends observed among the healthy and sick dolphins, WBC parameters did not usually represent outlier values when considering the first blood samples examined upon admission to DWH.

Among the other parameters considered in this study, creatinine concentrations did not show a clear pattern according to health status. It has been the experience at DWH that severe kidney disease is not common in stranded animals and hence creatinine is rarely elevated, but when it is elevated a grave prognosis is warranted. The levels of LDH and glucose were lower in the healthy group, suggesting that these may be useful indicators of health status (Table 1). This is supported by our findings from the first blood

samples, where LDH was represented by high outlier values in 6/9 dolphins that died but was also high in 4/5 that survived. Glucose was represented by high outlier values in 9/9 dolphins that died and in 2/5 that survived. Although glycolysis may have played a role, we do not anticipate this impacted our first blood sample findings because the collection and preservation methods were consistent throughout the study. High glucose upon admission to the DWH is believed to be a reflection of the stress response initiated by glucocorticoid activity, suggesting that the blood values obtained from a single blood sample soon after stranding may be driven by a combination of both stress and health status.

Comparison between facilities

Very few significant differences were observed in the biochemistry parameters (AP and total bilirubin) when comparing values from healthy animals between facilities. It is not surprising that there were more differences for red cell and white cell parameters between the two facilities. This is likely related to the shorter period of care for the dolphins at DWH, where all had been on antibiotics for a time prior to being considered healthy. It is likely that the difference in eosinophils was related to parasite loads because the dolphins at DWH were generally older than the DQFP animals and not routinely treated for parasites.

Other factors

Blood chemistry and hematology values appear to be species specific, and rough-toothed dolphin values were somewhat different when compared to other cetacean species (Bossart et al. 2001). Health status is one of several factors that may affect blood chemistry and hematology in cetaceans, with stress being another potential driving factor when considering stranded animals. In bottlenose dolphins, blood chemistry and hematology values vary according to total body length as well as seasonally (Hall et al. 2007; Schwacke et al. 2009), suggesting important factors to refine the healthy rough-toothed

dolphin reference intervals presented in this study if greater sample sizes become available. Length of time in captivity also has an impact on hematology and blood chemistry values (Sokolova 2004). Although medications may affect blood values, the effect of medication regime was not considered in this study. Additionally, reference ranges are ideally generated from analyses that represent a large number of individual animals, but the reference ranges presented in this study are limited to multiple samples obtained from only eight individuals. Regardless of these potential influences, this study provides valuable reference data that can be used by wildlife and animal care staff to interpret general health status of the poorly known rough-toothed dolphin during stranding events or when undergoing rehabilitation.

Based on satellite transmitter and sighting data, we conclude that the five rehabilitated and released dolphins in this study survived beyond the initial 3–6 wk postrelease period used to evaluate survival success in rehabilitated dolphins (Wells et al. 1999; Wells and Gannon 2005; Wells et al. 2008). Findings from this study validate the use of blood chemistry and hematology data in decision making during stranding events and rehabilitation efforts for rough-toothed dolphins although, in most cases, a single blood sample is less useful than a series of samples for establishing trends. Specific parameters such as glucose, liver enzymes (especially AP), WBCs, and ESR have value as indicators of health status and in predicting the likelihood of survival. Normal and abnormal reference data for rough-toothed dolphins are provided for veterinarians and biologists involved in responding to stranding events and providing care for this species.

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SUPPLEMENTARY MATERIAL

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