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Hematology, Serum Chemistry, and Cytology Findings from Apparently Healthy Atlantic Bottlenose Dolphins (*Tursiops truncatus*) Inhabiting the Estuarine Waters of Charleston, South Carolina

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Abstract

This study reports comprehensive baseline data for hematology, serum chemistry, and cytology variables in 63 apparently healthy Atlantic bottlenose dolphins (*Tursiops truncatus*) inhabiting the estuarine waters of Charleston (CHS), South Carolina (SC). Blood and cytology samples were collected from bottlenose dolphins in August 2003 and August 2004 during capture-release health assessment studies. Means, medians, standard deviations, 95% CI, and ranges were calculated for the population's hematology, serum biochemistry, and serum protein electrophoresis parameters. All of the parameters for which a published range was available were close to or within the ranges previously reported. Comparisons by age, gender, and pregnancy status revealed statistically significant differences ($p < 0.05$) for several hematology and serum chemistry parameters. Blowhole, rectal, and gastric swabs were examined for cytologic abnormalities, utilizing light microscopy. There were no pathologic findings in the samples; however, 4% (2/51) of the dolphins sampled had mild or moderate blowhole inflammation. The prevalence of gastric inflammation was 26% (13/50), and severe gastric inflammation was present in three of the dolphins sampled (6%). The majority of animals with gastric inflammation were male (10/13, 77%). In fact, all cases of severe inflammation occurred in males, and none were present in 2004. These data provide a baseline from which to compare hematological, serum chemistry, and cytological parameters in wild dolphin populations.

Key Words: Bottlenose dolphin, *Tursiops truncatus,* hematology, serum chemistry, cytology, Charleston, South Carolina

Introduction

Hematologic and serum biochemical analyses are valuable tools in evaluating the health and physiological status of wild and captive animals. Diagnostic laboratory tests complement clinical examination and are among the most useful tools for diagnosing disease in dolphins (Bossart et al., 2001). Few comprehensive health assessments have been performed on wild dolphins, however, largely due to the logistical difficulties in obtaining samples. As part of a comprehensive Bottlenose Dolphin Health and Risk Assessment (HERA) Project between the National Ocean Service Center for Coastal Environmental Health and Biomolecular Research in Charleston, South Carolina, and the Harbor Branch Oceanographic Institution (HBOI) in Fort Pierce, Florida, hematology, serum chemistry, and cytological parameters were measured in Atlantic bottlenose dolphins (*Tursiops truncatus)* from two southeast U.S. sites: the estuarine waters of Charleston (CHS), South Carolina (SC), and the Indian River Lagoon (IRL), Florida. Our study summarizes the hematology, serum analytes, and cytological data for the CHS dolphins (see Goldstein et al., 2006, for IRL results).

Marine mammals, such as dolphins, are important key species in coastal areas. They may reflect the effects of natural and anthropogenic stressors and are often viewed as sentinels for environmental and ecosystem health (Fair & Becker, 2000). Thus, defining the health status of bottlenose dolphins is not only important for future management of this species, but it would provide insight into the health of the ecosystem as well. This study expands the knowledge of baseline values for the hematology, serum chemistry, and

cytology of Atlantic bottlenose dolphins and can serve as a reference for monitoring dolphin populations.

Materials and Methods

Study Site

The study site was located in the estuarine waters of CHS, SC (32º 54' 0" N, 80º 1' 47" W), situated in the central region of the coastal zone. The site includes the Charleston Harbor as well as portions of the main channels and creeks of the Ashley River, Cooper River, Wando River, and the Stono River Estuary (Figure 1).

Animal Collection

Sixty-three Atlantic bottlenose dolphins were captured, examined, sampled, and released in August of 2003 and August of 2004 (Figure 1). Of the 63 dolphins sampled for this study, not all dolphins had all tests performed and, thus, all dolphins were not included in every analysis. Water temperature averaged 29º C $\pm$ 1.5º C and 28º C $\pm$ 0.05º C in 2003 and 2004, respectively. Dolphins were captured by encircling them with a large mesh seine net. Blood samples were collected from dolphins in the water once the animals were restrained by experienced handlers. Only dolphins that appeared clinically healthy, as assessed during physical examination based on the criteria of overt signs of disease and body condition (see Table 1), were used or included in this study. Age was determined by examining the postnatal dentine layers in an extracted tooth (Hohn et al., 1989). Pregnancy was determined by ultrasound (SonoSite 180plus, Bothell, WA). Only the first set of data from recaptured dolphins was used in the analysis, so the data set analyzed contained one value per parameter per dolphin. All animal capture and sampling protocols were approved by the HBOI Institutional Animal Care and Use Committee (IACUC).

Sampling

Hematology, Serum Chemistry, and Serum Protein Electrophoresis—Blood samples were drawn from the periarterial venous rete in the flukes as soon as possible after the dolphin was restrained to reduce variation as a result of time-dependent changes in blood constituents. The actual time between blood collection and the animals being captured in the net had a mean of 21 min and median of 16 min. The site was prepared aseptically with a surgical scrub (2% chlorhexidine gluconate), using an alcohol-soaked gauze pad and a 19-gauge needle, and a 1.9-cm butterfly catheter with a vacutainer attachment (Becton, Dickinson, and Co., Franklin Lakes, NJ) was utilized (Bossart et al., 2001).

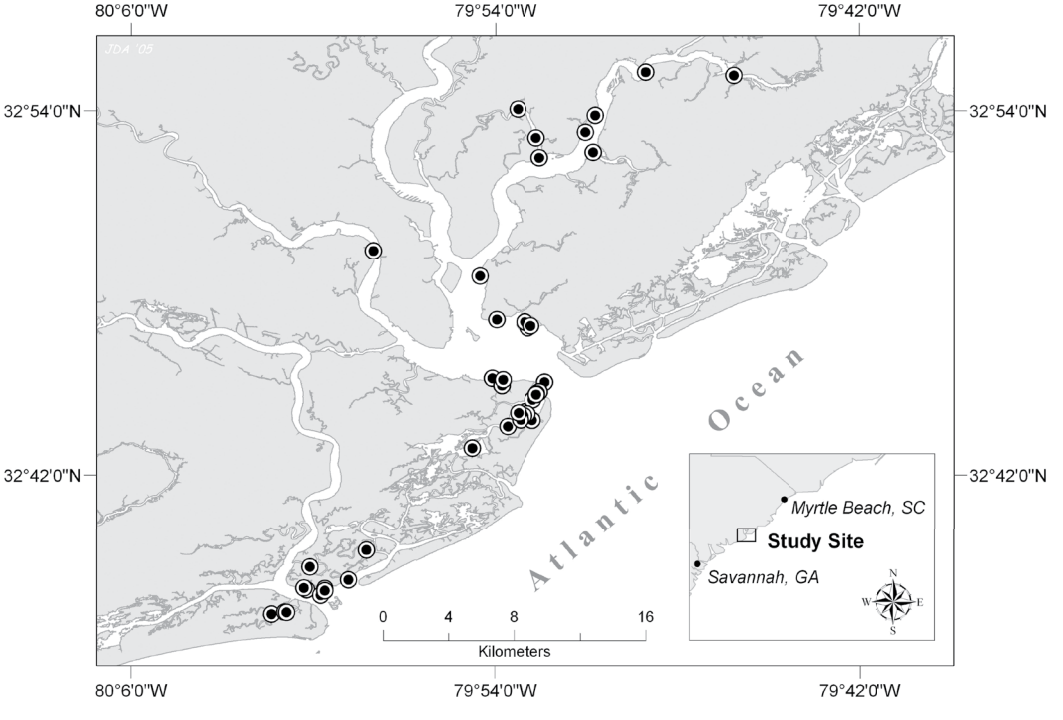

Figure 1. Capture-and-release locations for bottlenose dolphins sampled in Charleston, SC, during August of 2003 and 2004

Serum was collected in a 10-ml serum separator vacutainer tube (Beckon, Dickinson, and Co., Franklin Lakes, NJ), placed at room temperature for 20 to 40 min, then centrifuged for 15 min at 1,200 rpms. The serum was removed and placed in a cryovial (Corning Inc., Acton, MA) on ice. Samples for hematology were collected in a vacutainer tube with ethylenediaminetetraacetic acid (Becton, Dickinson, and Co., Franklin Lakes, NJ). Samples collected for determination of hematologic and biochemical parameters were shipped overnight on cold packs to Cornell University Veterinary Diagnostic Laboratory in Ithaca, New York, for a complete blood count and a serum chemistry panel. Differential leukocyte counts were performed by microscopic examination of modified Wright-Giemsa-stained blood smears (Bayer Healthcare, Tarrytown, NY). The microhematocrit tube, utilized for manual packed cell volume (PCV) determination, was centrifuged 5 min at 11,700 rpm and interpreted by visual inspection against a standard calibration. Automated hematocrit (HCT), hemoglobin (Hb), red blood cells (RBC), mean corpuscular platelet volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), white blood cells (WBC), platelets, and mean platelet volumes (MPV) were determined by using an automated analyzer (Bayer ADVIA 120, Bayer Diagnostics, Tarrytown, NY). The following serum chemistry analytes were measured with an automated analyzer (Roche Hitachi 917, Indianapolis, IN): sodium, potassium, chloride, bicarbonate, anion gap, blood urea nitrogen (BUN), creatinine, uric acid, calcium, phosphate, magnesium, total protein, albumin, globulin, albumin/globulin ratio, glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), sorbital dehydrogenase (SDH), creatinine phosphokinase (CPK), gamma-glutamyl transferase (GGT), total bilirubin, direct bilirubin, indirect bilirubin, amylase, lipase, cholesterol, triglycerides, creatinine kinase, lactate dehydrogenase (LDH), iron, total iron binding capacity (TIBC), and percent saturation (PSAT). Serum protein electrophoresis (SPEP) analytes were also determined with an automated analyzer ("rep" – Rapid Electrophoresis, Helena Laboratories, Beaumont, TX). Schalm fibrinogen was determined by heat precipitation. No hemolyzed or lipemic samples were included in this study. Erythocyte sedimentation rate (ESR) was measured within 10 min of sample collection using the Sediplast system (Polymedco, NY).

Cytology—Blowhole, gastric, and rectal swabs were taken for cytologic evaluation using methods described in Sweeney & Reddy (2001) and Sweeney et al. (2003). Slides for cytologic evaluation were air-dried, stained with modified Wright-Giemsa (Jorgensen Laboratories, Inc., Loveland, CO), and evaluated at the HBOI Marine Mammal Research laboratory. The following parameters were evaluated for each slide: cellular morphologic preservation; overall cellularity; epithelial cells; presence and type of inflammatory cells; and presence of bacteria, fungi, parasites, and noncellular and cellular debris. Ten high-powered (400x) fields (hpf) were examined and assigned an average for each parameter. Mild inflammation was classified as 5 to 10 cells/hpf, moderate inflammation as 10 to 16 cells/hpf, and severe inflammation as >16 cells/hpf.

Statistical Analyses—Descriptive statistics were calculated for the hematologic, serum analyte, and SPEP data for the entire population using *SAS*. Data provided included mean, standard deviations, median, 95% CI, and range. Outcome data (hematology, serum chemistry, or cytology) were not available for all animals due to varying circumstances (e.g., incomplete sampling, sample/lab error, etc.). As such, the sample size of each comparison may vary as it reflects the number of animals with complete data for that specific outcome. Differences between means of two groups were assessed using a Student's *t*-test. Differences between means of three or more groups were assessed using ANOVA, and a $p < 0.05$ was considered statistically significant. Dolphins < 6 y of age were classified as juveniles while those ≥ 6 y of age were categorized as adults. Multiple linear regression was employed to examine the difference between age and ALP. The log of ALP was used to eliminate the heteroscadacity at the younger age groups; a regression correlation of $r^2 = 42$ was observed (Figure 2). Stratified analysis frequently resulted in cells with small samples in which comparison of outcomes could have been influenced by outliers. Using the Grubbs (1969) and Dixon (Dixon et al., 2001) test for outliers, fibrinogin values in nonpregnant and pregnant females were assessed. If confirmed by both tests, it was removed for comparison.

Results

A total of 63 (46 in 2003 and 17 in 2004) apparently healthy dolphins were included in the various analyses after excluding data from two recaptured dolphins and two dolphins with confirmed papilloma virus. No dolphins examined were emaciated, according to the criteria in Table 1, and 70% of the dolphins exhibited ideal body condition scores. Of the 63 apparently healthy dolphins, blood chemistry data were available for 62 animals, and hematology data were available for 58 animals. Forty of the

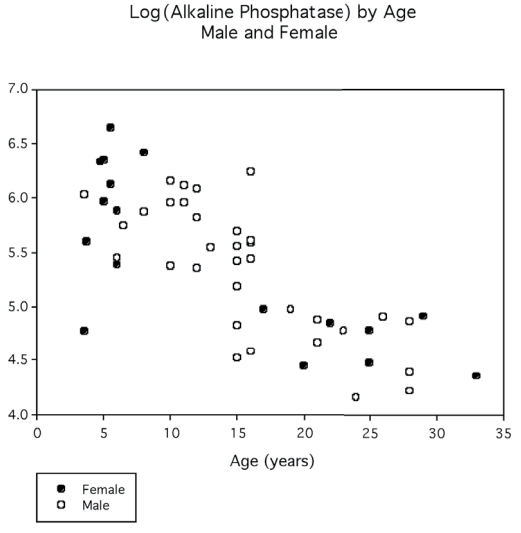

Figure 2. Regression model for alkaline phosphatase concentrations vs age for captured dolphins in estuarine waters of Charleston, SC; $r^2 = 51$, ALP (log) = 6.2 - 0.06 (Age), $p < 0.0001$.

animals were male, 18 were nonpregnant females, and five were pregnant females. Cytology data were available for a total of 46 possible dolphins for the two years, although not all animals had all cytology samples taken, so the actual numbers for gastric, blowhole, and rectal samples vary per animal (Table 2). Ages for 50 animals were determined from examination of dental enamel and ranged from 3.5 to 33 y; only known ages were included in the age comparison data. A wide variety of age categories have been utilized for categorizing sexual maturity in bottlenose dolphins, ranging from 5 to 12 y for females and from 10 to 13 y for males (Mead & Potter, 1990). Adults were operationally defined as ≥ 6 y old. We compared the data using both 10 y and 6 y of age for categorizing adults and found no differences in the hematology and serum chemistry parameters among these two groups. The reported values in this paper include 41 adults (≥ 6 y) and 9 juveniles (< 6 y).

Means, medians, standard deviations, 95% CI, ranges, and sample size for the collected dolphins' hematologic, serum biochemistry, and SPEP values are given in Tables 3a and 3b, respectively. Hematology and serum chemistry values partitioned by gender and pregnancy

Table 1. Dolphin body condition scoring based on a numerical system (1-5)

Score	Description
1	Emaciated body condition – Concavity of epaxial muscles. Severe overt post-nuchal depression (i.e., peanut-shaped head) and palpable minimal post-nuchal fat turgidity; bony structures such as ribs and peduncular vertebrae visually evident. Integument freely slides over underlying muscle.
2	Underweight body condition – Slight concavity to epaxial muscles. Slight to moderate overt post-nuchal depression (i.e., +/- peanut-shaped head). Palpable decreased post-nuchal fat turgidity. Ribs and peduncular vertebrae only visually evident with positional changes of body.
3	Ideal body condition – No concavity of epaxial surfaces and post-nuchal fat pad. Palpable moderate post-nuchal fat turgidity. Ribs and peduncular vertebrae not visually evident. Sleek body contours.
4	Overweight body condition – No concavity of epaxial surfaces and post-nuchal fat pad. Firm palpable post-nuchal fat pad turgidity. Small amount of rolls of fatty tissue around neck. Dorsal epaxial mild convexity. Slight dorsal epaxial convexity with slight depression of dorsal midline.
5	Obese body condition – No concavity of epaxial surfaces and post-nuchal fat pad. Very firm palpable post-nuchal fat pad. Overt multiple rolls of fatty tissue around neck. Eyes deeply set within orbits. Dorsal epaxial convexity with depression of dorsal midline.

Table 2. Cytology results of blowhole, rectal, and gastric samples in Atlantic bottlenose dolphins in Charleston, SC

		Inflammation			
Sample	Total no. samples	None (%) (*n*)	Mild (%) (*n*)	Moderate (%) (*n*)	Severe (%) (*n*)
Blowhole – 2003	35	94 (33)	3 (1)	3 (1)	--
Blowhole – 2004	16	100 (16)	--	--	--
Fecal – 2003	30	100 (30)	--	--	--
Fecal – 2004	14	100 (14)	--	--	--
Gastric – 2003	29	76 (22)	7 (2)	10 (3)	7 (2)
Gastric – 2004	21	71 (15)	19 (4)	5 (1)	5 (1)

are included in Tables 4a and 4b. Comparisons between juvenile dolphins and adults for hematologic, serum chemistry, and SPEP values are provided in Table 5. While the values for the majority of parameters were similar among gender and ages, some differences were noted. Males had higher creatinine values ($p < 0.03$), RBC ($p < 0.0003$), and RDW ($p < 0.002$) than females (both pregnant and nonpregnant). In contrast, males exhibited lower MCV ($p < 0.003$), MCH ($p < 0.003$), and BUN/creatinine values ($p < 0.006$) than females. Males had higher Hb ($p < 0.03$) than pregnant females. Nonpregnant females had higher uric acid ($p < 0.04$) and higher gamma globulins ($p < 0.02$) than males, higher CPK ($p < 0.02$) compared to pregnant females, and higher triglycerides ($p < 0.005$) compared to both groups. Pregnant females had lower Hct ($p < 0.01$) compared to both nonpregnant females and males. Initial comparison of fibrinogen between pregnant and nonpregnant females suggested the difference was not statistically significant. The one nonpregnant animal with a fibrinogen of 500 was determined to be an outlier according to both the Grubbs (1969) and Dixon (Dixon et al., 2001) tests for outliers. After removal, the mean for that group was reduced to 126. The differences between pregnant and nonpregnant animals was then statistically significant ($p = 0.04$).

Significant differences were observed between adult and juvenile dolphins (Table 6). Adults had higher mean values for creatinine ($p < 0.0001$), total protein ($p < 0.0093$), AST ($p < 0.004$), α-1 globulins and α-2 globulins ($p < 0.01$), γ- globulins ($p < 0.004$), total protein ($p < 0.009$), as well as relative ($p < 0.004$) and absolute percentage ($p < 0.008$) of neutrophils. Juveniles had higher mean values for glucose ($p < 0.01$), albumin/globulin ratio ($p < 0.02$), triglycerides ($p < 0.007$), TIBC ($p < 0.0001$), and CPK ($p < 0.001$). With increasing age, a significant decrease was observed in concentrations of alkaline phosphatase (ALP), with a regression correlation of $r^2 = 51$ (Figure 2).

Erythrocyte sedimentation rate (ESR) had a mean value of 48 +18.4 mm/h, median of 46 mm/h, and range of 21 to 95 mm/h. Sixty-five percent of all ESR measurements were less than 50 mm/h, while 35% exceeded this value.

No pathologic findings were present in the rectal cytologic examinations. Two of 51 blowhole samples (4%) showed evidence of mild or moderate inflammation; however, the prevalence of gastric inflammation was 26% (13/50), and severe gastric

Table 3a. Hematology values of Atlantic bottlenose dolphin population in Charleston, SC (n = 58: 43 in 2003 and 15 in 2004)

Hematology	Units	n	Mean ± SD	Median	95% CI	Range
White blood cells (WBC)	10^3/ul	58	11.7 (2.8)	11.7	10.9-12.4	7.0-19.1
Red blood cells (RBC)	10^6/ul	58	3.5 (0.3)	3.5	3.5-3.6	2.3-4.1
Hemoglobin (Hb)	g/dl	58	14.0 (0.9)	14.1	13.8-14.2	10.8-15.6
Packed cell volume (PCV)	%	15	40 (3)	41	39-42	34-44
Hematocrit (Hct)	%	58	40 (2.9)	40	39-41	29-47
Mean corpuscular volume (MCV)	Fl	58	114 (9)	113	111-116	97-149
Mean corpuscular hemoglobin (MCH)	pg	58	40 (3)	40	39-41	35-48.0
Mean corpuscular hemoblobin concentration (MCHC)	%	58	35 (1.1)	36	35-36	32-37
Red cell distribution width (RDW)	%	58	12.9 (1.2)	12.8	12.7-13.3	11-16
Basophils	10^6/ul	58	0.1 (0.1)	0	3.7-4.5	0-0.6
Segmented neutrophils – relative	% of WBC	58	36 (10)		33-38	12-62
Segmented neutrophils – absolute	10^3/ul	58	4.1 (1.5)		3.7-4.5	1.5-9.9
Bands – relative	% of WBC	58	0	0		
Bands – absolute	10^3/ul	58	0	0		
Lymphocytes – relative	% of WBC	58	23 (11)		20-26	3-58
Lymphocytes – absolute	10^3/ul	58	2.7 (1.3)		2.3-3.0	0.6-7.1
Monocytes – absolute	10^3/ul	58	0.3 (0.2)		0.2-0.3	0-0.8
Eosinophils – absolute	10^3/ul	58	4.6 (2.1)		1.3-10.3	4.0-5.1
Nucleated red blood cells (nRBC)		5	1.4 (0.9)		1.0-3.0	0.3-2.5
Erythrocyte sedimentation rate (ESR)	mm/hr	20	48 (18)	46	39-57	21-95
Platelets	10^3/ul	57	212 (55)	206	197-226	108-434
Mean platelet volume (MPV)	Fl	57	11.7 (1.5)	11.4	11.3-12.1	9.2-16.0
Total protein – refractometer	g/dl	58	7.4 (0.4)	7.4	7.3-7.5	6.6-8.3
Fibrinogen	mg/dl	58	153 (106)	100	125-181	50-600

Table 3b. Serum chemistry and serum protein electrophoresis of Atlantic bottlenose dolphin population in Charleston, SC (n = 62: 45 in 2003 and 17 in 2004)

Blood chemistry	Units	*n*	Mean ± SD	Median	95% CI	Range
Glucose	mg/dl	62	93 (12)	92	90-96	72-128
Sodium	mEq/l	62	156 (5)	156	155-157	140-180
Potassium	mEq/l	62	3.8 (0.4)	3.7	3.7-3.9	3.3-4.9
Sodium/potassium	mEq/l	17	43 (3)	42	41-44	38-48
Chloride	mEq/l	62	113 (4)	113	112-114	106-133
Bicarbonate	mEq/l	62	21 (4)	21	20-22	13-29
Anion gap	mEq/l	62	26 (5)	26	24-27	17-37
Blood Urea Nitrogen (BUN)	mg/dl	62	61 (8)	61	59-63	40-80
Creatinine	mg/dl	62	1.1 (0.3)	1.0	1.0-1.2	0.4-2.1
BUN/creatinine		62	61.4 (21.6)	56.9	55.9-66.9	28.6-147.5
Total protein	g/dl	62	7.1 (0.5)	7.1	6.9-7.2	5.6-8.6
Albumin	g/dl	62	4.4 (0.3)	4.5	4.3-4.5	3.7-5.8
Globulin	g/dl	62	2.6 (0.5)	2.7	2.5-2.8	1.1-4.0
Albumin/globulin	g/dl	62	1.8 (0.5)	1.7	1.6-1.8	0.9-4.2
Total bilirubin	mg/dl	62	0 (0)	0.1	0.10-0.10	0-0.2
Direct bilirubin	mg/dl	62	0.1 (0)	0	0-0	0-0.1
Indirect bilirubin	mg/dl	62	0.1 (0.1)	0.1	0.1-0.1	0-0.1
Calcium	mg/dl	62	9.3 (0.5)	9.3	9.1-9.4	7.3-10.5
Phosphorus	mg/dl	62	4.7 (0.9)	4.7	4.5-4.9	0.6-6.6
Magnesium	mg/dl	62	1.5 (0.1)	1.5	1.4-1.5	1.1-1.8
Uric acid	mg/dl	17	0.9 (0.6)	0.7	0.5-1.2	0.3-2.9
Alkaline phosphatase (ALP)	U/l	62	271 (199)	218	221-322	68-1,012
Alanine aminotransferase (ALT)	U/l	17	41 (13)	37	34-48	24-66
Aspartate aminotransferase (AST)	U/l	62	233 (80)	219	213-253	118-586
Sorbital dehydrogenase (SDH)	U/l	62	13 (11)	10	11-16	2-62
Lactate dehydrogenase (LDH)	U/l	17	178 (58)	475	426-486	346-534
Creatinine phosphokinase (CPK)	U/l	62	178 (48)	174	166-190	94-307
Amylase	U/l	17	1.6 (.04)	1.5	1.4-1.8	1.5-3.0
Lipase	U/l	17	9 (5)	10	7-12	1-16
Gamma-glutamyl transferase (GGT)	U/l	62	25 (5)	25	24-26	15-36
Cholesterol	mg/dl	17	157 (34)	151	139-174	114-232
Triglyceride	mg/dl	62	93 (29)	85	86-101	46-177
Iron	ug/dl	62	110 (32)	108	102-118	33-224
Total iron binding capacity (TIBC)	ug/dl	62	247 (51)	243	234-260	171-477
Transferrin saturation	%	62	45 (13)	44	42-49	17-85
Serum protein electrophoresis						
Total protein	g/dL	62	7.1 (0.5)	7.1	6.9-7.2	5.6-8.6
Albumin	g/dL	62	3.7 (0.4)	3.7	3.6-3.9	3.1-5.3
Total globulin	g/dl	62	3.3 (0.6)	3.4	3.2-3.5	1.9-4.4
Total alpha globulins	g/dl	62	1.2 (0.2)	1.2	1.2-1.3	0.8-1.7
Alpha-1	g/dl	62	0.5 (0.2)	0.6	0.5-0.6	0.1-0.9
Alpha-2	g/dl	62	0.7 (0.3)	0.5	0.6-0.8	0.3-1.3
Total beta globulins	g/dl	62	0.5 (0.1)	0.5	0.5-0.5	0.3-0.7
Beta-1	g/dl	45	0.2 (0.1)	0.2	0.2-0.3	0.1-0.5
Beta-2	g/dl	45	0.2 (0.1)	0.2	0.2-0.3	0.1-0.4
Total gamma globulins	g/dl	62	1.6 (0.5)	1.6	1.5-1.7	0.5-2.7
Albumin/globulin ratio	g/dl	62	1.2 (0.3)	1.1	1.1-1.3	0.8-2.3

Table 4a. Hematology values of Atlantic bottlenose dolphin population in Charleston, SC, by gender

		Males			Nonpregnant females			Pregnant females			
Hematology	Units	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*p*
White blood cells (WBC)	10^3/ul	35	11.9	2.9	17	11.4	2.4	5	11.5	3.4	0.757
Red blood cells (RBC)	10^6/ul	35	3.7[a]	0.2	17	3.4[b]	0.3	5	3.3[b]	0.3	0.000
Hemoglobin (Hb)	g/dl	35	14.2[a]	0.6	17	13.9[a,b]	1.1	5	13.2[b]	1.5	0.039
Packed cell volume (PCV)	%	7	41	2	7	40	3	0	--	--	0.182
Hematocrit (Hct)	%	35	40[a]	2	17	40[a]	3	5	36[b]	4	0.014
Mean corpuscular volume (MCV)	Fl	35	111[a]	6	17	119[b]	11	5	112[a,b]	10	0.003
Mean corpuscular hemoglobin (MCH)	pg	35	39	2	17	42	3	5	41	4	0.003
Mean corpuscular hemoglobin concentration (MCHC)	%	35	35	1	17	35	1	5	36	1	0.122
Red cell distribution width (RDW)	%	35	13.5[a]	1.1	17	12.1[b]	1	5	13[a,b]	1	0.000
Basophils	10^3/ul	35	0.1	0.1	17	0.1	0.2	5	0	0	0.214
Segmented neutrophils – relative	% of WBC	35	0	0	17	0	0	5	0	0	0.114
Segmented neutrophils – absolute	10^3/ul	35	4.4	1.5	17	3.5	1.5	5	4.1	1.0	0.096
Bands – relative	% of WBC	35	0	0	17	0	0	5	0	0	0
Bands – absolute	10^3/ul	35	0	0	17	0	0	5	0	0	0
Lymphocytes – relative	% of WBC	35	0	0	17	0	0	5	0	0	0.539
Lymphocytes – absolute	10^3/ul	35	2.6	1.4	17	2.9	1	5	2.6	1.7	0.787
Monocytes – relative	% of WBC	35	0	0	17	0	0	5	0	0	0.575
Monocytes – absolute	10^3/ul	35	0	0	17	0	0	5	0	0	0.501
Eosinophils – relative	% of WBC	35	37	12	17	4	15	5	4	4	0.759
Eosinophils – absolute	103/ul	35	5	2	17	5	2	5	5	2	0.996
Nucleated red blood cells (nRBC)		2	1	0	3	2	1	0	--	--	0.495
Erythrocyte sedimentation rate (ESR)	mm/hr	11	42	17	9	55	18	0	--	--	0.110
Platelets	10^3/ul	34	208	45	17	213	53	5	227	117	0.777
Mean platelet volume (MPV)	Fl	34	11.8	1.5	17	11.5	1.6	5	12.3	0.6	0.549
Total protein – refractometer	g/dl	35	7	0	17	8	0	5	8	0	0.703
Fibrinogen	mg/dl	35	150	111	17	147	110	5	200	71	0.601
Fibrinogen	mg/dl	--	--	--	16	126	63	5	200	71	0.040*

* Upper value of 500 in the nonpregnant group was an outlier by both the Grubbs and Dixon tests and was therefore removed.

[a, b] Means with different subscripts are statistically different.

inflammation was present in three (6%) of the dolphins sampled (Table 2). The majority of animals with some degree of gastric inflammation were male (10/13, 77%); all cases of severe inflammation occurred in males. The average age of dolphins with evidence of gastric inflammation was 14 y (+ 7 y).

Discussion

Reference values for several hematological and biochemical parameters have been previously reported for both wild (Asper et al., 1990; Bossart et al., 2001) and captive bottlenose dolphins (Ridgway et al., 1970; St. Aubin et al., 1996; Shirai & Sakai, 1997; Bossart et al., 2001; Terasawa et al., 2002; Terasawa & Kitamura, 2005). Little data are available on health measures of populations of wild bottlenose dolphins, and it is widely recognized that captivity imposes several environmental and physiologic adjustments that may alter clinical laboratory values (Medway & Geraci, 1986; Bossart et al., 2001). This study expands upon previously reported blood parameters for bottlenose dolphins and establishes hematology and serum

Table 4b. Serum chemistry and serum protein electrophoresis of Atlantic bottlenose dolphin population in Charleston, SC, by gender

		Males			Nonpregnant females			Pregnant females		
Blood chemistry	Units	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	*p*
Glucose	mg/dl	39	92	12	18	98	12	5	85	0.085
Sodium	mEq/l	39	156	3	18	156	7	5	155	0.765
Potassium	mEq/l	39	3.8	0.4	18	3.8	0.3	5	3.9	0.535
Sodium/potassium	mEq/l	10	43	4	7	42.0	3.2	0	--	0.516
Chloride	mEq/l	39	113	3	18	114	6	5	114	0.847
Bicarbonate	mEq/l	39	21	3	18	22	4	5	19	0.225
Anion gap	mEq/l	39	26	5	18	25	6	5	26	0.663
Blood Urea Nitrogen (BUN)	mg/dl	39	60	9	18	62	6.4	5	65	0.383
Creatinine	mg/dl	39	1.2[a]	0.3	18	0.9[b]	0.4	5	0.9[a,b]	0.032
BUN/creatinine	--	39	54.9[a]	17.0	18	73.3[b]	26.1	5	68.9[a,b]	0.006
Total protein	g/dl	39	7.1	0.5	18	6.9	0.6	5	7.3	0.492
Albumin	g/dl	39	4.4	0.3	18	4.5	0.4	5	4.2	0.188
Globulin	g/dl	39	2.7	0.5	18	2.5	0.5	5	3.1	0.056
Albumin/globulin	g/dl	39	1.8	0.5	18	1.9	0.5	5	1.4	0.159
Total bilirubin	mg/dl	39	0.1	0	18	0.1	0.1	5	0.1	0.706
Direct bilirubin	mg/dl	39	0	0	18	0	0	5	0	0.933
Indirect bilirubin	mg/dl	39	0.1	0.1	18	0.1	0.1	5	0.1	0.634
Calcium	mg/dl	39	9.3	0.3	18	9.4	0.7	5	8.8	0.057
Phosphate	mg/dl	39	4.8	0.7	18	4.6	1.3	5	4.7	0.633
Magnesium	mEq/l	39	1.5	0.1	18	1.5	0.2	5	1.4	0.064
Uric acid	mg/dl; det. limit 0.2	10	0.5[a]	0.2	7	1.3	0.8[b]	0	--	0.011
Alkaline phosphatase (AP)	U/l	39	285	202	18	262	189	5	200	0.652
Alanine aminotransferase (ALT)	U/l	10	38	12	7	45	15	0	--	0.253
Aspartate aminotransferase (AST)	U/l	39	235	68	18	236	111	5	202	0.681
Sorbital dehydrogenase (SDH)	U/l	39	14	12	18	12	10	5	13	0.839
Lactic dehydrogenase (LDH)	U/l	10	453	65	7	461	51	0	--	0.784
Creatine phosphokinase (CPK)	U/l	39	172[a,b]	49	18	201[a]	39	5	144[b]	0.022
Amylase	U/l	10	1.7	0.5	7	1.5	0	0	--	0.420
Lipase	U/l	10	8	4	7	11	5	0	--	0.263
Gamma-glutamyl transferase (GGT)	U/l	39	26	5	18	24	5	5	26	0.278
Cholesterol	mg/dl	10	149	34	7	167	34	0	--	0.311
Triglyceride	mg/dl	39	85[a]	24	18	111[b]	33	5	97[a,b]	0.006
Iron	ug/dl	39	111	29	18	109	33	5	106	0.938
Total iron binding capacity (TIBC)	mg/dl	39	241	56	18	262	40	5	238	0.344
Transferrin saturation	%	39	47	10	18	42	15	5	45	0.464
Serum protein electrophoresis										
Total protein	g/dl	39	7	1	18	7	1	5	7	0.533
Albumin	g/dl	39	3.7	0.4	18	3.8	0.5	5	3.8	0.815
Total globulins	g/dl	39	3	1	18	3	1	5	4	0.388
Total alpha globulins	g/dl	39	1.2	0.2	18	1.3	0.2	5	1.1	0.062
Alpha-1	g/dl	39	0.5	0.2	18	0.6	0.2	5	0.6	0.279
Alpha-2	g/dl	39	0.7	0.3	18	0.8	0.3	5	0.5	0.272
Total beta globulins	g/dl	39	0.5	0.1	18	0.5	0.1	5	0.5	0.185
Beta-1	g/dl	29	0.2	0.1	11	0.3	0.1	5	0.2	0.197
Beta-2	g/dl	29	0.2	0.1	11	0.2	0.1	5	0.3	0.121
Total gamma globulins	g/dl	39	1.7[a]	0.5	18	1.4[b]	0.4	5	1.9[a,b]	0.026
Albumin/globulin ratio	g/dl	39	1.2	0.3	18	1.2	0.3	5	1.1	0.649

[a, b] Means with different subscripts are statistically different.

Table 5. Hematology values of Atlantic bottlenose dolphin population in Charleston, SC by age

Hematology	Units	Adults (> 6 y)			Juvenile (< 6 y)			*p*
		n	Mean	SD	*n*	Mean	SD	
White blood cells (WBC)	10^3/ul	38	12.1	2.8	8	11.2	2.4	0.417
Red blood cells (RBC)	10^6/ul	38	3.5	0.2	8	3.5	0.2	0.562
Hemoglobin (Hb)	g/dl	38	14.0	0.8	8	13.9	1.0	0.645
Packed cell volume (PCV)	%	5	41	2	5	40	2	0.351
Hematocrit (Hct)	%	38	40	3	8	40	3	0.629
Mean corpuscular volume (MCV)	fl	38	112	6	8	116	10	0.373
Mean corpuscular hemoglobin (MCH)	pg	38	40	2	8	40	3	0.827
Mean corpuscular hemoglobin concentration (MCHC)	%	38	35	1	8	35	1	0.107
Red cell distribution width (RDW)	%	38	13.1	1.1	8	12.2	1.1	0.055
Basophils	10^6/ul	38	0.1	0.1	8	0	0.1	0.004
Segmented neutrophils – relative	% of WBC	38	37	10	8	25	10	0.005
Segmented neutrophils – absolute	10^3/ul	38	4	2	8	3	1	0.008
Bands – relative	% of WBC	38	0	0	8	0	0	--
Bands – absolute	10^3/ul	38	0	0	8	0	0	--
Lymphocytes – relative	% of WBC	38	0	0	8	0	0	0.156
Lymphocytes – absolute	10^3/ul	38	2.5	1.1	8	3.0	1.1	0.202
Monocytes – relative	% of WBC	38	2	2	8	2	0	0.688
Eosinophils – relative	% of WBC	38	0	0	8	0	0	0.537
Nucleated red blood cells (nRNB)	--	2	1	0	1	1	--	--
Erythrocyte sedimentation rate (ESR)	mm/hr	8	40	15	7	56	20	0.100
Platelets	10^3/ul	37	214	50	8	198	64	0.438
Mean platelet volume (MPV)	fl	37	11.8	1.3	8	11.7	2.0	0.939
Total protein – refractometer	g/dl	38	8	0	8	7	0	0.260
Fibrinogen	mg/dl	38	149	107	8	144	152	0.913

chemistry reference values for wild bottlenose dolphins from the estuarine waters of CHS, SC.

Photo-identification research from 1994 to 1996 in the CHS area established the presence of a resident dolphin community (Zolman, 2002). Subsequent research expanded the geographic scope of the study area to include more of the estuarine and near coastal areas for which we are now beginning to develop information in regards to the distribution and abundance of dolphins. At this early stage, the population is considered to be < 1,000 dolphins (Todd Speakman, pers. comm.). Thus, the hematology data would represent a sample size of at least 6% of the wider CHS-area dolphin population.

In this study and in our companion study for dolphins in the IRL (Goldstein et al., 2006), we report on an expanded suite of parameters, which includes red cell distribution width (RDW), mean platelet volume (MPV), bicarbonate, direct and indirect bilirubin, magnesium, uric acid, sorbital dehydrogenase (SDH), percentage of transferrin saturation (PSAT), Schalm fibrinogen, serum protein electrophoresis (SPEP), and the total protein and albumin/globulin ratio. The current study also includes cytologic findings, which had not previously been described for this specific population of dolphins. In addition to establishing mean baseline values for each parameter, the effects of gender, age, and pregnancy status were examined to determine their potential relationships to each parameter.

Hematological and biochemical values observed for the CHS dolphins were very close to or within the ranges previously published for these parameters in wild dolphins (Asper et al., 1990; Bossart et al., 2001). These previously published results, however, were not as extensive as those in this study or those for IRL dolphins (Goldstein et al., 2006). Nor did these prior studies include a comparable level of assessment relative to gender and age. Mean values for CHS dolphins were within the ranges for IRL dolphins, and most IRL means fell within or close to the CHS 95% CI; however, the CHS 95% CI for segmented neutrophils, relative and absolute, and lymphocytes, in addition to platelets and nRBC, were higher than mean IRL values. While differences found in some of these parameters may not be biologically relevant, others may be indicative of differences in the prevalence of subclinical disorders within these two populations.

Table 6. Serum chemistry and serum protein electrophoresis of Atlantic bottlenose dolphin population in Charleston, SC, by age

Blood chemistry	Units	Adults (> 6 y)			Juveniles (< 6 y)			*p*
		n	Mean	SD	*n*	Mean	SD	
Glucose	mg/dl	41	91	11	9	102	13	0.011
Sodium	mEq/l	41	156	3	9	153	5	0.196
Potassium	mEq/l	41	3.7	0.3	9	3.9	0.3	0.279
Sodium/potassium	mEq/l	7	45	3	5	43	4	0.319
Chloride	mEq/l	41	113	3	9	111	3	0.169
Bicarbonate	mEq/l	41	21	4	9	22	4	0.299
Anion gap	mEq/l	41	26	5	9	23	7	0.223
Blood Urea Nitrogen (BUN)	mg/dl	41	62	8	9	62	4	0.787
Creatinine	mg/dl	41	1.1	0.2	9	0.7	0.2	<.000
BUN/creatinine		41	57.7	13.9	9	92.5	27	0.005
Total protein	g/dl	41	7.1	0.4	9	6.7	0.5	0.009
Albumin	g/dl	41	4.4	0.3	9	4.3	0.3	0.739
Globulin	g/dl	41	2.8	0.4	9	2.4	0.4	0.016
Albumin/globulin	g/dl	41	1.6	0.3	9	1.8	0.3	0.028
Total bilirubin	mg/dl	41	0.1	0	9	0.1	0.1	0.310
Direct bilirubin	mg/dl	41	0	0	9	0	0	0.723
Indirect bilirubin	mg/dl	41	0.1	0.1	9	0.1	0	0.498
Calcium	mg/dl	41	9.2	0.4	9	9.2	0.8	0.961
Phosphate	mg/dl	41	4.7	0.6	9	4.6	0.8	0.526
Magnesium	mEq/l	41	1.5	0.1	9	1.4	0.2	0.839
Uric acid	mg/dl	7	0.5	0.2	5	1.5	0.9	0.071
Alkaline phosphatase (ALP)	U/l	41	227	136	9	344	226	0.167
Alanine aminotransferase (ALT)	U/l	7	40	13	5	38	11	0.756
Asparate aminotransferase (AST)	U/l	41	248	84	9	196	33	0.005
Sorbital dehydrogenase (SDH)	U/l	41	13	11	9	10	8	0.526
Lactate dehydrogenase (LDH)	U/l	7	467	48	5	453	60	0.674
Creatine phosphokinase (CPK)	U/l	41	160	37	9	218	35	<.000
Amylase	U/l	7	1.7	0.6	5	1.5	0	0.356
Lipase	U/l	7	9	2	5	10	6	0.630
Gamma-glutamyl transferase (GGT)	U/L	41	25	4	9	23	5	0.145
Cholesterol	mg/dl	7	158	38	5	165	38	0.780
Triglyceride	mg/dl	41	83	20	9	126	36	0.007
Iron	ug/dl	41	100	26	9	109	27	0.396
Total iron binding capacity (TIBC)	mg/dl	41	231	33	9	283	33	<.000
Transferrin saturation	%	41	44	12	9	39	9	0.210
Serum protein electrophoresis								
Total protein	g/dl	41	7	0	9	7	1	0.009
Albumin	g/dl	41	3.7	0.4	9	3.5	0.3	0.112
Total globulin	g/dl	41	3.4	0.5	9	3.2	0.5	0.202
Total alpha globulins	g/dl	41	1.2	0.2	9	1.4	0.3	0.013
Alpha-1	g/dl	41	0.6	0.2	9	0.6	0.1	0.879
Alpha-2	g/dl	41	0.6	0.3	9	0.8	0.4	0.087
Total beta globulins	g/dl	41	0.5	0.1	9	0.5	0.1	0.791
Beta-1	g/dl	34	0.2	0.1	4	0.2	0	0.783
Beta-2	g/dl	34	0.3	0.1	4	0.2	0.1	0.126
Total gamma globulin	g/dl	41	1.8	0.4	9	1.3	0.3	0.005
Albumin/ globulin ratio	g/dl	41	1.1	0.2	9	1.1	0.2	0.951

Most hematology and serum chemistry studies in wildlife are comprised of single samples which may not represent an accurate portrayal of an individual's condition. Variability associated with single sampling often makes it difficult to determine whether the blood parameter is improving or worsening. Thus, caution must be used when interpreting data based on single-values. Reference values are generally established for species to assist in monitoring the health of individuals (Cornell, 1983; Koopman et al., 1999). Gender and age are known to affect a number of hematological and serum chemistry measures in wildlife species, including marine mammals (Williams et al., 1992; St. Aubin et al., 1996). Hematological and biochemical parameters can also be affected by nutrition (Kuiken, 1985; Trites & Donnelly, 2003) and season (Terasawa et al., 2002). Additionally, capture stress and handling have been shown to affect blood parameters in several species, including harp seals (*Pagophilus groenlandicus*) (St. Aubin et al., 1979), sea otters (*Enhydra lutris*) (Williams et al., 1992), and beluga whales (*Delphinapterus leucas*) (St. Aubin & Geraci, 1988). As a result of these aforementioned variables affecting laboratory results, reference values can be difficult to determine in wild species.

Proper collection and sample handling protocols are of prime importance when measuring hematological parameters for accurate clinical interpretation. Serum and plasma should be separated within 20 min of collection and kept refrigerated as prolonged contact of erythocytes with serum can cause a decrease in glucose at a rate of 10%/h (Bossart et al., 2001). Our study employed standardized procedures similar to those used in other dolphin studies (Asper et al., 1990; Goldstein et al., 2006). For bottlenose dolphins, reference values reported here and for IRL dolphins (Goldstein et al., 2006) were derived from standardized protocols between sites, and studies were conducted under similar seasonal regimes and water temperatures. A comparison of the CHS dolphins' hematology and serum chemistry values with those from IRL dolphins with respect to other health parameters will contribute to the interpretation of clinical findings in dolphins from these populations.

Gender and age influences on some hematology and serum analyte parameters have been described previously in captive bottlenose dolphins (Asper et al., 1990) and wild dolphins in the IRL (Goldstein et al., 2006). Several statistically significant differences in hematological indices due to gender and/or age were found in the CHS dolphins as well. Males had higher Hb compared to the pregnant females, but RBC parameters of female dolphins had higher MCV, MCH, and lower RBC and RDW than males. Pregnant females had statistically lower Hct (the percentage of red blood cells relative to plasma volume) compared to both nonpregnant females and males, which may be due to the physiologic hemodilution of pregnancy. The increase in plasma volume during pregnancy is well-documented in humans, and erythrocyte production does not keep pace with the increased volume, resulting in decreased hematocrit (Hytten, 1985).

Female dolphins had higher mean triglyceride concentrations compared to males, although only nonpregnant females were statistically higher. Plasma triglyceride levels can vary widely with dietary changes, and higher triglyceride levels may reflect higher lipid intake. Elevated plasma lipid concentrations have also been observed during pregnancy in humans (Darmady & Postle, 1982), harbor seals (Kuiken, 1985), and also in dolphins in which triglycerides were elevated during the third and fourth stages of pregnancy (Terasawa & Kitamura, 2005). In humans, plasma triglycerides represent the most important source of fatty acids utilized by the fetus throughout pregnancy, with about 50% of these fatty acids being derived from maternal circulation (Nolan et al., 1995). The lack of statistically significant differences in triglycerides for pregnant dolphins, both in CHS and the IRL (Goldstein et al., 2006), may be due to the relatively small sample sizes of pregnant dolphins and also may have been affected by the stage of pregnancy. Triglycerides were significantly elevated in females from both the CHS and IRL sites, however, indicating consistent gender-based differences, which may be influenced by a number of variables, including prey, lipid metabolism, or perhaps hormonal factors. Juvenile CHS dolphins also had higher mean triglyceride values compared to adults, which may also be possibly related to diet and differences in lipid metabolism.

Fibrinolytic and coagulation systems undergo major alterations during pregnancy. Significantly higher fibrinogen values were observed in pregnant dolphins compared to nonpregnant dolphins. Elevated fibrinogen levels have been demonstrated in other mammals (Bunck et al., 2001), including humans (Manten et al., 2004), and this relationship is apparent in dolphins as shown in this study.

Creatinine, a product of muscle metabolism and cleared by renal excretion (Watnick & Morrison, 2005), was higher in the plasma of males compared to females, which would be plausible since males have higher muscle mass and plasma creatinine tends to increase with increased muscle mass. Creatinine kinase (CPK), an enzyme present in the heart, muscle, and brain, is elevated with muscle damage and physiologic stress. Younger

dolphins had higher mean CPK values compared to adults, which may have been related to enzyme leakage from muscle trauma during capture and restraint—and perhaps the higher enzyme levels in younger dolphins. In harp seals, CPK has been shown to increase with stress (St. Aubin et al., 1979), and significantly greater CPK values were also previously described in captive juvenile bottlenose dolphins (Asper et al., 1990). An interesting observation from females revealed they had twice the amount of uric acid, an end product of protein breakdown, when compared to males. It may be that the hyperuricaemia found in female dolphins may reflect normal levels of uric acid that may vary widely over time, but it could also be influenced by stress, high dietary purine, and renal disease (Daniels, 2003).

Adult dolphins had higher concentrations of AST, α-1 globulins, α-2 globulins, γ- globulins, and total protein, while juveniles had a higher albumin/globulin ratio and TIBC. Total protein, in addition to the albumin and globulin fractions, is important for the study of disease mechanisms and is receiving increased attention in marine mammals in terms of assessing health (Bossart et al., 2001). Adult dolphins also had higher segmented neutrophils, relative and absolute, although no statistically significant differences were observed in the absolute WBC. A stress-induced neutrophilia also cannot be ruled out. Granulocytes or segmented neutrophils are the main defender of the body against bacterial infection, and higher numbers may indicate an active infection while lower counts may indicate a compromised immune system. Both CHS and IRL adult dolphins (Goldstein et al., 2006) were found to have higher neutrophil counts (relative for IRL and both relative and absolute for CHS) than juveniles. Absolute, as opposed to relative, neutrophilia is generally accepted as an overall indicator of an inflammatory state in the body. The trend in increased neutrophil percentage in adults was also observed in captive dolphins by Asper et al. (1990), although they used age classifications of ≥ 12 y for adults and < 9 y for juveniles, which was higher compared to this study.

Alkaline phosphatase (ALP), an enzyme on cell membranes, has diagnostic significance for the hepatocellular and bone metabolism (Bossart et al., 2001). Serum ALP levels may be a useful prognostic indicator and is one of the best examples of a parameter associated with growing animals (Tryland et al., 2002). It is well known that ALP levels are age dependent, with higher values expressed during early growth associated with osseous activity (Robinson & Evans, 1996). Although ALP showed no age- or gender-related differences when tested as subgroups, ALP activity showed a clear trend in the full data set and was negatively correlated with age ($p < 0.0001$) (Figure 2).

Erythrocyte sedimentation rate (ESR) is a nonspecific indicator of inflammation acute-phase response and is used as a monitoring tool in veterinary and human medicine. In general, increases in ESR appear to be primarily associated with plasma fibrinogen (Bossart et al. 2001). The presence of elevated ESR generally indicates the need for further diagnostic tests, and a strong association exists between high ESR and infection, malignancy, and connective tissue disease (Saadeh, 1998). Normal ESR ranges in bottlenose dolphins are from 1 to 56 mm/h (Bossart et al., 2001) whereas the range in CHS dolphins was 21 to 95 mm/h. In our study, 50% of the dolphins had a mean ESR value of 46 mm/h. The significance and applications of ESR measurements in dolphins likely correlate with disease and other measures of inflammation and may be a useful prognostic indicator.

There were several differences in the hematology and serum chemistry between males and females, both pregnant and nonpregnant, as well as juveniles and adults. Consistent findings were obtained in both the CHS and IRL sites in juvenile dolphins having higher concentrations of BUN/creatinine, triglycerides, TIBC, and CPK than adults, which may have general inferences for age-related differences. It should be noted, however, that samples sizes of pregnant females and juvenile dolphins were small and may not have been representative of the entire population. Further monitoring of this population will help to increase sample size in these underrepresented groups. All parameters for which statistically significant differences were found between age and gender subgroups remained within the range considered clinically normal.

Cytology can be useful in detecting and monitoring pathological processes in cetaceans (Sweeney & Reddy, 2001). The degree of gastric inflammation may be an indication of subclinical disease as only apparently healthy animals were included in these analyses. The occurrence of gastric inflammation in CHS dolphins was also similar during the two years: 26% in 2003 and 29% in 2004. The prevalence of severe gastric inflammation was similar in CHS (3/50, 6%) and IRL dolphins (4/53, 7.5%; Goldstein et al., 2006). Older males comprised the majority of the animals exhibiting severe gastric inflammation; the mean age was 14 y in both populations. Gastric inflammation may be an indicator of stress, either physiologic or pathologic.

The relatively complete suite of hematology, serum chemistry, and cytology parameters obtained in this study can be used in assessing

health and in evaluating the effects of exposure to pathogens and contaminants as reported for other marine mammals (St. Aubin et al., 2001; Hanni et al., 2003; Reif et al., 2004). In the current study, with few exceptions, values for these parameters fell within the range reported in previous studies of captive and wild bottlenose dolphins. Our data were based on "apparently healthy" CHS dolphins, which are likely to exhibit the blood parameters of a healthy animal; however, blood parameters are but one measurement in establishing the overall health of a population. We plan to compare the data reported on the hematology, serum chemistry, and cytology with the comprehensive suite of health assessment measures, including contaminant body burdens, immunological profiles, and serological data that were collected during the Bottlenose Dolphin HERA Project. Between the years 1997 and 2003, bottlenose dolphin strandings along the SC coastal region averaged 43 per year with a range of 28 to 68 (McFee et al., 2006). Of the 302 stranded dolphins in SC during this time, 25% showed evidence of human interaction, but the causes of the majority of strandings are unknown. Long-term comprehensive studies are needed to assess the health status of dolphins in the CHS area and may provide insight into anthropogenic risk factors for mortality. This study represents one of the largest sets of hematological and biochemical analyses for wild cetaceans. For bottlenose dolphins, this study of CHS dolphins combined with the IRL dolphins, provides a unique data set collected over a relatively short period of time under similar environmental factors using standardized methods and a single reference laboratory. Thus, the new data presented in this study, when combined with that previously reported, should serve as an important reference point for future health-related and diagnostically integrated health studies of Atlantic bottlenose dolphins.

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