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HEMATOLOGY AND SERUM CHEMISTRY IN STRANDED AND WILD-CAUGHT HARBOR SEALS IN CENTRAL CALIFORNIA: REFERENCE INTERVALS, PREDICTORS OF SURVIVAL, AND PARAMETERS AFFECTING BLOOD VARIABLES

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ABSTRACT: Blood was collected from stranded harbor seal (*Phoca vitulina*) pups at admission ($n=64$) and release ($n=45$) from rehabilitation in 2007 and 2008 and from wild-caught harbor seal pups, subadults, and adults ($n=110$) in 2004, 2007, and 2008. Blood values measured at the time of admission were not predictive of survival during rehabilitation. Mass was associated with survival until release, and all pups that died weighed less than 10 kg at the time of admission. Döhle bodies were observed in leukocytes from 15% to 22% of the pups in rehabilitation, but not in the wild pups. Thresholds (95% confidence intervals) among wild pups were less than those in the released pups for leukocytes, neutrophils, total cholesterol, alanine aminotransferase (ALT), glucose, phosphorus, sodium, potassium, total protein, albumin, and globulin; thresholds were greater in wild pups than in released pups for hemoglobin (HGB), hematocrit (HCT), and glucose. Thresholds among released pups were less than those in wild pups for HGB, HCT, mean cell volume, chloride, and creatine kinase; thresholds among released pups were greater than those in wild pups for neutrophils, platelets, total cholesterol, triglycerides, ALT, aspartate aminotransferase, sorbitol dehydrogenase, bilirubin, phosphorus, potassium, total protein, and albumin. Age, girth, and geographic location affected the blood variables from wild-caught pups; age class, geographic location, sex, and body condition affected the blood variables of wild-caught, subadult and adult harbor seals.

Key words: Harbor seal, hematology, *Phoca vitulina*, reference intervals, rehabilitation, serum chemistry, stranding.

INTRODUCTION

Hematology and serum chemistry variables are used to help diagnose disease in marine mammals that are in rehabilitation and to assess the health of animals in wild populations. However, age, sex, season, reproductive status, captivity, diet, geographic location, and individual variability have all been reported to affect results, as well as laboratory methodology (Thompson et al., 1997; Morgan et al., 1998; Bossart et al., 2001; Trumble et al., 2006). When sampling the same individual repeatedly over time, changes in variables may reflect health trends; however, the results from a single blood sample can be difficult to interpret without baseline reference intervals. Age- and sex-specific reference intervals have been developed for humans, livestock, and domestic animals, but few exist for wild mammals, especially marine mammals, where sufficient samples are often scarce (e.g., Bossart et al., 2001).

Human medicine offers a growing body of literature on the choice of an appropriate reference population and on the most appropriate methods to calculate reference intervals for blood values from those groups (Fraser, 2004). Reference populations can vary by age, sex, or geographic location, depending on the research question. For example, hematology and serum chemistry reference intervals from local populations were established for clinical trials in Africa because using reference intervals from Americans as inclusion criteria resulted in the rejection of clinically healthy Africans from the study (Karita et al., 2009). When calculating reference intervals, The International Federation of Clinical Chemistry (IFCC) recommends using nonparametric statisti-

cal techniques (Solberg, 2004), which have recently been applied to terrestrial (riparian brush rabbit, *Sylvilagus bachmani riparius*; Black et al., 2009) and marine (bottlenose dolphin, *Tursiops truncatus*; Schwacke et al., 2009) wildlife. The resulting reference intervals have been used to evaluate differences in blood profiles among dolphins from different geographic locations, as well as between sexes and among age classes (Hall et al., 2007; Schwacke et al., 2009). Such reference intervals for other wildlife species, such as seals (family Phocidae), however, remain limited, yet are needed for health assessments of animals in veterinary care and in free ranging populations.

Harbor seal (*Phoca vitulina*) pups undergo a short, but intensive, lactation period lasting 3–5 wk. Each spring, harbor seal pups that are still dependent on maternal nutrition are found alone on the beach and admitted to The Marine Mammal Center (TMMC) for rehabilitation, and release back to the wild: Approximately 35 dependent pups are admitted each year. In the 10-yr period from 1992 to 2001, the most common health problems observed at admission among 940 harbor seals were malnutrition (52%), respiratory disease (10%), and trauma (8%); 78% of the harbor seals admitted were preweaned pups, but it was unclear whether maternal separation occurred naturally or as a result of human disturbance (Colegrove et al., 2005). On arrival at the hospital, each pup is examined and blood is drawn for hematology and serum chemistry tests. A treatment plan is devised based on blood results, age, and clinical signs. Previous work has shown that harbor seal pup blood values change during rehabilitation as they mature and their nutritional status improves (Lander et al., 2003). For example, bilirubin levels are often elevated in neonatal harbor seals but decline to levels similar to those in weaned wild pups by the time of release (Dierauf et al., 1984; Lander et al., 2003). Because of differences in age and diet between newborn pups admitted to rehabilitation and their recently weaned, wild conspecifics, reference intervals generated from the wild, weaned population are not useful for assessing the individual health of dependent pups admitted to rehabilitation.

The objectives of this study were 1) to use blood variables from stranded harbor seals (at admission to, and release from, rehabilitation); recently weaned, wild-caught harbor seals; and older, wild-caught harbor seals to provide age-specific ranges for evaluating harbor seal health in these four different groups of animals; 2) to evaluate the ability of blood variables measured at admission to predict survival during rehabilitation; and 3) to determine the effect of age class, sex, date (Julian day), location, size, and body condition on blood variables from wild-caught harbor seals.

MATERIALS AND METHODS

Capture and sampling

Wild harbor seals of all age classes were sampled in San Francisco Bay, California, USA (SF; 37°55′58″N, 122°25′3″W), and Tomales Bay, Point Reyes National Seashore, California, USA (TB; 38°13′9″N, 122°57′42″W), in May and June 2004, 2007, and 2008, after the pups were weaned (to avoid disturbing nursing animals). Blood was drawn from the epidural venous sinus into blood collection tubes containing ethylenediaminetetraacetic acid or serum-separation gel (Vacutainer, Becton Dickinson, Franklin Lakes, New Jersey, USA) and stored at 4 C from within 5 min of collection until processing. In 2007 and 2008, stranded harbor seal pups along the California coast from Mendocino (40°0′0″N, 124°1′24″W) to San Luis Obispo (35°0′0″N, 120°38′25″W) counties were brought to TMMC for rehabilitation, where they had blood drawn within 5 days of admission and within 48 hr of release. At admission, all pups were rehydrated for varying periods of 1 to 5 days with subcutaneous fluids and oral electrolytes and were treated with oral amoxicillin based (West-Ward Pharmaceutical Corp., Eatontown, New Jersey, USA) on clinical signs and blood work.

Sample analyses

A complete blood cell count (CBC) was obtained using an automated hematology

analyzer (Vet ABC Heska, Loveland, Colorado, USA) and included leukocytes (white blood cells [WBCs]); erythrocytes (red blood cells [RBCs]); hemoglobin (HGB); hematocrit (HCT); mean cell volume (MCV), from which mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) were derived; and platelets. White cell differential counts and cell morphology were read manually from a blood smear stained with Wright-Giemsa stain (EMD Chemicals Inc., Gibbstown, New Jersey, USA). All differentials were read by the same person (C.A.R.) knowing only the seal's identification number and species. The hematology analyzer, calibrated for canines (*Canis* spp.), was recalibrated when needed using Minocal whole blood hematology calibrator (scil veterinary excellence, Gurnee, Illinois, USA). Three commercial hematology controls—ABX Diagnostics Minotrol 16, Veterinary Hematology control, and scil Vet ABC+ (all from scil veterinary excellence)—were tested every Monday, then alternated (1/day) Tuesday through Friday, as recommended by the manufacturer.

Serum separator tubes were centrifuged, and 0.5 ml of serum was used for chemistry analytes determined with an automated chemistry analyzer (VET-ACE 1016 Quantitive Multistation Chemistry Analyzer, Alfa Wassermann Diagnostic Technologies, West Caldwell, New Jersey, USA). Chemistry variables measured were iron, cholesterol, triglycerides, γ-glutamyltransferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALK), creatine kinase (CK), sorbitol dehydrogenase (SDH), bilirubin, glucose, blood urea nitrogen (BUN), creatinine, phosphorus, calcium, sodium, potassium, chloride, magnesium, total protein, and albumin. Globulin was calculated as total protein minus albumin. The sample size for the SDH results was smaller than for the other variables because this enzyme was added to the chemistry panel midstudy. The sample size for the serum chemistries from wild-caught seals was smaller than for hematology because only hematologic variables were determined in 2004. The chemistry analyzer was designed for human use and is calibrated once a month using a calibrator from the manufacturer. Two controls of lyophilized human serum from the manufacturer were run every day as were two separate controls for SDH (Genzyme Diagnostics, Framingham, Massachusetts, USA). If a control failed, it was repeated, and if it failed again, the machine was recalibrated and the reagents were checked. Monthly quality control data were sent to the Bio-Rad Unity Interlaboratory Program (Bio-Rad Laboratories, Inc., Irvine, California, USA), which compared results with peer groups using the same instrument. Quality control results remained within the range generated for these peer groups for all analytes tested.

Data analyses

Data were separated into four data sets: 1) pups at admission, 2) pups at release, 3) wild-caught pups, and 4) wild-caught subadults and adults. Upper and lower thresholds consisting of 95% confidence intervals, with 90% confidence intervals for each threshold, were generated for each data set using a nonparametric bootstrap analysis as recommended by the IFCC (Solberg, 2004). Bootstrap analyses were performed using the R programming language (http://www.r-project.org; R Development Core Team, 2009) after Schwacke et al. (2009). For sample sizes of less than 40, the minimum and maximum values were reported as the upper and lower thresholds, and the 90% confidence intervals were not calculated. Three values were considered to be outliers because the difference between those values and the next lowest value was greater than one-third of the range of all values for that variable (Schwacke et al., 2009): one band neutrophil count (2,303/μl), a magnesium concentration (3.4 mg/dl), and a CK concentration (11,935 U/l) were deleted. One pup had a partial chemistry panel but was retained in the data set. Two values were deleted from the data set on wild-caught pups: an albumin concentration (0.1 g/dl) that was an outlier and the derived globulin. Three animals with obvious injuries were omitted from the wild-caught subadult and adult data set because they had potential to affect the boundaries of the intervals.

Binary logistic-regression models were used to test whether blood variables measured at admission were predictive of whether an animal would survive rehabilitation until release. Eight animals were omitted from this data set because they were given antibiotics based on their blood values at admission, which had the potential to affect their outcome. Date of stranding, mass at stranding, and sex were also tested as predictors of survival through rehabilitation. A χ^2 test was used to determine whether prematurity (defined after Dierauf et al. [1986] as birth before 15 April and presence of a lanugo coat) was predictive of survival.

Differences in lower and upper thresholds for pups treated at TMMC just before release were compared with those of wild-caught, recently weaned pups, again, using a bootstrap approach. Values for each group were esti-

mated, and the difference was calculated 1,000 times; when the 95% confidence intervals around the difference did not include zero, the differences were considered statistically significant (Schwacke et al., 2009).

Generalized linear models (GLMs) were used to evaluate the effects of different morphologic measurements (standard length, mass, axillary girth, and a body condition index), age (using capture date in Julian days as a proxy), sex, and location (SF versus TB) on the wild-caught, harbor seal blood variables. The body condition index was the residuals from the linear relationship between mass (in kilograms) and length (in centimeters): $mass = length \times 0.53 - 26.47$ ($R^2 = 0.57$, $P < 0.005$).

Length, mass, and girth were significantly related to each other, so girth was chosen as the most reproducible of the measurements and a good indicator of body condition in phocid seal pups (Hall and McConnell, 2007). Sex was not considered a relevant factor for the pup age class because no differences in physiology and behavior were expected until puberty (Hall, 1998). There was a temporal relationship between location and pup age because, in all 3 years, pup capture effort began in SF and then moved to TB; thus, to investigate the effect of both location and age (Julian day), the starting model for wild-caught pups was additive and included location, age, and girth: *blood variable*~(*location*+*age*+*girth*). Models were fitted to the data with a gamma error structure and logarithmic link function, such that predicted $Y = \exp(intercept + coefficient \times x_1 + coefficient \times x_2)$. Akaike's Information Criterion (AIC) was used to compare models and choose the most parsimonious model for each blood variable. Residual analysis was used to assess goodness of fit. Blood variables with zeroes could not be fitted using the gamma error structure, so bands, monocytes, eosinophils, and basophils were fitted using a compound Poisson distribution (Tweedie model).

Because length, girth, and mass are significantly and positively correlated with age, they were not used in the model for the older age classes. Instead, we chose location, sex, age class, and condition (which was not associated with age, $R^2 = 0.024$, $P = 0.20$). Zero values were deleted from the data set for monocytes ($n = 3$), eosinophils ($n = 1$), and basophils ($n = 1$). Band neutrophil data were fitted with a compound Poisson distribution (Tweedie model). The fully parameterized model for the wild-caught, subadult and adult data set was *blood variable*~(*location*+*sex*+*age*+ *body condition*). Parameters were considered significant at $P = 0.05$, but nonsignificant parameters were retained in some models based on AIC.

RESULTS

Predictors of survival

Hematology and serum chemistry variables were measured in dependent pups ($n = 64$) on admission to TMMC in 2007 and 2008, and the intervals are summarized in Table 1. Of the 64 seals, 42 (66%) were released back to the wild, and 22 (34%) died during rehabilitation; one seal did not receive an admission chemistry, and one seal did not receive an admission hematology. For the 22 pups that died, the mean number of days in rehabilitation was 20 (SD = 19, range = 1–70). Causes of death included septicemia and umbilical infection ($n = 13$; 59%), malnutrition ($n = 2$; 9%), encephalitis ($n = 2$; 9%), and other (23%; enteritis, pyelonephritis, euthanasia because of blindness, herpesvirus, and congenital defects).

Two variables at the time of admission were significantly associated with survival through rehabilitation, but neither model would have been significant had a Bonferroni correction for multiple tests approach been used, and both models were poor fits to the data. Decreased platelets were associated with decreased survival ($disposition = platelets \times 0.005 - 1.353$, $P = 0.03$) and decreased levels of protein were associated with decreased survival ($disposition = protein \times 1.3896 - 6.939$, $P = 0.01$). Neither date of stranding nor sex affected survival through rehabilitation; however, mass at stranding was predictive of survival ($probability\ of\ survival = e^{(mass \times 0.956 - 6.802)}/[1 + e^{(mass \times 0.956 - 6.802)}]$, $P = 0.004$), with all the pups that died during treatment weighing under 10 kg at stranding. Prematurity was not predictive of survival during rehabilitation ($X^2 = 1.675$, df = 1, $P = 0.20$).

Döhle bodies, which are areas of dissolving, rough, endoplasmic reticulum within the neutrophil cytoplasm (Prokocimer and Potasman, 2008), were observed in 10 blood

TABLE 1. Intervals for blood variables from dependent harbor seal pups at admission to The Marine Mammal Center, California, USA, 2007–2008.[a]

Blood variable	*n*	90% CI on lower threshold	Lower threshold	Median	Upper threshold	90% CI on upper threshold
WBC (/μl)	63	2,000–4,500	2,800	8,100	25,000	13,300–32,900
Neutrophils (mature)	63	1,120–2,173	1,680	5,440	22,000	9,842–26,978
Neutrophils (band)	62	0–0	0	56	864	570–990
Lymphocytes	63	663–1,127	720	2,130	3,888	3,626–5,088
Monocytes	63	0–0	0	153	648	567–749
Eosinophils	63	0–0	0	0	384	124–492
Basophils	63	0–0	0	0	344	255–352
RBC (10^6/μl)	63	4.22–4.73	4.44	5.81	7.09	6.95–7.33
HGB (g/dl)	63	14.0–17.3	14.7	19.8	24.4	23.6–25.2
HCT (%)	63	39.9–49.2	42.5	57.6	68.8	68.1–73.4
MCV (fl)	63	90–93	92	102	113	110–114
MCH (pg)	63	29.7–31.8	30.8	35.2	40.8	38.5–40.8
MCHC (g/dl)	63	31.7–32.6	31.8	34.5	37.7	36.2–37.8
Platelets (10^3/μl)	63	59–216	91	375	695	638–763
Iron (μg/dl)	62	45–55	46	181	562	423–585
Total cholesterol (mg/dl)	63	144–174	150	277	446	410–456
Triglycerides (mg/dl)	63	31–66	47	112	276	239–328
GGT (U/l)	63	9–15	13	34	242	130–304
ALT (U/l)	63	19–26	20	71	325	220–340
AST (U/l)	63	15–29	24	61	462	154–621
ALK (U/l)	63	43–92	59	177	404	306–511
CK (U/l)	61	71–84	72	194	2,569	1,389–3,140
SDH (U/l)	33		1.8	26.1	87.5	
Total bilirubin (mg/dl)	63	0.3–0.7	0.6	2.4	24.1	13.6–24.8
Glucose (mg/dl)	62	40–79	64	149	331	286–361
BUN (mg/dl)	63	7–13	11	26	59	47–70
Creatinine (mg/dl)	63	0–0.1	0	0.4	0.6	0.6–0.6
Phosphorus (mg/dl)	62	4.1–4.9	4.7	6.3	8.6	8.1–8.6
Calcium (mg/dl)	62	7.2–8.0	7.5	9.0	10.2	9.9–10.6
Sodium (mmol/l)	63	125–138	131	147	162	154–164
Potassium (mmol/l)	63	3.1–3.9	3.3	4.7	5.8	5.4–6.5
Chloride (mmol/l)	63	88–98	96	108	124	118–133
Magnesium (mg/dl)	61	1.4–1.5	1.5	1.7	2.3	2.2–2.4
Total protein (g/dl)	62	4.0–4.5	4.3	5.6	6.5	6.4–6.6
Albumin (g/dl)	62	2.1–2.8	2.4	3.5	4.2	4.1–4.3
Globulin (g/dl)	62	1.4–1.6	1.5	2.0	3.1	3.0–3.3

[a] CI = confidence interval; WBC = white blood cell count; RBC = red blood cell count; HGB = hemoglobin; HCT = hematocrit; MCV = mean cell volume; MCH = mean cell hemoglobin; MCHC = mean cell hemoglobin concentration; GGT = γ-glutamyltransferase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; ALK = alkaline phosphatase; CK = creatine kinase; SDH = sorbitol dehydrogenase; BUN = blood urea nitrogen.

smears (16%), including samples with three of the four highest WBC concentrations. Large lymphocytes were observed in 18 blood smears (28%). There was a wide range of HGB and HCT values, which was likely a reflection of the animal's hydration status because MCHC was not as variable, but these variables were not associated with survival. Leukocytes also ranged dramatically, with the highest and lowest WBC concentrations in pups that died within 24 hr of admission. Because diseased individuals were included in the data set, the intervals for this group do not represent true reference intervals for dependent harbor seal pups (Geffré et al., 2009).

Released pups compared with recently weaned wild pups

Harbor seal pups were released to the wild after an average of 84 days in rehabilitation (SD=20 days, range=30–

TABLE 2. Reference intervals for blood variables from harbor seal pups at release from the Marine Mammal Center, Sausalito, California, USA, 2007–2008.[a]

Blood variable	*n*	90% CI on lower threshold	Lower threshold	Median	Upper threshold	90% CI on upper threshold
WBC (/μl)	45	4,900–7,300	6,200[b]	9,700	15,300	13,600–16,000
Neutrophils (mature)	45	3,080–3,735	3,348[b]	6,077	11,250[b]	8,925–11,520
Neutrophils (band)	45	0–0	0	0	565	93–1,224
Lymphocytes	45	972–1617	1,170	2,790	4,900	4,150–5,194
Monocytes	45	0–0	0	74	900	480–918
Eosinophils	45	0–0	0	136	1,308	837–1,332
Basophils	45	0–0	0	238	666	558–721
RBC (10^6/μl)	45	4.23–4.80	4.54	5.33	6.03	5.74–6.70
HGB (g/dl)	45	15.9–17.2	15.9[b]	19	21.9[b]	21.3–22.1
HCT (%)	45	45.5–48.1	46.1[b]	54.4	62.0[b]	61.7–62.7
MCV (fl)	45	90–95	93[b]	103	112	109–115
MCH (pg)	45	33.0–33.9	33.2	36.4	39.1	38.6–40.1
MCHC (g/dl)	45	32.9–33.6	33.0	35.3	38.2	37.9–38.5
Platelets (10^3/μl)	42	268–463	334	689	1,130[b]	900–1,490
Iron (μg/dl)	43	65–111	95	199	494	417–566
Total cholesterol (mg/dl)	43	243–257	248[b]	306	422[b]	370–535
Triglycerides (mg/dl)	43	40–62	55	210	436[b]	392–550
GGT (U/l)	43	7–15	14	18	49	28–81
ALT (U/l)	43	22–41	28[b]	56	99[b]	78–176
AST (U/l)	43	30–45	32	75	191[b]	142–505
ALK (U/l)	43	43–83	62	136	307	265–434
CK (U/l)	43	72–103	79[b]	247	1986	1,122–8,472
SDH (U/l)	23		16.3	68.9	127.1[b]	
Total bilirubin (mg/dl)	43	0.2–0.3	0.3	0.8	1.9[b]	1.7–2.3
Glucose (mg/dl)	43	117–128	121[b]	145	176[b]	162–177
BUN (mg/dl)	43	25–35	29	48	75	61–77
Creatinine (mg/dl)	43	0.3–0.4	0.3	0.5	0.8	0.7–1.0
Phosphorus (mg/dl)	43	4.8–5.4	4.8[b]	7.3	10.1[b]	8.8–10.6
Calcium (mg/dl)	43	8.8–9.1	8.9	9.9	10.4	10.3–10.4
Sodium (mmol/l)	43	144–146	145[b]	150	160	152–166
Potassium (mmol/l)	43	4.0–4.3	4.0[b]	4.9	5.8[b]	5.6–5.9
Chloride (mmol/l)	43	99–103	100[b]	108	118	112–124
Magnesium (mg/dl)	42	1.5–1.7	1.6	2.0	2.6	2.3–2.6
Total protein (g/dl)	43	6.0–6.8	6.6[b]	7.5	8.9[b]	8.2–9.0
Albumin (g/dl)	43	3.0–3.2	3.1[b]	3.5	4.0[b]	3.7–4.3
Globulin (g/dl)	43	2.7–3.2	3.0[b]	4.0	5.2	4.7–5.4

[a] CI = confidence interval; WBC = white blood cell count; RBC = red blood cell count; HGB = hemoglobin; HCT = hematocrit; MCV = mean cell volume; MCH = mean cell hemoglobin; MCHC = mean cell hemoglobin concentration; GGT = γ-glutamyltransferase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; ALK = alkaline phosphatase; CK = creatine kinase; SDH = sorbitol dehydrogenase; BUN = blood urea nitrogen.

[b] Significant difference from the blood variable thresholds in wild pups in Table 3. See "Methods" for explanation of statistical testing.

143 days). Reference intervals for each blood variable were generated from 45 hematology and 43 serum chemistry samples collected just before release (Table 2) and from 40 recently weaned pups captured in May and June 2004, 2007, and 2008 (Table 3). Döhle bodies were present in 10 of the prerelease seals (22%) and large lymphocytes were present in four (9%). Among the wild-caught pups, no Döhle bodies were noted, and large lymphocytes were observed in six seals (15%). There were some significant differences between the released pup reference intervals and the wild pup reference intervals. Lower thresholds

TABLE 3. Reference intervals for blood variables from recently weaned, wild-caught harbor seal pups, California, USA, 2004, 2007, and 2008.[a]

Blood variable	*n*	90% CI on lower threshold[b]	Lower threshold	Median	Upper threshold	90% CI on upper threshold[b]
WBC (/μl)	40	4,300–4,800	4,300[c]	7,550	13,300	11,300–13,600
Neutrophils (mature)	40	1,968–2,464	1,968[c]	4,193	8,214[c]	6,901–8,296
Neutrophils (band)	40	0–0	0	0	309	266–360
Lymphocytes	40	1,088–1,364	1,088	2,354	4,070	3,294–4,407
Monocytes	40	0–0	0	142	812	486–960
Eosinophils	40	0–0	0	236	1,596	1,224–2,730
Basophils	40	0–0	0	185	928	540–1088
RBC (10^6/μl)	40	4.70–4.82	4.70	5.76	6.43	6.07–6.70
HGB (g/dl)	40	17.3–17.9	17.3[c]	21.6	23.9[c]	22.9–24.5
HCT (%)	40	49.4–52.8	49.4[c]	60.6	68.7[c]	64.9–69.9
MCV (fl)	40	99–100	99[c]	106	113	111–113
MCH (pg)	40	33.6–34.2	33.6	36.9	40.7	40.1–42.2
MCHC (g/dl)	40	32.8–33.3	32.8	34.5	39.0	37.4–39.2
Platelets (10^3/μl)	40	153–301	153	485	653[c]	622–795
Iron (μg/dl)	35		68	184	646	
Total cholesterol (mg/dl)	35		146[c]	245	361[c]	
Triglycerides (mg/dl)	35		35	88	157[c]	
GGT (U/l)	35		5	17	81	
ALT (U/l)	35		19[c]	29	58[c]	
AST (U/l)	35		27	47	92[c]	
ALK (U/l)	35		37	126	540	
CK (U/l)	35		127[c]	309	1403	
SDH (U/l)	30		13.2	34.3	74.4[c]	
Total bilirubin (mg/dl)	35		0.2	0.5	1.0[c]	
Glucose (mg/dl)	35		99[c]	152	217[c]	
BUN (mg/dl)	35		25	36	62	
Creatinine (mg/dl)	35		0.3	0.6	1	
Phosphorus (mg/dl)	35		3.7[c]	4.8	6.5[c]	
Calcium (mg/dl)	35		8.8	9.7	10.6	
Sodium (mmol/l)	35		143[c]	149	157	
Potassium (mmol/l)	35		3.8[c]	4.4	5.1[c]	
Chloride (mmol/l)	35		105[c]	108	117	
Magnesium (mg/dl)	35		1.6	2.1	2.8	
Total protein (g/dl)	35		5.2[c]	5.8	7.7[c]	
Albumin (g/dl)	34		2.3[c]	3.3	3.6[c]	
Globulin (g/dl)	34		2.0[c]	2.5	5.4	

[a] CI = confidence interval; WBC = white blood cell count; RBC = red blood cell count; HGB = hemoglobin; HCT = hematocrit; MCV = mean cell volume; MCH = mean cell hemoglobin; MCHC = mean cell hemoglobin concentration; GGT = γ-glutamyltransferase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; ALK = alkaline phosphatase; CK = creatine kinase; SDH = sorbitol dehydrogenase; BUN = blood urea nitrogen.

[b] 90% CI not calculated for sample sizes <40 (See "Methods").

[c] Significant difference from the blood variable thresholds in wild pups in Table 2. See "Methods" for explanation of statistical testing.

among the wild pups were less than those for the released pups for WBCs, neutrophils, cholesterol, ALT, glucose, phosphorus, sodium, potassium, total protein, albumin, and globulin: lower thresholds among the released pups were less than those for the wild pups for HGB, HCT, MCV, chloride, and CK. Upper thresholds were greater among the wild than the released pups for HGB, HCT, and glucose, whereas upper thresholds were greater among the released than the wild pups for neutrophils, platelets, cholesterol, triglycerides, ALT, AST, SDH, bilirubin,

phosphorus, potassium, total protein, and albumin (Tables 2 and 3).

Predictors of blood variables in wild harbor seals

Samples were obtained from 31 pups from SF and 9 pups from TB. The most consistent factor affecting wild-caught pup blood variables was age: monocytes ($t=6.03$, $P<0.005$), eosinophils ($t=4.48$, $P<0.005$), RBCs ($t=2.33$, $P=0.03$), HGB ($t=5.05$, $P<0.005$), HCT ($t=3.02$, $P<0.005$), MCH ($t=2.28$, $P=0.03$), MCHC ($t=2.94$, $P=0.01$), ALT ($t=2.10$, $P=0.04$), and creatinine ($t=3.70$, $P<0.005$) all increased with age; whereas iron ($t=[-2.93]$, $P=0.01$), ALK ($t=[-4.40]$, $P<0.005$), CK ($t=[-3.13]$, $P<0.005$), SDH ($t=[-2.70]$, $P=0.01$), sodium ($t=[-4.01]$, $P<0.005$), chloride ($t=[-4.33]$, $P<0.005$), and magnesium ($t=[-2.63]$, $P=0.01$) decreased with age. Location was retained in several models: HCT ($t=[-2.41]$, $P=0.02$) and glucose ($t=[-4.02]$, $P<0.005$) were greater in SF than TB, and concentrations of MCH ($t=2.02$, $P=0.05$), MCHC ($t=4.95$, $P<0.005$), and SDH ($t=3.25$, $P<0.005$) were greater in TB than SF. Eosinophils ($t=[-2.42]$, $P=0.02$) and GGT ($t=[-2.97]$, $P=0.01$) were negatively associated with girth, whereas creatinine ($t=2.11$, $P=0.04$) was positively associated with girth. Increased numbers of monocytes (>200/μl) and eosinophils (>500/μl) appeared around Julian day 150, about 25 days after the pups had been weaned and toward the end of the sampling period.

Samples were obtained from 9 subadult and 10 adult harbor seals in SF and 24 subadult and 27 adult harbor seals in TB. Reference intervals for these animals are reported in Table 4. Age class was a significant variable for blood results from subadult and adult harbor seals: WBCs, lymphocytes, eosinophils, basophils, RBCs, HGB, HCT, and ALK were greater among the subadults, whereas MCH and BUN were greater among adults. Erythrocytes, HGB, and HCT were greater in TB, whereas monocytes, MCV, and MCH were greater in SF. Males had more neutrophils, whereas females had greater concentrations of MCH. Eosinophils and GGT increased and SDH decreased with increased body condition (Table 5).

DISCUSSION

Although typically used to evaluate health and to direct clinical management, the blood variables measured in this study at the time of admission were not predictive of whether seal pups would survive rehabilitation. Half of the seals that died during rehabilitation were septic, with the umbilicus as the suspected entry point of infection, although there were rarely signs of inflammation on the initial blood panel to guide treatment. This suggests that the immediate handling for hematologic assessment of neonates using the current, routinely available tests may be less important than other features of the admission examination. Although we support the use of hematology and serum chemistry to help diagnose illness and to inform treatment during rehabilitation, the lack of prognostic changes in these data leads us to recommend that the initial treatment be based on mass and stage of development (e.g., presence of an umbilicus) of the seal. It should thus focus on minimizing the risk of umbilical infection and septicemia in neonates by cleansing the umbilical area regularly and using broad-spectrum antibiotics.

The significant relationship between mass at stranding and the probability of survival through rehabilitation was consistent with the recognized association between mass and survival at various life stages in phocid seals: Mass at weaning has been linked to first year survival in grey seals (*Halichoerus grypus*; Hall et al., 2002) and survival to 2 yr in monk seals (*Monachus schauinslandi*; Craig and Ragen, 1999); and autumnal mass has been linked to overwinter survival in harbor seals (Harding et al., 2005). Low birth weight and prematurity are associated with decreased survival in humans (Gold-

TABLE 4. Reference intervals for blood variables from wild-caught, subadult and adult harbor seals, California, USA, May and June 2004, 2007, and 2008.[a]

Blood variable	*n*	90% CI on lower threshold	Lower threshold	Median	Upper threshold	90% CI on upper threshold
WBC (/μl)	70	5,300–6,800	5,600	10,900	18,000	15,500–19,100
Neutrophils (mature)	70	1,276–2,546	1,520	5,610	10,325	9,300–11,050
Neutrophils (band)	70	0–0	0	0	350	145–465
Lymphocytes	70	440–1,458	1,140	3,006.5	5,940	4,797–6,000
Monocytes	70	0–96	0	475.5	1,562	1,170–2,397
Eosinophils	70	0–464	408	1,264.5	3,441	2,875–5,157
Basophils	70	0–109	91	397.5	900	764–1,170
RBC (10^6/μl)	70	2.9–4.09	3.75	4.92	5.84	5.64–5.97
HGB (g/dl)	70	12.7–17	15.8	20.3	23.3	22.8–24.4
HCT (%)	70	34.7–48.5	43.3	56.65	63.2	62.4–65
MCV (fl)	70	97–107	100	115	126	122–131
MCH (pg)	70	35.9–37.6	36.3	41.4	46.7	43.9–47.7
MCHC (g/dl)	70	34–34.4	34.1	35.5	39.1	38.4–39.5
Platelets (10^3/μl)	69	155–329	178	462	806	670–823
Iron (μg/dl)	57	35–71	58	146	312	258–330
Total cholesterol (mg/dl)	57	157–182	178	233	342	324–369
Triglycerides (mg/dl)	57	23–46	38	86	245	174–487
GGT (U/l)	56	0–7	1	11	29	21–39
ALT (U/l)	57	20–32	22	54	119	101–159
AST (U/l)	57	53–66	59	113	326	251–510
ALK (U/l)	56	0–9	4	38	80	66–88
CK (U/l)	57	154–230	205	659	5125	2,806–9,462
SDH (U/l)	16	15.7–28.7	15.7	48.0	87.2	66.2–87.2
Total bilirubin (mg/dl)	57	0.2–0.3	0.2	0.5	1.8	1.4–1.9
Glucose (mg/dl)	57	86–95	87	120	161	145–172
BUN (mg/dl)	57	20–29	20	41	74	67–86
Creatinine (mg/dl)	57	0.5–0.6	0.5	0.8	1.1	1–1.1
Phosphorus (mg/dl)	57	1.9–2.7	2.5	4.8	7.2	6.8–11.3
Calcium (mg/dl)	57	7.7–8.1	7.9	9.1	9.9	9.8–9.9
Sodium (mmol/l)	57	145–147	146	149	154	153–162
Potassium (mmol/l)	57	3.9–3.9	3.9	4.4	5.2	4.9–5.2
Chloride (mmol/l)	57	104–106	106	109	117	113–120
Magnesium (mg/dl)	57	1.4–1.5	1.5	1.8	2.2	2.1–2.4
Total protein (g/dl)	57	7–7.3	7	7.9	9.5	9–9.9
Albumin (g/dl)	57	2.5–2.5	2.5	3	3.4	3.3–3.5
Globulin (g/dl)	57	4.1–4.4	4.3	5.0	6.7	6.1–6.7

[a] CI = confidence interval; WBC = white blood cell count; RBC = red blood cell count; HGB = hemoglobin; HCT = hematocrit; MCV = mean cell volume; MCH = mean cell hemoglobin; MCHC = mean cell hemoglobin concentration; GGT = γ-glutamyltransferase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; ALK = alkaline phosphatase; CK = creatine kinase; SDH = sorbitol dehydrogenase; BUN = blood urea nitrogen.

enberg and Culhane, 2007) and red deer (*Cervus elaphus*; Clutton-Brock et al., 1987). Interestingly, among free-ranging harbor seals on Sable Island, Nova Scotia, Canada, birth weight was not associated with survival to weaning (Coltman et al., 1998). Prematurity, rather than mass, has previously been associated with poor survival of seals in rehabilitation (Dierauf et al., 1986), but that was not the case in the current study.

Decreased HGB and HCT in the released pups, compared with the wild pups, may be a function of underdeveloped diving abilities. Pups in rehabilitation are housed in pools up to 1.2 m deep, so they have not experienced water depths needed to develop the dive capabilities

TABLE 5. Model results for significant GLMs fitted to blood variables from wild caught subadult and adult harbor seals, California, USA.[a]

Blood variable	Model parameter	Estimate	SE	*t* value	*P* value
WBC (/μl)	Intercept	9.241	0.043		
	age_sa	0.175	0.063	2.78	0.01
Neutrophils (mature)	Intercept	8.573	0.054		
	sex_m	0.266	0.107	2.49	0.02
Lymphocytes	Intercept	7.926	0.062		
	age_sa	0.201	0.090	2.23	0.03
Monocytes	intercept	6.735	0.139		
	location_TB	−0.574	0.164	−3.49	0.00
Eosinophils	intercept	7.080	0.081		
	age_sa	0.412	0.118	3.51	0.00
	condition	0.022	0.010	2.21	0.03
Basophils	intercept	5.920	0.084		
	age_sa	0.310	−0.121	2.56	0.01
RBC (10^6/μl)	intercept	1.469	0.237		
	location_TB	0.107	0.025	4.30	0.00
	age_sa	0.077	0.022	3.45	0.00
HGB (g/dl)	intercept	2.925	0.023		
	location_TB	0.083	0.024	3.40	0.00
	sex_m	−0.045	0.025	−1.81	0.07
	age_sa	0.054	0.022	2.50	0.01
HCT (%)	intercept	3.940	0.022		
	location_TB	0.078	0.023	3.41	0.00
	age_sa	0.057	0.020	2.79	0.01
MCV (fl)	intercept	4.778	0.012		
	location_TB	−0.026	0.012	−2.11	0.04
	sex_m	−0.018	0.013	−1.46	0.15
	age_sa	−0.021	0.011	−1.95	0.06
MCH (pg)	intercept	3.758	0.013		
	location_TB	−0.027	0.013	−2.00	0.05
	sex_m	−0.030	0.014	−2.23	0.03
	age_sa	−0.023	0.012	−1.99	0.05
GGT (U/l)	intercept	2.390	0.084		
	age_sa	0.203	0.118	1.73	0.09
	condition	0.028	0.011	2.53	0.01
ALK (U/l)	intercept	3.472	0.093		
	age_sa	0.318	0.133	2.39	0.02
SDH (U/l)	intercept	3.832	0.090		
	condition	−0.314	0.015	−2.16	0.05
BUN (mg/dl)	intercept	3.950	0.057		
	sex_m	−0.132	0.083	−1.59	0.12
	age_sa	−0.265	0.074	−3.56	0.00

[a] WBC = white blood cell count; RBC = red blood cell count; HGB = hemoglobin; HCT = hematocrit; MCV = mean cell volume; MCH = mean cell hemoglobin; GGT = γ-glutamyltransferase; ALK = alkaline phosphatase; SDH = sorbitol dehydrogenase; BUN = blood urea nitrogen.

that wild pups have. Increased oxygen stores in wild, weaned pups, compared with wild, nursing pups described by Clark et al. (2007), are consistent with the increases in RBCs, HGB, HCT, and MCHC with age observed in the recently weaned, wild pups in this study.

Even though wild pups were captured during a relatively short time period (31 days), age was an important explanatory variable for several blood results. These trends over time may indicate a switch from nursing to fasting, the development of increased dive capabilities, or

growth and maturation in general. In an experimental study with recently weaned, harp seal (*Phoca groenlandica*) pups, Worthy and Lavigne (1982) found that ALT, ALK, sodium, and CK were lower in fasting seals than in feeding seals. In this study, ALK, sodium, and CK decreased over time, suggesting a transition from nursing to fasting, but ALT increased over time. In domestic animals, the release of the enzyme ALT from tissues into the blood is associated with liver disease; however, in harbor seals, ALT is also found in skeletal muscle and kidneys, such that its presence in blood is not specific to the liver (Fauquier et al., 2008). The ALT levels also may be related to specific food sources: In captive harbor seals, ALT was increased in those seals eating pollock, as opposed to those eating herring (Trumble et al., 2006). Although changes in hematologic variables with diet have been noted in harbor seals (Thompson et al., 1997), our study cannot directly address effects of diet changes in wild seals because prey consumption at the time of sampling was not known. Eosinophils, which have been associated with parasitism, and monocytes and which can indicate acute or chronic inflammation (Bossart et al., 2001; Piché et al., 2010), increased dramatically in the weaned harbor seal pups after 25 days. This may provide an initial estimate of the time from weaning to an inflammatory reaction to parasite acquisition from prey items.

The significance of the Döhle bodies noted in the neutrophils of harbor seal pups in rehabilitation is not known; Döhle bodies were not noted in the wild-caught, weaned pups. In humans, the presence of Döhle bodies is an early indicator of infection and sepsis (Prokocimer and Potasman, 2008), but their role in marine mammal neutrophils has not been documented. A greater percentage of harbor seals exhibited Döhle bodies on their blood smears at release than at admission, and their presence was not associated with survival; thus, we suspect that they are unlikely to be indicators of sepsis in newborn harbor seals. The meaning of the large lymphocytes in harbor seals at admission is also unknown, although in humans, large lymphocytes can result from bacterial, viral, or helminthic infection (Prokocimer and Potasman, 2008).

A number of hematologic variables varied with age, sex, condition, and location among the wild-caught, subadult and adult harbor seals. The slight hematologic variation with location may reflect exposure to different infectious agents with location, as evidenced by the increased monocytes in SF. Chemistry variables were not affected by location and were fairly robust to differences in age, sex, and condition with the exception of GGT, BUN, and SDH. These variables, as well as the hematologic variables, should be interpreted with caution in the field and in clinical settings when only a single sample is available. In addition, these seals were sampled only during the breeding season, when adult seals are in relatively poor body condition compared with their condition during the winter months; this study did not test for the effect of season on hematology and serum chemistry values.

In summary, the intervals generated by this study can help clinicians evaluate blood variables from stranded harbor seals, and the effects of age, sex, condition, and location on wild harbor seal blood variables may inform study designs for future health-assessment studies of these animals. However, this study indicates that, at the time of admission, the standard panel of variables used in veterinary practice today has little effect on treatment plan or rehabilitation outcome. Recently characterized markers of inflammation, such as C-reactive proteins (Funke et al., 1997) and interleukins (Fonfara et al., 2008), may be more sensitive earlier in treatment and should be evaluated clinically. Furthermore, applications of proteomics, genomics, and metabolomics (Abu-Asab et al., 2008) to

marine mammal health evaluations are needed because of the limitations of using conventional hematology and serum chemistry panels alone to assess health status from a single sample.

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