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# SEASONAL HEMATOLOGY AND SERUM CHEMISTRY OF WILD BELUGA WHALES (*DELPHINAPTERUS LEUCAS*) IN BRISTOL BAY, ALASKA, USA

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ABSTRACT: We collected blood from 18 beluga whales (*Delphinapterus leucas*), live-captured in Bristol Bay, Alaska, USA, in May and September 2008, to establish baseline hematologic and serum chemistry values and to determine whether there were significant differences in hematologic values by sex, season, size/age, or time during the capture period. Whole blood was collected within an average of 19 min (range=11–30 min) after the net was set for capture, and for eight animals, blood collection was repeated in a later season after between 80–100 min; all blood was processed within 12 hr. Mean hematocrit, chloride, creatinine, total protein, albumin, and alkaline phosphatase were significantly lower in May than they were in September, whereas mean corpuscular hemoglobin concentration, monocytes, phosphorous, magnesium, blood urea nitrogen, alanine aminotransferase, aspartate aminotransferase, γ-glutamyltranspeptidase, and creatinine kinase were significantly higher. Mean total protein, white blood cell count, neutrophils, and lymphocytes were significantly higher early in the capture period than they were later. No significant differences in blood analyte values were noted between males and females. Using overall body length as a proxy for age, larger (older) belugas had lower white blood cell, lymphocyte, and eosinophil counts as well as lower sodium, potassium, and calcium levels but higher creatinine levels than smaller belugas. These data provide values for hematology and serum chemistry for comparisons with other wild belugas.

*Key words:* Beluga whale, Bristol Bay, *Delphinapterus leucas*, health assessment, hematology, serum chemistry.

## INTRODUCTION

Bristol Bay, Alaska, USA, a large shallow bay on the southeastern margin of the Bering Sea, is inhabited by one of Alaska's five recognized populations of beluga whales (*Delphinapterus leucas*; O'Corry-Crowe et al., 1997; Lowry et al., 2008). Bristol Bay belugas (BBB) are present in the bay year-round and spend much of their time in the nearshore waters of Kvichak and Nushagak Bays, Alaska, USA, and are hunted locally by Alaska Native subsistence hunters (Frost et al., 1984; Frost and Lowry, 1990). This population is growing at a mean annual rate of 4.8% and, thus is considered a healthy population occupying a relatively pristine habitat (O'Corry-Crowe et al., 1997; Lowry et al., 2008). Changes due to increased human activity, including mining, oil and gas exploration, and varying climate, may affect the health of this ecosystem and possibly this population (Moore et al., 2000; Lowry et al., 2006). Documenting hematology and serum chemistry values will provide a baseline for comparisons that will allow researchers to monitor the effects of habitat

changes through time and will provide a reference for comparison with other wild belugas.

Bristol Bay, Alaska, USA, includes the tidal estuaries of two large river systems (the Kvichak and the Nushagak rivers), which support several large salmon (*Oncorhynchus* spp.) runs that compose one of the largest fisheries in the world. In spring (April–May), BBB feed on rainbow smelt (*Osmerus mordax*) traveling upriver to spawn and on salmon smolt migrating from river to sea. In June and July, BBB feed on adult salmon returning to spawn. During the autumn and winter months, BBB move to the outer bay, remaining east of 162° W longitude (Lowry et al., 2008), where they presumably feed on lower-quality nonanadromous fish, such as cod (*Gadus* spp.) and flounder (*Lepidopsetta* spp.), and invertebrate prey (e.g., isopods [*Saduria* spp. and *Saduria entomon*] and shrimp [*Crangon franciscorum*]; Frost et al., 1983). Such a feeding pattern may result in seasonal differences in blood values.

Hematology and serum chemistry values have been reported for captive belugas and for a few wild beluga populations in Canada and Norway but have not been reported in Alaska, USA (Cornell et al., 1988; St. Aubin et al., 2001; Tryland et al., 2006). Blood analytes have been opportunistically evaluated in other cetaceans, such as harbor porpoise (*Phocoena phocoena*; Koopman et al., 1995, 1999), bowhead (*Balaena mysticetus*; Heidel et al., 1996), fin (*Balaenoptera physalus*; Lambertsen et al., 1986; Kjeld, 2001), minke (*Balaenoptera acutorostrata*; Tryland and Brun, 2001), and killer whales (*Orcinus orca*; Cornell, 1983). Monitoring the health of cetaceans that are consumed by humans (e.g., beluga and bowhead whales in Alaska, USA) is especially important, and thus, assessment of blood analytes will provide information regarding the health of animals that is relevant to human health.

Hematology and serum chemistry can be used to evaluate physiologic, reproductive, and pathologic conditions important in assessing animal health (Duffield et al., 1995; St. Aubin et al., 2001). Combined with other biologic, habitat, and population information, hematology and serum chemistry may help detect health problems early so that mitigation can be considered before major compromises in ecosystem health occur (Harvell et al., 1999). Stress related to capture and handling of free-ranging cetaceans may result in significant variability in some blood values, depending on when during the processing period blood was drawn. Researchers working on captive animals have measured blood values over a longer period (~70 days), with samples drawn before and after taking the animals into captivity (St. Aubin and Geraci, 1989). No known studies, however, have sampled blood analytes in free-ranging cetaceans at multiple times postcapture to determine how analytes vary across the capture period. Our objectives were to establish baseline hematologic and serum chemistry ranges for wild BBB by sex and season and to evaluate changes that occur during the capture period.

## MATERIALS AND METHODS

### Capture

Belugas were captured in the Nushagak arm of Bristol Bay near Dillingham, Alaska, USA (59°1′44″N, 158°31′12″W). The capture area included the lower reaches of the Wood and Snake rivers, where they enter the Nushagak River and the Lower Nushagak River (Fig. 1). Belugas were captured, measured, examined, sampled, satellite-tagged for tracking, and released. Several small boats were used to direct the target animal parallel to the shore in water shallow enough for the net to reach bottom. When the boat was alongside the target animal, a drag buoy attached to one end of the net was thrown overboard to deploy the net across the whale's path. The whale was guided into the net by other boats, where it was removed from the net and taken to shallower water for handling. Belugas were either partially grounded and handled in water deep enough to support some of the animal's weight, yet shallow enough to allow handlers access for sampling, or were suspended in the water between two boats, partially supported by canvas slings. We did not categorize

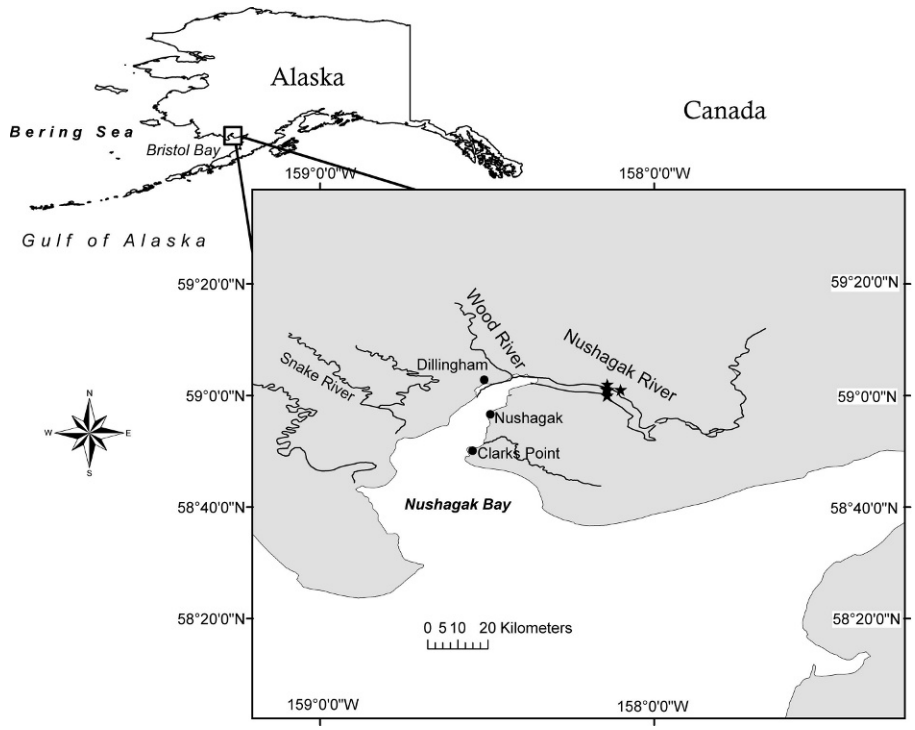


FIGURE 1. Study area of beluga whale (*Delphinapterus leucas*) capture and release health assessments and tagging in Bristol Bay, Alaska, USA, during May and September 2008 (black stars).

belugas by age class because of the difficulty of determining age based solely on length and coloration, which varies among beluga populations and is not well known for BBB. Skin color may provide some indication of age because white whales may be considered mature. However, gray females have been observed with calves, indicating they may reach sexual maturity before turning white (St. Aubin et al., 2001). To quantify variation in blood values between spring and late summer/early autumn (referred to, hereafter, as May and September, respectively), we collected blood in May, as close to the end of winter feeding as sea ice conditions allowed, and again, in September after the major salmon runs had ended.

### Sample collection and processing

Selected hematologic parameters were chosen for analysis because of their roles as markers of organ system disease, stress, or other health conditions (Bossart et al., 2001). These analytes included hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), erythrocyte sedimentation rate (ESR), erythrocyte and leukocyte counts. Sera were shipped to the Animal Health Diagnostic Center (Cornell University, Ithaca, New York, USA) and the following serum chemistry analytes were measured: sodium (Na), potassium (K), chloride (Cl), calcium (Ca), phosphorous (Phos), magnesium (Mg), blood urea nitrogen (BUN), creatinine (Creat), total protein (TP), albumin (Alb), globulin (Glob), fibrinogen (FIB), albumin/globulin ratio (A/G ratio), glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ-glutamyltranspeptidase (GGT), total bilirubin (Tbili), cholesterol, creatinine kinase (CK), and iron (Fe). Serum chemistry analytes were analyzed on a Hitachi 917 (Roche Diagnostics, Indianapolis, Indiana, USA) using photometric, enzymatic, and ion-selective electrode methods depending on the analyte. Controls were run daily for calibration, and the machine was calibrated daily using manufacturer recommended methods. Although the Hitachi 917 has not been validated for marine mammals, the Animal Health Diagnostic Center is the reference laboratory selected by the National Marine Fisheries Service's National Marine Mammal Health and Stranding Response Program.

Initial blood samples were drawn as soon after capture and restraint as possible ($n=18$). In September, to evaluate the variability in blood values across the capture-holding period, blood was also drawn immediately before release (late samples; $n=8$). All seasonal and sex comparisons are based only on the early draw samples. Blood was drawn from the periarterial venous rete on the dorsal side of the flukes using a 1.3-cm, 19-gauge butterfly catheter (Becton, Dickinson, and Co., Franklin Lakes, New Jersey, USA) after the skin was disinfected with an iodine solution or alcohol. Whole blood was collected into serum separator and ethylenediaminetetraacetic acid (EDTA) Vacutainer® tubes (BD Vacutainer, Becton Dickinson, Franklin Lakes, New Jersey, USA), placed immediately on ice in a cooler, then processed within 12 hr. Aliquots of serum were placed into sterile 2-ml polypropylene Cryovials® (Fisher Scientific, Pittsburgh, Pennsylvania, USA), frozen on dry ice in the field, then transferred to an ultralow freezer (−80 C) until they were shipped.

Manual hematologic techniques were used for leukocyte counts, using the 1:100 dilution, and red blood cell counts using a Unopette system (Becton, Dickinson). Duplicate blood smears were prepared using EDTA-treated blood and stained using the Three-Step Stain (Richard-Allan Scientific, Kalamazoo, Michigan, USA) to perform manual leukocyte differentials based on a count of 100 WBCs per slide. Microhematocrit determinations were done using a standard technique. Hemoglobin (Hb) was determined using a HemoCue® system (HemoCue AB, Angelholm, Sweden). The Hb results from the September season were run from samples frozen at −80 C. The ESR was determined 6–10 hr postcollection, using the Wintrobe method (International Council for Standardization in Hematology, 1993).

### Statistical Analyses

Hematology and serum chemistry reference ranges were characterized with descriptive statistics including mean, range, and standard deviation (Stata v10.1, StataCorp, College Station, Texas, USA). Two-sample *t*-tests were used to evaluate differences in mean analyte values by season and sex (Lehmann and Romano, 2005). Paired *t*-tests were used to compare means between selected hematologic analytes collected early and late during the capture-holding period (Petrie and Watson, 2006). Linear regression was used to evaluate the relationship between standard length (i.e., straight linear length from tip of rostrum to fluke notch), as a proxy for age, and early blood analytes, and then for differences of blood analytes between early and late in the processing period (R: A Language and Environment for Statistical Computing, http://www.R-project.org).

Coefficients of biological variation (CV) were calculated for all blood analytes. Analytes with nonnormal distributions were log-transformed or analyzed with nonparametric methods, such as the Mann-Whitney *U*-test (Petrie and Watson, 2006). Outliers (±3 SD) were assessed using Grubb's test (Grubbs, 1969). Alpha levels of $P<0.05$, unadjusted for multiple comparisons, were used for all statistical tests.

## RESULTS

Ten BBB were captured in May (eight females, two males) and eight were captured in September 2008 (three females and five males; Table 1). Mean length was 317.7 cm (SD=46.9, range=254–412 cm). Mean handling time in minutes (mean± SD) from initial chase to release was 80±4.2, range=50–129. Blood sampling averaged 19 min (SD=6.2, range=11–30 min) after the net was set and 77 min (SD=13.3, range=25–125 min) for the second blood draw collected from belugas captured in September. Time to first blood sample did not differ significantly between seasons ($P=0.34$) or sexes ($P=0.82$). Complete hematology data were collected early in the processing period for 13 of the 18 whales (72%). Sample sizes, means, medians, standard deviations, and ranges for all 18 belugas are reported in Tables 2 and 3, for May and September collections combined and separated. The greatest variation was observed for HCT, ESR, and WBC (CV>10%).

Although the values for most of the analytes were similar between the seasons, some differences were noted between May and September (Tables 2 and 3). Hematocrit, Cl, Creat, TP, Alb, and ALP were significantly lower in May compared with September. Conversely, MCHC, monocytes, Phos, Mg, BUN, ALT, AST, GGT, and CK were significantly greater in May samples. Mean CK in May was more

TABLE 1. Characteristics of beluga whales (*Delphinapterus leucas*) captured in Bristol Bay, Alaska, USA, May and September 2008. Mean±SD standard length=317.7 ±46.9 cm.

| ID Number | Month captured | Length (cm) | Sex[a] | Color |
|---|---|---|---|---|
| BBN0108 | May | 310 | F | Grey |
| BBN0208 | May | 305 | F | White |
| BBN0308 | May | 335 | F | Grey-white |
| BBN0408 | May | 307 | F | Grey |
| BBN0508 | May | 312 | F | Grey |
| BBN0608 | May | 412 | M | White |
| BBN0708 | May | 320 | F | White |
| BBN0808 | May | 254 | M | Grey |
| BBN0908 | May | 279 | F | Grey |
| BBN1008 | May | 295 | F | Grey-white |
| BBN1108 | September | 257 | M | Grey-white |
| BBN1208 | September | 328 | M | White |
| BBN1308 | September | 342 | F | White |
| BBN1408 | September | 396 | M | White |
| BBN1508 | September | 274 | F | Grey-white |
| BBN1608 | September | 384 | M | White |
| BBN1708 | September | 340 | F | White |
| BBN1808 | September | 269 | M | Light grey |

[a] M = male; F = female

than twice that measured in September (Table 3). Elevated mean ALP was observed in September with outliers (>300 IU/L) occurring in a small 274-cm female and a large 384-cm male. Other analytes did not differ significantly between seasons.

Significant differences between early and late samples were observed for some hematology values. Mean values for WBC (mean difference=$4.8\times10^3$ cells/μl, [95% confidence interval=$2.2–7.4\times10^3$ cells/μl], $P=0.004$), neutrophils ($1.3\times10^3$ cells/μl, [−0.005 to 2.7], $P=0.05$), lymphocytes ($2.8\times10^3$ cells/μl, [1.2–4.3], $P=0.005$), and TP (1.0 mg/dl, [0.6–1.3], $P=0.001$) were significantly greater early in the processing period. Other hematologic values did not vary significantly among seasons. Several analytes varied significantly with beluga length (Fig. 2). White blood cells ($P=0.01$), lymphocytes ($P=0.004$), eosinophils ($P=0.04$), Na ($P=0.04$), K ($P=0.009$), and Ca ($P=0.006$) were higher in shorter (younger) belugas. Increasing beluga length was significantly associated with decreases in both WBCs ($P=0.010$) and lymphocytes ($P=0.004$). Each additional meter of length was associated, on average, with a $6.1\times10^3$/μl decrease in WBC, accounting for about a third of the variation in WBC across animals (Fig. 2). An additional meter in length was also associated with a $3.1\times10^3$/μl decrease in lymphocytes, accounting for about 42% of the variation in lymphocyte counts across animals (Fig. 2). The opposite trend was observed for Creat ($P=0.03$), where levels were higher in longer (older) whales. There was no significant relationship between the length of the whale and differences in blood analyte values early versus late in the handling period. None of the analytes differed significantly between males ($n=7$) and females ($n=11$).

## DISCUSSION

The hematology and blood chemistry presented here are from apparently healthy, wild belugas and provide a frame of reference for evaluating health in wild belugas. The variability observed in blood analytes by season, age class, and sampling time was within ranges previously reported

TABLE 2. Hematology values of wild beluga whales (*Delphinapterus leucas*) captured in Bristol Bay, Alaska, USA, by season.

| Analyte (units) | Sample[a] | *n* | Mean±SD | Median | Range | *P*[b] |
|---|---|---|---|---|---|---|
| Red blood cells ($10^6$/μl) | Combined | 18 | 3.7±0.5 | 3.6 | 3.4–4.4 | 0.751 |
| | May | 10 | 3.7±0.5 | 3.7 | 2.8–4.5 | |
| | September | 8 | 3.7±0.5 | 3.6 | 2.8–4.3 | |
| Hemoglobin (g/dl) | Combined | 17 | 19.9±1.6 | 20.0 | 16.5–22.8 | 0.192 |
| | May | 10 | 19.4±1.7 | 20.0 | 16.5–21.9 | |
| | September | 7 | 20.5±1.2 | 20.0 | 19.5–22.8 | |
| Hematocrit (%) | Combined | 18 | 53.0±4.7 | 53.3 | 43.0–60.0 | <0.001 |
| | May | 10 | 50.1±3.9 | 51.0 | 43.0–54.5 | |
| | September | 8 | 56.6±2.4 | 57.0 | 53.5–60.0 | |
| Mean corpuscular volume (fl) | Combined | 18 | 145.4±23.8 | 140.9 | 100.0–204.2 | 0.065 |
| | May | 10 | 136.2±22.1 | 135.1 | 100.0–183.7 | |
| | September | 8 | 156.8±21.7 | 156.2 | 134.1–204.2 | |
| Mean corpuscular hemoglobin (pg) | Combined | 17 | 54.4±8.3 | 52.3 | 44.3–71.4 | 0.362 |
| | May | 10 | 52.8±8.3 | 49.9 | 44.3–71.4 | |
| | September | 7 | 56.6±8.3 | 56.3 | 46.6–70.4 | |
| Mean corpuscular hemoglobin concentration (g/dl) | Combined | 17 | 37.8±2.8 | 37.7 | 34.5–46.2 | 0.025 |
| | May | 10 | 38.9±3.0 | 38.7 | 35.1–46.2 | |
| | September | 7 | 36.2±1.5 | 36.4 | 34.5–38.6 | |
| Erythrocyte sedimentation rate (mm/hr) | Combined | 16 | 24.2±9.2 | 23.0 | 10.0–40.0 | 0.452 |
| | May | 8 | 26.0±10.0 | 20.0 | 10.0–40.0 | |
| | September | 8 | 22.4±8.7 | 22.0 | 11.0–35.0 | |
| White blood cells ($10^3$/μl) | Combined | 18 | 18.5±4.8 | 15.5 | 12.0–27.8 | 0.627 |
| | May | 10 | 19.0±1.4 | 19.1 | 12.0–24.3 | |
| | September | 8 | 17.9±1.9 | 15.0 | 13.0–27.8 | |
| Differentials | | | | | | |
| Neutrophils ($10^3$/μl) | Combined | 18 | 6.6±1.9 | 7.4 | 4.4–9.9 | 0.151 |
| | May | 10 | 7.1±1.9 | 7.2 | 4.4–9.9 | |
| | September | 8 | 5.8±1.8 | 5.3 | 3.3–8.6 | |
| Lymphocytes ($10^3$/μl) | Combined | 18 | 6.5±2.2 | 5.4 | 4.4–7.7 | 0.345 |
| | May | 10 | 6.1±1.7 | 6.3 | 3.6–9.2 | |
| | September | 8 | 7.1±2.7 | 6.8 | 3.4–11.1 | |
| Monocytes ($10^3$/μl) | Combined | 18 | 0.6±0.3 | 0.6 | 0.2–1.6 | 0.016 |
| | May | 10 | 0.8±0.4 | 0.7 | 0.2–1.6 | |
| | September | 8 | 0.4±0.2 | 0.5 | 0.2–0.6 | |
| Eosinophils ($10^3$/μl) | Combined | 18 | 3.6±1.5 | 3.8 | 2.2–4.7 | 0.374 |
| | May | 10 | 4.3±1.4 | 4.1 | 2.3–7.8 | |
| | September | 8 | 4.2±1.8 | 3.5 | 2.2–6.5 | |

[a] May = spring, September = late summer/autumn, combined = spring and late summer/autumn.

[b] Significance at $P \leq 0.05$ (difference between seasons).

for healthy, wild belugas in Canada and Norway (Cornell et al., 1988; St. Aubin and Geraci, 1989; Bossart et al., 2001; St. Aubin et al., 2001; Tryland et al., 2006).

The seasonal differences in BBB HCT levels were not observed in Canadian belugas but were seen in bottlenose dolphins (*Tursiops truncatus*) from Sarasota Bay, Florida, USA (St. Aubin et al., 2001; Hall et al., 2007). Lower HCT in other studies has been related to seasonal changes in diving patterns (Martin and Smith, 1992, 1999); however, water depths in Bristol Bay, Alaska, USA, are shallow and do not allow for extreme changes in diving depths. Lower HCT observed in BBB in May could be more indicative of dive frequency than depth or may reflect normal seasonal or nutritional variation (Ridgway and Johnson, 1966; Ridgway et al., 1984; Hendrick and Duffield, 1991). Lymphocytes, monocytes, and eosinophils were also

TABLE 3. Serum chemistry values of wild beluga whales (*Delphinapterus leucas*) captured in Bristol Bay, Alaska, USA, by season.

| Parameter (units) | Sample[a] | *n* | Mean±SD | Median | Range | *P*[b] |
|---|---|---|---|---|---|---|
| Sodium (mmol/l) | Combined | 18 | 165.1±3.9 | 166.0 | 157.0–171.0 | 0.898 |
| | May | 10 | 165.0±3.1 | 166.0 | 160.0–169.0 | |
| | September | 8 | 165.3±5.0 | 165.0 | 157.0–171.0 | |
| Potassium (mmol/l) | Combined | 18 | 5.3±1.4 | 5.1 | 2.9–8.5 | 0.202 |
| | May | 10 | 5.7±1.5 | 5.1 | 3.5–8.5 | |
| | September | 8 | 4.9±1.1 | 5.1 | 2.9–6.6 | |
| Chloride (mmol/l) | Combined | 18 | 109.5±4.6 | 108.5 | 101.0–118.0 | <0.001 |
| | May | 10 | 106.5±2.7 | 107.0 | 101.0–110.0 | |
| | September | 8 | 113.3±3.7 | 113.0 | 107.0–118.0 | |
| Calcium (mg/dl) | Combined | 18 | 12.1±1.0 | 12.1 | 10.7–14.6 | 0.283 |
| | May | 10 | 11.8±0.8 | 11.8 | 10.7–13.1 | |
| | September | 8 | 12.5±1.1 | 12.6 | 10.7–14.6 | |
| Phosphorus (mg/dl) | Combined | 18 | 9.1±1.3 | 9.5 | 6.4–10.8 | 0.014 |
| | May | 10 | 9.8±0.9 | 9.9 | 8.1–10.8 | |
| | September | 8 | 8.3±1.3 | 8.2 | 6.4–10.5 | |
| Magnesium (mg/dl) | Combined | 18 | 2.6±0.3 | 2.6 | 2.0–3.2 | 0.005 |
| | May | 10 | 2.8±0.3 | 2.8 | 2.3–3.2 | |
| | September | 8 | 2.4±0.2 | 2.4 | 2.0–2.7 | |
| Blood urea nitrogen (mg/dl) | Combined | 18 | 72.5±12.3 | 75.0 | 52.0–94.0 | 0.001 |
| | May | 10 | 80.0±7.8 | 80.5 | 69.0–94.0 | |
| | September | 8 | 63.1±10.3 | 60.0 | 52.0–80.0 | |
| Creatinine (mg/dl) | Combined | 18 | 1.7±1.1 | 1.3 | 0.1–3.8 | <0.001 |
| | May | 10 | 1.0±0.4 | 1.1 | 0.1–1.6 | |
| | September | 8 | 2.5±1.0 | 2.9 | 1.0–3.8 | |
| Total protein (mg/dl)[c] | Combined | 18 | 2.2±0.1 | 2.1 | 2.0–2.4 | 0.026 |
| | May | 10 | 2.1±0.0 | 2.1 | 2.0–2.2 | |
| | September | 8 | 2.2±0.0 | 2.2 | 2.1–2.4 | |
| Albumin (g/dl) | Combined | 18 | 4.6±0.4 | 4.7 | 3.7–5.3 | <0.001 |
| | May | 10 | 4.4±0.3 | 4.4 | 3.7–4.8 | |
| | September | 8 | 4.9±0.3 | 5.0 | 4.5–5.3 | |
| Globulin (g/dl) | Combined | 18 | 4.1±0.7 | 4.0 | 2.9–6.0 | 0.328 |
| | May | 10 | 3.9±0.1 | 0.4 | 3.3–4.5 | |
| | September | 8 | 4.3±0.4 | 1.0 | 2.9–6.0 | |
| Fibrinogen (mg/dl) | Combined | 18 | 115.2±34.8 | 108.5 | 76.0–240.0 | 0.502 |
| | May | 10 | 110.1±15.1 | 110.5 | 88.0–135.0 | |
| | September | 8 | 121.6±50.6 | 108.5 | 76.0–240.0 | |
| Albumin:globulin ratio | Combined | 18 | 1.2±0.3 | 1.2 | 0.8–1.8 | 0.409 |
| | May | 10 | 1.1±0.2 | 1.1 | 0.9–1.5 | |
| | September | 8 | 1.2±0.3 | 1.2 | 0.8–1.8 | |
| Glucose (mg/dl) | Combined | 18 | 126.2±18.8 | 131.0 | 99.0–159.0 | 0.805 |
| | May | 10 | 125.2±21.8 | 131.0 | 99.0–159.0 | |
| | September | 8 | 127.5±15.7 | 129.5 | 100.0–145.0 | |
| Alanine aminotransferase (IU/l)[c] | Combined | 18 | 2.4±0.7 | 2.2 | 1.4–3.8 | 0.002 |
| | May | 10 | 2.9±4.5 | 2.6 | 2.1–3.8 | |
| | September | 8 | 1.9±1.0 | 1.9 | 1.4–2.6 | |
| Aspartate aminotransferase (IU/l) | Combined | 18 | 109.0±39.5 | 102.5 | 58.0–188.0 | 0.001 |
| | May | 10 | 134.2±34.7 | 119.0 | 84.0–188.0 | |
| | September | 8 | 77.5±14.3 | 76.0 | 58.0–103.0 | |
| Alkaline phosphatase (IU/l)[c] | Combined | 18 | 4.9±0.8 | 5.1 | 3.3–6.1 | 0.028 |
| | May | 10 | 4.5±0.7 | 4.5 | 3.3–5.5 | |
| | September | 8 | 5.3±0.6 | 5.4 | 4.1–6.1 | |
| γ-glutamyltranspeptidase (IU/l)[c] | Combined | 18 | 2.9±0.5 | 2.9 | 1.9–4.3 | 0.026 |
| | May | 10 | 3.0±0.6 | 3.0 | 1.9–4.3 | |
| | September | 8 | 3.7±0.2 | 2.6 | 2.5–3.0 | |

TABLE 3. Continued.

| Parameter (units) | Sample[a] | *n* | Mean±SD | Median | Range | *P*[b] |
|---|---|---|---|---|---|---|
| Total bilirubin (mg/dl) | Combined | 18 | 0.1±0.01 | 0.1 | 0.0–0.3 | 0.708 |
| | May | 10 | 0.1±0.0 | 0.1 | 0.0–0.3 | |
| | September | 8 | 0.1±0.0 | 0.0 | 0.1–0.1 | |
| Cholesterol (mg/dl) | Combined | 18 | 172.8±34.7 | 158.0 | 126.0–254.0 | 0.404 |
| | May | 10 | 179.1±42.0 | 169.0 | 126.0–254.0 | |
| | September | 8 | 164.9±23.0 | 158.0 | 138.0–209.0 | |
| Creatinine kinase (IU/l) | Combined | 18 | 285.3±158.3 | 226.5 | 70.0–664.0 | <0.001 |
| | May | 10 | 383.2±141.9 | 7.2 | 4.4–9.9 | |
| | September | 8 | 163.0±64.9 | 197.0 | 70.0–219.0 | |
| Iron (μg/dl) | Combined | 18 | 240.7±83.1 | 237.5 | 117.0–442.0 | 0.799 |
| | May | 10 | 245.4±81.5 | 244.5 | 117.0–396.0 | |
| | September | 8 | 234.9±90.4 | 214.0 | 161.0–442.0 | |

[a] May = spring, September = late summer/autumn, combined = spring and late summer/autumn.

[b] Significance at $P \leq 0.05$ (difference between seasons).

[c] Log-transformed.

comparable to those reported for wild belugas in Canada (St. Aubin et al., 2001). The higher monocyte levels seen in May versus September may reflect normal physiologic, seasonal variation in this cell type (Maes et al., 1994). Higher WBC count indices in wild animals may reflect their immunologic responses to a broad range of antigenic stimuli, such as parasitism. Phosphorous ranges were slightly higher than those reported for other beluga populations (Bossart et al., 2001); however, other clinical abnormalities and indicators of illness that could be associated with elevated blood Phos levels, such as renal failure, elevated Ca, dietary Phos excess or thyroid dysfunction, were not observed (Bossart et al., 2001).

Seasonal differences in BUN were not observed in Canadian wild belugas or in the Sarasota bottlenose dolphins (St. Aubin et al., 2001; Hall et al., 2007). Elevated BUN in the presence of a relatively normal Creat may reflect a physiologic response to a relative decrease of blood flow to the kidney without any indication of kidney injury, or it may also be seen with high protein diets in which urea production is increased in response to elevated protein metabolism (Willard and Tvedten, 2003). Bristol Bay belugas typically feed on rainbow smelt and juvenile salmon (i.e., smolt) in May, which contain lower protein levels than the salmon they feed on in the summer, resulting in lower springtime BUN values (NMFS, 2011). Seasonal differences in protein and energy content have been reported in Chinook salmon (*Oncorhynchus tshawytscha*) (MacFarlane, 2010) and smelt (Foltz and Norden, 1977). Creatinine, which is an indicator of muscle mass and normal muscular metabolism, was significantly greater during September, which may reflect increased muscle mass from summer feeding on higher quality prey (Bossart et al., 2001). Overall, mean Creat was lower in BBB than in Canadian wild belugas, the latter sampled during summer and autumn, at which time they were likely feeding on high protein fish (St. Aubin et al., 2001). Elevated BUN and lowered Creat, along with elevated CPK and lower TP and ALB in May, may be suggestive of fasting metabolism in some species of marine mammals (Rea et al., 2009).

Although analyte levels varied considerably between individual belugas, differences in the average level by beluga length, which may be considered a proxy for age, accounted for as much as 40% of

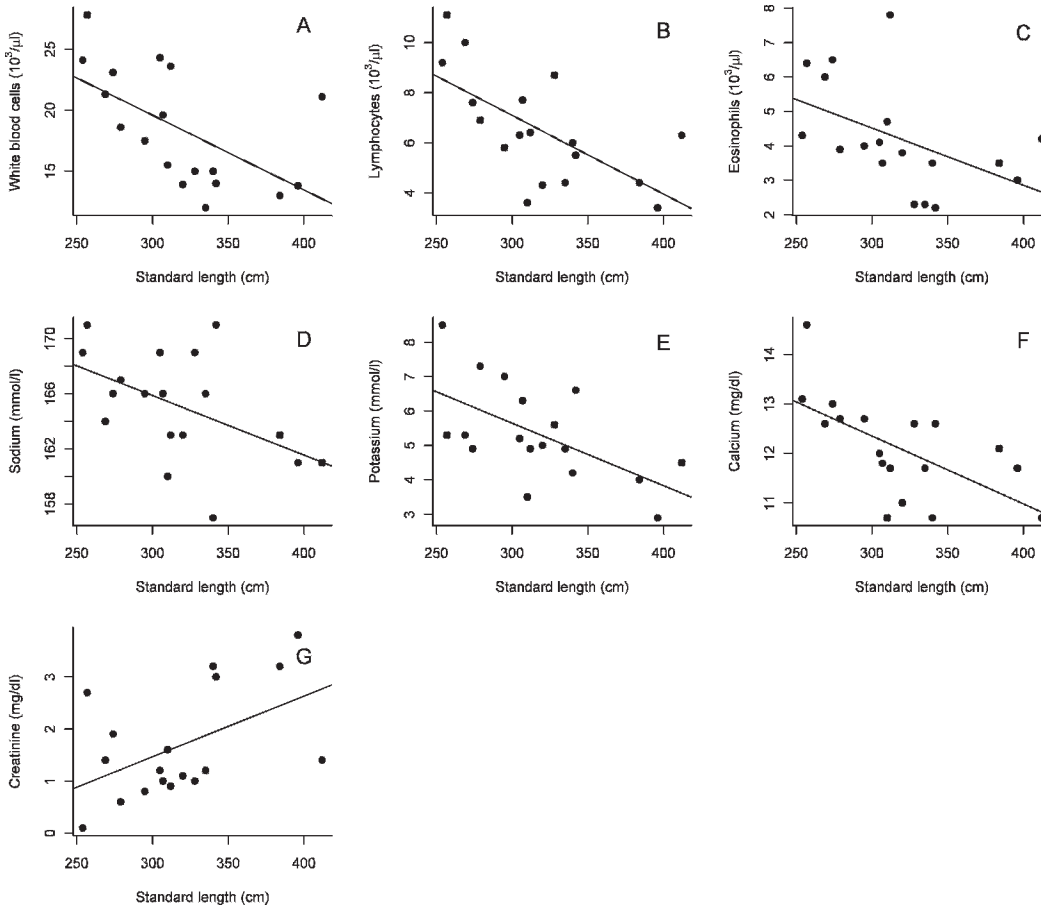


FIGURE 2. Relationship between standard length (cm) and (A) white blood cells ($y=-0.061x+37.987$, $r^2=0.35$, $P=0.01$), (B) lymphocytes ($y=-0.031x+16.436$, $r^2=0.42$, $P=0.004$), (C) eosinophils ($y=-0.016x+9.444$, $r^2=0.24$, $P=0.040$), (D) sodium ($y=-0.043x+178.850$, $r^2=0.252$, $P=0.040$), (E) potassium ($y=-0.018x+11.109$, $r^2=0.354$, $P=0.009$), (F) calcium ($y=-0.014x+16.475$, $r^2=0.388$, $P=0.006$), and (G) creatinine ($y=0.012x-2.040$, $r^2=0.255$, $P=0.03$) measured early in the capture period, in 18 beluga whales (*Delphinapterus leucas*) sampled in Bristol Bay, Alaska, USA, May and September 2008.

the variation of some analytes. Smaller, presumably younger, belugas had higher levels of WBC, lymphocytes, eosinophils, Na, K, and Ca than did larger (older) ones. Similar relationships between age and blood analytes were also noted in Canadian wild belugas (St. Aubin et al., 2001). Higher leukocyte counts in shorter (younger) individuals may reflect the still-developing immune systems of the younger whales as they mount immunologic responses to a broad range of largely novel, antigenic stimuli. The decrease seen with increasing length represents a 25–30% decrease in leukocytes across the range of lengths observed in the study. Mammalian lymphocyte counts are often higher at birth, the clinical significance of which is unknown (Willard and Tvedten, 2003). The decrease in electrolyte concentrations with increasing size may imply a changing capacity for electrolyte and fluid management with maturation (St. Aubin et al., 2001). Although length (proxy for age) leaves much of the variance in analyte levels unexplained, there are moderate to strong correlations between length and analyte levels. This suggests that interpretation of between-animal differences in analyte levels should be

made with caution if the animals are of different sizes/ages.

Chloride and Mg showed significant seasonal differences, with higher levels of Mg in September and elevated Cl in May (Yokus and Cakir, 2006). Total protein and ALB values were higher in September than they were in May, which would be consistent with whales consuming more high-protein prey during the summer but does not correspond with the higher BUN values noted in May. Creatinine kinase levels were higher in May and were higher overall than in the wild, beluga populations in Canada and Norway (St. Aubin et al., 2001; Tryland et al., 2006). Creatinine kinase values reflect muscle metabolism and mass and may increase in response to physical exertion (e.g., activity immediately before or during blood draws; Bossart et al., 2001).

Mean ALT and AST values were most similar to those observed in Canadian and Norwegian stocks (Bossart et al., 2001; Tryland et al., 2006), whereas mean ALP levels were most similar to those found in Canadian belugas (Cornell, 1983; St. Aubin et al., 2001). Alanine transferase (CV=30) and AST (CV=36.3) results were more variable in BBB than they were in the other wild beluga populations. Although elevated ALP values have been associated with liver disease, intestinal inflammation, or bone growth (i.e., in young mammals), we do not think these levels of ALP indicate disease (Willard and Tvedten, 2003). Bristol Bay belugas appear to be a healthy, growing population with little evidence of unusual disease incidence or deaths, as demonstrated by a lack of obviously diseased belugas reported by subsistence hunters or as indicated by increased numbers of strandings or unusual mortality events; therefore, it is possible that animals in our study were younger overall compared with those sampled in other wild populations.

Belugas may habituate to capture and handling within the handling period, with stress levels decreasing rather than increasing through time (St. Aubin and Dierauf, 2001). Blood sampling in autumn was performed both early and late in the handling period to examine the variability in hematologic values that are sensitive to stress. White blood cell and neutrophil counts decreased significantly during the handling period, which may be related to the stress during capture, followed by compensation or adjustment to the stress during the processing time.

Few published studies on blood analytes exist for wild belugas. This is the first study, to our knowledge, on the hematology and serum chemistry in a wild beluga whale population of Alaska, USA. Our study adds to previous studies by confirming the overall range of variability observed in healthy animals in other beluga populations and by demonstrating some similarity to other free-ranging cetacean populations. The most striking differences observed were seasonal, rather than between male and female or by length (age) of the belugas. This finding suggests that even within a clinically healthy, free-ranging population, seasonal differences in diet, behavior, or other variables may alter hematologic and serum chemistry levels measurably and should be considered in planning future studies and in assessing the health of individual animals. Differences within the capture period were less marked, possibly reflecting a small sample size, or the need for additional laboratory measures, such as indicators of stress or immune function. Our results are useful as a baseline for continued efforts to monitor the health status of this population of beluga whales, for comparison should an unusual mortality event occur, and as a control in evaluating the health of endangered beluga populations, such as those in Cook Inlet, Alaska, USA.

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## LITERATURE CITED

Bossart, G. D., T. H. Reidarson, L. A. Dierauf, and D. A. Duffield. 2001. Clinical pathology. *In* CRC handbook of marine mammal medicine, L. A. Dierauf and F. M. D. Gulland (eds.). CRC Press, Boca Raton, Florida, pp. 383–436.

Cornell, L. H. 1983. Hematology and clinical chemistry values in the killer whale, *Orcinus orca* L. Journal of Wildlife Diseases 19: 259–264.

———, D. S. Duffield, B. E. Joseph, and B. Stark. 1988. Hematology and serum chemistry values in the beluga (*Delphinapterus leucas*). Journal of Wildlife Diseases 24: 220–224.

Duffield, D. A., D. K. Odell, J. F. Mcbain, and B. Andrews. 1995. Killer whale (*Orcinus orca*) reproduction at Sea World. Zoo Biology 14: 417–430.

Foltz, J. W., and C. R. Norden. 1977. Seasonal changes in food consumption and energy content of smelt (*Osmerus mordax*) in Lake Michigan. Transactions of the American Fisheries Society 106: 230–234.

Frost, K. J., and L. F. Lowry. 1990. Distribution, abundance and movements of beluga whales, *Delphinapterus leucas*, in coastal waters of western Alaska. In: Canadian Bulletin of Fisheries and Aquatic Sciences 224: Advances in research on the beluga whale, *Delphinapterus leucas*: pp. 39–57.

———, ———, and R. R. Nelson. 1983. Investigations of belukha whales in coastal waters of western and northern Alaska, 1982–1983: Marking and tracking of whales in Bristol Bay. U.S. Department of Commerce, National Oceanic and Atmospheric Administration, Outer Continental Shelf Environmental Assessment Program Final Report 43 (1986): 461–585.

———, ———, and ———. 1984. Belukha whale studies in Bristol Bay, Alaska. *In* Proceedings of the workshop on biological interactions among marine mammals and commercial fisheries in the southeastern Bering Sea, Anchorage, Alaska, 18–21 October 1983, Alaska Sea Grant Report 84-1, pp. 187–200.

Grubbs, F. E. 1969. Procedure for detecting outlying observations in samples. Technometrics 11: 1–21.

Hall, A. J., R. S. Wells, J. C. Sweeney, F. I. Townsend, B. C. Balmer, A. A. Hohn, and H. L. Rhinehart. 2007. Annual, seasonal and individual variation in hematology and clinical blood chemistry profiles in bottlenose dolphins (*Tursiops truncatus*) from Sarasota Bay, Florida. Comparative Biochemistry and Physiology, Series A 148: 266–277.

Harvell, C. D., K. Kim, J. M. Burkholder, R. R. Colwell, P. R. Epstein, D. J. Grimes, E. E. Hofmann, E. K. Lipp, A. D. M. E. Osterhaus, R. M. Overstreet, J. W. Porter, G. W. Smith, and G. R. Vasta. 1999. Emerging marine diseases—Climate links and anthropogenic factors. Science 285: 1505–1510.

Heidel, J. R., L. M. Philo, T. F. Albert, C. B. Andreasen, and B. V. Stang. 1996. Serum chemistry of bowhead whales (*Balaena mysticetus*). Journal of Wildlife Diseases 32: 75–79.

Hendrick, M. S., and D. A. Duffield. 1991. Haematological and rheological characteristics of blood in seven marine mammal species: Physiological implications for diving behaviour. Journal of Zoology, London 225: 273–283.

International Council For Standardization In Haematology (Expert Panel on Blood Rheology). 1993. ICSH recommendations for measurement of erythrocyte sedimentation rate. Journal of Clinical Pathology 46: 198–203.

Kjeld, M. 2001. Concentrations of electrolytes, hormones, and other constituents in fresh postmortem blood and urine of fin whales (*Balaenoptera physalus*). Canadian Journal of Zoology 79: 438–446.

Koopman, H. N., A. J. Westgate, and A. J. Read. 1995. Blood chemistry of wild harbor porpoises *Phocoena phocoena* (L.). Marine Mammal Science 11: 123–135.

———, ———, ———, and D. E. Gaskin. 1999. Hematology values of wild harbor porpoises (*Phocoena phocoena*) from the Bay of Fundy, Canada. Marine Mammal Science 15: 52–64.

Lambertsen, R. H., B. Birnir, and J. E. Bauer. 1986. Serum chemistry and evidence of renal failure in

the North Atlantic fin whale population. Journal of Wildlife Diseases 22: 389–396.

LEHMANN, E. L., AND J. P. ROMANO. 2005. Testing statistical hypotheses (Springer texts in statistics). 3rd Edition. Springer, New York, New York, 800 pp.

———, K. J. FROST, A. ZERBINI, D. DEMASTER, AND R. R. REEVES. 2008. Trend in aerial counts of beluga or white whales (*Delphinapterus leucas*) in Bristol Bay, Alaska, 1993–2005. Journal of Cetacean Research and Management 10: 201–207.

LOWRY, L., G. O'CORRY-CROWE, AND D. GOODMAN. 2006. *Delphinapterus leucas* (Cook Inlet population). *In* International Union for Conservation of Nature 2006. 2006 IUCN Red List of Threatened Species. IUCN, Gland, Switzerland, www.iucnredlist.org. Accessed September 2011.

MACFARLANE, R. B. 2010. Energy dynamics and growth of Chinook salmon (*Oncorhynchus tshawytscha*) from the Central Valley of California during the estuarine phase and first ocean year. Canadian Journal of Fisheries and Aquatic Sciences 67: 1549–1565.

MAES, M., W. STEVENS, S. SCHARPÉ, E. BOSMANS, F. DE MEYER, P. D'HONDT, D. PEETERS, P. THOMPSON, P. COSYNS, L. DE CLERCK, C. BRIDTS, H. NEELS, A. WAUTERS, AND W. COOREMAN. 1994. Seasonal variation in peripheral blood leukocyte subsets and in serum interleukin-6, and soluble interleukin-2 and -6 receptor concentrations in normal volunteers. Experientia 50: 821–829.

MARTIN, A. R., AND T. G. SMITH. 1992. Deep diving in wild, free-ranging beluga whales, *Delphinapterus leucas*. Canadian Journal of Fisheries and Aquatic Sciences 49: 462–466.

———, AND ———. 1999. Strategy and capability of wild belugas, *Delphinapterus leucas*, during deep, benthic diving. Canadian Journal of Zoology 77: 1783–1793.

MOORE, S. E., K. W. SHELDEN, D. J. RUGH, B. A. MAHONEY, AND L. K. LITZKY. 2000. Beluga, *Delphinapterus leucas*, habitat associations in Cook Inlet, Alaska. Marine Fisheries Review 62: 60–80.

NATIONAL MARINE FISHERIES SERVICE [NMFS]. 2011. FishWatch – U.S. Seafood Facts. Available at http://www.nmfs.noaa.gov/fishwatch/#. Accessed May 2011.

O'CORRY-CROWE, G. M., R. S. SUYDAM, A. ROSENBERG, K. J. FROST, AND A. E. DIZON. 1997. Phylogeography, population structure and dispersal patterns of the beluga whale *Delphinapterus leucas* in the western Nearctic revealed by mitochondrial DNA. Molecular Ecology 6: 955–970.

PETRIE, A., AND P. WATSON. 2006. Statistics for veterinary and animal science. 2nd Edition. Blackwell Publishing Ltd., Oxford, UK, 299 pp.

REA, L. D., M. BERMAN-KOWALEWSKI, D. A. S. ROSEN, AND A. W. TRITES. 2009. Seasonal differences in biochemical adaptation to fasting in juvenile and subadult Steller sea lion (*Eumatopias jubatus*). Physiological and Biochemical Zoology 82: 236–247.

RIDGWAY, S. H., AND D. G. JOHNSTON. 1966. Blood oxygen and ecology of porpoises of three genera. Science 151: 456–458.

———, C. A. BOWERS, D. MILLER, M. L. SCHULTZ, C. A. JACOBS, AND C. A. DOOLEY. 1984. Diving and blood oxygen in the white whale. Canadian Journal of Zoology 62: 2349–2351.

ST. AUBIN, D. J., AND J. R. GERACI. 1989. Adaptive changes in hematologic and plasma chemical constituents in captive beluga whales, *Delphinapterus leucas*. Canadian Journal of Fisheries and Aquatic Sciences 46: 796–803.

———, AND L. A. DIERAUF. 2001. Stress and marine mammals. *In* L. A. Dierauf and F. M. D. Gulland (eds.). CRC handbook of marine mammal medicine, 2nd Edition. CRC Press, Boca Raton, Florida, pp. 253–269.

———, S. DEGUISE, P. R. RICHARD, T. G. SMITH, AND J. R. GERACI. 2001. Hematology and plasma indicators of health and ecological status in beluga whales, *Delphinapterus leucas*. Arctic 54: 317–331.

TRYLAND, M., AND E. BRUN. 2001. Serum chemistry of the minke whale from the northeastern Atlantic. Journal of Wildlife Diseases 37: 332–341.

———, S. I. THORESEN, K. M. KOVACS, AND C. LYDERSEN. 2006. Serum chemistry of free-ranging white whales (*Delphinapterus leucas*) in Svalbard. Veterinary Clinical Pathology 35: 199–203.

WILLARD, M. D., AND H. TVEDTEN. 2003. Small animal clinical diagnosis by laboratory medicine, 4th Edition. Elsevier Health Sciences, Philadelphia, Pennsylvania, 432 pp.

YOKUS, B., AND U. D. CAKIR. 2006. Seasonal and physiological variations in serum chemistry and mineral concentrations in cattle. Biological Trace Element Research 109: 255–266.

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