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Original Study

Effects of Season, Location, Species, and Sex on Hematologic and Plasma Biochemical Values and Body Mass in Free-ranging Grebes (*Aechmophorus* species)

Nancy L. Anderson, DVM, ABVP (Avian), PhD, Susan E. W. De La Cruz, PhD, Joseph K. Gaydos, VMD, PhD, Michael H. Ziccardi, DVM, MPVM, PhD, and Danielle J. Harvey, PhD

Abstract: The effects of season, location, species, and sex on body weight and a comprehensive array of blood chemistry and hematology analytes were compared for free-ranging western (*Aechmophorus occidentalis*) and Clark's (*Aechmophorus clarkii*) grebes. Birds (n = 56) were collected from Puget Sound, WA, and Monterey Bay and San Francisco Bay, CA, from February 2007 to March 2011. The data supported generalization of observed ranges for most analytes across *Aechmophoru*s grebe metapopulations wintering on the Pacific coast. Notable seasonal and location effects were observed for packed cell volume (winter 6% greater than fall; winter California [CA] 5% greater than Washington [WA]), total white blood cell count (CA 3.57×10^3 cells/μL greater than WA), heterophils (WA 10% greater than CA), lymphocytes (winter 19% greater than fall), heterophil to lymphocyte ratio (fall 5.7 greater than winter), basophils (CA greater than WA), plasma protein (WA about 10 g/L [1.0 g/dL] greater than CA), plasma protein to fibrinogen ratio (winter about 15 greater than fall), potassium (CA 2 mmol/L greater than WA), and liver enzymes (alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase: WA greater than CA). Within California, season had a greater effect on body mass than sex (mean winter weights about 200 g greater than fall), whereas within a season, males weighed only about 80 g more than females, on average. These data give biologists and veterinarians quantitative reference values to better assess health at the individual and metapopulation level.

Key words: seasonal effects, location effects, blood chemistry, hematology, reference values, avian, western grebe, *Aechmophorus occidentalis*, Clark's grebe, *Aechmophorus clarkii*

INTRODUCTION

Grebes of the genus *Aechmophorus* are piscivorous, aquatic birds best known for their elaborate courtship displays. Due to precipitous population declines across their winter and breeding ranges, *Aechmophorus* grebes are included in the conservation plans of several states and provinces.[1] The *Aechmophorus* genus includes 2 grebe species, the western (*Aechmophorus occidentalis*) and Clark's (*Aechmophorus clarkii*), that have overlapping home ranges (International Union for the Conservation of Nature Red List Status: least concern).[2] Both the western and Clark's grebes breed on freshwater lakes in western North America and winter primarily in nearshore habitats along the Pacific coast, but also in large open-water lakes.[3,4] The 2 species have similar behaviors and physical appearances and are commonly grouped together for research studies.[5–8]

Assessing the health of individual animals by hematologic analysis within defined metapopulations can be useful for understanding the health of entire populations of this grebe genus and may also

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reveal previously unknown causes for population declines. When available, this information can be used to improve the efficacy of conservation programs.[9,10] Hematologic analysis is also a valuable tool used during rehabilitation to evaluate the health and overall condition of the birds before release back into the wild. This is particularly beneficial for thousands of oiled grebes after oil spill events and hundreds that are annually admitted to wildlife hospitals in California, USA.[11,12] Although grebes are frequently the subject of rehabilitative efforts after coastal oil spills, very little information exists regarding normal variability in their health-related hematologic parameters.[6,13]

To address the disparity between the lack of published reference intervals and the need to better understand the health of individuals and populations for conservation and rehabilitation purposes, this study analyzed historical data from 3 free-ranging grebe (*Aechmophorus* species) metapopulations during the nonbreeding period for effects of season, location, species, and sex on body weight and a comprehensive array of plasma chemistry analytes and complete blood count (CBC) values.

MATERIALS AND METHODS

Animals and sampling

All capture and handling of grebes was carried out under the guidance and approval of the US Department of the Interior, Geological Survey, Western Ecological Research Center, University of California at Davis Institutional Animal Care and Use Committee (protocols 06-12401 and 15110), with permits from California Department of Fish and Wildlife (SC-003855), US Department of the Interior, Fish and Wildlife Service (MB-146942-0 and MB-102896-0), and US Geological Survey Bird Banding Laboratory (BBL-22911). Western and Clark's grebes were captured and identified with leg bands, and hematologic analysis was performed as described for 2 telemetry studies.[14,15] Briefly, free-ranging birds were captured by a modified neutrally buoyant gill net technique.[16] Samples from Washington (WA) birds (n = 24) were collected on Puget Sound, USA, during February 2007 and from California (CA) birds on Monterey Bay, USA (n = 4), during January 2011 and on San Francisco Bay, USA (n = 28), during November–December 2010 and March 2011.

Birds were examined at time of capture and were not included in the study if abnormalities were present. The body weight and beak length from the tip of the upper mandible to the end of the culmen were used to sex birds.[17,18] Sex was verified on postmortem examination or through DNA extracted from a blood sample (Zoogen Services, Davis, CA, USA). Postmortem examination was performed on birds belonging to a subset that had surgical implantation of coelomic radio transmitters that died before release. All study birds were examined and sampled after hatch year with the age being determined by plumage.[19,20] Blood (<2.5 mL) was collected from the right jugular vein with a 25-gauge needle and 3-mL syringe. Experienced personnel made hematology smears with fresh blood. The rest of the sample was placed in lithium heparin blood tubes (Microtainer™ 365965, Becton, Dickinson, and Company, Franklin Lakes, NJ, USA).[14,15] Within 4.2 hours of capture, all samples were centrifuged for 15 minutes at 1000*g*–1300*g*, and the resultant plasma was separated, refrigerated, and, except for the plasma protein electrophoresis, analyzed the same day at the William R. Pritchard Veterinary Medical Teaching Hospital, Clinical Diagnostic Laboratories, University of California, Davis, CA, USA. For grebes collected on California waters, blood chemistry analyses were performed on a Roche Cobas c501 (Roche Diagnostics, Indianapolis, IN, USA) and fibrinogen on a Stago STA Compact fibrometer (Diagnostica Stago Inc, Parsippany, NJ, USA) with a STA Fibrinogen kit (00674, Diagnostica Stago Inc) that used the method of Clauss. For birds collected on Puget Sound, blood chemistry analyses were performed on a Hitachi 917 (Roche Diagnostics) and fibrinogen on a BBL fibrometer (Becton, Dickinson, and Company) with test kit 00674/886-10 from Trinity Biotech (Berkeley Heights, Bray, Ireland) that used the method of Clauss. Tables 1 and 2 list test methodologies for the Roche analyzers. Standard techniques were used except for those listed here.[14,15,21] Blood for the packed cell volume (PCV) and total white blood cell (TWBC) count was obtained from the heparinized tubes. Leukocyte counts were determined manually with Natt and Herrick solution with 0.5% new methylene blue added. Plasma for protein electrophoresis was shipped overnight on ice packs to the University of Miami, Avian and Wildlife Laboratory, and analyzed on a Beckman Paragon SPEP-II gel system (Beckman Coulter, Fullerton, CA, USA).[22]

The following parameters were determined for all birds: PCV, cell counts (TWBC, heterophil [H], lymphocyte [L], monocyte [M], eosinophil [E], basophil [B]), percentage of total count for each white blood cell type (%H, %L, %M, %E, %B),

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Table 1. Test methodologies for the Roche Cobas c501 chemistry analyzer used to analyze samples from free-ranging western and Clark's grebes collected on Monterey Bay and San Francisco Bay, CA, USA.

Analyte	Method/principle	Manufacturer, kit
Alanine aminotransferase	Substrate-linked reaction without pyridoxal phosphate	Roche, ALTL
Alkaline phosphatase	*p*-Nitrophenyl phosphate, colorimetric	Roche, ALP2
Aspartate aminotransferase	NADH oxidation, colorimetric	Roche, ASTL
Bicarbonate	Phosphoenolpyruvate, colorimetric	Roche, CO2-L
Calcium	NM-BAPTA binding, photometric	Roche, CA2
Cholesterol	Enzymatic/cholesterol esterase, colorimetric	Roche, CHOL2
Creatine kinase	Enzymatic/NADPH, photometric	Roche, CKL
Electrolytes (sodium, potassium, chloride)	Ion-selective electrodes, electromagnetic force	Roche, ISE Indirect Gen 2
Glucose	Hexokinase, photometric	Roche, GLUC3
Glutamate dehydrogenase	Enzymatic/α-oxoglutarate NADH coupled, photometric	Randox, GL441
Inorganic phosphorus	Molybdate UV, photometric	Roche, PHOS2
Lactate dehydrogenase	Enzymatic, photometric	Roche, LDHI2
Total protein	Biuret, colorimetric	Roche, TP2
Triglycerides	Enzymatic, colorimetric	Roche, TRIG
Urea nitrogen	Enzymatic urease, photometric	Roche, UREAL
Uric acid	Enzymatic (uricase-peroxidase coupled), colorimetric	Roche, UA2

Abbreviations: NADH indicates nicotinamide adenine dinucleotide plus 1 hydrogen; NM-BAPTA, 5-nitro-5′-methyl-1,2-bis(*o*-aminophenoxyethane-*N*,*N*,*N*′,*N*′-tetraacetic acid; NADPH, nicotinamide adenine dinucleotide phosphate plus 1 hydrogen; UV, ultraviolet.

heterophil to lymphocyte ratio (H : L), total solids (TS) by refractometer, sodium, calcium, blood urea nitrogen, glucose, creatine phosphokinase (CK), potassium (K), uric acid (UA), cholesterol (CHOL), triglycerides (TRIG), bicarbonate (HCO_3), inorganic phosphorus (IP), total protein (TP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase

Table 2. Test methodologies for the Hitachi 917 chemistry analyzer (made by Roche Diagnostics) used to analyze samples from free-ranging western and Clark's grebes collected on Puget Sound, WA, USA.

Analyte	Method/principle	Manufacturer, kit number
Alanine aminotransferase	Substrate-linked enzymatic, colorimetric	Roche, 450065
Aspartate aminotransferase	Substrate linked enzymatic, colorimetric	Roche, 450064
Bicarbonate	PEPC/MDH, oxamate added, kinetic, rate with sample blank, UV	Roche, 450057
Calcium	*o*-cresolphthalein complexone, endpoint with sample blank	Roche, 1125621
Cholesterol	Enzymatic, colorimetric	Roche, 450061
Creatine kinase	Substrate-linked enzymatic, UV, NAC-activated, kinetic	Roche, 450060
Electrolytes (sodium, potassium)	Ion-selective electrodes, electromagnetic force	Roche, ISE Indirect Gen 2
Glucose	UV, hexokinase enzymatic, endpoint with sample blank	Roche, 450058
Inorganic phosphorus	UV, ammonium molybdate, endpoint with sample blank	Roche, 1040898
Lactate dehydrogenase	Lactate substrate, rate of NADH formation, UV, optimized, Tris buffer, kinetic	Roche, 1039044
Total protein	Biuret, endpoint with sample blank	Roche, 1040901 or 1553836
Triglycerides	GPO/PAP, enzymatic, colorimetric, endpoint	Roche, 1488899
Urea nitrogen	Enzymatic urease, photometric	Roche, 1489321
Uric acid	Uricase, endpoint with sample blank	Roche Plus, 1661850 or 1661868

Abbreviations: PEPC/MDH indicates phosphoenolpyruvate carboxylase/malate dehydrogenase; UV, ultraviolet; NAC, activated *N*-acetyl-cysteine; NADH, nicotinamide adenine dinucleotide plus 1 hydrogen; Tris buffer, tris(hydroxymethyl)aminomethane buffer; GPO, glycerol phosphate oxidase; PAP, enzymatic colorimetric method.

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(LDH), fibrinogen (Fb), plasma total protein to fibrinogen ratio (TP : Fb), and total solids to fibrinogen ratio (TS : Fb). Chloride (Cl), alkaline phosphatase, and glutamate dehydrogenase were measured only in CA birds. A limited subset of these blood chemistry data (mean, SD, minimum (MIN), and maximum (MAX) values for sodium, K, Cl, and inorganic phosphorus from 23 birds) were previously published.[13] Plasma protein electrophoresis values (prealbumin, albumin, α_1-globulin [α_1-GLOB], α_2-globulin [α_2-GLOB], β-globulin [β-GLOB], γ-globulin [γ-GLOB]) were determined only for WA birds. Hemolysis (HI) and lipemia (LI) indices were only available for the grebes collected in California.

Statistics

Statistical significance was set at $P < .05$. Outliers were identified with Dixon-Reed and Tukey interquartile fences tests, histograms, and quantile-quantile plots by Reference Value Advisor v2.1 statistical software (Biostatistiques, l'École Nationale Vétérinaire de Toulouse, France).[23] Laboratory report notes indicated that TRIG values could be affected by LI > 1 and ALT and AST values by indices >150. HI > 15 could affect LDH, HI > 40 affect AST, and HI > 100 affect CK. Data were checked for index values that could affect results. When indices exceeded cut off values, a Pearson correlation coefficient (PCC) was used to assess systematic correlation between index and affected analyte values. Data were also visually checked for relationships between outliers and magnitude of HI or LI. Per American Society for Veterinary Clinical Pathology guidelines, descriptive statistics (mean, median, SD, MIN, MAX) and reference intervals, including 90% confidence interval (CI) for the lower reference limit and 90% CI for the upper reference limit, were calculated for merged populations of birds captured in Washington and California, birds captured in Washington, all birds captured in California, birds captured in California during the fall, and birds captured in California during the winter. The Anderson-Darling test was used to assess normality of data sets for reference interval calculations. Standard and robust methods were used for data sets with Gaussian or symmetric distributions, respectively, either initially or after Box-Cox transformation.[24] When data did not meet these criteria, reference intervals and CIs were computed by a nonparametric method if $n \geq 40$. For parametric data, CIs were calculated by a parametric bootstrap method if $n < 20$. Otherwise a nonparametric bootstrap was used. When outliers persisted after these procedures, analysis was repeated without outliers. Outliers were only removed when they disproportionately influenced statistical significance between populations and when values were outside the expected clinical ranges for seabirds in general.[23–25] When data did not fit these criteria, observed ranges were reported (mean, median, SD, MIN, MAX).

Unless stated otherwise, all other statistical analyses were performed by IBM SPSS Version 24 for Macintosh (IBM Corp., released 2016, Armonk, NY, USA). Data were visually (color coded dot plots) and statistically assessed for differences between collection location (Puget Sound, San Francisco Bay, Monterey Bay), season collected (fall: November 17–December 9; winter: January 25–March 28), sex, and species (Clark's versus western). Because all grebes captured in Washington were collected during the winter, a season effect was only evaluated for grebes captured in California. All data were evaluated for normality with visual plots (histogram, dot, box, spread versus level, and normal quantile-quantile plots) and Shapiro-Wilk, Kolmogorov-Smirnov, skewness, and kurtosis statistics. If data were not normally distributed on all tests, nonparametric techniques were used. Comparison of independent means of normally distributed data were performed by *t* test or analysis of variance. To provide a conservative analysis, all *t* test results were supported by nonparametric Mann-Whitney tests.

Multifactorial comparisons were made by a stepwise general linear model starting with all variables and interactions. Bonferroni correction was applied to all multiple comparisons. Insignificant variables and interactions were removed stepwise until best "fit" was achieved. When only a single parameter was significant, data were analyzed with a *t* test, a nonparametric Mann-Whitney test, or both.

RESULTS

Hemolysis and lipemia indices effects

LI values were 1–30 for birds sampled in California (data not available for other birds). There was no correlation between LI and TRIG values ($P = .161$; $r = .184$). The one extreme outlier (LI = 25, TRIG = 709 mg/dL) did not have the highest LI (=30, TRIG = 133 mg/dL). HI ranged from 0 to 370, (8 samples <15, no effects; 10 samples 15–40, possible LDH effects; 12 samples 41–99, possible LDH or AST effects; 2 samples

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>100, possible LDH, AST, or CK effects). There was no correlation between HI and AST ($P = .104$; $r = -.229$) or CK ($P = .261$; $r = -.117$). The PCC was statistically significant for LDH ($P = .020$; $r = .366$), but the correlation was weak and the one extreme outlier for HI (=370, LDH = 427 IU/L) was not associated with the highest LDH value (HI = 12, LDH = 751 IU/L).

Location effects within California and species or sex effects

Differences were not statistically significant between grebes captured in Monterey Bay and San Francisco Bay, Clark's and western, or male and female for any plasma chemistry analyte or hematology value. Data from both sexes, species, and California locations were combined for further analysis (Tables 3–5).

Season effects: fall versus winter

Because all Washington grebes were collected during winter, season effect was only evaluated for California birds. Six of the hematology values were statistically different. Values for California grebes captured during winter were greater than those captured during the fall for TP:Fb and TS:Fb, PCV (mean difference 6%), L, and %L ($P_{TP:Fb} = .001$, $P_{TS:Fb} = .001$, $P_{PCV} = .002$, $P_{L} = .010$, $P_{\%L} = .002$) (Tables 4 and 5). Although general linear model analysis was not significant for H:L ($P = .444$), inspection of plotted data and L and %L results indicated a seasonal difference existed. A targeted Mann-Whitney analysis for season showed H:L was greater for grebes captured in California during the fall than those collected in the winter ($P = .034$) (Table 4).

Location effects: Washington versus California

Washington compared with California populations with no seasonal effect: No differences were statistically significant between California and Washington grebes for sodium, calcium, glucose, blood urea nitrogen, Fb, CK, H, M, and %E, but values for Washington birds were greater than for California birds for TP, TS, inorganic phosphorus, HCO_3, AST, ALT, LDH, %H, %M, and values for California birds were greater than for Washington birds for K, uric acid, CHOL, TRIG, TWBC, E, B, and %B ($P_{\text{all values}} < .045$; Tables 3–5). In particular, the mean (4.3 mmol/L) and MAX (7.5 mmol/L) for K were greater in grebes captured in California compared with Washington (mean = 2.3 mmol/L, MAX = 3.4 mmol/L). All 3 analytes associated with liver tissue damage (ALT, AST, LDH) were greater in grebes captured in Washington compared with California. Of these, LDH showed the most pronounced relative difference (WA: median=514 U/L, range= 289–1161 U/L; CA: median=143 U/L, range= 37–751 U/L).

Washington compared with California populations with seasonal effects: Values for Washington grebes for TP:Fb and TS:Fb were not significantly different from all California grebes ($P_{TP:Fb} = .527$, $P_{TS:Fb} = .648$) or those collected only during winter ($P_{TP:Fb} = .347$, $P_{TS:Fb} = .097$). Mean PCV for Washington birds was not significantly different than for all California birds ($P = .183$). However, PCV for birds captured in Washington was 5% lower than for California birds captured in the winter only ($P < .001$). The values for L and %L, for all California birds ($P_L = .001$, $P_{\%L} = .038$) and only those captured during winter ($P_L < .001$; $P_{\%L} < .001$) were greater than for Washington grebes. Values for H:L for all California birds were greater than for Washington birds ($P = .042$). However, H:L for California birds captured only during the winter was less than for Washington birds ($P = .001$; Tables 4 and 5).

Body mass

For grebes captured in Washington, the males weighed 250 g more, on average, than females ($P = .001$). For grebes captured in California, the winter birds weighed 201 g more, on average, than the fall birds ($P = .011$). Although no differences in body weight between male and female grebes captured in California were statistically significant, historical reference intervals and Washington results indicated that differences should be expected.[26] A general linear model by sex and season showed a trend for males to be about 80 g heavier, on average, than females in both fall and winter populations of grebes captured in California ($P = .054$; Tables 6 and 7).

DISCUSSION

Given the lack of information about hematologic values for grebes (*Aechmophorus* species, a genus of birds with significant conservation concerns), our results provide a valuable resource for biologists, veterinarians, and wildlife rehabilitators.[10,11] Published reference intervals cannot only be used to assess the health of individual animals undergoing rehabilitation better, thus improving convalescent care and release potential, but also to investigate previously unknown causes of popula-

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Table 3. Reference intervals (n $\geq$ 20) or observed ranges (n < 20) for 17 plasma chemistry parameters in free-ranging western and Clark's grebes from the Pacific coast adjacent to Washington and California, USA, 2007–2011. Conversion to SI units for Na, K, Cl, HCO_3 is 1 mmol/L = 1 mEq/L.

Parameter, unit	n	Mean (median) ± standard deviation[a]	Range (removed outlier)	Reference interval[b] or observed range[c]	LRL 90% CI	URL 90% CI	Distribution/method
Washington and California combined							
GLUC, mmol/L	47	15.1 (14.7) ± 2.76	10.9–24.0	9.2–20.3[b]	7.7–10.7	18.8–21.8	G/R
GLUC, mg/dL		272 (265) ± 49.7	196–433	165–365[b]	138–192	338–393	
Na, mmol/L	56	154 (154) ± 3.2	146–161	147–160[b]	146–148	159–162	G/R
K, mmol/L[d]	56	3.4 (3.1) ± 1.47	1.4–7.5	1.4–7.1[b]	1.2–1.6	6.1–8.1	NG/RT
HCO_3, mmol/L[d]	53	24 (24) ± 3.5	15–30	16–31[b]	15–18	29–32	G/P
Ca, mmol/L	51	2.6 (2.6) ± 0.13	2.3–2.9 (1.8)	2.3–2.8[b]	2.3	2.8–2.9	G/P
Ca, mg/dL		10.2 (10.2) ± 0.50	9.1–11.5 (7.3)	9.1–11.2[b]	9.0–9.3	11.0–11.4	
IP, mmol/L[d]	55	1.1 (1.11) ± 0.34	0.4–1.9	0.4–1.8[b]	0.3–0.6	1.7–1.9	G/P
IP, mg/dL		3.5 (3.5) ± 1.05	1.1–5.9	1.3–5.6[b]	1.0–1.7	5.2–6.0	
BUN, mmol/L	48	1.1 (0.7) ± 0.64	0.4–3.2	0.4–3.2[b]	0.4	1.8–3.2	NG/NP
BUN, mg/dL		3 (2) ± 1.8	1–9	1–9[b]	1	5–9	
UA, mmol/L[d]	55	297 (238) ± 174.5	59–952	59–833[b]	59–119	654–1011	NG/PT
UA, mg/dL		5 (4) ± 3.0	1–16	1–14[b]	1–2	11–17	
CHOL, mmol/L[d]	48	7.3 (7.3) ± 1.63	4.1–10.9	4.0–10.5[b]	3.4–4.6	9.7–11.2	G/RT
CHOL, mg/dL		283 (280) ±63.1	157–419	154–407[b]	131–176	375–434	
TRIG, mmol/L[d]	45	1.3 (1.2) ± 0.42	0.6–2.5 (8.0)	0.4–2.1[b]	0.3–0.6	1.9–2.3	G/P
TRIG, mg/dL		113 (110) ± 36.9	54–217 (709)	38–188[b]	23–53	172–204	
CK, U/L	54	2130 (985) ± 2921.7	436–13 046	436–12 817[b]	436–507	8859–13 046	NG/NP
AST, U/L[d]	55	445 (374) ± 247.4	188–1407	189–1053[b]	179–208	844–1280	NG/RT
ALT, U/L[d]	46	15 (8) ± 15.3	0–54	0–53[b]	0–3	48–54	NG/NP
LDH, U/L[d]	54	331 (287) ± 266.6	37–1161 (4780)	38–1162[b]	25–55	914–1476	NG/RT
Washington							
GLUC, mmol/L	15	14.8 (14.5) ± 1.75	12.4–17.9	10.9–18.7[c]	9.6–12.4	17.2–20.1	G/P
GLUC, mg/dL		267 (261) ± 31.5	223–323	197–337[c]	172–224	310–362	
Na, mmol/L	24	154(154) ± 4.1	148–161	145–162[b]	143–147	159–164	G/R
K, mmol/L[d]	24	2.3 (2.2) ± 0.56	1.4–3.4	1.1–3.4[b]	0.8–1.4	3.0–3.7	G/R
HCO_3, mmol/L[d]	21	25 (25) ± 2.8	20–30	19–31[b]	17–21	29–33	G/P
Ca, mmol/L	20	2.6 (2.6) ± 0.10	2.4–2.7	2.4–2.8[b]	2.3–2.4	2.7–2.8	G/R
Ca, mg/dL		10.2 (10.2) ± 0.38	9.4–10.7	9.4–11.0[b]	9.1–9.7	10.8–11.2	
IP, mmol/L[d]	23	1.3 (1.3) ± 0.25	0.7–1.8	0.8–1.8[b]	0.6–0.9	1.7–2.0	G/P
IP, mg/dL		4.1 (4.0) ± 0.78	2.3–5.7	2.4–5.7[b]	1.9–2.9	5.2–6.2	
BUN, mmol/L	16	0.7 (0.7) ± 0.21	0.7–1.4	ND	ND	ND	NG/ND
BUN, mg/dL		2 (2) ± 0.6	2–4	ND	ND	ND	
UA, mmol/L[d]	23	238 (178) ± 166.6	59–833	59–773[b]	59–119	476–1249	NG/RT
UA, mg/dL		4 (3) ± 2.8	1–14	1–13[b]	1–2	8–21	

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Table 3. Continued.

Parameter, unit	n	Mean (median) ± standard deviation[a]	Range (removed outlier)	Reference interval[b] or observed range[c]	LRL 90% CI	URL 90% CI	Distribution/method
CHOL, mmol/L[d]	16	6.3 (5.9) ± 1.05	5.2–9.5	4.8–9.1[c]	4.5–5.2	7.6–11.0	NG/PT
CHOL, mg/dL		243 (229) ± 40.4	199–366	186–351[c]	173–201	295–426	
TRIG, mmol/L[d]	14	1.0 (0.9) ± 0.31	0.6–1.5	0.3–1.7[c]	0.1–0.6	1.5–2.0	G/P
TRIG, mg/dL		91 (80) ± 27.2	54–136	30–152[c]	10–55	130–173	
CK, U/L	21	2128 (1200)±2130.0	539–8619 (13 046)	452–13 606[b]	398–553	5299–32 386	NG/PT
AST, U/L[d]	23	556 (457) ± 299.0	277–1407	0–1078[b]	0–156	735–1318	NG/R
ALT, U/L[d]	14	32 (36) ± 14.3	11–54	0–64[c]	0–12	53–76	G/P
LDH, U/L[d]	22	561 (514) ± 209.9	289–1161 (4780)	77–951[b]	0–244	766–1146	NG/RT
California, fall and winter combined							
GLUC, mmol/L	32	15.2 (14.8) ± 3.15	10.9–24.0	8.4–21.3[b]	6.9–10.2	19.0–23.0	G/R
GLUC, mg/dL		274 (267) ±56.8	196–433	151–383[b]	124–184	343–415	
Na, mmol/L	32	154 (154) ± 2.6	146–159	ND	ND	ND	NG/ND
K, mmol/L[d]	32	4.3 (4.1) ± 1.37	2.5–7.5	2.3–7.5[b]	2.1–2.6	6.1–8.3	NG/RT
HCO_3, mmol/L[d]	32	23 (24) ± 3.6	15–29	15–30[b]	13–17	28–32	G/P
Ca, mmol/L	31	2.6 (2.6) ± 0.14	2.3–2.9 (1.8)	2.3–2.8[b]	2.2–2.3	2.8–2.9	G/P
Ca, mg/dL		10.2 (10.2) ± 0.57	9.1–11.5 (7.3)	9.0–11.3[b]	8.7–9.2	11.0–11.6	
IP, mmol/L[d]	32	1.0 (0.9) ± 0.32	0.4–1.9	0.5–1.8[b]	0.4–0.6	1.5–2.0	G/RT
IP, mg/dL		3.0 (2.9) ± 1.00	1.1–5.9	1.4–5.5[b]	1.1–1.8	4.7–6.3	
BUN, mmol/L	32	1.1 (0.7) ± 0.71	0.4–3.2	ND	ND	ND	NG/ND
BUN, mg/dL		3 (2) ± 2.0	1–9	ND	ND	ND	
UA, mmol/L[d]	32	357 (297) ± 190.4	119–952	59–833[b]	59–119	654–1011	NG/RT
UA, mg/dL		6 (5) ± 3.2	2–16	1–14[b]	1–2	11–17	
CHOL, mmol/L[d]	32	7.8 (8.0) ± 1.57	4.1–10.9	4.6–11.1[b]	3.8–5.3	10.2–11.9	G/P
CHOL, mg/dL		302 (307) ± 60.5	157–419	177–428[b]	145–205	395–458	
TRIG, mmol/L[d]	31	1.4 (1.3) ± 0.44	0.9–2.5 (8.0)	0.4–2.2[b]	0.2–0.7	1.9–2.5	G/RT
TRIG, mg/dL		123 (116) ± 39.0	76–217 (709)	37–196[b]	20–59	170–220	
CK, U/L	32	1790 (881) ± 2848.9	436–12 436	429–11 826[b]	396–490	4609–41 470	NG/RT
AST, U/L[d]	32	365 (317) ± 179.9	188–913	171–843[b]	157–193	623–1165	NG/RT
ALT, U/L[d]	31	6 (6) ± 3.0	0–15 (50)	0–12[b]	0–1	11–14	G/P
LDH, U/L[d]	32	173 (143) ± 137.0	37–751	45–625[b]	37–62	426–925	NG/RT
Cl, mmol/L	31	118 (118) ± 2.7	112–124 (105)	112–124[b]	111–114	122–125	G/P
ALP, U/L	32	35 (29) ± 22.7	7–89	8–95[b]	7–11	71–120	NG/RT
GDH, U/L	32	3 (3) ± 3.0	0–15	2–90[b]	1–5	67–116	NG/RT
California, fall							
GLUC, mmol/L	16	16.7 (17.0) ± 3.32	11.7–24.0	10.7–25.0[c]	9.2–12.3	21.5–28.5	G/PT
GLUC, mg/dL		300 (307) ± 59.8	210–433	192–451[c]	165–221	387–514	
Na, mmol/L	16	154 (154) ± 1.7	151–158	150–158[c]	149–152	157–159	G/P
K, mmol/L	16	4.0 (3.5) ± 1.3	2.5–6.3	2.2–7.7[c]	1.9–2.5	5.9–9.9	NG/PT

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Table 3. Continued.

Parameter, unit	n	Mean (median) ± standard deviation[a]	Range (removed outlier)	Reference interval[b] or observed range[c]	LRL 90% CI	URL 90% CI	Distribution/ method
HCO_3, mmol/L	16	24 (25) ± 3.3	18–29	17–32[c]	15–20	29–34	G/P
Ca, mmol/L	15	2.6 (2.6) ± 0.17	2.3–2.9 (1.8)	2.2–2.9[c]	2.0–2.3	2.8–3.0	G/PT
Ca, mg/dL		10.3 (10.4) ± 0.69	9.1–11.5 (7.3)	8.6–11.7[c]	7.9–9.3	11.2–12.1	
IP, mmol/L	16	1.0 (1.0) ± 0.31	0.4–1.8	0.4–1.7[c]	0.1–0.6	1.4–1.9	G/P
IP, mg/dL		3.2 (3.2) ± 0.95	1.1–5.6	1.1–5.2[c]	0.3–1.9	4.4–6.0	
BUN, mmol/L	16	1.1 (0.7) ± 0.82	0.4–3.2	2.5–4.3[c]	ND–3.2	3.9–4.3	NG/PT
BUN, mg/dL		3 (2) ± 2.3	1–9	7–12[c]	ND–9	11–12	
UA, mmol/L	16	238 (297) ± 107.1	59–416	0–535[c]	0–119	416–595	G/PT
UA, mg/dL		4 (5) ± 1.8	1–7	1–9[c]	0–2	7–10	
CHOL, mmol/L	16	7.6 (7.4) ± 1.53	4.1–10.5	3.9–10.7[c]	2.3–5.5	9.7–11.7	G/PT
CHOL, mg/dL		294 (284) ± 59.1	157–406	152–414[c]	89–211	373–452	
TRIG, mmol/L	16	1.2 (1.2) ± 0.34	0.9–1.9	0.7–2.3[c]	0.6–0.9	1.8–3.0	G/PT
TRIG, mg/dL		109 (103) ± 29.8	76–165	65–202[c]	57–75	155–261	
CK, U/L	16	1967 (1091) ± 2871	436–12 436	430–17 680[c]	347–584	3607–ND	NG/PT
AST, U/L	16	430 (377) ± 209.5	188–913	158–1098[c]	130–212	722–1506	NG/PT
ALT, U/L	16	9 (6) ± 11.4	2–50	0–30[c]	ND	ND	NG/RT
LDH, U/L	16	212 (172) ± 171.5	37–751	33–780[c]	19–63	441–1279	NG/PT
Cl, mmol/L	16	119 (120) ± 2.4	115–124	114–124[c]	112–116	123–126	G/P
ALP, U/L	16	34 (24) ± 24.6	7–89	6–135[c]	3–9	75–238	NG/PT
GDH, U/L	16	3 (3) ± 2.5	0–8	0–8[c]	0	6–10	G/P
California, winter							
GLUC, mmol/L	16	13.7 (13.9) ± 1.82	10.9–16.6	9.8–17.7[c]	8.32–11.2	16.3–19.0	G/P
GLUC, mg/dL		247 (250) ± 32.8	196–299	176–319[c]	150–201	293–343	
Na, mmol/L	16	153 (153) ± 3.2	146–159	146–161[c]	144–149	158–163	G/P
K, mmol/L	16	4.6 (4.4) ± 1.21	3.3–7.5	3.0–10.0[c]	2.7–3.3	6.4–24.7	G/PT
HCO_3, mmol/L	16	21 (21) ± 2.9	15–25	14–27[c]	12–17	25–29	G/P
Ca, mmol/L	16	2.5 (12.5) ± 0.11	2.3–2.7	2.2–2.7[c]	1.9–2.4	2.7	G/PT
Ca, mg/dL		10.0 (10.1) ± 0.43	9.2–10.6	8.7–10.8[c]	7.5–9.4	10.6–10.9	
IP, mmol/L	16	0.9 (0.8) ± 0.34	0.5–1.9	0–1.7[c]	ND–0.5	1.5–2.0	G/PT
IP, mg/dL		2.9 (2.6) ± 1.04	1.5–5.9	0–5.2[c]	ND–1.5	4.5–6.2	
BUN, mmol/L	16	1.1 (0.7) ± 0.64	0.4–1.8	ND	ND	ND	NG/ND
BUN, mg/dL		3 (2) ± 1.8	1–5	ND	ND	ND	
UA, mmol/L	16	416 (357) ± 226.0	119–952	59–1071[c]	59–119	714–1368	G/PT
UA, mg/dL		7 (6) ± 3.8	2–16	1–18[c]	1–2	12–23	
CHOL, mmol/L	16	8.1 (8.3) ± 1.62	5.4–10.9	4.4–11.5[c]	3.0–5.8	10.4–12.7	G/PT
CHOL, mg/dL		311 (320) ± 62.6	210–419	169–445[c]	114–223	400–489	
TRIG, mmol/L	15	1.6 (1.4) ± 0.44	1.0–2.5 (8.0)	0.9–2.8[c]	0.8–1.0	2.2–3.5	G/PT
TRIG, mg/dL		138 (126) ± 38.9	87–217 (709)	76–247[c]	66–92	196–311	

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Table 3. Continued.

Parameter, unit	n	Mean (median) ± standard deviation[a]	Range (removed outlier)	Reference interval[b] or observed range[c]	LRL 90% CI	URL 90% CI	Distribution/method
CK, U/L	16	1613 (801) ± 2758.5	436–11 561	393–26 297[c]	350–469	2657–ND	NG/PT
AST, U/L	16	301 (295) ± 83.6	196–507	173–548[c]	154–205	427–697	G/PT
ALT, U/L	16	6 (6) ± 3.6	0–15	0–14[c]	0–2	11–17	G/P
LDH, U/L	16	134 (130) ± 65.6	55–261	43–320[c]	ND–61	228–401	G/PT
Cl, mmol/L	15	117 (117) ± 2.5	112–121 (105)	111–122[c]	108–113	120–123	G/PT
ALP, U/L	16	36 (31) ± 19.2	14–88	14–97[c]	12–17	65–136	G/PT
GDH, U/L	16	4 (3) ± 3.4	1–15	ND	ND	ND	NG/ND

Abbreviations: ASVCP indicates American Society for Veterinary Clinical Pathology; LRL, lower reference limit; CI, confidence interval; URL, upper reference limit; GLUC, glucose; G, Gaussian; R, robust; Na, sodium; K, potassium; NG, non-Gaussian; RT, robust, transformed; HCO_3, bicarbonate; P, parametric; Ca, calcium; IP, inorganic phosphorus; BUN, blood urea nitrogen; NP, nonparametric; UA, uric acid; PT, parametric, transformed; CHOL, cholesterol; TRIG, triglycerides; CK, creatine kinase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; Cl, chloride; ND, not done; ALP, alkaline phosphatase; GDH, glutamate dehydrogenase.

[a] Standard deviation and median: parametric values except robust method values reported if used to calculate reference interval.

[b] Please see ASVCP Reference Interval Guidelines[24] for outlier detection and reference interval construction. Negative lower bound of the CI listed as 0.

[c] $n < 20$: values reported in "reference interval" column are "observed range" values.

[d] Statistically significant difference ($P < .05$) between Washington and California populations.

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Table 4. Reference intervals (n > 20) or observed ranges (n < 20) for 13 complete blood count parameters in free-ranging western and Clark's grebes from the nearshore Pacific coast in Washington and California, USA, 2007–2011.

Parameter	n	Mean (median) ± SD[a]	Range (removed outlier)	Reference interval[b] or observed range[c]	LRL 90% CI	URL 90% CI	Distribution/ method
Washington and California combined							
PCV, %[d]	54	49 (49) ± 4.6	38–60	40–58[b]	38–42	56–60	G/R
TWBC, 10^3/μL[e]	54	7.42 (6.71) ± 3.826	2.50–19.50	2.75–18.91[b]	2.58–3.15	15.38–22.36	NG/RT
H, 10^3/μL	54	4.68 (3.88) ± 2.953	1.23–14.25	1.54–13.81[b]	1.33–1.82	9.86–17.63	NG/RT
%H[e]	54	63 (65) ± 15.7	30–95	31–95[b]	26–37	89–101	G/P
L, 10^3/μL[d]	54	1.93 (1.50) ± 1.519	0.30–6.88	0.41–6.61[b]	0.35–0.50	4.71–8.73	NG/RT
%L[d]	54	28 (25) ± 15.0	2–68	5–62[b]	2–7	53–71	NG/RT
H : L, %[d,e]	54	4.2 (2.7) ± 6.9	0.5–47.5	0.6–21.6[b]	0.6–0.8	12.3–35.5	NG/RT
E, 10^3/μL[e]	54	0.45 (0.18) ± 0.702	0–3.88	0–3.26[b]	0	1.34–3.88	NG/NP
%E	54	5 (3) ± 6.1	0–31	0–26[b]	0	17–31	NG/NP
M, 10^3/μL	53	0.26 (0.22) ± 0.177	0.03–0.85 (1.16)	0.04–0.70[b]	0.03–0.06	0.58–0.84	NG/RT
%M[e]	53	4 (3) ± 1.9	1–8 (12)	1–8[b]	1	7–8	NG/NP
B, 10^3/μL[e]	53	0.08 (0.05) ± 0.127	0–0.66	0–0.57[b]	0	0.32–0.66	NG/NP
%B[e]	53	1 (1) ± 1.3	0–6	0–5[b]	0	3–6	NG/NP
Washington							
PCV, %[d]	24	48 (48) ± 2.9	43–54	43–55[b]	41–44	53–56	G/P
TWBC, 10^3/μL[e]	24	5.43 (4.94) ± 1.752	3.54–8.92	3.24–11.63[b]	3.04–3.57	8.03–19.04	NG/RT
H, 10^3/μL	24	3.75 (3.43) ± 1.332	2.46–6.46	2.05–7.49[b]	1.82–2.30	5.70–9.54	NG/RT
%H[e]	24	69 (69) ± 9.7	49–86	49–90[b]	42–54	84–95	G/P
L, 10^3/μL[d,e]	24	1.24 (1.12) ± 0.600	0.52–3.06	0.50–3.08[b]	0.42–0.61	2.21–4.24	NG/RT
%L[d,e]	24	23 (21) ± 7.8	11–41	5–37[b]	2–10	31–43	G/R
H : L, %[d,e]	24	3.5 (3.3) ± 1.58	1.3–7.8	1.1–7.5[b]	0.9–1.6	6.1–8.9	G/RT
E, 10^3/μL[e]	24	0.18 (0.13) ± 0.221	0–0.96	0–0.68[b]	0	0.46–0.94	NG/RT
%E	24	3 (2) ± 3.9	0–17	ND	ND	ND	NG/ND
M, 10^3/μl	24	0.24 (0.23) ± 0.114	0.05–0.54	0.05–0.52[b]	0.03–0.09	0.43–0.61	G/PT
%M[e]	24	5 (5) ± 1.7	1–8	1–8[b]	0–2	7–9	G/P
B, 10^3/μL[e]	24	0.03 (0) ± 0.042	0–0.13	ND	ND	ND	NG/ND
%B[e]	24	1 (0) ± 0.8	0–2	ND	ND	ND	NG/ND
California, fall and winter combined							
PCV, %[f]	30	50 (50) ± 5.4	38–60	39–62[b]	36–42	58–64	G/P
TWBC, 10^3/μL[e]	30	9.00 (8.75) ± 4.175	2.50–19.50	3.20–17.68[b]	0–2.41	15.49–19.96	G/P
H, 10^3/μL	30	5.43 (4.63) ± 3.663	1.23–14.25	1.34–21.95[b]	1.23–1.69	14.31–30.40	NG/RT
%H[e]	30	59 (58) ± 18.5	30–95	20–96[b]	10–29	87–105	G/RT
L, 10^3/μL[e,f]	30	2.48 (1.88) ± 1.793	0.30–6.88	0–5.77[b]	0–0.08	4.50–7.30	NG/RT
%L[e,f]	30	31 (31) ± 17.6	2–68	0–67[b]	0–4	58–76	G/R
H : L, %[e,f]	30	4.9 (2.0) ± 9.38	0.5–47.5	0.5–27.7[b]	0.5–0.6	13.2–57.9	NG/RT
E, 10^3/μL[e]	30	0.67 (0.35) ± 0.867	0–3.88	ND	ND	ND	NG/ND
%E	30	6.7 (6.0) ± 7.1	0–31	ND	ND	ND	NG/ND
M, 10^3/μL	29	0.28 (0.23) ± 0.209	0.03–0.85 (1.16)	0.03–0.88[b]	0.03–0.05	0.64–1.15	NG/PT

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Table 4. Continued.

Parameter	n	Mean (median) ± SD[a]	Range (removed outlier)	Reference interval[b] or observed range[c]	LRL 90% CI	URL 90% CI	Distribution/ method
%M[e]	29	3 (3) ± 1.9	1–7 (12)	ND	ND	ND	NG/ND
B, 10^3/μL[e]	29	0.12 (0.07) ± 0.157	0–0.660	ND	ND	ND	NG/ND
%B[e]	29	1 (1) ± 1.5	0–6	ND	ND	ND	NG/ND
California, fall							
PCV, %[f]	16	47 (48) ± 4.8	38–55	37–58[c]	33–41	54–62	G/P
TWBC, 10^3/μL	16	8.72 (8.25) ± 3.469	3.30–15.00	2.67–17.33[c]	ND–4.35	13.42–20.50	G/PT
H, 10^3/μL	16	5.96 (5.47) ± 3.644	2.07–14.25	0–13.96[c]	0–0.80	11.10–16.55	G/P
%H	16	65 (67) ± 18.8	38–95	23–106[c]	9–39	91–120	G/P
L, 10^3/μL[f]	16	1.64 (1.70) ± 0.847	0.30–2.53	0–3.51[c]	ND	ND	NG/R
%L[f]	16	23 (23) ± 14.0	2–50	0–54[c]	0–3	44–65	G/P
H : L, %[f]	16	7.5 (3.1) ± 12.05	0.8–47.5	0.6–164.3[c]	0.4–0.9	20.0–1 687 364.3	NG/PT
E, 10^3/μL	16	0.67 (0.22) ± 1.039	0–3.88	ND	ND	ND	NG/ND
%E	16	7 (4) ± 8.8	0–31	ND	ND	ND	NG/ND
M, 10^3/μL	15	0.31 (0.30) ± 0.224	0.03–0.85 (1.16)	0–0.93[c]	0–0.05	629–1266	G/PT
%M	15	4 (3) ± 2.1	1–7 (12)	0–8[c]	0–1	7–10	G/P
B, 10^3/μL	16	0.09 (0.07) ± 0.110	0–0.34	ND	ND	ND	NG/ND
%B	16	1 (1) ± 1.4	0–4	ND	ND	ND	NG/ND
California, winter							
PCV, %[d,f]	14	53 (53) ± 4.5	47–60	43–63[c]	40–47	59–67	G/P
TWBC, 10^3/μL	14	9.33 (9.85) ± 4.979	2.50–19.50	0–20.46[c]	0–2.82	16.28–24.36	G/P
H, 10^3/μL	14	4.83 (4.28) ± 3.362	1.23–14.24	0.41–15.34[c]	0–1.37	9.26–20.84	NG/PT
%H	14	52 (50) ± 15.1	30–75	23–91[c]	17–33	73–111	G/PT
L, 10^3/μL[d,f]	14	3.44 (3.04) ± 2.054	0.45–6.88	0–8.03[c]	0–0.71	6.37–9.81	G/P
%L[d,f]	14	42 (43) ± 15.2	15–68	8–76[c]	0–21	62–89	G/P
H : L (%)[d,f]	14	1.8 (1.2) ± 1.38	0.5–4.9	0.4–10.7[c]	0.3–0.6	4.0–39.8	NG/PT
E, 10^3/μL	14	0.68 (0.58) ± 0.657	0.03–2.21	0–4.10[c]	0–0.04	1.89–7.67	G/PT
%E	14	6 (7) ± 4.6	1–17	0–16[c]	0	12–21	G/P
M, 10^3/μL	14	0.24 (0.20) ± 0.194	0.05–0.78	ND	ND	ND	NG/ND
%M	14	3 (2) ± 1.6	1–6	0–7[c]	ND–1	5–10	NG/RT
B, 10^3/μL	13	0.15 (0.05) ± 0.201	0–0.66	ND	ND	ND	NG/ND
%B	13	2 (1) ± 1.7	0–6	ND	ND	ND	NG/ND

Abbreviations: ASVCP indicates American Society for Veterinary Clinical Pathology; LRL, lower reference limit; CI, confidence interval; URL, upper reference limit; PCV, packed cell volume; G, Gaussian; R, robust; TWBC, total white blood cell count; NG, non-Gaussian; RT, robust, transformed; H, heterophil count; P, parametric; L, lymphocyte count; H : L, heterophil to lymphocyte ratio; E, eosinophil count; NP, nonparametric; ND, not done; M, monocyte count; PT, parametric, transformed; B, basophil count.

[a] Standard deviation and median: parametric values, except robust method values reported if used to calculate reference interval.

[b] Please see ASVCP Reference Interval Guidelines[24] for outlier detection and reference interval construction. Negative lower bound of the CI listed as 0.

[c] n < 20: Values reported in "reference interval" column are "observed range" values.

[d] Statistically significant difference ($P < .05$) between Washington and California, winter only populations.

[e] Statistically significant difference ($P < .05$) between Washington and California populations.

[f] Statistically significant difference ($P < .05$) within California between fall and winter populations.

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Table 5. Reference intervals (n > 20) or observed ranges (n < 20) for 16 plasma protein values determined by plasma electrophoresis, total solid values determined by refractometer, and fibrinogen values determined by fibrometer in free-ranging western and Clark's grebes from the nearshore Pacific coast in Washington and California, USA, 2007–2011. Conversion from SI units: g/L = mg/dL × 10.

Parameter	n	Mean (median) ± SD[a]	Range (removed outlier)	Reference interval[b] or observed range[c]	LRL 90% CI	URL 90% CI	Distribution/method
Washington and California combined							
TP, g/L[d]	55	41 (40) ± 6.8	29–58	27–55[b]	25–30	52–57	G/P
TS, g/L[d]	52	54 (55) ± 5.4	40–62	43–65[b]	41–45	62–67	G/P
Fb, g/L	46	2.94 (3.00) ± 1.806	0–8.00	0.18–7.83[b]	0–1.00	5.83–8.00	NG/NP
TP : Fb, %	45	18.5 (12.7) ± 14.23	4.5–55.0	4.2–60.1[b]	3.7–5.1	43.5–81.1	NG/RT
TS : Fb, %	45	24.5 (18.3) ± 16.42	5.9–61.0	6.1–60.9[b]	5.9–8.6	54.0–61.0	NG/NP
Washington							
TP, g/L[d]	23	47 (48) ± 5.0	40–58	37–58[b]	33–40	55–61	G/P
TS, g/L[d]	24	56 (56) ± 3.6	49–62	49–64[b]	47–51	62–66	G/P
Fb, g/L	18	3.22 (3.00) ± 2.211	0–8.00	0–8.02[c]	0–0.02	6.43–9.55	G/P
TP : Fb, %	17	20.6 (13.7) ± 16.20	4.5–55.0	4.5–111.6[c]	4.5–5.1	56.8–200.0	NG/PT
TS : Fb, %	17	24.0 (17.3) ± 17.64	5.9–60.0	5.9–94.9[c]	ND–6.7	56.5–147.3	NG/PT
PreALB, g/L	23	4.5 (4.3) ± 1.58	2.3–8.0	1.1–7.8[b]	0.3–2.1	6.9–8.8	G/P
PreALB, %	22	9.1 (8.7) ± 2.70	4.6–14.2 (19.9)	3.4–14.9[b]	1.9–5.0	13.1–16.5	G/P
ALB, g/L	23	24 (24) ± 3.3	16–29	17–31[b]	15–19	30–34	G/P
ALB, %	23	51.8 (51.7) ± 4.78	41.1–60.7	41.6–61.9[b]	39.0–44.5	58.9–64.8	G/P
α_1-GLOB, g/L	23	1.0 (1.0) ± 0.30	1.0–2.0	1.0–2.0[b]	0.6–1.0	1.9–2.2	G/P
α_1-GLOB, %	23	3.0 (2.9) ± 0.45	2.1–3.8	2.0–3.9[b]	1.8–2.3	3.7–4.2	G/P
α_2-GLOB, g/L	23	2.0 (2.0) ± 0.50	2.0–3.0	1.0–3.0[b]	1.0–2.0	3.0–4.0	G/P
α_2-GLOB, %	23	5.2 (5.3) ± 0.84	3.8–6.2	2.2–6.4[b]	ND–4.0	6.2–6.6	NG/RT
β-GLOB, g/L	23	8.0 (8.0) ± 1.40	6.0–11.0	5.0–11.0[b]	4.0–6.0	10.0–12.0	G/P
β-GLOB, %	23	16.8 (16.8) ± 2.72	11.6–21.4	11.0–22.6[b]	9.5–12.7	21.0–24.1	G/P
γ-GLOB, g/L	23	6.0 (6.0) ± 1.50	4.0–10.0	3.0–10.0[b]	2.0–4.0	9.0–11.0	G/P
γ-GLOB, %	23	13.6 (13.3) ± 2.38	10.2–19.5	8.6–18.7[b]	7.2–10.1	17.1–20.2	G/P
California, fall and winter combined							
TP, g/L[d]	32	37 (37) ± 3.8	29–44	29–44[b]	27–31	43–46	G/P
TS, g/L[d]	28	52 (52) ± 5.8	40–62	40–64[b]	37–43	60–67	G/P
Fb, g/L	28	2.75 (2.69) ± 1.560	1.00–6.00	0–5.90[b]	0–2.23	4.94–6.69	NG/RT
TP : Fb, %[e]	28	17.2 (12.6) ± 11.20	4.7–43.0	4.4–62.2[b]	3.3–5.8	38.2–100.9	NG/PT
TS : Fb, %[e]	28	24.8 (16.4) ± 17.11	7.7–61.0	7.9–81.3[b]	7.6–8.7	55.3–109.1	NG/RT
California, fall							
TP, g/L	16	35 (35) ± 3.3	29–41	27–42[c]	23–31	40–43	G/PT
TS, g/L	16	51 (52) ± 5.4	40–60	38–62[c]	31–43	58–65	G/PT
Fb, g/L	16	3.31 (3.00) ± 1.250	1.00–6.00	0.57–6.06[c]	0–1.68	4.98–7.02	G/P
TP : Fb, %[e]	16	11.6 (10.3) ± 6.68	4.7–33.0	4.8–40.0[c]	4.2–5.8	21.5–87.8	NG/PT
TS : Fb, %[e]	16	17.2 (16.0) ± 9.50	7.7–49.0	7.5–57.4[c]	6.5–9.0	30.6–147.2	NG/PT

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Table 5. Continued.

Parameter	n	Mean (median) ± SD[a]	Range (removed outlier)	Reference interval[b] or observed range[c]	LRL 90% CI	URL 90% CI	Distribution/ method
California, winter							
TP, g/L	16	38 (38) ± 4.0	32–44	29–47[c]	27–32	44–50	G/PT
TS, g/L	12	52 (51) ± 6.5	43–62	40–70[c]	37–44	61–83	G/PT
Fb, g/L	12	2.00 (1.50) ± 1.537	1.00–6.00	ND	ND	ND	NG/ND
TP : Fb, %[e]	12	24.7 (24.8) ± 11.83	6.0–43.0	0–51.8[c]	0–8.2	40.6–62.5	G/P
TS : Fb, %[e]	12	34.9 (34.0) ± 17.51	9.2–61.0	0–75.0[c]	0–10.5	58.5–90.9	G/P

Abbreviations: ASVCP indicates American Society for Veterinary Clinical Pathology; LRL, lower reference limit; CI, confidence interval; URL, upper reference limit; TP, total protein; G, Gaussian; P, parametric; TS, total solids; Fb, fibrinogen; NG, non-Gaussian; NP, nonparametric; RT, robust, transformed; TP : Fb, total protein to fibrinogen ratio; TS : Fb, total solids to fibrinogen ratio; PT, parametric, transformed; PreALB, prealbumin; ALB, albumin; α_1-GLOB, α_1-globulin; α_2-GLOB, α_2-globulin; β-GLOB, β-globulin; γ-GLOB, γ-globulin; ND, not done.

[a] Standard deviation and median: parametric values, except robust method values reported if used to calculate reference interval.

[b] See ASVCP Reference Interval Guidelines[24] for outlier detection and reference interval construction. Negative lower bound of the CI listed as 0.

[c] n < 20: Values reported in "reference interval" column are "observed range" values.

[d] Statistically significant difference ($P < .05$) between Washington and California populations.

[e] Statistically significant difference ($P < .05$) within California between fall and winter populations.

tion declines, increasing the options available to improve the efficacy of conservation programs.[9,10] Although our sample size was not large enough to provide definitive ranges for all hematologic analytes and values potentially affected by sex, location, season, or species differences, the data support generalization of observed ranges for most hematologic analytes and values across *Aechmophorus* species grebe metapopulations wintering on the Pacific coast. We also found location and season effects for a small number of hematologic analytes and values and that a sex effect was limited to body weight in this nonbreeding population.[27]

In an ideal world, plasma chemistry reference interval studies should be designed to describe the effects of all significant factors such as chemistry analyzer, body condition, species, population, season, time of day, health, recent and longitudinal diet, time since last meal or physical activity, reproductive cycle, sex, molt, captivity, stress, and method of capture, among others.[28–30] However, the level of detail required concerning each individual, coupled with the sample sizes needed to attain adequate power to detect and characterize differences between populations, makes the ideal unachievable for most wild populations.[28–30] Although this level of detail is best, it may not be necessary to make informed clinical or management decisions and a lack of additional measurements from a greater number of individuals should not delay judicious use of these available data. In some clinical situations, such as at wildlife rehabilitation centers, the specific population of origin is often unknown, so characterization of health must be based on the general population of grebes. In the field, even when sampling occurs within a previously studied population, it is virtually impossible to complete the work under the exact same conditions or with controlled modification of only a few well-defined parameters. Thus, having knowledge of the expected range of hematologic analytes and values occurring in the general population is useful to inform interpretation of data from subpopulations.

LI values were too low to affect ALT and AST values. TRIG values could have been affected, but LI and TRIG values were not correlated, and the one extreme outlier did not have the highest LI. Lipemia was not considered to have a significant effect on TRIG values in this study. HI could have affected LDH, AST, or CK values. However, there was no correlation between HI and AST or CK. Although the PCC was statistically significant for LDH, the correlation was weak and the one

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Table 6. Body mass (g) for western grebes collected in Washington and Clark's and western grebes collected in California.

	n	Mean	Median	SD	2.5th percentile	97.5th percentile	Min	Max
Washington								
All	24	1288	1253	176.5	935	1641	1000	1604
Males	10	1434	1449	109.1	1185[a]	1596[b]	1185	1604
Females	14	1183	1199	136.6	909	1456	1000	1484
California								
All	32	1362	1383	229.9	902	1822	865	1895
Winter	16	1463	1480	215.5	1032	1894	1070	1895
Fall	16	1262	1248	203.2	1257	1668	865	1592

Abbreviations: The 2.5th and 97.5th percentiles indicate calculations for normally distributed data (mean ± 2 SD); Min, minimum value; Max, maximum value.
[a] 5th percentile.
[b] 90th percentile.

extreme outlier for HI was not associated with the highest LDH value. Hemolysis was not considered to have a significant effect on AST, CK, or LDH values in this study. However, because LI and HI were not determined for Washington birds, a potential effect could not be completely ruled out in this metapopulation.

Interpretation of PCV and TWBC results from this study must take into consideration a minor difference in methodology between California and Washington birds. In this study, aside from analyses being performed within 8 hours of sample collection, the actual time from blood draw to analysis was not recorded. Thus, differences between metapopulations are likely for mean time between making blood smears to reading slides and from placing blood into anticoagulant until performing diagnostic testing. However, any adverse effects were not likely to be clinically important because storage of blood in heparin for less than 12 hours did not affect hematology values in macaws (*Ara* species) or PCV values in pigeons or ostriches (*Struthio camelus*).[31–33] Moreover, in whooping cranes (*Grus americana*), storage of blood in heparin for 4–6 hours resulted in only modest changes when interpreting the samples that were not expected to affect clinical decisions.[34]

Comparable to findings in owls (Strigiformes), use of Natt and Herrick technique for TWBC in this study may have produced reference intervals that would be statistically different from those generated by the Phloxine B technique.[35] An advantage of the Natt and Herrick technique is all white blood cell (WBC) types are directly counted. The Phloxine B technique only identifies granulocytes and thus is dependent on an accurate WBC differential count. Consequently, the Phloxine B technique is more prone to error in birds that have normally high lymphocyte percentages.[36] Given that the upper limit of the reference interval for %L reported in this study is 62%, how the TWBC count is determined should be a consideration for *Aechmophorus* species of grebes. The main disadvantage of the Natt and Herrick technique is a wider coefficient of variation compared with the Phloxine B technique. When using the Natt and Herrick technique, it can be more difficult to differentiate between small lymphocytes and thrombocytes, especially if there is clumping of cells.[35–37] The laboratory used in this study took the following steps to minimize errors associated with misidentification of cells: use of certified clinical laboratory scientists, addition of 0.5% methylene blue to standard Natt and Herrick solution (facilitated identification of leukocytes and differentiation between lymphocytes and thrombocytes), and cross-checking hemocytometer-determined lymphocyte and thrombocyte counts with smear estimate values.[21]

Table 7. Body mass (g) for Clark's and western grebes collected in California by sex and season captured.

	Males		Females	
	Fall	Winter	Fall	Winter
n	12	12	4	4
Mean	1270	1494	1238	1370
SD	178.6	216.7	297.6	211.8

Use of lithium heparin as an anticoagulant may have produced reference intervals for TS, PCV, and L that could be statistically different from those generated with dipotassium ethylenediaminetetraacetic acid (K_2EDTA). In ostriches, PCV was 4%–7% lower in heparinized samples than samples stored in K_2EDTA.[33] However, in 2 pigeon studies, PCV in heparinized samples remained

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equivalent to or less than 3% greater than those stored in K_2EDTA for at least 6 hours.[31,38] In Hispaniolan Amazon parrots (*Amazona ventralis*), even though heparin caused greater clumping of lymphocytes and thrombocytes that resulted in statistically lower PCV, lower TS determined by refractometer, and greater L values compared with samples placed in K_2EDTA or calcium EDTA as anticoagulants, the differences were not considered clinically significant.[31,39] Given the modest magnitude and apparent species-specific nature of anticoagulant effects on hematologic values, the reference intervals reported in this study are expected to be accurate and precise enough to satisfy the recommendation that published reference intervals for these analytes and values should be used as guidelines in birds.[32,40] As with any reference intervals, to follow trends in one individual or population precisely, it is necessary to use the same technique in a sequential manner.[39]

The study of avian TP:Fb is in its infancy; therefore, its clinical value in birds is unknown. However, the magnitudes of the differences between median values attributed to season for TP:Fb (CA, fall = 10.3, CA, winter = 24.8) and TS:Fb (CA, fall = 16.0, CA, winter = 34.0) are large compared with the values used in raptors to diagnose abnormalities (Plasma protein to fibrinogen ratios [PP:Fb] < 1.5 indicates infection or inflammation; PP:Fb > 5 can indicate dehydration).[41] Thus, PP:Fb is likely worth further investigation in grebes. Because the birds in this study were healthy and hypofibrinogenemia is almost always associated with end-stage disease such as liver failure or severe coagulopathy, it is unlikely that the observed seasonal variation observed in this study was due to unusually low fibrinogen levels.[42] Consequently, because the plasma protein levels obtained from the subject birds were not statistically different between seasons, the primary difference in PP:Fb was due to a larger denominator created by the 65% greater mean Fb level found in fall compared with winter grebes. In birds, increased Fb levels have been reported to be associated with inflammation.[42] One possible interpretation for the higher Fb levels found in the fall blood samples was that grebes were recovering from increased levels of antigenic stimulation associated with migration or environmental conditions experienced at inland lakes during the summer, which resulted in higher levels of fibrinogen relative to the environmental conditions experienced after birds arrived at wintering grounds along the Pacific coast.[42] However, even in domestic animals, caution is warranted when interpreting PP:Fb, because the clinical value may be limited. For example, in horses, although PP:Fb < 15 most frequently indicates inflammation and values > 20 can indicate dehydration, values between 15 and 20 do not provide any additional information regarding the presence of inflammation compared with fibrinogen values alone.[43–45]

The lymphocyte count was only 1.80×10^3 cells/μL higher for California birds captured in the winter compared with the fall samples. However, because of cumulative changes in other WBC types, the result was a 19% increase in mean %L and a reduction of H:L by 5.7. Given that capture stress was correlated with increases in H:L from 1.02 to 1.73 in boat-tailed grackles (*Quiscalus major*) and from 0.5 to 1.15 in Dalmatian pelicans (*Pelecanus crispus*), further investigation into seasonal and other effects on %L and H:L in grebes is warranted.[46,47] The H:L is used by clinicians and biologists to assess stress, inflammation, and disease.[47–49] Increases in this ratio are usually the result of increased H, decreased L, or both because of elevated corticosterone, epinephrine, inflammation, or infection.[48–50] Heterophilia is also associated with fasting in some species.[51] However, H:L is not always a reliable indicator for stress, inflammation, or disease. In the previously mentioned study involving boat-tailed grackles, house sparrows (*Passer domesticus*), and mourning doves (*Zenaida macroura*), the latter 2 species failed to show significant changes in H:L associated with capture stress.[46] Moreover, although not common, H:L can be significantly affected by changes in other WBCs and, thus, should always be interpreted in context with a full CBC. The H:L values for the grebes sampled in this study were within reference intervals published for "birds" in general.[52]

Mean PCV was 6% higher in California birds captured in the winter compared with those sampled in the fall. Although it is tempting to attribute the increase in PCV to a response related to improving body condition (ie, weight gains of approximately 200 g after foraging on wintering grounds), caution is still warranted because many factors influence PCV in adult birds, including hydration status, diet, migration, molt, reproductive cycle, sex, changes in aerobic demand, and ambient temperature.[48,53,54] Additionally, PCV was not reliably correlated with body mass in Wilson's storm petrels (*Oceanites oceanicus*) nor when studied over time in the same individuals in American kestrels (*Falco sparverius*).[50,55] Furthermore, in this grebe study, TP, TRIG, and CHOL,

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which are often used to evaluate body condition, showed no or low levels of correlation with seasonal changes in PCV (all $P \geq .081$ and PCC $\leq .395$, except for TRIG in California grebes captured in the fall, $P = .018$, PCC = .528).[50,51]

Mean PCV for California grebes captured in the winter was 5% greater than for the birds sampled in Washington. Because birds from both metapopulations were captured during the same time of year, one or more of the non–season-related factors discussed previously were the most likely sources for the difference. Mean TWBC for all grebes sampled in California (9.00×10^3 cells/μL) was not only greater than the mean value for grebes tested in Washington (5.43×10^3 cells/μL), but also greater than the maximum value for any grebe captured in Washington (MAX: 8.92×10^3 cells/μL). Greater E in all birds sampled from California and greater L in the California grebes collected only in winter accounted for most of the difference. Increased E can be observed in birds as a response to parasitism or foreign antigen exposure.[48,56] Increased L can indicate chronic antigen stimulation or an acute stress response in birds, and decreased L can be associated with chronic stress, viral infection, or septicemia.[48] Although H was not different between populations, the differences in E and L mentioned above resulted in a difference for %H (WA: mean = 69%, range = 49%–86%; CA: mean = 59%, range = 30%–95%). It is clinically important to note that although mean %H for the grebes sampled in Washington was greater, the California birds had a wider range of values. Although %M was statistically greater in grebes sampled in Washington than all birds captured in California (WA: median = 5%, range = 1%–8%; CA: median = 3%, range = 1%–7%), the overlap of the range of values made the difference clinically insignificant. Furthermore, this conclusion is supported by the lack of difference for M because this cell line has a tendency to increase with acute or chronic infection.[48] In birds in general, clinically relevant M increases are associated with aspergillosis, chlamydiosis, mycobacteriosis, tissue necrosis, and overwhelming bacterial infection.[48] The B counts and %B were greater in the California grebes (range: $0–0.66 \times 10^3$ cells/μL, 0%–6%) when compared with those sampled in Washington (range: $0–0.13 \times 10^3$ cells/μL, 0%–2%). Basophilia in birds is associated with acute inflammation, hypersensitivity reactions, induced molting, and chronic stress.[48,57]

Further discussion of the effects of location on plasma chemistry values between the California grebes compared with the Washington birds is constrained by the use of different chemistry analyzers and the lack of HI and LI information for Washington populations. Additionally, variations in diet, food availability, time since last meal, level of physical activity, and stress were likely between the 2 metapopulations that may have affected blood chemistry values. Differences in the plasma chemistry values found in this study between the California and Washington grebe metapopulations may be due in part to these potentially confounding factors. However, to inform future researchers or clinicians evaluating hematologic values for grebes, it is worth briefly highlighting 3 analytes with differences that were statistically significant and have apparent clinical relevance. Mean, MIN, and MAX TP values for grebes sampled in Washington (47 and 40–58 g/L) were 10 g/L greater than values for the California birds (37 and 29–44 g/L) despite the biuret method used by both analyzers. The difference is also supported by TS results obtained using the same methodology (refractometer). For grebes sampled in Washington (56 and 49–62 g/L), mean and MIN TS were 4 and 9 g/L greater than for the California birds (52 and 40–62 g/L). Because a low TP or TS is commonly used as a clinical decision point for triaging birds during oil spills or other mass casualty responses, it is important for clinicians and rehabilitators to be aware of potential differences between avian metapopulations of the same species.[12]

The mean and MAX for K were also considerably greater in grebes sampled in California than in the Washington birds. Although the upper reference limit for K for grebes sampled in California has been reported in a few species, it is greater than that observed for most.[25,48,49,58] All 3 analytes primarily associated with liver tissue damage in birds (ALT, AST, LDH) were higher in the Washington grebes than in the California birds.[41] The LDH values showed the most pronounced relative elevation from birds sampled in Washington (median = 514 U/L) compared with the California grebes (median = 143 U/L).

Although body weights between males and females sampled in California were not statistically significantly different, historical reference intervals and results from the Washington birds suggested that it should be expected.[26] Within the relatively homogeneous metapopulation of *Aechmophorus* species sampled in Washington (same location and season), our data were consistent with previous observations indicating male *Aechmophorus* species weigh, on average, about 250 g more than females.[26] The mass difference in metapopulations

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Table 8. Expected packed cell volume (%) ranges for western and Clark's, red-necked, and eared grebes.

Population	Range	n
Aechmophorus species: CA and WA combined	38–60	54
Aechmophorus species: CA, fall	38–55	16
Aechmophorus species: CA, winter	47–60	14
Aechmophorus species: WA	43–54	24
Eared grebe: captive on seawater	28–52	7
Eared grebe: wild from Mono Lake, CA	32–56	19
Red-necked grebe: female	45–53	13–15
Red-necked grebe: male	50–55	11–16

Abbreviations: CA indicates captured in California, USA; WA, captured in Washington, USA.

wintering on the Pacific coast was identical to that measured for *Aechmophorus* species nesting on freshwater lakes in California.[59] An interesting finding from the California grebe samples was that season had a statistically significant and much larger effect on body weight than sex. Winter birds weighed about 200 g more than fall birds, on average, but within a season, males weighed, on average, only about 80 g more than females.

Analytes such as calcium, inorganic phosphorus, TP, and CHOL are usually elevated in female birds compared with males. The lack of sex-related differences found during this study was most likely observed because the birds were sampled during the winter (ie, nonbreeding season), so hormonal effects associated with egg laying should not have been present.[27,42]

When comparing published reference intervals developed from a wide variety of sampling protocols (eg, selection of target metapopulation, blood collection, sample handling, choice of analyzers, statistical methods), it is critical to avoid overinterpretation of apparent differences. However, with proper caution, some general trends may become apparent. Although no values reported in this report were outside preexisting reference intervals published for many avian species, it is notable that one of the greatest differences involving hematologic values occurred between *Aechmophorus* species and red-necked grebes (*Podiceps grisegena*) when compared with eared grebes (*Podiceps nigricollis*).[41,49,58,60,61] The MIN PCV of wild *Aechmophorus* species and red-necked grebes was 38%, which was 10% greater than captive eared grebes undergoing experimental exposure to hypersaline water (Table 8).[60,61] Additionally, the MAX K (9 mmol/L) reported for eared grebes is considered to be incompatible with life in birds. Values this high are usually attributed to sample hemolysis. Hemolysis was also considered to be a likely cause of the concurrent low MIN PCV value found in eared grebes (28%) when compared with the *Aechmophorus* species.[62] However, one must consider that the MIN for PCV for eared grebes was within the lower range reported for seabirds, and this population of eared grebes was being held in captivity and undergoing physiologic experiments.[63] Alternatively, it is possible that the MIN PCV and MAX K values for eared grebes were valid, and physiologic differences from life history (eg, diving behavior) could be responsible for PCV difference between these grebe species.

This study provides reference and observed ranges for a comprehensive array of hematologic analytes and values during the nonbreeding period (September–March) for *Aechmophorus* species, a genus that includes western and Clark's grebes of conservation concern.[27] Access to a complete set of plasma chemistry and CBC data is important, because it gives veterinarians and biologists a quantitative means to better assess health at both the individual and population level. The combination of quantitative hematologic assessment with current methods, such as population demographics or physical examination results, has a synergistic effect that can provide a superior level of health assessment. Addition of plasma chemistry and CBC data reported herein, can improve sensitivity to detect effects such as differences in sex, location, season, or species, providing valuable information that can help design, implement, and monitor the most effective treatment or conservation protocols.

Sample collection only occurred during the nonbreeding portion of the annual cycle (November–March), which limited the inference of this study. However, these reference values reflect the time of the year when *Aechmophorus* species are at sea and most susceptible to oil spills and, thus, have a greater likelihood for needing veterinary care.[7,11] Future studies would benefit from collecting data from the same metapopulation throughout an entire year. Currently, this is hindered by the lack of knowledge regarding migration destinations of *Aechmophorus* species metapopulations

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and the difficulties associated with the use of satellite transmitters to track their movements.[15] The results obtained in this study are important because they provide the most comprehensive reference intervals for plasma chemistry and CBC data at this time.

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