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REFERENCE RANGES AND AGE-RELATED AND DIVING EXERCISE EFFECTS ON HEMATOLOGY AND SERUM CHEMISTRY OF FEMALE STELLER SEA LIONS (*EUMETOPIAS JUBATUS*)

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Abstract: Decreased health may have lowered the birth and survival rates of Steller sea lions (*Eumetopias jubatus*) in the Gulf of Alaska and Aleutian Islands over the past 30 yr. Reference ranges for clinical hematology and serum chemistry parameters needed to assess the health of wild sea lion populations are limited. Here, blood parameters were serially measured in 12 captive female Steller sea lions ranging in age from 3 wk to 16 yr to establish baseline values and investigate age-related changes. Whether diving activity affects hematology parameters in animals swimming in the ocean compared with animals in a traditional aquarium setting was also examined. Almost all blood parameters measured exhibited significant changes with age. Many of the age-related changes reflected developmental life history changes, including a change in diet during weaning, an improvement of diving capacity, and the maturity of the immune system. Mean corpuscular hemoglobin and mean corpuscular volume were also higher in the ocean diving group compared with the aquarium group, likely reflecting responses to increased exercise regimes. These data provide ranges of hematology and serum chemistry values needed to evaluate and compare the health and nutritional status of captive and wild Steller sea lions.

Key words: Diving, *Eumetopias jubatus*, hematology, marine mammal, serum chemistry, Steller sea lion.

INTRODUCTION

Hematology and serum chemistry analyses are well-established diagnostic tests that help evaluate several health parameters including inflammation, hydration, nutritional status, and organ function. However, accurate interpretation requires having species-specific reference ranges obtained from populations known to be healthy. These data can be difficult to obtain from wild animals because undocumented health and nutritional issues can alter normal values. Animals maintained at public display aquaria are representatives of wild populations, and they can provide such data because their ages are known, and their health and nutritional statuses are well monitored.

Several studies have used hematology and serum chemistry as indicators of health status in wild pinnipeds.[5,9,13,15,16,20,34] They have yielded reference ranges for several phocid ("true" seal) species,[1,9,22,25,36,37] but fewer ranges for otariids (sea lions and fur seals), which are likely physiologically distinct from phocids.

For Steller sea lions (*Eumetopias jubatus*), some hematology and serum chemistry values have been published for wild and rehabilitated pups[12] as well as for young, free-ranging Steller sea lions.[5,20,24] However, whether these values reflect a normal range is unclear, because the health status of these young wild individuals was unknown. There are also no reference ranges published for older, known-age Steller sea lions. The lack of such values for older animals is critical, because age-related changes may occur in reference values due to developmental life history changes, including a change in diet during weaning, an improved diving capacity, and the maturity of the immune system. Steller sea lions have a long developmental period, and young can wean from 1 to 3 yr,[4,32] gradually incorporating fish into their diet while still drinking milk. Switching from a diet high in fat (mother's milk) to a diet higher in protein (fish) will potentially impact serum chemistry parameters. Diving behavior also changes with age and is likely facilitated by changes in hematology parameters that increase blood oxygen storage capacity.[23]

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Table 1. Birth year (year sampling began), oldest age blood samples were collected, total samples collected, and the year female Steller sea lions began diving at the Open Water Research Laboratory, University of British Columbia, Canada.

Animal ID	Birth year	Oldest age sampled (yr)	Year began diving	Total samples (samples while diving)
F97SI	1997	15.8	2003	54 (21)
F97HA	1997	15.8	2005	54 (15)
F00BO	2000	13.2	2003	49 (27)
F00YA	2000	13.2	2008	58 (11)
F00TS	2000	9.6	—	44
F00ED	2000	8.2	—	39
F03AS	2003	10.4	—	68
F03IZ	2003	10.4	—	66
F03RO	2003	10.4	—	63
F03WI	2003	10.4	—	75
F03MA	2003	5.4	—	21
F03NU	2003	5.8	—	34

Significant declines in Steller sea lion and other pinniped populations in Alaska[6,29,30] accentuate the need for healthy reference ranges for comparative studies of animals in the wild. Steller sea lion populations in Alaska have experienced significant population declines since the 1970s.[30] Several theories have been proposed to explain the decline of Steller sea lion populations, including predation, disease, parasites, and nutritional stress,[3,6,31] many of which may affect hematology and serum chemistry parameters. The use of hematology and serum chemistry as a tool in field and captive studies investigating potential causes for the decline of Steller sea lions requires age-specific reference ranges for accurate interpretation of results.

The objective of the study was to report healthy reference ranges, and changes with age, in hematology and serum chemistry parameters of captive-raised female Steller sea lions living under human care. How diving activity affected hematology parameters was also examined by comparing adult female nondiving aquarium individuals with sea lions that were regularly engaged in open-ocean research diving activities. Results from this study aid in establishing reference values for this species and help to determine the health status of wild populations of Steller sea lions as well as individuals under human care.

MATERIALS AND METHODS

Animal subjects

Multiple blood samples were taken from 12 captive female Steller sea lions between June 1997 and October 2013 (Table 1). Individual animals were serially sampled 1 to 16 times per year from about 1 wk of age up until they were 5–16 yr old (Table 1) for routine clinical monitoring or as part of ongoing research projects. Any samples obtained when the animals did not seem healthy or within 1 mo of inclusion into research that might impact physiologic status were not included in this study, leaving 625 samples in total taken over 16 yr. Data were separated into four age groups, based on natural life history stages, with an additional division at 3 mo representing the early weaning date of the captive animals (all animals were weaned at the same age of 3 mo). Newborn pups ranged in age from 0 to 3 mo (mean = 7.6 wk, $n = 53$ blood samples), weanlings from 3 to 12 mo (mean = 6 mo, $n = 47$), juveniles from 1 to 4 yr (mean = 2.5 yr, $n = 166$), and adults from 4 to 15.8 yr (mean = 7.4 yr, $n = 359$). Not all parameters were measured for each sample obtained, resulting in varying sample size between parameters and age groups (Table 2).

All animals were captured as pups (~2–4 wk old) from the Scott Islands (Triangle Island and Maggot Island) located north of Vancouver Island, British Columbia, and were brought to the Vancouver Aquarium to take part in a long-term research project under animal care permits from the University of British Columbia and the Department of Fisheries and Oceans, Canada. The sea lions were fed an artificial milk formula that approximated maternal milk (Zoologic Milk Matrix 30/55, PetAG, Hampshire, Illinois 60140, USA) for between 2 and 3 mo. Weaned animals were fed a diet of various fish and squid species, although the predominant base of the diet was Pacific herring (*Clupea pallassi*). Animals were normally fed to satiation within handling and training constraints.

Table 2. Age-related changes in hematology and blood chemistry of Steller sea lions. Mean and SD, number of samples (*n*), minimum, median, maximum, and 95% range (0.025–0.975 quantile) are presented for pups (0–3 mo), weanlings (3–12 mo), juveniles (1–4 yr), and adults (>4 yr).

Parameter[a]	Age group	Significance[b]	*n*	Mean	SD	Minimum	Lower 95%	Median	Upper 95%	Maximum
RBC count	Pup	B	52	3.76	0.53	2.94	2.97	3.68	4.9	5.16
($\times 10^{12}$/L)	Weanling	B	47	3.89	0.35	3.29	3.33	3.88	4.48	4.51
	Juvenile	A	163	4.26	0.34	3.36	3.64	4.26	4.78	5.14
	Adult	A	359	4.28	0.33	3.4	3.6	4.3	4.91	5.2
Hemoglobin	Pup	D	52	133	18	101	110	129	177	182
(g/L)	Weanling	C	47	142	15	109	120	138	169	173
	Juvenile	B	163	147	15	109	121	148	177	184
	Adult	A	359	152	14	110	125	151	179	204
Hematocrit	Pup	C	52	0.39	0.05	0.28	0.31	0.37	0.5	0.55
	Weanling	C	47	0.4	0.04	0.31	0.34	0.39	0.48	0.49
	Juvenile	B	163	0.43	0.04	0.32	0.35	0.43	0.51	0.53
	Adult	A	359	0.44	0.04	0.31	0.35	0.44	0.52	0.6
Mean corpuscular	Pup	A	52	103	5	93	94	103	112	117
volume (fl)	Weanling	A	46	105	5	93	95	106	112	113
	Juvenile	B	163	100	6	83	87	101	111	113
	Adult	A	359	103	6	88	92	102	113	120
Mean corpuscular	Pup	B	52	35.6	1.9	31	31.7	35.7	38.8	40.3
hemoglobin (pg)	Weanling	A	46	36.7	2.1	31.2	32.8	37.3	39.3	40.2
	Juvenile	C	162	34.6	2.3	29.2	29.9	34.5	38.8	39.8
	Adult	B	359	35.5	2	29.5	31.8	35.6	39.3	41
Mean corpuscular	Pup	AB	52	346	12	316	318	345	369	374
hemoglobin	Weanling	A	47	351	8	330	333	350	366	368
concentration	Juvenile	B	163	345	9	318	329	345	361	370
(g/L)	Adult	A	359	347	7	320	333	347	362	367
RBC distribution	Pup	A	52	17.1	3.9	7.8	8.2	17.7	22.3	22.7
width (%CV,	Weanling	A	47	15.9	2.7	9.5	9.8	16.7	19	20.6
$P = 0.060$)	Juvenile	A	163	16.7	2.6	7.2	9.1	16.7	21.6	25.5
	Adult	A	359	16.8	1.6	13.7	14.1	16.7	19.8	23
Platelets	Pup	B	47	359	132	24	70	370	541	569
($\times 10^{9}$/L)	Weanling	A	46	445	104	260	291	435	647	697
	Juvenile	B	163	363	79	78	248	346	527	551
	Adult	C	359	294	65	62	168	290	430	532
Mean platelet	Pup	A	39	11.4	2.2	9	9	11	17	17
volume (fl)	Weanling	B	39	9.8	1.5	8	8	9	13.1	15
	Juvenile	B	158	9.5	1.2	7	8	9	12	13
	Adult	B	359	9.5	1.4	7	7.4	9.1	12.9	13.8
WBC count	Pup	A	50	11.6	3.7	2.6	4	11.6	19.2	19.5
($\times 10^{9}$/L)	Weanling	B	47	8	3.1	3.8	4.1	7.2	14.5	15.6
	Juvenile	D	163	5.5	1.6	3.3	3.5	5.2	8.7	13.9
	Adult	C	359	6	1.5	3.1	4.3	5.7	9.7	14.9
Neutrophils	Pup	A	44	8.2	3.6	0.6	1.1	8.2	15.3	17.8
($\times 10^{9}$ L^{-1})	Weanling	B	44	5.5	2.6	2.2	2.4	4.9	11.5	12.6
	Juvenile	C	152	3.6	1.2	1.9	2.1	3.3	6.4	9.5
	Adult	C	357	3.7	1.3	1.6	2.3	3.4	7.1	12.5
Lymphocytes	Pup	A	43	2.09	0.94	0.27	0.34	2.09	3.98	4.16
($\times 10^{9}$/L)	Weanling	B	44	1.56	0.57	0.64	0.75	1.44	2.81	2.97
	Juvenile	B	150	1.51	0.48	0.06	0.68	1.44	2.53	2.95
	Adult	B	357	1.65	0.49	0.26	0.73	1.64	2.71	3.94
Monocytes	Pup	A	44	0.93	0.55	0.12	0.2	0.72	2.29	2.34
($\times 10^{9}$/L)	Weanling	B	44	0.65	0.45	0.13	0.14	0.54	1.75	2.3
	Juvenile	C	152	0.38	0.25	0.05	0.1	0.34	1	1.95
	Adult	C	357	0.32	0.17	0.02	0.06	0.3	0.78	1.4

Table 2. Continued.

Parameter[a]	Age group	Significance[b]	*n*	Mean	SD	Minimum	Lower 95%	Median	Upper 95%	Maximum
Eosinophils	Pup	B	44	0.17	0.23	0	0	0.01	0.74	1.12
(× 10^9/L)	Weanling	B	44	0.07	0.09	0	0	0.03	0.26	0.37
	Juvenile	B	152	0.09	0.15	0	0	0.03	0.55	1.03
	Adult	A	356	0.31	0.43	0	0	0.12	1.51	1.9
Basophils	Pup	A	43	0.071	0.134	0	0	0	0.501	0.512
(× 10^9/L)	Weanling	B	44	0.037	0.044	0	0	0.029	0.14	0.184
	Juvenile	B	152	0.03	0.051	0	0	0.02	0.128	0.42
	Adult	B	356	0.023	0.032	0	0	0.014	0.12	0.254
Glucose	Pup	A	52	7	1.2	3.7	3.9	7.1	9.1	10.4
(mmol/L)	Weanling	B	45	6.6	0.7	4.7	5.1	6.7	7.8	8.4
	Juvenile	A	166	7	0.9	4.7	5.5	6.9	9	10.2
	Adult	B	359	6.7	0.5	5.2	5.8	6.7	7.8	8.2
Urea	Pup	B	52	4.8	2.2	1.3	2.3	4.2	12.1	13.2
(mmol/L)	Weanling	A	45	7.6	1.9	4.5	5	7.1	11.4	13.3
	Juvenile	A	166	7.9	1.1	4	5.9	7.9	10	12.3
	Adult	A	358	7.9	1.1	3.9	5.7	7.8	10.2	11.6
Creatinine	Pup	C	52	66	11	39	43	67	85	97
(μmol/L)	Weanling	C	45	60	11	35	36	62	75	76
	Juvenile	B	166	86	14	50	56	85	117	133
	Adult	A	359	89	14	59	68	86	118	132
BUN : creatinine	Pup	D	52	18.3	7.5	4.1	8.9	17.1	41	47.4
ratio	Weanling	A	45	32.5	12.3	15.7	20	29.9	62.7	75
	Juvenile	B	166	23.4	4.8	11.5	15.4	22.9	33.8	43.5
	Adult	C	358	22.5	4.4	7.4	14.8	22.3	32.1	39
Sodium	Pup	B	51	148	2.81	141	142	148	153	153
(mmol/L)	Weanling	A	44	151	2.68	146	147	150	157	158
	Juvenile	B	165	148	2.15	143	144	148	153	155
	Adult	B	359	148	1.75	143	144	148	151	154
Potassium	Pup	A	52	4.74	0.35	4	4.2	4.7	5.37	5.7
(mmol/L)	Weanling	B	45	4.43	0.42	3.7	3.81	4.4	5.29	5.5
	Juvenile	C	166	3.86	0.32	3.2	3.3	3.8	4.49	5.6
	Adult	C	358	3.84	0.25	3.2	3.4	3.8	4.4	5.2
Na : K ratio	Pup	C	19	31.2	2.1	27	27.5	31	35	35
	Weanling	B	12	35.8	1.8	33	33.3	35	38	38
	Juvenile	AB	56	37.4	3	26	30.5	37	42.6	44
	Adult	A	285	38.4	2.7	24.3	33.2	38.7	43.5	46
Chloride	Pup	C	52	105	3	98	100	105	110	115
(mmol/L)	Weanling	AB	45	109	2.6	103	104	109	113	115
	Juvenile	B	166	109	2.8	96	102	109	113	119
	Adult	A	359	109	2.5	101	105	109	114	117
Bicarbonate	Pup	C	52	21.1	4.4	9	10.1	22	27.7	28
(mmol/L)	Weanling	B	45	23.5	5.3	13	13	24	30	32
	Juvenile	A	166	25.5	3.7	11	16	26.6	30.1	32
	Adult	B	354	24.3	2.8	15	19	24.2	29	33
Anion gap	Pup	A	52	26.1	5.7	17	18	27	36	37
	Weanling	B	45	23	7.5	12	13	23	35	35
	Juvenile	C	165	17.8	5.3	10	12	16	32	35
	Adult	C	359	18.4	3.2	10	12	18.5	25	28.7
Calcium	Pup	A	52	2.56	0.12	2.39	2.41	2.51	2.84	2.91
(mmol/L)	Weanling	A	45	2.51	0.16	2.21	2.22	2.51	2.83	2.88
	Juvenile	B	166	2.43	0.11	2.03	2.24	2.42	2.66	2.77
	Adult	C	359	2.31	0.08	1.99	2.17	2.31	2.48	2.63
Phosphorus	Pup	A	52	2.52	0.41	1.52	1.66	2.53	3.16	3.3
(mmol/L)	Weanling	AB	45	2.43	0.28	1.66	1.98	2.42	2.92	2.93
	Juvenile	B	166	2.32	0.28	1.6	1.81	2.29	2.87	3.19
	Adult	C	359	1.96	0.29	1.32	1.47	1.93	2.55	3.21

Table 2. Continued.

Parameter[a]	Age group	Significance[b]	*n*	Mean	SD	Minimum	Lower 95%	Median	Upper 95%	Maximum
TP (g/L)	Pup	C	52	64.2	5.2	55	55.6	64	75.5	78
	Weanling	B	45	69.3	4.7	57	62.1	69	78.9	80
	Juvenile	A	166	75	4.1	64	67	75	82	86
	Adult	A	358	75	3.8	63	68	75	82.1	86
Albumin (g/L)	Pup	B	52	40.5	3.4	34	34	41	46	48
	Weanling	A	45	43.2	2.2	38	38.2	43	46.9	48
	Juvenile	A	166	42.2	1.9	37	38	42	46	48
	Adult	B	359	39.9	2.7	33	35	40	45.6	47
Globulin (g/L)	Pup	D	52	23.7	3.7	16	19	23	32	34
	Weanling	C	45	26.1	3.8	19	20.1	26	33	33
	Juvenile	B	166	32.8	4	23	25	33	40	45
	Adult	A	359	35.2	4.2	25	27	35	43	46
Albumin : globulin ratio	Pup	A	51	1.72	0.26	1.2	1.23	1.7	2.18	2.3
	Weanling	A	45	1.69	0.25	1.4	1.4	1.6	2.27	2.3
	Juvenile	B	166	1.31	0.18	0.9	1	1.3	1.7	2
	Adult	C	359	1.16	0.2	0.8	0.8	1.1	1.6	1.7
Total bilirubin (μmol/L)	Pup	AB	47	3.64	1.33	1	1	4	6	7
	Weanling	A	43	4.09	1.09	2	2	4	6	7
	Juvenile	B	163	3.4	1.02	1	2	3	5	8
	Adult	B	358	3.39	0.92	1	2	3	5	6
ALP (IU/L)	Pup	B	52	110	37	46	56	107	180	236
	Weanling	A	44	130	38	72	73	121	215	239
	Juvenile	A	166	124	37	62	71	117	211	228
	Adult	C	359	61	22	25	31	58	119	157
ALT (IU/L)	Pup	B	50	41	18	18	19	35	85	100
	Weanling	B	43	43	21	17	20	35	93	114
	Juvenile	A	159	65	22	21	31	63	125	179
	Adult	A	355	65	22	25	34	60	118	187
AST (IU/L)	Pup	A	50	31	20	12	13	26	78	116
	Weanling	B	43	20	9	8	9	20	44	53
	Juvenile	C	164	16	12	2	5	13	38	121
	Adult	C	335	16	6	5	6	15	31	50
GGT (IU/L)	Pup	A	41	113	41	51	52	109	200	206
	Weanling	BC	39	77	21	48	49	76	139	140
	Juvenile	B	166	81	18	46	52	80	119	136
	Adult	C	359	72	17	36	43	71	109	127
Creatinine kinase (IU/L)	Pup	A	46	435	549	76	83	258	2298	2421
	Weanling	AB	40	333	430	67	82	190	1661	2290
	Juvenile	B	148	212	238	42	50	120	879	1579
	Adult	B	352	213	256	37	52	125	1049	1857
Calculated osmolality (mmol/kg)	Pup	C	52	295	7	281	284	295	309	316
	Weanling	A	44	302	6	292	294	301	317	318
	Juvenile	B	165	298	4	286	290	297	307	311
	Adult	B	358	297	4	286	289	297	305	309
Serum Fe	Pup	A	50	35.2	16.2	11	12	32.5	72.8	75
	Weanling	B	44	27.5	11.8	12	13.1	24.5	60.5	64
	Juvenile	C	161	20.8	8.8	10	10	20	41	78
	Adult	C	111	21.3	6.5	8	11	21	33.8	53
IBCT	Pup	A	50	81	15	57	59	79	115	123
	Weanling	B	44	74	13	50	53	72	100	111
	Juvenile	B	161	74	12	48	55	72	100	110
	Adult	C	112	63	11	43	48	60	90	97
Fe % saturation	Pup	A	50	29.4	9.2	9	11	30	45.6	47
	Weanling	AB	44	26.8	8.4	12	14	25.5	46.6	49
	Juvenile	C	161	22.1	7.5	9	11	22	38	48
	Adult	B	112	25.3	6.1	9	12	26	36.6	46

Table 2. Continued.

[a] RBC indicates red blood cell, WBC, white blood cell; BUN, blood urea nitrogen; TP, total protein; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyltransferase; IBCT, iron-binding capacity.
[b] Letters represent significant differences between groups (A–D, representing highest to lowest values, respectively). For all parameters except red blood cell distribution width, there were significant differences between age groups ($P < 0.001$).

Most of the samples were obtained from animals maintained within the aquarium environment. A subset of these animals ($n = 4$ sea lions) moved as adults to a facility where they regularly dove and swam in the open ocean. The sea lions typically dove to 40–50 m, which is characteristic of the majority of dives of wild female Steller sea lions.[14,17]

Blood collection and analysis

Blood samples from young pups were obtained from interdigital veins of the rear flippers or from the caudal gluteal vein while under physical restraint. Samples from older animals were drawn from the caudal gluteal vein while anesthetized using isoflurane in 100% oxygen administered under veterinary supervision. Blood samples were either drawn directly into Vacutainer™ tubes (BD, Franklin Lakes, New Jersey 07417, USA) or into syringes and then transferred to Vacutainer tubes. Samples for blood biochemistry were drawn into a serum separator tube and centrifuged (1,500 *g* for 5 min) to isolate serum for analysis, and samples for hematology were drawn into a tube containing EDTA. All sampling was conducted under permits from the University of British Columbia Animal Care Committee.

Blood samples were sent on ice to a commercial veterinary diagnostic laboratory (Idexx Laboratory, Delta, British Columbia V3M 6M2, Canada) for hematology and serum chemical analysis within 8 hr of sampling. Hematology parameters that were reported included hemoglobin (Hb) concentration, hematocrit (HCT), red blood cell (RBC) count, white blood cell (WBC) count, platelet count, mean platelet volume (MPV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red blood cell distribution width (RDW). Differential leukocyte counts of neutrophils, lymphocytes, monocytes, eosinophils, and basophils were also manually determined. Standard clinical blood chemistry parameters that were assessed included glucose, blood urea nitrogen (BUN), creatinine, sodium, potassium, calcium, phosphorus, chloride, bicarbonate, albumin, globulin, total protein (TP), bilirubin, alkaline phosphatase (ALP), creatinine phosphokinase (CK), alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyltransferase (GGT), serum Fe, total iron-binding capacity (IBCT), and Fe percent saturation. The BUN : creatinine ratio was also calculated.

Statistical analysis

Studies have used varying measures to define the reference range of serum parameters among different species of marine mammals.[2,18] For this study, mean, median, SD, 95% range (0.025–0.975 quantile) as well as minimum and maximum values were determined for each parameter and age group (adult group included both diving and nondiving animals). A value was considered an outlier and removed from the data set if the difference between that value and the next highest or lowest value was greater than one-third of the total range of that variable.[27] Linear mixed effects models (using the package 'nlme' in R[19]) were used to determine whether age group significantly affected each parameter, with animal ID included as a random effect (to account for repeated measures). An analysis of variance (ANOVA) was performed on two models with and without age group as a fixed factor by using the maximum log-likelihood method. If age group was significant ($P < 0.05$), the Bonferroni method was then used to determine individual differences between each of the four age groups, denoted by different letters A–D (highest to lowest values, respectively) in Table 2.

Hematology parameters (RBC count, Hb, HCT, MCV, MCH, MCHC, and RDW) were also tested with diving activity as a fixed factor for the adult group only. An ANOVA was performed on two models with age (in years, if significant) and dive activity as fixed effects by using the log-likelihood method. Reported *P*-values are from the comparison of the final model to a model without dive activity.

RESULTS

Summary statistics and reference ranges for all parameters are presented for each age group in

Table 2. For all parameters except RDW ($P = 0.06$), age group was found to be a significant factor ($P \leq 0.001$). Parameters that increased with age included RBC count, Hb, HCT, ALT, creatinine, BUN, Na : K ratio, chloride, bicarbonate level, globulin, and TP. Parameters that generally decreased with age included most WBC counts, MPV, potassium, phosphorus, calcium, albumin : globulin ratio, AST, CK, IBCT, serum Fe, and Fe percent saturation. Some parameters, MCV and MCH, showed oscillating values between age groups, with the lowest values in measured juveniles before increasing again to adult levels. ALP increased in weanlings and juveniles and was lowest in adults.

The adult sea lion group was analyzed separately to examine the effect of regular diving activity on hematology parameters. MCV, MCH, and MPV were significantly higher in actively diving animals (all $P < 0.001$; Fig. 1); similar trends for Hb ($P = 0.15$) and HCT ($P = 0.24$) were not statistically significant. However, Hb, HCT, MCV, MCH, and MPV all increased with age within the adult group (platelet count increased with age, but it was not higher in diving animals). The apparent changes because of diving exercise could not be absolutely distinguished from age-related changes because most of the diving animals were older than any of the nondiving animals. RDW showed the opposite trend, decreasing with age, and this parameter was significantly lower in diving animals ($P = 0.004$).

DISCUSSION

Analysis of hematology and blood chemistry is an invaluable tool to assess the health of individuals and populations, provided appropriate species-specific reference ranges are available. Previous studies have used hematology and serum chemistry to evaluate the health of pinniped populations in the wild or in rehabilitation facilities. For example, differences were reported in some blood chemistry parameters between the western (declining population) and eastern (increasing population) stocks of Steller sea lion pups[12] as well as between a decreasing and increasing population of harbor seals (*Phoca vitulina*).[34] Significant differences have also been reported between free-ranging and rehabilitated harbor seal pups.[13] These studies demonstrate that blood values can vary between populations. However, interpretation of these differences is problematic because of the participant's presumed health status and, in the case of Steller sea lions, inadequate knowledge of appropriate reference ranges.

Although a few studies have reported some blood parameters for Steller sea lion pups and neonates,[2,12,20,24] none have reported normal values or ranges for juvenile and adult Steller sea lions. As a result, there have been no indications of how these parameters change with age. This study provides a reference range from female animals that were regularly monitored and known to be healthy at the time of sampling. Although captive animals may not be exposed to the same environment as wild animals, they likely experience the same physiologic and developmental changes and can provide a long-term dataset that can express the changes seen in individuals over a lifetime.

Noteworthy is that the pups in this study were fed an artificial milk formula that may contain different nutrients than natural mother's milk. In particular, formula may have higher Fe content, possibly resulting in some RBC-related parameters in pups being higher in this study than what would be seen in the wild. However, RBC count and Hb levels were still lowest in pups than in other age groups. In general, in the wild, the switch from a diet of mother's milk to fish, which is higher in Fe content, may contribute to the increase seen in most RBC parameters with age.

Several physiologic changes are known to occur in marine mammals as they progress through life history stages and develop environmental adaptations, such as increased capacity for diving and thermoregulation, and they could significantly impact these parameters. The longitudinal sampling design of this study addresses developmental changes and shows significant differences in at least one age group compared with the others for nearly all parameters measured. These changes are assumed to relate to a range of natural physiologic changes, such as changes in food sources, immune development, reproductive maturity, growth rate, and diving behavior.

Hematology and diving

Some of the most evident changes in hematology parameters relate to the development of diving ability and blood oxygen storage capacity in Steller sea lions. Development of oxygen storage capacity of the blood depends on Hb concentration and HCT[7] and affects diving behavior (duration and depth) in maturing otariids.[10] RBC count, Hb, and HCT all increased from low levels in pups to significantly higher levels in juveniles and adult females (Fig. 1). Previous research with young Steller sea lions found these

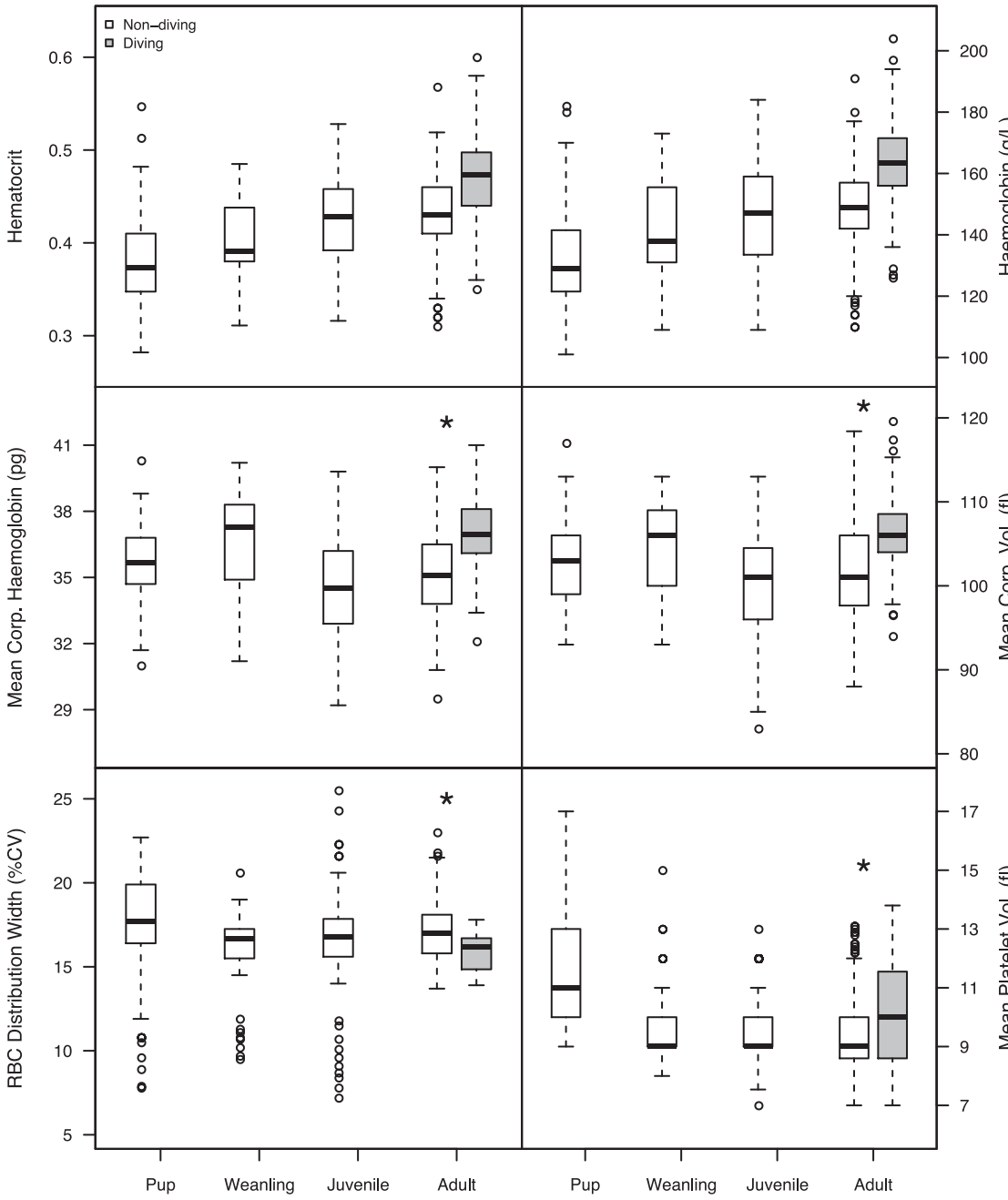

Figure 1. Age-related changes and the effect of diving activity (in adults) on hematocrit, hemoglobin concentration, mean corpuscular hemoglobin concentration, mean corpuscular volume, red blood cell distribution width, and mean platelet volume in female Steller sea lions. Nondiving individuals (white) are compared with individuals diving regularly at the Open Water Research Laboratory, University of British Columbia, Canada (gray). Mean corpuscular volume, mean corpuscular hemoglobin, and mean platelet volume were all significantly higher in the diving group, whereas red blood cell distribution width was significantly lower. Hematocrit and hemoglobin also showed this pattern, but diving status was not significantly different than age-related changes. RBC indicates red blood cell.

parameters reach adult levels at about 9 mo of age.[23] Past studies have been unable to separate cause and effect regarding physiologic changes and diving behavior. Thus, to differentiate the extent to which changes in blood oxygen capacity permit changes in diving behavior and to what degree increased physical activity induces changes in relevant hematologic parameters have been impossible. These factors were able to be partially differentiated by examining developmental changes within nondiving animals and by comparing differences between older individuals either engaging in natural diving behaviors or limited to relatively shallow pools.

Previous studies reported that most diving-related blood parameters reach peak levels in juveniles. which was the oldest age group other studies examined. RBC counts in this study were at peak levels during the juvenile stage; however, Hb and HCT did not reach statistical maximum levels until the sea lions were adults. The values in this study for RBC, HCT, Hb, and MCHC were all similar to those seen in other studies for the pup, weanling, and juvenile age groups.[12,15,20,24] Because only females were examined, the results for some diving-related parameters may differ in males, and there also may be seasonal variation in some parameters.

Most of the animals in this study were raised at an aquarium in relatively shallow pools and were not exposed to deep-water environments at a young age; thus, they did not experience the depths and durations of dives that would typically be performed by wild sea lions during the weaning period. However, some of the sea lions that participated in this study were regularly diving in the ocean as adults, which may have affected the development of some diving-related parameters later in life. The adult females actively diving in the open ocean for 5–10 yr in this study had significantly higher MCH and MCV values compared with those living in an aquarium (Fig. 1). They also had higher HCT and Hb levels among the diving sea lions, although these parameters also increased, in general, with age. The diving animals were also older animals, so the trends observed in HCT and Hb might simply reflect a general age-related increase rather than an effect of diving activity. Although no comparable data exist for wild adult female Steller sea lions, it is notable that years of diving, even only as adults, resulted in increased oxygen stores in the ocean diving animals, despite a lack of diving as pups and juveniles. Steller sea lions in the wild would begin diving at a younger age; hence, the values for some diving-related parameters in juveniles, and possibly weanlings, may be under estimated as well.

WBC counts

Total WBC counts showed a complex trend overall, with the highest values recorded in pups. The values decreased through the weanling and juvenile stages and then slightly increased again in adult females (although still lower than for the pup stage). Examination of the differential distribution of WBC types showed neutrophil and monocyte counts with a steady decline with age, whereas lymphocytes and basophils showed a more dramatic decline between the pup and weanling stages. The exception to this declining trend was the increase in eosinophils found among adult samples, likely accounting for the overall higher WBC count in adults.

One advantage of using captive animals in controlled environments for formulating reference ranges is that their health status is more likely to be known. For example, WBCs (leukocytes), which are a major component of the immune response, are assumed to be lower in captive animals because they are healthier. Previously reported values for pups in the wild are generally higher than those seen in this study,[11,12,15] suggesting that disease or infection increased the values seen in wild pups. However, these lower WBC counts may also be the result of less exposure to potential pathogens in animals in an aquarium setting compared with those in the wild. Mellish et al[15] found that Steller sea lion pups had significantly lower WBC counts after several months in captivity (dropping lower even than the values reported in this study). However, the opposite trend was seen in both harbor seal pups[13] and elephant seals[8] that had significantly higher WBC counts after entering a rehabilitation facility, despite being believed to be clinically healthy.

Blood chemistry

Pups have physiologic adaptations to store energy during nursing and weaning, when they undergo natural periods of fasting while females are on foraging trips. BUN has been used as an indicator for overall health or nutritional stress[20] because it is mostly unaffected by handling and capture stress or drugs. Fasting can result in decreased levels of BUN, although increased levels are exhibited during prolonged fasts.[21] BUN levels may also increase in underfed ani-

mals, possibly because of a combination of food protein intake and elevated skeletal muscle catabolism.[22] However, BUN levels are also generally higher in nonfasting marine mammals, probably because of a diet high in protein. In this study, BUN levels were significantly lower in pups than in all other age groups, and they were similar to those seen in Steller sea lion pups from Alaska.[20] These lower BUN levels likely reflect the change in their diet during weaning—in particular, in the relative amounts of protein and fat as they begin feeding on their own—as has been demonstrated with protein intake in adults.[22,33] Significantly lower levels of BUN were found in 6-wk-old fasting pups compared with 9-mo-old fed pups (whose values were comparable to weanlings and juveniles in this study), likely corresponding to decreases in protein intake and protein catabolism.[21] Because BUN is a product of both protein intake and protein tissue catabolism, studies have suggested that absolute levels may not be a useful indicator of nutritional stress.[26]

Both BUN and creatinine are indicative of kidney function and can be significantly affected by renal failure.[2] Creatinine levels also increased with age in the animals in this study, and they were within the range reported for harbor seals.[34,35] Creatinine is a function of skeletal muscle metabolism and is less sensitive to changes in diet; hence, the BUN : creatinine ratio may be more useful than BUN alone in examining nutritional stress. The BUN : creatinine ratio increased significantly from the pup-to-weanling stage, likely reflecting the change in diet to whole fish at 3–4 mo of age in the captive animals. This increase in protein intake would have caused BUN levels to increase much faster than those of creatinine. The BUN : creatinine ratio then declined to juvenile and adult levels as creatinine continued to increase, whereas BUN levels remained constant.

The TP value, which includes both globulins and albumin, increased with age in female Steller sea lions. Juan Fernandez fur seals (*Arctocephalus philippi*) showed the same trend, with juveniles and adults having a higher TP value than pups.[28] TP is influenced by hydration state and also correlates with diet. Albumin was slightly higher in weanlings and juveniles than in adults or pups in this study, whereas globulins increased steadily with age. This increase in globulins is consistent with the general trend for immature marine mammals to have lower globulin concentrations,[2] which is likely related to the lower levels of immunoglobulin reflecting a developing immune system. Greater values of globulins were also reported in rehabilitated harbor seal pups compared with wild pups, which was postulated to reflect increased immune stimulation during recovery from disease.[13] Mellish et al[15] found similar TP values for both Steller sea lion pups and juveniles, although albumin was slightly higher and globulins were lower than seen in this study.

ALT, AST, ALP, and GGT are enzymes generally used to assess liver and other organ function. Elevated levels of these enzymes may be associated with hepatocellular injury or induction.[2] A period of nutritional stress also resulted in a decrease in ALP and an increase in GGT levels in Steller sea lions.[26] Age significantly affected all of these liver enzymes in the present study. AST decreased with age and can be affected by handling and restraint as well as muscle damage and hemolysis. Whether the observed decrease in AST was a developmental trend or an effect of increased ease in obtaining blood samples is unknown (eg, better training and a switch from physical restraint to anesthesia). ALP increased in weanlings before decreasing to adult levels, a trend that is likely associated with growth. ALP tends to be higher in younger, growing animals because its involvement in bone growth; therefore, it decreases with age.[2,34] ALT levels were higher in juveniles and adults than in pups and weanlings, consistent with the findings of Mellish et al,[15] who also found ALT were higher in juveniles than in pups, although slightly lower than the values in this study overall. They hypothesized that higher ALT levels may be because of an increased incidence of parasitism in juveniles that are now feeding on fish and the observation that most juveniles sampled had some form of parasite load.[15] However, this factor is unlikely to have contributed to the increased ALT in the sea lions in this study, because they were consuming previously frozen fish that was largely free of active parasites.

Electrolyte levels in serum were all affected by age. Potassium, calcium, and phosphorus all decreased, whereas sodium and chloride slightly increased. However, these differences were minor. Reference ranges for electrolytes are usually very narrow because of physiologic dependence on electrolyte levels to maintain cellular function and osmotic gradients.[22] Levels higher than reference ranges may indicate dehydration.[34] Higher values of phosphorus and calcium in wild pups could also correspond to skeletal growth and protein levels, respectively.[2]

CONCLUSIONS

Hematology and serum chemistry reference ranges are necessary to monitor health and to interpret values taken from wild or captive Steller sea lion individuals. Thus, the reference ranges obtained for hematology and serum chemistry provide a much-needed baseline for assessing the health of different ages of female Steller sea lions. The longitudinal nature of this study also reveals how developmental changes related to key life history events, such as weaning and physical maturity, affect hematology and serum chemistry. In addition, this study differentiated which changes in hematology associated with increased diving ability were innate and which were likely related to increased diving activity. Only females were examined in this study; hence, the values for some parameters may differ for males.

Because of significant declines in Steller sea lion populations, it is especially important to be able to monitor the health of individuals of all ages. However, most studies to date assessing the health of Steller sea lions have only reported values for pups, which are significantly different than those of adults. Thus, the reference ranges obtained for hematology and serum chemistry can be used to assess the health of Steller sea lion populations—from pups to adult females. These values can, in turn, be used to examine the factors affecting the decline of Steller sea lions as well as be used to predict how hematology and blood chemistry should respond to shifts in prey populations or changing ecologic conditions.

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LITERATURE CITED

1. Boily F, Beaudoin S, Measures LN. Hematology and serum chemistry of harp (*Phoca groenlandica*) and hooded seals (*Cystophora cristata*) during the breeding season, in the Gulf of St. Lawrence, Canada. J Wildl Dis. 2006;42(1):115–132.

2. Bossart GD, Reidarson TH, Dierauf LA, Duffield DA. Clinical pathology. In: Dierauf LA, Gulland FMD (eds.). Handbook of marine mammal medicine. 2nd ed. Boca Raton (FL): CRC Press; 2001. p. 383–436.

3. Burek KA, Gulland FMD, Sheffield G, Beckmen KB, Keyes E, Spraker TR, Smith AW, Skilling DE, Evermann JF, Stott JL, Saliki JT, Trites AW. Infectious disease and the decline of Steller sea lions (*Eumetopias jubatus*) in Alaska, USA: insights from serological data. J Wildl Dis. 2005;41(3):512–524.

4. Calkins DG, Pitcher KW, Schneider KB, Murray N. 1982. Population assessment, ecology and trophic relationships of Steller sea lions in the Gulf of Alaska. Anchorage (AK): Outer Continental Shelf Environmental Assessment Program, US Department of the Interior, Bureau of Land Management.

5. Castellini M, Davis RW, Loughlin TR, Williams TM. Blood chemistries and body condition of Steller sea lion pups at Marmot Island, Alaska. Mar Mamm Sci. 1993;9(2):202–208.

6. DeMaster DP, Trites AW, Clapham P, Mizroch S, Wade P, Small RJ, Ver Hoef J. The sequential megafaunal collapse hypothesis: testing with existing data. Prog Oceanogr. 2006;68(2–4):329–342.

7. Fowler SL, Costa DP, Arnould JPY, Gales NJ, Burns JM. Ontogeny of oxygen stores and physiological diving capacity in Australian sea lions. Funct Ecol. 2007;21(5):922–935.

8. Goldstein T, Johnson SP, Werner LJ, Nolan S, Hilliard BA. Causes of erroneous white blood cell counts and differentials in clinically healthy young northern elephant seals (*Mirounga angustirostris*). J Zoo Wildl Med. 1998;29(4):408–412.

9. Greig DJ, Gulland FM, Rios CA, Hall AJ. Hematology and serum chemistry in stranded and wild-caught harbor seals in central California: reference intervals, predictors of survival, and parameters affecting blood variables. J Wildl Dis. 2010;46(4):1172–1184.

10. Horning M, Trillmich F. Development of hemoglobin, hematocrit, and erythrocyte values in Galapagos fur seals. Mar. Mamm. Sci. 1997;13(1):100–113.

11. Keogh MJ, Maniscalco JM, Atkinson S. Steller sea lion (*Eumetopias jubatus*) pups undergo a decrease in circulating white blood cells and the ability of T cells to proliferate during early postnatal development. Vet Immunol Immunopathol. 2010;137(3):298–304.

12. Lander ME, Fadely BS, Gelatt TS, Rea LD, Loughlin TR. Serum chemistry reference ranges for Steller sea lion (*Eumetopias jubatus*) pups from Alaska: stock differentiation and comparisons within a North Pacific sentinel species. EcoHealth 2013;10(4):376–393.

13. Lander ME, Harvey JT, Gulland FMD. Hematology and serum chemistry comparisons between free-ranging and rehabilitated harbor seal (*Phoca vitulina richardsi*) pups. J Wildl Dis. 2003;39(3):600–609.

14. Loughlin TR, Perlov AS, Baker JD, Blokhin SA, Makhnyr AG. Diving behavior of adult female Steller sea lions in the Kuril Islands, Russia. Biosph Conserv. 1998;1(1):21–31.

15. Mellish JE, Calkins DG, Christen DR, Horning M, Rea LD, Atkinson S. Temporary captivity as a research tool: comprehensive study of wild pinnipeds under controlled conditions. Aquat Mamm. 2006; 32(1):58–65.

16. Mellish JE, Hindle AG, Horning M. Health and condition in the adult Weddell seal of McMurdo Sound, Antarctica. Zoology 2011;114(3):177–183.

17. Merrick RL, Loughlin TR. Foraging behavior of adult female and young-of-year Steller sea lions in Alaskan waters. Can J Zool. 1997;75(5):776–786.

18. Norman SA, Beckett LA, Miller WA, Leger JS, Hobbs RC. Variation in hematologic and serum biochemical values of belugas (*Delphinapterus leucas*) under managed care. J Zoo Wildl Med. 2013;44(2): 376–388.

19. Pinheiro J, Bates D, DebRoy S, Sarkar D, The R Core Development Team. nlme: linear and nonlinear mixed effects models. R package version 3.1–111. Veinna (Austria): R Core Team; 2013.

20. Rea LD, Castellini MA, Fadely BS, Loughlin TR. Health status of young Alaska Steller sea lion pups (*Eumetopias jubatus*) as indicated by blood chemistry and hematology. Comp Biochem Physiol A Mol Integr Physiol. 1998;120(4):617–623.

21. Rea LD, Rosen DAS, Trites AW. Metabolic response to fasting in 6-week-old Steller sea lion pups (*Eumetopias jubatus*). Can J Zool. 2000;78(5):890–894.

22. Reif JS, Bachand A, Aguirre AA, Borjesson DL, Kashinsky L, Braun R, Antonelis G. Morphometry, hematology and serum chemistry in the Hawaiian monk seal (*Monachus schauinslandi*). Mar Mamm Sci. 2004;20(4):851–860.

23. Richmond J, Burns J, Rea L. Ontogeny of total body oxygen stores and aerobic dive potential in Steller sea lions (*Eumetopias jubatus*). J Comp Physiol B Biochem Syst Environ Physiol. 2006;176(6):535–545.

24. Richmond JP, Burns JM, Rea LD, Mashburn KL. Postnatal ontogeny of erythropoietin and hematology in free-ranging Steller sea lions (*Eumetopias jubatus*). Gen Comp Endocrinol. 2005;141(3):240–247.

25. Roletto J. Hematology and serum chemistry values for clinically healthy and sick pinnipeds. J Zoo Wildl Med. 1993;24(2):145–157.

26. Rosen DAS, Hastie GD, Trites AW. Searching for stress: hematologic indicators of nutritional inadequacies in Steller sea lions. In: Proc Comp Nutr Soc; 2004. p. 145–149.

27. Schwacke LH, Hall AJ, Townsend FI, Wells RS, Hansen LJ, Hohn AA, Bossart GD, Fair PA, Rowles TK. Hematologic and serum biochemical reference intervals for free-ranging common bottlenose dolphins (*Tursiops truncatus*) and variation in the distributions of clinicopathologic values related to geographic sampling site. Am J Vet Res. 2009;70(8):973–985.

28. Sepulveda MS, Ochoa-Acuna H, Homer BL. Age-related changes in hematocrit, hemoglobin and plasma protein in Juan Fernandez fur seals (*Arctocephalus philippii*). Mar Mamm Sci. 1999;15(2):575–581.

29. Trites AW. Northern fur seals: why have they declined? Aquat Mamm. 1992;18(1):3–18.

30. Trites AW, Larkin PA. Changes in the abundance of Steller sea lions (*Eumetopias jubatus*) in Alaska from 1956 to 1992: how many were there? Aquat Mamm. 1996;22(3):153–166.

31. Trites AW, Miller AJ, Maschner HDG, Alexander MA, Bograd SJ, Calder JA, Capotondi A, Coyle KO, Lorenzo ED, Finney BP, Gregr EJ, Grosch CE, Hare SR, Hunt GL, Jahncke J, Kachel NB, Kim H-J, Ladd C, Mantua NJ, Marzban C, Maslowski W, Mendelssohn ROY, Neilson DJ, Okkonen SR, Overland JE, Reedy-Maschner KL, Royer TC, Schwing FB, Wang JXL, Winship AJ. Bottom-up forcing and the decline of Steller sea lions (*Eumetopias jubatus*) in Alaska: assessing the ocean climate hypothesis. Fish. Oceanogr. 2007;16(1):46–67.

32. Trites AW, Porter BT. Attendance patterns of Steller sea lions (*Eumetopias jubatus*) and their young during winter. J Zool. 2002;256(4):547–556.

33. Trumble SJ, Castellini MA. Blood chemistry and morphometric comparisons between harbor seal pups from Tugidak Island and within Prince William Sound, Alaska: using cluster analysis to assess health status. In: Harbor Seal Investigations. Anchorage (AK): Alaska Department of Fish and Game Annual Report, NOAA NA87FX0300; 2001. p. 324–344.

34. Trumble SJ, Castellini MA. Blood chemistry, hematology, and morphology of wild harbor seal pups in Alaska. J Wildl Manage. 2002;66(4):1197–1207.

35. Trumble SJ, Castellini MA, Mau TL, Castellini JM. Dietary and seasonal influences on blood chemistry and hematology in captive harbor seals. Mar Mamm Sci. 2006;22(1):104–123.

36. Tryland M, Krafft BA, Lydersen C, Kovacs KM, Thoresen SI. Serum chemistry values for free-ranging ringed seals (*Pusa hispida*) in Svalbard. Vet Clin Pathol. 2006;35(4):405–412.

37. Yochem PK, Stewart BS, Mazet JA, Boyce WM. Hematologic and serum biochemical profile of the northern elephant seal (*Mirounga angustirostris*): variation with age, sex, and season. J Wildl Dis. 2008;44(4): 911–921.

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