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Effects of Physiological Factors and Seasonal Variations on Hematology and Plasma Biochemistry of Beluga Whales (*Delphinapterus leucas*) Managed in Pingtung, Taiwan

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Abstract

This is the first investigation of physiological baseline values using hematology and plasma biochemical data from managed beluga whales (*Delphinapterus leucas*) in Asia. Samples from eight clinically healthy individuals were analyzed and used as reference values for belugas managed in Pingtung, Taiwan. From 2002 to 2013, the effects of season as well as each beluga's age and gender were evaluated with respect to the hematological and plasma chemical characteristics through a series of linear mixed-effects models. In these fitted models, age was the most influential factor affecting blood analytes of the belugas housed in Taiwan. Many hematology parameters, including packed cell volume, hemoglobin, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration, lymphocytes, eosinophils, platelets, and erythrocyte sedimentation rate (ESR), were significantly higher in juveniles. On the other hand, higher segmented neutrophil and monocyte values were reported in adults than in juveniles. For plasma biochemistry, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, gamma-glutamyltransferase, creatine kinase, lactate dehydrogenase (LDH), glucose, blood urea nitrogen, potassium ion, lactate, and fibrinogen values were higher in juveniles than in adults; whereas high levels of albumin, cholesterol, triglycerides, creatinine, inorganic phosphorus, sodium ion, chloride ion, and total carbon dioxide (TCO_2) were reported in adults. Seasonal variations were observed in mean corpuscular volume, MCH, AST, ALT, cholesterol, some electrolytes, TCO_2, and lactate. Among gender-related differences, males had higher values in some red blood cell parameters, liver function indicators, LDH, and plasma iron, while females had higher levels of ESR, triglycerides, and proteins.

Key Words: cetacean, hematology, plasma biochemistry, beluga whale, *Delphinapterus leucas*

Introduction

A beluga whale (*Delphinapterus leucas*) is an Arctic and sub-Arctic cetacean which belongs to the family Monodontidae (Perrin et al., 2009). Beluga whales have a life span of 40 to 80 y, and their sexual maturity occurs around 5 to 10 y of age (Perrin et al., 2009). Similar to other animals, hematology and plasma biochemistry values of beluga whales provide important clinical references for health evaluation, disease diagnosis, and prognosis (Duffield et al., 1995; St. Aubin et al., 2001). However, there are only a few published papers related to the hematology and plasma biochemistry of belugas (Dhindsa et al., 1974; MacNeill, 1975; Engelhardt, 1979; Medway & Geraci, 1986; Cornell et al., 1988; St. Aubin et al., 2001; Tryland et al., 2006; Norman et al., 2012, 2013). Most of the studies prior to 2001 described the differences of blood analytes (mainly hematological data) between managed belugas and other cetacean populations in North America (MacNeill, 1975; Engelhardt, 1979; Cornell et al., 1988). Later studies reported on hematologic and serum chemistry values in wild belugas captured in the Canadian Arctic, Svalbard, and Alaska (St. Aubin et al., 2001; Tryland et al., 2006; Norman et al., 2012).

Besides the establishment of blood reference intervals, some potential environmental (e.g., season and habitat) and biological (e.g., age and gender) factors were investigated to understand the annual cycle of belugas and these factors' influences on blood constituents (St. Aubin et al., 2001; Tryland et al., 2006; Norman et al., 2012). Norman et al. (2013) recently published the results of a 22-y program on 31 managed belugas at three different locations in the U.S. The geographic location of the belugas had the dominant effect on blood values compared to the other covariates (Norman et al., 2013). These findings suggested that wild or managed belugas at different facilities and locations showed variations in blood data, which reflected the physiological plasticity in animals. Hence, establishment of the baseline profile of blood parameters for belugas at different locations can be beneficial to meaningful animal management.

The present study established the hematologic and plasma biochemical baseline profile for beluga whales managed in Taiwan. Samples were collected from eight clinically healthy belugas over a 12-y period, and the effects of seasonal variation as well as the whale's age and gender on the blood parameters were evaluated. This study builds upon the work of Norman et al. (2013) by adding baseline data from another site in Asia; the influence of these factors on blood indexes will allow a greater scope of analysis for interannual variability and geographical differences.

Methods

Eight beluga whales were recruited for this study, including three males ("Babu," "Ginbo," and "Baby") and five females ("Angel," "Blue," "Green," "Red," and "Yellow"). These belugas were acquired offshore of Russia at 2 to 5 y of age between the years 2002 and 2006. They were then managed at the National Museum of Marine Biology and Aquarium (22° 2' 47" N, 120° 41' 52" E) in Pingtung, the southernmost county of Taiwan (Table 1). The belugas were housed in indoor pools with 17 to 18° C filtered and ozone sterilized natural sea water. The lighting of the facility was controlled to provide 10 h of daylight daily (0800 to 1800 h). The belugas were fed various kinds of fish, mainly Pacific saury (*Cololabis saira*), Atlantic horse mackerel (*Trachurus japonicas*), bonito (*Katsuwonus pelamis*), and flathead asp (*Pseudaspius leptocephalus*), combined with vitamin and mineral supplementation.

During monthly routine medical procedures, technicians drew blood from the superficial fluke vessels using 19-gauge butterfly needles. The blood samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes and then sent to the laboratory while maintained at 4° C. All samples were sent to the Animal Hospital of National Pingtung University of Science and Technology, Pingtung, and analyzed within 24 h of being collected. Complete blood count (CBC) and plasma biochemical indexes were measured and recorded monthly when routine physical examinations were performed. The characteristics of CBC, such as packed cell volume (PCV), hemoglobin (Hb), total red blood cell (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total white blood cell (WBC), and platelet, were examined by using a Sysmex F-820 analyzer (Kobe, Hyōgo Prefecture, Japan). Erythrocyte sedimentation rate (ESR) was detected via the Wintrobe method, and manual counting was used to perform white blood cell classification. Fujifilm Dri-Chem 3500s (Tokyo, Japan) was used to measure the following plasma biochemical values: total plasma protein (TPP), albumin, globulin, A/G ratio, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP),

Table 1. Demographic characteristics of the eight belugas (*Delphinapterus leucas*) at the National Museum of Marine Biology and Aquarium in Pingtung, Taiwan, from 2002 to 2013

Beluga	Gender	Sampled year	Age	No. of samples
Angel	Female	2002-2013	3-14	107
Babu	Male	2002-2013	3-14	102
Baby*	Male	2002-2009	3-10	64
Ginbo	Male	2002-2013	3-14	108
Blue	Female	2006-2013	4-11	70
Green*	Female	2006-2009	2-5	31
Red*	Female	2006-2008	2-4	25
Yellow*	Female	2006-2008	2-4	27

*Beluga died during the study period.

gamma-glutamyltransferase (GGT), creatine kinase (CK), lactate dehydrogenase (LDH), cholesterol, triglyceride, glucose, creatinine, blood urea nitrogen (BUN), inorganic phosphorus (iP), and plasma fibrinogen. Electrolytes of calcium (Ca^{2+}), sodium (Na^+), potassium (K^+), and chloride (Cl^-) were examined by using EasyLyte PLUS (Medica Corporation, Bedford, MA, USA), while total carbon dioxide (TCO_2), lactate, and plasma iron were tested by Kodak Ektachem DT60 Analytical System complete with DTE Module and DTSC II-Module.

Only blood values collected from apparently clinically healthy belugas were recruited into the study database. During this study period, any blood data that indicated diseases, bacterial infections, inflammation, and data within 30 d before any animal's death were excluded from the baseline data and further analyses. The scrubbed dataset was then analyzed and used to build reference values for the belugas managed in Taiwan. The reference intervals of each index was calculated and set as mean ± 3 SD (Norman et al., 2013). Statistical analyses were also performed by utilizing *SAS Enterprise Guide 6.1* (SAS Institute Inc., Cary, NC, USA). Age was classified into juvenile (less than 5 y old), subadult (5 to 10 y old), and adult (over 10 y old). Seasons were defined as the following: spring (March to May), summer (June to August), autumn (September to November), and winter (December to February). Data of each CBC and plasma biochemical index were first examined for its normality by using Q-Q plots. Seasonal and age-related differences between means were then determined with one-way ANOVAs followed by Tukey tests. A Student's *t*-test was performed to compare the means of each index between genders. A p value less than 0.05 was considered statistically significant. Further, a series of linear mixed-effects models were used to evaluate the relationships between the values and three factors: (1) season, (2) age, and (3) gender. We also compared variations attributable to inter- and intraindividual differences in these models.

Results

A total of 603 hematology and 594 plasma biochemistry screened values from a 12-y period were included in this analysis. The hematology and plasma biochemistry reference intervals (mean and SD) are summarized in Tables 2 and 3. The values of each parameter were either normally distributed or normally distributed after ln transformation. On the basis of the results of ANOVA and Student's *t*-test, several hematology and plasma biochemistry values were found significantly different with age (Table 4). Several hematology parameters, specifically PCV, Hb, MCH, MCHC, lymphocytes, eosinophils, platelets, and ESR, were significantly higher in juveniles. Higher segmented neutrophil and monocyte values were reported in adults than in juveniles. In plasma biochemistry, AST, ALT, ALP, GGT, CK, LDH, glucose, BUN, K^+, lactate, and fibrinogen values were higher in juveniles than in adults, whereas high levels of albumin, cholesterol, triglycerides, creatinine, iP, Na^+, Cl^-, and TCO_2 were found in adults. Significant seasonal variations were observed in MCV, MCH, AST, ALT, cholesterol, iP, Ca^{2+}, K^+, Cl^-, TCO_2, and lactate (Table 4). The concentrations of plasma calcium and chloride were higher in summer than in winter, while AST, ALT, cholesterol, and TCO_2 values were higher in winter. High levels of MCV, MCH, iP, and plasma potassium were observed in autumn. In gender-related differences, some RBC parameters (e.g., PCV, total RBC, Hb, and MCV), liver function indicators (AST, ALT, and ALP), LDH, and plasma iron were higher in males than in females. On the other hand, females had high levels of ESR, triglycerides, and proteins (Table 4).

Linear mixed-effects models were built to predict the value of each blood parameter with different status of managed belugas in Taiwan (Tables 5 & 6). In these models, "Juvenile," "Male," and "Winter," respectively, were set as the references for the three variates—age, gender, and season. Akaike's Information Criterion (AIC) was used to compare inter- and intraindividual variation, and the results showed that intraindividual variation was larger than interindividual variation in all parameters. The individual beluga whale, therefore, was considered to be a random effect in the linear mixed-effects models. AIC was then used for model comparison, and the parsimonious model for each blood parameter was chosen as the model with the best fit. In these models, age was the most influential factor affecting blood analytes of the belugas housed in Taiwan. Cholesterol, triglycerides, iP, Na^+, and plasma iron increased with age, while some WBC parameters, platelets, ESR, ALT, ALP, GGT, LDH, and creatinine decreased with age. Significant seasonal effects were presented in total RBCs, Hb, MCV, MCH, total WBCs, segmented neutrophils, ESR 1 h, AST, ALT, ALP, CK, LDH, cholesterol, triglycerides, BUN, electrolytes, TCO_2, lactate, and plasma iron. Compared to females, male belugas had high levels of PCV, ALT, and K^+, and low TPP values (Tables 5 & 6).

Table 2. Hematology reference values for whole group and individual belugas at the National Museum of Marine Biology and Aquarium in Pingtung, Taiwan

		Whole group		Angel		Babu		Blue		Ginbo		Baby		Green		Red		Yellow	
Hematology	Units	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
PCV	%	52.76	2.71	51.05	1.96	53.28	1.77	51.61	4.29	53.74	2.09	54.01	2.51	52.73	1.70	52.84	2.03	53.91	2.72
RBCs	10^6/µL	3.36	0.17	3.21	0.15	3.36	0.14	3.47	0.14	3.43	0.16	3.38	0.14	3.38	0.10	3.25	0.13	3.46	0.16
Hb	g/dL	21.09	1.05	20.44	0.79	21.02	0.86	20.75	1.15	21.34	0.82	21.67	1.28	21.16	0.82	21.50	0.99	21.98	1.01
MCV	fL	162.8	4.8	165.8	3.5	163.9	3.9	157.1	3.8	162.5	4.6	164.9	3.7	159.6	2.6	164.2	5.0	160.0	3.9
MCH	pg	62.8	3.2	63.8	2.5	62.7	2.3	59.8	3.1	62.4	3.0	64.1	3.6	62.5	2.5	66.4	3.0	63.6	1.9
MCHC	g/dL	38.6	1.7	38.5	1.6	38.2	1.4	38.2	2.0	38.4	1.6	38.8	1.9	39.1	1.5	40.5	1.5	39.8	1.4
WBCs	µL	9,772	2,022	8,892	1,262	8,740	1,816	11,995	2,013	11,076	1,469	9,511	1,715	7,613	957	9,572	1,471	10,445	1,340
Segmented neutrophils	µL	6,003	1,531	5,664	1,021	5,170	1,193	7,845	1,584	6,729	1,302	6,084	1,199	4,218	991	5,541	1,152	5,895	1,026
Lymphocytes	µL	2,714	864	2,335	609	2,595	1,012	2,732	918	3,152	853	2,526	631	2,495	551	2,933	669	3,409	897
Monocytes	µL	504	226	517	193	471	216	650	296	547	233	468	206	381	135	393	137	424	149
Eosinophils	µL	554	326	389	254	507	297	759	389	628	305	438	216	579	268	714	437	709	330
Platelets	10^3/µL	128	65	137	66	111	44	136	55	123	73	140	82	125	37	143	78	126	69
ESR 30 s[a]	mm/30 s	3.30	3.85	4.53	3.05	3.28	2.70	4.61	4.99	0.41	1.27	0.65	1.17	8.00	4.47	5.47	3.24	2.33	3.29
ESR 1 h[b]	mm/1 h	10.97	8.63	15.36	5.37	11.12	5.95	14.95	8.52	1.66	1.73	2.77	3.25	23.52	4.16	18.68	6.37	9.58	6.93

[a]ESR 30 s: ESR in 30 seconds
[b]ESR 1 hr: ESR in an hour

Table 3. Plasma biochemistry reference values for whole group and individual belugas at the National Museum of Marine Biology and Aquarium in Pingtung, Taiwan

Plasma biochemistry	Unit	Whole group		Angel		Babu		Blue		Ginbo		Baby		Green		Red		Yellow	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
TPP	g/dL	6.97	0.51	6.95	0.41	7.07	0.37	7.42	0.38	6.62	0.54	6.59	0.42	7.20	0.37	7.24	0.30	7.23	0.39
Albumin	g/dL	4.03	0.29	4.08	0.28	4.05	0.29	4.06	0.21	4.02	0.32	3.82	0.31	4.11	0.22	4.08	0.18	4.09	0.24
Globulin	g/dL	2.93	0.47	2.87	0.45	3.00	0.41	3.35	0.34	2.60	0.45	2.77	0.43	3.09	0.31	3.16	0.29	3.13	0.34
A/G ratio		1.43	0.32	1.46	0.26	1.38	0.30	1.19	0.16	1.61	0.41	1.42	0.31	1.35	0.17	1.30	0.15	1.34	0.18
AST	U/L	75.8	21.3	70.2	22.5	79.0	20.1	67.2	11.0	68.9	18.0	103.8	20.7	81.5	9.9	65.6	10.0	71.3	10.1
ALT	U/L	13.8	9.0	11.1	9.3	19.4	10.2	8.2	3.1	13.6	8.0	21.0	8.1	7.1	1.6	9.5	1.5	12.0	2.8
ALP	U/L	212	99	143	77	209	74	137	57	231	70	228	63	392	105	348	75	247	60
GGT	U/L	34.3	14.0	27.9	8.7	25.5	7.2	44.6	7.2	31.0	7.2	60.6	14.8	27.9	3.8	30.1	4.3	28.3	3.6
CK	U/L	193	66	169	54	167	40	174	56	170	34	209	58	294	73	288	46	272	56
LDH	U/L	368	199	442	284	411	178	221	53	379	137	477	211	278	37	219	38	251	49
Cholesterol	mg/dL	176	43	185	43	166	37	165	24	199	51	139	30	180	32	200	38	173	26
Triglycerides	mg/dL	158	85	138	72	151	87	204	63	132	80	114	70	166	35	329	44	180	34
Glucose	mg/dL	104	13	101	11	101	12	105	14	103	12	102	10	101	10	113	10	121	13
Creatinine	mg/dL	1.00	0.24	1.04	0.18	0.98	0.18	1.17	0.19	1.15	0.19	0.74	0.22	0.94	0.19	0.81	0.14	0.76	0.10
BUN	mg/dL	57.0	7.1	57.8	6.4	54.0	6.8	56.3	6.3	55.1	6.4	60.1	8.9	65.2	4.8	57.5	2.9	56.7	4.4
iP	mg/dL	6.08	0.85	6.04	0.75	6.69	0.84	6.15	0.76	6.00	0.78	5.42	0.74	6.42	0.54	5.91	0.60	5.37	0.59
Ca^{2+}	mg/dL	10.29	0.77	10.12	0.75	10.17	1.04	10.45	0.50	10.34	0.76	9.84	0.72	10.74	0.67	10.51	0.63	10.26	0.50
Na^{+}	mEg/L	156.6	4.0	156.1	4.1	155.4	3.8	158.0	2.4	155.8	4.3	158.0	5.1	157.1	2.8	156.2	2.3	158.0	2.4
K^{+}	mEg/L	3.98	0.35	3.86	0.36	4.06	0.30	3.93	0.30	4.13	0.30	4.01	0.42	3.96	0.32	3.78	0.27	3.78	0.24
Cl^{-}	mEg/L	122.0	4.5	121.1	4.2	121.8	4.1	124.2	3.8	122.9	5.3	123.2	4.3	118.5	2.7	120.3	3.2	119.9	3.2
Total CO_2	mEg/L	26.0	5.1	27.4	4.7	27.4	4.9	25.5	4.9	25.3	5.2	28.5	4.5	23.8	4.2	21.1	3.1	21.2	3.6
Lactate	mg/dL	2.62	2.09	2.54	2.86	2.08	1.23	2.33	1.61	2.00	1.06	2.66	1.55	3.92	2.49	3.21	1.57	5.12	3.05
Plasma iron	μg/dL	386	87	347	64	423	99	307	55	418	75	389	72	395	68	444	96	390	80
Fibrinogen	mg/dL	80.4	20.4	77.8	18.1	81.5	21.7	69.1	15.6	79.6	21.6	84.1	17.2	87.3	16.5	108.3	16.1	93.8	17.6

Table 4. Seasonal and age-related differences, determined with one-way ANOVA followed by Tukey test, and gender-related differences, performed by Student's *t* test, on blood analytes for the belugas at the National Museum of Marine Biology and Aquarium in Pingtung, Taiwan

	Age[b]		Season[c]		Gender[d]	
Parameter	ANOVA (*p* value)	Tukey	ANOVA (*p* value)	Tukey	Student's *t* test (*p* value)	
PCV	0.0331	J > S	0.7609		<0.0001	M > F
RBCs	0.0711		0.0472		<0.0001	M > F
Hb	0.0020	J > A, S	0.0867		<0.0001	M > F
MCV	0.0003	A, J > S	<0.0001	Au > Sp, Su	<0.0001	M > F
MCH	<0.0001	J > A, S	0.0163	Au > Sp, Su	0.7307	
MCHC	<0.0001	S, J > A	0.4358		0.0093	F > M
WBCs	0.3503		0.0557		0.6086	
Segmented neutrophils	<0.0001	A, S > J	0.0323		0.7848	
Lymphocytes	<0.0001	J > S > A	0.1476		0.0366	M > F
Monocytes	<0.0001	A > S, J	0.1626		0.6224	
Eosinophils[a]	<0.0001	J > S > A	0.8055		0.6935	
Platelets[a]	<0.0001	J > A, S	0.8912		0.0010	F > M
ESR 30 s[a, e]	<0.0001	J > A, S	0.0713		<0.0001	F > M
ESR 1 h[a, f]	<0.0001	J > A, S	0.1707		<0.0001	F > M
TPP	0.2994		0.0454		<0.0001	F > M
Albumin	<0.0001	A > S, J	0.0505		0.0001	F > M
Globulin	0.4159		0.1095		<0.0001	F > M
AST	<0.0001	S > J > A	0.0035	W > Sp, Su, Au	<0.0001	M > F
ALT[a]	<0.0001	S, J > A	0.0088	W > Sp, Su	<0.0001	M > F
ALP	<0.0001	J > S > A	0.8430		0.0265	M > F
GGT[a]	<0.0001	S, J > A	0.7121		0.1494	
CK	<0.0001	J > S > A	0.7613		<0.0001	F > M
LDH[a]	<0.0001	S, J > A	0.0553		<0.0001	M > F
Cholesterol	<0.0001	A > S, J	0.0036	Au, W > Su	0.0694	
Triglycerides[a]	<0.0001	A > S > J	0.0592		<0.0001	F > M
Glucose	<0.0001	J > A, S	0.1537		0.0019	F > M
Creatinine	0.0001	A > S, J	0.1502		0.3861	
BUN	<0.0001	S > J > A	0.0585		0.0002	F > M
iP	<0.0001	A > S > J	0.0003	Au > Sp, Su, W	0.2296	
Ca^{2+}	0.0236	J > S	0.0002	Su > Sp, W	0.0360	F > M
Na^{+}	<0.0001	A, S > J	0.0577		0.0286	F > M
K^{+}	<0.0001	S, J > A	0.0087	Au > Sp	<0.0001	M > F
Cl^{-}	<0.0001	A > S > J	0.0032	Su, Au > W	0.0037	M > F
TCO_2	<0.0001	A, S > J	0.0092	W > Su, Au	0.0003	M > F
Lactate[a]	<0.0001	J > S > A	0.0222	Su > Sp	0.0002	F > M
Plasma iron	0.4161		0.1898		<0.0001	M > F
Fibrinogen	<0.0001	S, J > A	0.0379		0.4139	

[a]ln transformed
[b]A: adult, S: subadult, and J: juvenile
[c]Sp: spring, Su: summer, Au: autumn, and W: winter
[d]F: female and M: male
[e]ESR 30 s: ESR in 30 seconds
[f]ESR 1 h: ESR in an hour

Table 5. Results of linear mixed-effects models fitted to hematology parameters for the belugas at the National Museum of Marine Biology and Aquarium in Pingtung, Taiwan

					Gender[a]			Adult[b]			Subadult[b]			Spring[c]			Summer[c]			Autumn[c]		
Hematology	Units	Intercept	SE	*p* value	Estimate	SE	*p* value	Estimate	SE	*p* value	Estimate	SE	*p* value	Estimate	SE	*p* value	Estimate	SE	*p* value	Estimate	SE	*p* value
PCV	%	54.20	0.47	<0.0001	-1.75	0.52	0.0313	-0.33	0.39	0.3949	-0.90	0.37	0.0195	--	--	--	--	--	--	--	--	--
RBCs	10^6/μL	3.41	0.04	<0.0001	--	--	--	--	--	--	--	--	--	-0.03	0.02	0.0887	-0.06	0.02	0.0004	-0.07	0.02	<0.0001
Hb	g/dL	21.26	0.19	<0.0001	--	--	--	--	--	--	--	--	--	-0.09	0.11	0.4152	-0.28	0.11	0.0108	0.04	0.11	0.7254
MCV	fL	164.87	1.87	<0.0001	-3.30	2.28	0.196	-0.93	0.82	0.2616	-2.36	0.76	0.0026	-0.19	0.43	0.6647	-0.42	0.43	0.3361	1.58	0.44	0.0004
MCH	pg	63.06	0.71	<0.0001	--	--	--	-0.94	0.62	0.1323	-0.57	0.56	0.3134	0.08	0.32	0.8094	0.21	0.32	0.5056	1.29	0.33	<0.0001
MCHC	g/dL	38.61	0.43	<0.0001	0.57	0.49	0.3159	-0.50	0.31	0.1175	0.09	0.29	0.7545	--	--	--	--	--	--	--	--	--
WBCs	μL	10578.00	1079.57	<0.0001	-618.86	1324.76	0.6573	-1525.69	417.64	0.0006	-1147.12	404.70	0.0064	267.66	179.23	0.136	581.64	179.87	0.0013	289.80	181.54	0.1111
Segmented neutrophils	μL	5552.35	648.57	<0.0001	-16.61	785.94	0.9838	574.29	292.88	0.0551	134.38	283.98	0.6379	225.37	142.70	0.115	474.54	143.33	0.001	261.39	144.66	0.0715
Lymphocytes	μL	3623.32	314.68	<0.0001	-433.36	375.05	0.2967	-1526.10	166.21	<0.0001	-835.51	160.95	<0.0001	-18.38	77.51	0.8127	88.78	77.82	0.2546	-11.77	78.56	0.8809
Monocytes	μL	503.96	56.61	<0.0001	-5.20	62.36	0.9376	67.29	41.29	0.1097	-7.30	40.06	0.8563	-34.56	26.90	0.1995	-43.70	27.06	0.107	-19.21	27.33	0.4826
Eosinophils	μL	780.66	123.09	0.0003	-33.23	139.74	0.8215	-450.99	86.51	<0.0001	-297.05	83.41	0.0008	60.49	29.62	0.0417	43.99	29.67	0.1389	50.92	29.96	0.0899
Platelets	10^3/μL	151.81	10.78	<0.0001	--	--	--	-56.38	11.17	<0.0001	-48.11	10.50	<0.0001	10.42	7.31	0.1549	9.73	7.45	0.1923	12.21	7.53	0.1056
ESR 30 s[d]	mm/30 s	4.08	1.58	0.027	1.88	1.63	0.2943	-3.07	1.10	0.0071	-2.33	1.07	0.0327	--	--	--	--	--	--	--	--	--
ESR 1 h[e]	mm/1 h	8.74	3.65	0.0363	9.24	4.01	0.0571	-4.22	2.17	0.0541	-1.97	2.02	0.3314	-0.27	0.71	0.7086	-1.58	0.72	0.029	-0.97	0.75	0.1935

[a]Reference variates of gender was male.
[b]Reference variates of age was juvenile.
[c]Reference variates of season was winter.
[d]ESR 30 s: ESR in 30 seconds.
[e]ESR 1 h: ESR in an hour.

Table 6. Results of linear mixed-effects models fitted to plasma biochemistry parameters for the belugas at the National Museum of Marine Biology and Aquarium in Pingtung, Taiwan

Plasma biochemistry	Units	Intercept	SE	p value	Gender[a]			Adult[b]			Subadult[b]			Spring[c]			Summer[c]			Autumn[c]		
					Estimate	SE	p value	Estimate	SE	p value	Estimate	SE	p value	Estimate	SE	p value	Estimate	SE	p value	Estimate	SE	p value
TPP	g/dL	6.76	0.13	<0.0001	0.44	0.17	0.0401	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Albumin	g/dL	3.97	0.05	<0.0001	0.12	0.06	0.1197	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Globulin	g/dL	2.79	0.11	<0.0001	0.31	0.14	0.07	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
AST	U/L	90.61	6.67	<0.0001	-11.25	7.82	0.1986	-10.14	4.27	0.0204	5.10	3.86	0.1889	-6.98	1.89	0.0003	-8.80	1.93	<0.0001	-4.83	1.92	0.0122
ALT	U/L	23.39	2.65	<0.0001	-10.93	3.12	0.0148	-10.52	1.70	<0.0001	-5.16	1.49	0.0007	-0.52	0.67	0.436	-0.30	0.68	0.6586	2.20	0.68	0.0013
ALP	U/L	257.87	20.08	<0.0001	--	--	--	-112.20	16.14	<0.0001	-35.10	13.58	0.0107	15.22	5.41	0.0051	8.11	5.51	0.1416	20.05	5.47	0.0003
GGT	U/L	34.85	4.45	<0.0001	--	--	--	-5.70	2.32	0.0162	3.96	1.89	0.0377	-0.64	0.68	0.3419	0.58	0.69	0.4002	0.62	0.68	0.3621
CK	U/L	180.89	27.31	0.0005	53.68	33.31	0.1642	-19.27	13.08	0.1449	27.83	11.34	0.0156	-12.51	4.95	0.0118	-13.57	5.03	0.0072	-2.75	4.95	0.5791
LDH	U/L	532.05	76.47	0.0002	-198.04	92.36	0.0772	-215.34	43.04	<0.0001	-93.18	35.91	0.0103	21.98	13.99	0.1168	29.15	14.06	0.0387	30.22	13.98	0.0312
Cholesterol	mg/dL	154.92	16.63	<0.0001	30.20	20.04	0.1873	48.96	9.25	<0.0001	20.75	7.92	0.0098	-13.50	3.26	<0.0001	-26.08	3.32	<0.0001	-10.32	3.30	0.0019
Triglycerides	mg/dL	76.12	45.02	0.1408	104.87	55.77	0.1123	102.26	17.15	<0.0001	64.21	14.50	<0.0001	-8.44	5.68	0.1377	-15.15	5.79	0.0092	-8.00	5.74	0.1642
Glucose	mg/dL	104.22	3.78	<0.0001	4.82	4.36	0.322	-5.02	2.52	0.0516	-1.14	2.63	0.6651	1.43	1.67	0.3911	-1.54	1.70	0.366	-0.33	1.63	0.8408
Creatinine	mg/dL	1.01	0.07	<0.0001	--	--	--	-0.09	0.04	0.0166	-0.13	0.03	0.0003	--	--	--	--	--	--	--	--	--
BUN	mg/dL	54.42	2.46	<0.0001	3.42	2.88	0.2911	1.18	1.70	0.4915	4.77	1.44	0.0012	0.28	0.58	0.6361	-1.31	0.60	0.0288	-1.12	0.59	0.0592
iP	mg/dL	5.67	0.17	<0.0001	--	--	--	0.82	0.17	<0.0001	0.42	0.15	0.0059	--	--	--	--	--	--	--	--	--
Ca^{2+}	mg/dL	10.09	0.11	<0.0001	--	--	--	--	--	--	--	--	--	0.06	0.10	0.5761	0.39	0.11	0.0003	0.22	0.10	0.0349
Na^{+}	mEg/L	154.72	0.90	<0.0001	--	--	--	4.72	0.76	<0.0001	3.45	0.69	<0.0001	--	--	--	--	--	--	--	--	--
K^{+}	mEg/L	4.09	0.06	<0.0001	-0.17	0.06	0.0048	-0.16	0.07	0.0226	0.07	0.06	0.2388	-0.08	0.03	0.0152	-0.02	0.03	0.589	0.09	0.03	0.0091
Cl^{-}	mEg/L	119.96	0.97	<0.0001	-1.13	1.02	0.3151	3.73	0.89	<0.0001	0.45	0.80	0.5728	0.94	0.43	0.0306	1.40	0.44	0.0017	1.41	0.44	0.0014
TCO_2	mEg/L	27.48	1.38	<0.0001	-2.70	1.58	0.1502	1.52	0.95	0.115	0.02	0.89	0.9846	-0.78	0.57	0.1686	-1.96	0.58	0.0007	-1.68	0.57	0.0034
Lactate	mg/dL	2.54	0.60	0.0074	1.08	0.59	0.1823	-0.69	0.50	0.1796	0.35	0.47	0.4651	-0.62	0.25	0.013	-0.02	0.26	0.9361	-0.03	0.26	0.8982
Plasma iron	µg/dL	377.90	21.73	<0.0001	--	--	--	48.29	17.38	0.007	57.75	15.78	0.0004	-15.79	8.38	0.0601	-36.74	8.58	<0.0001	-25.01	8.56	0.0036
Fibrinogen	mg/dL	97.24	7.88	<0.0001	-3.87	6.84	0.6041	-20.54	6.70	0.0059	-8.25	6.31	0.2032	2.09	2.75	0.4491	0.95	2.80	0.7352	-1.42	2.81	0.6132

[a]Reference variates of gender was male.
[b]Reference variates of age was juvenile.
[c]Reference variates of season was winter.

Discussion

This study established a physiological baseline profile of hematologic and plasma biochemical characteristics for beluga whales managed in Asia and also investigated the effects of seasonal variation as well as the beluga's age and gender on these values. Many, but not all, of the baseline hematologic and plasma biochemical values reported in this study were in accordance with those reported previously for managed belugas in other geographical areas (Engelhardt, 1979; Cornell et al., 1988; Norman et al., 2013). Higher total RBCs, AST, ALT, ALP, GGT, CK, and LDH were observed in belugas housed in Taiwan than in North America, whereas belugas housed in Taiwan had lower PCV, MCV, cholesterol, and triglycerides (Engelhardt, 1979; Cornell et al., 1988; Norman et al., 2013). Previous studies on captive beluga whales in North America were housed in a variety of sites, and the blood testing equipment differed from laboratory to laboratory (Engelhardt, 1979; Cornell et al., 1988; Norman et al., 2013). Belugas' blood values were also possibly affected by water temperature, water components of natural/artificial sea water, and an indoor/outdoor raising environment (Norman et al., 2013). Therefore, geographic location of the belugas and possible analyzer differences can affect blood parameters and may have resulted in the occurrences of location-related variations on hematologic and plasma biochemical values.

Compared with managed belugas, greater total RBCs, total WBCs, total protein, globulin, AST, glucose, creatinine, BUN, iP, Ca^{2+}, Na^+, K^+, and fibrinogen as well as lower MCV and MCH were observed in wild beluga populations (Engelhardt, 1979; Cornell et al., 1988; St. Aubin et al., 2001; Tryland et al., 2006; Norman et al., 2012, 2013). Natural sea water generally has more microbes than the water used for managed belugas. The value of leukocytes, important defenses against antigens, can be elevated when encountering stimuli; this may explain why wild belugas had greater total WBC values than managed populations (St. Aubin et al., 2001). The different seawater components and salinities may also result in higher Ca^{2+}, Na^+, and K^+ concentration in wild belugas than in managed animals.

Concern regarding the effects of chemical pollutants to the marine environment has been increasing since the 20th century. The potential toxicity of chemical pollutants, especially persistent organohalogen contaminants, polychlorinated biphenyls (PCBs), and heavy metals, have been investigated for their influences on physiological systems of marine mammals, including belugas (Norstrom et al., 1998; Houde et al., 2005). However, studies focused on the relationship between marine contaminants and marine mammals' blood characteristics are limited. It has been found that lipophilic characteristics of these contaminants would result in changes in levels of triglycerides and some hormones in cetaceans (Houde et al., 2005). More recently, elevated levels of hepatic enzymes, electrolytes, LDH, and magnesium were observed in bottlenose dolphins with PCB exposure. Anemia, hypothyroidism, and immunosuppression were also strongly associated with these marine contaminants (Schwacke et al., 2011). The effect of exposure to environmental contaminants in wild and managed beluga whales would affect both their physical health and blood characteristics, and could contribute to observed differences between populations.

A number of studies in mammals, reptiles, fish, and birds have documented changes in hematologic and biochemical values at different age stages (Bush et al., 1981; Tocidlowski et al., 2000; Hrubec et al., 2001; Ihrig et al., 2001; Howlett et al., 2002; Villegas et al., 2002; Kakizoe et al., 2007). Erythrocyte parameters are typically related to oxygen transport and metabolic processes ensuring cell survival, and are usually increased with age in fish, birds, turtles and various mammals (Bush et al., 1981; Tocidlowski et al., 2000; Hrubec et al., 2001; Ihrig et al., 2001; Howlett et al., 2002; Villegas et al., 2002; Kakizoe et al., 2007). However, the values of PCV, Hb, MCH, and MCHC in belugas from Taiwan decreased with age, and this trend contradicted what was found in North America (Norman et al., 2013).

In leukocytes, higher levels of total WBC in juvenile belugas compared to adults were observed in the present and several previous reports (St. Aubin et al., 2001; Norman et al., 2012, 2013). The leukocyte kinetics in juveniles can represent the function of body defense and may reflect the response of still-developing immune systems, which react to a wide variety of stimuli, including microbes and parasites (St. Aubin et al., 2001). Moreover, significantly higher neutrophil and monocyte values and significantly lower levels of lymphocyte and eosinophil were found in adult individuals compared to juveniles in this study. The increasing stress leukograms, which result from an elevation of adrenocorticosteroid, were shown along with the increase of age in this study (Dierauf & Gulland, 2001; Clark et al., 2006; Davis et al., 2008; Schmitt et al., 2010). This finding raises the concern that the belugas seemed to have been undergoing stress even when they presented to be clinically healthy.

Generally, increased stress can be related to certain health conditions in belugas, such as impaired growth and reproduction, frequent infections, and disease occurrences (Dierauf & Gulland, 2001). Several stimuli, including noise, aggression, separation, chronic exposure to organohalogens, and other environmental factors, are also linked to stress by causing adrenal hyperfunction (Thomas et al., 1990; Dierauf & Gulland, 2001; Castellote & Fossa, 2006; Clark et al., 2006; Jiang et al., 2007; Wright et al., 2011). However, the chronic stress effects are not easily diagnosed and are even more difficult to trace back to specific factors or events (Dierauf & Gulland, 2001). In Taiwan, all pools at the National Museum of Marine Biology and Aquarium for housing belugas, including those used for separation for medical treatment and medical training, conform to the Animal Welfare Act and Animal Welfare Regulations (Animal Welfare Act and Regulations "Blue Book") by the U.S. Department of Agriculture. Each beluga has a keeper in charge of daily caring who leads the medical and nonmedical training. Staff also created various toys to enrich the belugas' daily life. Therefore, further investigations will be needed to understand and verify the cause of this apparent stress response. Ways of improving stress recognition and management in a managed beluga colony are significant issues.

In the present study, significant age-related differences were seen with higher platelet numbers in juveniles than in adults. In other studies of cetaceans, the same results were found in managed bottlenose dolphins, while no age-related differences in platelets were reported in wild bottlenose dolphins and harbor seals (Fair et al., 2006; Hall et al., 2007; Venn-Watson et al., 2007; Greig et al., 2010).

AST and ALT are enzymes present in both cytoplasm and mitochondria of cells. Both enzymes are found mainly in the liver, but also are found in the heart, skeletal muscle, and kidney (Latimer, 2011). Our study showed low AST and ALT levels in adult belugas, similar to the findings of a study in adult managed belugas (Norman et al., 2013). Alkaline phosphatase (ALP) can be an indicator for liver or bone disorders since it is produced primarily in these structures (Latimer, 2011). Cetacean ALP enzyme activity has been shown to decrease with age as seen in the present study, and it is associated with rapid bone development (St. Aubin et al., 2001; Fair et al., 2006; Venn-Watson et al., 2007).

Plasma calcium, controlled by parathormone, calcitonin, and calcitriol, is active in bone formation, neuromuscular activity, blood coagulation, and cellular biochemical processes (Moe, 2008; Latimer, 2011). Calcium concentration was observed to be higher in juveniles than adults in the present study, similar to previous cetacean studies (St. Aubin et al., 2001; Fair et al., 2006; Venn-Watson et al., 2007). The dynamic status of calcium in juveniles is likely connected with bone growth, which showed a trend similar to ALP enzyme activity. Besides Ca^{2+}, iP concentration is also mediated by parathormone, which elevates Ca^{2+} and decreases iP (Moe, 2008; Latimer, 2011), and may explain the low iP and high Ca^{2+} concentrations in juveniles in this study.

GGT is an enzyme found in high concentration in the liver and kidney. The GGT enzyme present in the serum is mainly from the hepatobiliary system (Latimer, 2011). High levels of GGT in managed juveniles reported in Taiwan and the U.S. were inconsistent with those observed in wild belugas in which higher GGT values were found in adults (St. Aubin et al., 2001; Norman et al., 2013). In this study and previous ones of belugas, high levels of cholesterol and triglycerides were found in adults (Cornell et al., 1988; St. Aubin et al., 2001). The concentration of cholesterol and triglycerides is affected by diet, lipid metabolism, and reproductive hormones (Cohn et al., 1988; Latimer, 2011). In humans, higher cholesterol and triglyceride values were observed in adults compared to in younger individuals (Cohn et al., 1988). Plasma triglyceride concentrations have been reported to increase significantly after an individual consumed a fat-rich meal, and they remained elevated for 9 h (Cohn et al., 1988).

The values of total RBC, PCV, Hb, and WBC parameters showed no significant seasonal differences in this study. However, these RBC parameters have been reported to be higher during summer in some species of wild and managed cetaceans (Hall et al., 2007; Macchi et al., 2011; Norman et al., 2013). Certainly, stress leukograms have been observed in summer for captive belugas (Norman et al., 2013). Seasonal effects in RBC and WBC parameters may be due to nutritional differences, erythropoietic activity, seasonal reproductive hormones, or the stress from seasonal reproductive events (Hall et al., 2007; Macchi et al., 2011; Norman et al., 2013). For wild belugas, prey quality may vary seasonally. Prey availability and food intake are usually higher in the summer compared to the winter (Hall et al., 2007).

The food type and the component of diet supplied for captive belugas can also differ seasonally and could be a factor in any seasonal fluctuations of blood values observed. Autumn and winter ALT values peaked in both managed bottlenose dolphins and belugas, including those in the present study, but ALT values were high in spring in wild belugas (St. Aubin et al., 2001; Macchi et al., 2011; Norman et al., 2013). Creatinine values are usually reported to be higher in summer due to the change of seasonal muscle mass as reported in both wild and managed bottlenose dolphins and belugas (Hall et al., 2007; Macchi et al., 2011;

Norman et al., 2012, 2013). However, no seasonal changes in plasma creatinine were seen in the present study. In wild bottlenose dolphins and belugas in the present study, the effect of season in Ca^{2+} concentration was high in summer, which may result from the influence of certain hormones (Hall et al., 2007).

Previous studies have demonstrated that seasonal differences in blood parameters may be associated with photoperiod, diet, water temperature, ambient temperature, seasonal changes in body condition, seawater components, and other factors (Domingo-Roura et al., 2001; Terasawa et al., 2002; Sergent et al., 2004; Norman et al., 2013). The belugas in this study were housed in a facility with constant water temperature (17 to 18° C) and lighting (0800 to 1800 h daily), and the amount as well as nutritional content of food they ate remained consistent for the most part throughout the year (only 10% difference between seasons). Thus, the seasonal differences in blood values observed may possibly be connected to ambient temperature and seawater components. There are three main currents around Taiwan: (1) the South China Sea Warm Current, (2) the China Coastal Current, and (3) the Kuroshio Current (Hu et al., 2000). These currents, driven by monsoon winds, seasonally change the salinities, nutrients, and other components of sea water in the south of Taiwan where the museum is located (Hu et al., 2000; Liu et al., 2000; Jan et al., 2002; Chen & Chen, 2006). The somatosensory sensations of belugas may have been induced by the chemicals or other unknown factors which led to seasonal changes in body condition and the blood values observed.

In addition to season and age, gender is also an important factor that can influence hematologic and biochemical values. In managed belugas in Taiwan and the U.S., RBC parameters (i.e., PCV, total RBCs, Hb, and MCV) were higher in males compared to females (Norman et al., 2013). Factors such as pregnancy, reproductive hormones, or other physiologic effects may influence the values of RBC parameters in cetaceans (St. Aubin et al., 2001; Fair et al., 2006; Norman et al., 2013). In the present study, TPP values in females were significantly higher than in males as noted in managed bottlenose dolphins, but the reasons remain unknown (Venn-Watson et al., 2007). The higher plasma triglyceride concentrations seen in female belugas in the present study as well as in female dolphins (Fair et al., 2006) may be attributed to food consumption, lipid metabolism, and hormones.

The establishment of animal blood reference values requires correspondingly large sample sizes, which is usually not feasible in wild populations, particularly those of a known health status. Recruiting available subjects and using obtainable information to build reference values for both managed and wild populations in different geographical locations is necessary and meaningful for proper animal management. Our study is the first long-term investigation of hematology and plasma biochemistry of belugas in Asia. Data collection from belugas in neighboring regions is recommended to overcome the high individual variation of the values due to the relatively small sample size in the present study. The effects of season, the beluga's age and gender, photoperiod, diet, components of sea water, and a host of unknown factors can all contribute to variation specific to different geographical areas. The reference values and analyses in this study supply valuable information that will ultimately contribute to our understanding and health monitoring of belugas in Asia.

Conflict of Interest Statement

None of the authors have any financial and personal relationships with other people or organizations that could inappropriately influence or bias their work.

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Hematology and Plasma Chemistry as Indicators of Health and Ecological Status In Beluga Whales, Delphinapterus Leucas Arctic The Arctic Institute of North America 0004-0843 1923-1245 10.14430/ARCTIC791.Â If you want to receive offers and information about our related products and services from us, please tick the box. Connect with: Register as an organisation for even more detailed and powerful intelligence. Fill the form and we will be in touch to set up your trial accessâ€¦ for free! Or if you have a question, ask us here. Fill the form and we will be in touch to set up your trial accessâ€¦ for free! The study objective was to identify the influences of partitioning characteristics on hematology and serum chemistry analytes of apparently healthy managed beluga (Delphinapterus leucas). Blood values from 31 managed belugas, at three facilities, collected over 22 yr, were assessed for seasonal variation and aging trends, and evaluated for biologic variation among and within individuals. Linear mixed effects models assessed the relationship between the analytes and sex, age, season, facility location, ambient air temperature, and photoperiod. Sex differences in analytes and associations with i