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Haematology and clinical blood chemistry in harbour porpoises (*Phocoena phocoena*) from the inner Danish waters

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ABSTRACT

Harbour porpoises *(Phocoena phocoena)* in the Baltic Sea are under increasing pressure from anthropogenic activities, which affect the overall health of populations. Individuals' haematologic and biochemistry parameters are known to be linked to a population's health status and are therefore useful tools for cross-population comparisons and to assess health patterns of a population through time. However, it is often difficult to acquire data representing the full range of values and assess the influence of intrinsic and extrinsic factors. Here, we present the range of haematology and blood chemistry values obtained from 46 wild (n = 54 blood samples) individuals incidentally caught in pound-nets and five porpoises in long-term human care (n = 449 blood samples) from an outdoor semi-open facility in Denmark. Although it was not possible to formally test the differences between samples from free-ranging and captive individuals, lymphocyte values were lower for free-ranging animals whereas eosinophils and white blood cell values were higher in captive individuals. Aspartate aminotransferase and alanin aminotransferase values were also lower for captive individuals compared to free-ranging ones. Age group did not influence any of the blood parameters tested for free-ranging individuals. Sodium values were higher for males compared to females. Values were higher and lower in the fall for platelets and lactic acid dehydrogenase, respectively, compared to the other seasons. Based on samples yielded by individuals in long-term human care, haemoglobin, mean cell volume, white blood cells, absolute lymphocyte count, and alkaline phosphatase values were all influenced by health status based on clinical examination. These are therefore candidate parameters to assess health status of wild porpoises. Our results underline that it is essential to obtain ranges of reference values for all haematologic and biochemistry markers in order to assess health status of free-ranging individuals. Individuals in human care provide the opportunity to observe biological and ecological determinates (e.g. age, season) of long-term biomarker response patterns and to assess the suite of biomarkers best suited to predict individual health status.

1. Introduction

The harbour porpoise (*Phocoena phocoena*) is the most abundant cetacean species in the Kattegat, Belt Seas and Baltic Sea regions (Siebert et al., 2006; Hammond et al., 2002, 2013; Viquerat et al., 2014). These waters are exposed to increasing anthropogenic activities, including shipping, construction of offshore wind farms, fisheries, seismic surveys, oil and gas activities, military operations, chemical pollution, and marine litter (Southall et al., 2007, 2008; Siebert et al., 2012a, 2012b; Russell et al., 2014). These activities are affecting

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Table 1
Summary of the number of individuals and samples per level of the factors considered in the present study. NA stands for no information.

Independent factor	Levels	N samples	N individuals
Group	Free-ranging	54	46
	Permanent	449	5
Sex	Females	335	24
	Males	166	25
	NA	2	2
Age group	Adults	336	45
	Juveniles	167	9
Season	Spring	144	29
	Summer	126	18
	Fall	105	14
	Winter	128	5
Health status (only permanent animals)	Healthy	277	4
	Sick	79	4
	NA	147	46
Total		**503**	**51**

dramatically the Baltic harbour porpoise population through habitat loss, by-catch, food depletion, underwater noise, and chemical insult. Disturbances can cause acute or chronic stress, which is known to affect negatively individual health reducing survival and ultimately affecting population size (Bailey et al., 2014; DeMaster et al., 2001; Parsons et al., 2008, Jepson et al., 2005, 2016; Beest et al., 2018).

Systematic pathological investigations of stranded and by-caught harbour porpoises from the North Sea through the Danish straits to the Baltic Sea indicate that harbour porpoises suffer from infectious diseases, mainly caused by a variety of bacteria and parasites (Jauniaux et al., 2002; Jepson et al., 2000; Lehnert et al., 2005; Siebert et al., 2001, 2009). Harbour porpoises from those areas suffer from a higher rate of chronic infectious diseases when compared to animals from less industrialized areas around Iceland, Greenland and Norway (Bruhn et al., 1999; Siebert et al., 2001, 2006; Thron et al., 2004). Harbour porpoises with higher levels of bioaccumulated environmental contaminants, such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), have documented lymphoid depletion in the thymus and spleen (Beineke et al., 2005), and impairment of lymphoid function resulting in a higher susceptibility to disease (Das et al., 2006). However, health assessments of wild harbour porpoise populations are extremely difficult and have often been limited to pathological investigations of stranded individuals that represent a biased picture of the population health (Jepson et al., 1999; Jauniaux et al., 2002; Siebert et al., 2001, 2006).

Analysis of blood and assessment of clinical biomarkers is a widely recognized method in marine mammal medicine to assess the health of an individual (Dierauf and Gulland, 2001). However, samples from large numbers of individuals are needed in order to estimate the natural biological variation and the environmental and individual parameters influencing this (Schwacke et al., 2014). Rehabilitation, long-term tagging, and permanent captive programs have previously provided such samples for bottlenose dolphins (*Tursiops truncatus*) and rough-toothed dolphins *(Steno bredanensis)* (Hall et al., 2007). For harbour porpoises, limited data exists for complete blood count and biochemistry values for free-ranging animals from the United States (Koopman et al., 1995, 1999; Siebert et al., 2012a, 2012b), while single animals in human care in Denmark and the Netherlands have provided information on haematology and biochemistry (Andersen, 1966, 1968; Kastelein et al., 1990; Nielsen and Andersen, 1982).

A long-term tagging study to investigate free-ranging harbour porpoise movements in Danish and adjacent waters (Teilmann et al., 2007; Sveegaard et al., 2011, 2015), and porpoises in human care for research purposes at a Danish aquarium (Desportes et al., 2003) provided a unique opportunity to gather both cross-sectional and longitudinal health data for up to16 years. Here we present haematology and biochemistry values for these two groups of porpoises, representing 51 individuals and over 500 blood samples. The aims of the study were to: 1) determine the possible range of haematology and blood biochemistry parameters values based on the two datasets, 2) to assess which parameters reflect an individual's health status, and 3) to explore the effects of season, sex, age and health status on these blood parameters.

2. Materials and methods

2.1. Animals

Five hundred and three blood samples (n = 503) from 51 porpoises belonging to two groups were collected between 1997 and 2012 (Table 1). The free-ranging animals (animals: n = 46; blood samples: n = 54) were by-caught alive in pound nets in the Kattegat and the Belt seas (*free-ranging group*). The animals could swim freely in the net without being entangled. These animals were captured inside the net typically within 3–8 h of the animals being discovered by a fisherman, sampled, instrumented and released (Teilmann et al., 2007; Eskesen et al., 2009). Only individuals with a healthy clinical appearance (apparent good body condition, no detectable respiratory symptoms) and a standard length of at least 100–110 cm were handled (Ruser et al., 2016). Long-term captive animals from a semi-open outdoor facility in Denmark (animals: n = 5; blood samples: n = 449; *permanent group*) (Desportes et al., 2003, 2007) originated from the same region and were taken into manage-care on a permanent basis for research purposes (see Desportes et al. 2003 and Table 1 for details). Four individuals were wild-caught whereas the fifth was born in captivity. All five animals were housed in an outdoor enclosure with naturally flowing seawater (Desportes et al., 2003; Lockyer et al., 2003). The age of each animal at capture was estimated based on their length following values from Lockyer et al. (2003). Animals were sorted into two age groups: juveniles (< 135 cm for males and < 143 cm for females) and adults (≥ 135 cm for males and ≥ 143 cm for females). The ages of the individuals in the permanent group were based on their size upon arrival, assuming birth occurred on 1 July each year Lockyer et al. (2003).

Animals from the permanent group were considered "healthy" if they did not show clinical alterations (normal behaviour and appetite, no clinical pathologies e.g. for respiratory and digestive diseases) or were not under pharmaceutical treatment. Animals were considered "sick" if they showed clinical alterations or were under pharmaceutical treatment. This information was only available for four out of the five permanent animals.

2.2. Blood sampling

Blood samples were obtained from the dorsal fluke veins using the method described by Desportes et al. (2003, 2007) and by Eskesen et al. (2009). The handling and sampling of the free-ranging group was carried out directly on board the fishermen's boat, after the harbour porpoises had been lifted out of the pound net and placed onto a padded stretcher (Eskesen et al., 2009). Permission for harbour porpoise handling and sampling was obtained from the Danish Forest and Nature Agency NST 3446/-0016 and Danish Ministry of Justice 2010-561-1801. One to three samples were taken from the free-ranging group animals as part of various studies: the first directly after the animal was removed from the pound nets, the second around two hours later and the third around three and a half hours later (Müller et al., 2013). Animals in the permanent group were sampled regularly during routine medical examinations over a 16 year period. Blood samples were

Table 2
Summary statistics for each blood parameter for a. *permanent group* and b. *free-ranging group* individuals. The number of samples and individuals for each blood parameter is also presented. The 10th and 90th percentiles are presented for each blood parameter with their 95% bootstrapped Confidence Intervals (CI). NA: not available.

Blood parameter	units	n samples	n individuals	minimum	10th	CI10low	CI10high	median	CImedlow	CImedhigh	90th	CI90low	CI90high	maximum
a														
Red blood cells	RBC (T/l)	434	5	2.69	4.61	4.40	4.82	5.74	5.65	5.83	6.40	6.34	6.46	7.61
Haemoglobin	HGB (g/dl)	432	5	7.40	14.20	13.51	14.79	17.80	17.61	17.92	19.40	19.17	19.57	22.30
Haematocrit	HTC (%)	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Mean cell volume	MCV (fl)	427	5	51.00	81.00	80.00	81.57	88.60	87.82	89.53	93.00	92.23	93.59	130.00
Mean corpuscular haemoglobin concentrate	MCHC (g/dl)	427	5	22.90	33.00	32.89	33.13	35.10	34.85	35.31	38.40	37.74	39.22	77.30
Platelets	PLT (G/l)	430	5	11.00	108.80	100.13	119.18	159.00	154.63	162.30	239.30	222.66	253.53	666.00
Mean platelet volume	MPV (um3)	294	4	6.00	8.50	8.38	8.70	9.70	9.58	9.89	10.80	10.57	10.99	12.80
Eosinophilc granulocytes	eosin (%)	226	4	0.00	0.00	0.00	0.00	6.00	4.39	7.03	18.50	14.78	22.28	49.30
White blood cells	WBC (G/l)	433	5	1.40	2.30	2.16	2.40	3.50	3.28	3.77	8.78	7.32	10.12	17.10
Lymphocyte	lymphocyt (%)	423	5	0.00	27.00	24.64	29.55	44.40	42.61	45.91	62.00	58.93	65.03	82.00
Monocyte	monocyte (%)	416	5	0.00	0.00	0.00	0.00	2.65	2.29	3.04	4.90	4.20	5.61	12.00
Absolute lymphcyte count	abslym (%)	286	4	0.00	0.00	−0.33	0.22	1.50	1.33	1.67	4.10	3.26	4.79	7.40
Absolute monocyte count	absmo (%)	286	4	0.00	0.00	NA	NA	0.10	NA	NA	1.40	1.07	1.98	2.80
Absolute granulocyte counts	absgran (%)	249	4	0.80	1.40	1.27	1.49	2.50	2.30	2.72	5.10	4.71	5.61	13.70
Calcium	Ca (mmol/l)	324	5	0.50	2.20	2.16	2.25	2.47	2.43	2.51	2.86	2.78	2.94	3.61
Iron	Fe (µmol/l)	196	5	5.00	21.65	18.20	24.57	43.15	39.61	46.59	64.35	59.69	69.01	114.30
Chlorine	Cl (mmol/l)	42	4	18.92	88.20	55.50	132.68	109.50	104.84	114.96	129.50	108.76	147.43	201.00
Potassium	K (mmol/l)	316	5	1.50	3.05	2.80	3.29	3.98	3.90	4.07	4.80	4.72	4.96	8.94
Magnesium	Mg (mmol/l)	79	4	0.48	0.61	0.55	0.64	0.73	0.69	0.76	0.90	0.80	1.02	1.08
Sodium	NA (mmol/l)	293	5	115.00	149.00	147.75	151.04	155.00	154.57	155.40	160.00	159.41	161.00	174.00
Phosphorus	phos (mmol/l)	320	5	0.41	1.35	1.29	1.41	1.86	1.79	1.95	2.36	2.23	2.46	8.76
Gamma glutamyl transferase	GGT (U/l)	197	4	5.00	8.00	7.45	8.98	13.00	12.29	14.47	28.40	25.18	31.63	188.00
Alkaline phosphatase	ALP (U/l)	297	5	37.00	119.60	99.87	137.37	305.00	268.47	334.15	675.80	613.55	730.19	1812.00
Aspartate aminotransferase	AST (U/l)	201	4	52.00	109.00	105.97	113.07	137.00	132.14	141.97	322.00	291.38	349.59	937.00
Alanin aminotransferase	ALT (U/l)	354	5	5.00	32.00	30.22	33.47	50.00	47.00	53.00	115.10	99.67	131.17	848.00
Lactic acid dehydrogenase	LDH (U/l)	201	5	171.00	382.00	369.66	394.15	466.00	451.06	480.24	654.00	589.50	711.85	1154.00
Triglyceride	trigl (mg/dl)	202	4	8.00	72.20	65.70	78.77	116.50	104.14	128.14	250.90	219.83	286.14	673.00
Cholesterol	cholest (mg/dl)	222	4	63.00	137.10	128.85	143.49	174.00	168.77	179.56	207.90	201.29	214.08	274.00
Urea	urea (mg/dl)	200	4	33.00	71.00	68.06	74.04	92.00	88.77	95.11	117.10	111.78	122.74	145.00
Creatinine	crea (mg/dl)	338	5	0.20	0.36	0.32	0.39	0.60	0.57	0.62	0.90	0.87	0.94	2.00
Albumin	ALB (U/l)	149	4	19.00	27.80	26.87	29.47	31.00	30.03	31.59	37.00	35.28	38.24	44.00
Blood urea nitrogen	Bun (mmol/l)	148	4	7.00	11.00	9.29	12.20	14.00	12.69	14.54	18.30	17.10	19.35	26.00
Globuline	Glo (g/l)	149	4	3.80	34.80	32.40	38.20	41.00	39.05	41.92	51.00	48.76	53.76	58.00
Total protein	TP (g/l)	340	5	22.60	65.88	64.72	67.49	72.00	71.57	72.60	80.10	77.55	81.87	97.00
b.														
Red blood cells	RBC (T/l)	47	41	2.75	4.26	3.13	5.81	5.77	5.55	6.00	6.59	6.20	6.87	7.20
Haemoglobin	HGB (g/dl)	47	41	8.80	13.04	10.82	16.08	16.90	16.19	17.45	19.34	18.44	20.26	20.50
Haematocrit	HTC (%)	47	41	0.25	0.37	0.30	0.47	0.50	0.48	0.55	0.57	0.53	0.60	0.66
Mean cell volume	MCV (fl)	47	41	71.00	79.60	76.41	82.97	86.00	83.39	87.95	92.00	88.14	95.16	128.00
Mean corpuscular haemoglobin concentrate	MCHC (g/dl)	47	41	26.20	31.00	29.54	31.82	34.00	33.62	34.64	36.08	31.11	39.41	56.20
Platelets	PLT (G/l)	47	41	9.00	118.60	100.18	137.34	169.00	155.75	184.19	326.80	138.36	491.11	851.00
Mean platelet volume	MPV (um3)	9	9	7.80	9.08	7.64	10.73	10.60	7.36	13.00	14.60	12.52	17.43	15.40
Eosinophilc granulocytes	eosin (%)	42	39	4.00	6.00	5.18	6.79	13.50	10.27	17.14	23.94	12.03	32.46	53.90
White blood cells	WBC (G/l)	47	41	2.70	3.56	3.22	3.85	4.90	4.05	5.55	7.94	6.73	9.01	16.00
Lymphocyte	lymphocyt (%)	43	40	2.00	9.20	3.52	12.75	20.00	12.05	24.53	56.80	42.85	74.79	75.00
Monocyte	monocyte (%)	42	39	0.00	0.00	−0.22	0.19	2.00	1.47	2.90	7.60	3.41	13.79	16.00
Absolute lymphcyte count	abslym (%)	23	17	0.50	0.84	0.44	1.12	1.60	1.27	2.12	3.28	2.61	4.11	3.80
Absolute monocyte count	absmo (%)	23	17	0.00	0.00	−0.07	0.05	0.10	0.09	0.11	0.20	0.15	0.31	0.20
Absolute granulocyte counts	absgran (%)	23	17	0.90	1.74	0.79	2.56	3.20	2.53	3.76	6.22	4.95	8.26	7.50
Calcium	Ca (mmol/l)	31	31	1.44	2.01	1.75	2.23	2.42	2.35	2.52	2.63	2.52	2.73	2.75
Iron	Fe (µmol/l)	29	29	4.30	11.92	4.82	16.64	29.80	20.91	39.51	50.04	42.05	61.67	69.90
Chlorine	Cl (mmol/l)	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

(*continued on next page*)

Table 2 (*continued*)

Blood parameter	units	n samples	n individuals	minimum	10th	CI10low	CI10high	median	CImedlow	CImedhigh	90th	CI90low	CI90high	maximum
Potassium	K (mmol/l)	28	28	2.34	3.35	2.92	3.89	4.08	3.60	4.47	4.96	1.69	7.21	13.10
Magnesium	Mg (mmol/l)	7	7	0.66	0.70	0.62	0.76	0.78	0.67	0.87	0.95	0.74	1.16	1.11
Sodium	NA (mmol/l)	32	32	135.00	146.00	141.50	151.58	153.00	150.93	155.46	157.90	149.36	164.97	179.00
Phosphorus	phos (mmol/l)	35	35	0.64	0.92	0.69	1.12	1.61	1.22	1.90	3.44	0.16	5.94	6.91
Gamma glutamyl transferase	GGT (U/l)	34	34	5.00	7.30	5.19	8.58	12.50	9.95	14.47	34.40	−22.20	73.12	231.00
Alkaline phosphatase	ALP (U/l)	29	29	38.00	56.00	−29.44	110.52	390.00	267.20	554.68	751.60	586.88	997.56	994.00
Aspartate aminotransferase	AST (U/l)	7	7	228.00	261.00	191.56	315.43	350.00	280.39	440.33	432.40	326.08	543.32	511.00
Alanin aminotransferase	ALT (U/l)	7	7	48.00	73.20	26.19	109.96	111.00	88.32	132.57	134.60	119.45	156.97	143.00
Lactic acid dehydrogenase	LDH (U/l)	36	36	359.00	440.00	406.67	468.69	602.00	544.35	670.56	765.00	653.36	871.87	1021.00
Triglyceride	trigl (mg/dl)	37	37	46.00	64.60	49.40	79.09	105.00	81.13	123.27	165.20	145.26	193.75	216.00
Cholesterol	cholest (mg/dl)	39	39	89.00	109.80	90.54	127.40	150.00	134.49	164.85	202.80	147.48	248.33	300.00
Urea	urea (mg/dl)	39	39	7.41	9.89	8.89	11.34	12.36	11.19	13.20	16.80	13.93	18.80	21.04
Creatinine	crea (mg/dl)	39	39	0.46	0.67	0.57	0.83	0.89	0.76	1.02	1.35	1.10	1.60	1.80
Albumin	ALB (U/l)	2	2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Blood urea nitrogen	Bun (mmol/l)	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Globuline	Glo (g/l)	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Total protein	TP (g/l)	38	38	62.00	63.47	60.50	65.89	74.95	70.29	78.77	86.62	73.73	96.01	109.00

obtained according to perceived necessity based on the animal's health, ranging from daily for very sick animals to once every three months for seemingly healthy animals. Blood samples were taken by voluntary procedure or by moving the animal from the pool onto a foam mat for the duration of the sampling procedure (Desportes et al., 2003, 2007). The broad spectrum of parameters to be analysed required approximately 10 ml of fresh blood, although attaining this quantity was not always possible. Due to this limitation, not every sample was assessed for full haematological and biochemical biomarker profiles.

2.3. Differential haematology and serum chemistry

For differential haematology and serum chemistry, venous whole blood was collected in tubes with ethylenediaminetetraacetic acid (EDTA) anticoagulant and tubes with coagulation gel for serum extraction. All samples were analysed within 24 h. Differential haemogram profiles were generated with a ScilVet ABC™ Animal Blood Counter (Scil Animal Care Company GmbH, 68519 Viernheim, Germany) which included RBC = red blood cells, Hgb = haemoglobin, HCT = haematocrit, MCV = mean cell volume, MCHC = mean corpuscular haemoglobin concentrate, PLT = platelets, MPV = mean platelet volume, Eosin = eosinophilic granulocytes, WBC = white blood cells, ALC = absolute lymphocyte count, AMC = absolute monocyte count, AGC = absolute granulocyte count. Serum separator tubes were centrifuged for 15 min after blood was clotted (Hettich™ EBA I centrifuge, Andreas Hettich GmbH & Co. KG, 78,532 Tuttlingen, Germany). Serum was extracted, frozen at −20 °C and later sent to the veterinary laboratory Synlab Vet in Geesthacht, Germany, for the determination of blood chemistry parameters, which included Ca = calcium, Fe = iron, Cl = chlorine, K = potassium, Mg = magnesium, Na = sodium, P = phosphorus, GGT = gamma glutamyl transferase, ALP = alkaline phosphatase, AST = aspartate aminotransferase, ALT = alanin aminotransferase, LDH = lactic acid dehydrogenase, Trigl = tryclyceride, Cholest = cholesterol, Crea = creatinine, ALB = albumin, BUN = blood urea nitrogen, Glo = Globuline, Bili = bilirubin, TP = total protein, and Gluc = glucose.

2.4. Statistical analysis

All data processing, analyses and graphic representations were conducted using the R statistical framework (R Core, 2012). In order to present the range of values for each blood parameter (n = 33), we calculated the minimum, 10th percentile, median, 90th percentile and maximum with their 95% confidence intervals (CI). The CI were calculated using bootstrapping procedures with 1000 repetitions. These summary statistics are presented separately for each group (free-ranging and permanent animals) due to an unbalanced dataset. Summary statistics for blood parameters with at least nine samples are presented. We then present the same information but this time partitioned between healthy and sick samples based on four animals in human care.

We then explored the potential factors influencing the blood parameter values of free-ranging individuals. The random effect (animal identity) was included in order to account for dependency between repeated sampling from the same individual. In the first part, blood samples from *free-ranging group* were examined for effects of season (spring, summer and fall), sex, and age group (adult vs juvenile). No individuals were captured in winter. We used linear-mixed effect models (LMEs). Seasons were separated into three categories: spring (March-May), summer (June-August) and fall (September-November). We considered interactions between sex, season, and age group when the sample size allowed it. The full model was blood parameter ~ season* sex * age group, random = ~1|id.

We then explored whether the blood parameters were influenced by their clinical status (healthy vs sick). For this part of the analysis, we only considered samples from the permanent facility for which we had

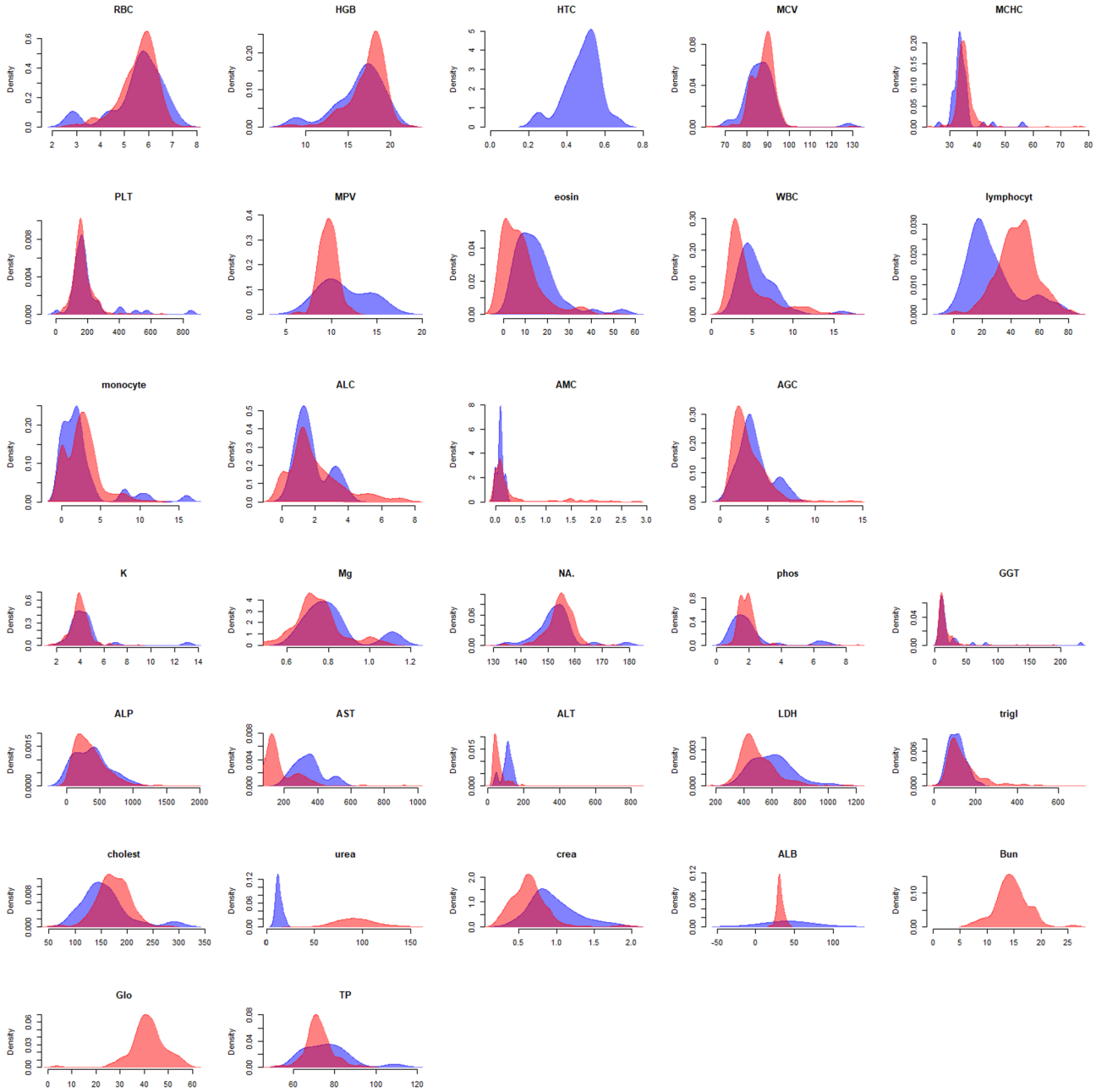

Fig. 1. Density curves for each blood parameter. Values from free-ranging individuals are in blue and values from permanent individuals are in red. The area where both curves overlap is purple. Note that no value for HTC were available for permanent animals and no value for Bun and Globuline were available for free-ranging individuals. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

a corresponding health status (healthy vs sick). We considered the effects of age group and season as random variables and health status as predictor and we included a first order autocorrelation term. The effect of sex was not considered because there was only one male in this subset of the data. The full model was blood parameter ~ health, random = ~1|id/season/age group, correlation = corAR1 (value = x, form = ~1|id/season/age group).

In the third part of this study, long-term variation of haematology and biochemical parameters were examined as a function of season and age, exclusively using samples of animals under human care deemed healthy. In order to have meaningful time series, only blood parameters with at least 100 sample points were examined. We used Generalized Additive Mixed-effect Models (GAMMs) or LMEs according to the shape of the relationship (linear or not). The effect of sex was not considered because there was only one male in this subset of the data. Age was entered as a smooth term. Season was entered as a cyclic smooth term as a factor with 4 levels and 4 knots. As above animal ID was included as a random effect to take into account the lack of independence between multiple samples from the same individual and a first order temporal autocorrelation term within each animal was added.

The full model was the following: Blood parameter ~ age + season, random = ~1|id, correlation = corAR1(value = x, form = ~1|id). In all parts of the analysis, models were selected using the Bayesian information criterion (BIC), starting with full models. Data were log-

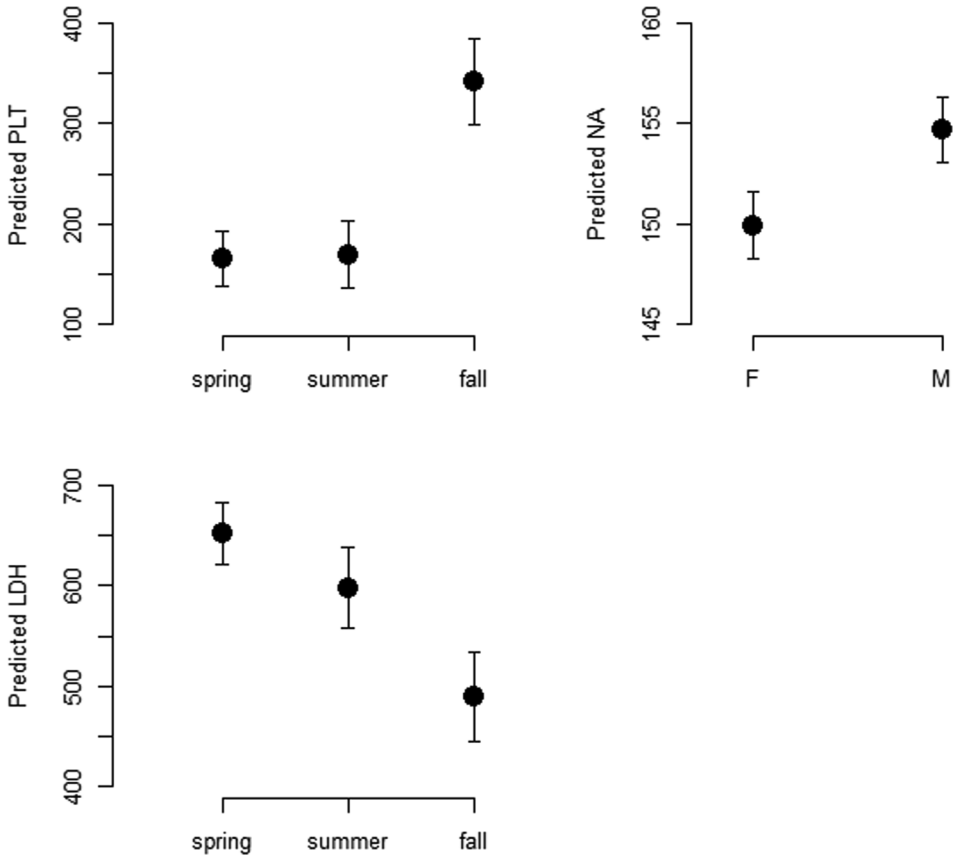

Fig. 2. Predicted values (mean ± 95% CI) based on the best fitted LMEs for the blood parameters considering only the samples from the permanent group (see Table 3 for details on each model).

transformed prior to analyses if necessary. Predicted plots based on the best-fitted models are presented.

3. Results

3.1. Summary statistics for haematology and biochemistry parameters

A total of 503 blood samples taken from 51 individuals were analysed for haematology and biochemistry parameters. Some free-ranging individuals were sampled twice; at the beginning and at the end of the capture which explains the higher number of samples compared to the number of individuals. Samples spanned nearly 15 years from 22nd April 1997 to 7th August 2012. The number of individuals and samples for each blood parameter are presented in Tables 1 and 2 a and b. The number of samples was greater for females (n = 335) compared to males (n = 166), and for adults (n = 336) compared to juveniles (n = 167) (Table 1). The majority of the samples came from the permanent animals (n = 449) compared to *free-ranging group* animals (n = 54). The samples were collected equally between the four seasons except for the free-ranging animals for which no samples were collected in winter. More samples from healthy (n = 277) animals were collected compared to sick ones (n = 79) in the *permanent group*.

Summary statistics for each blood parameter for *free-ranging group* and *permanent group* individuals are presented in Table 2 a and b. Although it is not possible to test statistically the differences between both groups due to biased sampling, the distribution of several parameters differed between free-ranging and permanent animals (Fig. 1, Table 2). The percent of lymphocytes was higher for *permanent group* animals with a median of 44% compared to 20% for *free-ranging group* animals. Concentrations for AST and ALT were greater, and urea smaller, for free-ranging animals compared to permanent ones (Fig. 1 and Table 2).

3.2. Effects of sex, season, and age group on haematology and blood biochemistry in free-ranging animals

Most of the haematology and biochemistry values, with the exception of PLT, LDH and Na, were not influenced by sex, season or age group. PLT and LDH varied significantly across seasons with values from the fall being greater and lower in spring and summer, respectively (Fig. 2 and Table 3). Values for Na were significantly greater for males compared to females. It is worth noting that the Δ BIC between these best-fitted models and the null models were lower than 1 suggesting that the influence of the tested parameters were minimal (Table 3). Similarly, six null models were competing with models including sex, suggesting that this variable might influence RBC, HGB, eosin, triglycerides, cholesterol and creatinine to some extent.

3.3. Influence of health status on haematology and blood biochemistry in permanent animals

HGB, MCV, ALC, WBC, and ALP values were all influenced by individual health status (Fig. 3, Tables 4, 5). Sick individuals had lower HGB, MCV, and ALP values but higher WBC and ALC. As previously, it is worth noting that AGC and creatinine were marginally influenced by the health status of the individuals as shown by the Δ BIC less than 1 between both models (Tables 4 and 5).

Table 3
Best-fitted linear mixed-effect models (LMEs) structure for each blood parameter for the free-ranging group. The full models include age group (juvenile or adult), sex (male or female) and season (spring, summer or fall). The difference in Bayesian Information Criteria (Δ BIC) is presented between the best-fitted model and the next competing model. The sample size is also indicated.

Response variable	Best fitted model structure	DF	Delta BIC	Sample size
Red blood cells	~1	3	< 1	47
Haemoglobin	~1	3	< 1	47
Mean cell volume	~1	3	1.8	47
Mean corpuscular haemoglobin concentrate	~1	4	3.4	47
Platelets	~season	5	1.5	47
Eosinophilc granulocytes	~1	3	< 1	47
White blood cells	~1	5	1.2	47
Lymphocyte	~1	3	3.4	43
Monocyte	~1	3	5.5	43
Absolute monocyte count	~1	4	3.8	23
Absolute granulocyte counts	~1	3	2.3	23
Calcium	~1	3	3.2	31
Iron	~1	3	1.9	29
Potassium	~1	3	3.2	28
Sodium	~1	4	< 1	32
Phosphorus	~1	3	2.2	35
Gamma glutamyl transferase	~1	3	3.3	34
Alkaline phosphatase	~1	6	3.2	29
Lactic acid dehydrogenase	~season	5	< 1	36
Triglyceride	~1	3	< 1	37
Cholesterol	~season + age group	6	< 1	39
Urea	~1	3	2.2	39
Creatinine	~1	3	< 1	39
Total protein	~1	3	2.2	38

3.4. Long term patterns of haematology and blood biochemistry in permanent animals

MCV, monocyte, lymphocyte, ALP, and AST were influenced by age (Fig. 3b, c, d, f, Table 6). MCV and ALP decreased with age, while Mono and AST decreased or stayed stable for younger animals, but increased from ca 1500 days old (ca 4 years) (Fig. 3d and f). Around the same age, lymp showed the opposite trend (Fig. 3c). HGB and cholesterol were influenced by season; HGB was highest in spring and winter and lowest in summer and fall while cholesterol was highest in fall (Fig. 3a and g, Table 6).

4. Discussion

Reference interval values for haematology and biochemistry biomarkers constitute important diagnostic tools to assess the health status of wild and captive individuals. However, reference values for free-ranging populations are usually scarce and limited to few individuals, which impairs overall validity. Here, we combine the values based on point samples from free-ranging individuals and longitudinal samples from managed-care individuals allowing use to evaluate the influence of inter and intra individual variability as well as biological and ecological factors. This constitutes the largest dataset of harbour porpoises to date and thus the most comprehensive summary of possible values for this species.

The range of haematology and biochemistry values of the free-ranging harbour porpoises in the present dataset (Table 1) were comparable to those of harbour porpoises from the Bay of Fundy, Canada (Koopman et al., 1995, 1999) and of animals in human care from previous studies (Andersen, 1966, 1968; Nielsen and Andersen, 1982; Kastelein et al., 1990). The range of absolute WBC for six harbour porpoises studied in the 1960′s was 8.0 G/l higher than the results presented in this study (Andersen, 1966). Differences were also seen in the biochemistry values of 19 captive animals presented by Andersen (1968), including a cholesterol mean of 32.2 mg/100 ml compared to 0.0495 mg/100 ml (1.28 μmol/L) in the present study. Other values were similar between the Andersen study and the present study, including Fe, with a mean of 0.103 mg/100 ml (Andersen, 1968) to 0.103 mg/100 ml (18.38 μmol/l) in the present study. Erythrocyte values measured in 19 harbour porpoises kept in Strib, ranging from a period of a few days to three years, were also similar (mean: 5.2 T/l) to the animals investigated in this study (5.28 T/l) (Nielsen and Andersen, 1982). Differences between these studies may also be the result of several decades of analytical and medical progress in laboratory techniques and marine mammal husbandry. But it can also not be excluded that some of the samples from the older studies were deteriorated.

The highest values for eosinophils were found in the free-ranging group, most likely reflecting their higher parasitic burden, which has been confirmed in previous pathological investigations (Siebert et al., 2001, Lehnert et al., 2005). Lower levels of WBC and eosinophils were found in the animals permanently kept. This suggests that the animals were less exposed to environmental pathogens, such as parasites, bacteria and viruses, as well as environmental stressors, including noise and contaminants, which are known to affect general stress levels via endocrine disruption and immune suppression (Beineke et al., 2005; Southall, 2005; Das et al., 2006). The permanent group was regularly given anti-parasitic treatments, which likely contributed to decreased eosinophils. The regular feeding and controlled food quality removed any chronic nutritional stress in the permanent animal group. It is hypothesized that the permanent animals enclosed in a semi-natural environment had lower levels of chronic environmental stress compared to free-ranging individuals in the highly industrialized Baltic Seas. Higher WBC levels were also observed in wild belugas (*Delphinapterus leucas*) (St. Aubin and Geraci, 1989; Norman et al., 2012) and killer whales (*Orcinus orca*) (Cornell 1983), when compared to animals in human care.

The seasonality of several variables showing lowest values in autumn have also been observed in a study of wild belugas in Bristol Bay, Alaska, USA, where animals captured in September showed lower WBC, neutrophils, lymphocytes, HCT, and BUN than animals captured in May (Norman et al., 2012). Seasonal variations in blood values have also been found in a long-term study of bottlenose dolphins in Sarasota Bay (Hall et al., 2007). It was assumed that this might be a result of hydration, feeding, diving behaviour, and changes in water temperature, all of which affect reproduction and survival.

The reason for lower WBC levels in free-ranging adult females in the present study remains unclear, but may be influenced by the combined energetic demand of pregnancy and lactation in late summer and early autumn. This has also been observed in managed-care animals as harbour porpoises had their highest energetic demands during this period, while their body weight was lowest (Lockyer et al., 2003). Lower WBC and higher energetic demands would also support female harbour porpoises' vulnerability to pathogens and stress during and after pregnancy. This is consistent with a general lower immune function in pregnant mammals, reducing the risk of spontaneous abortion (Bainbridge, 2000; Barratclough et al., 2020).

Regardless of group and season, juveniles had higher blood values for RBC and eosin than adults. Age related RBC differences have also been reported for bottlenose dolphins from Sarasota Bay (Hall et al., 2007; Venn-Watson et al., 2007), but not for harbour porpoises from the Bay of Fundy (Koopman et al., 1999). It has been suggested for

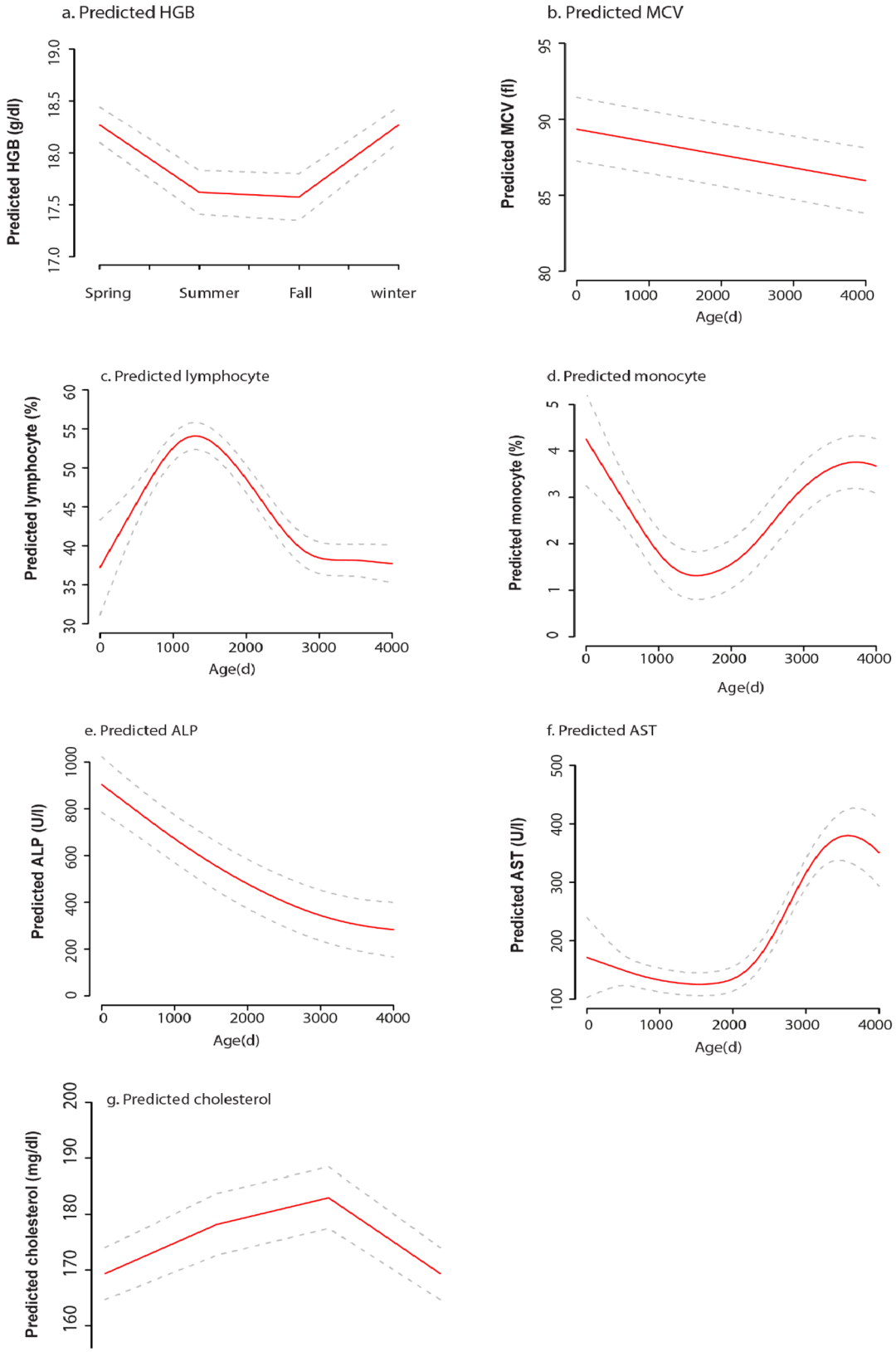

Fig. 3. Predicted values (mean ± 95% CI) based on the best fitted LMEs or GAMMs for the blood parameters considering only the samples from the permanent group of animals deemed healthy (see Table 5 for details on each model).

Table 4
Summary statistics of the blood parameters according to health status. Health status was only available for some samples of the permanent animals. Health status was defined as follows: Healthy: animals clinically healthy and non-treated. Sick: animals clinically unhealthy and pharmaceutically treated.

	Healthy												
Blood parameter	n samples	n individuals	min	10th	CI10low	CI10high	median	CImedlow	CImedhigh	90th	CI90low	CI90high	max
Red blood cells	264	4	2.98	5.05	4.93	5.18	5.80	5.72	5.85	6.40	6.35	6.49	7.13
Haemoglobin	262	4	12.90	15.80	15.34	16.23	18.20	18.00	18.33	19.60	19.36	19.77	22.30
Mean cell volume	258	4	61.00	84.70	83.82	86.02	89.00	88.20	89.50	93.00	92.52	93.85	130.00
Mean corpuscular haemoglobin concentrate	258	4	22.90	32.97	32.66	33.53	35.00	34.76	35.35	38.53	37.74	39.47	77.30
Platelets	262	4	31.00	120.10	112.31	128.45	159.00	155.15	162.64	226.00	210.72	240.32	666.00
Mean platelet volume	138	3	6.00	9.00	8.81	9.15	9.80	9.63	9.94	10.63	10.45	10.83	11.60
Eosinophilc granulocytes	191	4	0.00	0.00	0.00	0.00	6.00	4.49	7.84	17.00	12.69	20.42	49.30
White blood cells	264	4	1.40	2.20	2.09	2.31	3.00	2.89	3.14	4.67	3.85	5.29	11.80
Lymphocyte	260	4	2.00	28.00	24.10	30.57	44.75	41.72	46.93	64.00	60.93	66.92	82.00
Monocyte	253	4	0.00	0.00	0.00	0.00	2.00	1.97	2.03	5.80	4.62	7.60	12.00
Absolute lymphcyte count	137	3	0.00	0.00	0.00	0.00	1.20	1.14	1.35	2.30	2.07	2.57	6.80
Absolute monocyte count	137	3	0.00	0.00	0.00	0.00	0.10	0.09	0.11	1.70	1.38	1.97	2.80
Absolute granulocyte counts	100	3	1.20	1.40	1.28	1.54	2.00	1.80	2.17	3.40	2.76	4.02	9.20
Calcium	191	4	1.47	2.20	2.16	2.27	2.44	2.39	2.49	2.79	2.71	2.87	3.49
Iron	158	3	5.00	21.75	17.50	25.24	42.75	39.26	46.21	62.63	58.16	66.27	93.70
Chlorine	34	3	86.00	96.60	91.23	102.49	110.00	104.50	115.35	133.50	107.32	156.22	201.00
Potassium	184	4	1.80	2.80	2.52	3.01	3.80	3.69	3.92	4.60	4.46	4.74	8.94
Magnesium	72	3	0.48	0.61	0.55	0.65	0.73	0.69	0.76	0.90	0.78	1.00	1.08
Sodium	168	4	115.00	149.00	145.27	152.11	156.00	155.43	157.06	160.00	159.45	161.23	163.00
Phosphorus	190	4	0.41	1.34	1.25	1.45	1.89	1.80	1.97	2.48	2.38	2.65	8.76
Gamma glutamyl transferase	169	3	5.00	7.80	7.11	9.05	12.00	11.26	13.25	22.20	16.24	26.47	188.00
Alkaline phosphatase	179	4	37.00	130.00	114.96	147.01	305.00	263.53	338.36	672.20	578.51	769.29	1812.00
Aspartate aminotransferase	173	3	52.00	107.40	103.63	110.64	134.00	130.12	138.35	293.00	258.86	320.61	937.00
Alanin aminotransferase	214	4	5.00	31.00	29.91	32.73	43.00	39.45	45.54	103.70	82.50	127.08	848.00
Lactic acid dehydrogenase	171	3	171.00	384.00	373.13	393.05	467.00	449.24	484.21	635.00	580.11	679.50	899.00
Triglyceride	175	3	8.00	74.00	67.70	82.13	114.00	100.10	124.30	230.00	194.88	268.76	673.00
Cholesterol	187	4	63.00	137.60	128.93	144.63	174.00	169.17	181.62	205.40	201.29	210.48	245.00
Urea	173	3	33.00	73.00	69.83	76.76	93.00	89.90	95.86	118.80	114.03	125.91	145.00
Creatinine	209	4	0.20	0.45	0.37	0.52	0.69	0.66	0.73	0.90	0.86	0.93	2.00
Albumin	38	3	25.00	29.00	27.44	32.06	31.00	29.52	32.44	36.30	34.75	38.46	37.00
Blood urea nitrogen	38	3	8.00	10.70	8.75	12.48	14.00	12.90	15.32	18.00	16.40	19.85	19.00
Globuline	38	3	26.00	34.40	29.59	39.88	43.50	40.62	47.07	52.00	48.55	55.70	56.00
Total protein	205	4	22.60	66.04	64.83	67.22	71.50	70.73	72.33	79.60	77.94	81.76	92.00
	Sick												
Blood parameter	n samples	n individual	min	10th	CI10low	CI10high	median	Cimedlow	CImedhigh	90th	CI90low	CI90high	max
Red blood cells	78	4	2.74	4.76	4.03	5.50	6.00	5.90	6.11	6.68	6.57	6.88	7.61
Haemoglobin	78	4	8.80	14.08	12.56	15.75	17.50	17.09	17.87	19.03	18.78	19.32	20.20
Mean cell volume	77	4	51.00	80.00	78.75	81.37	82.00	81.62	82.39	88.00	84.02	91.34	97.00
Mean corpuscular haemoglobin concentrate	77	4	29.00	33.00	32.42	33.70	35.10	34.68	35.52	37.44	35.72	38.78	65.20
Platelets	76	4	11.00	74.50	56.00	94.48	151.50	139.20	164.10	250.00	235.09	274.23	405.00
Mean platelet volume	65	3	6.40	8.14	7.77	8.40	8.80	8.57	8.95	10.30	9.67	10.88	11.50
Eosinophilc granulocytes	14	3	0.00	3.30	0.64	6.51	8.00	0.94	13.72	29.92	15.06	50.37	38.60
White blood cells	78	4	2.60	3.87	2.85	4.61	7.75	6.56	8.67	12.30	11.42	13.26	17.10
Lymphocyte	76	4	6.00	22.80	17.88	28.10	41.00	37.86	43.55	51.80	48.06	54.95	72.00
Monocyte	76	4	0.00	1.20	NA	2.48	3.00	2.94	3.07	4.00	2.77	4.64	11.40
Absolute lymphcyte count	62	3	1.00	1.21	0.55	1.60	3.10	2.55	3.50	5.40	4.60	6.14	7.40
Absolute monocyte count	62	3	0.00	0.10	0.04	0.21	0.20	0.16	0.25	0.49	0.41	0.66	1.10
Absolute granulocyte counts	62	3	1.50	2.31	1.88	2.72	4.20	3.67	4.77	6.19	4.91	7.21	13.70
Calcium	55	4	0.50	2.28	2.16	2.42	2.49	2.44	2.53	2.76	2.51	2.95	3.33
Iron	8	2	5.00	12.77	NA	24.17	24.55	15.91	32.27	46.15	NA	85.44	82.90
Chlorine	4	1	90.00	95.70	75.64	109.25	112.00	99.39	128.90	118.50	112.40	128.43	120.00
Potassium	55	4	1.70	3.34	2.93	3.89	4.10	3.94	4.30	4.98	4.29	5.65	6.60
Magnesium	6	2	0.70	0.71	0.66	0.74	0.77	0.73	0.81	0.80	0.78	0.82	0.80
Sodium	51	3	142.00	147.00	143.34	149.23	153.00	151.82	154.03	162.00	159.51	166.65	164.00
Phosphorus	53	3	1.04	1.40	1.29	1.52	1.66	1.49	1.79	2.13	1.70	2.46	7.07
Gamma glutamyl transferase	10	2	15.00	16.80	11.81	20.58	25.50	18.63	33.14	42.90	28.83	62.42	51.00
Alkaline phosphatase	51	3	42.00	195.00	79.22	336.20	385.00	276.77	456.76	743.00	595.15	839.96	1127.00
Aspartate aminotransferase	10	2	99.00	112.50	77.53	142.11	177.50	79.15	259.68	391.10	289.84	572.67	419.00
Alanin aminotransferase	59	4	35.00	44.00	41.36	46.31	60.00	55.81	66.66	118.20	74.33	172.83	208.00
Lactic acid dehydrogenase	10	2	388.00	440.20	375.20	509.44	510.00	434.56	573.10	678.30	545.09	849.34	762.00
Triglyceride	10	2	63.00	64.80	50.42	74.54	94.50	55.35	131.01	195.70	105.15	307.30	256.00
Cholesterol	14	3	116.00	131.30	98.17	155.35	188.00	162.31	216.28	223.40	172.77	260.44	274.00
Urea	10	2	66.00	69.60	63.15	75.22	76.50	66.43	83.33	99.30	88.53	120.18	102.00
Creatinine	52	4	0.24	0.35	0.30	0.40	0.49	0.42	0.54	0.78	0.66	0.93	1.01
Albumin	48	3	19.00	27.00	25.55	28.52	31.00	30.14	32.25	36.00	34.76	38.50	38.00
Blood urea nitrogen	48	3	7.00	11.70	10.31	13.06	14.00	13.05	14.62	17.00	16.30	17.90	19.00

(*continued on next page*)

Table 4 (*continued*)

	Healthy												
Blood parameter	n samples	n individuals	min	10th	CI10low	CI10high	median	CImedlow	CImedhigh	90th	CI90low	CI90high	max
Globuline	48	3	3.80	34.40	30.24	39.54	40.00	38.15	41.06	45.30	43.08	47.22	56.00
Total protein	59	4	55.00	64.80	61.29	68.20	72.00	70.54	73.32	77.20	73.11	80.37	85.00

Table 5
Best-fitted linear mixed-effects models (LMEs) structure for each blood parameter using data from permanent group of animals. All models are fitted with a correlation and random structures. The difference in Bayesian Information Criteria (Δ BIC) is presented between the best fitted model and the next competing model. The number of samples for each blood parameter can be found in Table 4.

Response variable	Best fitted model structure	Δ BIC with the null model
Red blood cells	Null model	3.7
Haemoglobin	~health	5.9
Mean cell volume	~health	< 1
Mean corpuscular haemoglobin concentrate	Null model	4.7
Platelets	Null model	5.4
Eosinophilc granulocytes	Null model	3
White blood cells	~health	27
Lymphocyte	Null model	3.3
Monocyte	Null model	5.8
Absolute lymphcyte count	~health	7.7
Absolute monocyte count	Null model	5.2
Absolute granulocyte counts	Null model	< 1
Calcium	Null model	3
Iron	Null model	1.3
Potassium	Null model	4.7
Magnesium	Null model	4.3
Sodium	Null model	5.1
Phosphorus	Null model	4.4
Chlorine	Null model	3.5
Gamma glutamyl transferase	Null model	5
Alkaline phosphatase	~health	22.8
Aspartate aminotransferase	Null model	4.5
Alanin aminotransferase	Null model	4.3
Albumin	Null model	3
Lactic acid dehydrogenase	Null model	5.2
Triglyceride	Null model	5.3
Cholesterol	Null model	4.3
Urea	Null model	3.2
Creatinine	Null model	< 1
Total protein	Null model	5
Globuline	Null model	5
Blood urea nitrogen	Null model	5

different dolphin species that this may be connected to changes in diving physiology, as the animal's ability and adaptation to diving may evolve with age, but it is also possible this is an effect of the growth and development of the animal's immune system (Bossart et al., 2001). However, telemetry investigations on porpoises from Denmark and Greenland have shown that mother and calf pairs, juveniles, and adults all had similar dive durations and depths (Teilmann et al., 2007; Nielsen et al., 2018).

Lower HGB counts were observed on *permanent group* animals in the summer and fall compared to other seasons, like in *free-ranging group*. In free-ranging animals, this was likely explained by nutritional stress associated with the decreasing water temperatures and the building up of blubber reserves before the winter (Hall et al., 2007). Although permanent animals were fed according to their energetic demand and were not nutritionally stressed, they still experienced seasonal changes in air and water temperature, and consequentially in blubber thickness (Lockyer et al., 2003), and these changes might also be reflected in the RBC values. The age-related changes for MCV, lymphocytes, monocytes, AST and ALP were most likely due to changes in the development of the immune system and metabolic capacity, as already suggested for bottlenose dolphins (Hall et al., 2007). Parameters most strongly associated with the health status of the individual were WBC, GGT, and LDH. These biomarkers are therefore suggested for further investigation for use as health indicators in managed-care and free-ranging harbour porpoises. Understanding when and why harbour porpoises exhibit changes in haematology and biochemistry values is essential to conduct successful husbandry and medical care of captive marine mammals (Bossart et al., 2001). This knowledge is also crucial when assessing health of free-ranging porpoise populations.

5. Conclusions

HGB, MCV, WBC, ALC and ALP values were all influenced by animal health status in managed-care harbour porpoises and are therefore candidate parameters for assessing the health status of wild porpoises. Seasonality and age also influenced the range of hæmatologic and biochemistry parameters, including HGB, MCV, lymphocytes, monocytes, ALP, AST and cholesterol. The presented results underline that seasonality and animal age are important to consider when assessing health status based on blood analysis. This present study strengthens and presents further nuances and insights of the factors influencing blood biomarkers in a large dataset from free-living harbour porpoises. The identification of reference ranges and suitable health indicators are crucial for future assessments of health impacts in wild harbour porpoises in the Baltic, which face important conservation threats.

Authors contributions statement

US designed the study, sampled porpoises in Denmark, conducted analyses, treated results and wrote the manuscript. MAB contributed equally to the study and is shared first co-author, sampled porpoises in Denmark, conducted statistical analyses, treated results and wrote the manuscript. GD sampled porpoises and designed sampling in Denmark and approved the manuscript. JT sampled free-living porpoises and approved the manuscript. KAH samples porpoises in Denmark, treated results, wrote the manuscript and as native speaker approved the english. JK sampled porpoises and designed sampling in Denmark and approved the manuscript.

CS did data treatment and interpretation of results and approved the manuscript. RD sampled free-living porpoises and approved the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Table 6
Best-fitted linear mixed-effects models (LMEs or GAMMs) structure for each blood parameter using data from healthy captive animals. All models are fitted with a correlation and random structures. The difference in Bayesian Information Criteria (Δ BIC) is presented between the best fitted model and the next competing model. The number of samples for each blood parameter is also presented as is the number of degrees of freedom for each chosen model (DF).

Response variable	Best fitted model structure	DF	Delta BIC	Sample size	Next best competing model	Model
Red blood cells	~1	4	4.4	264	~season	GAMM
Haemoglobin	~season	5	1.9	262	~1	GAMM
Mean cell volume	~age	6	4	258	~1	LME
Mean corpuscular haemoglobin concentrate	~1	4	5	258	~age	GAMM
Platelets	~1	4	5.9	262	~season	GAMM
Eosinophilc granulocytes	~1	4	3.8	191	~season	GAMM
White blood cells	~1	4	5.4	264	~season	GAMM
Lymphocyte	~age	6	4.3	260	~season + age	GAMM
Monocyte	~age	6	6.1	253	~season + age	GAMM
Absolute monocyte count	~1	4	10	137	~season	GAMM
Calcium	~1	4	5.2	191	~season	GAMM
Iron	~1	4	4.1	158	~season	GAMM
Potassium	~1	4	1.2	184	~age	GAMM
Sodium	~1	4	5.1	168	~season	GAMM
Phosphorus	~1	4	4.2	190	~season	GAMM
Gamma glutamyl transferase	~1	4	3.2	168	~age	GAMM
Alkaline phosphatase	~age	6	4.2	179	~season + age	GAMM
Aspartate aminotransferase	~age	6	6.4	173	~1	GAMM
Alanin aminotransferase	~1	4	< 1	274	~age	GAMM
Lactic acid dehydrogenase	~1	4	1.2	171	~age	GAMM
Triglyceride	~1	4	5	175	~season	GAMM
Cholesterol	~season	5	< 1	187	~1	GAMM
Urea	~1	4	5.5	173	~season	GAMM
Creatinine	~1	4	2.2	209	~season	GAMM
Total protein	~1	4	2	205	~age	GAMM

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