Differences in haematology and blood chemistry between the lesser mouse-eared bat (*Myotis blythii*) and its sibling species the greater mouse-eared bat (*Myotis myotis*)

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Abstract

While bats are important reservoirs of infectious agents, they play a unique and irreplaceable role in the ecosystem. Nevertheless, they are now threatened by a wide range of negative influences and are increasingly becoming the subject of strict protection. A collection of reference haematological and biochemical indicator ranges can significantly contribute to the assessment of both individual and population health status. Thus, the aim of this study was to compare blood parameters of two sibling species, the lesser mouse-eared bat *Myotis blythii* and the greater mouse-eared bat *Myotis myotis*, to assess any differences between males and females and to establish reference intervals for blood parameters. A total of 51 bats were captured (22 × *M. blythii*, 29 × *M. myotis*). Reference ranges were established for haematocrit, haemoglobin, potassium, urea, glucose, pH, partial dissolved carbon dioxide, total dissolved carbon dioxide, bicarbonate, base excess, alanine aminotransferase, calcium, creatinine, total protein and globulin. For most parameters, there was no influence of species or sex; however, species differences were found for albumin, sodium, chloride, phosphorus and anion gap, and sex differences for total bilirubin, alkaline phosphatase, and amylase. The data obtained will prove useful in clinical diagnostics and care of *M. myotis* and *M. blythii* in wildlife rescue centres and in research into the effects of infectious diseases, toxic substances or other negative factors affecting these bat species.

Chiroptera, haematology, swarming, reference ranges

Being the only actively flying mammals, bats play a unique and irreplaceable role in the ecosystem (Kunz et al. 2011). Like most bats, the metabolisms of insectivorous temperate zone bats shift from extremely low during hibernation to extremely high during active flight or reproduction. As a result, these bats have developed numerous adaptations to ensure their fitness throughout their annual life cycle (Davis and Reite 1967; Boyles et al. 2016). In recent years, researchers have shown growing interest in bats, especially their physiology and immunology, as they are important reservoirs for numerous infectious agents (Calisher et al. 2006). Nevertheless, very little is known about pathogenic processes in bats. Moreover, many bat species are now of conservation concern, partly due to widespread outbreaks of white nose syndrome (Frick et al. 2010) but also because of the negative impacts of chemical and light pollution and wind power plants (Rydell et al. 2010; Stone et al. 2015; Zukal et al. 2015; Zimmerling and Francis 2016; Kaňová et al. 2022; Bandouchova et al. 2024).

Haematology and blood chemistry indices reflect the internal status of an organism and thus represent an important tool for health assessment for both domestic and wild animals (Wobeser 1994), whether from an individual perspective in wildlife rescue

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centres or from a scientific perspective, such as when monitoring the overall health status of a population (Maceda-Veiga et al. 2015), assessing the effects of pathogenic agents (Brock et al. 2013) or monitoring physiological (López-Olvera et al. 2006), ecological or nutritional (Brock et al. 2013) status. Several intrinsic and extrinsic factors can affect blood parameters (Tryland 2006) and numerous studies have been undertaken to assess haematology and blood chemistry reference ranges for wildlife species, though very few on insectivorous bats. Those that have been published were based on a low number of samples (Wolk and Bogdanowicz 1987; Wolk and Ruprecht 1988; Albayrak et al. 2016) and focused mainly on haematological and/or acid base parameters (Bandouchova et al. 2018; Bandouchova et al. 2020) or blood urea (Bassett 2004). The only study to date assessing blood chemistry parameters, such as total protein, albumin, creatinine or liver enzymes, was based on just 10 greater mouse-eared bats (*Myotis myotis*; Paksuz 2022).

The low body weight of vespertilionid bats, ranging from 4 to 63 g, represents a serious obstacle when determining blood chemistry values (Macdonald and Barrett 1993; Thabah et al. 2007). As sampling more than 10% of total blood volume (~1% of body weight; Joslin 2009) is not recommended in bats, the maximum sample volume that can be taken in larger insectivorous bats is approximately 250–300 µl and, until recently, procedures allowing analysis of such small blood samples were unavailable. Recent technological advances, however, mean it is now possible to perform such analyses on small volumes of blood (Verant et al. 2014; Bandouchova et al. 2018; Linhart et al. 2020; Linhart et al. 2022), allowing us to determine reference ranges for haematological and blood chemistry parameters in bats. Reference ranges from active animals can be used to assess the overall health status of an individual or be compared with values from animals at different life-stages, such as hibernation or lactation, to evaluate their impact on metabolic status (Wimsatt et al. 2005).

The aim of this study was to (i) determine reference intervals for selected haematological and blood chemistry parameters in *M. myotis*, one of the largest bat species in Europe weighing up to 35 g, and the lesser mouse-eared bat (*Myotis blythii*), its slightly smaller sibling species weighing up to 29 g (Arlettaz et al. 1991), (ii) to compare results between the two species, and (iii) to compare results between males and females of each species. We hypothesised that there would be no significant differences between the two species for most parameters. On the other hand, we expected there to be slight sex differences as the females would have weaned their young shortly before capture; hence, it could be expected that some indicators in females would deviate slightly from those in males due to previous gestation and lactation, as had been documented in lactating noctule bats (Pikula et al. 2017).

Materials and Methods

Collection and analysis of blood samples

Bats were caught using mist nets during the swarming period in late summer and early autumn of 2015 and 2016, *M. myotis* being caught in front of the Katerinska Cave (49.3607006N, 16.7102508E) in the Moravian Karst and Ledové sluje in the Podyji National Park (48.8846406N, 15.8448672E), Czech Republic (Bandouchova et al. 2020), and in front of the Lednitsata Cave (41.6489419N, 24.5262217E) in Western Rhodopes, Bulgaria (Dundarova 2018), while *M. blythii* were caught in front of the Orlova chuka Cave (43.5933164N, 25.9600819E) in Bulgaria. Species identification in the field was determined based on forearm length and shape of the face and ears (Arlettaz et al. 1997a). Sampling complied with Czech Law No. 114/1992 on Nature and Landscape Protection and was based on permits 01662/MK/2012S/00775/MK/2012, 866/JS/2012 and 00356/KK/2008/AOPK issued by the Nature Conservation Agency of the Czech Republic and permits 645/13.08.2015 and 683/04.07.2016 issued by the Bulgarian Ministry of Environment and Water.

Once caught, each bat was quickly and carefully removed from the net and a sample of blood collected from a punctured uropatagial vein using a micropipette, as described in Bandouchova et al. (2020). Blood parameters were measured using the i-STAT analyser (Abaxis, Union City, USA) fitted with EC8+ cartridges (Abaxis, Union City, USA) for haemoglobin, haematocrit, glucose, urea, sodium, potassium, chloride, total dissolved carbon dioxide (tCO_2), partial dissolved carbon dioxide (pCO_2), bicarbonate (HCO_3), pH, base excess (BE) and anion gap

(AnGap), and the VetScan analyser (Abaxis, Union City, USA) with the Comprehensive Diagnostic Profile rotor (Abaxis, Union City, USA) for albumin, alkaline phosphatase, alanine aminotransferase, amylase, total bilirubin, calcium, phosphorus, creatinine, total protein and globulin. From the total of 29 *M. myotis* (17 male, 12 female) and 22 *M. blythii* (9 male, 13 female) caught, blood samples were taken from 29 *M. myotis* using iSTAT and 17 using VetScan, and from 21 *M. blythii* using iSTAT and 18 using VetScan. After sampling, the animals were given a 5% glucose solution (B. Braun, Melsungen, Germany) orally and released.

Statistical analysis

To assess the influence of sex and species on blood parameters, we used either general linear models (GLM) or generalised linear models (GLZ), performed in R v.4.2.1 (R Core Team 2022). Prior to fitting the model, outliers were identified using the Grubbs test and removed from the dataset. For each blood parameter, we used the GLM formula parameter_values ~ species*sex as the maximal model. Based on the results, non-significant factors were removed one-by-one and the minimal model was used for analysis. In cases of heteroscedasticity, a GLZ was used with the setting Gamma(link = log) or a GLM with logarithmic transformation of data. For each parameter, data normality was tested using Shapiro-Wilks test and based on the result, reference ranges were set as described in Cray (2015), using the Reference Value Advisor v.2.1 (Geffré et al. 2011) for parameters that did not differ between *Myotis* species. In each case, significance was set as $P < 0.05$.

Results

A significant difference was recorded between *M. blythii* and *M. myotis* blood sodium (GLZ: Na ~ species; $P < 0.05$, model explains 11.16% of variability in the data; Fig. 1), chloride (GLZ: Cl ~ species; $P < 0.05$, model explains 8.70% of variability in the data; Fig. 2), anion gap (GLZ: AnGap ~ species; $P < 0.05$, model explains 12.06% of variability in the data; Fig. 3), albumin (GLZ: albumin ~ species; $P < 0.05$, model explains 11.81% of variability in the data; Fig. 4) and phosphorus (GLZ: P ~ species; $P < 0.01$, model explains 24.35% of variability in the data; Fig. 5), with values measured in *M. blythii* higher in each case. Significantly higher levels of alkaline phosphatase were recorded in females (GLZ: ALP ~ sex; $P < 0.05$, model explains 11.94% of variability in the data; Fig. 6), and of total bilirubin in males (GLZ: t_bilirubin ~ sex; $P < 0.05$, model explains 11.94% of variability in the data; Fig. 7), while the amylase model showed significant interaction between sex and species in *M. myotis* (GLZ: amylase ~ sex*species; $P < 0.05$, model explains 18.44% of variability in the data; Fig. 8). In all models GLZ was used due to heteroscedasticity. Descriptive statistical characteristics determined for *M. blythii* (Table 1) and *M. myotis* (Table 2) were used to determine reference intervals for those parameters that did not differ between sexes or species (Table 3).

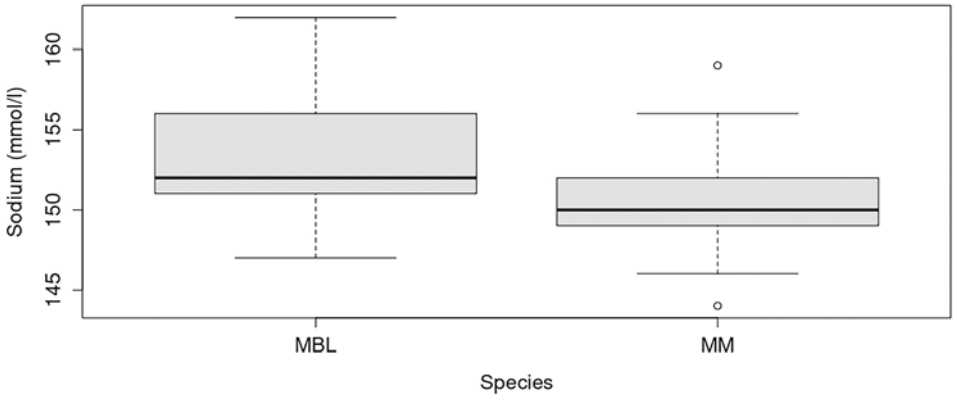

Fig.1. Difference in blood sodium between *M. blythii* (MBL) and *M. myotis* (MM). GLZ: sodium ~ species; $P < 0.05$; box plots show 1st and 3rd quartiles and median value, whiskers indicate 1.5 times interquartile range and circles indicate outliers.

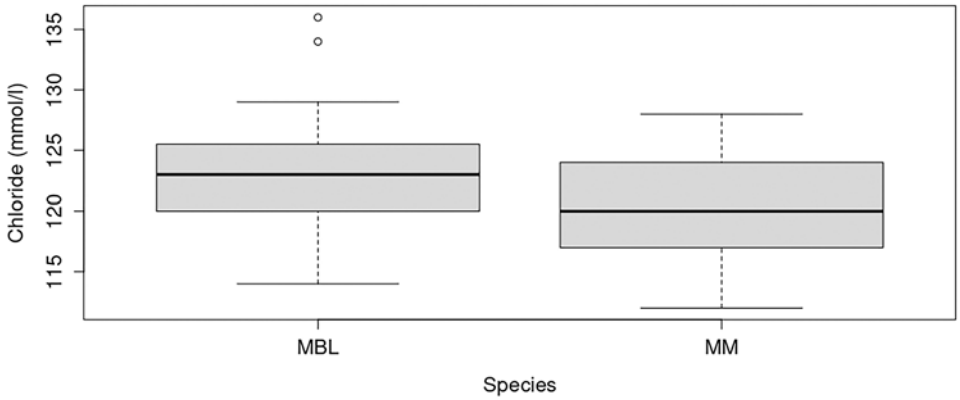

Fig.2. Difference in blood chloride between *M. blythii* (MBL) and *M. myotis* (MM). GLZ: chloride ~ species; $P < 0.05$; box plots show 1st and 3rd quartiles and median value, whiskers indicate 1.5 times interquartile range, circles indicate outliers.

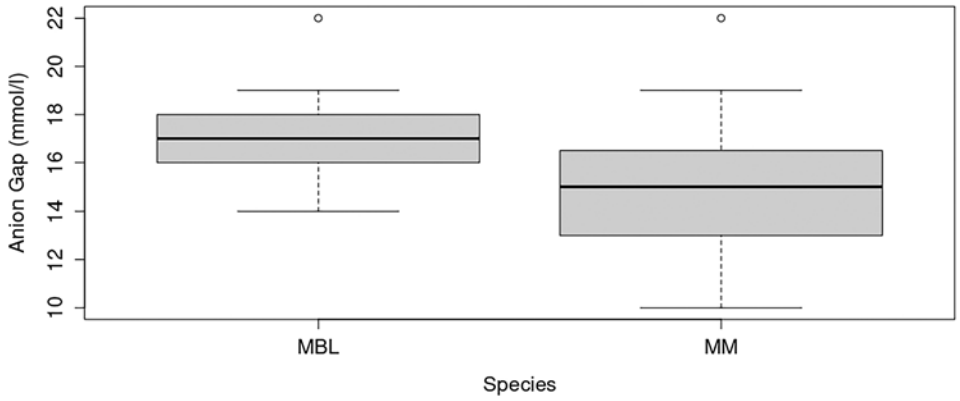

Fig.3. Difference in blood anion gap between *M. blythii* (MBL) and *M. myotis* (MM). GLZ: AnionGap ~ species; $P < 0.05$; box plots show 1st and 3rd quartiles and median value, whiskers indicate 1.5 times interquartile range, circles indicate outliers.

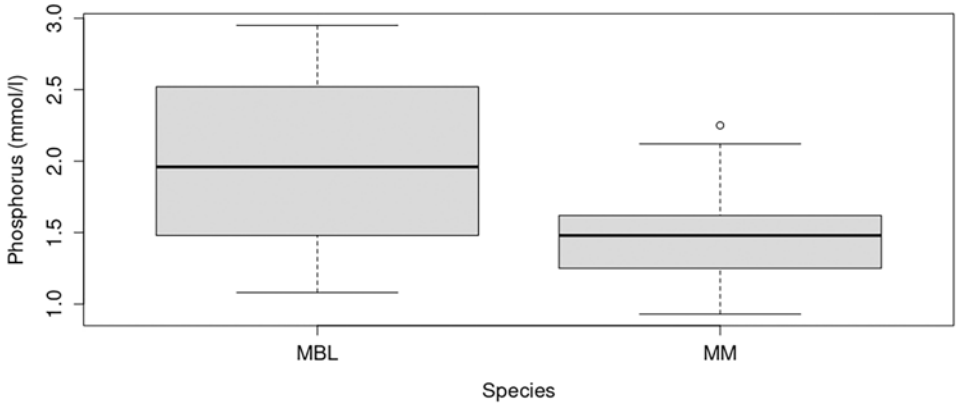

Fig.4. Difference in blood phosphorus between *M. blythii* (MBL) and *M. myotis* (MM). GLZ: phosphorus ~ species; $P < 0.01$; box plots show 1st and 3rd quartiles and median value, whiskers indicate 1.5 times interquartile range, circles indicate outliers.

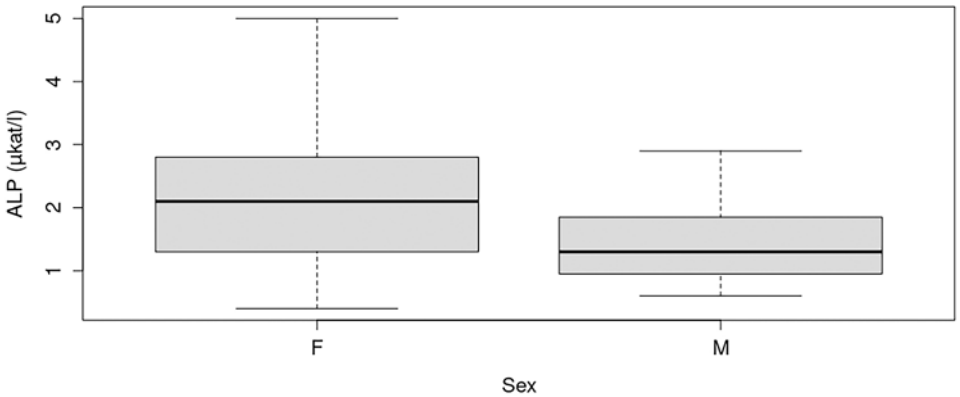

Fig.5. Difference in blood alkaline phosphatase between male (M) and female (F) *M. blythii* and *M. myotis*. GLZ: alkaline_phosphatase ~ sex; $P < 0.05$; box plots show 1st and 3rd quartiles and median value, whiskers indicate 1.5 times interquartile range.

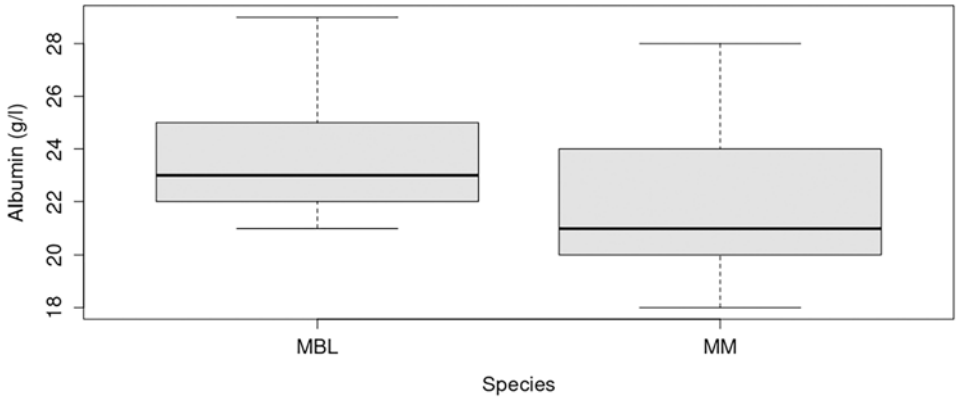

Fig.6. Difference in blood albumin between *M. blythii* (MBL) and *M. myotis* (MM). GLZ: albumin ~ species; $P < 0.05$; box plots show 1st and 3rd quartiles and median value, whiskers indicate 1.5 times interquartile range.

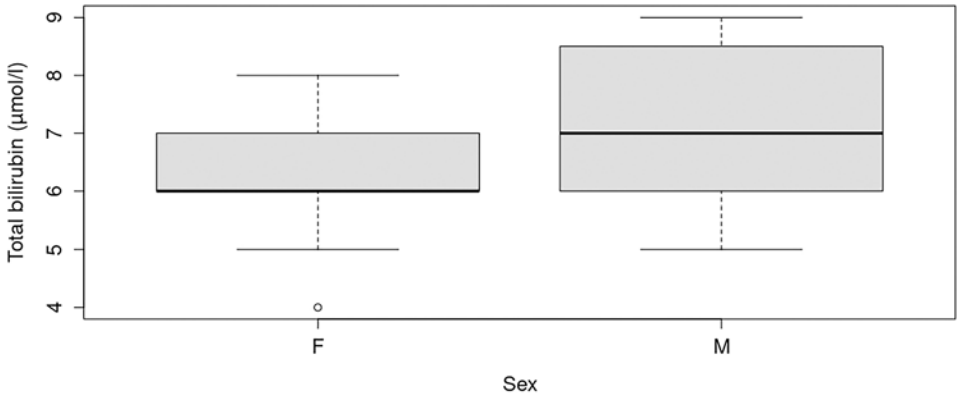

Fig.7. Difference in blood total bilirubin between male (M) and female (F) *M. blythii* and *M. myotis*. GLZ: total_bilirubin ~ sex; $P < 0.05$; box plots show 1st and 3rd quartiles and median value, whiskers indicate 1.5 times interquartile range and circles indicate outliers.

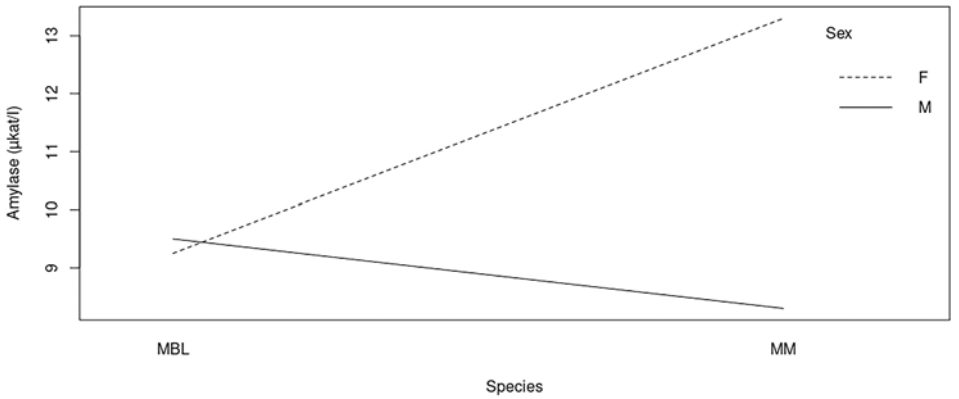

Fig.8. Interaction plot of blood amylase indicating a significant difference between *M. myotis* males and females. GLZ: amylase ~ sex * species; $P < 0.05$; MBL = *Myotis blythii*, MM = *Myotis myotis*, M = male, F = female.

Table 1. Haematology and blood chemistry parameters for the lesser mouse-eared bat (*Myotis blythii*).

Indicator	n	Mean	SD	Min	1stQ	Median	3rdQ	Max	CI (95%)	
Hct (l/l)	20	52.35	3.12	47.00	50.00	52.00	54.25	58.00	50.89	53.81
Hgb (g/l)	20	178.10	10.63	160.00	170.00	177.00	184.80	197.00	173.07	183.03
Na (mmol/l)	18	153.30	4.38	147.00	151.00	152.00	156.00	162.00	151.16	155.51
K (mmol/l)	20	6.83	1.76	3.70	5.65	7.05	8.60	9.00	6.00	7.65
Cl (mmol/l)	19	123.20	5.41	114.00	120.00	123.00	125.50	136.00	120.55	125.76
Urea (mmol/l)	20	24.34	10.42	10.10	17.50	22.15	29.20	50.00	19.47	29.22
Glu (mmol/l)	20	6.55	2.79	1.70	4.38	6.90	8.23	10.90	5.24	7.85
pH	20	7.23	0.05	7.14	7.21	7.24	7.26	7.34	7.21	7.26
pCO_2 (kPa)	20	6.76	0.96	4.86	5.96	6.82	7.29	8.54	6.31	7.20
tCO_2 (mmol/l)	21	23.00	3.18	15.00	22.00	24.00	25.00	28.00	21.55	24.45
HCO_3 (mmol/l)	21	20.90	3.20	13.30	19.50	21.70	23.30	25.50	19.44	22.36
BE (mmol/l)	20	-5.75	2.55	-12.00	-7.00	-6.00	-3.75	-2.00	-6.94	-4.56
AnGap (mmol/l)	16	16.88	1.96	14.00	16.00	17.00	18.00	22.00	15.83	17.92
Alb (g/l)	18	23.89	2.17	21.00	22.00	23.00	25.00	29.00	22.81	24.97
ALP (µkat/l)	16	1.72	0.90	0.40	1.15	1.65	2.13	3.90	1.24	2.20
ALT (µkat/l)	16	6.50	0.83	5.40	5.90	6.40	7.18	8.10	6.06	6.94
Amy (µkat/l)	17	9.92	2.53	6.70	8.00	9.50	12.10	15.80	8.62	11.22
Tbil (µmol/l)	17	6.47	1.28	4.00	6.00	6.00	7.00	9.00	6.33	7.19
Ca (mmol/l)	18	2.30	0.12	2.06	2.24	2.29	2.37	2.48	2.24	2.36
P (mmol/l)	17	2.02	0.59	1.08	1.48	1.96	2.52	2.96	1.72	2.32
Cre (µmol/l)	18	39.78	16.57	18.00	26.00	37.00	52.75	66.00	31.54	48.02
TP (g/l)	17	69.18	4.72	60.00	66.00	70.00	73.00	76.00	66.75	71.60
Glob (g/l)	18	46.44	5.27	38.00	43.25	46.00	49.50	61.00	43.82	49.07

Hct = haematocrit, Hgb = haemoglobin, Na = sodium, K = potassium, Cl = chloride, Glu = glucose, pCO_2 = partial pressure of carbon dioxide, tCO_2 = total carbon dioxide, HCO_3 = bicarbonate, BE = base excess, AnGap = anion gap, Alb = albumin, ALP = alkaline phosphatase, ALT = alanine aminotransferase, Amy = amylase, Tbil = total bilirubin, Ca = calcium, P = phosphorus, Cre = creatinine, TP = total protein, Glob = globulin.

Table 2. Haematology and blood chemistry parameters for the greater mouse-eared bat (*Myotis myotis*).

Indicator	n	Mean	SD	Min	1stQ	Median	3rdQ	Max	CI (95%)	
Hct (l/l)	29	52.21	3.21	46.00	50.00	52.00	55.00	58.00	50.99	53.43
Hgb (g/l)	29	177.40	10.88	156.00	170.00	177.00	187.00	197.00	173.31	181.59
Na (mmol/l)	29	150.70	3.17	144.00	149.00	150.00	152.00	159.00	149.52	151.93
K (mmol/l)	29	5.96	1.45	3.60	5.00	5.70	6.70	9.00	5.40	6.51
Cl (mmol/l)	29	120.20	4.45	112.00	117.00	120.00	124.00	128.00	118.48	121.86
Urea (mmol/l)	29	20.94	8.69	10.80	15.30	19.20	24.20	43.60	17.64	24.25
Glu (mmol/l)	29	7.93	3.05	2.90	6.20	7.80	9.40	14.80	6.77	9.09
pH	29	7.25	0.04	7.16	7.24	7.25	7.28	7.32	7.24	7.27
pCO_2 (kPa)	29	6.55	0.68	5.16	6.09	6.59	6.72	8.22	6.29	6.80
tCO_2 (mmol/l)	29	23.14	2.68	18.00	21.00	23.00	25.00	29.00	22.12	24.16
HCO_3 (mmol/l)	29	21.63	2.54	16.40	19.70	21.60	23.10	26.90	20.66	22.59
BE (mmol/l)	29	-5.62	2.97	-11.00	-8.00	-6.00	-5.00	0.00	-6.75	-4.49
AnGap (mmol/l)	27	15.04	2.91	10.00	13.00	15.00	16.50	22.00	14.01	16.07
Alb (g/l)	17	22.06	2.93	18.00	20.00	21.00	24.00	28.00	20.55	23.56
ALP (µkat/l)	16	2.12	1.30	0.80	1.05	1.85	2.80	5.00	1.43	2.81
ALT (µkat/l)	16	6.83	1.55	4.50	5.76	7.00	7.63	9.90	6.01	7.66
Amy (µkat/l)	16	10.76	4.00	6.00	8.25	9.25	12.10	18.80	8.63	12.89
Tbil (µmol/l)	17	7.06	1.14	5.00	6.00	7.00	8.00	9.00	6.47	7.65
Ca (mmol/l)	17	2.26	0.24	1.85	2.10	2.26	2.41	2.69	2.14	2.38
P (mmol/l)	17	1.50	0.36	0.93	1.25	1.48	1.62	2.25	1.31	1.68
Cre (µmol/l)	17	32.18	13.02	18.00	23.00	30.00	38.00	63.00	24.48	38.87
TP (g/l)	15	68.13	5.46	61.00	64.00	67.00	74.00	76.00	65.11	71.16
Glob (g/l)	16	47.56	5.27	42.00	43.75	46.00	49.25	61.00	44.76	50.37

Hct = haematocrit, Hgb = haemoglobin, Na = sodium, K = potassium, Cl = chloride, Glu = glucose, pCO_2 = partial pressure of carbon dioxide, tCO_2 = total carbon dioxide, HCO_3 = bicarbonate, BE = base excess, AnGap = anion gap, Alb = albumin, ALP = alkaline phosphatase, ALT = alanine aminotransferase, Amy = amylase, Tbil = total bilirubin, Ca = calcium, P = phosphorus, Cre = creatinine, TP = total protein, Glob = globulin.

Table 3 Haematology and blood chemistry reference intervals for *M. blythii* and *M. myotis*

Indicator	n	Reference interval		Method
Hct (l/l)	49	46.00	58.00	NP
Hgb (g/l)	49	156.00	197.00	NP
K (mmol/l)	49	3.60	9.00	NP
Urea (mmol/l)	48	10.30	43.50	NP
Glu (mmol/l)	49	1.80	14.80	NP
pH	50	7.10	7.33	NP
pCO_2 (kPa)	50	4.90	9.20	NP
tCO_2 (mmol/l)	50	18.80	28.70	NP
HCO_3 (mmol/l)	50	13.70	26.50	NP
BE (mmol/l)	49	-11.80	-0.30	NP
ALT (µkat/l)	31	4.40	9.00	BCR
Ca (mmol/l)	35	1.90	2.60	BCR
Cre (µmol/)	35	3.60	66.60	PR
TP (g/l)	34	59.80	88.10	BCP
Glob (g/l)	35	39.00	68.00	BCP

(Hct = haematocrit, Hgb = haemoglobin, K = potassium, Glu = glucose, pCO_2 = partial pressure of carbon dioxide, tCO_2 = total carbon dioxide, HCO_3 = bicarbonate, BE = base excess, ALT = alanine aminotransferase, Ca = calcium, Cre = creatinine, TP = total protein, Glob = globulin, NP = nonparametric, BCR = Box-Cox transformed robust, PR = parametric robust, BCP = Box-Cox transformed parametric.

Discussion

Determination of blood parameters is an important tool, not just for assessing the health status of individual animals but also for assessing the influence of factors on specific organ systems and on homeostasis in general, right up to population level (Maceda-Veiga et al. 2015). To assess whether the blood parameters of a specific individual are within their 'normal' range, it is necessary to know the range in which the given parameter varies in healthy individuals. Ideally, the reference range should be determined using a non-parametric method based on values obtained from 120 or more clinically healthy individuals (Friedrichs et al. 2012). However, in the case of wild species, especially when they are endangered, it can be very difficult to obtain sufficient samples; thus, datasets with fewer individuals are often used, though it will then be necessary to use a suitably robust method to determine the reference interval (Cray 2015). Another problem with establishing blood reference intervals in bats is their low body weight, which significantly limits the amount of blood that can be collected. It is generally stated that it is safe to collect 10% of total blood volume, which corresponds to about 1 ml for an individual with a body weight of 100 g (Joslin 2009). Vespertilionid bats, however, have significantly lower body weight (Macdonald and Barrett 1993; Thabah et al. 2007); thus, for a long time, it was not possible to collect a sufficient amount of blood without harming the individual. Owing to the recent development of micro-analytical methods, however, it is now possible to analyse blood parameters in very small volumes of whole blood. In the case of the iSTAT and VS analysers used in this study, the minimum volume is 80 to 100 µl per analysis. For larger species of vespertilionid bat weighing more than 20 g, it is therefore possible to collect a sufficient volume of blood for analysis of blood parameters without causing life-threatening harm. Blood parameters can be affected by a whole range of pathological conditions, such as infectious diseases, poisoning or metabolic disorders caused by internal or external factors (Washington and Hoosier 2012; Etim et al. 2014). In addition, changes can also occur as a result of physiological stress, when the same pathological homeostasis-regulating mechanisms are applied, though these are generally milder deviations compared to pathological influences (Billman 2020). Generally speaking, reversibility of such deviations in a given parameter, or group of parameters, will be determined by the intensity of stress factor causing it. Some blood parameters, however, are strictly maintained within predetermined physiological ranges and their regulation controlled precisely (Clancy and McVicar 1997), while the physiological ranges of others can be relatively wide and small deviations caused by external environmental conditions or internal factors are unlikely to represent any serious problem (Kotas and Medzhitov 2015). As *M. blythii* and *M. myotis* are sibling species with similar morphology, it was assumed that the species would not differ in most blood indices, and that it would be plausible to determine blood parameter reference intervals for both species at the same time. Indeed, studies have shown that these closely related vespertilionid bat species also have similar mitochondrial lineages and, in areas where both species overlap, hybridisation has been recorded (Berthier et al. 2006; Bachanek and Postawa 2010). Other studies, however, have revealed differences in allelic frequencies at several polymorphic protein and microsatellite loci (Ruedi et al. 1990; Arlettaz et al. 1997a; Castella et al. 2000; Berthier et al. 2006). Thus, for a long time, it has not been entirely clear whether these are two distinct species or just two sub-species (Bogdanowicz et al. 2009). A newly proposed evolutionary scenario for *M. myotis* and *M. blythii* s.l. in the Western Palaearctic, based on more complex highly-variable nuclear and mitochondrial markers, suggests an initial, ancient divergence of both species in isolated glacial refugia in Western Europe and Asia, respectively. During this period of geographic isolation, both species adapted to distinct ecological niches and diverged morphologically

and genetically, reaching partial reproductive isolation (Furman et al. 2014). While these two species are difficult to distinguish morphologically, they do display significant differences in ecology, hunting strategy and choice of prey/diet composition (Arlettaz et al. 1991; Arlettaz 1996; Arlettaz et al. 1997a; Arlettaz et al. 1997b), with differences in composition determined primarily by the prey available in a given locality. Nevertheless, it is possible to observe general patterns in diet, with prey taken by *M. myotis* in southwestern Switzerland, for example, largely consisting of beetles (mainly carabidae, except in May and September when numbers are lower), with mole crickets and lepidopteran larvae also taken to a lesser degree in many areas. In the case of *M. blythii*, bush crickets (Tettigoniidae) often represent a major prey item, often replaced by cockchafers (*Melolontha melolontha*) from May to June when bush crickets are unavailable (Arlettaz 1996). In the area where we carried out trapping and sampling, next generation sequencing of droppings indicated presence of carabid beetles in the diet of *M. blythii*, though at a much lower proportion than for *M. myotis* from the same location (Hubancheva et al. 2023). Thus, while *M. blythii* also hunt carabid beetles, the diet range of the two species is significantly different, allowing them to avoid interspecific competition and coexist in the same area (Arlettaz et al. 1997b).

Diet composition can also play a role in terms of availability of certain nutrients. Calcium, for example, is an important dietary component that helps regulate a wide range of metabolic processes, ensures mechanical skeletal properties, and plays a role in signal transmission in the cell (Breitwieser 2008; Carafoli and Krebs 2016). In general, the ratio of calcium and phosphorus in the diet of insectivorous species is low (Adams et al. 2003); however, some bat species are known to compensate for this deficiency by consuming mineral licks or drinking clay-saturated water (Voigt et al. 2008). Lowered calcium intake can be a problem, especially during the growth period and in females during gestation and lactation, when females may be unable to cover the calcium requirements for foetus formation and subsequent milk production. In such cases, they are capable of mobilising calcium from the bones through activation of osteoclasts (Booher and Hood 2010); however, this may be associated with an increase in bone turnover and bone ALP isoenzyme activity, levels of which remain elevated after the young are weaned, only gradually returning to normal sometime later. A similar situation occurs in human females, where a slightly increased ALP level can persist for several months after the end of breastfeeding (Kent et al. 1990). The female bats captured in this study had significantly higher ALP concentrations in blood than males, possibly as a consequence of increased turnover in bone tissue during gestation and lactation, with elevated levels persisting even during swarming. A further cause of enriched ALP may be an increase in bone tissue metabolism during growth (Fernandez and Kidney 2007). In the present study, *M. myotis* females had significantly higher concentrations of amylase than males, which may have been physiologically related to amylase leakage from the pancreas or salivary glands, or decreased excretion of amylase by the urinary tract (Koop 1984). Higher amylase in females than males has previously been described in the Siberian weasel (*Mustela sibirica*; Ha et al. 2023), humans (Segawa et al. 1989), red howler monkeys (*Alouatta seniculus*; Vie et al. 1998) and in lactating common noctule (*Nyctalus noctula*) females (Pikula et al. 2017). Conversely, male *M. myotis* had higher total bilirubin concentrations than females in this study. Previous studies have described a physiological increase in bilirubin levels during intense physical exertion, starvation, stress or dehydration (Thrall et al. 2012; Thomas et al. 2022). Since mating occurs during swarming, and males are significantly more active than females at this time (Burns and Broders 2015), increased total blood bilirubin could be related to stress and exhaustion caused by fights for females. A higher level of total bilirubin is also associated with a higher level of testosterone in human males (Ling et al. 2024), again corresponding to the physiological state in which bat males find themselves

during swarming. Surprisingly, increased bilirubin can also have a positive effect as an antioxidant (Sedlak and Snyder 2004; Wu and Storey 2016), potentially playing a role as an adaptation to the increase in active flight-induced oxidative stress (Hulbert et al. 2007) in males during swarming. Differences between *M. blythii* and *M. myotis* were recorded for albumin, sodium, chloride, anion gap and phosphorus, with concentrations for all these parameters significantly higher in *M. blythii*. This could be a consequence of hypertonic dehydration due to water deficit, the common denominator for the increase in all the above-mentioned blood parameters (Mutlu et al. 2006; Thrall et al. 2012).

Unfortunately, it was not possible to compare the values obtained for most indicators with those for insectivorous bats in other studies as most previous studies dealing with bat blood parameters have focussed on haematocrit, haemoglobin, and urea measurements. Exceptions include the study of Pikula et al. (2017), who described haematology and blood chemistry for seven *N. noctula* females during the early post-hibernation period and during lactation, and that of Paksuz (2022), where haematocrit, haemoglobin, urea, total protein, albumin, creatinine, glucose and ALT values ere obtained for 10 *M. myotis* (unfortunately, it is unclear at what time of year the samples were taken in the latter study). While the haematocrit and haemoglobin values measured here correspond with those for bats in other studies (e.g. Wolk and Ruprecht 1988; Albayrak et al. 2016; Pikula et al. 2017; Paksuz 2022), the values are significantly higher than those for mice (Silva-Santana et al. 2020), most likely due to the higher capacity required by bats for oxygen transport, especially during flight (Jürgens et al. 1981). In the case of total protein, albumin and glucose, the values in this study were comparable to the ranges reported for *M. myotis* by Paksuz (2022) and for laboratory mice (Silva-Santana et al. 2020). On the other hand, glucose and total protein values were lower and albumin values higher in noctule females (Pikula et al. 2017) compared to our study. Conversely, values for urea provided by Paksuz (2022) were significantly lower than our own, and for those of laboratory mice (Silva-Santana et al. 2020). Generally speaking, higher urea values are typical for insectivorous bats, being related to urea function in postprandial urine concentration (Bassett 2004), while urea values in laboratory mice often tend to be at the lower end of the range found in bats. Pikula et al. (2017) also noted lower urea values in noctule females than those found in our study, potentially explained by sampling of the captive bats in the former study taking place prior to feeding. Creatinine values in *M. myotis* obtained by Paksuz (2022), and in euthermic *N. noctula* females by Pikula et al. (2017), were higher than those measured for *M. blythii* and *M. myotis* in the present study, the latter being closer to the values found normally in frugivorous bats (Hall et al. 2014; Selig et al. 2016) and lactating noctule females (Pikula et al. 2017).

In conclusion, no significant differences were observed between most blood parameters for the closely-related bat species *M. blythii* and *M. myotis*. As expected, therefore, it was possible to establish common reference intervals for both species. In the case of differences between the sexes, we recorded a probable influence of previous pregnancy and lactation on bone turnover. Furthermore, the difference in total bilirubin values between males and females (higher in males) and its possible association with protection against oxidative stress during intensive exercise is of interest and deserves further study. The data obtained here will contribute to improving the care for *M. myotis* and *M. blythii* in wild animal rescue centres and to research into the influence of infectious diseases, toxic substances or other negative factors on homeostasis of the internal environment of these bat species.

Conflict of interest

The authors declare that they have no competing interests.

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