Background: Establishment of haematological and biochemical reference intervals is important to assess health of animals on individual and population level. Reference intervals for 13 haematological and 34 biochemical variables were established based on 88 apparently healthy free-ranging brown bears (39 males and 49 females) in Sweden. The animals were chemically immobilised by darting from a helicopter with a combination of medetomidine, tiletamine and zolazepam in April and May 2006–2012 in the county of Dalarna, Sweden. Venous blood samples were collected during anaesthesia for radio collaring and marking for ecological studies. For each of the variables, the reference interval was described based on the 95% confidence interval, and differences due to host characteristics sex and age were included if detected. To our knowledge, this is the first report of reference intervals for free-ranging brown bears in Sweden.

Results: The following variables were not affected by host characteristics: red blood cell, white blood cell, monocyte and platelet count, alanine transaminase, amylase, bilirubin, free fatty acids, glucose, calcium, chloride, potassium, and cortisol. Age differences were seen for the majority of the haematological variables, whereas sex influenced only mean corpuscular haemoglobin concentration, aspartate aminotransferase, lipase, lactate dehydrogenase, β-globulin, bile acids, triglycerides and sodium.

Conclusions: The biochemical and haematological reference intervals provided and the differences due to host factors age and gender can be useful for evaluation of health status in free-ranging European brown bears.

Keywords: Brown bear, Ursus arctos, Haematology, Biochemistry, Reference intervals
characteristics age and sex influenced the haematological and biochemical variables.

**Methods**

**Study site and animals**

The study area was in the county of Dalarna, Sweden, approximately 61°N, 15°E, at altitudes of 300–700 m above sea level. The study included 88 free-ranging brown bears (39 males and 49 females), anaesthetised in April and May 2006 to 2012. Venous blood samples were collected during anaesthesia for radio collaring and marking for ecological studies. All bears were darted from a helicopter with a combination of medetomidine, tiletamine and zolazepam. The capture procedure is described in detail elsewhere [13]. All bears appeared clinically healthy as determined by clinical examination during anaesthesia. The bears were between 1 and 18 years of age. For animals that had not been followed from birth, age determination was performed by evaluation of an extracted rudimentary first maxillary premolar tooth [14]. Age was grouped into three categories: yearling (15 months), subadult (2–4 years old) and adult (≥5 years old). Fourteen bears were resampled during multiple years; in these cases, only one sample from each bear was included and randomly selected using a random number generator. A total of 69 samples for haematology and 88 samples for clinical chemistry were analysed. The capture and sampling were approved by the Ethical Committee on Animal Experiments, Uppsala, Sweden (application numbers: C 7/12, C 47/9 and C 59/6).

**Sampling procedure**

Blood was collected from the jugular vein using a vacutainer system (BD Vacutainer®, BD Diagnostics, Preanalytical Systems, Franklin Lakes, NJ, USA). Blood was sampled approximately 60 minutes after darting. Blood for haematological analyses was collected in 4 mL tubes, with EDTA (ethylenediaminetetraacetic acid) as anticoagulant (Vacutette®, Greiner Bio-One International AG, Kremsmünster, Austria). Two slides with blood smears, from each bear, were performed just after collection. The blood was kept refrigerated until shipment, and kept cool by cooling elements during the transport to the Clinical Chemistry Laboratory at the Swedish University of Agricultural Sciences, Uppsala, Sweden. The time from sampling to analysis was two days. After arrival to the laboratory, the blood slides were stained with May–Grünwald–Giemsa stain.

Blood for biochemistry was collected in 9 mL tubes with gel and clot activating factor (Vacuette®, Greiner Bio-One International AG, Kremsmünster, Austria). The blood tubes were kept at room temperature for 1–2 hours to ensure complete clotting, and then centrifuged at 1500 × g for 10 minutes to separate the serum. The serum was stored in 2 mL cryogenic vials (Nalgene, Nalge Company, Rochester, NY, USA) and kept at −20°C until shipment to the Central Laboratory, Norwegian School of Veterinary Science, Oslo, Norway. The blood tubes were kept cool by ice packs during shipment, and stored at −80°C until analysis.

**Laboratory analyses**

The haematology profile included red blood cell (RBC) count, white blood cell (WBC) count, haemoglobin, haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and platelet count. The Cell-Dyn 3500 Hematology Analyser (Abbott Laboratories, Abbott Park, Illinois, USA) was used for the haematological analyses in 2006 and 2007. From 2008, the ADVIA®2120 Hematology System was used (Bayer HealthCare, Diagnostics Division, Tarrytown, NY, USA). In addition, a white blood cell differential count (percentage of total) for band shaped neutrophils, segmented neutrophils, eosinophils, basophils, lymphocytes and monocytes, was carried out by manual examination of a blood smear, performed by the same technician. The technician counted 100 white blood cells.

The biochemical profile included aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (AP), γ-glutamyl transpeptidase (GGT), glutamate dehydrogenase (GD), creatinine kinase (CK), lactate dehydrogenase (LD), amylase, lipase, total protein, urea, creatinine, uric acid, bile acids, total bilirubin, cholesterol, triglycerides, free fatty acids (FFA), β-hydroxybutyrate (β-HBA), glucose, inorganic phosphorous, calcium, magnesium, sodium, potassium, chloride, iron, and cortisol. Serum protein electrophoresis yielded five protein fractions; albumin, α1-globulin, α2-globulin, β-globulin, γ-globulin, and the albumin-globulin ratio. All clinical chemistry analyses, except cortisol analysis, were carried out with an ADVIA®1650 Chemistry System (Siemens Healthcare, Tarrytown, NY, USA) from 2006 to 2011. Thereafter it was replaced by the ADVIA®1800 Chemistry System (Siemens Healthcare). The analytical methods used for the analysis are given in Table 1. Cortisol was measured with an Immulite system (Diagnostic Products Corporation, Los Angeles, CA, USA) and the protein fractions were measured by serum protein electrophoresis. Reagents from Siemens Medical Solutions Diagnostics were used for all analytes except FFA (Wako Chemicals GmbH, Neuss, Germany), β-HBA and GD (Randox Laboratories Ltd., Dublin, Ireland) [15].

**Statistical analysis**

The guidelines from the American Society for Veterinary Clinical Pathology’s Quality Assurance and Laboratory Standard Committee were used for the establishment of
reference intervals in this study [2]. To establish reference intervals for the biochemical and haematological variables, and to determine if these should be stratified based on age and sex, the following strategy was used: haematological and biochemical variables were included as continuous variables and their distributions determined. Descriptive statistics are presented with upper and lower 95% confidence interval for all variables, and mean and standard deviation for variables that were normally distributed. The distributions of the haematological and biochemical variables were assessed using histograms and QQ-plots, including an assessment if they had a Normal distribution. Those with an approximate Normal distribution were kept on the direct scale, whereas Box-Cox transformation was used for variables that did not appear with a Normal distribution of the direct scale.

The formula for the Box-Cox transformation, where $\bar{y}$ is the geometric mean [16];

$$y'_{\lambda} = \frac{y^\lambda - 1}{\lambda \cdot \bar{y}^{\lambda-1}}, \text{for } \lambda \neq 0,$$

$$y'_{\lambda} = \bar{y} \cdot \log (y), \text{for } \lambda = 0.$$

When >90% of the values for a given variable were 0, transformation was deemed non-sensible. Therefore the reference intervals for these variables are presented the 90th, 97.5th and 100th percentiles. For variables including observed values of 0, a factor of 0.05 or 0.5 was added to all the values before transformation to avoid impossible mathematical operations. In case a transformation was used, the transformation parameter $\lambda$ was reported.

Comparisons between age and sex were performed on the transformed variables using analysis of variance. A significant F-test was followed by a simultaneous comparison of paired means using the Tukey-Kramer test, to evaluate if there was a significant difference between age groups and genders. After calculating the reference intervals, the transformed data were back-transformed to the original scale. The added values of 0.05 or 0.5 were subtracted from the variables where they had been added. Resulting reference intervals were presented in sex and age cohorts if significantly different or otherwise combined. Data were presented as 95% confidence intervals.

Statistical analyses were performed using JMP®10.0.0 statistical software (SAS Institute, Cary, North Carolina, USA), and a $p$-value <0.05 was considered significant.

**Results**
Sixty-nine haematological specimens (44 females and 25 males) were included in the statistical analysis. These were from 23 adults, 19 subadults and 27 yearlings. In 21 of the specimens there was platelet aggregation, so because of the small sample size in each group, the variable platelet count was not included in the analysis of variance. As >90% of the values obtained for band-shaped neutrophils and basophils were 0, they were presented as 90th, 97.5th and 100th percentiles. For variables including observed values of 0, a factor of 0.05 or 0.5 was added to all the values before transformation to avoid impossible mathematical operations. In case a transformation was used, the transformation parameter $\lambda$ was reported.

Comparisons between age and sex were performed on the transformed variables using analysis of variance. A significant F-test was followed by a simultaneous comparison of paired means using the Tukey-Kramer test, to evaluate if there was a significant difference between age groups and genders. After calculating the reference intervals, the transformed data were back-transformed to the original scale. The added values of 0.05 or 0.5 were subtracted from the variables where they had been added. Resulting reference intervals were presented in sex and age cohorts if significantly different or otherwise combined. Data were presented as 95% confidence intervals.

Statistical analyses were performed using JMP®10.0.0 statistical software (SAS Institute, Cary, North Carolina, USA), and a $p$-value <0.05 was considered significant.

**Discussion**
This study provides the first comprehensive report on reference intervals for free-ranging brown bears in
Sweden. Variation caused by the capture and immobilisation procedure (stress, helicopter pursuit and anaesthetics), blood collection, and analytic procedures may affect the results, but are an inherent challenge in studies involving free ranging animals.

Both chasing prior to immobilisation and the anaesthetic drugs can potentially induce stress in addition to other factors such as fear, shock, pain and acid–base imbalance which can occur during immobilisation [17]. Stress increases the release of glucocorticoid hormones (cortisol and corticosterone) which results in increased gluconeogenesis and decreases use of glucose, decreased sensitivity to insulin, and fat and protein metabolism [18]. This results in increased blood glucose levels during stress. Plasma concentrations of cortisol have been used as an indicator of stress in different animals, but the correlation between visually evaluated amounts of handling stress and plasma cortisol levels are controversial [19-21]. The shift in the composition of white blood cells, known as a stress leukogram, characterised with a mild neutrophilia accompanied by a lymphopenia and eosinophilia and, less frequently with a monocytosis are well known [22]. The anaesthetics used can potentially affect the blood variables. Medetomidine has been shown to increase the blood glucose level in different species of animals [23-26]. A decrease in the total protein level is also reported after administration of medetomidine [23,25]. The effect of medetomidine on cortisol levels in the blood of different animals is controversial, as some studies reports an increase, some a decrease and some reports that there is no change [24,26-29]. To the authors’ knowledge, only few studies have looked at the effects of ketamine or tiletamine/zolazepam on blood variables. In dogs, the effect of ketamine has been linked to effects caused by stress, with increased plasma values of glucose and cortisol [30]. Lower haemoglobin, haematocrit and MCV in yearlings compared to subadult and adult animals are well described phenomena in brown bears [5,12,31]. Hypochromic microcytic anaemia in young, growing bears has been a suggested cause, attributable to iron deficiency caused by growth demands [31,32]. Lower values of iron in yearlings and subadults compared to adults were found, but RBC counts were similar, so the yearlings in this study were not deemed to be anaemic. It is possible that the differences observed relate to differences in diet among the age groups [33].

The variation in white blood cell differential count in different age groups could reflect maturation of the immune system, because younger animals are known to

| Variable  | Unit       | n   | $\lambda$ | 2.5 - 97.5 interval | Sex | Age group | Reference interval | $p$-value |
|-----------|------------|-----|-----------|---------------------|-----|------------|-------------------|----------|
| Haemoglobin | g/L       | 69  | 1.8       | 142-189             | B   | Y          | 137-171           | $<$0.0001²²|
| RBC       | $\times 10^{12}$/L | 69  | 0         | 6.2-8.0             | B   | YSA        | 6.2-8.0           |          |
| Haematocrit | L/L       | 69  | 0.6       | 0.41-0.54           | B   | Y          | 0.40-0.49         | $<$0.0001²²|
| MCV       | fL         | 69  | 2         | 57-75               | B   | Y          | 57-70             | $<$0.0001²²|
| MCHC      | g/L        | 69  | 0         | 334-373             | F   | YSA        | 332-366           | 0.0062²²²³ |
| WBC       | $\times 10^{9}$/L | 69  | −0.2      | 3.6-11.4            | B   | YSA        | 3.9-11.5          |          |
| Segmented neutrophils | %        | 69  | 2         | 57.8-92.5           | B   | Y          | 62.2-93.6         | 0.0185²² |
| Eosinophils | %        | 69  | 0         | 0.0-9.6             | B   | Y          | 0.0-4.3           | $<$0.0001²²|
| Lymphocytes | %        | 69  | 0.4       | 2.5-32.5            | B   | YS         | 4.8-32.6          | 0.001²² |
| Monocytes | %         | 69  | 0         | 0.8-11.3            | B   | YSA        | 0.7-11.7          |          |
| Platelet count | $\times 10^9$/L | 48  | 0.4       | 103-811             | B   | YSA        | 141-520           |          |

¹: Presented stratified by age group, sex or presented overall, depending on if a difference between groups was detected. ²: RBC=red blood cell count; MCV=mean corpuscular volume; MCHC=mean corpuscular haemoglobin concentration; WBC=white blood cell count. $\lambda$: the transformation value from the Box-Cox transformation. ³: 2.5 and 97.5 percentiles on untransformed data. ⁴: B=both female and male; F=female; M=male. ⁵: Y=yearling; S=subadult; A=adult. ⁶: $p$-values are given for significantly different groups: $a=p$-value for age and $aS=p$-value for age-sex interaction.
have a higher lymphocyte count than adult animals [22]. The higher mean eosinophil levels in yearlings and subadults could potentially be explained by parasites in some individuals, however, this was not evaluated.

Alkaline phosphatase (AP) and inorganic phosphorous were highest in yearlings and lowest in adults, which probably reflected elevated levels in young growing animals due to increased osteoblastic activity during rapid bone growth [34]. Similar results for AP have been reported previously for other bears [6,32,35,36] and for inorganic phosphorous in grizzly bears, black bears and wolves [6,36,37]. Only juvenile mammals are able to mobilise magnesium from the skeleton in the face of inadequate dietary intake, which could explain why younger bears had significantly higher serum magnesium concentrations than subadults and adults [38].

The higher values of lipase in females compared to males is in agreement with findings in polar bears [39]. Increased serum lipase activity could be associated to renal or pancreatic disease [34], while decreased serum amounts of lipase could be associated to lipaemic specimens [40]. However, none of the brown bear specimens in the present study, or the polar bear specimens in earlier mentioned study, were classified as lipaemic. Increased serum values of LD and AST could be a result of hepatocyte or muscle damage [34]. An intensive chase before immobilisation and dart trauma could induce acute skeletal muscle injury, and increased serum values.

Higher levels of total serum protein and albumin in older brown bears compared to younger brown bears have been previously described in other bear species [33,35]. A correlation between primary food source and total protein levels has been described [33], and may explain the differences we found between yearlings and subadult and adult bears. Total serum protein and particularly albumin levels are indicators of dietary protein levels, so an increase from yearlings to subadults to adults could reflect better physiological condition in older bears [35]. The higher albumin:globulin ratio in subadult and adult bears compared to yearlings is also likely due to the higher level of albumin and total

| Table 3 Enzyme reference intervals for brown bears (*Ursus arctos*) presented as 95% confidence interval
| Variable | Unit | n | λ | 2.5 - 97.5 interval | Sex | Age group | Reference interval | p-value |
|----------|------|---|---|-------------------|-----|----------|-------------------|---------|
| AST      | U/L  | 88 | 1.2 | 45-215            | F   | YA       | 56-168            | 0.0034  |
|          |      |    |     |                   |     | S        | 46-114            |         |
|          |      |    |     |                   |     | M        | 46-114            |         |
| ALT      | U/L  | 88 | −1  | 11-47             | B   | YS       | 12-41             |         |
| AP       | U/L  | 88 | 0.2 | 15-166            | B   | Y        | 65-185            | <0.0001 |
|          |      |    |     |                   |     | S        | 31-101            |         |
|          |      |    |     |                   |     | A        | 10-92             |         |
| GGT      | U/L  | 88 | 0.2 | 3-91              | B   | YS       | 3-51              | 0.0126  |
|          |      |    |     |                   |     | A        | 5-82              |         |
| GD       | U/L  | 88 | −0.8 | 2-18            | B   | YS       | 3-8               | 0.0003  |
|          |      |    |     |                   |     | A        | 3-16              |         |
| CK       | U/L  | 88 | −0.4 | 53-690          | B   | Y        | 81-631            | <0.0001 |
|          |      |    |     |                   |     | SA       | 44-419            |         |
| Amylase  | U/L  | 88 | 0   | 22-192           | B   | YS       | 24-143            |         |
| Lipase   | U/L  | 88 | −0.4 | 14-247          | F   | YA       | 16-NE*            | 0.0063  |
|          |      |    |     |                   |     | S        | 10-NE*            | <0.0001 |
|          |      |    |     |                   |     | M        | 11-159            |         |
|          |      |    |     |                   |     | Y        | 11-159            |         |
|          |      |    |     |                   |     | S        | 9-58              |         |
| LD       | U/L  | 88 | 0.2 | 434-1066         | F   | Y        | 587-1060          | 0.0184  |
|          |      |    |     |                   |     | SA       | 421-875           |         |
|          |      |    |     |                   |     | M        | 587-1060          |         |
|          |      |    |     |                   |     | Y        | 421-875           |         |
|          |      |    |     |                   |     | A        | 421-875           |         |

1: Presented stratified by age group, sex or presented overall, depending on if a difference between groups was detected. *: AST=aspartate aminotransferase; ALT=alanine transaminase; AP=alkaline phosphatase; GGT=γ-glutamyl transpeptidase; GD=glutamate dehydrogenase; CK=creatine kinase; LD=lactate dehydrogenase; A:G=albumin:globulin ratio. λ: λ=the transformation value from the Box-Cox transformation. 2.5 and 97.5 percentiles on untransformed data. ^: B=both female and male; F=female; M=male. ^=yearling; S=subadult; A=adult. ^: p-values are given for significantly different groups; ^=p-value for age, ^=p-value for sex and ^=p-value for age-sex interaction. *NE: Non-estimable because estimates were above the range of the transformation.
protein in the older bears. The increase of gamma globulins with increasing age may indicate a still developing antibody producing mechanism in younger individuals [6,35,41]. Similar differences have been demonstrated previously in brown bears and black bears [10,35].

The higher values of cholesterol and triglycerides in yearlings compared to subadult and adult brown bears stand in contrast to a study of sloth bears, where the heaviest sloth bears had the highest values of cholesterol and triglycerides. This could be caused by an increased calorie intake or a decreased physical activity in the heaviest bears, or be due to an age related variation in diet [42]. The sloth bears were kept under semi-captive conditions with access to natural food resources. In studies of black bears, body fat mass correlated better with triglyceride and cholesterol values, than body weight. Significant differences in blood lipid concentrations among populations may reflect differences in diet [35,43]. The higher cholesterol and triglyceride values in yearlings compared to subadult and adult brown bears in the present study could be a result of differences in diet in the age-groups, and a potential correlation between body fat mass and blood lipid concentrations would be an interesting avenue for further study.

The higher creatinine in adults compared to subadults, and in subadults compared to yearlings is in agreement with previous reports in bears [6,36]. It has been concluded that the higher amounts of creatinine was due to larger muscle mass in older bears [6], although higher values would then be expected in males than in females, especially in adult bears, but this was not documented in the present study. Creatinine values have been reported to decrease during immobilisation, and the elevated levels in the first specimen could have been caused by exercise prior to the immobilisation [6].

Urea is a product of the protein metabolism, so differences in protein contents in the diet could be reflected in the blood values of urea. In early spring when the bears have left the den they need to increase their muscle mass, and protein rich food is the main energy source [44]. The lower urea values in yearlings may be due to different dietary preferences in yearlings.

Table 4 Metabolite and cortisol reference intervals for brown bears (Ursus arctos) presented as 95% confidence interval

| Variable | Unit | n | λ | 2.5 - 97.5 interval | Sex | Age group | Reference interval | p-value |
|----------|------|---|---|----------------------|-----|-----------|-------------------|---------|
| β-HBA    | mmol/L | 88 | −0.2 | 0.0-1.9 | B | YSA | 0.0-1.1 |          |
| Bile acids | μmol/L | 88 | −0.6 | 5-65 | F | YA | 5-60 |          |
|          |       |    |     |          |    | M | YA | 6-46 |          |
|          |       |    |     |          |    |  S |  S | 7-13 |          |
| Bilirubin (total) | μmol/L | 88 | 0.2 | 0-6 | B | YSA | 0-5 |          |
| Cholesterol | mmol/L | 88 | 1.2 | 4.5-12.2 | B | Y | 5.9-13.2 | <0.0001 A |
|           |       |    |     |          |    | SA |     | 4.7-10.4 |        |
| Creatinine | μmol/L | 88 | 1 | 66-218 | B | Y | 57-160 | <0.0001 A |
|           |       |    |     |          |    | S |  101-190 |        |
|           |       |    |     |          |    |  A | 107-235 |        |
| Free fatty acids | mmol/L | 88 | 0.2 | 0.1-1.4 | B | YSA | 0.1-1.3 |          |
| Glucose   | mmol/L | 88 | 0.4 | 3.3-11.5 | B | YSA | 3.3-10.9 |          |
| Triglycerides | mmol/L | 88 | 0.8 | 1.7-5.4 | F | Y | 1.5-5.4 | 0.0220 A, 0.0122 S |
|           |       |    |     |          |    | SA | 1.7-4.4 |        |
|           |       |    |     |          |    |  M |  3.2-8.6 |        |
|           |       |    |     |          |    |  S |  1.9-7.9 |        |
| Urea      | mmol/L | 88 | −0.4 | 0.5-11.9 | B | Y | 0.4-4.0 | 0.0255 A |
|           |       |    |     |          |    | SA | 0.4-NE * |        |
| Uric acid | μmol/L | 88 | −0.8 | 68-209 | B | Y | 73-111 | <0.0001 A |
|           |       |    |     |          |    | SA | 70-222 |        |
| Cortisol  | nmol/L | 88 | 0.4 | 30-564 | B | YSA | 45-565 |          |

1: Presented stratified by age group, sex or presented overall, depending on if a difference between groups was detected. 2: β-HBA=β-hydroxybutyrate 3: λ=the transformation value from the Box-Cox transformation. 4: 2.5 and 97.5 percentiles on untransformed data. 5: B=both female and male; F=female; M=male. 6: Y=yearling; S=subadult; A=adult. 7: p-values are given for significantly different groups: ^p-value for age and *p-value for sex 8: NE: Non-estimable because estimates were above the range of the transformation.
A small sample size is often a limitation when sampling wild animals for research studies. Where significant differences among age groups or genders were detected, it was not possible to establish reference intervals. This was often because of a large standard error due to a small sample size and a large distribution of the values. The standard error could have been decreased with removal of outliers, but elimination of apparent outliers or unexpected values should not be done indiscriminately [2], and the large standard error might have been influenced by the preanalytical factors. Consequently, this could not really be addressed further except noting the presence of differences.

**Table 5** Mineral and protein reference intervals for brown bears (*Ursus arctos*) presented as 95% confidence interval

| Variable<sup>a</sup> | Unit     | n  | λ<sup>b</sup> | 2.5 - 97.5 interval<sup>d</sup> | Sex<sup>c</sup> | Age group<sup>e</sup> | Reference interval | p-value<sup>f</sup> |
|----------------------|----------|----|--------------|-------------------------------|--------------|---------------------|--------------------|-------------------|
| Calcium              | mmol/L   | 88 | 2            | 1.8-2.7                       | B            | YSA                 | 1.8-2.6            | <0.0001<sup>A</sup> |
| Chloride             | mmol/L   | 88 | 2            | 88-104                        | B            | YSA                 | 92-105             |                   |
| Iron                 | μmol/L   | 88 | 0.2          | 13-68                         | B            | YS                  | 14-50              |                   |
| Magnesium            | mmol/L   | 88 | 1            | 0.70-1.21                     | B            | Y                  | 0.70-1.27          | 0.0167<sup>A</sup> |
| Inorganic Phosphorous| mmol/L   | 88 | 1.4          | 0.6-2.6                       | B            | Y                  | 1.1-2.7            | 0.0002<sup>A</sup> |
| Potassium            | mmol/L   | 88 | -0.2         | 3.2-5.3                       | B            | YSA                 | 3.3-5.1            |                   |
| Sodium               | mmol/L   | 88 | 2            | 128-141                       | F            | YSA                 | 132-141            | 0.0135<sup>S</sup> |
|                      |          |    |              |                               | M            | YSA                 | 123-145            |                   |
| Albumin              | g/L      | 86 | 1.4          | 27.2-49.6                     | B            | Y                  | 24.5-40.6          | <0.0001<sup>A</sup> |
|                      |          |    |              |                               | SA           |                     | 34.2-49.1          |                   |
| α<sub>1</sub>-globulin| g/L     | 86 | 0.4          | 2.6-79                        | B            | Y                  | 3.0-10.6           | <0.0001<sup>A</sup> |
|                      |          |    |              |                               | SA           |                     | 1.7-8.3            |                   |
| α<sub>2</sub>-globulin| g/L     | 86 | -0.4         | 2.9-10.2                      | B            | Y                  | 3.1-13.4           | 0.0004<sup>A</sup> |
|                      |          |    |              |                               | SA           |                     | 2.7-9.3            |                   |
| β-globulin           | g/L      | 86 | 0            | 3.0-9.2                       | F            | Y                  | 3.2-6.0            | <0.0001<sup>A</sup>, 0.0096<sup>S</sup> |
|                      |          |    |              |                               | SA           |                     | 3.7-9.1            |                   |
|                      |          |    |              |                               | M            | Y                  | 2.2-8.6            |                   |
|                      |          |    |              |                               | SA           |                     | 5.1-10.0           |                   |
| γ-globulins          | g/L      | 86 | 0.4          | 1.6-79                        | B            | Y                  | 1.4-5.0            | <0.0001<sup>A</sup> |
|                      |          |    |              |                               | S            |                     | 2.2-5.8            |                   |
|                      |          |    |              |                               | A            |                     | 3.2-8.1            |                   |
| A:G                  |          | 86 | 0            | 1.13-2.89                     | B            | Y                  | 1.03-2.70          | <0.0001<sup>A</sup> |
|                      |          |    |              |                               | SA           |                     | 1.38-3.07          |                   |
| Total protein        | g/L      | 88 | 2            | 48-72                         | B            | Y                  | 39-64              | <0.0001<sup>A</sup> |
|                      |          |    |              |                               | S            |                     | 50-68              |                   |
|                      |          |    |              |                               | A            |                     | 53-74              |                   |

<sup>a</sup>: Presented stratified by age group, sex or presented overall, depending on if a difference between groups was detected.  
<sup>b</sup>: λ=the transformation value from the Box-Cox transformation.  
<sup>c</sup>: 2.5 and 97.5 percentiles on untransformed data.  
<sup>d</sup>: B=both female and male; F=female; M=male.  
<sup>e</sup>: Y=yearling; S=subadult; A=adult.  
<sup>f</sup>: p-values are given for significantly different groups: A=p-value for age; S=p-value for sex.

**Conclusion**
To conclude, we provided haematological and biochemical reference intervals, which can be useful in health assessments of free-ranging brown bears. Differences in some host and ecological factors exist, which should be considered when interpreting diagnostic data.

**Competing interests**
The authors have declared that they have no competing interests.

**Authors’ contributions**
ARG analyzed the data and drafted the manuscript. JMA, ALE and ÅF conceived, designed and performed the experiments. SSN and MFB supervised data analysis. All authors participated in writing the manuscript and approved the final version.
Acknowledgements
This is paper number 170 of the Scandinavian Brown Bear Research Project (SBBRP). The captures were conducted by personnel of the SBBRP including Sven Brunberg. Funding for biochemical and haematological analysis came from the Conservation Medicine Group at Hedmark University College, Norway and the Directorate for Nature Management in Norway. Wads Kjelgaard-Hansen is thanked for provision of critical comments in the course of the project.

Author details
1 Department of Large Animal Sciences, University of Copenhagen, Giønnegårdsvej 8, DK-1870 Frederiksborg C, Denmark. 2 Center for Zoo and Wild Animal Health, Copenhagen Zoo, Roskildevej 38, DK-2000 Frederiksberg, Denmark. 3 Department of Forestry and Wildlife Management, Faculty of Applied Ecology and Agricultural Sciences, Hedmark University College, Campus Evenstad, NO-2418 Elverum, Norway. 4 Department of Clinical Sciences, Faculty of Veterinary Medicine and Animal Science, Swedish University of Agricultural Sciences, P.O. Box 7054, SE-750 07 Uppsala, Sweden. 5 Department of Animal Sciences, University of Copenhagen, Græsli Campus Evenstad, NO-2418 Elverum, Norway. 4 Department of Clinical Sciences, Faculty of Veterinary Medicine and Animal Science, Swedish University of Agricultural Sciences, SE-901 83 Umeå, Sweden.

References
1. Geffen A, Friedrichs K, Harr K, Concordet D, Trumel C, Braun JP. Reference values: a review. Vet Clin Path 2009, 38:288–298.
2. Friedrichs KR, Harr KE, Freeman KP, Szladovits B, Walton RM, Barnhart KF, Bianco-Chavez J. AVSCP reference interval guidelines: determination of de novo reference intervals in veterinary species and other related topics. Vet Clin Path 2012, 41:441–453.
3. Ramsay EC. Ursidae and hyaenidae. In: Zoo and Wild Animal Medicine. 5th edition. Edited by Fowler ME, Miller RE. USA: Elsevier Science; 1999:523–528.
4. Kindberg J, Svensson JE, Ericsson G, Bellermann E, Michel C, Taberlet P. Estimating population size and trends of the Swedish brown bear (Ursus arctos) population. Wild Boi 2011, 17:114–123.
5. Pearson AM, Halloran DW. Hematology of the brown bear (Ursus arctos) from southwestern Yukon Territory, Canada. In: J Zoo J 1972, 50:279–286.
6. Brannon RD. Serum chemistry of central and northern Alaska grizzly bears. J Wild Manage 1985, 49:993–900.
7. Hissa R, Seikkinen J, Hohtola E, Saarela S, Hakala A, Pudas J. Effects of sex, age, capturing method, and season on serum chemistry values of brown bears in Croatia. Dis Zoo and Wild Animal Medicine. 2009, 136:53–58.
8. Karjalainen M, Surcel HM, Hissa R. Effects of immobilization with medetomidine and atipamezole on serum glucose and cortisol levels in captive reindred (Rangifer tarandus tarandus). Rangifer 1999, 19:85–89.
9. Soveti T, Sankari S, Salonen JS, Nieminen M. Effects of immobilization with medetomidine and reversal with atipamezole on blood chemistry of semi-domesticated reindred (Rangifer tarandus tarandus) in autumn and late winter. Acta Vet Scand 1999, 40:333–349.
10. Ambrisko TD, Hikasa Y. Neuroendocrine and metabolic effects of medetomidine compared with xylazine in beagle dogs. J Vet J Res 2002, 66:42–49.
11. Carroll GL, Hartshfield SM, Champey TH, Geller SC, Martinez EA, Haley EL. Effect of medetomidine and its antagonism with atipamezole on stress-related hormones, metabolites, physiologic responses, sedation, and mechanical threshold in goats. Vet Anesth Analg 2005, 32:147–157.
12. Kallio A, Koulu M, Scheinin H, Viljan M, Scheinin M. Acute effects of medetomidine, a selective alpha-2-adrenoceptor agonist, on anterior pituitary hormone and cortisol secretion in man. Acta Endocrinol 1988, 119:11–15.
13. Ranheim B, Horsberg TE, Solli NE, Ryeng KA, Arnemo JM. The effects of medetomidine and its reversal with atipamezole on plasma glucose, cortisol and noradrenalin in cattle and sheep. J Vet Pharmacol Ther 2000, 23:379–387.
14. Ambrisko TD, Hikasa Y, Sato K. Influence of medetomidine on stress-related neuroendocrine and metabolic effects caused by butorphanol, fentanyl, and ketamine administration in dogs. Am J Vet Res 2005, 66:606–612.
15. Seal US, Swain WR, Erickson AW. Hematology of ursidae. Comp Biochem Physiol A 1967, 22:451.
16. Matula GJ, Linderz JS, Rothenbacher H. Sex, age, and seasonal differences in the blood profile of black bears captured in northeastern Pennsylvania. Bears: Their Biol Manage 1980, 449–56.
17. Hellgren EC, Rogers LL, Seal US. Serum chemistry and hematology of black bears: physiologic indices of habitat quality or seasonal patterns? J Mammal 1993, 74:304–315.
18. Stockman SL, Scott MA. Fundamentals of veterinary clinical pathy. 2nd edition. Ames, Iowa, USA: Blackwell Publishing; 2008.
19. Schroder MT. Blood chemistry, hematolgy, and condition evaluation of black bears in northcoastal California. Bears: Their Biol Manage 1987, 733–349.
20. Storm GL, Alt GL, Matula GJ, Nelson RA. Blood chemistry of black bears from Pennsylvania during winter dormancy. J Wildl Dis 1988, 24:515–521.
21. Thoresen SJ, Arnemo JM, Liberg O. Hematology and serum clinical chemistry reference intervals for free-ranging Scandinavian gray wolves (Canis lupus), Vet Clin Path 2003, 38:224–229.
22. Nosil TJ, Capen CC. Calcium-regulating hormones and diseases of abnormal mineral (calcium, phosphorus, magnesium) metabolism. In: Clinical Biochemistry of domestic Animals. 5th edition. Edited by Kaneko JJ, Harvey JW, Bruss ML. San Diego, California, USA: Academic Press; 1997:619–702.
23. Tryland M, Brun E, Derocher AE, Arnemo JM, Kierulf P, Olberg RA, Wiig O. Plasma biochemical values from apparently healthy free-ranging polar bears from Svalbard. J Wildl Dis 2002, 38:566–575.
40. Tryland M, Brun E: Serum chemistry of the minke whale from the northeastern Atlantic. J Wild Dis 2001, 37:332–341.

41. Rostal MK, Evans AL, Solberg EJ, Arnemo JM: Hematology and serum chemistry reference ranges of free-ranging moose (Alces alces) in Norway. J Wild Dis 2012, 48:548–559.

42. Shanmugam AA, Kumar JK, Selvaraj I, Selvaraj V: Effects of body weight and season on serum lipid concentrations in sloth bears (Melusus ursinus ursinus). J Zoo Wildl Med 2011, 42:373–381.

43. Frank N, Elliott SB, Allin SB, Ramsay EC: Blood lipid concentrations and lipoprotein patterns in captive and wild American black bears (Ursus americanus). Am J Vet Res 2006, 67:335–341.

44. Swenson JE, Jansson A, Riiq R, Sandegren F: Bears and ants: myrmecophagy by brown bears in central Scandinavia. Can J Zool 1999, 77:551–561.

doi:10.1186/s12917-014-0183-x
Cite this article as: Græsli et al: Haematological and biochemical reference intervals for free-ranging brown bears (Ursus arctos) in Sweden. BMC Veterinary Research 2014 10:183.