Journal of Wildlife Diseases, 48(2), 2012, pp. 282–294

FACTORS AFFECTING HEMATOLOGY AND PLASMA BIOCHEMISTRY IN THE SOUTHWEST CARPET PYTHON (*MORELIA SPILOTA IMBRICATA*)

Gillian L. Bryant,[1,3] Patricia A. Fleming,[1] Leanne Twomey,[2] and Kristin A. Warren[1]

[1] School of Veterinary & Biomedical Sciences, Murdoch University, South Street, Murdoch, Western Australia 6150, Australia
[2] Vetpath Laboratory Services, 39 Epsom Avenue, Ascot, Western Australia 6104, Australia
[3] Corresponding author (email: g.bryant@murdoch.edu.au)

ABSTRACT: Despite increased worldwide popularity of keeping reptiles as pets, we know little about hematologic and biochemical parameters of most reptile species, or how these measures may be influenced by intrinsic and extrinsic factors. Blood samples from 43 wild-caught pythons (*Morelia spilota imbricata*) were collected at various stages of a 3-yr ecological study in Western Australia. Reference intervals are reported for 35 individuals sampled at the commencement of the study. As pythons were radiotracked for varying lengths of time (radiotransmitters were surgically implanted), repeated sampling was undertaken from some individuals. However, because of our ad hoc sampling design we cannot be definitive about temporal factors that were most important or that exclusively influenced blood parameters. There was no significant effect of sex or the presence of a hemogregarine parasite on blood parameters. Erythrocyte measures were highest for pythons captured in the jarrah forest and at the stage of radiotransmitter implantation, which was also linked with shorter time in captivity. Basophil count, the only leukocyte influenced by the factors tested, was highest when the python was anesthetized, as was globulin concentration. Albumin and the albumin:globulin ratio were more concentrated in summer (as was phosphorous) and at the initial stage of radiotransmitter placement (as was calcium). No intrinsic or extrinsic factors influenced creatinine kinase, aspartate aminotransferase, uric acid, or total protein. This study demonstrates that factors including season, location, surgical radiotransmitter placement, and anesthetic state can influence blood parameters of *M. s. imbricata.* For accurate diagnosis, veterinarians should be aware that the current reference intervals used to identify the health status of individuals for this species are outdated and the interpretation and an understanding of the influence of intrinsic and extrinsic factors are limited.

Key words: Anesthetic, captivity, hemoparasite, radiotransmitter, reference intervals, reptile, season, snake.

INTRODUCTION

Monitoring hematologic measures and serum or plasma biochemical analytes is important for evaluating the health status of reptiles kept in captivity for research, as pets, or in zoos (Nordøy and Thoresen, 2002). Evaluating hematologic and biochemical responses can facilitate the diagnosis of stress and disease states in reptile species (Christopher et al., 1999). Blood analysis can be used to detect conditions such as anemia, inflammatory disease, parasitemia, hematopoietic disorders, and hemostatic alterations by comparing individual samples with a clinically normal sample population (Campbell and Ellis, 2007). Reptilian blood analysis has not been evaluated for clinical application to the same extent as in mammals, although various blood values are known to be influenced by factors such as age, sex, and nutritional status (Campbell, 2004). Reference intervals have typically not accounted for these variations in intrinsic and environmental factors, making interpretation difficult (Campbell, 2004, 2006).

The International Species Information System (ISIS) reports hematologic measures and plasma biochemical analytes for samples collected from 25 pythons (*Morelia spilota*) from 10 institutions. However, no details are provided on source of the animals, nor is any information given to indicate sex, season, or length of time in captivity before blood sampling (Teare, 2002). The Near-Threatened southwest

carpet python (*Morelia spilota imbricata*) that inhabits southwest Western Australia measures up to 2.4 m snout-to-vent length (SVL) and can weigh up to 6 kg (IUCN, 1998; Pearson et al., 2002). Since 2003 it has been legal to obtain a license and keep reptiles as pets in Western Australia, including the southwest carpet python (Edwards, 2003). As part of a wider ecology study on wild-caught *M. s. imbricata*, blood samples were collected to assess whether the health of pythons was adversely affected by having a radiotransmitter surgically implanted into their coelomic cavity, which enabled in situ monitoring of individuals. This study therefore presented a unique opportunity to carry out repeated sampling for this population of wild pythons over time, before and after surgery. We present hematologic and biochemical data collected from 43 individuals and analyze the effects of season, sex, study site, anesthesia, duration of time in captivity, radiotransmitter placement over time, and the presence of parasites (*Haemogregarina* sp.) within red blood cells.

MATERIALS AND METHODS

Study animals and sample collection

Forty-three southwest carpet pythons were collected through opportunistic hand capture for an ecology and thermal biology research project. Individuals were captured in the southwest of Western Australia from two habitat types (referred to elsewhere as study site): 1) coastal woodland: included animals captured and monitored at Martin's Tank (32°51′S, 115°40′E) and Leschenault Peninsula Conservation Park (33°26′S, 115°41′E) and 2) jarrah forest: areas surrounding Dwellingup Township (32°43′S, 116°4′E). This project was approved by the Animal Ethics Committees of Murdoch University (W2028/07) and Department of Environment and Conservation, Western Australia (DEC AEC/55/2006 and DEC AEC54/2006).

Body mass (M_b) was determined with a calibrated spring balance (±0.2 kg), and SVL was measured with a tape measure in a straight line along the ventral surface of the python from the tip of the mouth to the cloaca. The sex of each python was determined by eversion of hemipenes or by insertion of a lubricated blunt probe into the cloaca and then directed toward the tail to determine the presence or absence of hemipenes. Sex was determined by depth of the probe insertion, as measured by the number of overlying subcaudal scales. Females probed to between one and five scales, whereas males probed to depths equivalent to 7–20 scales. Adult male pythons weighed an average 694±(SD)285 g (range 298–1,500 g) and measured SVL 134±17 cm (range 97–170 cm). Adult female pythons weighed 1,420±554 g (range 103–3,731 g) and measured SVL 179±119 (range 90–200 cm).

Upon initial capture, pythons were brought into a holding facility at the DEC Research Center, Dwellingup, Western Australia between December 2006 and November 2008 to undergo surgical implantation of radiotransmitters (for surgical details see Bryant et al., 2010). When held at the research center, the animals were housed in a 25 C temperature-controlled room in purpose-built ventilated enclosures with flooring lined to approximately 2-cm depth with recycled newspaper kitty litter (Old News Cat Litter, Ciber Cycles Pty Ltd., Toowoomba, Queensland, Australia). The enclosures contained a hide box as a cardboard box with an entrance hole cut into one side, cage furniture including a branch and rocks, ad libitum water, and an external heat pad positioned under the plywood flooring that heated that portion of the cage floor above the heat pad to approximately 30 C. All cages were kept in a room under natural lighting provided by a large window. If they were held for >3 wk during warmer seasons, pythons were fed dead laboratory mice and rats that were stored frozen and thawed before feeding. Pythons were not fed during winter. Animals were held for varying lengths of time because of logistic reasons, including the necessity of customizing a radiotransmitter for surgical implantation. Length of time each python spent in captivity before sample collection was recorded and scored for statistical analyses as 0–30 days (n=33), 30–60 days (n=18), and >60 days (n=7).

We recorded repeated samples from pythons at three time points; however, the number of samples collected from pythons varied between these points. At the initial stage of presurgical implantation of the radiotransmitter 38 blood samples were collected in the laboratory (pre-TM, n=38 individuals). Twenty four of these 38 pythons were anesthetized via inhalation of 1.5% isoflurane gas. The remaining 14 pythons were manually restrained by holding carefully within calico

bags, with the tail exposed for blood collection from the ventral coccygeal vein. Animals were opportunistically allocated to these two treatments on the basis of logistics around the transportation of blood samples to the clinical laboratory for analysis. The reference intervals were calculated from the samples collected from these 38 animals. Pythons were released approximately 2 wk after surgical implantation and were radiotracked weekly to monitor feeding behavior and habitat use. When pythons could be captured by hand from their retreat site, 3–12 mo (average 6.85±4.86 mo) postimplantation of radiotransmitters (post-TM, $n=22$), a second blood sample was collected from manually restrained conscious animals in the field. One python was moved back into temporary captivity for surgical replacement of a radiotransmitter due to battery failure, and the posttransmitter (second) blood sample was collected under anesthesia. Pythons were anesthetized at the time of radiotransmitter removal, allowing a third (removal-TM, $n=20$) blood sample. Twelve individuals lost their transmitters during the period of tracking and 13 died over the 3-yr study (postmortem examinations were performed when possible and the results are presented elsewhere; Bryant, 2012). In total, we collected 77 blood samples from 43 animals. Sixteen pythons were sampled once, 17 were sampled twice, and 10 were sampled three times.

A range of needle sizes (depending on the size of the python; 25 G for SVL<100-cm pythons, 25 G, 23 G for intermediate sizes, and 21 G for SVL>150 cm) and a 2-ml syringe (BD PrecisionGlide™ Needle; BD Slip Tip Syringe; Becton Dickinson, Singapore) were used to collect blood samples from the ventral coccygeal vein. Fresh blood smears without anticoagulate were made immediately after collection; the remainder of the sample was carefully transferred immediately into 2-ml (13×75 ml) lithium heparin vacutainers (BD Vacutainer®, Becton Dickinson, Plymouth, UK) and kept refrigerated at approximately 4 C during transportation to a commercial laboratory. Analysis was completed by Vetpath Laboratory Services (Ascot, Western Australia, Australia) within 48 hr of collection. Samples collected in the field were kept on ice in an insulated container or refrigerated when possible before transport to the laboratory. Whenever possible, a complete set of hematologic and plasma biochemical data was collected from each blood sample. Incomplete blood analysis was common, generally due to insufficient volumes of plasma for the full biochemistry panel.

Hematology analysis

Blood samples were analyzed using a CELL-DYN™ (Cell Dyn 3700, Abbott Diagnostics, North Ryde, New South Wales, Australia) analyzer using the reptilian/avian setting to take nucleated erythrocytes into account. The blood variables analyzed included hemoglobin (Hb), packed cell volume (PCV), red blood cell count (RBC), mean cell hemoglobin (MCH), mean cell volume (MCV), mean cell hemoglobin concentration (MCHC), and total white blood cell count (WBC) using the automated system. Manual calculations for PCV were made to correct hemoglobin values (Hb, MCH, MCV, and MCHC) and if these measures did not correspond (8/77 samples), they were removed from further analysis and only PCV was included.

Blood smears were air-dried and stained with Wright's–Giemsa stain by an automated slide stainer (Hematek, Siemens, Osborne Park, Western Australia). The proportions of heterophils (including potential eosinophils, which could not be definitively identified by morphology alone; Stirk et al., 2007), lymphocytes, basophils, and combined monocytes/azurophils (Fig. 1a–d) were classified through manual counts of blood smears. Parasites in red blood cells that were morphologically consistent with a *Hemogregarina* species were found in some samples (Fig. 1e). Following descriptions provided by Mackerras (1961) of the seven *Hemogregarina* species found in Australian Boidae (O'Donoghue and Adlard, 2000), the species in this study appears to be *Hemogregarina moreliea*. Samples were categorized according to the presence or absence of the hemoparasites in smears.

Plasma biochemical analysis

A minimum of 150 µl of plasma from heparinized blood samples was used for biochemical analysis. Plasma was analyzed for creatinine kinase (CK), aspartate aminotransferase (AST), uric acid, total protein, albumin, globulin, albumin-to-globulin ratio (A/G), calcium, phosphorous, and glucose using an automated chemical analyzer (Olympus AU 400, Integrated Science, Tokyo, Japan). Because of varying lengths of time before laboratory analysis (up to 48 hr) and incomplete data collection, glucose results are variable and therefore, although range of data is indicated, caution should be taken when using the values presented here. For these reasons, multiple regression analyses were not performed on glucose.

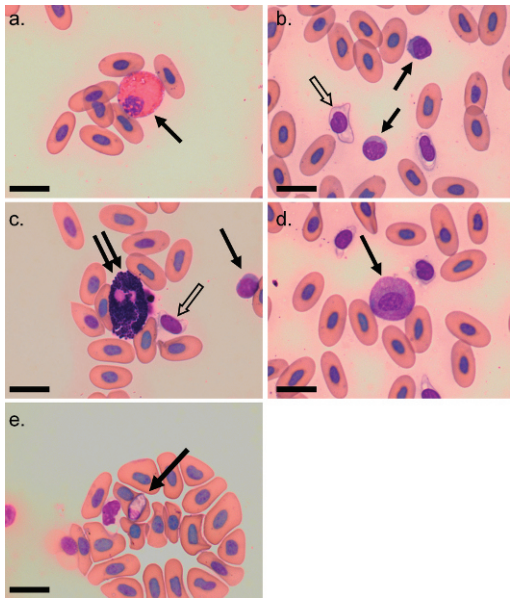

FIGURE 1. Peripheral blood films of a southwest carpet python (*Morelia spilota imbricata*) showing: a) heterophil (black arrow); b) lymphocytes (black arrows) and thrombocytes (open arrow); c) basophil (double black arrow); a lymphocyte (single black arrow) and thrombocyte (open arrow) are also visible; d) azurophil (black arrow); and e) erythrocyte containing a *Hemogregarina moreliea* hemoparasite (black arrow). Images (a–e) stained with Wright–Giemsa stain, 1,000×, bar = 15 μm.

Statistical analysis

Reference intervals for *M. s. imbricata* were investigated for the first time period sampling point only (pre-TM implantation) for 38 individuals. Blood samples from three female pythons were removed as they were considered outliers using Dixon's range test within the program Reference Value Advisor V 1.4 (Anonymous, 2010); reference intervals were therefore calculated using blood samples for 35 individuals. Standard descriptive statistics include sample size, mean, standard deviation, minimum, median, and the maximum values. Normality tests were performed and if required, data were transformed with Box-Cox transformation. The lower and upper limit of the reference interval as well as the 2.5, 5, 90, and 97.5% confidence intervals are given where possible using the program Reference Value Advisor V 1.4 (Anonymous, 2010).

Data from our study (*M. s. imbricata*) are expressed as a proportion of reported ISIS average values (*M. spilota*) following the formula:

$$\text{Proportional difference} = \frac{(\text{Average } M.\ s.\ imbricata - \text{average } M.\ spilota)}{\text{average } M.\ spilota}$$

Multiple regression analysis (Statistica 9.0, Statsoft Inc.) was carried out separately for each blood measure (dependent variable) against the same independent factors: sex (male or female), study site (coastal woodland or jarrah forest), season (summer: December–February; autumn: March–May; winter: June–August; spring: September–November), anesthesia (anesthetized or conscious), the experimental phase of sample collection (three categories: pre-TM, post-TM, and removal-TM), the time in captivity from capture until sample collection (three categories: 0–30 days, 31–60 days, and >60 days; animals sampled in the field were ascribed as 0 days), and hemoparasite presence (positive or negative). The blood variables examined included: Hb, PCV, RBC, MCH, MCV, MCHC, WBC, heterophil, lymphocyte, basophil, and combined monocyte and azurophil counts, CK, AST, uric acid, total protein, albumin, globulin, A/G ratio, calcium, and phosphorus.

Chi-square analysis was used to determine variation in hemoparasite prevalence across seasons with the expected values calculated assuming an equal proportion of the population was positive each season. A similar analysis was carried out for *H. moreliea* hemoparasite prevalence at each sampling period, assuming an equal proportion of samples collected pre-TM, post-TM, or removal-TM were positive (i.e., expected values calculated assuming an equal distribution across these three time points). Chi-square analysis was also used to test for a significant difference in *H. moreliea* prevalence between study sites.

RESULTS

Reference intervals

Reference intervals are shown for 35 pythons for blood collected before surgical implantation of radiotransmitters (pre-TM) only (Table 1). Raw, untransformed data are shown for reference intervals. Most average values found in this study were similar (<20% difference) to published ISIS values for *M. spilota* (Teare, 2002). However, MCV were twice those of published ISIS values, WBC counts were

TABLE 1. Hematology and plasma biochemical values for 35 apparently healthy, wild-caught southwest carpet pythons (*Morelia spilota imbricata*) sampled before radiotransmitter implantation. Sample size varies among measures because of differences in the volume of blood that could be collected (some tests not conducted because of small sample size), due to variation in time to analysis (not all samples were analyzed for glucose because of transport delays), or due to discrepancies in erythrocyte counts (see text for further detail). Values were calculated using Reference Value Advisor v1.4 program and given to three significant digits, or were interpreted as zero for negative results.

							Confidence intervals					
Value	*n*	Mean	Median	SD	Minimum	Maximum	Transformation[a]	Lower limit	Upper limit	2.5%	5%	90%	97.5%
Hemoglobin (g/l)	26	76.5	79.0	17.1	28.0	105	BoxCox	30.4	104	0	48.7	97.4	109
Packed cell volume	30	0.232	0.240	0.051	0.080	0.30	BoxCox	0.090	0.307	0	0.155	0.293	0.319
Red blood count ($\times 10^{12}$/l)	26	0.669	0.700	0.174	0.200	0.900	BoxCox	0.027	0.905				
Mean cell hemoglobin concentration (g/l)	26	343	342	17.7	315	374	UT	305	380	297	314	369	389
Mean cell hemoglobin (pg)	26	117	111	19.8	96.0	178	UT	67.2	152	51.0	86.1	133	170
Mean cell volume(fl)	26	339	327	49.5	279	484	UT	219	430	188	260	386	465
White blood cell count ($\times 10^9$/l)	35	14.9	13.4	6.94	4.90	29.3	UT	0	29.4	0	2.70	24.8	32.2
Heterophils ($\times 10^9$/l)	31	7.72	7.13	5.17	0.410	24.8	BoxCox	0.620	21.7	0.141	1.79	16.8	27.1
Lymphocytes ($\times 10^9$/l)	35	3.09	2.59	2.19	0.400	10.3	BoxCox	0.367	9.07	0.275	0.603	6.91	11.3
Basophils ($\times 10^9$/l)	28	0.232	0.155	0.246	0.00	0.940	BoxCox	0	1.217	0	0	0.778	1.83
Monocytes & azurophils ($\times 10^9$/l)	32	4.57	3.94	2.76	1.08	12.3	BoxCox	1.08	12.9	0.768	1.50	9.77	17.0
Creatinine kinase (U/l)	32	1,880	1,651	1,115	365	5,147	BoxCox	379	4,767	293	542	3,726	5,746
Aspartate aminotransferase (U/l)	32	84.8	63.0	63.8	17.0	259	BoxCox	17.8	296	13.9	23.6	187	445
Uric acid (mmol/l)	28	0.186	0.184	0.061	0.081	0.333	UT	0.047	0.301	0.019	0.088	0.267	0.342
Total protein (g/l)	32	73.0	71.5	11.7	56.0	105	UT	47.0	96.0	41.0	53.6	88.2	103
Albumin (g/l)	32	20.9	21.0	3.03	16.0	27.0	UT	14.5	27.1	13.0	15.7	25.3	28.8
Globulin (g/l)	32	52.0	50.5	9.25	39.0	78.0	UT	31.5	70.4	26.3	37.0	64.2	76.0
Albumin-to-globulin ratio	32	0.407	0.420	0.045	0.280	0.490	BoxCox	0.296	0.485	0.251	0.343	0.469	0.499
Calcium (mmol/l)	32	3.43	3.46	0.30	2.90	4.41	UT	2.79	4.02	2.60	3.00	3.82	4.19
Phosphorus (mmol/l)	32	1.14	1.14	0.316	0.430	1.86	UT	0.468	1.78	0.337	0.643	1.57	1.94
Glucose (mmol/l)	28	2.84	2.10	1.94	0.100	8.30	BoxCox	0.189	8.21	0.051	0.491	5.75	10.3

[a] UT = untransformed robust data; BoxCox = Box Cox robust transformation.

40%, and heterophil counts were 172% greater than ISIS values. All other leukocyte values were lower than those reported in ISIS for *M. spilota*. Creatinine kinase (+292%) and AST (+239%) were higher than reference values and uric acid (−57%), albumin (−30%), calcium (−46%), and phosphorous (−59%) concentrations were lower than average values reported by ISIS.

Factors affecting hematologic measures and plasma biochemistry

Pythons from the jarrah forest had greater RBC ($t_{56}=2.04$, $P<0.05$) and lower concentrations of MCH ($t_{56}=-2.41$, $P<0.05$) and MCV ($t_{56}=-2.18$, $P<0.05$) compared with pythons from coastal woodland. There was no statistical difference in any hematologic variable or plasma biochemical analytes between males and females (all $P>0.05$, Tables 2 and 3).

Season, anesthesia, surgical implantation, and time in captivity significantly influenced some hematologic and biochemical analytes; however, due to the lack of independence of the factors tested, the effects of season and radiotransmitter placement cannot be differentiated. Season significantly influenced values for MCH ($t_{56}=3.33$, $P<0.01$), MCV ($t_{56}=2.55$, $P<0.01$), albumin ($t_{65}=2.07$, $P<0.05$), A/G ratio ($t_{65}=2.42$, $P<0.05$), and phosphorous ($t_{65}=3.23$, $P<0.01$). For MCH, MCV, albumin, and phosphorous, the lowest average values were recorded in winter (Tables 2 and 3), whereas the A/G ratio peaked in summer (Table 3). Blood values were apparently influenced by anesthetic state, with anesthetized animals having higher average basophil counts ($t_{55}=2.10$, $P<0.05$) and globulin concentrations ($t_{65}=2.01$, $P<0.05$; Tables 2 and 3). The A/G ratio was also lower for anesthetized animals ($t_{65}=-2.09$, $P<0.05$; Table 3). The time of blood collection (categorized by three stages of radiotransmitter implantation) influenced several hematologic parameters (Hb: $t_{57}=2.16$, $P<0.04$; PCV: $t_{56}=2.78$, $P<0.01$; RBC: $t_{56}=3.03$, $P<0.01$; and basophil count: $t_{55}=-2.44$, $P<0.05$) and plasma biochemical analytes (albumin: $t_{65}=3.54$, $P<0.001$; A/G ratio: $t_{65}=4.24$, $P<0.001$; and calcium: $t_{65}=3.50$, $P<0.001$). All these measures were highest at the pre-TM implantation sampling period. The length of time in captivity before blood sample collection (0–30 days, 30–60 days, or >60 days) significantly influenced only MCH ($t_{56}=-2.09$, $P<0.05$) and MCV measures ($t_{56}=-2.78$, $P<0.05$; Table 2), where values were higher for animals that had spent less time in captivity.

The presence of *H. moreliea* was only marginally associated with reduced total protein ($t_{65}=1.88$, $P>0.06$), albumin ($t_{65}=1.85$, $P>0.07$), and globulin ($t_{65}=1.88$, $P>0.06$; Table 3) measures. There was no significant difference in the occurrence of the intracellular hemoparasite between pythons captured from coastal woodland or jarrah forest ($\chi^2_1=2.43$, $P>0.05$). A greater proportion of blood samples collected from pythons during spring (September–November) were positive for *H. moreliea* ($\chi^2_1=8.44$, $P<0.01$; Fig. 2). Significantly more pythons sampled at removal-TM were positive for hemogregarines compared with pre-TM and post-TM ($\chi^2_1=8.30$, $P<0.01$; Fig. 2).

DISCUSSION

We examined the influence of intrinsic and extrinsic factors on hematologic parameters and plasma biochemical analytes in a Western Australian python species. Health screening provides useful baseline data for conservation management programs of wild populations of threatened species and for species held in captivity for zoologic collections and as pets (Espinosa-Avilés et al., 2009). We provide reference intervals for wild-caught southwest carpet pythons that could contribute to health screening of these animals for conservation management. There was no significant

TABLE 2. Average±SD values for each hematologic measure analyzed by multiple regression for all factors. Bold values indicate a significant (* $P<0.05$, ** $P<0.005$, *** $P<0.001$) difference between the categories in the analysis. Values to three significant digits.

Factor	Category, n=number of pythons (N=number of samples)	Hb (g/l), N=65	PCV (l/l), N=64	RBC ($\times 10^{12}$/l)[a], N=64	MCH (pg)[b], N=64
Study site	Coastal, n=28 (54)	69.6±16.3	0.209±0.050	***0.600±0.155**	***118±17.5**
	Jarrah forest, n=13 (23)	74.3±9.62	0.227±0.038	**0.672±0.102**	**113±14.7**
Sex	Female, n=19 (33)	68.7±16.7	0.203±0.053	0.581±0.159	122±21.7
	Male, n=22 (44)	72.7±13.0	0.225±0.039	0.649±0.128	113±10.7
Season	Summer, n=23 (25)	80.2±10.0	0.241±0.031	0.681±0.117	****120±23.1**
	Autumn, n=17 (17)	63.7±18.0	0.190±0.062	0.536±0.169	**121±14.1**
	Winter, n=11 (12)	65.0±12.2	0.195±0.042	0.618±0.133	**107±12.5**
	Spring, n=20 (23)	69.7±13.4	0.210±0.046	0.617±0.138	**115±9.32**
State during sampling	Anesthetized, n=29 (42)	72.0±15.7	0.211±0.047	0.623±0.154	117±16.2
	Conscious n=28 (35)	69.8±13.7	0.220±0.048	0.617±0.136	116±17.8
Radiotransmitter	Pre, n=35 (35)	***76.5±17.1**	***0.232±0.051**	****0.669±0.174**	116±19.8
	Post, n=22 (22)	**66.6±12.6**	**0.199±0.042**	**0.575±0.107**	118±18.4
	Removal, n=20 (20)	**68.1±11.3**	**0.199±0.036**	**0.600±0.119**	115±9.39
Time in captivity	0–30 d, n=31 (48)	71.0±14.3	0.215±0.045	0.610±0.137	***120±19.0**
	30–60 d, n=18 (21)	69.1±17.5	0.212±0.058	0.624±0.179	**112±11.0**
	>60 d, n=7 (8)	76.7±9.14	0.220±0.025	0.683±0.075	**111±10.2**
Parasite presence	Negative, n=37 (62)	72.6±13.3	0.221±0.042	0.642±0.131	115±15.8
	Positive, n=11 (15)	65.0±18.4	0.192±0.057	0.543±0.170	121±20.2

Hb = hemoglobin; PCV = packed cell volume; RBC = red blood cell count; MCH = mean cell hemoglobin; MCV = mean cell volume; MCHC = mean cell hemoglobin concentration; WBC = total white blood cell count.
[a] Nonnormal distribution for Box-Cox transformed data.
[b] Box-Cox transformed data with normal distribution.

effect of sex or the presence of a hemogregarine parasite, but season, time in captivity, anesthesia, and the stage of radiotransmitter implantation did influence blood parameters.

Because of the physiologic flexibility of reptiles (e.g., poikilothermy, long-term fasting, and rapid up-regulation of the digestive system upon feeding; Bedford and Christian, 2001; Secor and Ott, 2007), reference intervals are difficult to establish (Campbell and Ellis, 2007). The overall average leukocyte counts in this study for *M. s. imbricata* varied substantially from ISIS reports for the species (all values presented as *M. spilota*). This discrepancy may reflect differences between subspecies of *Morelia* that include varying diet and potential climatic influences such as rainfall, temperature, and humidity, but also reflects that ISIS values do not follow the current Clinical and Laboratory Standards Institute (CLSI) guidelines for calculation and presentation (C28-A3) and should be updated (CLSI, 2008). Leukocyte counts for *M. s. imbricata* differed from ISIS values in a manner consistent with heterophilia and lymphopenia, which can occur with a stress response to capture and handling in wild-caught pythons (Campbell, 2004, 2006; Campbell and Ellis, 2007). Alternatively, there may be differences in leukocyte identification between individual pathologists. The high heterophil numbers by comparison with low lymphocytes, basophils, and monocytes/azurophils may suggest the latter. However, we also recorded higher CK and AST in *M. s. imbricata* compared with ISIS average values, which may suggest muscle damage associated with handling of wild-caught individuals (assuming that the majority of samples used for the ISIS values are from captive animals that may be habituated to human presence and handling). The

TABLE 2. Extended.

MCV (fl)[b], N=64	MCHC (g/l), N=65	WBC ($\times 10^9$/l)[b], N=77	Heterophils ($\times 10^9$/l)[b], N=77	Lymphocytes ($\times 10^9$/l)[b], N=77	Basophils ($\times 10^9$/l)[a], N=63	Monocytes & Auzurophils ($\times 10^9$/l)[b], N=73
***348±59.4**	338±23.0	14.9±6.85	7.05±0.75	3.11±2.31	0.309±0.326	4.28±2.39
327±46.0	346±20.7	14.9±6.23	6.62±4.02	2.94±2.51	0.345±0.240	4.87±2.69
358±65.5	342±20.9	15.8±8.08	7.28±5.49	3.45±2.64	0.238±0.186	4.45±2.83
331±46.4	339±23.8	14.2±5.29	6.66±3.68	2.76±2.10	0.393±0.354	4.44±2.21
****351±72.7**	343±22.0	13.2±5.86	6.65±4.11	2.85±2.20	0.233±0.282	4.23±2.03
355±48.5	340±30.3	13.8±7.14	6.07±3.68	2.98±2.33	0.345±0.278	4.38±3.29
323±54.1	333±17.8	15.5±9.02	6.54±5.77	2.51±2.49	0.207±0.234	4.81±3.00
333±38.5	341±19.4	18.2±6.93	8.06±4.85	3.62±2.51	0.478±0.315	4.85±2.48
336±45.4	345±19.1	14.4±6.15	7.23±4.65	3.15±2.26	***0.368±0.319**	4.19±2.39
350±67.3	334±25.3	15.5±7.20	6.56±4.41	2.94±2.50	**0.270±0.267**	4.78±2.58
339±49.5	343±17.7	14.9±6.94	6.40±3.76	3.09±2.19	***0.232±0.246**	4.57±2.76
356±75.2	334±29.6	13.8±7.40	6.76±5.01	2.54±2.38	**0.286±0.286**	4.13±2.29
332±38.9	342±19.4	16.1±5.11	8.02±5.18	3.56±2.61	**0.521±0.314**	4.59±2.25
***356±61.0**	337±23.3	14.4±6.71	7.12±4.95	2.94±2.39	0.266±0.214	4.12±2.25
320±39.8	343±21.6	16.4±6.79	5.84±3.87	3.34±2.50	0.366±0.375	5.11±2.93
313±30.9	354±14.4	13.7±5.70	8.57±2.85	3.00±1.92	0.553±0.414	4.72±2.44
341±57.6	338±22.8	14.8±6.81	6.54±4.54	2.96±2.42	0.313±0.305	4.54±2.59
348±53.5	347±20.4	15.2±6.05	8.52±4.22	3.45±2.10	0.371±0.257	4.11±2.01

differences between values presented in this study and reported ISIS values may therefore reflect real differences for wild-caught animals.

Although multiple regression analysis should simultaneously take multiple factors into consideration in its computation, we nevertheless urge caution in interpretation of data where there are clearly unequal samples collected for each factor and where multiple factors could influence the results. Multiple regression analysis did not distinguish between samples collected from male and female *M. s. imbricata* for either hematologic or plasma biochemical analytes tested. *Morelia spilota imbricata* are capital breeders and will breed only every second year, at best (Pearson, 2002). Over the 3-yr study, only seven females were identified as gravid once over this time; only two of these were sampled while gravid (none were sampled postoviposition) and they did not stand out as outliers. Therefore, to our knowledge, the majority of female *M. s. imbricata* sampled were nonreproductive. Similarly, serum biochemistry does not vary between male and female black diamond water snakes (*Nerodia rhombifera rhombifera*; McDaniel et al., 1984), a viviparous species. By contrast, other studies have identified sex differences in plasma analytes for various species of snakes, with elevations of plasma calcium, phosphorus, and protein concentrations in females during estrus and egg production (Dessauer, 1970; Campbell, 2004). It is therefore likely that blood measures only differ between the sexes under specific reproductive states.

We found significant seasonal differences for MCH, MCV, albumin, A/G ratio, and phosphorous concentration. Wojtaszek (1992) noted a significant decrease in RBC, PCV, and Hb concentration in spring, the mating period for the grass snake (*Natrix natrix natrix*), and attributed the hematologic changes to a hormonal influence produced by decreased erythropoietic activity and from some RBC breakdown during winter. The reduction in albumin and phosphorous concentrations in winter for *M. s. imbricata* is most likely associated with fasting (Campbell, 2004).

TABLE 3. Average±SD values for each plasma biochemical measure analyzed by multiple regression for all factors. Bold values indicate a significant (* $P<0.05$, *** $P<0.001$) difference between the categories in the analysis. Values to three significant digits.

Factor	Category, n = number of pythons (N=number of samples)	CK (U/l), N=71	AST (U/l)[a], N=73	Uric acid (mmol/l)[a], N=68	Total protein (g/l), N=73	Albumin (g/l), N=73	Globulin (g/l), N=73	Albumin/ globulin ratio, N=73	Ca (mmol/l)[b], N=73	P (mmol/l), N=73
Study site	Coastal, n=28 (54)	1,630±889	61.7±34.0	0.295±0.238	71.6±12.8	17.9±3.75	54.0±11.4	0.340±0.076	3.21±0.359	1.06±0.295
	Jarrah forest, n=13 (23)	1,910±1,200	88.6±84.1	0.185±0.069	76.6±13.4	21.0±3.25	55.7±11.7	0.387±0.077	3.26±0.788	1.21±0.437
Sex	Female, n=19 (33)	1,740±1,190	66.6±61.2	0.315±0.266	72.9±14.0	19.2±4.18	54.2±12.2	0.363±0.086	3.33±0.526	1.18±0.388
	Male, n=22 (44)	1,710±841	73.0±56.7	0.221±0.140	73.4±12.6	18.6±3.60	54.8±10.9	0.349±0.073	3.14±0.519	1.05±0.310
Season	Summer, n=23 (25)	2,050±1,260	104±72.0	0.211±0.136	73.7±11.5	***21.1±2.85**	52.6±9.55	***0.409±0.062**	3.37±0.309	***1.32±0.395**
	Autumn, n=17 (17)	1,860±907	61.7±33.3	0.284±0.213	72.8±13.3	**19.4±4.10**	53.4±10.9	**0.370±0.080**	3.13±0.841	**1.13±0.279**
	Winter, n=11 (12)	1,410±776	45.2±23.4	0.365±0.321	67.7±14.4	**16.5±4.36**	51.2±11.4	**0.326±0.064**	3.04±0.375	**0.781±0.255**
	Spring, n=20 (23)	1,410±715	53.1±54.7	0.245±0.187	75.8±14.0	**17.4±3.02**	59.2±12.8	**0.302±0.061**	3.23±0.502	**1.05±0.219**
State during sampling	Anesthetized, n=29 (42)	1,525±709	53.1±33.7	0.223±0.158	74.3±13.3	18.9±3.59	***55.8±12.2**	***0.351±0.080**	3.37±0.463	1.06±0.211
	Conscious n=28 (35)	1,940±1,210	88.7±72.8	0.301±0.246	72.0±13.0	18.9±4.16	**53.1±10.5**	**0.360±0.078**	3.06±0.551	1.16±0.453
Radio-transmitter	Pre, n=35 (35)	1,880±1,120	84.8±63.8	0.186±0.061	73.0±11.7	***20.9±3.03**	52.5±9.15	*****0.407±0.045**	***3.43±0.298**	1.14±0.316
	Post, n=22 (22)	1,850±1,060	77.8±65.8	0.372±0.293	70.1±13.9	**17.5±4.27**	52.6±11.4	**0.338±0.086**	**2.93±0.667**	1.17±0.490
	Removal, n=20 (20)	1,330±569	38.8±15.7	0.255±0.198	76.7±14.3	**17.1±3.02**	60.5±12.9	**0.290±0.054**	**3.22±0.523**	1.00±0.168
Time in captivity	0–30 d, n=31 (48)	1,680±904	62.8±50.5	0.282±0.223	72.7±13.0	18.7±3.88	54.5±11.1	0.353±0.079	3.28±0.452	1.08±0.384
	30–60, n=18 d (21)	1,870±1,320	76.3±67.7	0.250±0.197	74.0±13.1	18.6±3.86	55.1±11.7	0.348±0.084	3.20±0.330	1.17±0.289
	>60 d, n=7 (8)	1,590±568	101±74.8	0.153±0.048	74.7±16.3	20.7±3.64	54.0±13.0	0.391±0.050	2.90±1.14	1.13±0.278
Parasite presence	Negative, n=37 (62)	1,830±1,030	77.9±62.4	0.245±0.146	74.5±12.1	19.4±3.70	55.5±10.7	0.360±0.082	3.23±0.508	1.10±0.383
	Positive, n=11 (15)	1,330±761	40.4±21.7	0.316±0.349	68.0±15.9	16.8±3.80	51.2±13.1	0.335±0.064	3.21±0.615	1.14±0.177

CK = creatinine kinase; AST = aspartate aminotransferase.

[a] Box-Cox-transformed data with normal distribution.

[b] Nonnormal distribution for Box-Cox-transformed data.

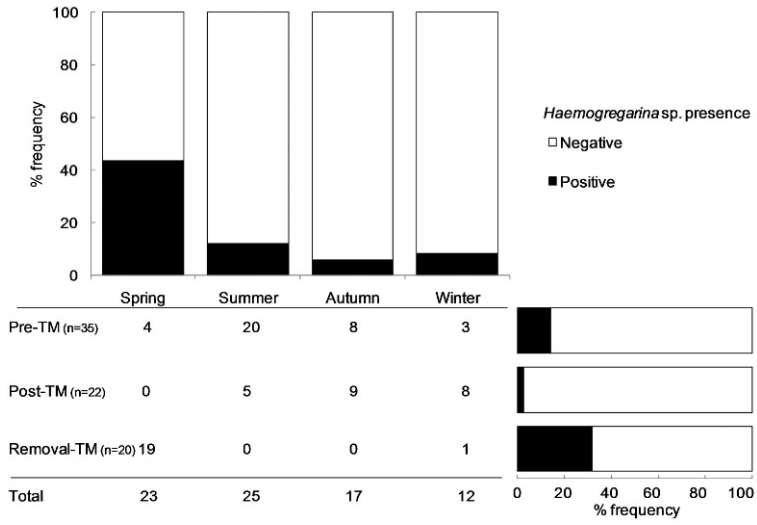

FIGURE 2. Of 77 blood samples collected from 43 southwest carpet pythons *Morelia spilota imbricata*, a greater proportion of blood samples collected from pythons during spring was positive for intracellular hemoparasites (September–November; $\chi^2_1=8.44$, $P<0.01$), whereas a greater proportion of samples collected at the time of radiotransmitter removal was positive ($\chi^2_1=8.30$, $P<0.01$) compared with preradiotransmitter and postradiotransmitter placement. The morphology of the hemoparasites seen in blood smears was consistent with *Haemogregarina moreliea*. The numbers shown in the table indicate sample size for each sample group.

The state of pythons during sampling (anesthetized or conscious) appeared to influence some blood measures. Basophil count and globulin concentration were elevated for anesthetized *M. s. imbricata* (and the reverse for the A/G ratio). We have little understanding of how anesthetics influence hematologic and biochemistry values in snakes (McDaniel et al., 1984). Further testing including perioperative, intraoperative, and postoperative stages of anesthesia and surgery would be beneficial in deciphering the interpretation of anesthetic effects on specific hematologic and plasma biochemical values.

The time individuals were sampled in relation to radiotransmitter placement appeared to affect several hematologic and plasma biochemical values. However, given that we opportunistically collected blood samples, it was not possible to control for the seasonal spread of samples collected at each radiotransmitter implantation stage. As pythons are most active during spring and summer, they were opportunistically captured at higher rates during that time (20 of 35 samples were collected in summer; Fig. 2), and the study concluded in spring of 2008 when most radiotransmitters were removed (19 of 20 removal-TM samples were collected in spring; Fig. 2). Hemoglobin, PCV, RBC, calcium, albumin, and the A/G ratio were all highest at the pre-TM stage of implantation. The majority of pre-TM samples were collected during summer, and although season did not statistically influence Hb, PCV, RBC, and calcium, the seasonal effect was significant for albumin and A/G ratio. These parameters may reflect hydration or nutritional status. A study by Lentini et al. (2011), specifically designed to test the inflammatory response of implanting radiotransmitters in rattlesnakes (*Sistrurus catenatus catenatus*), found that 33% of implanted snakes had grade 3 or higher reactions (on the basis of histopathologic tissue examination) with extensive and active inflammation. The reaction to the implant

was reflected with increases in counts of heterophils and monocytes and a decrease in globulin concentrations after 6 mo. Hemoglobin was significantly lower compared with snakes without implants (Lentini et al., 2011). Increases in these leukocyte counts were not as pronounced in our study at the three transmitter sampling stages; however, reduced Hb concentration at the last two sampling stages may indicate an anemic response, similar to that found by Lentini et al. (2011).

Hemogregarine parasites (Phylum Apicomplexa, Family Haemogregorinidae) require a vertebrate host (e.g., reptiles) and an intermediate invertebrate host (e.g., ticks, mites, mosquitoes, or leeches) to complete their life cycle (Diethelm, 2006). Infection with hemoparasites is often subclinical in reptiles; however, heavy burdens can result in anemia (Diethelm, 2006). Hemogregarines are reasonably common among snake species, including brown tree snakes (*Boiga irregularis*) and slatey-grey snakes (*Stegonotus cucullatus*) from Queensland (Caudell et al., 2002). In these two species, there was no significant difference between infected (less than 10% of RBCs) and noninfected snakes for Hb, PCV, albumin, calcium, phosphorus, protein, uric acid, AST, and CK (Caudell et al., 2002). Similarly, we found no significant differences in any hematologic or biochemical analytes for *M. s. imbricata* according to hemogregarine parasite status. The incidence of hemoparasites in *M. s. imbricata* did, however, vary significantly seasonally, with a greater prevalence in blood smears collected in spring. Changes in seasonal prevalence of hemoparasites have been observed for some lizards, where there is greater hemoparasite prevalence toward the end of the mating season in spring (Amo et al., 2005; Huyghe et al., 2010), particularly in females (Amo et al., 2005). In comparison, males often show consistent levels of hemoparasite prevalence across seasons, which has been attributed to the immunosuppressive effects of testosterone (e.g., Amo et al., 2005). Increasing numbers of invertebrate intermediate host vectors during spring would potentially drive an increase in the prevalence of the hemoparasites and hence the number of affected vertebrate (python) hosts (Huyghe et al., 2010). Furthermore, *M. s. imbricata* show greater movement and activity patterns during spring (Pearson et al., 2005), which may increase exposure to intermediate invertebrate hosts at this time. About 95% of samples were collected from pythons in spring at the time of radiotransmitter removal and there was a significant difference in the presence of hemoparasites during this sampling period. Although no conclusions can be drawn regarding the relative influences of diet, breeding activity, or stage of capture/sample collection, this seasonal pattern in hemoparasites warrants further investigation.

In conclusion, it is important to consider a variety of factors when interpreting hematologic and biochemical values for diagnosing diseases or evaluating health status of pythons. We found compounded seasonal differences in blood analytes and the time of sampling, which may be related to feeding and hydration status. Although our data suggest an effect of radiotransmitter placement, we recommend that additional research be undertaken. We hope to provide the impetus to further improve the understanding and interpretation of hematology and biochemical analytes in reptile species.

ACKNOWLEDGMENTS

We thank all staff at Vetpath Laboratory Services involved in analyzing samples for this study. Thank you to all volunteers involved in helping in fieldwork and assisting GLB with sample collection and delivery of samples to Vetpath Laboratory Services, in particular M. Connor, S. Dundas, J. Clarke, J. Bryant, and A. Bryant. This project was financially supported by the Department of Environment and Conservation, Murdoch University and the Australian Research Council LP0562099.

LITERATURE CITED

AMO, L., P. LÓPEZ, AND J. MARTÍN. 2005. Prevalence and intensity of haemogregarine blood parasites and their mite vectors in the common wall lizard, *Podarcis muralis*. Parasitology Research 96: 378–381.

ANONYMOUS. 2010. Reference guide for Reference Value Advisor v1.4, Rykkinn, Norway, 7 pp.

BEDFORD, G. S., AND K. A. CHRISTIAN. 2001. Metabolic response to feeding and fasting in the water python (*Liasis fuscus*). Australian Journal of Zoology 49: 379–387.

BRYANT, G. L. 2012. Biology of the south west carpet python (*Morelia spilota imbricata*): Is there evidence for mesopredator release in response to fox baiting? PhD Thesis, School of Veterinary and Biomedical Sciences, Murdoch University, Perth, Western Australia, 232 pp.

———, P. EDEN, P. J. DE TORES, AND K. S. WARREN. 2010. Improved procedure for implanting radiotransmitters in the coelomic cavity of snakes. Australian Veterinary Journal 88: 443–448.

CAMPBELL, T. W. 2004. Clinical chemistry of reptiles. *In* Veterinary hematology and clinical chemistry, M. A. Thrall (ed.). Blackwell Publishing Ltd, Oxford, UK, pp. 493–498.

———. 2006. Clinical pathology of reptiles. *In* Reptile medicine and surgery, 2nd Edition, D. Mader (ed.). Saunders, Elsevier, Philadelphia, Pennsylvania, pp. 453–470.

———, AND C. ELLIS. 2007. Hematology of reptiles. *In* Avian and exotic animal hematology and cytology, T. W. Campbell and C. Ellis (eds.). Blackwell Publishing Ltd, Oxford, UK, pp. 51–81.

CAUDELL, J. N., J. WHITTIER, AND M. R. CONOVER. 2002. The effects of haemogregarine-like parasites on brown tree snakes (*Boiga irregularis*) and slatey-grey snakes (*Stegonotus cucullatus*) in Queensland, Australia. International Biodeterioration & Biodegradation 49: 113–119.

CHRISTOPHER, M., K. BERRY, I. WALLIS, K. NAGY, B. HENEN, AND C. PETERSON. 1999. Reference intervals and physiologic alterations in hematologic and biochemical values of free-ranging desert tortoises in the Mojave Desert. Journal of Wildlife Diseases 35: 212–238.

CLINICAL AND LABORATORY STANDARDS INSTITUTE (CLSI). 2008. Defining, establishing, and verifying reference intervals in the clinical laboratory: Approved guideline, 3rd Edition. Clinical and Laboratory Standards Institute, Wayne, Pennsylvania, 59 pp.

DESSAUER, H. C. 1970. Blood chemistry of reptiles: Physiological and evolutionary aspects. *In* Biology of the reptilia, C. Cans and T. S. Parsons (eds.). Academic Press, New York, New York, pp. 1–72.

DIETHELM, G. 2006. Haemoparasites. *In* Reptile medicine and surgery, D. R. Mader (ed.). WB Saunders, Philadelphia, Pennsylvania, pp. 1103–1117.

EDWARDS, J. 2003. Wildlife conservation (reptiles and amphibians; pet herpetofauna) notice 2003. Government Gazette Wildlife Conservation Act 1950 CO301, The Department of Environment and Conservation, Perth, Western Australia, pp. 658–659.

ESPINOSA-AVILÉS, D., V. M. SALOMÓN-SOTO, AND S. MORALES-MARTÍNEZ. 2009. Hematology, blood chemistry, and bacteriology of the free-ranging Mexican beaded lizard (*Heloderma horridum*). Journal of Zoo and Wildlife Medicine 39: 21–27.

HUYGHE, K., A. VAN OYSTAEYEN, F. PASMANS, Z. TADIĆ, B. VANHOOYDONCK, AND R. VAN DAMME. 2010. Seasonal changes in parasite load and a cellular immune response in a colour polymorphic lizard. Oecologia 163: 867–874.

INTERNATIONAL UNION FOR CONSERVATION OF NATURE (IUCN). 1998. Guidelines for re-introductions. Prepared by the IUCN/SSC Re-introduction Specialist Group. Gland, Switzerland and Cambridge, UK, 10 pp.

LENTINI, A. M., G. J. CRAWSHAW, L. E. LICHT, AND D. J. MCLELLAND. 2011. Pathologic and hematologic responses to surgically implanted transmitters in eastern massasauga rattlesnakes (*Sistrurus catenatus catenatus*). Journal of Wildlife Diseases 47: 107–125.

MACKERRAS, M. 1961. The haematozoa of Australian reptiles. Australian Journal of Zoology 9: 61–122.

MCDANIEL, R., W. GRUNOW, J. DALY, AND M. PLUMMER. 1984. Serum chemistry of the diamond-backed water snake (*Nerodia rhombifera rhombifera*) in Arkansas. Journal of Wildlife Diseases 20: 44–46.

NORDØY, E. S., AND S. I. THORESEN. 2002. Reference values for serum biochemical parameters in free-ranging harp seals. Veterinary Clinical Pathology 31: 98–106.

O'DONOGHUE, P. J., AND R. D. ADLARD. 2000. Catalogue of protozoan parasites recorded in Australia. Memoirs of the Queensland Museum 45: 1–163.

PEARSON, D. 2002. The ecology and conservation of the south-western Australian carpet python, *Morelia spilota imbricata*. PhD Thesis, School of Biological Sciences, University of Sydney, Sidney, New South Wales, Australia, 152 pp.

———, R. SHINE, AND A. WILLIAMS. 2002. Geographic variation in sexual size dimorphism within a single snake species (*Morelia spilota*, Pythonidae). Oecologia 13: 418–426.

———, ———, AND ———. 2005. Spatial ecology of a threatened python (*Morelia spilota imbricata*) and the effects of anthropogenic habitat change. Austral Ecology 30: 261–274.

SECOR, S. M., AND B. D. OTT. 2007. Adaptive correlation between feeding habits and digestive physiology for boas and pythons. *In* Biology of the boas and pythons, R. W. Henderson and R.

Powell (eds.). Eagle Mountain Publishing, Eagle Mountain, Utah, pp. 257–268.

STIRK, N., I. R. ALLENMAN, AND K. E. HARR. 2007. Circulating and inflammatory cells. *In* Infectious diseases and pathology of reptiles, E. R. Jacobson (ed.). CRC Press: Taylor & Francis Group, Boca Raton, Florida, pp. 167–189.

TEARE, J. A. (ed.). 2002. Reference ranges for physiological values in captive wildlife: International Species Information System physiological data reference values. ISIS, Eagan, Minnesota, on CD-ROM.

WOJTASZEK, J. S. 1992. Seasonal changes of circulating blood parameters in the grass snake *Natrix natrix natrix*. Comparative Biochemistry and Physiology Part A: Physiology 103: 461–471.

Submitted for publication 4 August 2010.
Accepted 1 October 2011.