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Blood Parameters in Natural Populations of *Trichosurus* Species (Marsupialia : Phalangeridae) I. Age, Sex and Seasonal Variation in *T. caninus* and *T. vulpecula*

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Abstract

Age, sex and seasonal related changes in body weight and blood parameters were determined for *T. caninus* and *T. vulpecula*, whose distribution overlapped in part of the study area. In *T. caninus* age-related changes occurred in body weight, mean plasma glucose and protein concentrations and haematocrit. Seasonal changes occurred in plasma glucose and protein concentrations, haemoglobin concentration and red blood cell count. There were sexual dimorphisms in haematocrit, haemoglobin concentration and red blood cell count in both species; the mean values were greater in males. *T. vulpecula* also exhibited a sexual dimorphism in body weight (males > females). The only seasonal change in *T. vulpecula* was in haemoglobin concentration.

Comparison of the species as adults showed that *T. caninus* had higher mean levels of body weight, haematocrit and haemoglobin concentration, while plasma lipid concentration and red blood cell counts were greater in *T. vulpecula*. These species differences may be a reflection of the diets of the two species. In addition the measured parameters showed greater seasonal variation in *T. caninus*, a *K*-selected species, than in *T. vulpecula*, which is more *r*-selected.

Introduction

Natural populations of two species of congeneric phalangers, the mountain possum *Trichosurus caninus* (Ogilby) and the brush-tailed possum *T. vulpecula* (Kerr) have been studied since 1967 (How 1972, 1976). *T. caninus* young develop more slowly (How 1976) and mature later (Smith and How 1973) than those of *T. vulpecula*. *T. caninus* is heavier, lives longer and appears to form pair bonds, while *T. vulpecula* is polygamous (How 1972). *T. caninus* has a low rate of survival of dependent young and high survival of subadults and adults, whereas *T. vulpecula* has high survival as dependent young and low survival among dispersing subadults (How 1978). *T. vulpecula* is widespread in forest, woodland and urban areas while *T. caninus* is mainly confined to tall open and closed forest (Ride 1970), where it probably excludes *T. vulpecula* (How 1972). These species differences correlate closely with the *r*–*K* selection continuum of Pianka (1970), *T. caninus* being the more *K*-selected species.

This paper analyses age, sex and seasonal changes in blood parameters of both species and attempts to relate them to their contrasting population strategies.

Most data on blood parameters of mammals have been obtained from laboratory-maintained or reared animals (Francis and Strong 1938; Anderson and Gee 1958; Barnett *et al.* 1974) in which seasonal changes cannot be properly considered. For example an 8-month study of various blood parameters of the brown marsupial mouse, *Antechinus stuartii* Macleay, under captive conditions with natural

daylength, and of wild-caught animals showed more pronounced changes in the blood parameters in the latter (Barnett 1973).

Many techniques have been used to examine seasonal trends in various physiological characteristics of populations. Wild-caught animals have been acclimatized in the laboratory for a standard period prior to sampling (Newson 1962; Barnett 1973; Cheal *et al.* 1976; Middleton 1976) but variation detected could be due to the laboratory conditions. Alternatively, animals have been bled the morning of capture (Lee and Brown 1970; Maclean and Lee 1973; Middleton 1976) but the stress of being trapped and the period in the trap were not considered by these authors. However, the levels of corticosteroids are similar in the Australian bush rat, *Rattus fuscipes* (Waterhouse), bled the morning after capture or after 3 days confinement in the laboratory (unpublished data of J. L. Barnett), but bleeding on the morning after capture was considered to give a more accurate index of nutritional status in this species. Short-term deprivation of food did not result in increased levels of free fatty acids, whereas laboratory-held animals showed a marked response to diet by increasing their plasma lipid levels (Middleton 1976). Although large animals may be shot to obtain rapid blood samples (Wilber and Robinson 1958; Oddie *et al.* 1976) this technique is impractical for small or secretive animals and prevents the study of individuals through time.

The techniques used in the present study, namely bleeding the trapped animals in the field and releasing them, allowed individuals to be sampled sequentially, did not alter the age structure and had minimal effect on the social organization of the populations of both species in our study area. How (1972) provided known ages for the majority of individuals sampled.

We measured two groups of blood parameters. First, those which, in other mammals, often show seasonal changes (haemoglobin concentration, haematocrit and red cell count) and second, blood plasma determinants of nutritional status (plasma proteins, glucose and lipid concentrations) to indicate possible responses to changing food quality.

Materials and Methods

Study Area

The study area is at Clouds Creek (30°05'S., 152°37'E.), situated between altitudes of 570 and 730 m on the eastern escarpment of the New England Tableland in north-eastern New South Wales. *T. caninus* occurred in most forest types of the 118-km^2 study area but was mainly confined to tall open and closed forests (Barnett *et al.* 1976). Specht's (1970) vegetation classification is followed. These two forest types we considered as optimal habitats for the species, whereas open forest, woodland, grassland and pine plantations we considered suboptimal as no individuals exclusively inhabited these latter habitats. *T. vulpecula* was confined mainly to open forest and grazed woodland but also occurred in pine platations (Barnett *et al.* 1976).

Trapping and Blood Sampling

Animals were trapped on seven occasions during all seasons, spaced over 18 months, in two sizes of non-collapsible traps (Mascot Wire Works, Enfield, N.S.W.; 61 by 30 by 30 cm and 76 by 30 by 30 cm) and one size of collapsible trap (Tomahawk Live Trap Co., Tomahawk, Wisconsin, U.S.A.; 66 by 22 by 22 cm) baited with apple. On the morning after capture animals were weighed and the head length measured; this measurement, along with testis length or pouch condition, was used to assign an animal to a juvenile or adult age class (How 1976) if its age was not accurately known from the previous long-term population study. *T. caninus* is a seasonal breeder and 80% of births occur in March–April (How 1976); consequently we considered that all individuals aged by one year in April.

Blood samples were obtained at the site of capture by cutting the ear and collecting the blood either directly into heparinized vials or into heparinized haematocrit tubes from which it was transferred into vials. The vials were placed in an insulated container for transportation to the laboratory.

Measurements

One haematocrit tube of blood was kept to determine packed red cell volume; this tube was centrifuged on an International Micro-Capillary centrifuge at 13000 *g* for 3 min. A 20-μl sample of whole blood was mixed with a cyanide solution to determine the haemoglobin concentration by the cyanomethaemoglobin method (Boehringer, Mannheim, W. Germany). The blood in the vials was centrifuged (with a delay of 1–6 h) and the plasma stored frozen for later analysis. Plasma glucose and total plasma lipid concentrations were measured with appropriate kits (Boehringer) and plasma protein concentration by the biuret method (Gornall *et al.* 1949).

During the early stages of this study a sexual dimorphism in haematocrit was noticed; subsequently red and white cell counts were made; the results for the white cell counts will be reported elsewhere. Blood was collected in diluting pipettes and diluted with either Türks fluid (for white cell counts) or Hayem's solution (for red cell counts). The pipettes were shaken for 3 min, half the solution expelled and the rest transferred into vials. The cells were resuspended by 3 min mixing by inversion, and cell counts made in a counting chamber with a Neubauer ruling; there was a delay of 1–6 h between collection and counting.

Statistical Analyses

In seven sampling periods spaced over 18 months 296 blood samples were collected from *T. caninus*. We assumed that all sampling periods were independent. Ten of the blood samples were from animals of uncertain age class (either 2 or 3 y old). These samples were not included in the analyses as they could not be assigned with certainty to adult (> 3 y old) or subadult (< 3 y old) age classes.

Data were analysed by analysis of variance for differences between juveniles and adults, sex and season (Winer 1971). If significant differences were found between the age classes the data were further analysed by least squares regression against age, with only those samples from individuals of known age. Where a significant age effect was found the data were standardized for convenience to values of an animal 4 y old, by the gradient of the regression line (this method is discussed fully in Barnett *et al.* 1974). These age-corrected data were then analysed for differences between sexes and seasons, independently of age relationships, by analysis of variance, and the means compared using the Least Significant Difference (Winer 1971).

Data from *T. vulpecula* were insufficient to analyse on the basis of age. Of the 52 samples only one was from a subadult male (< 18 months old) and four from subadult females (*T. vulpecula* matures at an earlier age than *T. caninus*; Smith and How 1973). Data from adult *T. vulpecula* were analysed for sex and seasonal differences and compared with adult *T. caninus*.

Results

T. caninus

(i) *Body weight*

Subadult *T. caninus* were lighter than adults ($P < 0{\cdot}001$; Table 1), and body weight changed with season ($P < 0{\cdot}001$); also there was a significant interaction term between age and season ($P < 0{\cdot}01$). The regression between body weight and age was significant (where w is body weight in grams and y is age in years: $\ln w = 0{\cdot}218 \ln y + 7{\cdot}615$; $r = 0{\cdot}83, n = 171$). After correction for age differences by adjusting body weight (see above section on statistics for this method), significant changes in mean body weight with season were abolished (Table 2).

(ii) *Plasma glucose concentration*

Mean plasma glucose concentration changed with age class (subadult > adults;

Table 1. Changes in body weight and blood parameters in adult and subadult *T. caninus*

Females were weighed without their pouch young unless the young was < 3 months old when its weight was < 100 g; pouch young were present in May, August and October. Values are means ± standard errors, except those for samples of two, which are ranges; sample sizes are in parentheses

Date	Body weight (g)	Glucose (mg dl^{-1})	Proteins (g dl^{-1})	Lipids (mg dl^{-1})	Haematocrit (%)	Hb concn (g dl^{-1})	10^{-12} × red cell count per l
				Subadult males			
Feb. 1975	2440·0 ± 147·8 (5)	89·3 (1)	7·5 ± 0·2 (5)	417·8 (1)	49·3 ± 3·6 (5)	17·5 ± 1·3 (5)	–
May 1975	2250·0 ± 84·5 (7)	116·8 ± 17·3 (5)	7·3 ± 0·4 (7)	567·2 ± 53·2(5)	50·9 ± 2·6 (7)	14·1 ± 0·4 (7)	–
Aug. 1975	2000·0 – 2600·0 (2)	71·9 – 110·5 (2)	8·4 – 8·8 (2)	619·9 – 698·9 (2)	48·0 – 49·0 (2)	8·5 – 10·4 (2)	–
Oct. 1975	2396·4 ± 76·8 (14)	118·5 ± 6·4 (13)	7·8 ± 0·2 (14)	499·7 ± 37·1 (11)	46·3 ± 1·3 (13)	14·5 ± 0·4 (13)	5·66 ± 0·24 (13)
Feb. 1976	2478·6 ± 80·1 (7)	101·4 ± 12·4 (7)	8·5 ± 0·8 (7)	537·4 ± 65·0 (7)	51·1 ± 1·2 (7)	16·7 ± 0·4 (7)	6·39 ± 0·39 (7)
May 1976	2062·5 ± 213·5 (4)	96·6 ± 14·4 (4)	6·9 ± 0·3 (4)	494·0 ± 54·1 (4)	46·0 ± 2·9 (4)	17·8 ± 1·8 (4)	5·77 ± 0·59 (4)
Aug. 1976	2535·7 ± 85·7 (7)	128·2 ± 7·5 (7)	7·0 ± 0·3 (7)	477·1 ± 22·9 (7)	48·5 ± 0·8 (7)	16·2 ± 0·3 (7)	6·54 ± 0·40 (7)
				Adult males			
Feb. 1975	3054·2 ± 72·2 (12)	96·8 ± 8·8 (10)	8·1 ± 0·2 (10)	458·5 ± 15·6 (8)	50·3 ± 1·4 (12)	15·3 ± 0·7 (11)	–
May 1975	2925·0 ± 64·7 (16)	97·3 ± 6·4 (15)	7·9 ± 0·3 (16)	530·1 ± 23·6 (15)	52·3 ± 1·0 (16)	14·4 ± 0·4 (16)	–
Aug. 1975	2950·0 ± 27·4 (5)	126·7 ± 12·0 (3)	7·6 ± 0·3 (5)	539·5 ± 19·8 (3)	48·2 ± 1·8 (5)	13·9 ± 0·6 (5)	–
Oct. 1975	2989·5 ± 60·3 (14)	106·3 ± 9·2 (10)	8·0 ± 0·4 (12)	476·6 ± 48·6 (11)	50·5 ± 1·1 (13)	15·4 ± 0·6 (14)	5·47 ± 0·28 (14)
Feb. 1976	3091·7 ± 38·0 (18)	69·4 ± 6·1 (17)	8·9 ± 0·5 (18)	466·4 ± 24·6 (16)	52·9 ± 1·4 (17)	16·0 ± 0·6 (18)	6·58 ± 0·25 (18)
May 1976	2925·0 ± 62·9 (18)	81·9 ± 3·9 (18)	7·3 ± 0·4 (18)	553·8 ± 29·4 (18)	47·2 ± 1·1 (18)	17·2 ± 0·4 (18)	6·39 ± 0·13 (18)
Aug. 1976	2984·8 ± 71·2 (23)	97·7 ± 4·1 (23)	7·6 ± 0·2 (23)	474·6 ± 27·8 (23)	48·0 ± 1·0 (23)	16·5 ± 0·4 (23)	6·22 ± 0·16 (23)

Subadult females							
Feb. 1975	2116·7 ± 372·3 (3)	173·6 (1)	7·6 ± 0·1 (3)	542·2 (1)	46·0 ± 1·2 (3)	17·4 ± 1·4 (3)	—
May 1975	1500·0 – 1750·0 (2)	119·0 – 185·3 (2)	7·9 – 8·2 (2)	496·6 – 635·7 (2)	44·0 – 49·0 (2)	13·2 – 13·7 (2)	—
Aug. 1975	2250·0 – 2650·0 (2)	121·3 – 123·5 (2)	6·5 ± 0·8 (3)	510·3 – 537·6 (2)	45·0 ± 4·6 (3)	12·3 ± 0·7 (3)	—
Oct. 1975	2400·0 ± 200·0 (3)	108·3 ± 6·2 (3)	6·8 ± 0·9 (3)	503·2 ± 42·7 (3)	44·7 ± 2·0 (3)	13·0 ± 0·1 (3)	6·17 ± 0·36 (3)
Feb. 1976	2750·0 ± 73·4 (4)	78·6 ± 5·6 (4)	7·0 ± 1·3 (3)	567·6 ± 35·7 (3)	47·5 ± 2·7 (4)	15·8 ± 1·2 (4)	5·35 ± 0·34 (4)
May 1976	—	—	—	—	—	—	—
Aug. 1976	2270·0 ± 128·1 (5)	110·4 ± 7·2 (5)	6·8 ± 0·2 (5)	539·0 ± 62·2 (5)	46·4 ± 0·75 (5)	15·7 ± 0·6 (5)	5·81 ± 0·90 (5)
Adults females							
Feb. 1975	3013·6 ± 52·3 (11)	98·4 ± 10·0 (9)	8·2 ± 0·2 (11)	444·5 ± 36·6 (6)	42·0 ± 0·8 (11)	14·6 ± 0·6 (10)	—
May 1975	3028·6 ± 73·1 (14)	108·0 ± 4·8 (13)	7·9 ± 0·3 (14)	515·7 ± 26·8 (13)	44·8 ± 0·6 (14)	12·7 ± 0·4 (14)	—
Aug. 1975	3291·7 ± 127·4 (6)	106·9 ± 9·9 (5)	7·8 ± 0·2 (5)	581·7 ± 68·9 (5)	42·2 ± 2·0 (5)	14·5 ± 0·9 (5)	—
Oct. 1975	3023·9 ± 63·5 (23)	96·1 ± 5·5 (21)	7·8 ± 0·3 (22)	531·1 ± 38·8 (19)	41·2 ± 1·1 (22)	12·6 ± 0·3 (22)	4·36 ± 0·23 (17)
Feb. 1976	2983·3 ± 57·5 (12)	82·9 ± 5·9 (12)	8·3 ± 0·3 (12)	466·6 ± 38·7 (11)	40·3 ± 1·2 (12)	14·1 ± 0·4 (12)	5·30 ± 0·14 (12)
May 1976	3084·4 ± 91·8 (16)	82·5 ± 2·7 (16)	7·1 ± 0·2 (16)	506·5 ± 18·6 (15)	41·4 ± 0·8 (18)	14·9 ± 0·5 (16)	5·19 ± 0·16 (16)
Aug. 1976	2990·0 ± 55·5 (20)	100·0 ± 4·7 (20)	7·1 ± 0·2 (20)	494·6 ± 25·8 (17)	43·2 ± 0·9 (19)	15·2 ± 0·5 (20)	5·27 ± 0·14 (20)

$P < 0{\cdot}01$; Table 1) and differed between sexes ($P < 0{\cdot}05$; females > males). However, this sex difference was abolished after correction for age (Table 2) by the relationship $\ln g = -0{\cdot}199 \ln y + 4{\cdot}88$, where g is glucose concentration in mg dl^{-1}; $r = -0{\cdot}421$, $n = 148$. In both sexes there was a decrease ($P < 0{\cdot}05$) in corrected mean plasma glucose concentration between October 1975 and February 1976, followed by an increase ($P < 0{\cdot}05$) in corrected mean value by the end of winter 1976 (Table 2). The mean values in adult males and females were less in February 1976 (summer) and May 1976 (autumn) than in the corresponding periods in 1975 ($P < 0{\cdot}05$; Tables 1 and 2).

Table 2. Changes in body weight and plasma glucose concentration in *T. caninus*, corrected for age to 4 years old

Known-age animals only. Values are means ± standard errors, except those for samples of two, which are ranges. Sample sizes are in parentheses

Date	ln body weight (g)	ln plasma glucose (mg dl^{-1})
	Males	
Feb. 1975	7·92 ± 0·02 (15)	4·61 ± 0·09 (9)
May 1975	7·90 ± 0·02 (14)	4·63 ± 0·07 (11)
Aug. 1975	7·88 ± 0·04 (4)	4·17 – 4·49 (2)
Oct. 1975	7·91 ± 0·02 (22)	4·63 ± 0·04 (19)
Feb. 1976	7·91 ± 0·01 (16)	4·31 ± 0·09 (16)
May 1976	7·89 ± 0·03 (17)	4·42 ± 0·07 (17)
Aug. 1976	7·97 ± 0·02 (21)	4·62 ± 0·04 (21)
	Females	
Feb. 1975	7·92 ± 0·01 (8)	4·79 ± 0·08 (6)
May 1975	7·85 ± 0·06 (7)	4·74 ± 0·09 (6)
Aug. 1975	8·00 ± 0·06 (4)	4·63 ± 0·07 (3)
Oct. 1975	7·93 ± 0·02 (12)	4·58 ± 0·09 (11)
Feb. 1976	7·95 ± 0·03 (8)	4·30 ± 0·06 (8)
May 1976	7·90 ± 0·02 (7)	4·42 ± 0·06 (7)
Aug. 1976	7·92 ± 0·02 (4)	4·59 ± 0·05 (14)

(iii) *Plasma protein and lipid concentrations*

Initial analysis of plasma protein and total lipid concentrations showed no significant changes with age class, season or sex (Table 1). However, when data from the two sexes were combined the subadults had lower mean plasma protein concentrations than adults ($P < 0{\cdot}05$; Table 3). Mean combined plasma protein concentration also changed with season ($P < 0{\cdot}05$); in both age classes there was a peak value in February 1976 (summer) followed by a decrease in mean value by early winter (May 1976, $P < 0{\cdot}05$; Table 3). The pattern was similar in 1975 although these changes were not significant.

(iv) *Haematocrit*

There was a sexual dimorphism in the haematocrit (males > females; $P < 0{\cdot}0001$; Table 1) as well as differences between subadults and adults in females (subadults > adults; $P < 0{\cdot}05$) but not in males. Haematocrit was significantly

regressed with age in both males and females ($P < 0{\cdot}01$). Equations of the regression lines, where h is percentage haematocrit, are:

$$\text{Males: } h = 0{\cdot}537\, y + 46{\cdot}98;\ r = 0{\cdot}26,\ n = 106;$$
$$\text{Females: } h = -0{\cdot}774\, y + 48{\cdot}05;\ r = -0{\cdot}49,\ n = 58.$$

(v) *Haemoglobin concentration*

As in mean haematocrit, there was a sexual dimorphism in mean haemoglobin concentration (males > females; $P < 0{\cdot}01$; Table 1). There was no significant regression with age but there were seasonal changes that differed between years. In males there was a minimum mean value in winter 1975 (May–August) whereas in 1976 haemoglobin concentration was at a peak mean value in May ($P < 0{\cdot}05$; Table 1). A similar pattern occurred in females although the changes were not significant.

(vi) *Red blood cell count*

Red blood cell counts in males were higher than in females ($P < 0{\cdot}0001$; Table 1) and the only seasonal difference was a minimum mean value in males and adult females in October 1975 ($P < 0{\cdot}05$; Table 1).

Table 3. Changes in plasma protein concentration in *T. caninus* with age class and time of year

Sexes combined. Values are means ± standard errors, in grams per decilitre, with sample sizes in parentheses

Date	Subadults	Adults
Feb. 1975	7·5 ± 0·1 (8)	8·2 ± 0·1 (21)
May 1975	7·5 ± 0·3 (9)	8·0 ± 0·2 (30)
Aug. 1975	7·8 ± 0·4 (4)	7·7 ± 0·1 (10)
Oct. 1975	7·6 ± 0·3 (17)	7·9 ± 0·2 (36)
Feb. 1976	8·1 ± 0·7 (10)	8·7 ± 0·3 (30)
May 1976	6·9 ± 0·3 (4)	7·2 ± 0·2 (34)
Aug. 1976	7·0 ± 0·2 (12)	7·6 ± 0·1 (43)

T. vulpecula

(i) *Body weight*

There was an overall sexual dimorphism in body weight in *T. vulpecula* (males > females; $P < 0{\cdot}05$; Tables 4 and 5); no sexual dimorphism in body weight was found in *T. caninus*.

(ii) *Protein, glucose and lipid concentration*

No significant differences in mean plasma concentrations of protein, glucose and total lipids were found between sexes or within or between years (Tables 4, 5) even when data from the sexes were combined.

(iii) *Haematocrit and haemoglobin concentration*

As in *T. caninus* there was a significant sexual dimorphism in mean haemoglobin

Table 4. Changes in body weight and blood parameters in adults *T. vulpecula*

Females were weighed without their pouch young unless the young was <2 months old when its weight was < 100 g; pouch young were present in May and August. Values are means ± standard errors, except for samples of two, which are ranges; sample sizes are in parentheses

Date	Body weight (g)	Protein (g dl^{-1})	Glucose (mg dl^{-1})	Lipids (mg dl^{-1})	Haematocrit (%)	Hb concn (g dl^{-1})	10^{-12} × red cell count per l
			Males				
Feb. 1975	2200·0 (1)	7·9 (1)	115·0 (1)	473·1 (1)	38·0 (1)	12·3 (1)	–
May 1975	2787·5 ± 119·7 (4)	8·1 ± 0·5 (4)	123·2 ± 12·8 (4)	502·5 ± 47·1 (4)	41·1 ± 1·7 (4)	12·4 ± 0·6 (4)	–
Aug. 1975	2850·0 (1)	8·8 (1)	93·3 (1)	602·2 (1)	50·0 (1)	18·4 (1)	–
Oct. 1975	2830·0 ± 144·6 (5)	6·9 ± 0·8 (5)	85·0 ± 18·2 (4)	661·7 ± 35·2 (4)	44·4 ± 2·4 (5)	13·7 ± 0·5 (5)	7·02 ± 0·39 (5)
Feb. 1976	2850·0 – 3000·0 (2)	6·2 – 8·1 (2)	84·8 – 107·5 (2)	417·5 – 449·1 (2)	45·0 – 47·0 (2)	14·7 – 17·4 (2)	7·49 – 7·76 (2)
May 1976	2975·0 ± 85·4 (4)	8·2 ± 0·6 (4)	96·0 ± 14·8 (4)	673·7 ± 75·6 (4)	44·7 ± 1·0 (4)	16·9 ± 1·0 (4)	9·15 ± 3·07 (4)
Aug. 1976	2816·7 ± 101·4 (3)	8·4 ± 0·3 (3)	119·0 ± 29·1 (3)	582·3 ± 21·7 (3)	50·2 ± 3·4 (3)	15·9 ± 0·7 (3)	6·82 ± 0·26 (3)
			Females				
Feb. 1975	2400·0 – 2750·0 (2)	7·1 – 7·7 (2)	93·3 – 141·9 (2)	557·6 – 594·8 (2)	35·0 – 45·0 (2)	10·9 – 15·9 (2)	–
May 1975	2537·0 ± 106·8 (4)	8·2 ± 0·5 (4)	91·6 ± 18·2 (4)	515·8 ± 38·5 (4)	43·7 ± 1·8 (4)	11·4 ± 0·8 (4)	–
Aug. 1975	3000·0 (1)	8·1 (1)	90·8 (1)	867·4 (1)	41·5 (1)	13·5 (1)	–
Oct. 1975	2460·0 ± 60·0 (5)	7·6 ± 0·4 (6)	94·6 ± 6·3 (5)	643·3 ± 76·8 (6)	41·0 ± 1·4 (6)	13·2 ± 0·6 (6)	5·36 ± 0·29 (6)
Feb. 1976	2900·0 – 3000·0 (2)	7·6 – 8·4 (2)	107·3 – 114·8 (2)	564·9 – 571·9 (2)	39·0 (2)	11·6 – 15·0 (2)	5·57 – 6·18 (2)
May 1976	2675·0 ± 101·0 (4)	6·9 ± 0·6 (4)	70·6 ± 19·3 (4)	593·3 ± 69·7 (4)	41·0 ± 4·3 (4)	15·4 ± 0·6 (4)	5·91 ± 0·30 (4)
Aug. 1976	2685·0 ± 97·3 (5)	7·3 ± 0·3 (5)	124·8 ± 15·2 (5)	622·9 ± 109·0 (5)	39·2 ± 1·0 (5)	13·6 ± 0·5 (5)	6·18 ± 0·58 (5)

concentration and haematocrit (males > females; $P < 0{\cdot}01$; Tables 5, 6). There were also changes between years in haemoglobin concentration similar to those in *T. caninus*; the mean value in May 1976 (early winter) was higher than that in May 1975 in both males and females ($P < 0{\cdot}01$; Table 5).

(iv) *Red cell counts*

As in *T. caninus* the red blood cell count was higher in males than in females ($P < 0{\cdot}05$; Tables 4, 5) but there was no significant seasonal change in red cell counts (Table 4).

Table 5. Overall mean values of body weight and blood parameters in adult *T. caninus* and *T. vulpecula*

Values are means ± standard errors, with sample sizes in parentheses

Variable	Males		Females	
	T. caninus	*T. vulpecula*	*T. caninus*	*T. vulpecula*
Body weight (g)	2990·6 ± 25·0 (106)	2827·5 ± 57·5 (20)	3026·0 ± 28·4 (104)	2635·9 ± 47·4 (23)
Protein (g dl^{-1})	8·0 ± 0·1 (102)	7·8 ± 0·3 (20)	7·8 ± 0·1 (102)	7·5 ± 0·2 (24)
Glucose (mg dl^{-1})	91·4 ± 2·7 (96)	103·9 ± 7·3 (19)	95·4 ± 2·3 (97)	99·7 ± 6·7 (23)
Lipid (mg dl^{-1})	497·8 ± 12·3 (94)	581·1 ± 27·2 (19)	506·1 ± 11·9 (88)	606·9 ± 33·2 (23)
Haematocrit (%)	49·9 ± 0·5 (104)	46·4 ± 1·1 (20)	42·2 ± 0·4 (101)	40·8 ± 0·9 (24)
Haemoglobin (g dl^{-1})	15·9 ± 0·2 (105)	14·8 ± 0·5 (20)	14·0 ± 0·2 (101)	13·4 ± 0·4 (24)
10^{-12} × Red cell count per l	6·21 ± 0·11 (73)	7·67 ± 0·84 (14)	5·02 ± 0·10 (67)	5·79 ± 0·21 (17)

Species Comparison of Adults

T. caninus showed higher overall mean values of body weight ($P < 0{\cdot}0001$), haematocrit ($P < 0{\cdot}01$) and haemoglobin concentration ($P < 0{\cdot}05$) than *T. vulpecula* (Table 5). There was a sexual dimorphism in haematocrit, haemoglobin and red blood cell count in both species (males > females; $P < 0{\cdot}05$; Table 5). Overall mean levels of total lipids and red blood cell count were higher in *T. vulpecula* ($P < 0{\cdot}0001$; Table 5).

Effects of Trapping

In an attempt to assess the effects of trapping and trap experience of the possums on the measured parameters, we compared two categories of *T. caninus* for changes in haematocrit. First, to determine the effects of experience of blood sampling *per se*, possums previously trapped and handled (> 10 captures) but not bled were compared on the first and third occasion they were bled. The percentage haematocrits (mean ± standard error, with sample size in parentheses) were:

	Males	Females
First bleed	51·3 ± 1·3 (11)	42·2 ± 0·7 (13)
Third bleed	51·8 ± 1·7 (11)	41·8 ± 1·1 (13)

Second, to determine the effects of trapping and handling experience, possums caught and bled for the first time were compared with those previously trapped but not bled:

	Males	Females
Trap naive	52·1 ± 1·6 (12)	41·5 ± 1·1 (13)
Trap experienced	50·8 ± 1·2 (14)	42·2 ± 0·7 (13)

The results of both trials showed no significant effects.

Discussion

T. caninus

We have measured certain blood parameters throughout post-weaning life and can therefore detect changes related to both age and seasonal influences. Blood parameters of *T. caninus* have not previously been measured and there are no 'normal' values for comparison.

(i) *Age*

Changes with age occurred in body weight, in mean plasma protein and glucose concentrations and in haematocrit. Except for haematocrit in males these changes were probably developmental, as they were greatest as the animals reached maturity. Haematocrit increased linearly with age in males although the change was not large [mean percentage value ± SE at 1–2 y old = 47·8 ± 0·9 (n = 20) and at 8–9 y old = 49·9 ± 1·6 (n = 9)]; an increase with age has been recorded in other mammals (Kitts *et al.* 1956). The decrease in haematocrit with age in females is unusual and may be associated with the level of circulating sex hormones (these have still to be determined for *T. caninus*); an increase in haematocrit following gonadectomy has been observed in male goats (Wilkins and Hodges 1962), and in laboratory rats where the effect was greater in females than in males (Barnett and Phillips 1976). Plasma glucose concentration decreased with age in white-tailed deer *Odocoileus virginianus*, but was associated with the physiological development of the ruminant stomach (Johnson *et al.* 1968). Digestion is of the non-ruminant type in *Trichosurus* spp. (Tyndale-Biscoe 1973).

(ii) *Season*

There were seasonal changes in haemoglobin concentration and red blood cell count. Haemoglobin concentration in winter was high during the second year of the study but low during the first year. Haemoglobin concentration changes seasonally in mice (Maclean and Lee 1973) and voles (Newson 1962), being highest in winter. In the voles the changes were not correlated with age, breeding condition, weather or parasite infestation (Newson 1962). Other evidence suggests that haemoglobin concentration responds to abiotic factors (Sealander 1960) such as temperature, as found in *Mus musculus* (Maclean and Lee 1973). At Clouds Creek the seasons in 1975 were 'normal' with a wet summer and dry winter, but in 1976 an exceptionally wet summer was followed by a wet winter. In 1975 the transient reduction in winter of haemoglobin concentration was not associated with lowered haematocrit; this resulted in a decreased mean corpuscular haemoglobin concen-

tration suggestive of anaemia (Schalm *et al.* 1975). These changes in haematological parameters did not reflect reproductive state, as has been observed in goats (Holman and Dew 1966), because they were independent of age and sex.

The reduction of the level of plasma glucose and the increase in plasma protein in summer may indicate changed adrenal activity (Selye 1946).

(iii) *Sex*

In *T. caninus* the only sex differences observed are in haematocrit, haemoglobin concentration and red blood cell count, which are higher in males than females. Values of haematocrit are sufficiently different to be useful in determining sex. Sex-dependent differences in these parameters are variable between mammal species and where they occur are generally small (cf. haematocrit in this study). Other mammal species may show no sexual differences in haematocrit or males may have higher or lower haematocrit than females (see Schalm *et al.* 1975 for review). Contradictory reports exist for beagle dogs with either males (Anderson and Gee 1958) or females (Robinson and Ziegler 1968) having greater haematocrit.

T. vulpecula

Data for subadult *T. vulpecula* were insufficient to detect age-related changes over a period, similar to those in *T. caninus*. There were, however, similar sex differences in the haematocrit, haemoglobin concentration and red blood cell count which were greater in males than in females, and similar changes in mean haemoglobin concentration within and between years. In contrast to *T. caninus* a sexual dimorphism in body weight existed in *T. vulpecula* (males > females). The seasonal changes in plasma glucose and protein concentrations, and in red blood cell count in *T. caninus* were absent in *T. vulpecula*.

Effects of Trapping

In the present study possums were in traps between 4 and 18 h before the blood samples were taken. They were transferred into a sack and restrained manually while the blood sample was taken from an ear, a procedure lasting between 5 and 10 min. This procedure may be criticized, as the changes in blood variables we have noted may be in response to trapping and handling.

If an animal is stressed, an adrenal response in the form of increased glucocorticoid production occurs within 3 min (Urquhart 1966; Dallman and Jones 1973) and can result in changes in plasma levels of glucose and proteins. In *T. vulpecula* an injection of cortisol corresponding to the maximally ACTH-stimulated cortisol secretion rate caused a rapid increase in plasma glucose to near maximal levels of 150 mg dl^{-1} (Khin Aye Than and McDonald 1973, 1974). In the present study, levels of glucose in *T. caninus* and *T. vulpecula* were not significantly different from each other or (at most times of the year) from the 'normal values' of captive *T. vulpecula*, of about 100 mg dl^{-1}, measured by Khin Aye Than and McDonald (1974). This suggests that the changes in glucose concentration we recorded in *T. caninus* were not a maximal response to an increase in adrenal activity. Further, our levels for plasma glucose are well below those found in a *T. vulpecula* under anaesthesia or stressed by rough handling and partial suffocation (Parsons *et al.* 1971).

Gartner *et al.* (1969) showed that blood samples taken from naive cattle gave less reliable estimates of haemoglobin, haematocrit and red cell count than those taken from cattle previously exposed to the sampling techniques. Also, the haematocrit of *T. vulpecula* is very responsive to stress (Dawson and Denny 1968). In the present study we were unable to detect any effect of the sampling technique on the level of haematocrit; the other parameters (haemoglobin, red blood cells and glucose) could not be assessed for trapping effects as they changed seasonally. In any case, if any of the parameters were influenced by the trapping, handling or bleeding procedures, then the response of the possums to these procedures changed with season and in some cases between years.

Species Comparison

Five of the seven parameters had significantly different levels in the two species. Body weight, haematocrit and haemoglobin concentration were greater in *T. caninus*, red blood cell counts and plasma lipid concentration were greater in *T. vulpecula*; the latter parameter showed the greatest specific difference, being 20% greater in *T. vulpecula*. The level of four of the parameters showed significant seasonal change in *T. caninus* (glucose, haemoglobin and protein concentration and red blood cell count) but only haemoglobin changed with season in *T. vulpecula*.

The interspecific differences found for various parameters may reflect either different 'set points' for the species or their differing diet. Changes due to diet quality have been reported for haemoglobin concentration in mice (Francis and Strong 1938) and in serum protein and plasma lipid concentrations in dogs (Zeldis *et al.* 1945). The two species are generally spatially separated (Calaby 1966; How 1978) and have different dietary preferences (Owen and Thomson 1965); *T. caninus* feeds on numerous understorey species whereas *T. vulpecula* can feed on canopy, shrub and ground-layer species. These factors make it impossible to test the food hypothesis *per se* in natural populations.

Seasonal variation in the seven parameters we measured is greater in *T. caninus*, the more *K*-selected species, which occupies classically 'stable' habitat in which abiotic factors are less variable but biotic factors (e.g. competition for food and nest sites) may be intense. The lower seasonal response in the *r*-selected *T. vulpecula* may reflect its ability to ameliorate environmental stress and so occupy more diverse habitats. The relationship between population strategy and physiological variability may be coincidental, and requires testing by either a congeneric species comparison or intraspecifically where the species occupy different habitats with differing population parameters. The latter approach is being assessed for *T. caninus* by an analysis of the blood parameters from individuals occupying preferred and peripheral habitats. Similarly, comparison of peripheral *T. caninus* with *T. vulpecula* in the same habitat should show whether the differences are habitat- or species-specific and indicate the response of a *K*-selected species to a less stable habitat. These analyses will be reported in a subsequent paper. It is clear that the changes we have found for *T. caninus* are not a function of the population alone, because the seasonal variation differed between years while the population structure remained the same.

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