Chapter 3

Seasonal Changes in Renal Function in *Antechinus stuartii*

3.1 Introduction

Intact renal function in mammals is essential for conservation of body water, maintenance of body electrolyte concentration, and for the removal of urea, the main end product of nitrogen metabolism (Schmidt-Nielsen 1990). There are many influences ensuring appropriate renal excretion of electrolytes and urea. Low or high plasma urea concentrations may affect renal function as urea recycling by the loops of Henle is needed to maintain the medullary concentration gradient while keeping blood urea concentrations low (Alvestrand and Bergeström 1989, Choshniak and Arnon 1985). Fluctuating plasma concentrations of sodium and potassium are modified by appropriate renal excretion or conservation of electrolytes. The complex interactions between the kidney and antidiuretic hormone (ADH), the renin-angiotensin system and mineralocorticoid activity have been discussed (see Chapter 1). All are important for water conservation and electrolyte balance. The interactions of these hormones with the kidney enable mammals to respond to changing environmental stresses that may affect water and electrolyte balance.

Glomerular filtration of the plasma is an essential component of renal function in all vertebrates. Glomerular filtration rate (GFR) is often used as a measure of renal function and is a parameter frequently used to determine any changes in whole kidney function in mammals. Many diverse conditions will alter GFR including age, water stress and hibernation (Bakker and Bradshaw 1983, Brown *et al.* 1971, Denny and Dawson 1977, Remuzzi *et al.* 1988, Yokota *et al.* 1985, Zatzman and South 1972).

Moreover GFR can change with glucocorticoid action (Baylis and Brenner 1978, Campen *et al.* 1983) and, in the marsupial Quokka (*Setonix brachyurus*), the adrenal gland is necessary for the maintenance of GFR (McDonald and Bradshaw 1993). GFR can also

be different between sexes, due to the presence of testosterone (Corman *et al.* 1985, Munger and Baylis 1988, Remuzzi *et al.* 1988). The dramatic endocrine and life history changes in *A. stuartii* include marked changes in plasma glucocorticoid and testosterone concentrations (Bradley *et al.* 1980, Lee *et al.* 1977, McDonald *et al.* 1981). The renal function of males may be compromised by such endocrine changes and this may contribute to male mortality. Therefore GFR, urine potassium, sodium, chloride, urea, urine osmolality, plasma potassium, sodium, chloride, plasma osmolality, and faecal electrolytes were measured to establish any seasonal and sex differences in renal function in *A. stuartii*.

3.2 Materials and methods

3.2.1 Animals

Male and female *A. stuartii* were trapped from the New England Tablelands, N. S. W. (see Chapter 2, and Appendix II). Sample sizes were as follows, in February males n = 5, females n = 2; May males n = 7, females n = 4; July males n = 6, females n = 5; and August males n = 11, females n = 5. In February all individuals were juveniles. In May, although all animals were of adult body mass, they were sexually immature. In July animals were sexually mature, but not breeding. In August kidney function was measured twice, once prior to mating and then following mating activity in the laboratory. *Mus musculus* (males n = 5, females n = 7) were studied for comparisons with a similar sized placental mammal that does not undergo these life history changes. *Mus musculus* values were compared with GFRs determined by other methods to validate the method of GFR measurement. *Mus musculus* values were measured at a time independent of the seasonal measurements of *A. stuartii*. Low female numbers were due to a 2:1 male:female trapping ratio in the field area over the period of collection (see Appendix II).

A. stuartii and *M. musculus* were caged and handled as outlined in Chapter 2.

3.2.2 Measurement of Glomerular Filtration Rate

GFR was measured using the single injection method as outlined in Chapter 2 (Hall *et al.* 1977, Stacey and Thorburn 1966).

3.2.3 Haematocrit

Haematocrits were obtained from every animal when GFR experiments were performed (see Chapter 2).

3.2.4 Plasma samples

Plasma samples were collected as described in Chapter 2.

3.2.5 Urine and faecal collections

Urine and faecal samples were collected as described in Chapter 2.

3.2.6 Urine and plasma electrolytes

Urine and plasma potassium and sodium were analysed using a Corning 405 flame photometer with an internal lithium standard (see Chapter 2). Chloride, urea and osmolality were measured as outlined in Chapter 2. For *A. stuartii* both urine concentration ($mmol \cdot L^{-1}$) and total amount excreted (concentration x volume in nmol, see Chapter 2) are presented. Insufficient *M. musculus* samples were collected for calculation of total amount excreted.

3.2.7 Faecal electrolytes

Faecal samples collected during renal function experiments and when left overnight in metabolic cages, were analysed for sodium, potassium, calcium, magnesium and iron using the sealed chamber digestion method (see Chapter 2). The percentage of faecal water was calculated before digestion analysis. Concentrations were analysed ($mmol \cdot kg^{-1}$), as were total amounts excreted, (concentration x faecal mass in nmol, see Chapter 2).

3.2.8 Statistical methods

Two-way analyses of variance were used, followed by a Fisher PLSD test (Haycock *et al.* 1992, Zar 1984). Percentage values were arcsine transformed prior to analysis (Zar 1984). Where the assumption of equal variances was not met, data were transformed to give equal variances and then analysed (Zar 1984). However, means $\pm$ standard errors of untransformed data are presented in the results.

3.3 Results

3.3.1 Body mass

Body mass differed significantly across the year (sexes $P<0.001$, seasons $P<0.01$, interaction $P<0.02$, Table 3.1). Body mass did not change significantly over the course of the year in female *A. stuartii*, but body masses of males in May, July, and August were significantly higher than those of males captured in February (Table 3.1). Male body masses were higher than those of females at all times of year (Table 3.1). Body mass was not different between sexes for *Mus musculus*.

3.3.2 Glomerular filtration rate

The GFRs of *A. stuartii* differed significantly across the year (sex $P<0.02$, season $P<0.025$, interaction $P<0.05$, Figure 3.1, Table 3.1). The GFRs of males from July and both times in August were significantly lower than those from February and May. In July and post-breeding August the GFRs of males were significantly lower than values for females. The GFRs of *Mus musculus* did not differ from the GFR values for *A. stuartii* in February and May, but were significantly higher than those found for male *A. stuartii* in July, August pre-breeding and August post-breeding (Fisher's PLSD test $P<0.05$).

3.3.3 Haematocrit

Haematocrit differed between sexes ($P<0.02$, Figure 3.2, Table 3.1) and across seasons ($P<0.001$, Figure 3.2, Table 3.1). Males had higher haematocrits than females. Haematocrits from pre-breeding and post-breeding August animals were significantly higher than those in February and May (Fisher's PLSD $P<0.02$, Table 3.1). Haematocrits from both sexes in July were significantly lower than those from post-breeding August (Fisher's PLSD $P<0.02$) and higher than those in February (Fisher's PLSD $P<0.06$). Haematocrit did not differ between sexes in *M musculus* (Table 3.1).

3.3.4 Plasma analysis

Plasma potassium levels in *A. stuartii* differed significantly between seasons ($P<0.02$). Plasma potassium levels were significantly lower in *A. stuartii* in July and August (Fisher's PLSD test

Table 3.1 Body mass (g), Glomerular filtration rate ($mL \cdot min^{-1} \cdot kg^{-1}$) and Haematocrit (%)

	Body mass (g) Males	Body mass (g) Females	GFR ($mL \cdot min^{-1} \cdot kg^{-1}$) Males	GFR ($mL \cdot min^{-1} \cdot kg^{-1}$) Females	Haematocrit (%) Males	Haematocrit (%) Females
February	28.7 ± 2.1	24.5 ± 1.6	5.39 ± 1.07	6.26 ± 1.03	46.1 ± 1.9	45.5 ± 4.3
May	37.7 ± 2.6	21.4 ± 2.6	4.18 ± 0.53	5.46 ± 0.98	49.2 ± 1.1	46.3 ± 3.6
July	37.8 ± 2.1	21.3 ± 0.7	2.63 ± 0.52	5.00 ± 0.93	51.8 ± 1.1	46.5 ± 0.9
August pre- breeding	39.9 ± 2.2	22.7 ± 1.8	3.57 ± 0.54	3.65 ± 0.71	52.6 ± 1.1	51.1 ± 1.5
August post- breeding	39.3 ± 2.1	23.0 ± 1.2	2.36 ± 0.37	5.64 ± 0.87	54.5 ± 1.2	51.3 ± 1.7
M. musculus	35.1 ± 0.4	34.4 ± 0.9	5.98 ± 1.08	4.18 ± 0.47	51.3 ± 0.8	52.4 ± 1.3
Sex Season Interaction	$P<0.001$ $P<0.01$ $P<0.02$		$P<0.02$ $P<0.025$ $P<0.05$		$P<0.02$, $P<0.001$, NS	

Table 3.1. Body mass (g), glomerular filtration rates ($mL \cdot min^{-1} \cdot kg^{-1}$) and haematocrits (%) for both *A. stuartii* and *M. musculus*. Values are means ± standard errors of the mean. Significance levels for two-way analyses of variance are indicated at the base of the columns.

Figure 3.1

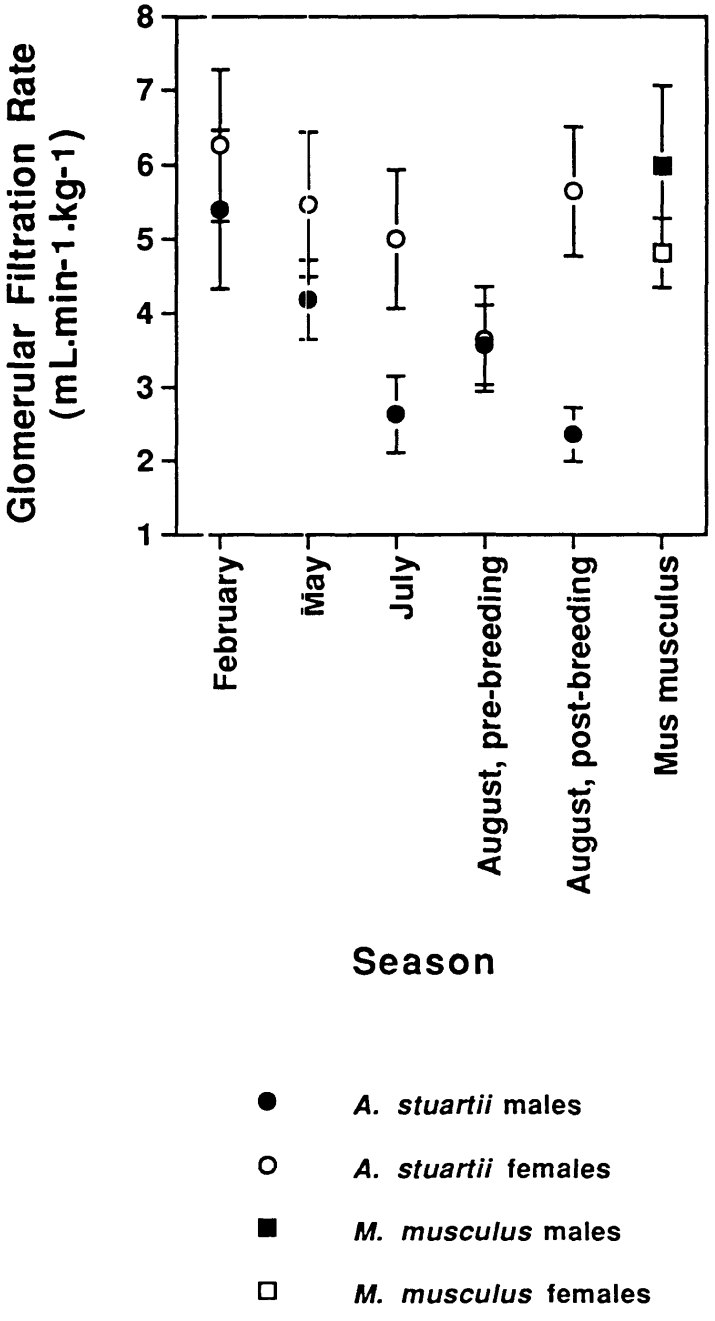


Figure 3.1 Seasonal changes in glomerular filtration rate in *A. stuartii* and *M. musculus*. Data are means ± standard errors of the mean.

Figure 3.2

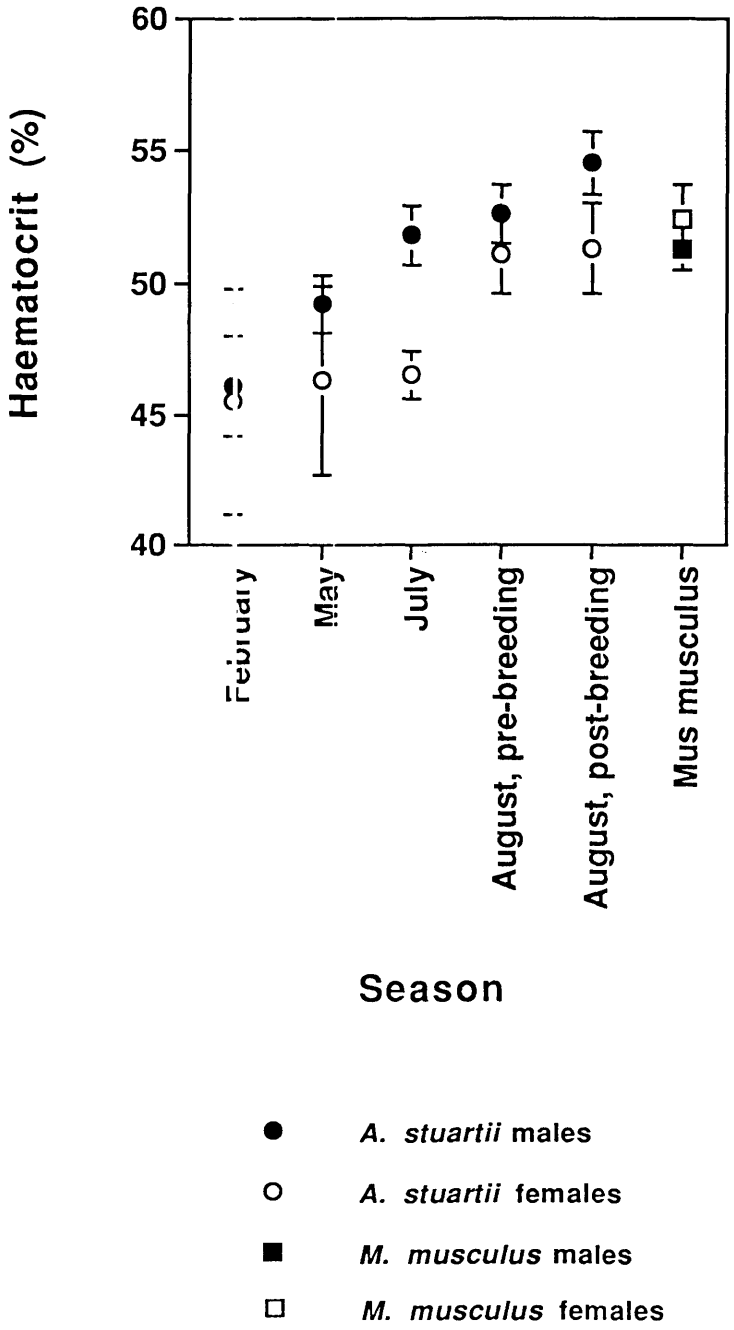


Figure 3.2 Seasonal changes in haematocrit (%) in *A. stuartii* and *M. musculus*. Data are mean ± standard error of the mean.

$P<0.05$, Figure 3.3a, Table 3.2). *M. musculus* plasma potassium levels were not significantly different from those obtained from *A. stuartii* in February and May, but were significantly higher than those from *A. stuartii* in July and August (Fisher's PLSD test $P<0.05$, Figure 3.3a, Table 3.2).

Plasma sodium levels differed significantly between sexes and seasons (sex, $P<0.005$, season, $P<0.0005$, interaction $P<0.16$, Figure 3.3b, Table 3.2). In general, male *A. stuartii* had significantly higher plasma sodium concentrations than females (Fisher's PLSD test $P<0.005$, Table 3.2), and animals from July and August had significantly higher plasma concentrations than those from February and May (Fisher's PLSD test $P<0.05$, Figure 3.3b, Table 3.2). Plasma sodium concentrations from *M. musculus* did not differ from those of *A. stuartii* from July and August but were significantly higher than plasma sodium levels in February and May (Fisher's PLSD test $P<0.05$, Table 3.2).

Plasma chloride levels differed significantly between sexes and seasons (sex, $P<0.01$, season, $P<0.02$, interaction NS, Figure 3.4a, Table 3.2). Male *A. stuartii* had significantly higher plasma chloride concentrations than females (Fisher's PLSD test $P<0.01$, Table 3.2), and animals from July and August had significantly higher plasma concentrations than those from May (Fisher's PLSD test $P<0.05$, Figure 3.4a, Table 3.2). Animals from August had significantly higher plasma concentrations than those from February (Fisher's PLSD test $P<0.05$, Figure 3.4a, Table 3.2) and those animals from July tended to be higher than those from February (Fisher's PLSD test $P<0.09$, Figure 3.4a, Table 3.2). Plasma chloride concentrations from *M. musculus* did not differ from those of *A. stuartii* from July and August, but were significantly higher than plasma chloride levels in February and May (Fisher's PLSD test $P<0.05$, Table 3.2).

Plasma osmolality differed significantly across seasons (sex $P<0.13$, season $P<0.05$, interaction NS, Figure 3.4b, Table 3.2). The plasma osmolality of animals in February was significantly higher than for animals from May and August (Fisher's PLSD test $P<0.025$). The plasma osmolalities of *M. musculus* did not differ from *A. stuartii* (Table 3.2).

Table 3.2 Plasma Electrolytes ($mmol \cdot L^{-1}$) and Osmolality ($mosmol \cdot kg^{-1}$)

	Potassium ($mmol \cdot L^{-1}$) Males	Potassium ($mmol \cdot L^{-1}$) Females	Sodium ($mmol \cdot L^{-1}$) Males	Sodium ($mmol.L^{-1}$) Females	Chloride ($mmol \cdot L^{-1}$) Males	Chloride ($mmol \cdot L^{-1}$) Females	Osmolality ($mosmol \cdot kg^{-1}$) Males	Osmolality ($mosmol \cdot kg^{-1}$) Females
February	7.3 ± 1.0	8.6 ± 0.3	135.3 ± 6.7	102.0 ± 18.0	105.4 ±5.1	98.0 ± 9.4	338.3 ± 11.0	359.7 ± 10.7
May	8.3 ± 1.0	8.7 ± 0.8	135.0 ± 4.6	113.3 ± 5.0	104.0 ± 4.6	94.3 ± 7.2	316.7 ± 27.9	290.8 ± 25.3
July	6.2 ± 0.6	5.0 ± 0.3	142.0 ± 5.3	135.3 ± 10.3	114.2 ± 1.3	108.4 ± 3.1	338.2 ± 5.8	317.6 ± 6.5
August post-breeding	5.0 ± 0.3	5.5 ± 0.7	130.4 ± 5.9	123.3 ± 10.8	116.5 ± 1.0	104.7 ± 11.4	311.4 ± 5.9	296.4 ± 27.5
M. musculus	7.9 ± 0.3	7.7 ± 0.6	145.2 ± 2.0	142.0 ± 2.3	114.5 ± 1.4	109.4 ± 1.0	336.6 ± 5.9	302.9 ± 8.4
Sex	NS		P<0.005		P<0.01		P<0.13	
Season	P<0.02		P<0.0005		P<0.02		P<0.05	
Interaction	NS		P<0.16		NS		NS	

Table 3.2 Concentration of plasma electrolytes ($mmol \cdot L^{-1}$) and plasma osmolality ($mosmol \cdot kg^{-1}$) for both *A. stuartii* and *M. musculus*. Values are means ± standard errors of the mean. Significance levels for two-way analyses of variance are indicated at the base of the columns.

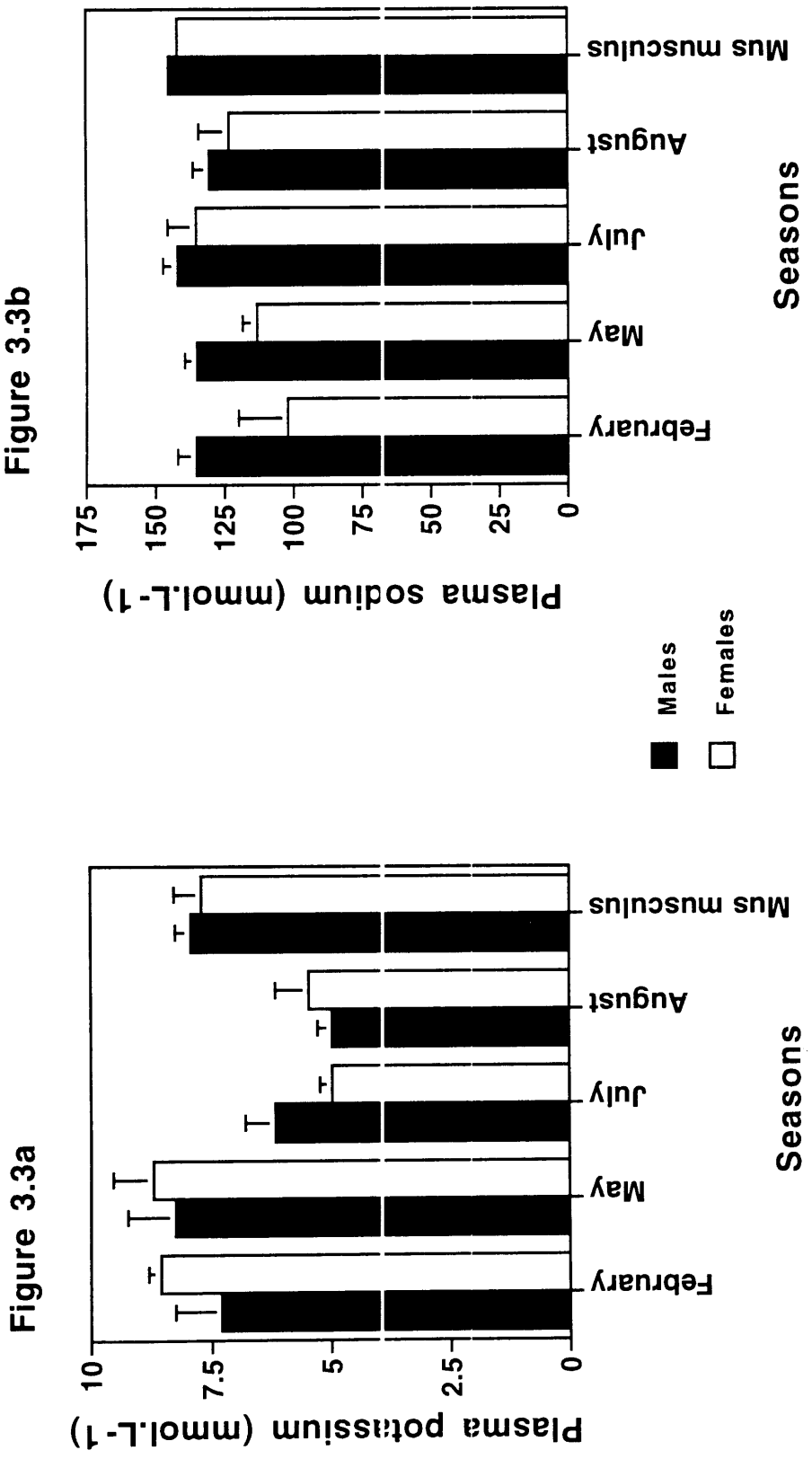


Figure 3.3 Plasma concentrations in mmol.L-1 of a) potassium and b) sodium

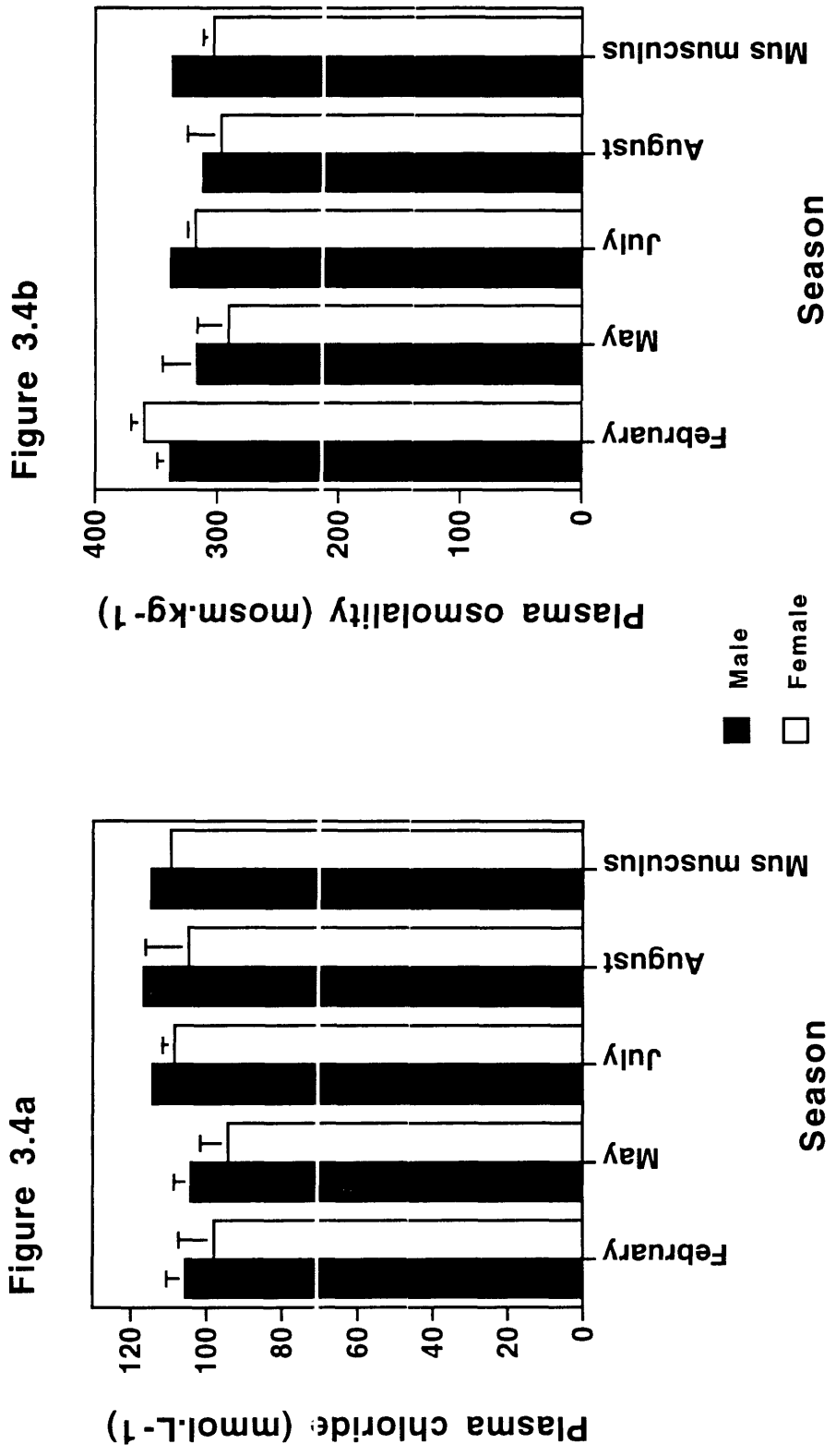


Figure 3.4 a) Plasma concentrations in mmol.L-1 of chloride. b) Plasma osmolality in mosm.kg-1.

3.3.5 Urine analysis

Urea concentration was significantly higher in female than in male *A. stuartii* (Data transformed for assumption of equal variances, sex $P<0.05$, Table 3.3). There were significant seasonal differences in urine osmolality in *A. stuartii* (season $P<0.05$, Table 3.3). Osmolality was significantly higher in urine from *A. stuartii* in July and August (Table 3.3). Urine potassium, sodium and chloride concentrations did not differ significantly in *A. stuartii* (Table 3.3). Urine potassium concentrations were significantly higher in *M. musculus* than in *A. stuartii* (Fisher's PLSD test $P<0.05$, Table 3.3), however, urine sodium and chloride concentrations were similar in the two species.

In *A. stuartii* the total sodium excreted differed between seasons ($P<0.02$). Values were significantly lower in February than in July and both August sampling periods (Fisher's PLSD test $P<0.05$, Table 3.4). May values were somewhat lower, although not statistically significantly, than those in July (Fisher's PLSD test $P<0.07$, Table 3.4). Total potassium excreted did not change over the year. Total chloride excreted differed between seasons ($P<0.02$, Table 3.4). July values were significantly higher than those in February and pre-breeding August (Fisher's PLSD test $P<0.05$, Table 3.4). The values for February were lower than those for August post-breeding animals, but this was not statistically significant (Fisher's PLSD test $P<0.06$, Table 3.4). Total urea concentrations were higher in females than in males (sex $P<0.06$, Table 3.4).

3.3.6 Faecal analysis

The percentage water in the faecal samples was significantly different across seasons $P<0.05$, Table 3.5). Samples from February and May contained a significantly higher percentage of water than those from July and August, and those from *M. musculus* were significantly drier than from *A. stuartii* (Fisher's PLSD test $P<0.05$, Table 3.5). Faecal sodium concentration was significantly higher in *M. musculus* than in *A. stuartii* (Fisher's PLSD test $P<0.05$, Table 3.5). No differences were found in *A. stuartii*. Faecal potassium concentration was significantly lower for *M. musculus* than for *A. stuartii* in July and August, and *A. stuartii* from February and May had

Table 3.3 Urine Electrolytes ($mmol \cdot L^{-1}$) and Osmolality ($mosmol \cdot kg^{-1}$)

Seasons and Sex	Sodium ($mmol \cdot L^{-1}$)	Potassium ($mmol \cdot L^{-1}$)	Chloride ($mmol \cdot L^{-1}$)	Urea ($mmol \cdot L^{-1}$)	Osmolality ($mosmol \cdot kg^{-1}$)
February males	62.7 ± 17.6	134.0 ± 34.5	100.2 ± 37.1	1402.0 ± 470.2	776.2 ± 169.7
February females	122.5 ± 77.2	164.0 ± 47.4	166.3 ± 35.3	2168.0 ± 181.5	1408.0 ± 360.0
May males	223.3 ± 108.3	191.0 ± 72.2	135.0 ± 33.3	1573.0 ± 403.5	1422.0 ± 189.7
May females	270.0 ± 115.9	78.0 ± 72.0	98.5 ± 39.5	744.5 ± 201.5	503.5 ± 227.5
July males	103.4 ± 25.4	126.2 ± 27.5	134.2 ± 33.0	1690.0 ± 327.3	1506.4 ± 224.8
July females	124.3 ± 39.2	125.2 ± 23.2	146.2 ± 24.3	3210.2 ± 554.6	1755.1 ± 270.6
August males prebreeding	146.4 ± 20.2	166.67 ± 19.75	107.8 ± 14.9	1216.3 ± 247.8	1716.0 ± 133.4
August females prebreeding	171.4 ± 33.5	180.2 ± 32.0	181.5 ± 43.5	2381.7 ± 266.9	2108.9 ± 236.7
August males post breeding	134.4 ± 26.6	163.8 ± 23.9	115.8 ± 15.6	2887.5 ± 345.8	1683.9 ± 119.4
August females post breeding	182.2 ± 55.2	141.7 ± 24.9	190.9 ± 25.4	2580.0 ± 35.0	1918.0 ± 148.6
Mus musculus males	110.8 ± 23.5	403.9 ± 50.3	77.5 ± 16.6	2571.0 ± 613.9	1584.2 ± 321.6
Mus musculus females	121.5 ± 30.0	645.5 ± 107.4	108.5 ± 42.5	-	1025.5 ± 974.6
Sex	NS	NS	NS	$P<0.05$	NS
Season	NS	NS	NS	NS	$P<0.05$
Interaction	NS	NS	NS	NS	NS

Table 3.3 Concentration of urinary electrolytes for both *A. stuartii* and *M. musculus*. Values are means ± standard errors of the mean. Significance levels for two-way analyses of variance are indicated at the base of the columns.

Table 3.4 Total excreted urinary electrolytes (nmol)

Seasons and Sex	Sodium (nmol)	Potassium (nmol)	Chloride (nmol)	Urea (nmol)
February males	2.2 ± 0.6	6.9 ± 1.7	7.5 ± 5.0	143.0 ± 72.9
February females	8.1 ± 3.3	10.7 ± 6.6	15.9 ± 10.7	362.9 ± 271.3
May males	4.2 ± 0.4	5.7 ± 3.1	20.9 ± 16.7	224.0 ± 95.4
May females	15.1 ± 3.1	11.5 ± 10.2	16.6 ± 3.5	127.8 ± 9.4
July males	29.5 ± 7.0	23.6 ± 6.7	33.6 ± 9.9	451.4 ± 232.5
July females	25.2 ± 4.8	31.3 ± 8.7	45.3 ± 9.4	906.9 ± 210.4
August males pre-breeding	22.0 ± 7.9	31.0 ± 16.8	24.9 ± 10.7	309.4 ± 148.3
August females pre-breeding	15.8 ± 4.8	10.8 ± 3.4	19.7 ± 6.6	478.1 ± 109.2
August males post- breeding	16.7 ± 3.5	22.6 ± 5.0	23.3 ± 5.6	457.7 ± 85.3
August females post-breeding	24.0 ± 9.7	36.7 ± 13.3	68.8 ± 23.9	740.3 ± 217.3
Sex	NS	NS	NS	P<0.06
Season	P<0.02	NS	P<0.05	NS
Interaction	NS	NS	NS	NS

Table 3.4 Total amounts of urinary electrolytes excreted for *A. stuartii*. Values are concentration (mmol) x volume (μL), and are means ± standard errors of the mean. Significance levels for two-way analyses of variance are indicated at the base of the columns.

Table 3.5 Faecal Electrolytes (mmol·kg^{-1})

Seasons and Sex	Sodium mmol·kg^{-1}	Potassium mmol·kg^{-1}	Calcium mmol·kg^{-1}	Magnesium mmol·kg^{-1}	Iron mmol·kg^{-1}	Faecal water % of wet weight
February males	241 ± 30	115 ± 16	358 ± 46	47 ± 6	13,972 ± 1,254	79.9 ± 2.1
February females	160 ± 46	80 ± 25	185 ± 58	54 ± 11	7821 ± 575	67.8 ± 6.6
May males	176 ± 71	48 ± 13	138 ± 67	31 ± 10	13,661 ± 4,693	82.4 ± 3.5
May females	141 ± 46	68 ± 15	362 ± 93	43 ± 9	13,584 ± 1,462	72.4 ± 3.1
July males	214 ± 37	113 ± 16	287 ± 53	47 ± 8	10,006 ± 1,350	65.2 ± 3.8
July females	242 ± 60	134 ± 27	530 ± 65	74 ± 12	10,513 ± 1,185	76.1 ± 3.1
August males pre-breeding	196 ± 23	93 ± 8	474 ± 67	57 ± 8	13,376 ± 1,173	71.5 ± 2.4
August females pre-breeding	197 ± 16	103 ± 10	299 ± 47	47 ± 8	13,965 ± 3,037	74.9 ± 3.3
August males post- breeding	296 ± 18	132 ± 6	264 ± 19	33 ± 2	15,254 ± 2,483	71.8 ± 1.8
August females post- breeding	222 ± 28	127 ± 13	341 ± 73	39 ± 4	20,081 ± 1,296	74.8 ± 2.9
Mus musculus males	60 ± 8	39 ± 4	712 ± 70	161 ± 15	9,504 ± 437	65.8 ± 1.4
Mus musculus females	66 ± 10	46 ± 5	694 ± 92	146 ± 17	7,933 ± 390	61.1 ± 2.2
Sex	NS	NS	NS	NS	NS	NS
Season	NS	$P<0.05$	NS	NS	NS	$P<0.05$
Interaction	NS	NS	NS	NS	NS	NS

Table 3.5 Concentration (mmol·kg^{-1}) of faecal electrolytes for both *A. stuartii* and *M. musculus*. Values are means ± standard errors of the mean. Significance levels for two-way analyses of variance are indicated at the base of the columns.

Table 3.6 Faecal Electrolytes- Total excreted (nmol)

Seasons and Sex	Sodium nmol	Potassium nmol	Calcium nmol	Magnesium nmol	Iron nmol
February males	8.1 ± 2.7	3.4 ± 0.9	11.4 ± 4.4	1.9 ± 0.5	451.6 ± 107.9
February females	4.1 ± 2.0	2.3 ± 1.5	18.3 ± 10.9	2.6 ± 2.0	398.7 ± 177.6
May males	3.4 ± 1.2	1.5 ± 0.6	5.7 ± 3.2	1.1 ± 0.4	255.8 ± 075.8
May females	2.6 ± 1.0	3.8 ± 1.3	15.4 ± 3.3	2.3 ± 0.4	595.1 ± 60.0
July males	31.1 ± 7.3	14.7 ± 3.1	28.9 ± 8.5	6.6 ± 1.7	912.0 ± 126.0
July females	49.7 ± 19.6	30.9 ± 9.0	78.6 ± 20.6	10.4 ± 2.8	1092.3 ± 203.2
August males prebreeding	17.3 ± 5.4	7.3 ± 2.2	45.0 ± 12.2	5.0 ± 0.9	917.4 ± 158.6
August females prebreeding	18.3 ± 6.3	9.0 ± 3.5	38.0 ± 10.7	5.7 ± 2.1	803.2 ± 161.2
August males post breeding	14.0 ± 2.3	8.5 ± 1.5	25.2 ± 4.2	3.2 ± 0.5	966.6 ± 133.6
August females post breeding	8.0 ± 1.2	5.3 ± 1.2	10.3 ± 2.4	1.7 ± 0.4	665.9 ± 87.6
Mus musculus males	7.6 ± 2.2	4.8 ± 1.1	87.1 ± 20.8	19.3 ± 4.4	976.0 ± 203.6
Mus musculus females	8.0 ± 2.0	6.4 ± 1.2	96.0 ± 16.3	21.0 ± 3.7	1029.4 ± 199.0
Sex	NS	NS	NS	NS	NS
Season	P<0.02	P<0.0001	P<0.02	P<0.002	P<0.005
Interaction	NS	NS	NS	NS	NS

Table 3.6 Total amounts of faecal electrolytes excreted for *A. stuartii* and *M. musculus*. Values are concentration (mmol) x mass (mg), and are means ± standard errors of the mean. Significance levels for two-way analyses of variance are indicated at the base of the columns.

lower potassium concentrations than July and post-breeding August individuals (seasons $P<0.05$, Fisher's PLSD test $P<0.05$, Table 3.5). Faecal calcium and magnesium concentrations were significantly higher in *M. musculus* than for *A stuartii* (Fisher's PLSD test $P<0.05$, Table 3.5) although no differences were found in *A. stuartii*. Faecal iron concentration was significantly lower in *M. musculus* than in *A. stuartii* ($P<0.05$, Table 3.5). No differences were found between sex and season in *A. stuartii*.

When the total amounts of electrolytes were calculated, some differences were found between seasons for *A. stuartii*. Total faecal sodium excreted was significantly higher in July than for all other times of the year and *M. musculus* values (season $P<0.002$, Fisher's PLSD test $P<0.02$, Table 3.6) and was higher in both periods of August than May, February and *M. musculus* values (Fisher's PLSD test $P<0.05$, Table 3.6). Total faecal potassium excreted was significantly higher in July than all other times of the year and for *M. musculus* values (season $P<0.0001$, Fisher's PLSD $P<0.0005$, Table 3.6). While values in pre-breeding and post-breeding August tended to be higher than February and May, these differences were not statistically significant (Fisher's PLSD test $P<0.08$ and $P<0.10$ respectively). Total faecal calcium excreted was significantly higher in July, both August samples and *M. musculus* values, than for February and May (season $P<0.02$, interaction $P<0.08$, Fisher's PLSD test $P<0.05$, Table 3.6). Total faecal magnesium excreted was significantly higher in July and pre-breeding August than in February, May and post-breeding August (season $P<0.002$, Fisher's PLSD $P<0.05$, Table 3.6). Total faecal magnesium excreted by *M. musculus* were significantly higher than all other groups (Fisher's PLSD $P<0.05$, Table 3.6). Total faecal excretion of iron was significantly higher in July, both periods in August and *M. musculus* than in February and May (season $P<0.005$, Fisher's PLSD $P<0.05$, Table 3.6).

3.4 Discussion

Renal function in *A. stuartii* was found to alter seasonally and some aspects differed between sexes. The GFRs of *A. stuartii* differed between sexes and across seasons. With the exception of the mating

period, GFRs of females remained unchanged throughout the year and those of males were similar to females in February and May. However, the GFRs of males in July and August were half of those from other times of the year. The GFR values for *M. musculus* in the present study were similar to those found by Stewart (1971) who used inulin clearance to determine GFR. This finding validated the use of the single injection slope intercept method for measurement of GFR in small mammals. The electrolyte values for *M. musculus* were also within the ranges found in other studies (Haines *et al.* 1973). The GFRs of *M. musculus* and those of *A. stuartii* in the non-reproductive period are similar to the values reported for other small mammals (Weisser *et al.* 1970, Edwards *et al.* 1983).

To the best of my knowledge this is the first time that GFR has been shown to have change seasonally. Fluctuations in GFR are usually attributed to water deprivation (Bakker and Bradshaw 1983, Etzion and Yagil 1986). Labile GFRs have been observed in desert mammals that reduce GFR in response to low water availability (Maloiy 1972, Denny and Dawson 1977, Etzion and Yagil 1986, Grenot 1992). These reductions are usually a response to an immediate environmental challenge, and the low GFRs are reversed by hydration. In kangaroos (*Macropus rufus* and *M. robustus*) but not wombats (*Lasiorhinus latifrons* and *Vombatus ursinus*), water deprivation significantly reduced GFR (Barboza 1993, Denny and Dawson 1977). However, similar to female *A. stuartii* and males from February and May, the GFR of possums (*Trichosurus vulpecula*) did not differ markedly from similar sized eutherians (Reid and McDonald 1968b).

Some of the other Australian marsupials that have been studied experience arid and unpredictable environments (see Denny and Dawson 1977, Morton 1980, Bakker and Bradshaw 1983) and this contrasts with *A. stuartii* which lives in a mesic environment with no severe seasonal environmental changes. Water availability was not considered to be a problem for wild *A. stuartii* by Nagy *et al.* (1978), and drinking was rarely observed in captive *A. stuartii* (Blair-West *et al.* 1983). The diet of *A. stuartii* consists of mostly insects and, although the prey type differs substantially throughout the year, it is predictable (Dickman 1986). Diet has been found to affect GFR in other mammals, with high protein diets increasing GFR and excreted urea

(Ladd *et al.* 1951, Alvestrand and Bergström 1989), and low protein diets inducing a reduction in GFR and excreted urea (Bakker and Bradshaw 1983, Choshniak and Arnon 1985).

However, the ready availability of food and water in the wild, throughout the time when GFR was observed to fall, further exclude the effect of diet and dehydration as the causes of the changes seen in GFR in *A. stuartii*. This was also true in the laboratory, where all animals were observed to eat and drink at all times of the year. Therefore other factors affecting GFR must be involved.

The seasonal reduction in GFR, noted in males in July and through August, and in females in August prior to breeding, suggests that the changes in the known hormone profiles exhibited by these mammals may be important in explaining the drop in GFR. Moreover, the study was performed over two years, and the same reduction in the GFRs from July and August was found in both years.

A previous study has found higher plasma cortisol levels in males in July and August than at earlier times of the year, and a small but non-significant rise during the breeding period in females (Bradley *et al.* 1980). Testosterone levels in males begin rising in July, continuing to rise until death, by which time they are eight-fold the concentration found in plasma in early June (Bradley *et al.* 1980, Kerr and Hedger 1983). Although plasma oestrogen and progesterone levels have not been measured for females throughout the year, the plasma progesterone levels in females rise during pregnancy (Hinds and Selwood 1990). The known hormone changes are coincident with changes in GFR.

The coincidence of changes in GFR with plasma hormone concentrations found by other studies may be a result of either a direct action of cortisol or testosterone on the kidney structure and function, or as a response to glucocorticoid actions on other physiological systems that results in renal compensation. Studies on other small mammals have found that renal structure can be modified by hormonal changes. These include proximal tubule hypertrophy due to the actions of testosterone (Oudar *et al.* 1991, Schiebler and Danner 1978) and glomerular and tubular damage due to adrenocortical activity (Christian *et al.* 1965, von Holst 1972a). In other mammals, testosterone administration can induce renal absorption of sodium and

water and cause GFR to decrease, increase, or remain unchanged, depending in the mamma (Munger and Baylis 1988, Welsh *et al.* 1942).

The adrenal gland secretes both the mineralocorticoid aldosterone and the glucocorticoid cortisol, both of which can compete for type I (aldosterone) receptors (Arriza *et al.* 1987, Funder *et al.* 1988, Krozowski and Funder 1983, Krozowski *et al.* 1990). High plasma corticosteroid binding globulin (CBG) levels that bind the high plasma concentrations of cortisol and reduce the amount of free hormone available for action, allow aldosterone better access to the type I receptors (Funder 1993, Meaney *et al.* 1992, Mercer and Krozowski 1992). Moreover, receptor specificity is believed to be maintained by the presence of 11ß-hydroxysteroid dehydrogenase in the target cells (Funder *et al.* 1988, Monder and Lakshmi 1990). In male *A. stuartii*, CBGs fall in response to rising plasma testosterone levels, and in females there is a decrease in CBGs during the mating period (Bradley *et al.* 1973, 1980). Thus the potential is present for the excess unbound cortisol to compete successfully with aldosterone for target cells in *A. stuartii*.

Renal aspects of glucocorticoid action include increased potassium excretion, although an increase in sodium reabsorption is not always observed (Marver 1992, van Buren *et al.* 1993). In some eutherians, administration of glucocorticoids increases GFR, even on a sodium free diet (Bayis and Brenner 1978, Marver 1992). Adrenalectomy causes a fall in GFR in another marsupial, the quokka (McDonald and Bradshaw 1993). In *A. stuartii* GFR decreases in July and August, which is not consistent with glucocorticoid action. However, plasma potassium concentrations are lower in July and August, and plasma sodium concentrations were higher in males than females and higher in July and August than February and May. These responses are consistent with glucocorticoid action. Thus some aspects of renal function are consistent with glucocorticoid action on the kidney, whereas others are not, perhaps implying testosterone involvement in renal function in *A. stuartii*.

High plasma potassium concentrations may be, in part, due to the method of collection. The blood was collected from the trunk, and cellular damage may significantly increase the potassium

concentrations. Because all animals were bled in the same manner, a significant seasonal decrease is indicated, although the absolute values may be a higher than bleeding from the orbital sinus or by cardiac puncture. The values for *M. musculus* are higher than the usual 4-6 $mmol \cdot L^{-1}$ range of many eutherians (Fetcher 1939, Guyton and Hall 1996) and, although the plasma potassium concentrations may be affected by the method of collection, the relative seasonal differences are still of significant interest.

Although the mean values for plasma potassium obtained for *A. stuartii* in February and May are high, they are not above values obtained by other bleeding methods for other mammals (Bakko 1975, 1977, Collins 1978, Jhala *et al.* 1992, Maloiy 1972, Pfeiffer *et al.* 1979, Scoggins *et al.* 1970, Urison and Buffenstein 1994, Wood and Lee 1985). Moreover, the plasma potassium concentration of the pygmy gerbil (*Gerbillus pusillus*), determined from blood taken from the orbital sinus, was 12.6 ± 1.9 $mmol \cdot L^{-1}$ when animals were acclimated to water deprivation and 7.4 ± 0.9 $mmol \cdot L^{-1}$ with *ad libitum* water (Buffenstein 1984). Similar results for blood samples obtained by cardiac puncture were found in gray squirrels (*Sciurus carolinensis*, mean 12.6 ± 1.0 $mmol \cdot L^{-1}$, Bakko 1975) and red squirrels (*Tamiasciurus hudsonicus*, mean 12.0 ± 1.0 $mmol \cdot L^{-1}$, Bakko 1975). Individual plasma concentrations of potassium of as much as 27 $mmol \cdot L^{-1}$ have been reported, and some of the high values that have been reported are summarised in Table 3.7. Clearly, the physiological tolerance of high plasma potassium concentrations above the commonly quoted eutherian values of 4-6 $mmol \cdot L^{-1}$ is a common phenomenon in mammals.

The seasonal change in plasma potassium and sodium concentrations in *A. stuartii* have not been as obvious in other mammals. While large changes have been observed in electrolyte concentrations in hedgehogs (*Erinaceus europaeus* and *Hemiechinus auritus*, Yaakobi and Shkolnik 1974), rabbits (*Oryctolagus cuniculus*, Scoggins *et al.* 1970, Wood and Lee 1985) camels (*Camelus dromedarius*, Maloiy 1972), squirrels (*Sciurus carolinensis* and *Tamiasciurus hudsonicus* Bakko 1975), prairie dogs (*Cynomys leucurus* and *C. ludovicianus*, Bakko 1977) and bats (*Rhinolophus ferrumequinum* and *Rhinopoma hardwickeri*, Vogel and Vogel 1972),

Table 3.7 Plasma sodium and potassium values ($mmol \cdot L^{-1}$) for some mammals. The table includes species and common names of the mammals, method to obtain blood, and the source of the data.

Mammals	Potassium ($mmol \cdot L^{-1}$)	Sodium ($mmol \cdot L^{-1}$)	Bleeding method	Authors
Primates, sheep, dogs	4-6	130-140	venipuncture	Fetcher 1939, Guyton and Hall 1996
Gerbillus pusillus (pygmy gerbil)	13.7 ± 1.9 food restriction 7.4 ± 0.9 *ad libitum* water & food	172.0 ± 10.7 food restriction 191.3 ± 8.3 *ad libitum* water & food	orbital sinus	Buffenstein 1984
Camelus dromedarius (camel)	8.8 ± 0.99 minimum water, 4.6 ± 0.21 *ad libitum* water	165.0 ± 1.7 minimum water	venipuncture	Maloiy 1972
Sciurus carolinensis (gray squirrels)	12.6 ± 1.0 (4.4-27.0)	140.2 ± 2.8	cardiac puncture	Bakko 1975
Tamiasciurus hudsonicus (red squirrels)	12.0 ± 1.0 (5.2-24.0)	142.0 ± 3.9	cardiac puncture	Bakko 1975
Cynomys leucurus (prairie dog)	8.5 ± 0.8 (5.3-17.3)	122.0 ± 6.7	cardiac puncture	Bakko 1977
Cynomys ludovicianus (prairie dog)	9.1 ± 0.7 (5-18.2)	137.6 ± 3.4	cardiac puncture	Bakko 1977
Rattus rattus (black rat)	9.1 ± 1.0	150.9 ± 4.1	orbital sinus	Collins 1978
Rattus villosissimus (long haired rat)	7.5 ± 0.7	152.3 ± 2.6	orbital sinus	Collins 1978
Oryctolagus cuniculus (rabbit)	7.2 ± 1.0 water deprived	157.0 ± 5.6 water deprived	cardiac puncture	Wood and Lee 1985
Rhinolophus ferrumequinum	5.9 ± 0.86 water deprived	227.0 ± 6.2 water deprived	cardiac puncture	Vogel and Vogel 1972
Setonix brachyurus (quokka)	7.2 ± 0.55	176 ± 1.0	venipuncture	Purohit 1971
Macropus robustus (euro)	6.5 ± 0.1 to 8.8 ± 0.3	169 ± 1.0	cardiac puncture	Dawson and Denny 1969
Macropus rufus (red kangaroo)	7.3 ± 0.3 to 10.9 ± 1.0	170 ± 1.5	cardiac puncture	Dawson and Denny 1969

these are usually a response to water restriction or dietary changes. There were no water restriction or dietary changes in the present study on *A. stuartii*.

In other marsupials fluctuations in plasma potassium similar to those reported for eutherians have been observed, often in response to water restriction. In wild kangaroos (*Macropus robustus* and *M. rufus*) plasma potassium concentrations ranged up to 10.9 ± 1.0 mmol·L^{-1}, and in the laboratory mean values were 7.1 mmol·L^{-1} (Dawson and Denny 1969). Smaller fluctuations in plasma potassium concentrations were also found in the koala (*Phascolarctos cinereus*), with values ranging from 3.9-7.0 mmol·L^{-1} (mean 5.1 mmol·L^{-1}, Canfield *et al.* 1989). Less variable plasma potassium concentrations have been observed in the pademelon (*Thylogale thetis*, Hume and Dunning 1979), the opossum (*Didelphis virginiana*, Hartman *et al.* 1943) and the brush-tailed possum (*Trichosurus vulpecula*, Reid and McDonald 1968b).

High mean plasma potassium concentrations have been observed for other marsupials (Bakker and Bradshaw 1989, Miller and Bradshaw 1979, Scoggins *et al.* 1970). The changes are coincident with similar fluctuations in concentrations of plasma sodium. Most marsupials have plasma sodium concentrations of 130-145 mmol·L^{-1} (Canfield *et al.* 1989, Hartman *et al.* 1943, Miller and Bradshaw 1979, Reid and McDonald 1968b, Scoggins *et al.* 1970). However, plasma sodium concentrations rise when the animals are exposed to water deprivation, both in the laboratory and in the wild (Bakker and Bradshaw 1983, 1989, Dawson and Denny 1969, Denny and Dawson 1977, Jones *et al.* 1990, Reid and McDonald 1968). In the quokka (*Setonix brachyurus*), whose only available drinking water during summer is the sea, plasma sodium concentrations can be as high as 176 ± 1.0 mmol·L^{-1} when drinking sea water (Purohit 1971). In water deprived kangaroos values can be nearly as high (*Macropus robustus* 169 ± 1.0 mmol·L^{-1}, *M. rufus* 170 ± 1.5 mmol·L^{-1}, Dawson and Denny 1977). Similar high plasma sodium concentrations are seen in some eutherians (Buffenstein 1984, Maloiy 1972, Malvin and Rayner 1968, Wesser *et al.* 1970), with plasma sodium concentrations reaching as high as 202.3 ± 10.5 mmol·L^{-1} in water deprived pygmy gerbils (*Gerbillus pusillus*, Buffenstein 1984) and 227

± 6.2 mmol·L^{-1} in water deprived bats (*Rhinolophus ferrumequinum*, Vogel and Vogel 1972). These high plasma sodium concentrations are associated with high plasma osmolalities (546 ± 14.3 mosm·kg^{-1} in *Rhinolophus ferrumequinum*, Vogel and Vogel 1972). Moreover, plasma osmolalities as high as 586 ± 18 mosm·kg^{-1} have been recorded (*Acomys subspinosus*, Buffenstein *et al.* 1985) and high values are not uncommon in many desert or carnivorous species (Buffenstein 1984, Buffenstein *et al.* 1985, Vogel and Vogel 1972).

Lower plasma sodium concentrations are seen in both eutherian and marsupial mammals under normal hydrated conditions (Jhala *et al.* 1992), and in a sodium deprived habitat (Dawson and Denny 1969, Scoggins *et al.* 1970). However, the low plasma sodium concentrations exhibited by *A. stuartii* in February and May are not generally observed. Concentrations as low as these have been observed in some adrenalectomised marsupials prior to saline or glucocorticoid treatment (Buttle *et al.* 1952, Reid and McDonald 1968a). However, the opossum (*Didelphis virginiana*, Hartman *et al.* 1943, Silvette and Britton 1936) and the red kangaroo (*Macropus rufus*, McDonald 1974) have relatively stable sodium concentrations after adrenalectomy, although plasma potassium concentrations are raised in all adrenalectomised marsupials studied (Buttle *et al.* 1952, Hartman *et al.* 1943, McDonald 1974, McDonald and Bradshaw 1993, Reid and McDonald 1968a). The values found for *A. stuartii* in February and May mimic those found for adrenal insufficiency, while those in July and August are more within the usual mammalian range.

Other studies have found that the sodium sparing effect of aldosterone is not always replicated by glucocorticoid action although potassium excretion is accelerated (Marver 1992). In *A. stuartii* in July and August the plasma concentration of sodium rises and that of potassium drops, as would be expected if there was a direct glucocorticoid action on the kidneys. However, the urine and faecal analyses do not support this notion. There were no significant seasonal or sex differences in urine concentrations of sodium, potassium and chloride, although total sodium and chloride excreted increased from July.

Urinary osmolar concentration increased in July and August and urea concentration and total urea excreted were higher in females than

males. The higher osmolal concentration as the seasons progress, suggests that urine concentrating ability is not particularly compromised in males even though GFR declines and urea concentrations are higher in females than males. However, there is a reduction in urea concentrations in the males, suggesting that other electrolytes not examined in the present study may be responsible for the high urine osmolalities. The urea concentrations in *A. stuartii* are, however, similar to those of many small mammals, especially the small carnivores (Carpenter 1969, Collins 1978, Heisinger and Breitenbach 1969, Morton 1980, Noll-Banholzer 1979, Studier and Wilson 1983, Wallace *et al*. 1984, Yaakobi and Shkolnik 1974).

Faecal potassium concentrations increased in July and August, but sodium remained unchanged. However, all the total amounts of the electrolytes increased in *A. stuartii* as the year progressed, and this was coupled with a decrease in the water content of the faeces. This suggests that more water is being absorbed from the faeces, at a time when GFR is decreasing. Drier faeces, lowering of GFR, and increasing urine osmolality occur in other mammals when water stressed or deprived (Bakker and Bradshaw 1983, Buffenstein 1985, Hume and Dunning 1979, Maloiy 1972, Pfeiffer *et al*. 1979). In this study *A. stuartii* were not water deprived.

Concurrent with faecal changes due to dehydration, a change in haematocrit often occurs. Many studies have found that water restriction significantly raises the haematocrit (Buffenstein 1984, Collins 1978, Edwards *et al*. 1983, Meir and Shkolnik 1984, Pfeiffer *et al*. 1979, Urison and Buffenstein 1994). In desert adapted Slughi dogs (*Canis familiaris*), the increase in the haematocrit of water restricted animals over that of hydrated animals are as much as ten per cent (from 55.6% ± 0.7 to 65.2% ± 1.7, Meir and Shkolnik 1984). A significant rise in haematocrit in July and August was observed in *A. stuartii*. Seasonal changes in haematocrit have been reported previously for marsupials, as has the higher haematocrit for males than females (Barnett *et al*. 1979, Cleva *et al*. 1994b, Spencer and Speare 1992). Lower haematocrits in males have been reported for *A. stuartii*, and this was associated with gastrointestinal bleeding just prior to death in these males (Barker *et al*. 1978). Gastrointestinal

bleeding did not occur in the present study, explaining the differences between the two studies.

Higher haematocrit could also be due to higher red cell production in males. However, the significantly higher haematocrit in males implies fluid loss, perhaps by higher urinary output, or by reduced faecal reabsorption. The drier faeces indicate that fluid is being conserved in the gastrointestinal tract. Urinary output was not measured in this study, and because not all the urine and faecal samples were collected overnight, but only during renal function experiments, firm conclusions cannot be drawn about electrolyte and fluid exchange and loss.

While mindful of the limitations of the electrolyte data, it is noted that the increase in total faecal electrolytes in July and August suggests that some of the changes may be due to the poorer gut absorption of nutrients in the males (Barnett 1973, Barker *et al.* 1978). Coupled with any cortisol induced compensatory diuresis and natriuresis (Guyton and Hall 1996) and negative nitrogen balance (Woollard 1971), this may contribute to the demise of male *A. stuartii*.

There were also changes in the plasma and urinary electrolytes in females, although there were few associated changes in GFR. This may be due partly to fewer female samples, especially in February and May, which may falsely indicate changes in urinary and faecal electrolytes. The plasma electrolytes change in a similar manner in both sexes. This suggests that there may be seasonal factors affecting both sexes, although the males are obviously unable to continue responding to the challenge of the seasonal cycle.

One pronounced feature of male "die-off" is the negative nitrogen balance (Woollard 1971), although Woollard's (1971) study did not report actual levels of urinary and faecal nitrogen so comparison with the present study is not possible. Unfortunately, the sealed chamber digestion method also means that faecal nitrogen could not be examined in our study. However, lower urinary urea concentration may be the result of protein loss in the faeces, malabsorption due to gastric ulceration and parasitic infection, or because of higher urine output in the males. Loss of protein in the gut coupled with muscle and connective tissue wastage from cortisol action (see Woollard 1971), may essentially mimic a low protein diet,

perhaps contributing to the lowering of GFRs. Certainly, the increased urine osmolality is not due to urinary urea concentrations in the male.

The precise mechanism controlling seasonal changes in GFR in *A. stuartii* is not clear. However, the changes are a most unusual physiological observation in any small mammal. The GFR and electrolyte values obtained for *M. musculus* are within the ranges found by other authors (Haines and Schmidt-Nielsen 1967, Haines *et al.* 1973, Stewart 1971) and for the few other small mammals that have been studied for GFR (Edwards 1975, Edwards *et al.* 1983, Weisser *et al.* 1970), indicating that renal function in the non-reproductive period for *A. stuartii* is not different from placental mammals. While the seasonal changes in renal function are profound in male *A. stuartii*, whether they are testosterone induced, cortisol induced, cortisol and testosterone dependent or secondary to hormonal action is the subject of chapter 6.

Chapter 4

Seasonal Changes in the Renal Morphology of *Antechinus stuartii*

4.1 Introduction

The previous chapter demonstrated that renal function in *A. stuartii* changes with season. In other mammals, such seasonal changes have not been observed, but fluctuations in kidney function are known to result from water restriction or adaption to an arid environment (Denny and Dawson 1977, Etzion and Yagil 1986, Grenot 1992, Maloiy 1972). The urine concentrating ability of mammals that are exposed regularly to water restriction is usually enhanced.

Coincident with increased urine concentrating ability are renal morphological adaptations (de Rouffignac 1990, Greenwald 1989). The most notable features are in the medullary region of the kidney, where the length and number of long-looped nephrons may increase in association with urine concentrating ability (Bankir and de Rouffignac 1985, de Rouffignac 1990). Plasticity of components of the nephron and its association with changes in the physiological environment have been demonstrated in rodents (Kaissling and Stanton 1992). Moreover, morphological plasticity can be moderated by hormonal mechanisms (Bankir *et al.* 1988, Crabtree 1941b,c, Trinh-Trang-Tan *et al.* 1987).

In *A. stuartii*, the reduction in GFR in males is coincident with the time of year when plasma testosterone and cortisol concentrations are significantly elevated. Changes in plasma testosterone and cortisol profiles have been associated with renal morphological changes in other mammals, in which renal structure and function are known to be affected by both androgen and glucocorticoid activity. The androgenic effects have been examined extensively, and include increasing organ mass, and hypertrophy of cortical regions, especially the proximal tubules (Bardin and Catterall 1981, Crabtree 1941b, Kochakian and Stettner 1948, MacKay and MacKay 1927, Oudar *et al.* 1991, Selye 1939). Androgens also stimulate enzymatic activity in the proximal

tubules (Bullock *et al.* 1978, Koibuchi *et al.* 1993, Mills *et al.* 1979, Mowszowicz *et al.* 1974).

Glucocorticoids are known to affect the proximal tubule by stimulating gluconeogenesis (Vandewalle *et al.* 1981). They also compete for aldosterone receptors in the epithelium of the glomerulus and the distal tubule and collecting duct of the nephron (Baylis and Brenner 1978, Funder 1993). Moreover, in other small mammals where high levels of adrenal activity are associated with social stress, renal pathology is a predominant contributor to mortality. Glomerular and tubular damage has been reported in wild rodent populations, usually when population numbers increase (Andrews 1968, Andrews *et al.* 1972, Andrews *et al.* 1975, Christian *et al.* 1965). The renal pathology observed after exposure to social stress in the deer mouse (*Peromyscus maniculatus*), the lemming (*Lemmus trimucronatus*) and the long-haired rat (*Rattus villosissimus*) are all similar, and include increasing sclerosis of the glomeruli, loss of tubular epithelium, hyalinization of the glomeruli, dilated distal convoluted tubule (DCT) and even disintegration of the tubules (Andrews and Belknap 1979, Barnett *et al.* 1975). The extent of the damage was correlated with the number of social interactions and with population density (Andrews and Belknap 1979, Barnett *et al.* 1975, Christian *et al.* 1965). Adrenal secretory reactivity was correlated with the renal pathology in the deer mouse and the lemming (Andrews and Belknap 1979).

The previous chapter has indicated that renal alterations occur during the life cycle of *A. stuartii*. The change in renal function may be associated with changes in renal structure. The structure-function relationship in the kidney of *A. stuartii* will be examined in this chapter, with an emphasis on the correlations with the hormonal changes that have been described previously in *A. stuartii* (Barnett 1973, Bradley *et al.* 1980, McDonald *et al.*1981).

4.2 Materials and methods

4.2.1 Animals

Male and female *Antechinus stuartii* were trapped from the New England Tablelands in February (males n = 7, females n = 3), May (males n = 9, females n = 5), mid July (males n = 6, females n =

5) and August (males n = 12, females n = 5). Animals collected in August were sacrificed after mating. Animals were maintained as outlined in Chapter 2.

4.2.2 Histology

All animals were sacrificed and tissues processed as outlined in Chapter 2. Renal nomenclature follows Kriz and Bankir (1988).

4.2.3 Morphometry

Kidney size and relative medullary thickness (RMT) were calculated using the formulae of Sperber (1944) using kidney size and medullary thickness (MT). Kidney size (KS) is the cube root of length x breadth x width.

$$RMT = \frac{MT \times 10}{KS}.$$

Length, height, and width were measured with vernier calipers. Medullary thickness and total thickness (TT) were measured with a calibrated eyepiece graticule, and by finding the mid-section of the kidney and measuring the thickness of the medulla and cortex (cortical thickness plus MT = TT). Because TT and MT were measured after processing for histology, the TT was compared to the height measured with vernier calipers and the MT and TT were adjusted to account for dehydration shrinkage for each individual. Percentage medullary thickness (PMT) was also calculated where MT is divided by TT and expressed as a percentage (Heisinger and Breitenbach 1969). Actual kidney values were also analysed by a two-way analysis of variance followed by pairwise Fisher PLSD tests to determine sex or seasonal differences. Significance levels were $P < 0.05$ (Zar 1984). Values expressed as a percentage of body weight were arcsine transformed prior to analysis, however, percentage values are presented in tables (Zar 1984).

The kidney mass and size for each individual were compared to predicted values. Initially kidney masses were compared to those predicted for each individual using the scaling equation of Calder (1984). However, all marsupial kidneys were found to fall well below the values predicted using the eutherian equation. Thus an allometric equation was calculated for marsupials. An equation was also

calculated for kidney size. Values for both equations were taken from the literature and also from unpublished observations (Barboza 1993, Barnes 1977, Brooker and Withers 1994, Crile and Quiring 1940, Denny and Dawson 1977, Freudenberger 1991, McAllan *et al.* 1995, Purohit 1974, Smith 1951, Sperber 1944, Yadav 1979).

For kidney masses the allometric equation was

Kidney mass (g) = $5.973M^{0.780}$

where M is body mass (kg), r^2 = 0.973, n = 17, $P<0.0001$, slope upper 95% confidence interval 0.838 and slope lower 95% confidence interval 0.722 (Figure 4.1a). The range for body mass was 0.006 to 39.5kg.

For kidney sizes the allometric equation was

Kidney size (mm) = $15.738M^{0.263}$

where M is body mass (kg), r^2 = 0.952, n = 34, $P<0.0001$, slope upper 95% confidence interval 0.284 and slope lower 95% confidence interval 0.243 (Figure 4.1b). The range for body mass was 0.006 to 39.5 kg. Table 4.1 gives the complete data used for the regression analyses.

The predicted kidney masses and sizes for each individual were calculated from these equations and compared with actual values using paired t-tests. Body masses used for predictions and for adjusting kidney parameters for body mass were the values obtained for renal function experiments, conducted 3-7 days after capture. These were used because initial body masses were obtained after the animals had been fasting (only baits available) overnight in Elliott traps and final body masses were obtained after at least one week in captivity and after some individuals had gained as much as 30 % on their initial body mass.

Light microscopy sections were analysed using either an Apple II digital analyser, a square lattice system of type A (Weibel 1979) or a calibrated eyepiece graticule. Sample sizes were determined using the t-distribution method of Aherne and Dunnill (1982). Systematic sampling of sections was used (Mayhew 1991). Counts were made of the following parameters using the point estimation method (Cruz-Orive and Weibel 1990, Gundersen *et al.* 1988a).

i) Number of glomeruli per mm^2 cortex (Gundersen *et al.* 1988b).

Table 4.1 Body mass (kg), kidney mass (g), kidney size (mm) and source of data for the allometric equations for marsupials.

ORDER **Family** *Species*	Body mass(kg)	Kidney mass (g)	Kidney size (mm)	Author(s)
POLYPROTODONTA **Dasyuridae**				
Antechinomys laniger	0.020		5.5	Sperber (1944) Brooker and Withers (1994)
Antechinus flavipes	0.056	0.43	7.5	Brooker and Withers (1994) Unpublished Data
Antechinus melanurus	0.048		5.5	Brooker and Withers (1994)
Antechinus stuartii agilis	0.035	0.26	6.79	Unpublished Data
Antechinus swainsonii	0.041	0.59	7.92	Unpublished Data, Brooker and Withers (1994)
Dasyuroides byrnei	0.120	1.21	10.44	Unpublished Data
Dasyurus viverrinus	1.0		15.0	Sperber (1944)
Ningaui ridei	0.006	0.13	4.76	Brooker and Withers (1994) Unpublished Data
Sarcophilus harrisii	7.0		31	Sperber (1944)
Sminthopsis crassicaudata	0.015	0.27	6.39	Brooker and Withers (1994) Unpublished Data
Sminthopsis macroura	0.023	0.36	6.44	Brooker and Withers (1994)
Sminthopsis murina	0.025		5.7	Purohit (1974)
Didelphidae				
Caluromys philander	0.222	4.0		Crile and Quiring (1940)
Didelphis marsupialis	1.147	7.5		Crile and Quiring (1940)
Didelphis virginiana	4.8 2.0		23 10.4*	Sperber (1944) * Smith (1951)
Marmosa robinsoni	0.080	1.064		Barnes (1977)
DIPROTODONTA **Petauridae**				
Acrobates pygmaeus	0.012		5.1	Sperber (1944)
Petaurus breviceps	0.120		8.2	Sperber (1944)
Phalangeridae				

Trichosurus vulpecula	2.0	9.44	18.97	Sperber (1944) Unpublished Data
Phascolarctidae				
Phascolarctos cinereus	6.91		29.6	Sperber (1944)
Vombatidae				
Lasiorhinus latifrons	24.63	64.12	36.59	McAllan *et al.* (1995)
Vombatus ursinus	39.5	145.82	48.11	McAllan *et al.* (1995)
Macropodidae				
Aepyprymnus rufescens	3.0		11.29	J. R. Roberts (Unpub.)
Bettongia lesueur	1.74		17.4	Yadav (1979)
Bettongia pencillata	1.3		14.5	Yadav (1979)
Dendrolagus matscheii	6.5		22.5	Yadav (1979)
Lagorchestes conspicillatus	1.7		20.1	Yadav (1979)
Lagorchestes hirsutus	1.74		17.4	Yadav (1979)
Lagostrophus fasciatus	1.5		12.0	Yadav (1979)
Macropus eugenii	2.93	17.78	26.0	Yadav (1979) Purohit (1971)
Macropus giganteus	40		49.0	Yadav (1979) Sperber (1944)
Macropus robustus erebuscens	17.63	57.69	35.2	Yadav (1979) Denny & Dawson (1977)
Macropus robustus robustus	20.88	84.3		D. Freudenberger (1991)
Macropus rufus	14.4		36.6	Yadav (1979)
Peradorcas concinna	1.4		15.0	Yadav (1979)
Potorous tridactylus	0.86	2.90		J. R. Roberts (Unpub.)
Setonix brachyurus	3.5		21.3	Yadav (1979)
Wallabia irma	7.65		31.2	Yadav (1979)

Figure 4.1a

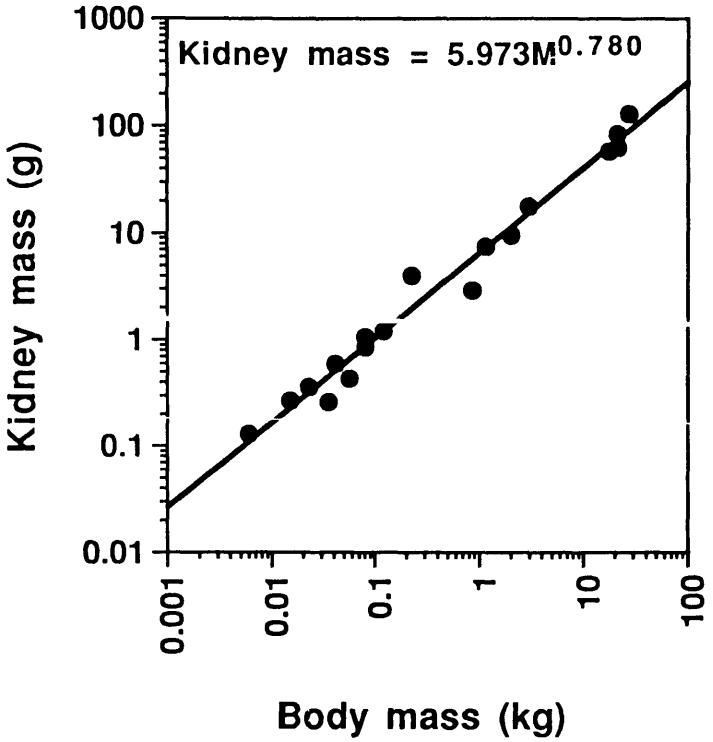


Figure 4.1a Regression equation for kidney mass (g) versus body mass (M in kg). Data are $\log_{10}$ transformed.

Figure 4.1b

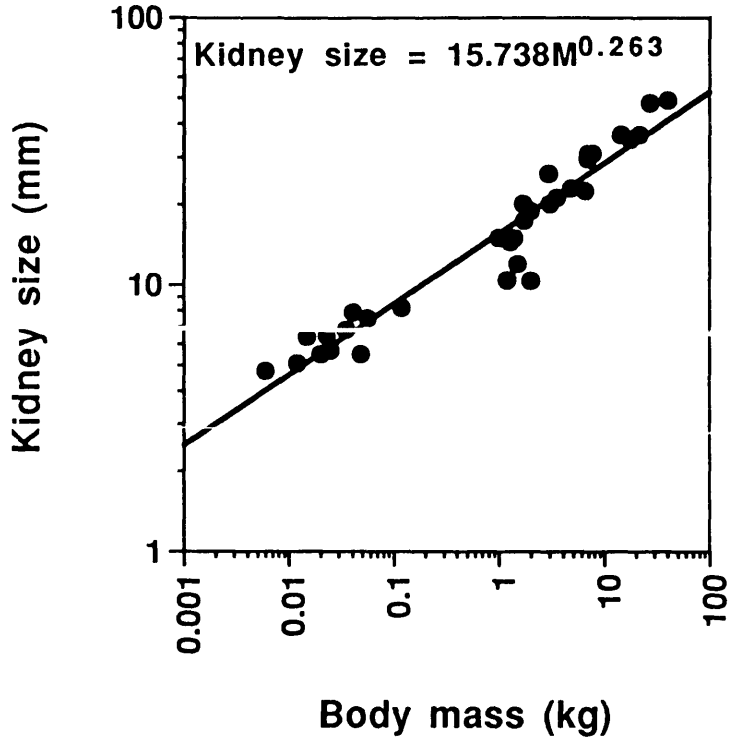


Figure 4.1b Regression equation for kidney size (mm) versus body mass (M in kg). Data are $\log_{10}$ transformed.

ii) Areas of glomeruli were made using the point estimation method and from this the volume was calculated using the formula $V = ß/k \cdot (A)^{3/2}$ where ß = 1.38, the shape coefficient for spheres, and k = 1.1, a size coefficient (Pagtalunan *et al.* 1995, Weibel 1979). The midpoints (widest diameters) of the glomeruli were used to determine area, and adjacent sections of tissue were assessed to ensure that the midpoint of each glomerulus was measured. Counts were made for both outer glomeruli and juxtamedullary glomeruli.

iii) Diameter of perpendicular cross-sections of proximal tubules (μm). Counts were made for proximal convoluted tubules of the outer cortex, and for proximal straight tubules in the outer stripe of the outer medulla.

iv) Diameter of perpendicular cross-sections of distal tubules (μm). Counts were made for distal convoluted tubules and for distal straight tubules (or thick ascending limb) in the outer medulla.

v) Diameter of perpendicular cross-sections of collecting tubules (μm). Counts were made for outer cortex tubules and for tubules in the outer medulla.

vi) Epithelial cell volumes (μm^3) of proximal tubules in the cortex were measured using the nucleator method (Gundersen *et al.* 1988a).

vii) Epithelial cell volumes (μm^3) of the thin loops of Henle were measured using the nucleator method (Gundersen *et al.* 1988a).

viii) Epithelial cell volumes (μm^3) of distal straight tubules were measured using the nucleator method (Gundersen *et al.* 1988a).

ix) Epithelial cell volumes (μm^3) of cortical collecting tubules were measured using the nucleator method (Gundersen *et al.* 1988a).

x) Epithelial cell volumes (μm^3) of collecting ducts were measured using the nucleator method (Gundersen *et al.* 1988a).

The above data were analysed by two-way analysis of variance followed by Fisher's PLSD pairwise tests where appropriate. Significance levels were $P < 0.05$ (Zar 1984).

Sections stained with PAS and Alcian blue pH 1.0 or 2.5 were analysed as follows. Structural components were examined for absence, very weak presence, presence or strong staining for each of the three stains used. The components examined were glomeruli, Bowman's capsule, cytoplasm of the proximal tubules, luminal border

of the proximal tubule, cytoplasm of the distal tubules, luminal border of the distal tubule, cytoplasm of the thick ascending limb, luminal border of the thick ascending limb, cytoplasm of the thin loops of Henle, luminal border of the thin loops of Henle, cytoplasm of the collecting tubules, luminal border of the collecting tubule, interstitial tissue of the cortex and interstitial tissue of the medulla.

The data for each component were then assigned ranks for each stain where absence = 0, very weak presence = 1, presence = 2, strong staining = 3. The ranks of each stain were then summed for each component and then Kruskall-Wallis analyses of variance were performed to determine differences in sex or season and were followed by pairwise Dunn's least square difference tests. Significance levels were $P < 0.05$ (Zar 1984).

4.3 Results

4.3.1 Gross morphology

The kidneys were unilobular with a single papillae. They were also sightly concave or flattened on one side. The papillae extended beyond the confines of the kidney in some individuals, however, this did not occur in all individuals. Mean paired kidney mass was larger in males than females (sex $P<0.0001$, interaction $P=0.09$, Table 4.2). Paired kidney mass increased over the year in males but not females and this trend was eliminated when kidney mass was divided by body mass. The percentage kidney mass/body mass of females was larger than the males (sex $P<0.0001$, Table 4.2) and showed no seasonal changes. Predicted kidney mass was not significantly different from those obtained for each individual, except for the August males, where actual kidney mass was significantly smaller than the predicted kidney mass for each individual (Paired t-test $P<0.05$, Table 4.2). Similar to kidney mass, the predicted values for kidney size were significantly larger than actual values in August males (Paired t-test $P<0.01$, Table 4.2) and kidney sizes of males were larger than females ($P<0.001$, Table 4.2).

The RMT showed considerable variation, ranging from 4.55 to 8.0. The RMT did not differ between seasons or sexes (Table 4.2). PMT and MT did not differ between sexes or seasons (Table 4.2).

Table 4.2 Gross renal morphology of *Antechinus stuartii*.

Season and Sex	Body mass (g)	Paired kidney mass (g)	Predicted kidney mass(g)	Paired kidney mass (% of body mass)	Mean kidney size (mm)	Predicted kidney size (mm)	Relative medullary thickness	Percentage medullary thickness (%)	Medullary thickness (mm)
February males	28.7 ± 2.1	0.328 ± 0.013	0.346 ± 0.009	1.252 ± 0.069	6.17 ± 0.07	6.18 ± 0.13	5.78 ± 0.60	78.1 ± 0.9	3.63 ± 0.30
February females	24.5 ± 1.6	0.280	0.267	1.471	5.74	5.76	7.04	79.2 ± 1.3	3.89 ± 0.16
May males	37.7 ± 2.6	0.402 ± 0.015	0.419 ± 0.022	1.216 ± 0.059	6.56 ± 0.09	6.63 ± 0.12	6.31 ± 0.68	74.5 ± 2.3	4.13 ± 0.48
May females	21.4 ± 2.6	0.257 ± 0.003	0.246 ± 0.020	1.574 ± 0.109	6.00 ± 0.25	5.72 ± 0.13	6.00 ± 0.53	74.8 ± 2.2	3.40 ± 0.24
July males	37.8 ± 2.1	0.407 ± 0.016	0.424 ± 0.021	1.254 ± 0.069	6.61 ± 0.20	6.65 ± 0.10	6.54 ± 0.77	77.9 ± 2.5	4.20 ± 0.46
July females	21.3 ± 0.7	0.256 ± 0.010	0.263 ± 0.007	1.373 ± 0.053	5.70 ± 0.09	5.72 ± 0.05	7.06 ± 0.53	79.2 ± 1.7	4.02 ± 0.29
August males	39.3 ± 2.1	0.393 ± 0.011 *	0.429 ± 0.018	1.204 ± 0.057	6.36 ± 0.05 **	6.70 ± 0.09	6.14 ± 0.29	78.2 ± 1.4	3.92 ± 0.19
August females	23.0 ± 1.2	0.282 ± 0.009	0.280 ± 0.011	1.370 ± 0.081	5.72 ± 0.05	5.74 ± 0.08	5.97 ± 0.49	76.2 ± 1.6	3.40 ± 0.30
Sex	P<0.001	P<0.0001	-	P<0.0001	P<0.001	-	NS	NS	NS
Season	P<0.01	NS	-	NS	P=0.16	-	NS	NS	NS
Interaction	P<0.02	P=0.09	-	NS	NS	-	NS	NS	NS

Table 4.2 Gross renal morphology of *Antechinus stuartii*. Data are means and standard errors. Body mass values differ slightly from those from the GFR study because of different sample sizes between the two studies. Paired kidney mass and mean kidney size was significantly larger in males than in females ($P<0.0001$). Paired kidney mass as a percentage of body mass was significantly larger in females than in males ($P<0.0001$). An asterisk indicates that the predicted kidney mass was larger than actual kidney mass for that group at $P<0.05$, two asterisks indicate significance at $P<0.01$. Relative medullary thickness, percent medullary thickness and medullary thickness did not differ between sex and season.

4.3.2 Light microscopy

The sections of the kidneys of *A. stuartii* revealed a histological organisation similar to that of most unipapillary kidneys of mammals. The cortex consisted of glomeruli, proximal convoluted tubules, distal convoluted tubules and cortical collecting ducts. The medulla consisted of two regions, the outer medulla and the inner medulla. The outer medulla was as thick as the cortex in most individuals and consisted of the proximal straight tubules, distal straight tubules, loops of Henle and the collecting ducts. The inner medulla consisted of the collecting ducts and the long loops of Henle. In general, there were no changes in the overall appearance of the kidneys in females throughout the year, however, in males some pathological changes were noted (Figure 4.2). In males from July and August the proximal tubules were hypertrophied and in many individuals the cytoplasm contained granular material which stained red with Masson's trichrome stain. Moreover, the glomeruli of the males in July and August showed evidence of hypertrophy and some sclerosis. Many glomeruli also showed a proliferation of proximal tubule-like cells within the Bowman's capsule, in some instances these cells continued around the whole capsule. The cellular proliferation was most pronounced in the juxtamedullary glomeruli (Figure 4.3). This phenomenon was observed infrequently in females and in males at other times of year.

4.3.3 Morphometry

i) The mean number of glomeruli/mm^2 differed significantly (sex $P<0.001$, season $P<0.001$, interaction $P=0.011$, Table 4.3) with males in July and August having significantly fewer glomeruli/mm^2 than all female groups and males in February and May (Table 4.3).

ii) The glomerular volume in superficial glomeruli differed significantly (sex $P=0.002$, season $P<0.0001$, interaction NS, Table 4.3). The glomerular volumes of February and May animals were significantly smaller than those of July and August animals (Fisher's PLSD test, $P<0.001$, Table 4.3). Those of the females were smaller than those of males (Fisher's PLSD test $P<0.002$, Table 4.3, Figure 4.4a).

Figure 4.2 Views of the cortex of the kidneys of *A. stuartii*, showing the reduction in numbers of glomeruli and hypertrophy of the cortex in the males over the year. Haematoxylin and Eosin or Masson's trichrome stain. Arrows indicate glomeruli, asterisks indicate slightly distended distal tubules. Note the pronounced hypertrophy of the proximal tubules in C) and D). Scale bars are 50 μm.

A) A male from February.

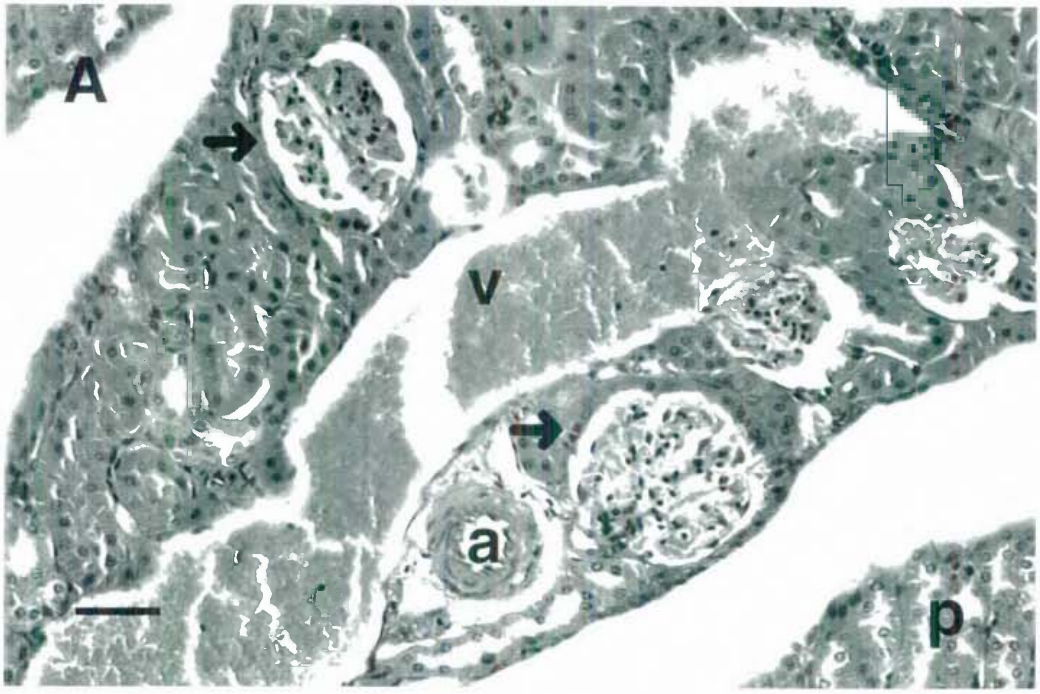
B) A male from May.

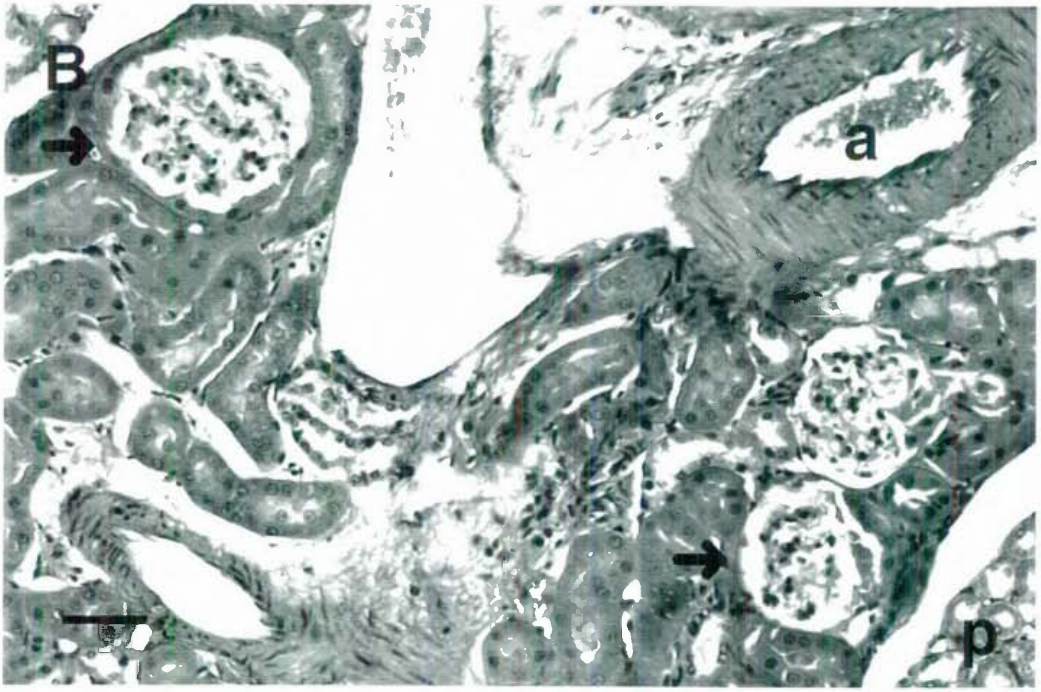
C) A male from July.

D) A male from August.

A
B
C
D

Figure 4.3 A) and B) Glomeruli from two August males showing the proliferation of the proximal tubule-like material into the Bowman's capsule of the juxtamedullary glomeruli. Arrows indicate glomeruli with the proximal tubule-like material within the Bowman's capsule, **a** indicates an artery, **v** indicates a vein, and **p** indicates the papilla. Scale bars are 50 μm.


A
v
a
p


B
a
p

The volumes of the juxtamedullary glomeruli were significantly larger than the superficial glomeruli in all individuals (Paired t-test, $P<0.001$, as compared in Figures 4.4a and 4.4b, and Table 4.3). The glomerular volume of juxtamedullary glomeruli differed significantly (sex $P<0.0001$, season $P=0.0002$, interaction NS). The glomerular volumes of individuals from February and May were significantly smaller than those of individuals in July and August, and those of males were significantly larger than those of females.

iii) The tubular diameter of the proximal convoluted tubules in the outer cortex differed significantly (sex $P<0.001$, season $P<0.001$, interaction $P=0.05$, Table 4.4). The tubular diameters of males in July and August were significantly larger than all female groups and males in February and May (Table 4.4). Female groups did not differ from each other or from males in February and May (Table 4.4). The diameters of the proximal straight tubules in the outer medulla were significantly smaller in females than in males (sex $P<0.001$, season $P=0.14$, Table 4.4). The interaction between sex and season ($P=0.066$) indicated that the tubular diameters of males in July and August approached being significantly larger than those from all other groups (Table 4.4).

iv) The tubular diameters of the distal straight tubules in the outer medulla differed between seasons ($P=0.004$, Table 4.4) and between sexes ($P=0.018$, interaction $P=0.069$, Table 4.4). The interaction between sex and season ($P=0.069$) indicated that the values of the males in July and August approached being significantly larger than those from all other groups (Table 4.4). The same was true of the tubular diameters of the distal convoluted tubules (sex $P<0.002$, season $P<0.001$, interaction $P=0.069$, Table 4.4).

v) The tubular diameters of the cortical collecting ducts in females were significantly smaller than those of males (sex $P<0.003$, Table 4.4). The tubular diameters of the collecting ducts of the outer medulla differed significantly (sex $P<0.003$, season NS, interaction $P=0.05$), with males in July and August having significantly larger tubular diameters than all female groups and males in February and May (Table 4.4).

vi) The volumes of the proximal convoluted tubule cells differed significantly (sex $P=0.002$, season $P=0.026$, interaction $P=0.016$,

Table 4.3 Glomerular measurements.

Season and Sex	Glomerular number per mm^2 of cortex	Superficial glomerular volumes ($\mu m^3 \times 10^5$)	Juxtamedullary glomerular volumes ($\mu m^3 \times 10^5$)
February males	17.9 ± 0.5	1.70 ± 0.07	2.61 ± 0.22
February females	17.4 ± 0.9	1.29 ± 0.07	2.07 ± 0.08
May males	18.2 ± 1.2	1.69 ± 0.25	2.80 ± 0.37
May females	19.7 ± 1.2	1.46 ± 0.32	2.09 ± 0.42
July males	12.8 ± 0.7	2.87 ± 0.25	4.76 ± 0.42
July females	18.5 ± 0.6	1.93 ± 0.15	2.82 ± 0.12
August males	13.2 ± 0.5	2.51 ± 0.11	4.08 ± 0.26
August females	17.7 ± 0.7	2.09 ± 0.13	2.85 ± 0.29
Sex	P<0.0001	P=0.002	P<0.0001
Season	P<0.0001	P<0.0001	P=0.0002
Interaction	P=0.01	NS	NS

Table 4.3. Glomerular measurements in *Antechinus stuartii*. The values are means ± standard errors of the mean. Significance levels for two-way analyses of variance are indicated at the base of the columns.

Figure 4.4a

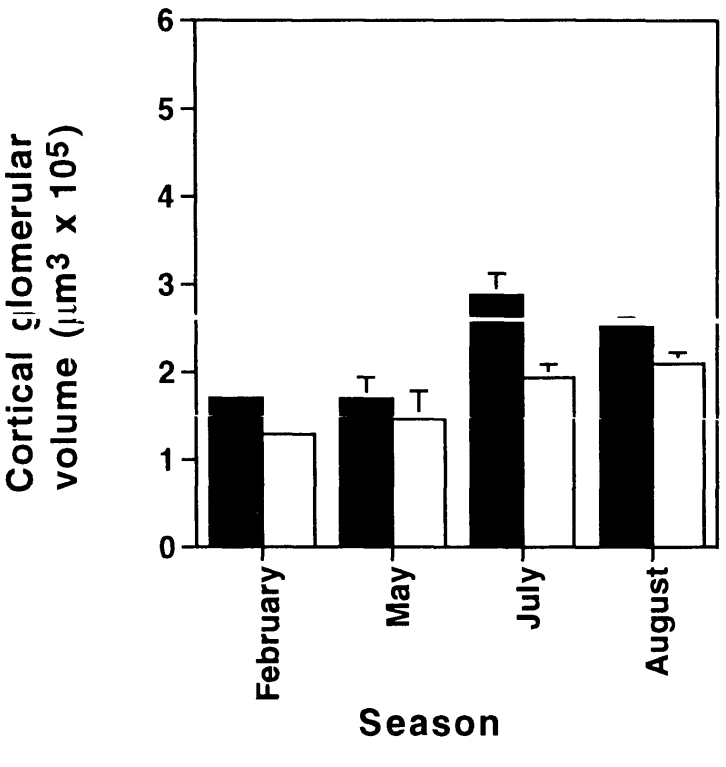
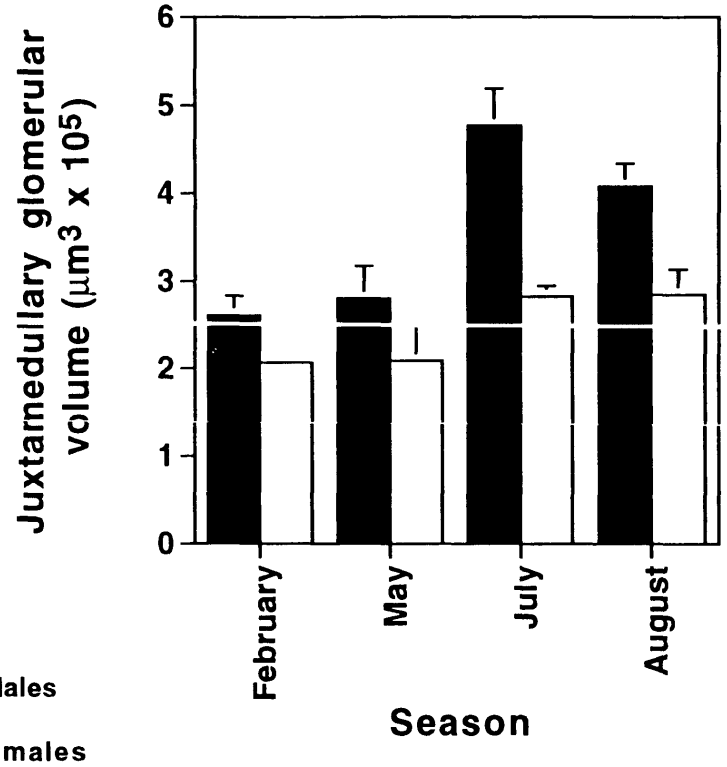


Figure 4.4b



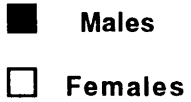


Figure 4.4 Mean glomerular volumes ($\mu m^3 \times 10^5$) in *Antechinus stuartii*, a) cortical glomerular volumes, b) juxtamedullary glomerular volumes. Values are means ± standard errors of the mean.

Table 4.4 Mean tubular diameters (μm).

Season and Sex	Diameter of proximal convoluted tubules (μm)	Diameter of proximal straight tubules (μm)	Diameter of distal straight tubules (μm)	Diameter of distal convoluted tubules (μm)	Diameter of collecting ducts (cortex μm)	Diameter of collecting ducts (outer medulla μm)
February males	29.5 ± 1.0	28.1 ± 0.6	22.4 ± 1.0	23.3 ± 1.4	21.6 ± 1.3	21.9 ± 1.3
February females	27.7 ± 0.8	28.8 ± 0.3	22.5 ± 0.6	23.1 ± 0.5	19.9 ± 1.0	22.3 ± 1.0
May males	30.4 ± 1.2	27.4 ± 1.4	22.0 ± 1.1	23.6 ± 1.4	20.6 ± 1.1	21.6 ± 1.2
May females	26.9 ± 0.8	24.6 ± 1.1	21.3 ± 1.4	20.9 ± 1.3	18.7 ± 1.0	19.5 ± 1.4
July males	34.9 ± 1.5	30.3 ± 1.4	26.3 ± 1.0	26.9 ± 0.8	21.9 ± 0.9	24.2 ± 1.1
July females	30.0 ± 1.0	26.1 ± 0.9	22.5 ± 0.6	24.5 ± 0.5	20.4 ± 0.6	21.3 ± 0.8
August males	36.1 ± 0.8	31.7 ± 1.1	26.5 ± 0.7	29.2 ± 0.6	23.4 ± 0.6	25.2 ± 0.7
August females	28.4 ± 0.6	25.3 ± 0.8	21.9 ± 0.5	22.9 ± 1.0	19.2 ± 0.3	19.2 ± 0.4
Sex	P<0.001	P<0.001	P=0.018	P<0.002	P<0.003	P<0.003
Season	P<0.001	P=0.14	P=0.004	P<0.001	NS	NS
Interaction	P=0.05	P=0.066	P=0.069	P=0.069	NS	P=0.05

Table 4.4. Mean diameters of the tubular regions of the kidney of *Antechinus stuartii*. Values are means ± standard errors of the mean. Significance levels for two-way analyses of variance are indicated at the base of the columns.

Figure 4.5, Table 4.5) such that the cell volumes were larger in males from July and August than those of all other groups (Figure 4.5, Table 4.5).

vii) The volumes of the cells of the thin loops of Henle did not differ between sex or season. However they were greater in females in February and May, but greater in males in July and August (interaction $P=0.022$, Table 4.5).

viii) The volumes of the cells of the distal straight tubules differed between sexes ($P=0.011$, interaction $P=0.073$, Figure 4.6, Table 4.5). The interaction ($P=0.073$) suggested that the cell volumes of males in July and August were significantly larger than all female groups and males in February and May (Figure 4.6, Table 4.5).

ix) The volumes of the cells of the cortical collecting tubule differed significantly (sex $P=0.14$, season $P=0.022$, interaction $P=0.031$, Figure 4.7, Table 4.5). Cell volumes increased in males from February and May to July and August but did not change in females (Figure 4.7, Table 4.5).

x) The volumes of the cells of the collecting ducts in the outer medulla were significantly larger in males than in the females (sex $P<0.025$, season $P=0.15$, Figure 4.8, Table 4.5).

The results of PAS-Alcian blue staining were relatively consistent for both sexes and seasons. The glomeruli stained strongly for all three stains and the Bowman's capsule stained with Alcian blue pH 1.0, with occasional weak reactions to pH 2.5. The brush border of the proximal tubules was strongly PAS positive, while the cytoplasm was more weakly stained. The luminal border of the distal convoluted tubules stained strongly with Alcian blue pH 2.5, whereas the cytoplasm was PAS positive and this reaction was significantly stronger in males and females in August than males at other times of the year ($P<0.05$). The luminal border of the distal straight tubule was stained strongly with Alcian blue pH 2.5, while the cytoplasm was PAS positive. The thin loops of Henle were stained strongly with Alcian blue pH 2.5 and pH 1.0. The collecting ducts in the inner medulla were strongly positive to both Alcian blue pH 2.5 and 1.0, the interstitial tissue of the cortex and medulla were PAS positive and the medullary tissue also stained with Alcian blue pH 1.0.

Table 4.5 Epithelial cell volumes (μm^3).

Season and Sex	Proximal convoluted tubules cellular volumes (μm^3)	Thin loops of Henle cellular volumes (μm^3)	Distal straight tubules cellular volumes (μm^3)	Cortical collecting duct cellular volumes (μm^3)	Collecting duct cellular volumes in outer medulla (μm^3)
February males	717.4 ± 76.6	21.9 ± 3.0	281.0 ± 28.2	125.7 ± 7.7	623.3 ± 112.2
February females	734.5 ± 148.8	32.6 ± 4.5	342.0 ± 96.6	130.9 ± 5.5	532.9 ± 110.0
May males	755.9 ± 82.8	23.5 ± 3.1	273.4 ± 45.0	98.3 ± 7.5	706.5 ± 101.0
May females	759.4 ± 110.3	32.3 ± 5.9	333.3 ± 55.8	114.8 ± 15.3	738.6 ± 264.0
July males	1416.3 ± 125.0	39.2 ± 3.3	425.8 ± 30.0	153.7 ± 11.4	1177.2 ± 140.2
July females	768.6 ± 97.2	31.5 ± 5.8	390.6 ± 42.1	112.5 ± 12.0	630.6 ± 100.3
August males	1198.2 ± 131.3	46.9 ± 5.5	478.4 ± 26.6	148.8 ± 8.6	1117.3 ± 144.9
August females	669.0 ± 37.8	27.7 ± 6.7	352.9 ± 29.4	122.4 ± 7.8	684.4 ± 97.6
Sex	$P=0.002$	NS	$P=0.011$	$P=0.14$	$P<0.025$
Season	$P=0.026$	NS	NS	$P=0.022$	$P=0.15$
Interaction	$P=0.016$	$P=0.022$	$P=0.073$	$P=0.031$	NS

Table 4.5. Epithelial cell volumes (μm^3) of different sections of the nephron. Values are means ± standard errors of the mean. Significance levels for two-way analyses of variance are indicated at the base of the columns.

Figure 4.5

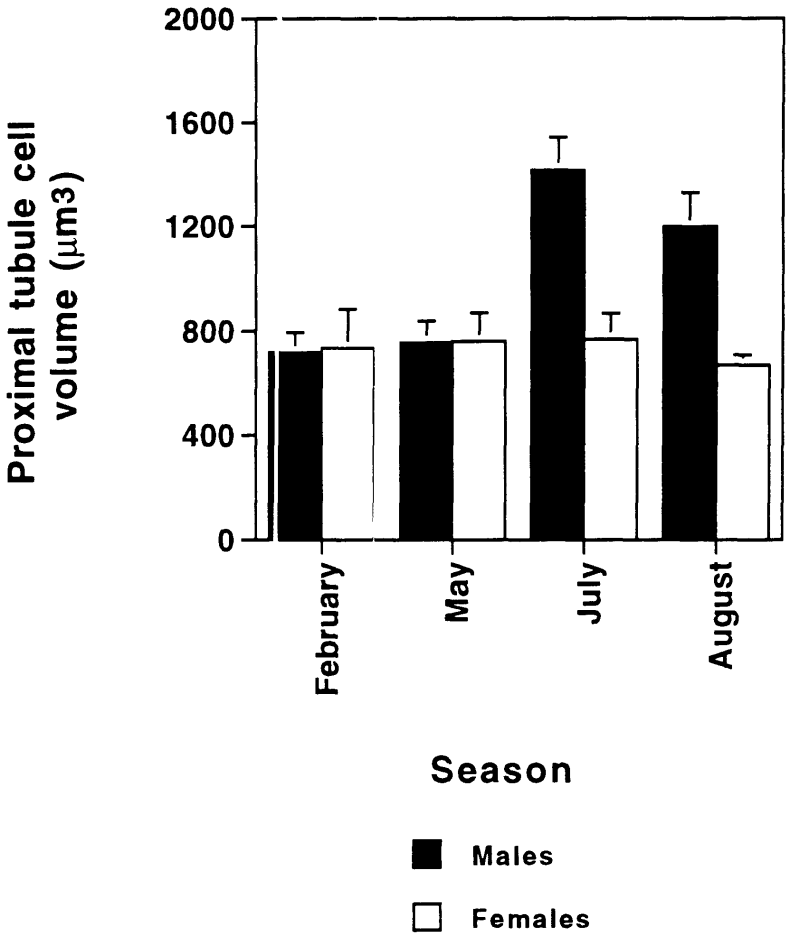


Figure 4.5 Means of proximal tubule cell volumes (μm^3). Values are means ± standard errors of the mean.

Figure 4.6

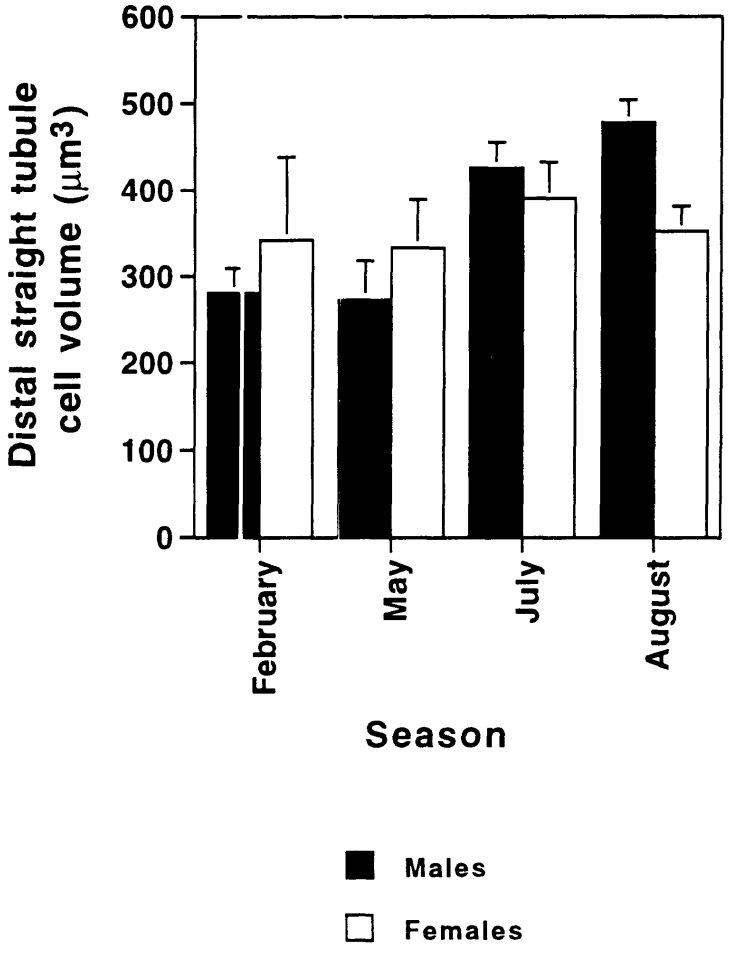


Figure 4.6 Mean volumes of distal straight tubule cell volumes (μm^3). Values are means ± standard errors of the mean.

Figure 4.7

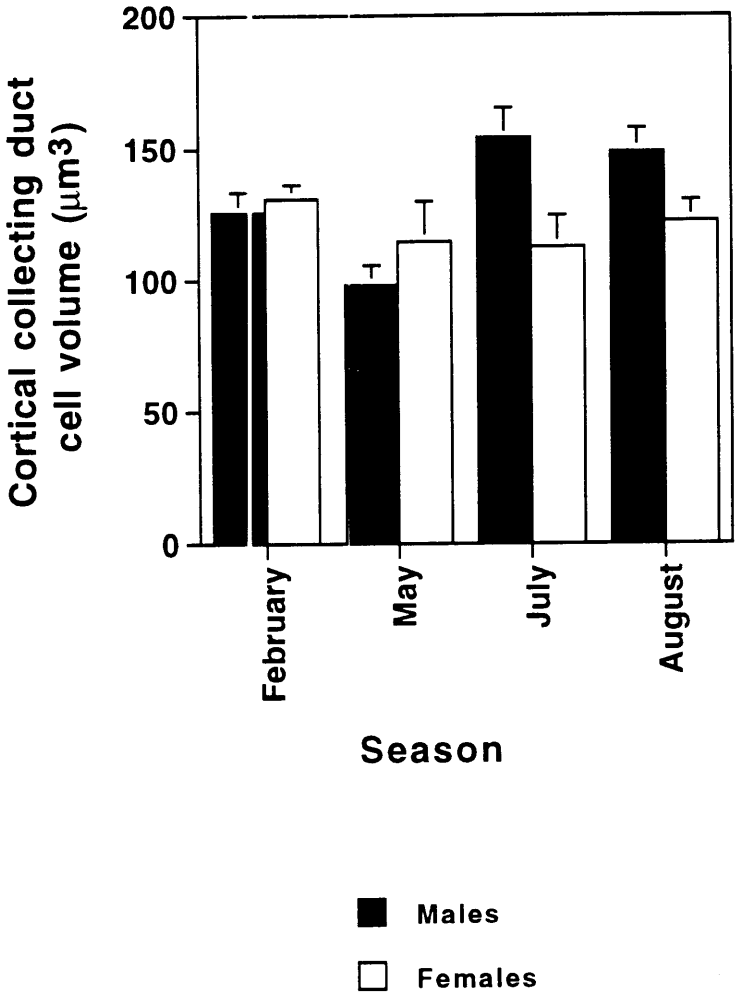


Figure 4.7 Mean volumes of the cells of the cortical collecting duct (μm^3). Values are means ± standard errors of the mean.

Figure 4.8

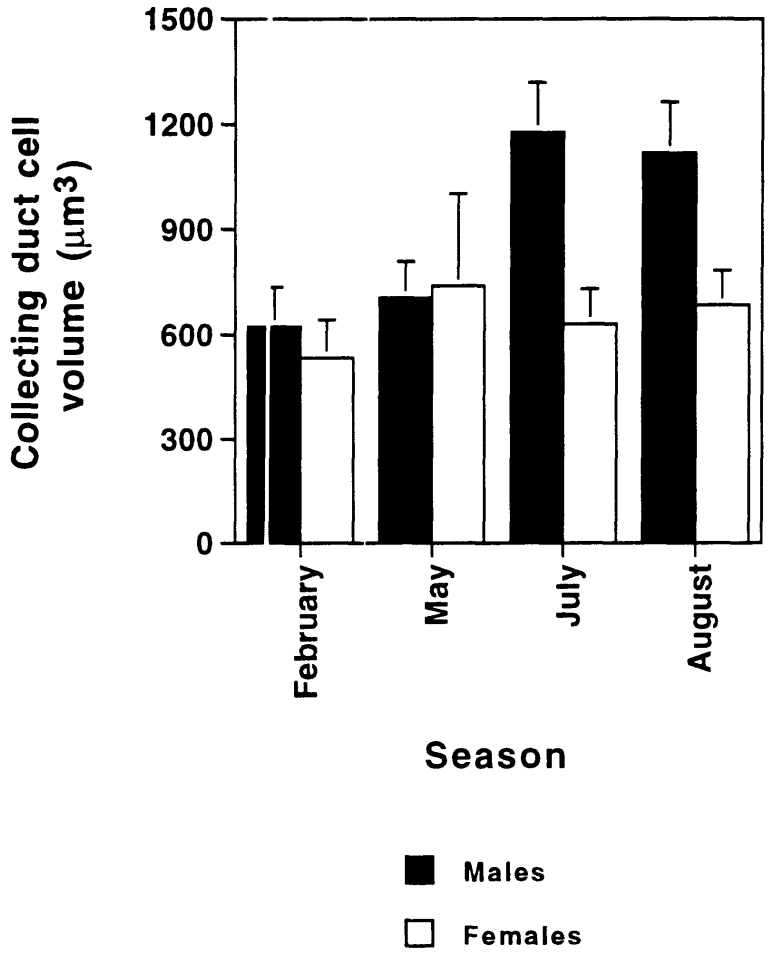


Figure 4.8 Mean volumes of the cells of the collecting ducts of the outer medulla (μm^3). Values are means ± standard errors of the mean.

4.4 Discussion

4.4.1 General changes in morphology

The present study demonstrates that there are pronounced seasonal changes in the renal morphology of male *A. stuartii* (Figure 4.2). Although gross morphological characteristics, such as RMT and PMT, did not differ between sexes or across seasons, the microscopic morphology of *A. stuartii* showed consistent seasonal changes. These changes occurred in the male groups, where July and August males were significantly different to males in February and May (Figure 4.2). In contrast, seasonal changes were not observed in females.

An increase in kidney mass in male *A. stuartii* has been reported previously (Moore 1974), although in the present study the increase was not significant. However, when adjusted for body mass, kidney mass shows no seasonal change in males. The percentage of total body mass contributed by the kidney is significantly higher in females. The relatively higher proportion of body mass contributed by the kidneys of the females (which are smaller than the males) has been reported for other small mammals, in which the proportion of body mass contributed by kidney mass increases as body size reduces (Calder 1984). This is presumably due to greater metabolic activity, and thus production of metabolic waste products, of smaller mammals. The mass-dependent differences were also found for the kidney size in *A. stuartii*.

Variation in the renal indices (e.g. RMT, PMT) similar to that observed in *A. stuartii* has been found in other mammals (Dawson and Denny 1969, Downs and Perrin 1991, Hulbert and Dawson 1974b, Lawler and Geluso 1986, Studier *et al.* 1983). Studier *et al.* (1983) found similar variations in renal indices in neotropical bats and suggested that some of this variation may be explained by imperfect placement of tissue within the paraffin blocks. However, in this study, as in that of Studier *et al.* (1983), all care was taken to avoid this artifact.

Many studies have had to rely on small sample sizes, sometimes only single individuals, to determine renal indices (Beuchat 1990a, Brooker and Withers 1994, Downs and Perrin 1991, Lawler and Geluso 1986, Sperber 1944, Studier *et al.* 1983). In studies of marsupials this is difficult to avoid as many species are rare or difficult

to obtain. While RMT and PMT are considered reliable predictors of concentrating ability (Beuchat 1990a, Sperber 1944), the variation found in RMT in *A. stuartii* suggests cautious use of this indicator if sample sizes are small.

The mean RMT is higher than might be expected for a mammal, such as *A. stuartii* from a mesic habitat. The high RMT of *A. stuartii* contrasts to the RMTs of desert adapted mammals such as the red kangaroo (*Macropus rufus*) and rabbit-eared bandicoot (*Macrotis lagotis*) which are lower than that obtained for *A. stuartii* (Denny and Dawson 1977, Hulbert and Dawson 1974b). However, this can be explained in part by the scaling of renal indices and concentrating ability for body size, where renal indices such as RMT decline with increasing body size, as does urine osmolality (Beuchat 1990a,b, 1993). These phenomena may be partly explained by the lower metabolic rate of larger mammals (Beuchat 1990b, 1993).

In marsupials, however, few studies have addressed the structure-function relationship in the kidneys. The gross morphological differences of kidneys in dasyurids have been related to habitat aridity (Brooker and Withers 1994), and similar relationships have been found in macropods (Yadav 1979 . In the wombats (*Lasiorhinus latifrons* and *Vombatus ursinus*) the gross and microscopic morphology of the kidney was related to the greater capacity of *L. latifrons* to conserve water in its arid environment (McAllan *et al.* 1995). When compared with other small dasyurids from both mesic and xeric environments, the RMT of *A. stuartii* is intermediate between the extreme RMTs found in the different environments (Brooker and Withers 1994). In general, however, while RMT is larger in mammals from xeric environments, the RMT is also dependent on body size and metabolic rate of mammals (Beuchat 1990b, 1993).

4.4.2 Effect of diet on renal indices

In addition to habitat and body size, diet is another factor that determines renal indices. High protein diets necessitate higher urea excretion to maintain plasma concentrations at an acceptably low level, and the relationship between high protein diets and increased urine osmolality due to high urinary urea is well established (Edwards *et al.* 1983, Leon *et al.* 1983, McFarland and Wimsatt 1969, Nagy *et*

al. 1976, Vogel and Vogel 1972, Schmidt-Nielsen *et al.* 1948). While presence of urea has been demonstrated to reduce the water required for a given amount of osmotically active solutes, this is true within a small range of urea: non-urea ratios (Crawford *et al.* 1959, Gamble *et al.* 1934). This is believed to function by the accumulation of urea in the concentration gradient in the medullary interstitium which contributes to the extraction of water from the collecting ducts (Bouby *et al.* 1988, Schmidt-Nielsen and Robinson 1970).

Low protein diets as are often experienced by herbivores, promote a lower excretion of urea as the urea is recycled around the loops of Henle to maintain the medullary concentration gradient (Choshniak and Arnon 1985, Kooyman and Drabek 1968, Schmidt-Nielsen 1990, Wallace *et al.* 1984). Some authors have claimed that urine concentrating mechanisms are not as important for carnivores because the carnivorous diet contains ample water, even though large amounts of urea must be excreted (Deavers and Hudson 1979, Leon *et al.* 1983, Noll-Banholzer 1979, Schmidt-Nielsen and Haines 1964, Schmidt-Nielsen and Newsome 1962). Other studies have shown that high protein diets induce hypertrophy of renal mass and especially of the medullary components of the nephron (Bouby *et al.* 1988, MacKay *et al.* 1928, 1931). Moreover, high protein diets also increase urine concentrating ability in carnivores and non-carnivores alike (Bouby *et al.* 1988, Hendrixx and Epstein 1958, Schmidt-Nielsen and Robinson 1970). Carnivores must conserve water and electrolytes while excreting urea. Therefore, relatively longer looped nephrons, which translate to higher medullary thicknesses, may be necessary for water balance and urinary excretion. Because of the high metabolic rates of the smallest carnivores, efficient renal function and water conservation are essential for survival, even for those living in mesic habitats, as does *A. stuartii*.

Previously, however, there has been some controversy concerning the necessity of an efficient urine concentrating mechanism in carnivores. Some studies have associated high urinary urea and urine osmolality with higher medullary indices (Studier and Wilson 1983, Studier *et al.* 1983), while others maintain that the high water content of a carnivorous diet obviates the need for efficient renal

concentrating ability (Deavers and Hudson 1979, Schmidt-Nielsen and Haines 1964, Schmidt-Nielsen and Newsome 1962).

Thus there is some controversy as to whether or not carnivores possess larger renal indices than other mammals matched for size and habitat. This can be examined if renal indices of mammals are separated for habitat and data. Data were collected for carnivorous and non-carnivorous mammals from the literature and analysed for body mass (kg), kidney mass (g), kidney size (mm), RMT and PMT in relation to their habitat type (see Appendix IV for full details of the data collected). Data for kidney mass, kidney size, RMT and PMT were regressed against body mass for mammals from xeric or mesic environments. The slopes and Y-intercepts (elevation) of the regression equations for these parameters for carnivorous and non-carnivorous mammals were compared (Zar 1984). Moreover, the kidney mass and kidney sizes were divided by body mass and arcsine transformed for analysis by two-way ANOVA for effects of diet and environment (Zar 1984). Data were not divided by body mass adjusted by the slope of the regression equation for pooled kidney mass against body mass similar to Calder (1984). This was because the slopes of the lines for regression equations were different between diet groups within environments. The RMTs and PMTs were also analysed by two-way ANOVA for effects of diet and environment (Zar 1984). Data for kidney mass adjusted for body mass are graphed as a percentage, and data for kidney size adjusted for body mass are graphed as an arcsine transformation of the percentage data and called "kidney size index".

The elevation of the regression equations for kidney mass for carnivores from mesic environments was significantly higher than those of non-carnivores from mesic environments ($P<0.0001$, Figure 4.9a). This relationship was also true of carnivores and non-carnivores from xeric environments ($P<0.01$ Figure 4.9b). The slopes were not different within or between environments for either diet group.

The elevation of the regression equations for kidney size for carnivores from xeric environments was significantly higher than those of non-carnivores from xeric environments ($P<0.0001$, Figure 4.10). This was also true for carnivores and non-carnivores from mesic environments ($P<0.001$, Figure 4.10). The slopes were not different

Figure 4.9. Regression equations for carnivorous and non-carnivorous mammals for

a) Kidney mass (g) versus body mass (M in kg) for mesic environments where for carnivores

Kidney mass = $8.945M^{0.904}$

r^2 = 0.981, P<0.0001, and the 95% confidence for slope, lower = 0.821, upper = 0.988, the 95% confidence interval for the Y-intercept lower = 6.998, upper = 11.429. For non-carnivores the equation is

Kidney mass = $7.064M^{0.749}$

r^2 = 0.924, P<0.0001, and the 95% confidence for slope, lower = 0.613, upper = 0.885, the 95% confidence interval for the Y-intercept lower = 4.966, upper = 10.023.

b) Kidney mass (g) versus body mass (M in kg) for xeric environments where for carnivores the equation is

Kidney mass = $9.785M^{0.884}$

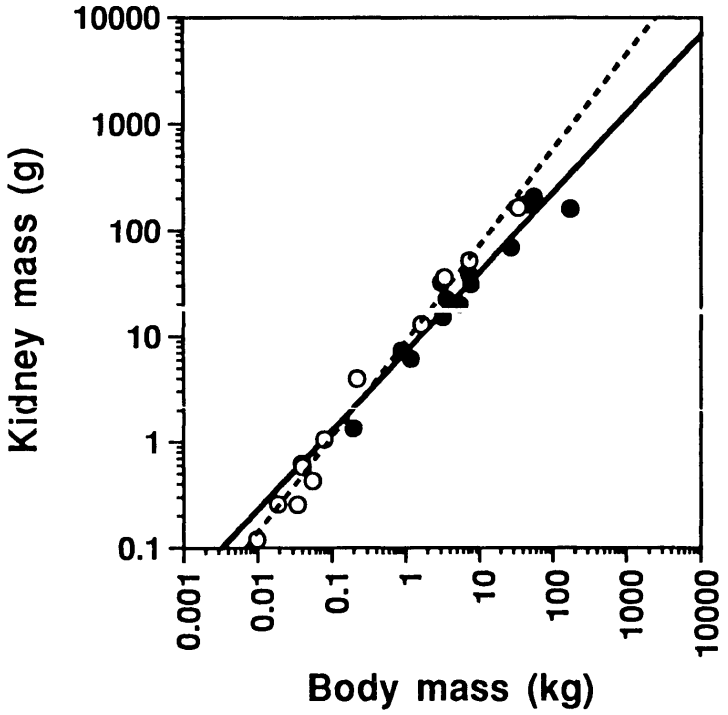
r^2 = 0.993, P<0.0001, and the 95% confidence for slope, lower = 0.841, upper = 0.926, the 95% confidence interval for the Y-intercept lower = 8.506, upper = 11.246. For non-carnivores the equation is

Kidney mass = $6.367M^{0.841}$

r^2 = 0.984, P<0.0001, and the 95% confidence for slope, lower = 0.802, upper = 0.879, the 95% confidence interval for the Y-intercept lower = 5.512, upper = 7.355.

Data are $\log_{10}$ transformed. The dotted line represents the equation for carnivores and the solid line represents the equation for non-carnivores. The regression equations for each diet are presented underneath their respective legends.

Figure 4.9a Mesic environments



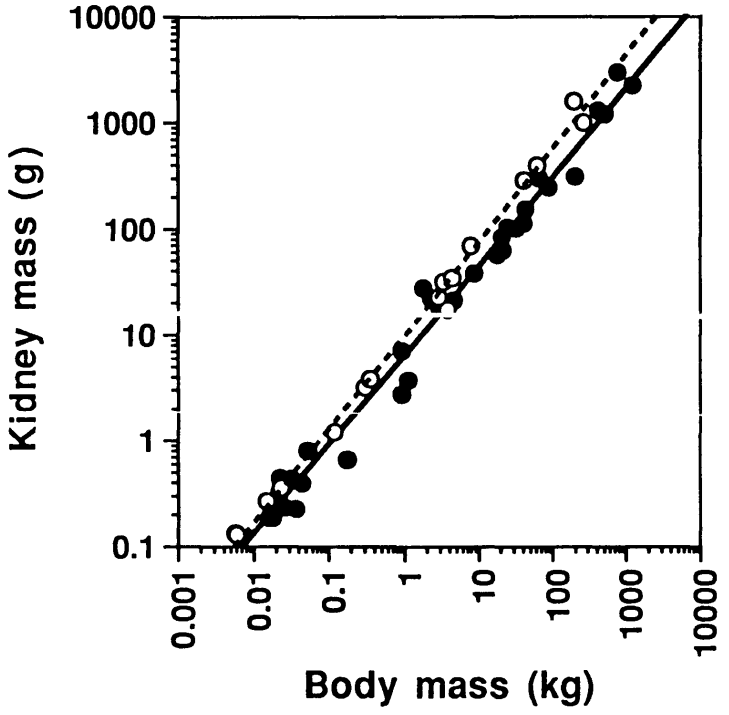
○ Carnivores

Kidney mass = $8.945M^{0.904}$

● Non-Carnivores

Kidney mass = $7.064M^{0.749}$

Figure 4.9b Xeric environments



○ Carnivores

Kidney mass = $9.785M^{0.884}$

● Non-carnivores

Kidney mass = $6.367M^{0.841}$

Figure 4.10. Regression equations for carnivorous and non-carnivorous mammals for

a) Kidney size (mm) versus body mass (M in kg) for mesic environments where for carnivores the equation is

Kidney size = $17.648M^{0.268}$

r^2 = 0.949, P<0.0001, and the 95% confidence for slope, lower = 0.244, upper = 0.291, the 95% confidence interval for the Y-intercept lower = 16.334, upper = 19.063. For non-carnivores the equation is

Kidney size = $15.171M^{0.281}$

r^2 = 0.901, P<0.0001, and the 95% confidence for slope, lower = 0.247, upper = 0.315, the 95% confidence interval for the Y-intercept lower = 13.871, upper = 16.592.

b) Kidney size (mm) versus body mass (M in kg) for xeric environments where for carnivores the equation is

Kidney size = $18.806M^{0.305}$

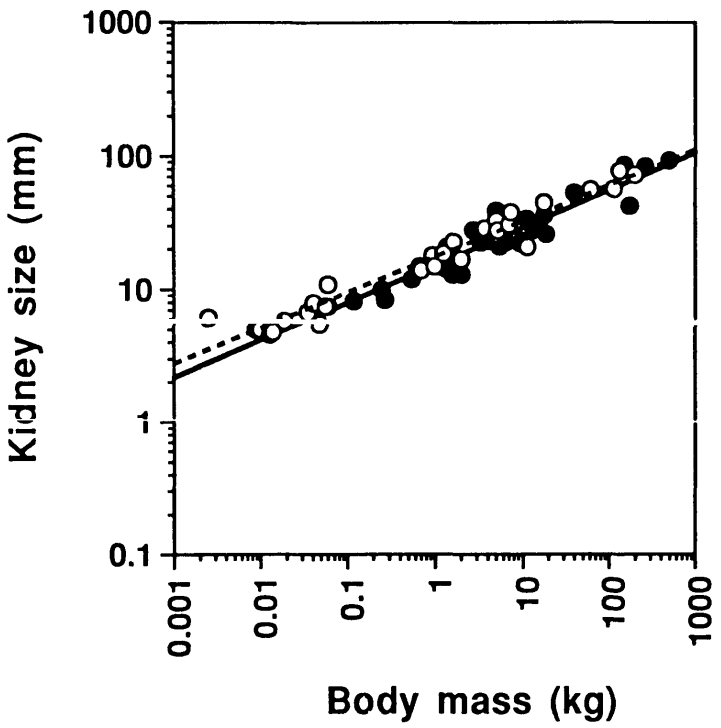
r^2 = 0.979, P<0.0001, and the 95% confidence for slope, lower = 0.281, upper = 0.328, the 95% confidence interval for the Y-intercept lower = 17.334, upper = 20.403. For non-carnivores the equation is

Kidney size = $15.072M^{0.289}$

r^2 = 0.942, P<0.0001, and the 95% confidence for slope, lower = 0.265, upper = 0.313, the 95% confidence interval for the Y-intercept lower = 13.980, upper = 16.252.

Data are $\log_{10}$ transformed. The dotted line represents the equation for carnivores and the solid line represents the equation for non-carnivores. The regression equations for each diet are presented underneath their respective legends.

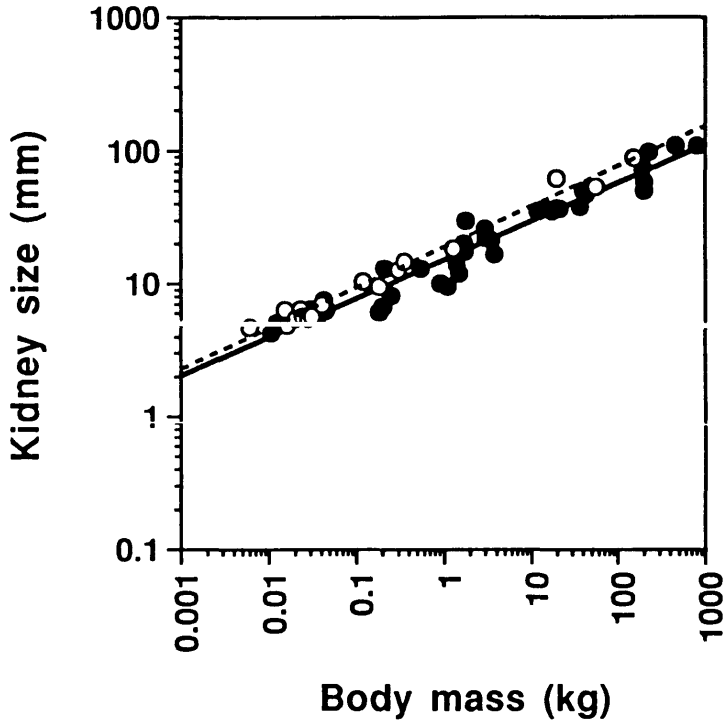
Figure 4.10a Mesic environments



○ Carnivores
Kidney size = $17.648M^{0.268}$

● Non-carnivores
Kidney size = $15.171M^{0.281}$

Figure 4.10b Xeric environments



○ Carnivores
Kidney size = $18.806M^{0.305}$

● Non-carnivores
Kidney size = $15.072M^{0.289}$

within or between environments, and the elevations of equations from non-carnivores did not differ between environments.

The relationship between body mass and RMT did not differ between carnivores and non-carnivores from the same environment (Figure 4.11a and 4.11b). However the elevation of the equations for carnivores from xeric environments were significantly higher than those of carnivores from mesic environments ($P<0.001$, Figure 4.11a and 4.11b). This relationship was also true for non-carnivores ($P<0.001$, Figure 4.11a and 4.11b). The slopes of the equations were not different within or between environments for either diet group.

The change in PMT with body mass was significantly different in carnivores than non-carnivores from both mesic and xeric environments (Figure 4.12a and 4.12b). The elevation of the equations for carnivores from xeric environments were significantly higher than those of carnivores from mesic environments ($P<0.05$, Figure 4.12a and 4.12b). This relationship was also true for non-carnivores ($P<0.001$, Figure 4.12a and 4.12b). The slope of the equation for xeric carnivores declined more slowly with increasing body mass than for carnivores from mesic environments ($P<0.05$ Figure 4.12a and 4.12b). This was also true for non-carnivores ($P<0.001$, Figure 4.12a and 4.12b). The slope of the equation for carnivores from mesic environments declined more slowly with body mass than non-carnivores ($P<0.001$) and the elevation of the equation was higher in carnivores than non-carnivores from mesic environments ($P<0.0001$, Figure 4.12a). These trends were not significant for carnivores and non-carnivores from xeric environments (slope $P<0.10$, elevation NS, Figure 4.12b)

Kidney mass, when adjusted for body mass, was significantly higher in carnivores than non-carnivores, and environment had little effect on this ratio (diet $P<0.0001$, environment $P=0.679$, interaction $P=0.220$, Figure 4.13a).

Both diet and environment affected kidney size adjusted for body mass (diet $P<0.0001$, environment $P<0.02$, interaction $P=0.318$, Figure 4.13b). Mammals from xeric environments had larger kidney sizes than mammals from mesic habitats (Figure 4.13b). Carnivores had larger kidney sizes than non-carnivores (Figure 4.13b).

Figure 4.11. Regression equations for carnivorous and non-carnivorous mammals for

a) RMT versus body mass (kg) for mesic environments where for carnivores the equation is

$RMT = 4.515M^{-0.066}$

$r^2 = 0.325$, $P<0.005$, and the 95% confidence for slope, lower = -0.108, upper = -0.024, the 95% confidence interval for the Y-intercept lower = 3.970, upper = 5.129. For non-carnivores the equation is

$RMT = 4.366M^{-0.123}$

$r^2 = 0.517$, $P=0.0001$, and the 95% confidence for slope, lower = -0.177, upper = -0.069, the 95% confidence interval for the Y-intercept lower = 3.837, upper = 4.965.

b) RMT versus body mass (kg) for xeric environments where for carnivores the equation is

$RMT = 6.431M^{-0.072}$

$r^2 = 0.465$, $P<0.0001$, and the 95% confidence for slope, lower = -0.102, upper = -0.042, the 95% confidence interval for the Y-intercept lower = 5.752, upper = 7.191. For non-carnivores the equation is

$RMT = 6.630M^{-0.062}$

$r^2 = 0.456$, $P<0.0001$, and the 95% confidence for slope, lower = -0.080, upper = -0.045, the 95% confidence interval for the Y-intercept lower = 6.269, upper = 7.011.

Data are $\log_{10}$ transformed. The dotted line represents the equation for carnivores and the solid line represents the equation for non-carnivores. The regression equations for each diet are presented underneath their respective legends.

Figure 4.11a Mesic environments

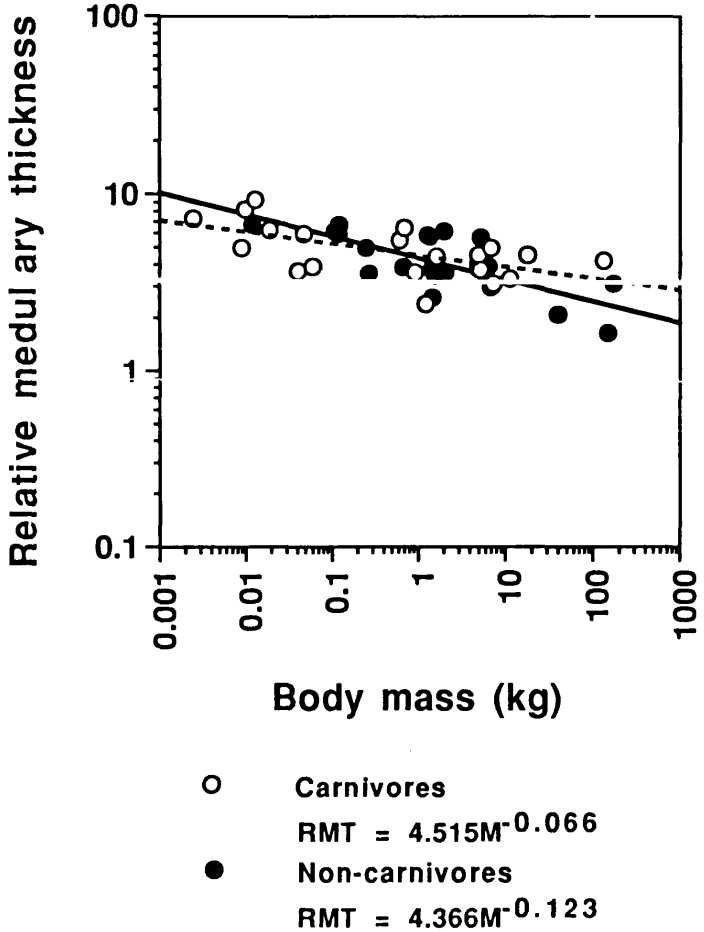


Figure 4.11b Xeric environments

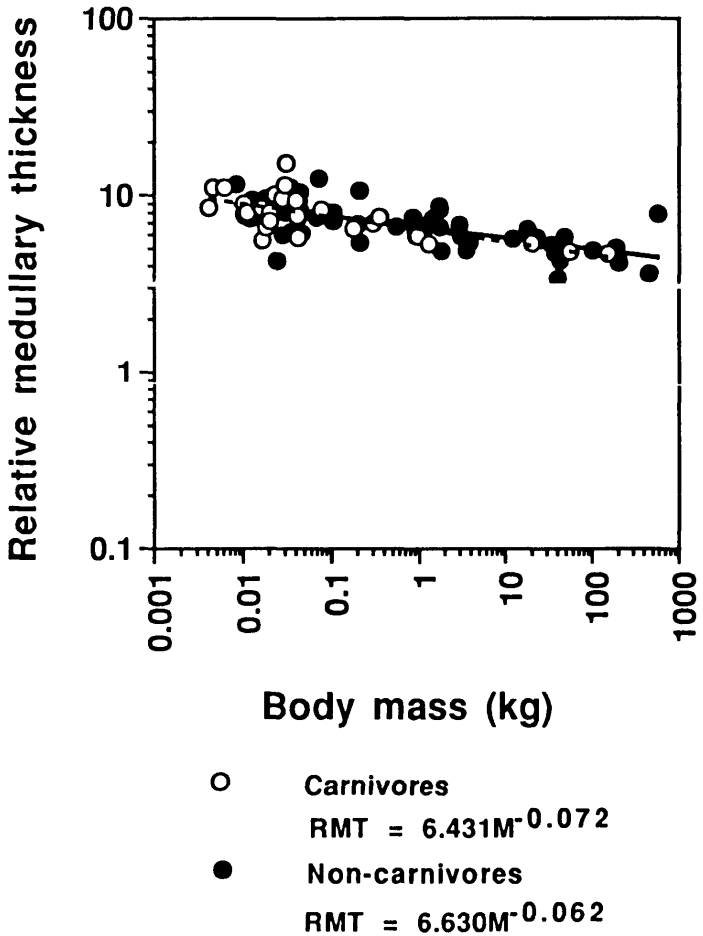


Figure 4.12. Regression equations for carnivorous and non-carnivorous mammals for

a) PMT versus body mass (kg) for mesic environments where for carnivores the equation is

$PMT = 75.037M^{-0.020}$

$r^2 = 0.364$, $P=0.0003$, and the 95% confidence for slope, lower = -0.029, upper = -0.010, the 95% confidence interval for the Y-intercept lower = 72.360, upper = 77.822. For non-carnivores the equation is

$PMT = 65.660M^{-0.057}$

$r^2 = 0.407$, $P<0.0001$, and the 95% confidence for slope, lower = -0.079, upper = -0.035, the 95% confidence interval for the Y-intercept lower = 62.445, upper = 69.040.

b) PMT versus body mass (kg) for xeric environments where for carnivores the equation is

$PMT = 83.683M^{-0.006}$

$r^2 = 0.155$, $P=0.032$, and the 95% confidence for slope, lower = -0.012, upper = -0.0006, the 95% confidence interval for the Y-intercept lower = 81.752, upper = 85.664. For non-carnivores the equation is

$PMT = 78.216M^{-0.019}$

$r^2 = 0.292$, $P<0.0001$, and the 95% confidence for slope, lower = -0.027, upper = -0.011, the 95% confidence interval for the Y-intercept lower = 76.190, upper = 80.297.

Data are $\log_{10}$ transformed. The dotted line represents the equation for carnivores and the solid line represents the equation for non-carnivores. The regression equations for each diet are presented underneath their respective legends.

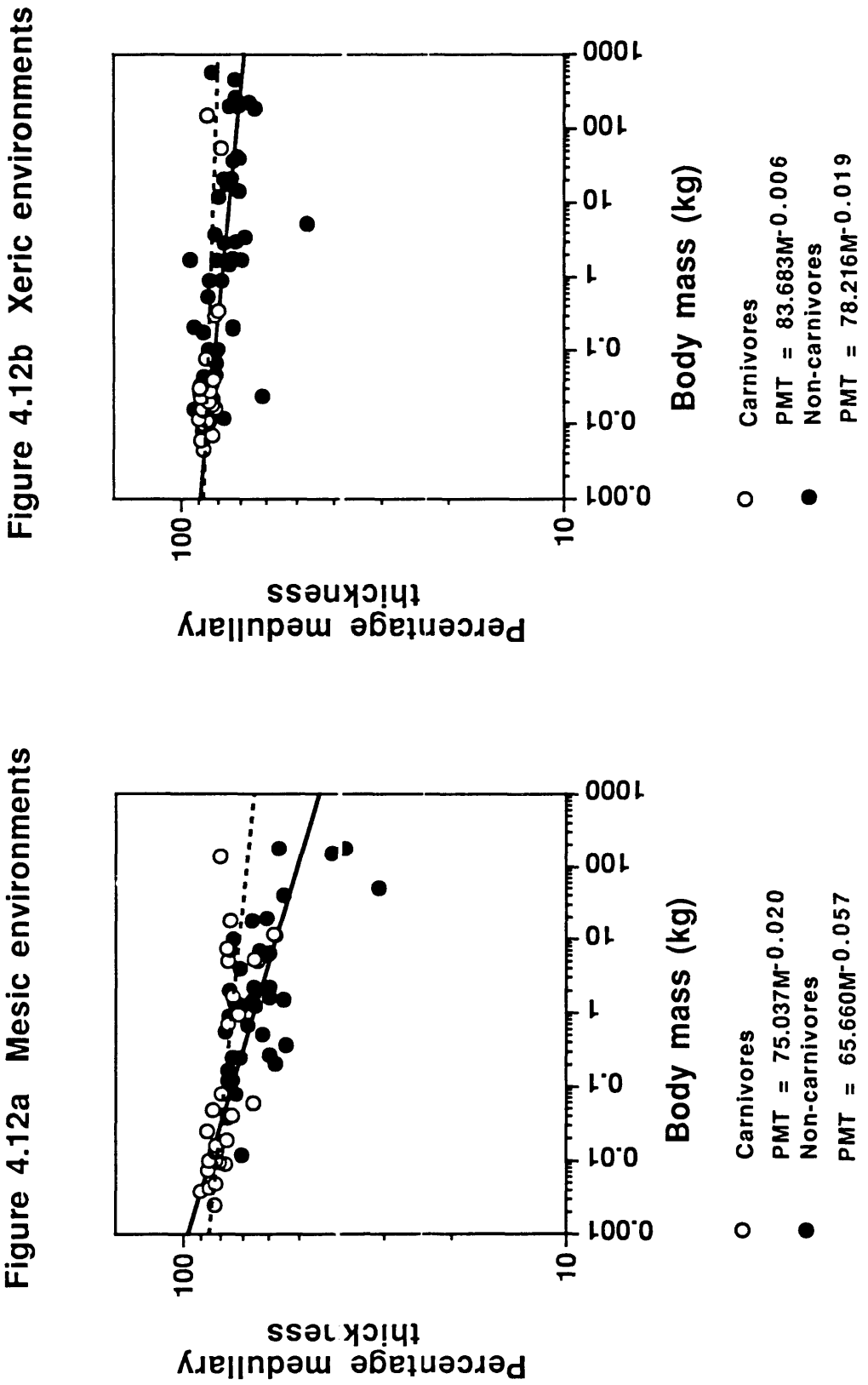


Figure 4.12b Xeric environments
Percentage medullary thickness
100
10
0.001
0.01
0.1
1
10
100
1000
Body mass (kg)
Carnivores
PMT = 83.683M-0.006
Non-carnivores
PMT = 78.216M-0.019
Figure 4.12a Mesic environments
Percentage medullary thickness
100
10
0.001
0.01
0.1
1
10
100
1000
Body mass (kg)
Carnivores
PMT = 75.037M-0.020
Non-carnivores
PMT = 65.660M-0.057

The same relationship was also true of the RMTs (diet $P<0.05$, environment $P<0.0001$, interaction $P=0.543$, Figure 4.14a). There were significant differences found in the analysis of PMTs (diet $P<0.0001$, environment $P<0.0001$, interaction $P<0.02$, Figure 4.14b). The PMTs of carnivores from xeric environments were significantly higher than non-carnivores from xeric environments, which were the same as PMTs of carnivores from mesic environments, who in turn had significantly higher PMTs than non-carnivores from mesic environments (Figure 4.14b).

The present analysis supports the view that renal size and gross morphology are significantly modified by diet, and for some parameters were little influenced by environment and increasing body mass. When matched for body size, it is apparent that renal indices are higher in carnivores than in non-carnivores. However, for most indices, the relationship between the parameter examined and increasing body size was similar between diet groups.

For the relationship between body mass and both kidney mass and kidney size, a carnivorous diet was the only parameter that was influential. This was also true for the analysis of variance of kidney mass adjusted for body mass, although environment was also important in determining kidney size when adjusted for body mass. The relationship between RMT and body mass demonstrated that environment was more important than diet, although the analysis of variance of RMT indicated that diet was also influential, independent of environment. The interaction of these parameters was apparent in the analyses of PMTs. The PMTs of carnivores from xeric environments did not decline as markedly with body mass as they did with carnivores from mesic environments and non-carnivores from xeric environments, although environment also influenced this relationship in non-carnivores. The interaction between diet and environment is more clearly seen in the analysis of variance of PMTs, with carnivores from xeric environments having the highest PMTs and non-carnivores from mesic environments exhibiting the lowest PMTs.

Thus the RMTs and PMTs exhibited by *A. stuartii* may appear high for a small mammal living in a mesic environment. However the above analysis demonstrates that carnivores, especially ones with low body mass, frequently exhibit higher renal indices than non-carnivores.

Figure 4.13a

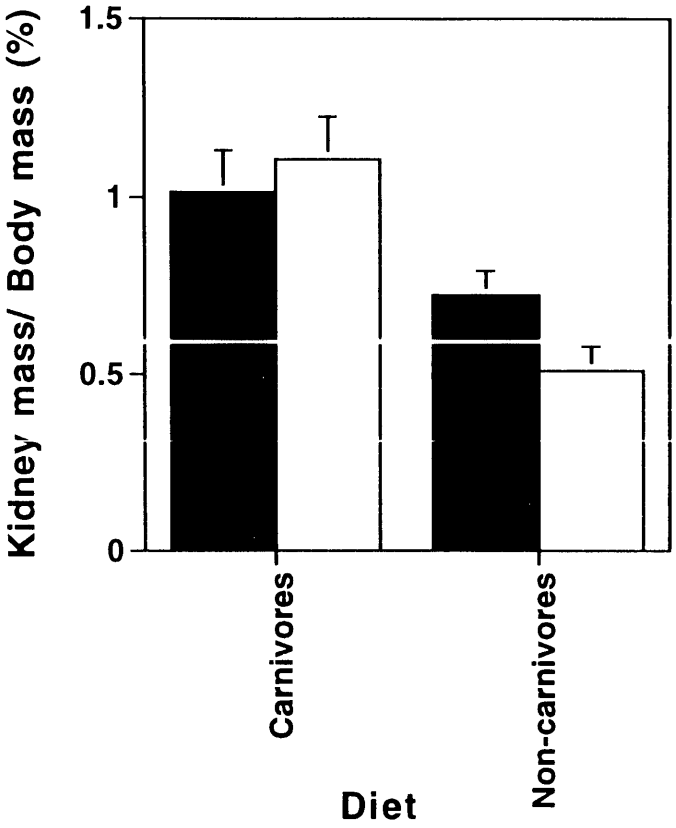


Figure 4.13b

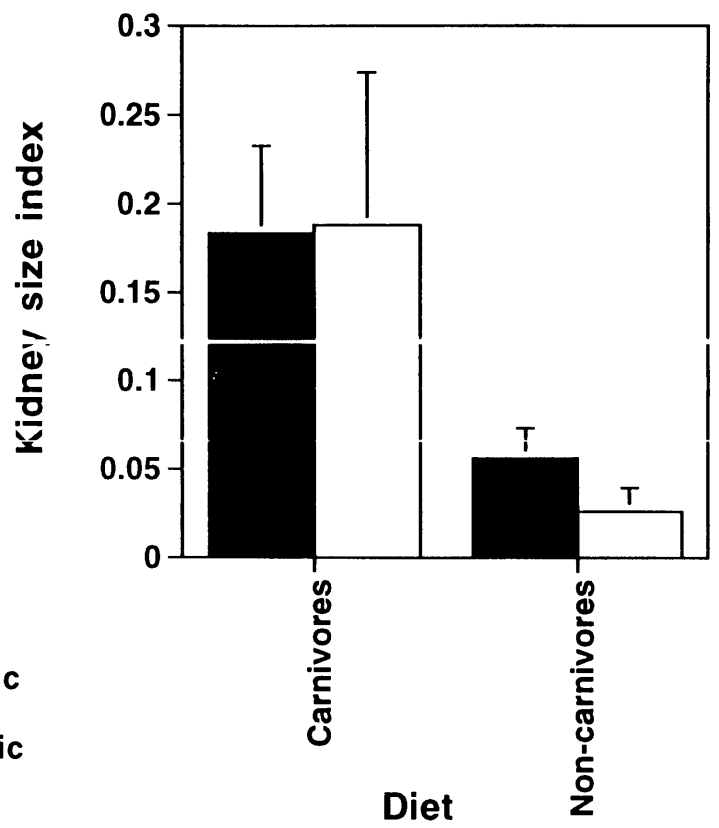


Figure 4.13 a) Kidney mass (g) / Body mass (g) (%), and b) Kidney size (mm) / Body mass (kg) index (arcsine transformed) for carnivores and non-carnivores from mesic and xeric environments. Values are means ± standard errors of the mean.

Figure 4.14a

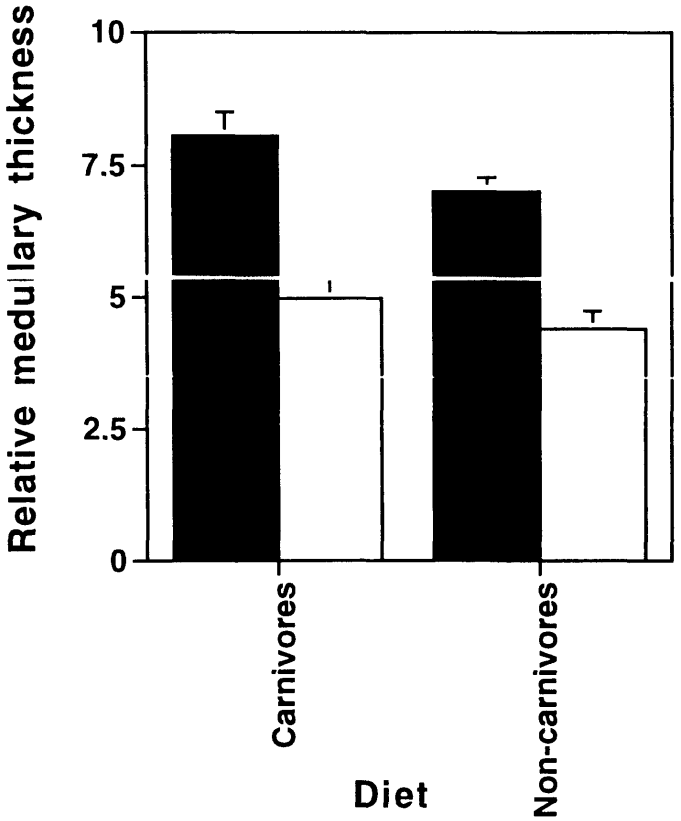


Figure 4.14b

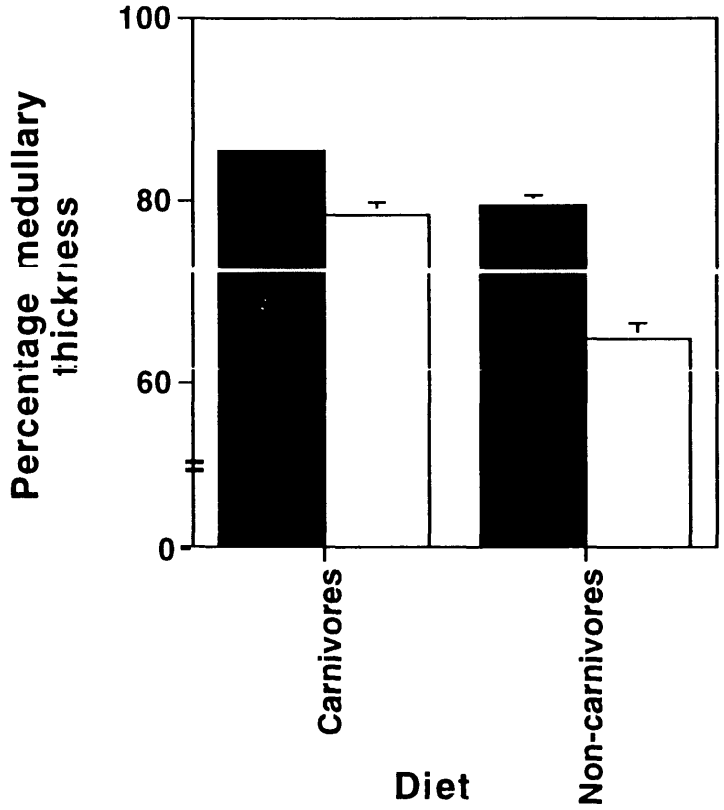


Figure 4.14a) RMT and b) PMT for carnivores and non-carnivores from mesic and xeric environments. Values are means ± standard errors of the mean.

For many of the indices, the changes are independent of environment. These higher indices would appear to be related to the conservation of water while excreting a urea load.

4.4.3 Comparative morphology in *A. stuartii*

The above analysis has demonstrated that the high renal indices observed in *A. stuartii* are not unusual for small carnivores. In the present morphometric analysis of renal anatomy of *A. stuartii*, there were no differences between seasons or sexes in RMT and PMT, and in males there were no significant differences in kidney mass, kidney size, RMT or PMT over the year. However, the number of glomeruli per unit area of cortex had, however, decreased in males from July and August, although the kidney mass and size had not changed. A possible explanation for this finding is that there had been a loss of nephrons. Loss of functional kidney mass is usually followed by hypertrophy of the remaining nephrons (Fine 1986). In this study the glomerular volumes, diameters and cellular volumes of the proximal convoluted tubules were significantly hypertrophied in the male *A. stuartii* in July and August. Moreover, there were glomerular profiles which were not partial sections of a larger glomerulus but small glomeruli, many of which did not continue into adjacent sections. These poorly defined, sclerotic glomeruli were only observed in males in July and August.

Aging can cause renal histological disturbances such as glomerulosclerosis and membranous nephritis, and loss of renal mass accelerates this process (Bolton *et al.* 1976, Corman *et al.* 1985, Haensly *et al.* 1982, Haley and Bulger 1983, Sakemi and Baba 1993). Furthermore male rats seem to be more susceptible to the development of proteinuria and glomerulosclerosis than do female rats (Remuzzi *et al.* 1988). Castration alleviates this process (Sakemi and Baba 1993).

The hypertrophy of most of the regions of the tubules examined in this study in the July and August males may be the result of increased circulating plasma concentrations of testosterone. Several studies have shown that testosterone is directly implicated in the hypertrophy of the kidney especially the proximal tubules (Bardin and Catterall 1981, Oudar *et al.* 1991, Koibuchi *et al.* 1993, Swank *et al.* 1978). The proliferation of proximal tubule-like epithelium into the

region of the Bowman's capsule has been seen in old mice of both sexes (Crabtree 1940, 1941a, Selye 1939). Testosterone was found to promote this phenomenon and when given to castrates of both sexes also produced hypertrophy of the proximal tubule (Crabtree 1941b, Deitert 1967, Selye 1939).

The lysosome pattern of the proximal tubule changes with testosterone treatment, with more lysosomes evident in the earlier part of the proximal tubule of males, and more in the later part of the tubule in females (Schiebler and Danner 1978). Examination of rats castrated as adults reveal that these sex differences remain, although the differences are reduced (Zabel and Schiebler 1980). There were also changes to the proximal tubule after hypophysectomy and this causes an atrophy of the proximal tubule cells (Daigeler 1981, Evan *et al.* 1972). The changes may be related to other hormone alterations besides the removal of sex hormones as urine volume is increased. However proteinuria is maintained if the anterior pituitary is retained (Daigeler 1981).

Proximal tubule hypertrophy is also reflected by higher activity of enzymes important for cellular growth and differentiation in these tubules (Koibuchi *et al.* 1993, Swank *et al.* 1978). Androgens have also been shown to increase cell size, but not number (Catterall *et al.* 1986). The enlargement of both tubule diameter and volumes in *A. stuartii* males from July and August may be the result of increased levels of testosterone at these times in males. Plasma cortisol levels do not begin to rise until three or four weeks before death (Bradley *et al.* 1980) and thus the hypertrophy cannot be attributed solely to cortisol action.

Cortisol action may also contribute to the relative reduction in overall kidney size and mass. The severe effects of cortisol on the general biology of male *A. stuartii* have been well documented (Barker *et al.* 1978, Barnett 1973, Cheal *et al.* 1976, Moore 1974, Woollard 1971). The reduction of kidney mass and size as a proportion of body mass may be a further sign of the debilitating effects of excess cortisol in this mammal. Kidney mass decreases in other mammals exposed to stress as a part of their life history (Andrews and Belknap 1979).

The gluconeogenic activity observed in the proximal tubule is the only other site outside the liver where glucose production occurs

(Marver 1992). Glucocorticoids also compete for aldosterone receptors and affect the smooth muscle of the glomerulus (Baylis and Brenner 1978) and the distal tubule and collecting duct of the nephron (Funder 1993). Some of the hypertrophy in these areas in the August males may be under the influence of cortisol as well as testosterone. However, many of the morphological changes seen in *A. stuartii* differed from those seen in other small mammals in which a massive stress response is part of the life history pattern (Andrews 1968, Andrews and Belknap 1979, Andrews *et al.* 1975, Barnett *et al.* 1975, Christian *et al.* 1965). The tubular damage, increasing sclerosis of the glomeruli, loss of tubular epithelium, hyalinization of the glomeruli, and dilated distal convoluted tubules were less obvious in *A. stuartii*, suggesting that cortisol may be less important than testosterone in initiating the renal changes observed in the present study.

There were also some seasonal changes in females. These were restricted to the increase in glomerular volumes in July and August. The reasons for this are unclear. There is evidence that the proximal tubule is directly affected by female sex hormones (Evan *et al.* 1973, Schiebler and Danner 1978, Zabel and Schiebler 1980). However there were no changes evident at the light microscope level in proximal tubules of female *A. stuartii*. These changes in the females, when coupled with the changes in PAS staining in the cytoplasm in the distal convoluted tubule of males and females in August, may indicate some changes in the functional integrity of A. *stuartii* as they age.

In general however, the most significant morphological changes occurred in males collected in July and August where hypertrophy of the remaining nephrons occurs at the time when plasma testosterone and cortisol profiles are their highest. The relative contributions of testosterone and cortisol to renal morphological changes in the males will be discussed in Chapter 7. In conclusion, however, the dramatic changes seen in renal morphology in *A. stuartii* males, but not females, may well contribute to the male decline observed after mating in these mammals.