

# HEMATOLOGIC AND SERUM BIOCHEMICAL REFERENCE RANGES AND AN ASSESSMENT OF EXPOSURE TO INFECTIOUS DISEASES PRIOR TO TRANSLOCATION OF THE THREATENED WESTERN RINGTAIL POSSUM (*PSEUDOCHÉIRUS OCCIDENTALIS*)

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**ABSTRACT:** Health screening of animals before translocation is important to minimize the risk of pathogen transmission between sites and species. Reintroduction has been incorporated into management of the endangered western ringtail possum (*Pseudocheirus occidentalis*) to mitigate for habitat loss within the species' core range in southwestern Australia. Between November 2005 and March 2008 we screened 47 wild and 24 captive *P. occidentalis* and 68 sympatric common brushtail possums (*Trichosurus vulpecula hypoleucus*) for infectious diseases that might compromise possum survival or fecundity at translocation sites. We found no evidence that infectious disease limits translocation success, and neither possum species showed evidence of infection with *Salmonella* spp., *Toxoplasma gondii*, *Leptospira* spp., or *Chlamydophila* spp. Antigen of *Cryptococcus gattii* was detected in one *T. v. hypoleucus* but was not of pathologic significance. Hematologic and serum biochemical reference ranges were determined for 81 wild and 24 captive *P. occidentalis*. Site differences were identified for red blood cell count, hemoglobin, albumin, urea, and globulin, suggesting that habitat quality or nutrient intake may vary among sites. Differences between wild and captive values were found for several parameters. These data are useful for health evaluations of injured *P. occidentalis* and the future monitoring of wild populations.

**Key words:** Biochemistry, *Cryptococcus*, hematology, possums, *Pseudocheirus occidentalis*, *Toxoplasma*, translocation, *Trichosurus vulpecula*.

## INTRODUCTION

Minimization of risk for disease transmission must be considered during reintroduction programs (Viggers et al., 1993; Woodford and Rossiter, 1994), and examination of hematologic and serum biochemical parameters complements clinical assessments during health surveys of wild animal populations (Kock et al., 2007). Translocation has featured in management of the western ringtail possum, *Pseudocheirus occidentalis*, which is listed as threatened under the Commonwealth of Australia's Environment Protection and Biodiversity Conservation Act, 1999 (Department of the Environment, Water, Heritage and the Arts, 2009) and by the

International Union for the Conservation of Nature (IUCN, 2009). Land clearing for building development threatens the last major coastal population stronghold around Busselton in southwestern Australia (de Tores, 2008).

Although there is no current evidence that disease limits persistence of marsupial populations in southwestern Australia, there are anecdotal historical reports of widespread disease-induced mortality of native species, including possums, soon after European colonization (Abbott, 2006). The unexplained decline of a translocated *P. occidentalis* population between 1998 and 2002 (de Tores et al., 2004) prompted this study in which we investigated pathogens most likely to

affect possum survival and fecundity: *Salmonella* spp., *Toxoplasma gondii*, *Leptospira* spp., *Cryptococcus* spp., and *Chlamydophila* spp. These organisms are capable of causing disease in stressed hosts, especially those immunosuppressed following capture.

Knowledge of species-specific reference ranges from healthy wild populations facilitates identification of diseased individuals. Data on hematologic and serum biochemical parameters are scarce for *P. occidentalis* (Clark, 2004); therefore, establishment of baseline reference ranges was important. Our aims were to 1) determine levels of exposure of *P. occidentalis* and coexisting *Trichosurus vulpecula hypoleucus* to the infectious diseases most likely to adversely affect their survival or fecundity, 2) assess fecal shedding of *Salmonella* spp. by these possums, and 3) establish hematologic and biochemical reference ranges for coastal populations of *P. occidentalis*.

## MATERIALS AND METHODS

### Animals and samples

We sampled 36, 22, and 15 clinically healthy wild *P. occidentalis* from Busselton (33°39'S, 115°21'E), Tuart Forest National Park (TFNP, 33°38'S, 115°25'E) and Gelorup (33°25'S, 115°37'E), respectively, between November 2005 and March 2008. Possums were captured by darting using 1:1 tiletamine and zolazepam (Zoletil 100®, Virbac, Milperra, New South Wales, Australia) delivered at 0.1 mg/kg. We also sampled 24 orphaned or displaced captive *P. occidentalis* held by local wildlife rehabilitators. Samples were collected from 19 Busselton-born *P. occidentalis*  $\geq 5$  mo after translocation 80–150 km north to Leschenault Peninsula Conservation Park (33°14'S, 115°41'E) and Yalgorup National Park (32°50'S, 115°40'E). Samples were collected from 68 *T. v. hypoleucus* at the translocation sites. Thirty of the *P. occidentalis* and 17 of the *T. v. hypoleucus* were sampled on 2–4 occasions, at least 3 mo apart.

Possums were anesthetized with isoflurane delivered via facemask using an anesthetic machine employing an open, non-rebreathing circuit system (Stinger®, Advanced Anaesthesia Specialists, Sydney, Australia). Blood samples,

to a maximum of 4 mL, were collected from the tail vein into ethylenediaminetetraacetic acid-coated tubes (Becton, Dickenson and Co., North Ryde, New South Wales, Australia) for hematology and into untreated tubes for serum biochemistry and antibody-antigen testing. Cloacal swabs for microbiologic culture were taken into MW 170 transport medium (Tran-swab®, Medical Wire and Equipment Co. Ltd., Corsham, Wiltshire, England). Epithelial cell samples for *Chlamydophila* testing were collected onto sterile dry rayon swabs (COPAN® Italia, Brescia, Italy) from the conjunctiva of eyes, pharynx, and cloaca. Wild and captive possums from Busselton and the translocation sites were sampled for all tests; some more than once. Samples from *P. occidentalis* at TFNP and Gelorup were collected for hematologic and serum biochemical analyses only.

Hematologic and serum biochemical analyses were carried out using an automated Advia 120 Hematology System® (Siemens Healthcare Diagnostics, Deerfield, Illinois, USA) and an RX Daytona™ (Randox Laboratories, Crumlin, Co. Antrim, UK) autoanalyzer, respectively, to determine parameters listed in Tables 1 and 2. Differential white cell counts were performed on blood smears stained with Wright's Giemsa stain, spun packed cell volume was measured manually, and total solids protein was read by refractometry. Fibrinogen was measured by heat precipitation (Schalm, 1980).

Cloacal swabs were plated onto sheep blood agar and MacConkey agar. Bacteria were identified by Gram reaction and standard biochemical tests and diagnostic kits (MB12A Microbact®, Oxoid Ltd., Cambridge, UK, and API-20E®, bioMérieux Clinical Diagnostics, worldwide). Each sample was tested for *Salmonella* spp. using Salmonella-Shigella agar and brilliant green agar. Any *Salmonella* spp. found were checked by Poly O (A–S) and Poly H antiserum agglutination testing and then identified to species. Antibody testing for *T. gondii* used direct agglutination (DAT) and modified agglutination (MAT) tests (Antigen Toxo AD kit, BioMérieux, Charbonnières les Bains, France). A titer of  $\geq 1/64$  was considered positive. Sera were screened, using standard microscopic agglutination tests (Levett, 2001), against a reference panel of 23 *Leptospira interrogans* serovars (pomona, hardjo, tarassovi, grippityphosa, celledoni, copenhageni, australis, zannoni, robinsoni, canicola, kremastov, szwajizak, medanensis, bulgarica, cynopteri, ballum, bataviae, djasiman, javanica, panama, shermani, topaz, and balcanica). Sera were tested for antigens of *Cryptococcus neoformans* and *Cryptococcus*

TABLE 1. Hematologic reference ranges for clinically healthy wild and captive western ringtail possums (*Pseudocheirus occidentalis*) >600 g. Gaussian tolerance intervals<sup>a</sup> and means±2 SD are provided for wild *P. occidentalis*; central 95% intervals are provided for parameters that did not conform to parametric distributions before or after transformation and for comparison with other studies. The minimum and maximum back-transformed values for each parameter are in bold. Means and ranges are provided for captive *P. occidentalis*. Parameters for which sex and lactation effects were found are indicated in footnotes, as are parameters that differed between wild and captive animals. (—) dashes indicate parameters that did not conform to parametric distributions before or after transformation.

Parameter <sup>b</sup> and unit	Data distribution or transformation	Wild <i>P. occidentalis</i>				Captive <i>P. occidentalis</i>			
		n	Mean <sup>c</sup>	Gaussian tolerance interval <sup>c</sup>	Mean±2 SD <sup>e</sup>	Central 95% interval <sup>c</sup>	n	Mean <sup>c</sup>	Range <sup>e</sup>
WBC (×10 <sup>9</sup> /L)	square root	115	5.31	2.10–9.99 <sup>d</sup>	2.28–9.61 <sup>d</sup>	2.07–8.72	23	5.12	3.20–7.40
RBC (×10 <sup>12</sup> /L) <sup>e</sup>	normal	115	5.30	4.06–6.55	4.15–6.46	3.98–6.42	23	5.35	4.01–6.47
Hemoglobin (g/L)	square root	115	139	110–171	112–169	112–169	23	135	109–167
PCV (l/L)	normal	111	0.40	0.33–0.48	0.33–0.48	0.32–0.47	23	0.40	0.31–0.50
Hematocrit (l/L)	normal	115	0.40	0.32–0.48	0.32–0.48	0.31–0.48	23	0.40	0.31–0.54
MCV (fl)	—	113	—	—	—	70–89	23	75	66–85
MCH (pg)	—	113	—	—	—	24.1–29.2	23	25.4	23.2–28.8
MCHC (g/L)	—	113	—	—	—	314–371	23	340	302–371
Platelets (×10 <sup>9</sup> /L)	square root	115	454	256–708 <sup>d</sup>	268–688	268–659	23	472	268–665
MPV (fl)	log	107	11.2	7.6–16.5 <sup>d</sup>	7.8–16.1 <sup>d</sup>	7.4–15.2	23	9.8	8.0–15.3
Neutrophils (×10 <sup>9</sup> /L) <sup>f</sup>	log	115	1.68	0.59–4.83	0.63–4.49	0.63–4.26	23	1.14	0.40–2.47
Lymphocytes (×10 <sup>9</sup> /L)	square root	115	3.05	0.82–6.68	0.93–6.38	1.00–6.41	23	3.69	1.58–6.22
Monocytes (×10 <sup>9</sup> /L)	log	115	0.08	0.01–0.43	0.02–0.28	0.00–0.33	23	0.07	0.00–0.26
Eosinophils (×10 <sup>9</sup> /L)	log	115	0.16	0.03–0.92 <sup>d</sup>	0.04–0.70	0.00–0.77	23	0.13	0.00–0.53
Basophils (×10 <sup>9</sup> /L)	log	115	0.06	0.01–0.26 <sup>d</sup>	0.03–0.11	0.00–0.16	23	0.01	0.00–0.14
Disintegrated (×10 <sup>9</sup> /L)	log	114	0.12	0.03–0.52	0.03–0.47	0.00–0.55	23	0.08	0.00–0.64
TSP (g/L) <sup>f</sup>	log	111	64	54–76	55–75	54–76	23	58	46–70
Fibrinogen (g/L) <sup>f</sup>	square root	111	2.7	1.2–5.0	1.3–4.8	1.0–5.2	23	2.3	1.0–4.0

<sup>a</sup> Gaussian tolerance intervals are more meaningful than means±2 SD because they carry an associated probability (0.90) of containing 95% of the sampled population.

<sup>b</sup> WBC = white blood cell count; RBC = red blood cell count; PCV = packed cell volume; MCV = mean cell volume; MCH = mean cell hemoglobin; MCHC = mean cell hemoglobin concentration; MPV = mean platelet volume; TSP = total solids protein.

<sup>c</sup> Back-transformed values.

<sup>d</sup> Values outside the sample range.

<sup>e</sup> Wald tests indicated sex and lactation effects for wild animals ( $P < 0.05$ ).

<sup>f</sup> Wald tests indicated differences between wild and captive animals ( $P < 0.05$ ).

TABLE 2. Serum biochemical reference ranges for clinically healthy wild and captive western ringtail possums (*Pseudocheirus occidentalis*) >600 g. Gaussian tolerance intervals<sup>a</sup> and means±2 SD are provided for wild *P. occidentalis*; central 95% intervals are provided for parameters that did not conform to parametric distributions before or after transformation and for comparison with other studies. The minimum and maximum back-transformed values for each parameter are in bold. Means and ranges are provided for captive *P. occidentalis*. Parameters for which sex and lactation effects were found are indicated in footnotes, as are parameters that differed between wild and captive animals.

Parameter <sup>b</sup> and unit	Data distribution or transformation	Wild <i>P. occidentalis</i>				Captive <i>P. occidentalis</i>			
		n	Mean <sup>c</sup>	Gaussian tolerance interval <sup>c</sup>	Mean±2SD <sup>c</sup>	Central 95% interval <sup>c</sup>	n	Mean <sup>c</sup>	Range <sup>c</sup>
ALP (IU/L) <sup>d,e</sup>	log	122	74	<b>19-285</b>	21-261	24-239	23	176	40-394
ALT (IU/L) <sup>e</sup>	log	122	63	<b>31-127</b>	33-121	<b>34-130</b>	23	47	33-88
AST (IU/L) <sup>e,f</sup>	log	122	31	<b>11-85</b>	12-79	<b>14-91</b>	23	19	10-31
CK (IU/L) <sup>e,g</sup>	log	121	6,114	<b>1,414-26,438<sup>h</sup></b>	1,559-23,977 <sup>h</sup>	<b>1,393-20,000</b>	23	3139	1,686-10,554
Albumin (g/L) <sup>g</sup>	normal	121	43	<b>35-50</b>	36-49	36-49	23	42	34-49
Bilirubin (µmol/L) <sup>d,e</sup>	log	119	13.7	<b>5.9-31.6</b>	6.3-29.8	6.5-30.4	19	11.1	5.0-17.3
Calcium (mmol/L)	log	122	2.74	<b>2.41-3.12</b>	2.43-3.09	2.45-3.03	23	2.76	2.55-3.13
Cholesterol (mmol/L) <sup>e</sup>	normal	120	2.5	<b>1.4-3.6</b>	1.5-3.5	1.5-3.6	23	2.1	0.9-3.5
Creatinine (µmol/L)	log	121	60	<b>41-90</b>	42-88	41-84	23	58	43-80
Glucose (mmol/L) <sup>e</sup>	log	122	4.9	<b>2.6-9.3</b>	2.7-8.9	<b>2.4-8.5</b>	23	7.5	4.3-13.2
Protein (g/L) <sup>e</sup>	log	121	60	<b>49-73</b>	50-72	49-72	23	52	40-59
Phosphorus (mmol/L) <sup>d</sup>	log	122	1.8	<b>0.9-3.6</b>	0.9-3.4	1.0- <b>3.7</b>	23	1.8	0.8-3.0
Urea (mmol/L) <sup>e,g</sup>	normal	122	7.0	<b>2.2-11.7</b>	2.6-11.3	<b>2.8-12.0</b>	23	3.4	0.7-6.9
Globulin (g/L) <sup>e</sup>	square root	121	17.0	<b>8.3-28.8</b>	8.8-27.8	<b>9.0-29.9</b>	23	10.2	3.6-18.2

<sup>a</sup> Gaussian tolerance intervals are more meaningful than means±2 SD because they carry an associated probability (0.90) of containing 95% of the sampled population.

<sup>b</sup> ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; CK = creatinine kinase.

<sup>c</sup> Back-transformed values.

<sup>d</sup> Wald tests indicated age effects ( $P<0.05$ ).

<sup>e</sup> Wald tests indicated differences between wild and captive animals ( $P<0.05$ ).

<sup>f</sup> Wald tests indicated sex effects ( $P<0.05$ ).

<sup>g</sup> Wald tests indicated sex and lactation effects ( $P<0.05$ ).

<sup>h</sup> Values outside the sample range.

*gattii* using slide latex cryptococcal antigen tests (Crypto-LA Test, Wampole Laboratories, Cranbury, New Jersey, USA). We detected *Chlamydothrix* spp. in mucosal swabs using PCR (Markey et al., 2007).

### Statistical analyses

We used only samples from clinically healthy possums >600 g to calculate reference ranges. Wild-caught and captive *P. occidentalis* were considered separately and juveniles (<600g) were not included. Outlying data points were identified if either  $(x_{(n)} - x_{(n-1)}) / (x_{(n)} - x_{(1)})$  or  $(x_{(2)} - x_{(1)}) / (x_{(n)} - x_{(1)})$  was >1/3 (Lumsden and Mullen, 1978) and then removed. Box plots, normal probability plots, and Shapiro-Wilk normality test statistics were assessed to check for parametric distribution of continuous variables. Nonnormal variables were logarithm or square-root transformed. For wild *P. occidentalis* means, SD, and Gaussian tolerance intervals (Lumsden and Mullen, 1978) were calculated for variables conforming to parametric distributions before or after transformation. Medians and central 95% intervals were calculated for all variables. Sample sizes of at least 120 are recommended for reliable inference from empirical quantiles (Reed et al., 1971); sample sizes in this study ranged from 107–122. Only means, medians, and ranges were calculated for variables from captive *P. occidentalis* because samples sizes were small (19–23).

We assessed effects of age (subadult [developing pouch or testicles] vs. adult), sex, lactation, and location on hematologic and serum biochemical parameters from wild *P. occidentalis* using multilevel mixed effects linear regression with restricted maximum likelihood model fitting (Bolker et al., 2009); animal ID was modeled as the random effect. Wald test statistics of fixed effects (age, sex, lactation, and location) were examined ( $\alpha=0.05$ ) to determine groupings for comparing variables. Effects of wild vs. captive status on hematologic and serum biochemical parameters were similarly assessed.

## RESULTS

### Hematology and serum biochemistry

We analyzed 138 blood and 145 serum samples from clinically healthy *P. occidentalis* for hematology and biochemistry, respectively. Three outlying data points were removed (a low creatinine value, a low albumin value, and a high number of

disintegrated cells in the differential white blood cell count). Hematologic and serum biochemical reference ranges from wild-caught and captive *P. occidentalis* are provided in Tables 1 and 2. Values for neutrophil count, total solids protein (TSP), fibrinogen, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine kinase (CK), bilirubin, cholesterol, protein, urea, and globulin for captive *P. occidentalis* were all lower than for wild *P. occidentalis* while alkaline phosphatase (ALP) and glucose values were higher (Wald tests,  $P<0.05$ ).

We found effects of sex, age, and lactation status for some parameters (Table 3). Red blood cell (RBC) counts were lower for nonlactating females than for lactating females (Wald statistic=7.25,  $P=0.007$ ,  $n=69$ ) or males (Wald statistic=9.21,  $P=0.002$ ,  $n=71$ ). Albumin levels were higher in lactating females than in males (Wald statistic=13.48,  $P<0.001$ ,  $n=93$ ) or nonlactating females (Wald statistic=19.54,  $P<0.001$ ,  $n=75$ ). Creatinine kinase levels were higher in nonlactating females than in males (Wald statistic=7.76,  $P=0.005$ ,  $n=75$ ) or lactating females (Wald statistic=10.88,  $P=0.001$ ,  $n=74$ ). Urea levels differed between lactating and nonlactating females (Wald statistic=12.41,  $P<0.001$ ,  $n=75$ ). Levels of ALP and phosphorus were higher in subadults than in adults (Wald statistic=20.23,  $P<0.001$ ,  $n=122$ ; Wald statistic=21.53,  $P<0.001$ ,  $n=122$ , respectively), while bilirubin levels were higher in adults than in younger animals (Wald statistic=7.45,  $P=0.006$ ,  $n=119$ ). Levels of AST were higher in males than in females (Wald statistic=7.61,  $P=0.006$ ,  $n=122$ ).

We identified site differences for RBC count (Wald statistic=20.85,  $P<0.001$ ,  $n=115$ ), hemoglobin (Wald statistic=14.30,  $P<0.001$ ,  $n=115$ ), albumin (Wald statistic=9.75,  $P=0.002$ ,  $n=121$ ), urea (Wald statistic=6.61,  $P=0.01$ ,  $n=122$ ), and globulin (Wald statistic=6.62,  $P=0.01$ ,  $n=121$ ; Table 4). Red



TABLE 3. Summary statistics for hematologic and biochemical parameters of wild western ringtail possums (*Pseudocheirus occidentalis*) for which Wald tests showed sex and lactation differences, age differences, and sex differences. For each parameter, uppercase letters A and B indicate subgroups that differed statistically from one another (see text).

Parameter <sup>a</sup>	Grouping	n	Mean	Median	Min–Max
Sex and lactation differences					
RBC ( $\times 10^{12}/L$ )	Male B	46	5.44	5.54	3.99–6.59
	Lactating female A	44	5.35	5.37	4.14–6.45
	Nonlactating female A,B	25	4.96	5.03	3.61–5.66
CK (IU/L)	Male B	47	6,513	5080	1,363–17,269
	Lactating female A	46	7,113	5255	424–>20,000 <sup>b</sup>
	Nonlactating female A,B	28	10,233	8739	2,267–>20,000 <sup>b</sup>
Albumin (g/L)	Male A	46	42	42	34–49
	Lactating female A,B	47	44	45	37–49
	Nonlactating female A	28	41	42	36–48
Urea (mmol/L)	Male	47	7.0	6.7	2.8–13.6
	Lactating female A	47	6.3	6.4	2.6–11.3
	Nonlactating female A	28	8.0	7.6	5.4–11.8
Age differences					
ALP (IU/L)	Subadult A	15	169	152	51–367
	Adult A	107	78	68	18–194
Bilirubin ( $\mu\text{mol}/L$ )	Subadult A	15	11.3	10.8	3.9–18.3
	Adult A	104	15.2	14.4	3.0–33.4
Phosphorus (mmol/L)	Subadult A	15	2.6	2.4	1.2–4.7
	Adult A	107	1.7	1.8	0.6–3.1
Sex differences					
AST (IU/L)	Male A	47	41	37	12–92
	Female A	75	31	25	14–107

<sup>a</sup> RBC = red blood cell count; CK = creatinine kinase; ALP = alkaline phosphatase; AST = aspartate aminotransferase.

<sup>b</sup> 20,000 was the upper limit that the analyzer would read, so this value was used in analyses.

blood cell counts and levels of hemoglobin and albumin followed similar patterns among the four sites with values lowest at TFNP and highest at Busselton development sites. Urea and globulin levels were lower at Busselton than at TFNP (Table 4).

#### Infectious diseases

We isolated bacteria from 79 of 99 swabs from *P. occidentalis* and from 96 of 97 cloacal swabs from *T. v. hypoleucus*. Bacterial growth was heavier for *T. v. hypoleucus* than for *P. occidentalis* and mixed bacterial growths were common. Coliform bacteria were most frequently isolated from *T. v. hypoleucus* samples while *Streptococcus* spp. and Coryneform bacilli were more common in *P. occidentalis*. No *Salmonella* spp. were cultured from either possum species.

We tested 99 and 95 serum samples from *P. occidentalis* and *T. v. hypoleucus*, respectively, for antibodies to *T. gondii*. Twenty-one individual *P. occidentalis* and 17 *T. v. hypoleucus* were tested more than once, at least 3 mo apart. All *T. v. hypoleucus* samples were negative on DAT and MAT tests. All *P. occidentalis* samples were negative on MAT; however, three samples (from two different *P. occidentalis*) gave mild positive reactions to DAT (titers of 1:64 and 1:256) without evidence of seroconversion. Neither of the two animals became ill and the MAT status of one retested after 3 mo remained negative.

We tested 89 serum samples from *P. occidentalis* and 89 from *T. v. hypoleucus* for antibodies to 23 serovars of *Leptospira interrogans*. No possums of either species were positive. Eighty-four and 92 serum

TABLE 4. Summary statistics for hematologic and biochemical parameters of wild western ringtail possums (*Pseudocheirus occidentalis*) for which field site differences were identified. Field sites are sorted by mean value within each parameter. For each parameter, uppercase letters A, B, C indicate sites that differed statistically from one another. Wald statistics and *P*-values are provided for these.

Parameter	Field site	<i>n</i>	Mean ( $\pm$ SE)	Wald statistic	<i>P</i>	Median	Range
RBC <sup>a</sup> ( $\times 10^{12}$ /L)	TFNP <sup>b</sup> A	32	4.97 (0.39)	17.19 A	<0.001	5.10	3.61–6.45
	Translocation sites B	21	5.22 (0.50)	8.47 B	0.004	5.25	4.22–6.17
	Gelorup	27	5.39 (0.45)			5.41	4.55–6.42
	Busselton A,B	35	5.59 (0.40)			5.64	4.69–6.59
Hemoglobin (g/L)	TFNP A	32	135 (14.33)	8.89 A	0.003	135	98–168
	Translocation sites B	21	136 (17.92)	7.87 B	0.005	139	112–159
	Gelorup	27	140 (16.06)			139	115–168
	Busselton A,B	35	145 (14.46)			144	117–171
Albumin (g/L)	TFNP A,B	32	41 (1.13)	11.54 A	0.001	41	34–49
	Translocation sites	22	42 (1.38)	12.19 B	0.001	42	37–48
	Gelorup B	31	44 (1.19)			44	37–49
	Busselton A	36	44 (1.10)			44	37–49
Urea (mmol/L)	Busselton A,C	36	5.4 (0.39)	47.21 A	<0.001	5.7	2.6–8.4
	Translocation sites B	23	6.2 (0.52)	16.44 B	<0.001	5.5	3.2–11.2
	Gelorup C	31	7.9 (0.50)	33.22 C	<0.001	7.7	4.7–11.8
	TFNP A,B	32	8.4 (0.51)			7.8	5.0–13.6
Globulin (g/L)	Busselton A,C	36	14.3 (2.55)	22.64 A	<0.001	14.5	8.5–19.1
	Translocation sites B	23	16.2 (3.55)	4.53 B	0.03	16.2	7.6–37.2
	Gelorup C	31	18.6 (3.36)	23.70 C	<0.001	18.9	9.9–25.9
	TFNP A,B	31	19.4 (3.47)			19.6	11.3–30.5

<sup>a</sup> RBC = red blood cell count.

<sup>b</sup> TFNP = Tuart Forest National Park.

samples from *P. occidentalis* and *T. v. hypoleucus*, respectively, were tested for antigen of serovars of *C. neoformans* and *C. gattii*. All, except one *T. v. hypoleucus*, were negative. The positive *T. v. hypoleucus* that had a low titer of 1:8 was negative when resampled after 10 and 14 mo. No chlamydial DNA was amplified by PCR from swabs from 95 *P. occidentalis* and 97 *T. v. hypoleucus*.

## DISCUSSION

### Hematology and serum biochemistry

We found age, sex, and lactation effects in several parameters. Age differences in ALP and phosphorus occur as a function of increased levels of the bone isoenzyme of ALP in growing animals (Willard and Tvedten, 2004). Age differences in bilirubin levels could be due to different degrees of fasting or differing levels of endoparasite-induced hemolysis, although evidence for either is lacking. Sex differences in red

cell counts are common in animals, with males usually having higher values (Clark, 2004). Nonlactating female *P. occidentalis* had lower red cell counts and albumin than did males and lactating females; this may have been partly an age effect, although we found no age differences in males. Lactating females had the highest albumin levels, which may have been partly a function of hydration status. Levels of urea in nonlactating females were higher than in males or lactating females. Urea values generally reflect protein intake for healthy animals in neutral or positive energy balance (Stirrat, 2003). Those in negative energy balance may catabolize their muscle protein, leading to high blood urea levels even with low protein intake (Reiss et al., 2008). Given the lack of sex and lactation differences in glucose levels, it is likely that the difference in urea levels between the three groups resulted from differing protein intake and nitrogen balance.

We found statistical differences between wild and captive populations and

variation between sites for wild *P. occidentalis*. Nutritional factors may explain most of the former. Captive animals cannot select foliage quality and are likely to have diets lower in protein and available nitrogen, resulting in lower blood values for protein- and nitrogen-breakdown products. Their diets are often supplemented with fruit and flowers which increase their blood glucose. Enzymes elevated during stress include AST and CK (Jain, 1993); wild *P. occidentalis* are likely to produce higher levels of these enzymes following capture than do captive individuals that are used to people. Similar differences in AST, CK, bilirubin, urea, and globulin were found between captive and wild populations of Gilbert's potoroos (*Potorous gilbertii*) (Vaughan et al., 2007).

Differences in hematologic and serum biochemical parameters may reflect variations in habitat quality and foliage nutritional status, as observed for other marsupials (Stirrat, 2003; Clark and Spencer, 2006). The Busselton development sites are in prime habitat that supports high population densities of *P. occidentalis* (Jones et al., 1994a, b) while densities at Gelorup, TFNP, and the translocation sites are lower than elsewhere (de Tores and Elscot, 2010; Clarke, 2011). Values for RBC, hemoglobin, and albumin were higher at the Busselton development sites than at TFNP and the translocation sites, and levels of urea and globulin were lower at Busselton than at TFNP and Gelorup. The variation in urea levels could represent lower protein intake at Busselton. Alternatively, *P. occidentalis* at TFNP and Gelorup could be in negative energy balance, having higher urea values due to muscle protein catabolism. Glucose levels at the four sites, although not statistically different, followed the reverse pattern to urea, supporting the latter explanation.

#### Infectious diseases

The variety of gastroenteric bacteria cultured from cloacal swabs was normal for small marsupials (Heard et al., 1997)

and none appeared associated with disease. Although several serovars of *Salmonella enterica*, including Typhimurium have been isolated from free-ranging possums elsewhere (Presidente, 1984), *Salmonella* spp. were absent in cultures from cloacal swabs of our animals. An asymptomatic carrier status for *Salmonella* spp. can exist in free-ranging possums (Johnson and Hemsley, 2008); this could develop into clinical disease under stress. It is therefore encouraging that we found no fecal evidence of such carrier status. These findings also suggest a low zoonotic risk of *Salmonella* transmission.

We found no evidence in either possum species of infection with *T. gondii*, *Leptospira* spp., or *Chlamydophila* spp., indicating that the health and survival of possums in this region are unaffected by these pathogens and that transmission between translocated and resident individuals is not a risk for either species, despite the prevalence of toxoplasmosis in cats in southern Western Australia (Jakob-Hoff and Dunsmore, 1983). Reaction to nonspecific immunoglobulin (Desmots and Remington, 1980) is the most likely reason for the low positive DAT titers observed in two *P. occidentalis*, as MAT titers were negative. *Leptospira* serovars occur in domestic stock in Western Australia but few wildlife surveys have occurred prior to this study. Recent surveys have increased the known wildlife host range for *Chlamydophila* spp.; however, in possums chlamydial DNA has only been detected in the mountain brushtail possum (*Trichosurus caninus*; Bodetti et al., 2003). Clinical disease has been recorded only in the koala (*Phascolarctos cinereus*), greater glider (*Petauroides volans*), and western barred bandicoot (*Perameles bougainville*; Warren et al., 2005).

Although one *T. v. hypoleucus* was positive for *Cryptococcus* antigen on the first of three sampling occasions, the titer was low and subsequent samples were negative, consistent with subclinical infection (Malik et al., 1999) or a false-positive



result. If present, the causal organism was most likely *C. gattii*, which has been found in association with tuart trees (Ellis and Pfeiffer, 1996), common at the field sites. Subclinical infection with *C. gattii* may reduce reproductive output in *T. v. hypoleucus*; the animal with the low titer in this study was in poor condition and nonbreeding. Alternatively, mild cryptococcal infection could follow immunosuppression due to parasitism and poor body condition. Our generally negative findings are encouraging for wildlife conservation and translocation outcomes, as there appears little likelihood of activation of latent disease by the stress associated with relocation of possums. The low translocation success of *P. occidentalis* in this region is related to inadequate control of introduced predators, high numbers of *T. v. hypoleucus*, and limited high-quality habitat (Clarke, 2011) rather than to infectious disease. However, the lack of evidence of exposure to *T. gondii*, *Leptospira*, *Cryptococcus*, and *Chlamydia* indicates that possums in this area may be particularly susceptible to introductions of these diseases. This emphasizes the need for vigilance and continued surveillance of wildlife health when managing threatened species.

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