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Ectoparasite infestation patterns, haematology and serum biochemistry of urban-dwelling common brushtail possums

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Urban environments support high concentrations of humans, domestic pets and introduced animals, creating conditions conducive to the transmission of parasites. This study compared patterns of ectoparasite infestation of the common brushtail possum *Trichosurus vulpecula* in urbanised Sydney (n = 161) to those from a remote woodland site (n = 18) from February 2005 – November 2006. We found differences in ectoparasite species prevalence between the two groups: the flea *Echidnophaga myrmecobii* was only found on urban possums and the tick *Ixodes trichosuri* was much more prevalent in the urban habitat, while the mite *Atellana papilio* was more prevalent on woodland possums. *E. myrmecobii* and *I. trichosuri* differed from other ectoparasites by showing an association with host sex and host age. Potential physiological costs of ectoparasitism to urban-dwelling possums were determined using multivariate analysis of haematology, serum biochemistry and body condition. Changes in serum iron levels were seen in the presence of both the tick *Ixodes trichosuri* and the flea *E. myrmecobii*, and *E. myrmecobii* was associated with elevated serum levels of the liver enzyme ALT. However, ectoparasite-related changes in haematology and serum biochemistry were not indicative of long-term pathology. In this urban possum population, the costs of ectoparasitism appear to be limited and unlikely to pose a major threat to the health of the population.

Urbanisation, the growth of cities to accommodate an increasing human population, is regarded as the most destructive anthropocentric pressure on wildlife, as it results in fragmentation or complete destruction of habitat (McKinney 2002, Garden et al. 2006). Less overt consequences of urbanisation such as changes to food webs, competition for resources and alterations in host–parasite relationships may also threaten the viability of wildlife populations (Patz et al. 2004, Spratt 2005, Bradley and Altizer 2007). In particular, urbanisation leads to a restructuring of species composition that may destabilise wildlife–pathogen relationships. Evidence of this exists in the growing number of studies in which urban areas act as a focal point for the emergence of zoonotic disease, including West Nile virus, Dengue fever (Dauphin et al. 2004, Weaver 2005), toxoplasmosis (Smith and Frenkel 1995, Meireles et al. 2004, Sukthana 2006), Lyme disease (Stanczak et al. 2004, Jobe et al. 2007) and leptospirosis (Ko et al. 1999). Despite the risks to the health of wildlife and humans, few studies have systematically investigated whether wildlife species experience a higher parasite burden as a result of living in an urban environment.

Ectoparasites are arthropods that complete at least one part of their life cycle on the surface of their host and include

the ticks, lice, fleas and mites. The survival and spread of ectoparasites may be favoured as a consequence of urbanisation (Spratt 2005, Bradley and Altizer 2007). Increasing population densities of wildlife that exploit anthropogenic sources of food and shelter may facilitate ectoparasite transmission, whilst the co-existence of humans, domestic pets and wildlife provides ectoparasites with a range of novel hosts (Dautel et al. 1991, Comer et al. 2001, Maetzel et al. 2005, Pearce and O’Shea 2007). Urban areas are also associated with warm microclimates due to lack of shade from vegetation, smog and effluent from industry, retention of heat by impervious surfaces (Saaroni et al. 2000, Baker 2002) and the high number of human occupants (Torok et al. 2001). The warmer temperatures and reduced seasonality caused by this ‘urban heat island effect’ may increase the survival, breeding success and activity of arthropod ectoparasites (Bradley and Altizer 2007).

As a consequence of inhabiting a disturbed environment with potentially higher rates of ectoparasite infection, wildlife inhabiting human settlements may be adversely impacted. By acquiring nutrients at the expense of the host, feeding on blood or skin tissue, ectoparasites impose a cost on the host (Price 1977). Ectoparasites may also inflict physical damage, such as creating skin lesions, injecting salivary toxins

into the wound and causing blood loss (Aeschlimann 1991); such physical damage may be costly to repair. Behavioural changes that deter ectoparasites, such as grooming or relocation of nest sites, can diminish reproductive success (Loehle 1995, Moore and Wilson 2002) and immune responses to infestation are energetically demanding (Brossard et al. 1991). Ectoparasites are also vectors and intermediate hosts for a range of highly-pathogenic viral, protozoan, bacterial and rickettsial diseases (Walker et al. 1996). Owing to these detrimental impacts, ectoparasites have the potential to influence the growth, survival and reproduction of their host and consequently act as an important selective agent (Renaud et al. 1996).

The common brushtail possum *Trichosurus vulpecula* is one of the most abundant native mammals in urban Australia, having adapted to utilise anthropogenic resources (Hill et al. 2007). Despite success in colonising urban habitats, there are limits to the adaptability of this species. Tree clearing and predation by the European red fox *Vulpes vulpes* has been responsible for the decline of possums from more than 50% of their former range (Kerle 1992, 2004, Statham and Statham 1997). This study sought to determine whether adaptation to the urban environment enhanced ectoparasite infestation and whether such infestations were associated with changes in host physiology including body condition, haematological and serum biochemical parameters. To achieve this, we compared two populations of possums, one from metropolitan Sydney and the other inhabiting woodland remote from urban areas. This is the first detailed investigation into multiple host–ectoparasite relationships of common brushtail possums, providing baseline data against which the health of the species may be compared as humans increasingly modify their original habitat.

Material and methods

Study sites

The principal urban study area was Taronga Zoological Park (33°50'S, 151°14'E) in the northern Sydney suburb of Mosman, Australia. The zoo is inhabited by an abundant free-ranging possum population that forage nightly on the leftover foodstuffs in the exhibits and on anthropogenic food from garbage bins. Also included in the 'urban' field site were the properties of Mosman residents who lived in proximity to the zoo (< 1 km) and had experienced possum activity. Possums inhabiting this urban area achieved a population density of approximately 5.03 ha⁻¹, one of the highest recorded in Australia (Hill and Deane unpubl.). For comparison, trapping took place at a non-urban site located within Jenolan Caves Reserve Trust land, Blue Mountains, Australia (33°49'S, 150°01'E). The reserve was characterised by open eucalypt woodland and was inaccessible to the public. A wire fence prevented invasion by exotic species and control of feral animals was routinely performed throughout the enclosure.

Climate information was obtained from the closest Australian Bureau of Meteorology weather station to the urban study site (Observatory Hill, Sydney; station number 066062) and the woodland site (Oberon, Springbank; station number 063063). Records included monthly averages

of rainfall, number of rainy days, maximum temperature and minimum temperature for each month of trapping (<www.bom.gov.au/climate/averages>).

Trapping

At the urban site, trapping was performed on a monthly basis between February 2005 and November 2006 (n = 22 trapping surveys). At the woodland site, the remote location limited trapping to November 2005, May 2006 and October 2006 (n = 3 trapping surveys). Treadle-operated cage traps (59 × 22 × 22 cm) were baited with apples and a rolled oats/peanut butter mixture and set at locations considered to be suitable possum 'runways' (ground routes used to travel between trees). The top of each trap was covered in durable plastic sheeting to provide protection from the elements. Captured animals were transported either to the Veterinary and Quarantine Centre, Taronga Zoo or sampled on site at Jenolan Caves. All possums were permanently implanted with a passive integrated transponder to enable identification of recaptured animals. Recaptured possums were included in the survey if the time since original capture exceeded three months (to avoid pseudo-replication). Ethics approval was obtained from the Macquarie University Animal Ethics Committee (no. 2004/16), the Zoological Parks Board of New South Wales Animal Care and Ethics Committee (no. 4c/08/04) and the NSW Dept of Environment and Conservation (no. S11107).

Animal handling

Possums were anaesthetised via a face-mask using 5% isoflurane in oxygen during sampling, eliminating the need for physical restraint and minimising stress for the animal. Age was estimated by observing the degree of molar tooth wear as per Winter (1980). Body measurements, weight and sex were recorded. Males were classified as 'juvenile' if their tooth wear class was 1 or 2 (corresponding to an age of < 2 years; Winter 1980) and their testis size was less than 15 mm long and less than 10 mm wide; 'adult' males had larger testes (i.e. > 15 mm long and > 10 mm wide) and a tooth wear class of 2 or more. (In this species males can reach sexual maturity as early as 14–15 months; Johnson et al. 2001). Females were classified as 'juvenile' if their pouch was undeveloped (indicating a lack of sexual maturity), which in all cases corresponded with a tooth wear class of 1 or 2. Females were considered 'adult' if they were lactating or had a fully developed (non-breeding) pouch; this typically corresponded with tooth wear classes of 3 or greater (i.e. an age of 2 + years), but a few individuals of tooth wear class 2 were found to be sexually mature. The age at which females reach sexual maturity varies between populations, but most females in most populations studied begin breeding sometime in their second year (How and Hilcox 2000). All females of breeding age were classified as either 'with young' if they carried offspring and/or were lactating, or 'without young' if they exhibited neither of these features.

Ectoparasite collection and identification

Ectoparasites were collected from six sites on the body: eyes, ears, nose, shoulders, rump and belly. To maximize

consistency in sampling effort, a single researcher (NJH) performed all ectoparasite collections. The number of ticks and fleas present at each site were counted and samples stored in sealed containers. Feeding mites were detected by parting the pelage on the rump in 5–6 locations and dousing with paraffin oil to allow collection with forceps. Fur-clasping mites were collected in hair samples removed with a fine-toothed comb. The small size of mites and the time-consuming methods necessary to locate them meant counts could not be performed. Ectoparasites were stored in 70% ethanol and later identified under a stereo microscope to species-level on the basis of shared morphology with published descriptions of ticks (Roberts 1970), fleas (Dunnet and Mardon 1974) and mites (Domrow 1987, 1992).

Estimation of body condition

Body mass (M_b) was measured on electronic scales to the nearest single gram. Skeletal body length (L) was measured from the tip of the nose to the cloaca. To ensure consistency, only a single researcher (NJH) performed measurements of possum skeletal structures. A typical body condition index (BCI) estimate, such as that used for the closely related mountain brushtail possum, *Trichosurus caninus* (Viggers et al. 1998), is to divide body mass by length: $BCI = M_b / L$. Possums are sexually dimorphic, with males significantly heavier than females (but not significantly longer), so the typical BCI estimation could lead to over-estimating the body condition of males. Therefore, we instead calculated a “scaled mass index”, SMI (Peig and Green 2009, 2010), for each population sub-group (adult males, adult females, juvenile males and juvenile females), where the body mass was scaled to the mean body length for each sub-group.

Blood collection and analyses

Approximately 5 ml of blood was drawn from the lateral tail vein and distributed between two vacuette tubes; one lined with ‘EDTA’ for haematological analysis and a serum clot activator tube for biochemical analysis. After clotting, the serum clot activator tube was centrifuged at $200 \times g$ for 10 min and serum collected in a microtube. All testing was conducted within 72 h of blood collection at PaLMS (Pacific Laboratory Medicine Services, NSW, Australia).

Haematological parameters were measured on a Beckman–Coulter counter. To limit erroneous measurements arising from the use of equipment designed for human blood cells (Whittington and Comer 1984), instrumentation was optimised for use with marsupial cells. Electronic white cell counts were verified against differential counts from blood films to confirm accuracy of the equipment. Red cell parameters measured included haemoglobin concentration, erythrocyte count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). White cell parameters measured were lymphocytes, neutrophils and eosinophils. Also measured were mean platelet volume (MPV) and iron parameters including iron and iron saturation.

Serum biochemical parameters were measured on a modular analyzer, optimised for use with marsupial serum. General biochemistry measurements included sodium, potassium,

creatinine, urate, total protein, albumin, total bilirubin, calcium, phosphate, magnesium, lipase and hormones, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine transaminase (ALT) and g-glutamyl transferase (GGT). Assays for bilirubin and GGT were colorimetric, whereas those for ALP, AST and ALT were UV based protocols. Also measured were lipid chemistry parameters including cholesterol and triglyceride.

Statistical analysis

Possum population demographics and climate data

Pearson’s χ^2 -tests were used to investigate whether the urban possum sub-groups (zoo and backyard) were similar with respect to age, gender and maternal status. The same test was used to assess similarity between the possum populations in urban and woodland habitats. One-way ANOVA was used to assess whether body condition (as estimated by SMI) varied between urban and woodland populations.

The average rainfall, number of rainy days, maximum temperature and minimum temperature at each habitat was determined for the period of trapping and compared with a one-way ANOVA.

Ectoparasite data

Prevalence, defined as the number of infected individuals expressed as a percentage of the study population (Bush et al. 1997), was determined for each ectoparasite species in each trapping area. Abundance, defined as the number of individual parasite specimens occurring on the host (Bush et al. 1997), was determined for ticks and fleas, but not mites (since mites were scored as present/absent but not counted). The abundance of each ectoparasite species was not normally distributed (Shapiro–Wilk test of normality, $p < 0.05$) and when plotted revealed an aggregated distribution, whereby the majority of animals were not infected with an individual species of ectoparasite. It was therefore more informative to use either prevalence or intensity (the number of parasite specimens per ‘infected’ individual; always a number > 0) for comparative purposes (e.g. across trapping sites).

Pearson’s χ^2 -tests were used to identify differences in prevalence of ectoparasites between the trapping sites. For three ectoparasite species with prevalence greater than 25% (in any trapping site), variables influencing the prevalence of ectoparasite species were investigated using generalized linear mixed models (GLMMs). In this analysis the individual microchip ID of each possum was included as subject variable, to control for possible effects of re-trapped individuals on the analysis. Factors included in each model were trapping site (woodland, zoo or backyard), host demography (sex, age and maternal status of females), host body condition (scaled mass index, SMI), and season. Factors were centred around their respective mean values (‘grand mean centering’) unless there was more variation within animals than between animals, in which case factors were centred around individual animal means (‘group mean centering’).

Species richness by host individual was defined as the total number of parasite species occurring on the host individual (Magurran 1988). The species richness of

ectoparasites per host individual was compared across habitats using ANOVA. In addition, the variables influencing species richness per host individual were examined using a GLMM with the factors trapping site, host sex, host age, maternal status and SMI.

The large number of health variables measured (29 in total; 11 haematological measurements, 17 serum biochemical parameters and scaled mass index), were reduced to limit statistical analysis to biologically meaningful groups of variables. We hypothesised that ectoparasite infestation was most likely to affect red blood cell parameters, immune function, iron levels and body condition, and that severe infestation could potentially compromise host liver function. Selection of the specific variables for investigation was assisted by using a Pearson's correlation matrix to identify variables that were correlated, although in some cases groupings were selected for their biological importance, rather than solely on correlations data (e.g. variables within both liver function parameters and immune parameters were only weakly, though significantly, correlated). The following groupings or single variables were selected for analysis: red blood cell parameters (variables: haemoglobin, erythrocyte count), iron status (variables: iron levels, iron saturation), immune parameters (variables: lymphocytes, neutrophils and eosinophils), liver function (variables: ALT, AST, total bilirubin) and scaled mass index.

Multivariate ANOVA (MANOVA) was used to examine the effect of ectoparasite prevalence on red blood cell parameters, iron status, immune parameters and liver function of urban possums only (the small study population in the woodland habitat precluded use of such statistics). The independent variables in this analysis were presence/absence of each of six ectoparasite species (analysed simultaneously to account for potential interactions between ectoparasites), species richness (to allow for potential interactions between species prevalence and the number of species parasitising a host to be assessed), age, a combined sex/female lactation status variable, season and number of re-captures (these variables are known to affect haematology and blood biochemistry of possums; Barnett et al. 1979, Presidente and Correa 1981, O'Keefe and Wickstrom 1998). Two-way interactions between ectoparasites were only included in the model if the number of individuals hosting both parasites was greater than twelve. MANOVA was also used to examine the effect of the abundance of the most abundant ectoparasite (a flea) on red blood cell parameters and iron status.

Univariate ANOVA was used to examine the effect of ectoparasite prevalence on scaled mass index of urban possums. Again, the independent variables were presence/absence of each of six ectoparasite species, species richness, age, combined sex/lactation status, season and number of re-captures. A seventh ectoparasite species (the flea *Ctenocephalides felis*) was excluded from these analyses as it was only found on a single possum in the urban habitat.

Statistical analysis was performed using SPSS Statistics, ver. 20. For all analyses, an alpha value ≤ 0.05 indicated a significant difference. All data are reported as mean \pm SEM (standard error of the mean), unless otherwise noted.

Results

Possum population

In total, across all trapping areas, there were 278 capture events of 179 individual possums. The majority of these were at Taronga Zoo, where 140 individuals were trapped over 219 capture events. The backyard group consisted of 21 individual possums, captured on 22 occasions. There were 37 capture events at the woodland site, consisting of 18 individual possums. Because of the large number of recapture events, only first capture data was used for comparisons of the sex structure of the populations. However, because both the age of individual possums and maternal status of females changed over the time-course of the study, all data points were used in comparisons of age and maternal status across the different possum populations. A summary of the demographic data is shown in Table 1.

The urban possum sub-groups (zoo and backyard) displayed no significant differences in the demographic profiles of the trapped individuals with respect to sex ($\chi^2 = 0.054$, DF = 1, $n = 172$, $p = 0.82$), age ($\chi^2 = 0.464$, DF = 1, $n = 252$, $p = 0.50$) or maternal status (lactating vs non-lactating; $\chi^2 = 0.152$, DF = 1, $n = 123$, $p = 0.70$). Therefore, the data from the two sub-groups were combined into a single 'urban' group for comparison with the woodland population. There was no significant difference between possums trapped in urban and woodland habitats with respect to sex ($\chi^2 = 1.812$, DF = 1, $n = 190$, $p = 0.18$) or age ($\chi^2 = 0.144$, DF = 1, $n = 289$, $p = 0.71$). Nor was there any significant difference between possums with respect to maternal status ($\chi^2 = 3.430$, DF = 1, $n = 148$, $p = 0.06$), even though the woodland population contained fewer lactating females than was expected (48% of females lactating, compared to 67% of females in the urban habitat).

The mean (\pm SEM) scaled mass index (SMI) of zoo possums was 2297 ± 52 g ($n = 227$); possums trapped in backyards had a mean SMI of 2617 ± 155 g ($n = 22$); and woodland possums had mean SMI of 2320 ± 145 g ($n = 27$). This index of body condition was not significantly different across the three trapping sites (one-way ANOVA; $F_{2,273} = 1.711$, $p = 0.183$).

Table 1. Demographic data from possums trapped in urban (zoo or backyard) and woodland habitats. Sex structure data is from first captures only; all other data includes recaptures of the same individuals (because age and maternal status changed over the time-course of the study).

	Site			Total
	Zoo	Backyard	Woodland	
Sex				
no. females	70	10	12	92
no. males	70	11	6	87
total	140	21	18	179
Age				
no. juveniles	30	2	6	38
no. adults	189	20	31	240
total	219	22	37	278
Maternal status				
no. non-lactating	32	3	13	48
no. lactating	74	8	12	94
total	106	11	25	142

Table 2. Ectoparasite species richness per individual possum: (a) woodland versus urban animals, (b) females versus males, and (c) seasonal changes.

Species richness (per host possum)	(a) Habitat				(b) Sex				(c) Season							
	Woodland		Urban		Female		Male		Summer		Autumn		Winter		Spring	
	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%
0	11	29.7	64	26.6	50	35.2	25	18.4	14	25.5	20	23.3	16	24.6	25	34.7
1	19	51.4	111	46.1	61	43.0	69	50.7	28	50.9	48	55.8	29	44.6	25	34.7
2	7	18.9	49	20.3	24	16.9	32	23.5	8	14.5	15	17.4	17	26.2	16	22.2
3	0	0	15	6.2	6	4.2	9	6.6	4	7.3	3	3.5	3	4.6	5	6.9
4	0	0	1	0.4	0	0	1	0.7	1	1.8	0	0	0	0	0	0
5	0	0	1	0.4	1	0.7	0	0	0	0	0	0	0	0	1	1.4
Total	37	100	241	100	142	100	136	100	55	100	86	100	65	100	72	100

Ectoparasite fauna of possums

In total, seven species of ectoparasites were identified from 179 possums (161 from urban and 18 from woodland habitat). The ectoparasite fauna of possums included three species of tick, *Ixodes trichosuri*, *Ixodes tasmani* and *Ixodes holocyclus*; two species of mite, one astigmatid, *Atellana papilio* and one mesostigmatid mite, *Trichosurolaelaps crassipes*; and two species of flea, *Echidnophaga myrmecobii* and *Ctenocephalides felis*. The two flea species were unique to urban-dwelling possums, but all other ectoparasite species were found in both urban and woodland possum populations. All ticks, fleas and mites detected in this study had been previously identified from this species of possum.

Species richness, prevalence and mean intensity of ectoparasites

Mean species richness of ectoparasites per host individual was not significantly different between the two urban subgroups (zoo and backyard; one-way ANOVA $F_{1,239} = 0.246$, $p = 0.62$). Therefore further comparisons of ectoparasite species richness per host individual were made between the urban and woodland populations. Urban possums were more likely than woodland possums to host three or more ectoparasite species (Table 2a), but the mean ectoparasite species richness per host was not significantly different between possums inhabiting urban and woodland habitat ($F_{1,276} = 1.648$, $p = 0.200$). The GLMM analysis of factors affecting the species richness per host confirmed that there was no significant effect of habitat, as well as no effect attributable to host age or host scaled mass index (SMI). However, species richness per host individual was significantly affected

by host sex (GLMM $F_{1,145.5} = 4.315$, $p = 0.028$) and season (GLMM $F_{1,79.6} = 6.859$, $p = 0.011$). More females than males were ectoparasite-free (35%, compared with 18% of males); and one or more ectoparasite species were more common on males than females (Table 2b). In most seasons, possums were most likely to host a single ectoparasite species (45–55% of the population affected; Table 2c). However, in spring the proportion of the population that was ectoparasite-free was equal to that hosting a single species (35%). Winter appeared to be the peak season for trapping possums hosting two species of ectoparasite, but possums hosting three or more parasites were most commonly trapped in spring or summer (Table 2c).

A summary of the prevalence of ectoparasite species in each trapping area (i.e. zoo, backyard or woodland) is presented in Table 3. χ^2 -analysis revealed significant differences in prevalence between trapping areas for the flea *E. myrmecobii*, which was not present in woodland habitat ($\chi^2 = 26.104$, $DF = 2$, $n = 278$, $p < 0.001$); for the mite *A. papilio*, which was more prevalent in woodland habitat ($\chi^2 = 24.196$, $DF = 2$, $n = 278$, $p < 0.001$); and for the tick *I. trichosuri*, which was most prevalent on backyard possums ($\chi^2 = 7.939$, $DF = 2$, $n = 278$, $p = 0.019$).

The prevalence of most ectoparasite species in most areas was low (<20%; Table 3). Species with prevalence greater than 25% of the population were the tick *I. trichosuri* (41% prevalence in backyard possums), the flea *E. myrmecobii* in urban possums (27% prevalence in backyards and 43% at the zoo) and the mite *A. papilio* (43% prevalence in woodland possums).

The mean intensity (mean number of parasites per infested individual) of each of the tick and flea species is presented in Table 4 (frequency histograms of intensity are available in

Table 3. Prevalence of ectoparasite species in the different trapping areas. Prevalence is presented as the percentage of the population with each ectoparasite species present.

Ectoparasite species	Urban areas (n = 241)					
	Zoo (n = 219)		Backyard (n = 22)		Woodland (n = 37)	
	Count	Prevalence (%)	Count	Prevalence (%)	Count	Prevalence (%)
<i>Ixodes tasmani</i>	42	19.2	5	22.7	5	13.5
<i>Ixodes trichosuri</i>	36	16.4	9	40.9	8	21.6
<i>Ixodes holocyclus</i>	9	4.1	0	0.0	1	2.7
<i>Echidnophaga myrmecobii</i>	94	42.9	6	27.3	0	0.0
<i>Ctenocephalides felis</i>	1	0.5	0	0.0	0	0.0
<i>Atellana papilio</i>	26	11.9	2	9.1	16	43.2
<i>Trichosurolaelaps crassipes</i>	34	15.5	0	0.0	3	8.1

Table 4. Mean intensity of ectoparasite species (ticks and fleas) in the different trapping areas. Mean intensity is defined as the mean number of conspecific parasites living on an infected host.

Ectoparasite species	Urban areas (N = 241)											
	Zoo (N = 219)				Backyard (N = 22)				Woodland (N = 37)			
	n	Mean	CI	V:M ratio	n	Mean	CI	V:M ratio	n	Mean	CI	V:M ratio
<i>Ixodes tasmani</i>	42	1.79	[1.39, 2.18]	0.89	5	1.60	[0.49, 2.71]	0.50	5	1.80	[0.18, 3.42]	0.94
<i>Ixodes trichosuri</i>	36	1.56	[1.18, 1.93]	0.79	9	2.11	[0.60, 3.62]	1.83	7	1.00	–	0
<i>Ixodes holocyclus</i>	9	1.33	[0.79, 1.88]	0.38	0	–	–	–	1	1.00	–	–
<i>Echidnophaga myrmecobii</i>	87*	18.54	[13.3, 23.8]	33.14	6	35.67	[-11.6, 83.0]	56.93	–	–	–	–
<i>Ctenocephalides felis</i>	1	1.00	–	–	0	–	–	–	–	–	–	–

'N' indicates the total number of hosts trapped in each trapping area; 'n' indicates the number of hosts infected with each parasite species in each trapping area. 'CI' indicates the lower and upper limits of the 95% confidence interval and 'V:M ratio' is the variance to mean ratio.

*There were 94 hosts with the presence of *E. myrmecobii* recorded, but only 87 with intensity of the parasite recorded (i.e. seven cases were missing).

Supplementary material Appendix 1 Fig. A1–A4). The tick species all had mean intensities in the range of one to two ticks per possum host (Table 4), with one tick per host being the most frequent intensity for all tick species in all trapping areas. The flea *E. myrmecobii* had the highest mean intensities, of 18.54 fleas per possum in the zoo habitat and 35.67 fleas per possum in backyards. However, the very high mean intensity of *E. myrmecobii* on backyard possums must be treated with caution, as only six individuals were hosts of this ectoparasite (Table 4), and the high mean intensity was principally due to a single possum hosting 115 individual fleas (hence the very high variance: mean ratio for the intensity of this ectoparasite). For possums trapped at Taronga Zoo, *E. myrmecobii* intensity was skewed towards lower intensities, with 60 out of 84 individual hosts (71%) infested with fewer than 15 fleas per possum, and 47 individuals (56% of infested hosts) having fewer than 10 fleas.

Factors affecting infestation by the most prevalent ectoparasite species

The GLMM analysis of factors affecting the presence or absence of *I. trichosuri* indicated that the presence of this species was affected by host sex (GLMM $F_{1,116,11} = 4.389$, $p = 0.038$), with males nearly twice as likely to harbour *I. trichosuri* than females (Table 5a). The presence of the flea *E. myrmecobii* was significantly affected by trapping site (GLMM $F_{1,188,99} = 18.283$, $p < 0.001$) and an interaction

between host sex and host age (GLMM $F_{1,229,91} = 4.733$, $p = 0.031$). Approximately half of adult male possums hosted *E. myrmecobii*, compared to one fifth of juvenile males, and around one quarter of juvenile and adult females (Table 5b). *E. myrmecobii* was found only on urban possums, with a higher prevalence on possums trapped at Taronga Zoo than in suburban backyards (Table 3). A trapping site effect was also found for the mite *A. papilio* ($F_{1,179,33} = 5.477$, $p = 0.020$), with this parasite significantly more prevalent in woodland habitat (43%) than in either urban habitat (Table 3). Separate analyses of females only indicated no effect of maternal status on prevalence of any of these three parasites.

Effect of ectoparasite prevalence and abundance on the health of urban-dwelling possums

A summary of the haematological and serum biochemical parameters of possums according to habitat type (urban or woodland) is presented in the Supplementary material Appendix 2 Table A1. Because of the low sample numbers for woodland possums, only the health parameters of urban possums were investigated using MANOVA or ANOVA.

Host sex and lactation status interacted with host age to significantly affect the red blood cell parameters haemoglobin and erythrocyte count (analysed together using MANOVA; Wilks $\lambda = 0.945$, $F_{2,187} = 5.407$, $p = 0.005$, partial eta-squared = 0.055). Adult male possums had the

Table 5. Factors significantly affecting the prevalence of (a) *I. trichosuri* (host sex); and (b) *E. myrmecobii* (interaction between host sex and host age, in addition to trapping site effect shown in Table 3).

(a)	No. of possums			Prevalence
	Without <i>I. trichosuri</i>	With <i>I. trichosuri</i>	Total	
Sex				
female	122	20	142	14.1%
male	103	33	136	24.3%
(b)	No. of possums			Prevalence
	Without <i>E. myrmecobii</i>	With <i>E. myrmecobii</i>	Total	
Female				
juvenile	14	4	18	22.2%
adult	89	35	124	28.2%
Male				
juvenile	16	4	20	20.0%
adult	59	57	116	49.1%

Table 6. Factors affecting the health parameters of common brushtail possums. (a) Mean haemoglobin concentration and erythrocyte count of urban-dwelling possums by age, sex and lactation groups (a significant interaction effect). (b) Mean serum iron concentration and iron saturation of urban-dwelling possums in the presence and absence of the tick *I. trichosuri* and the flea *E. myrmecobii* (a significant interaction effect), and by sex and lactation group. (c) Mean liver function parameters (ALT, AST, total bilirubin) of urban possums in the presence and absence of five species of ectoparasites, in relation to ectoparasite species richness per possum, and across seasons.

		(a) Red cell measures					
		Haemoglobin concentration (g l ⁻¹)			Erythrocyte count (× 10 ¹² l ⁻¹)		
		Mean	SEM	Valid n	Mean	SEM	Valid n
Juvenile males		99.5	3.922	16	4.654	0.234	16
Juvenile (non-lactating) females		104.5	3.695	10	4.875	0.155	10
Adult males		117.552	1.538	87	5.542	0.08	87
Adult non-lactating females		103.053	2.653	19	4.698	0.136	19
Adult lactating females		105.5	1.319	80	4.756	0.064	80

		(b) Serum iron parameters					
		Iron concentration (µmol l ⁻¹)			Iron saturation (%)		
		Mean	SEM	Valid n	Mean	SEM	Valid n
<i>I. trichosuri</i> absent	<i>E. myrmecobii</i> absent	37.134	1.51	97	56.684	2.116	95
	<i>E. myrmecobii</i> present	34.475	1.83	80	50.468	2.225	79
<i>I. trichosuri</i> present	<i>E. myrmecobii</i> absent	29.607	1.976	28	45.778	2.905	27
	<i>E. myrmecobii</i> present	32.867	3.219	15	55.533	5.546	15
Males		33.124	1.113	113	53.054	3.835	112
Non-lactating females		32.719	2.64	32	49.156	1.789	32
Lactating females		38.56	2.113	75	54.528	2.469	72

		(c) Immune cell parameters								
		Lymphocytes (× 10 ⁹ l ⁻¹)			Neutrophils (× 10 ⁹ l ⁻¹)			Eosinophils (× 10 ⁹ l ⁻¹)		
		Mean	SEM	Valid n	Mean	SEM	Valid n	Mean	SEM	Valid n
Juveniles		5.023	0.521	26	2.281	0.261	26	0.045	0.024	22
Adults		3.672	0.155	187	3.43	0.136	187	0.095	0.011	172

		(d) Liver function parameters								
		ALT concentration (IU l ⁻¹)			AST concentration (IU l ⁻¹)			Total bilirubin (µmol l ⁻¹)		
		Mean	SEM	Valid n	Mean	SEM	Valid n	Mean	SEM	Valid n
No ectoparasites		37.776	2.021	58	60.839	3.576	56	4.228	0.258	57
One species		41.05	1.399	101	68.475	4.101	99	4.13	0.205	100
Two species		38.4	2.422	45	60.422	4.175	45	4.222	0.224	45
Three species		36.769	3.055	13	54.769	3.976	13	3.846	0.337	13
Four species		21	.	1	34	.	1	4	.	1
Five species		33	.	1	42	.	1	4	.	1
<i>E. myrmecobii</i> absent		38.424	1.24	125	61.713	3.115	122	4.146	0.172	123
<i>E. myrmecobii</i> present		40.362	1.63	94	66.28	3.431	93	4.17	0.186	94
<i>A. papilio</i> absent		40.026	1.081	194	64.6	2.503	190	4.219	0.139	192
<i>A. papilio</i> present		33.28	2.038	25	56.76	5.585	25	3.68	0.229	25
<i>T. crassipes</i> absent		39.205	1.073	190	63.72	2.253	186	4.201	0.14	189
<i>T. crassipes</i> present		39.586	2.702	29	63.483	9.317	29	3.857	0.245	28
<i>I. tasmani</i> absent		39.791	1.176	177	64.989	2.644	174	4.114	0.139	175
<i>I. tasmani</i> present		37	1.504	42	58.171	4.49	41	4.333	0.306	42
<i>I. trichosuri</i> absent		39.61	1.145	177	63.933	2.374	208	4.181	0.13	210
<i>I. trichosuri</i> present		37.762	1.914	42	56.429	6.945	7	3.429	0.429	7
Summer		35.957	1.555	47	57.261	2.685	46	4.087	0.323	46
Autumn		46.25	2.37	68	76.364	6.001	66	4.701	0.266	67
Winter		35.867	1.258	60	56.881	3.069	59	3.967	0.173	60
Spring		36.591	1.785	44	60.523	3.833	44	3.659	0.208	44

highest haemoglobin and erythrocyte counts, and juvenile males had the lowest (Table 6a). Females, regardless of age or lactation status, had similar red blood cell parameter values, which were intermediate between adult and juvenile male values (Table 6a). Red blood cell parameters were unaffected by the presence of any of the haematophagus ectoparasites.

Iron status (iron levels and iron saturation) was significantly affected by an interaction between the haematophagus ectoparasites *I. trichosuri* and *E. myrmecobii*

(Wilks $\lambda = 0.965$, $F_{2,184} = 3.300$, $p = 0.039$, partial eta-squared = 0.035). When *I. trichosuri* was absent, both iron levels and iron saturation were lower in possums hosting *E. myrmecobii* than those without (Table 6b). When *I. trichosuri* was present, iron parameters were higher in hosts of *E. myrmecobii* compared to possums without the flea (Table 6b). Iron status was also affected by the sex and lactation status of the possums (Wilks $\lambda = 0.926$, $F_{4,368} = 3.618$, $p = 0.007$, partial eta-squared = 0.038), with lactating

females having generally higher iron parameters than both males and non-lactating females (Table 6b). Differences in iron concentration and iron saturation due to differences in ectoparasite presence were of similar magnitude to differences between demographic groups.

The abundance of the most abundant ectoparasite, the flea *E. myrmecobii*, had no significant effect on either red blood cell parameters or iron status.

The immune parameters lymphocyte count, neutrophil count and eosinophil count were significantly affected only by the age of the host possums, and not by the presence of any ectoparasites. Adults had lower numbers of lymphocytes, but higher numbers of neutrophils and eosinophils, than juveniles (Table 6c).

The liver function parameters (ALT, AST, total bilirubin) were significantly affected by the presence of *E. myrmecobii* (Wilks $\lambda = 0.949$, $F_{3,196} = 3.544$, $p = 0.016$, partial eta-squared = 0.051), *A. papilio* (Wilks $\lambda = 0.932$, $F_{3,196} = 4.735$, $p = 0.003$, partial eta-squared = 0.068), *T. crassipes* (Wilks $\lambda = 0.948$, $F_{3,196} = 3.568$, $p = 0.015$, partial eta-squared = 0.052), *I. tasmani* (Wilks $\lambda = 0.937$, $F_{3,196} = 4.425$, $p = 0.005$, partial eta-squared = 0.063) and *I. trichosuri* (Wilks $\lambda = 0.950$, $F_{3,196} = 3.415$, $p = 0.018$, partial eta-squared = 0.050). These significant effects were due to significant differences in ALT alone, rather than the grouped parameters. Possums hosting *E. myrmecobii* exhibited higher mean ALT values than possums without this ectoparasite (Table 6d). By contrast, possums infested with *A. papilio*, *I. tasmani*, *I. trichosuri* or *T. crassipes* had lower mean ALT measurements than possums without these species (Table 6d). Liver function parameters were also affected by season (Wilks $\lambda = 0.867$, $F_{9,477.163} = 3.195$, $p = 0.001$, partial eta-squared = 0.046) and species richness per host individual (Wilks $\lambda = 0.943$, $F_{3,196} = 3.964$, $p = 0.009$, partial eta-squared = 0.057). Seasonal differences were seen in all liver function parameters, with a peak in ALT, AST and total bilirubin observed in possums trapped during autumn. However, species richness only significantly affected ALT levels, with possums hosting one to three species of ectoparasites having a higher mean ALT measurements than those without any ectoparasites. The highest mean ALT was measured in possums hosting a single species of ectoparasite (Table 6d).

The scaled mass index (SMI) of urban possums was significantly affected by an interaction of sex and lactation status with the presence of the flea *E. myrmecobii* ($F_{2,205} = 7.437$, $p = 0.001$, partial eta-squared = 0.068). Males hosting *E. myrmecobii* were larger (mean SMI 2723.96 ± 86.7 g, $n = 61$) than males without this parasite (2067.36 ± 106.7 g, $n = 59$), but lactating females infested with *E. myrmecobii* were smaller (2185.91 ± 120.28 g, $n = 27$) than their flea-free counterparts (2538.06 ± 69.43 g, $n = 56$). Non-lactating females were of similar sizes when this parasite was absent (1938.15 ± 175.44 g, $n = 24$) and when it was present (1991.79 ± 363.53 g, $n = 11$).

Abiotic characteristics of each habitat

The mean maximum temperature at the urban site during the study season was 23.3°C , significantly higher than woodland habitat, 17.3°C ($F_{1,22} = 18.18$, $p < 0.01$). Similarly, the mean minimum temperature at the urban site

during the study season was 14.7°C , significantly higher than woodland habitat, 5.6°C ($F_{1,22} = 54.77$, $p < 0.01$).

Discussion

Previous studies have demonstrated that animals inhabiting human-managed habitat including game parks (Ezenwa 2003, 2004) and conservation reserves (Lebarbenchon et al. 2006) are susceptible to enhanced levels of parasite exchange due to the high density of sympatric species atypical in natural environments. In addition, the diversity of hosts in an environment can also be crucial in determining the species richness of ectoparasites and may even outweigh intrinsic factors such as body size of the host (Krasnov et al. 2004). In the present study, only urban-dwelling common brushtail possums were found to host three or more species of ectoparasites per host individual (Table 2a). This suggests that opportunities for ectoparasite transmission may be greater in the urban environment, in part due to higher possum population densities, but also potentially due to the concentration of other hosts in the study area, including humans, domestic pets, captive zoo animals and other urban-dwelling wildlife.

Most ectoparasites hosted by brushtail possums occur at low prevalence, but three species were present on more than 40% of the population in at least one trapping area: the mite *Atellana papilio*, the tick *Ixodes trichosuri* and the flea *Echidnophaga myrmecobii* (Table 3). The fur-clasping mite *A. papilio* occurred with higher prevalence in woodland compared to urban habitat; this concurs with New Zealand studies, where *A. papilio* was the most prevalent ectoparasite, occurring on 81% (Stankiewicz et al. 1996) and 98% (Clark et al. 1997) of possum populations examined. The cooler climate may explain the dominance of the mite on possums from woodland and New Zealand habitat, compared to possums from urban Sydney. The denser coat and woollier tail of possums inhabiting cooler terrain (Kerle 1991) may offer a more favourable 'habitat' for fur-clasping mites, compared to possums adapted to the urban environment and the associated warmer microclimate. The host's fur is used by *A. papilio* as an important site of attachment for all life stages from egg to adult (Clark 1993) implying that it may be the limiting factor causing the lower prevalence of the mite in urban-dwelling possums.

The flea *Echidnophaga myrmecobii* only infected possums from urban habitat (both at Taronga Zoo and in suburban backyards). It was the most prevalent ectoparasite in urban areas, and also had the highest mean intensity of all the ectoparasites in this study. Known as the common 'stickfast' flea, this species possesses a broad host range encompassing native wildlife and introduced mammals such as rabbits, cats and dogs (Dunnet and Mardon 1974). The ability of generalist parasites to exploit a range of unrelated host species enables them to succeed in habitats that support a diversity of hosts (Ezenwa 2003, Krasnov et al. 2007). The transmission of *E. myrmecobii* may be favoured in this particular urban environment because of the overlapping host populations from the metropolitan zoo and residential areas. The less prevalent flea, *Ctenocephalides felis*, originates from domestic cats and has shown an ability to colonise a variety of introduced and native mammals and also humans (Dunnet and Mardon 1974). The absence of *E. myrmecobii*

and *C. felis* from possums inhabiting woodland habitat in this study and non-urban environments in South Australia (O'Callaghan and Moore 1986), Victoria (Presidente et al. 1982) and New Zealand (Stankiewicz et al. 1996, Clark et al. 1997) highlights the role of human settlement in mediating transmission of these fleas.

The success of the flea *E. myrmecobii* among urban possum populations may also reflect the flea's adult male-biased pattern of infestation. The significantly higher prevalence of *E. myrmecobii* amongst adult males compared to females corresponds with a wide range of ectoparasite–host interaction studies (Mooring et al. 1996, Tälleklint-Eisen and Eisen 1999, Soliman et al. 2001). Sex-differences in behaviour, physical characteristics and immunocompetence have been proposed to explain this phenomenon. For example, males often maintain larger home ranges and engage in territorial behaviour more than females, facilitating heightened interaction with parasites (Zuk and McKean 1996). In support of this explanation, GPS-tracking of twelve urban-dwelling possums revealed that males occupied significantly larger home ranges (1.21–2.41 ha) than females (0.27–0.61 ha) (Hill and Deane unpubl.). Males typically inhabit home-ranges overlapping those of several females, and the polygamous mating system of brushtail possums means that adult males associate with multiple females, potentially exposing them to more ectoparasites. Sexual dimorphism often manifests in larger-bodied males, providing ectoparasites with a larger surface area and diversity of refugia (Moore and Wilson 2002); in the present study males hosting *E. myrmecobii* were significantly larger than their flea-free counterparts. Lastly, the inhibitory effect of testosterone on the immune system can lower the resistance of male hosts and make them more susceptible to parasite infestation (Folstad and Karter 1992). The lower prevalence of *E. myrmecobii* amongst juvenile male possums (compared to adult males) can also be explained by the above factors: juvenile males typically remain within the (smaller) maternal home range until maturity (Isaac 2005), are smaller and have lower testosterone levels than those of adult male possums, and are not engaging in mating activities that could promote the spread of ectoparasites.

The tick *Ixodes trichosuri*, sometimes known as the 'possum tick', similarly occurred with significantly higher prevalence in urban habitat, specifically in possums trapped in backyards. Like *E. myrmecobii*, *I. trichosuri* displayed a male-biased pattern of infestation, with sex differences in home-range size, body size and immune function again likely to explain the higher prevalence of this tick amongst male possums. While this tick species was originally isolated and described from a brushtail possum host (Roberts 1970), it has since been identified parasitising on other native mammal hosts, including bush rats *Rattus fuscipes* (Spratt and Haycock 1988), koalas *Phascolarctos cinereus* (Vilcins et al. 2009) and eastern bettongs *Bettongia gaimardi* (Portas et al. 2014). It has also been putatively identified, in the larval stage only, on domestic dogs and cats (Baxter et al. 2009). Like *E. myrmecobii*, *I. trichosuri* appears to flourish in an environment where multiple possible hosts are present. The ability of the larval stage to infest domestic dogs and cats may explain the higher prevalence of *I. trichosuri* in urban backyards, where domestic pets may serve as a parasite 'reservoir'.

The secondary focus of the present study was an examination of the haematological and biochemical health indicators of common brushtail possums. In urban possums, these

indicators were primarily affected by demographic variables such as sex, age and lactation status, as well as season (Table 6). Haemoglobin concentrations and erythrocyte count were higher in adult males than females; this was comparable with previous studies of this species (Barnett et al. 1979, Presidente and Correa 1981). However, in a previous study juvenile males had also higher haemoglobin levels and erythrocyte counts than juvenile females (Presidente and Correa 1981); the converse was true in the present study. Serum iron concentrations and iron saturation were highest in lactating females, which is consistent with a previous study of the marsupial quokka, *Setonix brachyurus* (Kaldor and Morgan 1986). Adult possums had lower numbers of lymphocytes but higher numbers of neutrophils than juveniles, which was similar to patterns seen in the tammar wallaby, *Macropus eugenii* (McKenzie et al. 2002), but contrasted with the possum population studied by Presidente and Correa, which did not exhibit age differences in differential white cell counts. The seasonal effect of a peak in all liver function parameters in autumn is most likely related to increased energetic demands of mating (urban possums breed in both autumn and spring), as well as the higher incidence of females with immature pouch young (autumn being the peak time for births in the urban population; Hill and M. Deane unpubl.). Bilirubin in particular is known to increase during human pregnancy (Lentner 1984), and in tammar wallabies, total bilirubin levels are significantly higher in autumn (a time of peak births) than summer (McKenzie et al. 2002).

In a few instances there was evidence of some impact of ectoparasite infestation on host haematology or serum biochemistry. Serum iron levels and iron saturation of the blood of urban possums were affected by an interaction between the presence of *E. myrmecobii* and *I. trichosuri*, two haematophagous parasites. When the tick alone was present, iron parameters were lower than in unparasitised possums (Table 6b); this was presumably due to the blood loss caused by the tick's blood-meals. A reduced blood iron concentration has previously been reported for tick-infested cattle (O'Kelly et al. 1971, O'Kelly and Kennedy 1981). The presence of *E. myrmecobii* was associated in a smaller decline in iron concentration and iron saturation than the presence of *I. trichosuri* (compared to possums that did not host either ectoparasite; Table 6b); this may have been due to increased blood production to replace blood lost from the potentially more frequent flea bloodmeals. In the presence of both ectoparasites, both iron parameters were higher than in the presence of the tick alone (Table 6b); this may be evidence of increased blood production in the presence of *E. myrmecobii* off-setting the decline in iron levels caused by *I. trichosuri* infestation. However, the mean changes in serum iron concentration and iron saturation seen in response to ectoparasite infestation were equivalent in magnitude to the differences between lactating females, non-lactating females and males. It therefore seems unlikely that infestation with either *E. myrmecobii* or *I. trichosuri* (or both) would result in changes in serum iron indicative of a clinical state of anaemia (or hyperaemia) in most possums.

In the present study, the liver function parameters analysed were alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin levels. Elevated levels of these three liver function parameters are often associated with liver damage and/or disease. Possums hosting one or two ectoparasite species had elevated ALT levels compared to possums with no parasites (Table 6d), indicating a possible

effect on liver metabolism of multiple species of ectoparasite. However, the exact effect of specific ectoparasites is difficult to determine, since possums hosting *E. myrmecobii* exhibited higher ALT levels than possums without this ectoparasite (Table 6d), but possums infested with *A. papilio*, *I. tasmani* or *I. trichosuri* had lower ALT levels than possums without these species (Table 6d). There was no evidence of elevated liver function parameters in association with any of the ixodid tick species, despite tick infestation being known to disrupt host liver metabolism of the host (Kaufman 1989), most likely as a direct effect of a toxin secreted by the parasite (O'Kelly et al. 1971). In humans, liver function may also be impacted by the pathogens spread by ectoparasites, such as the tick-borne bacteria *Babesia* spp. (Kjemtrup and Conrad 2000) and *Borrelia* spp., which cause Lyme disease (Horowitz et al. 1996). It appears that in this urban population of possums, ectoparasite infestation does not cause large changes in liver function parameters, since all ectoparasite effects were smaller in magnitude than the differences observed across seasons.

In conclusion, this study provides insight into multiple host-ectoparasite relationships, ectoparasite infestation patterns and the haematology and serum biochemistry of common brushtail possums, with a particular focus on an urban population successfully adapted to human settlement. Urban conditions favoured a higher prevalence of the flea *E. myrmecobii* and the tick *I. trichosuri* on urban-dwelling possums (compared to woodland possums). Both ectoparasites were associated with host sex and host age in a manner that facilitated transmission. Although the presence of several ectoparasite species was found to impact on health parameters of urban possums, such as iron status and liver function, there was no evidence of long-term pathology. These findings demonstrate that changes in host-ectoparasite dynamics resulting from adaptation to the urban environment do not pose a major threat to the health of possums in urban Sydney.

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Supplementary material (Appendix wlb.00027 at <www.wildlifebiology.org/readers/appendix>). Appendix 1–2.