



Land use, season, and parasitism predict metal concentrations in Australian flying fox fur



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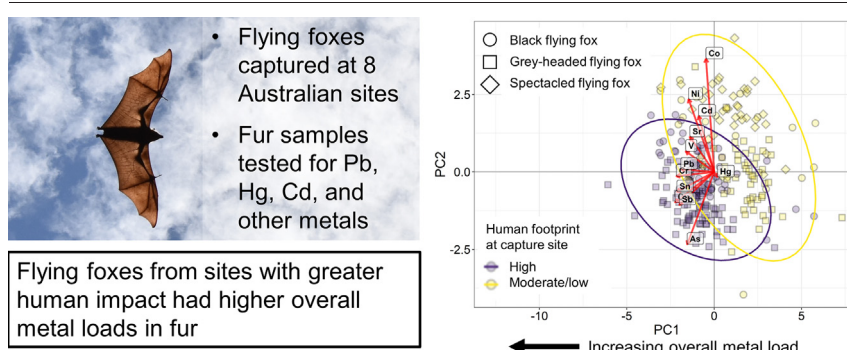
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HIGHLIGHTS

- We tested if Australian flying fox metal exposure is linked to roost site land use.
- Flying foxes captured at sites with greater human impact had higher metal loads.
- Copper, tin, and strontium concentrations were generally highest.
- Flying foxes from Adelaide typically had higher metal concentrations in winter.
- Some metal concentrations were associated with species, sex, age, and parasitism.

GRAPHICAL ABSTRACT



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ABSTRACT

Urban-living wildlife can be exposed to metal contaminants dispersed into the environment through industrial, residential, and agricultural applications. Metal exposure carries lethal and sublethal consequences for animals; in particular, heavy metals (e.g. arsenic, lead, mercury) can damage organs and act as carcinogens. Many bat species reside and forage in human-modified habitats and could be exposed to contaminants in air, water, and food. We quantified metal concentrations in fur samples from three flying fox species (*Pteropus* fruit bats) captured at eight sites in eastern

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Australia. For subsets of bats, we assessed ectoparasite burden, haemoparasite infection, and viral infection, and performed white blood cell differential counts. We examined relationships among metal concentrations, environmental predictors (season, land use surrounding capture site), and individual predictors (species, sex, age, body condition, parasitism, neutrophil:lymphocyte ratio). As expected, bats captured at sites with greater human impact had higher metal loads. At one site with seasonal sampling, bats had higher metal concentrations in winter than in summer, possibly owing to changes in food availability and foraging. Relationships between ectoparasites and metal concentrations were mixed, suggesting multiple causal mechanisms. There was no association between overall metal load and neutrophil:lymphocyte ratio, but mercury concentrations were positively correlated with this ratio, which is associated with stress in other vertebrate taxa. Comparison of our findings to those of previous flying fox studies revealed potentially harmful levels of several metals; in particular, endangered spectacled flying foxes (*P. conspicillatus*) exhibited high concentrations of cadmium and lead. Because some bats harbor pathogens transmissible to humans and animals, future research should explore interactions between metal exposure, immunity, and infection to assess consequences for bat and human health.

1. Introduction

Wildlife in urban areas face exposure to environmental toxicants (e.g. heavy metals, pesticides, persistent organic pollutants) through contaminated food, water, and air (Riley et al., 2014). For instance, landscape maintenance of parks and lawns can introduce fertilizers and pesticides into soil and waterways, facilitated by impervious surface cover (Arnold and Gibbons, 1996). Water can also be contaminated with point-source toxicants such as industrial wastewater and oil or chemical spills (Barron, 2011; Jusi, 1989). Pesticides used to poison nuisance wildlife can reach non-target species via secondary exposure (e.g. carnivores feeding on dead or dying rodents exposed to second-generation anti-coagulant rodenticides), bioaccumulation (i.e. age-related accumulation in tissues and organs), and biomagnification (i.e. increase in concentration with trophic position) (Hoekstra et al., 2002; Iyaniwura, 1991; Lavoie et al., 2013). Transportation and industrial activities can release airborne contaminants, sometimes across large distances, such as observed for atmospheric deposition of polychlorinated biphenyls (Eisenreich et al., 1981; Newman, 1979; Newman and Schreiber, 1988). Even if contaminant-generating sources are removed from an environment, toxicants often persist for months to years and continue to harm wildlife (Scheifler et al., 2006). Likely as a result of exposure to these multiple contamination sources, urban wildlife generally have significantly higher toxicant loads than non-urban conspecifics across diverse animal taxa (Murray et al., 2019).

Metals and metalloids such as mercury, lead, and arsenic are naturally occurring trace elements that can reach toxicity at relatively low levels of exposure from anthropogenic activities. Many of these metals, including cadmium, lead, and mercury, have no known biological function and represent systemic toxicants that can damage organs and disrupt DNA even at low exposure levels (O'Shea and Johnston, 2009). Other metals such as cobalt, copper, iron, and zinc can serve as micronutrients (essential to biochemical and physiological functions) yet become toxic at higher levels of exposure through their interactions with enzymes involved in metabolism, detoxification, and damage repair. Negative consequences of metal exposure for wildlife include altered foraging and other behaviors resulting from neurological damage, reduced body condition, physical deformities, reduced fecundity, and mortality (Eng et al., 2019; Henry et al., 2012; Ouellet et al., 1997; Sandheinrich and Atchison, 1990). Some animals exposed to toxicants also show lower immune function or reduced behavioral defenses such as grooming (Acevedo-Whitehouse and Duffus, 2009; Winans et al., 2011). As one example, female tree swallows (*Tachycineta bicolor*) breeding at mercury-contaminated sites had higher mercury concentrations in blood and weaker immune response than birds at non-contaminated sites (Hawley et al., 2009). Additionally, negative effects of metal exposure can be exacerbated by other stressors (e.g. competition, predation, food limitation, habitat alteration) (Liess et al., 2016; Porter et al., 1984). For instance, laboratory rats experimentally exposed to both concentrated air pollutants and chronic social stress exhibited elevated levels of inflammation biomarkers (Clougherty et al., 2010).

Bats as a group are well-suited to study biological and environmental predictors of metal exposure (Jones et al., 2009). Their long lifespans (in some cases over 40 years (Austad and Fischer, 1991)) permit metal accumulation in organs and tissues over time, and their high mobility and dietary breadth allow them to forage in natural and human-modified habitats. Most studies of bat exposure to metals and other toxicants have focused on insectivorous species (Bayat et al., 2014; O'Shea and Johnston, 2009; Zukal et al., 2015), likely because there is a clearer exposure route (i.e. uptake through insect prey). However, fruit bats (which feed on nectar, pollen, and fruits) can also be exposed to metals and toxicants while foraging. These bats are increasingly settling in urban and agricultural areas, where they consume introduced and cultivated plant species (Boardman et al., 2021; Kessler et al., 2018; Timmiss et al., 2021). Metals taken up by plants from soil can subsequently be passed to honey bees via pollen and nectar (Fakhimzadeh and Lodenius, 2000; Roman, 2010; van der Steen et al., 2012); similarly, fruit bats face exposure to metals and pesticides while foraging (Oliveira et al., 2017; Oliveira et al., 2018; Pulscher et al., 2020; Zukal et al., 2015). Atmospheric deposition and polluted waterways also pose potential risks (Hariono et al., 1993; Korine et al., 2015; Pulscher et al., 2020). Experimental dosing found that fruit bats (*Pteropus* spp.) absorb more lead than do other mammals (Hariono, 1991), which might make species in this group especially vulnerable to negative toxicant effects. Mechanistic modeling has suggested that the extent of toxic habitat on a landscape, combined with viral infection that causes morbidity or mortality, could cause bat population declines (Sánchez et al., 2020). Additionally, if toxicants impair immune function in bats, this could increase their susceptibility to (or slow their recovery from) pathogen infection. Because some bats host viruses that can be transmitted to domesticated animals and humans (e.g. paramyxoviruses, coronaviruses, filoviruses, lyssaviruses) (Calisher et al., 2006), impaired immune function owing to metal exposure could pose increased public health risks (Becker et al., 2017).

In this study, we examined the metal exposure of three *Pteropus* fruit bat species (flying foxes) captured at eight sites across Australia between 2015 and 2018. Specifically, we measured the concentrations of 13 metals in 641 fur samples (including mercury, lead, and cadmium, for which low levels of exposure are known to cause toxicity for vertebrates). Fur samples have been used in multiple studies to assess metal exposure in bats (Becker et al., 2018; Flache et al., 2015; Hernout et al., 2016a; Mina et al., 2019) as they are easy to collect and minimally invasive to the animal. Metal concentrations in fur and other tissue types generally correlate positively, though the strength of the correlation can depend on the metal and the bat species (Hariono et al., 1993; Hernout et al., 2016a; Hernout et al., 2016b; Timofieieva et al., 2021). We tested for relationships between metal concentrations and environmental predictors (season, land use surrounding bat capture site) and individual-level predictors (species, sex, age, body condition). We predicted that metal concentrations would be higher for bats captured in areas with greater human modification (e.g. urbanization, industrialization, agriculture), and in older bats and those in poorer body condition. We also assessed ectoparasite burden, haemoparasite infection status, paramyxovirus infection status, and

neutrophil:lymphocyte ratio (NL ratio) in a subset of bats, as additional indicators of health. High metal concentrations might predict infection burden or status, but the direction of this relationship could vary among parasites. For example, some metals might weaken bat immune defenses or reduce grooming behavior, leading to a positive relationship between metal concentrations and parasitism. Alternatively, if metals are toxic to parasites, such as might occur if enzymatic pathways in the parasites themselves are disrupted, then metal exposure could reduce infection for some parasite groups (Becker et al., 2021; Poulin, 1992). Based on previous work (Becker et al., 2017), we expected that higher metal concentrations would be associated with larger NL ratios.

2. Methods

2.1. Animal capture and sampling

Three species of pteropodid flying foxes (black flying fox, BFF, *Pteropus alecto*; spectacled flying fox, SFF, *P. conspicillatus*; grey-headed flying fox, GHFF, *P. poliocephalus*) were captured between June 2015 and September 2018 at eight sites across three Australian states (Queensland, New South Wales, and South Australia; Fig. 1, Table 1). Capture sites were chosen based on known bat roost locations, with colony sizes typically ranging from the low thousands to >50,000 (for additional details on sampling locations and colony sizes, see the National Flying-fox Monitoring Viewer: <https://www.environment.gov.au/webgis-framework/apps/ffc-wide/ffc-wide.jsf>). Flying foxes were captured pre-dawn using mist nets as they returned from nightly foraging and anesthetized under veterinary supervision with inhalant isoflurane (Jonsson et al., 2004). We recorded each bat's species, sex, weight (nearest g), and forearm length (nearest mm). Body condition was calculated as the ratio of weight to forearm length. Bats

were assigned to an age class (adult or non-adult) based on secondary sexual characteristics (Welbergen, 2010). The number of ectoparasites (bat flies, family *Nycteribiidae*) was recorded if present. A fur clipping (~20–80 mg) was taken from the chest or back of bats and stored in individual plastic bags at room temperature until analysis. For Adelaide bats, a passive integrated transponder (PIT) tag was injected subcutaneously between the scapulae. After all samples were collected, flying foxes were allowed to recover from anesthesia and released at the capture site.

Urine was collected from bats captured from four sites (Table 2). We palpated bats' abdomens gently to express urine and collected samples in 1.5 mL screw-cap tubes (Axygen, Union City, CA). Samples were placed on cooler packs in the field for up to 6 h and later stored at ~−20 °C. Thin blood smears were also prepared for a subset of bats captured from six sites (Table 2), using blood drawn from the cephalic vein with a 25-gauge needle.

Fieldwork in Queensland was authorized under section 173P of the Nature Conservation Act 1992. Fieldwork in New South Wales was authorized under section 132c of the National Parks & Wildlife Act, 1974 (SL101396). Fieldwork in South Australia was authorized by the Government of South Australia Department of Environment, Water and Natural Resources (M26371-4). Ethical approval was granted by the CSIRO Ecosystem Sciences Animal Ethics Committee (13-02), the University of Adelaide Animal Ethics Committee (S-2015-028), the University of Georgia Institutional Animal Care and Use Committee (A2015 03-028-R3), and the Griffith University Animal Ethics Committee (ENV/10/16/AEC).

2.2. Analyses of biological samples

Fur samples were analyzed at Baylor University for ten metals: cadmium, chromium, cobalt, copper, lead, nickel, selenium, strontium, tin, vanadium, and two metalloids (hereafter referred to as metals): antimony and arsenic, following slight modification of previously published methods (Rainwater et al., 2009) (see Supplementary Material for details). Briefly, fur samples were individually weighed and ~0.05 g was placed in borosilicate glass tubes (VWR International, Radnor, PA). Batches of 25–30 samples were digested with nitric acid and hydrogen peroxide in a series of heating and cooling steps, then filtered into acid-rinsed Erlenmeyer flasks (VWR International) and diluted in ultrapure water. Based on literature from previous analytical approaches showing little contribution of external metal contamination (Chételat et al., 2018), fur samples were not washed prior to the digestion step. Blanks were included for each batch. Human hair standard (Sigma-Aldrich, St. Louis, MO) was used as a standard reference material, with one reference sample included for each bat capture site. Samples were analyzed by inductively coupled plasma mass spectrometry using a 7900 ICP-MS (Agilent Technologies, Santa Clara, CA). Sample limits of detection were calculated as three times the standard deviation of response divided by the slope of the corresponding calibration curve for a given metal and sample run. Nearly all (97 %) selenium concentrations were below detection limits; we therefore deemed them unreliable and excluded selenium concentrations for all subsequent analyses. For other metals, concentrations that fell below a sample's limit of detection (arsenic: $n = 6$; cadmium: $n = 75$) were estimated as half of the detection limit. We report metal concentrations in ng/g.

Following these assays, remaining fur samples were sent to Texas Christian University and analyzed for total mercury (methylmercury + inorganic mercury; hereafter, mercury) using direct mercury analysis (DMA-80 Direct Mercury Analyzer, Milestone, Shelton, CT), which uses thermal decomposition, gold amalgamation, and atomic absorption spectroscopy (EPA US, 1998). Quality assurance included reference (National Research Council of Canada Institute for National Measurement Standards) and duplicate samples. Reference samples (DORM-4) were analyzed every 10 samples, and the mean recovery percentage for DORM-4 was 103 ± 4.31 % ($n = 81$). Duplicate samples were analyzed every 20 samples, and the mean relative difference percentage was 6.83 ± 7.05 % ($n = 44$). Limited amounts of fur available for analysis meant that

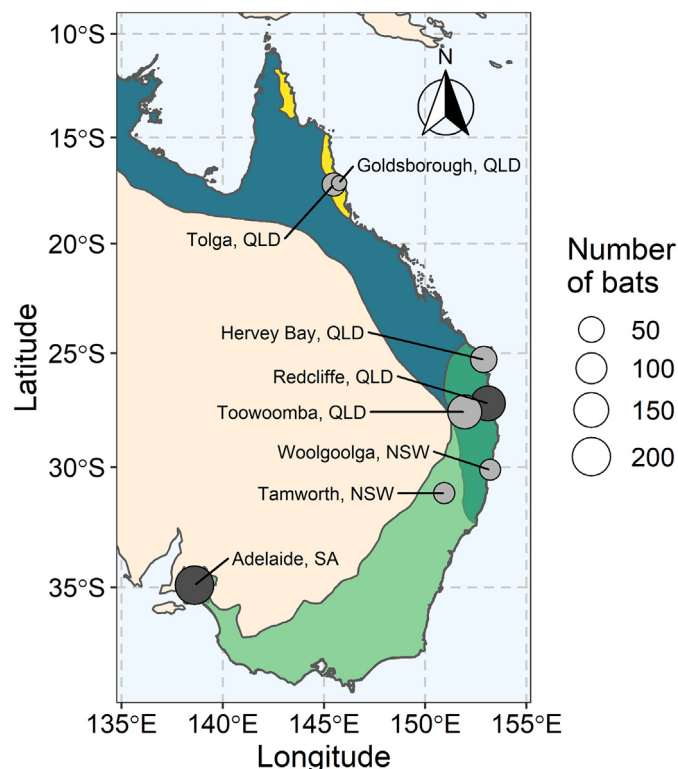


Fig. 1. Map of eastern Australia showing the eight sites where flying foxes were captured. Current range distributions (last updated March 2021, <https://www.ausbats.org.au/batmap.html>) are shaded blue for black flying foxes, green for grey-headed flying foxes, and yellow for spectacled flying foxes. The area of each bubble corresponds to the number of flying foxes at a site with at least one metal concentration reported, while the color of each bubble corresponds to the site's human footprint level (high = dark grey, moderate-low = light grey).

Table 1

Capture information for flying foxes for which ≥ 1 metal concentrations are reported. Numbers represent sample sizes by site, species, sex, and sampling interval. BFF: black flying fox, GHFF: grey-headed flying fox, SFF: spectacled flying fox.

State	Site	Lat.	Long.	Capture date	Species			Sex		Age class		Total
					BFF	GHFF	SFF	Female	Male	Adult	Non-adult	
New South Wales	Tamworth	−31.10161	150.93999	July 2015	0	24	0	8	16	10	14	24
	Woolgoolga	−30.10389	153.19764	July 2015	1	22	0	9	14	19	4	23
Queensland	Goldsborough	−17.15411	145.7372	June 2015	0	0	11	3	8	11	0	11
	Hervey Bay	−25.28886	152.89286	July 2018	14	43	0	27	30	39	18	57
	Redcliffe	−27.23145	153.09875	May 2018	49	4	0	29	24	36	17	53
				July 2018	24	11	0	20	15	12	23	35
				Sept. 2018	60	0	0	27	33	43	17	60
	Tolga	−17.2315	145.4804	June 2015	0	0	34	18	16	30	4	34
	Toowoomba	−27.60102	151.94297	June 2018	30	1	0	17	14	21	10	31
				July 2018	47	2	0	27	22	29 ^a	18 ^a	49
				Sept. 2018	56	1	0	26	31	26	31	57
South Australia	Adelaide	−34.91588	138.6065	Aug. 2016	0	49	0	28	21	31	18	49
				Feb. 2017	0	57	0	40	17	38	19	57
				Aug. 2017	0	48	0	33	15	41	7	48
				Feb. 2018	0	53	0	36	17	35	18	53
Total					281	315	45	348	293	421 ^a	218 ^a	641

^a Age class was not recorded for two bats, meaning that totals do not add up in the age class columns.

some samples ($n = 119$) weighed <0.01 g, the lower limit of reliability for the scale used; to be conservative, concentrations for these samples were excluded from subsequent analyses. All samples below the mercury detection limit (0.1 ng, ~ 0.6 ng/g; $n = 55$) were also below the lower limit of reliability for the scale and were thus excluded rather than estimated as half of the detection limit. We report mercury concentrations in ng/g.

Blood smears were examined at two different time points. In 2016, blood smears collected at three sites (Tamworth, Tolga, and Woolgoolga; $n = 81$) were examined at the University of Georgia for the presence of haemoparasites (order Haemosporida). Smears were stained with Modified Wright's stain and the monolayer of each blood smear was scanned at both $500\times$ and $1000\times$ (oil immersion) magnifications by a board-certified veterinary clinical pathologist. Samples for which one or more infected erythrocytes were detected were scored as positive for haemoparasites. In 2022, to address reviewer feedback, white blood cell differential counts (i.e. the average number of lymphocytes, neutrophils, eosinophils, basophils, and monocytes out of 100 leucocytes) were performed on the above blood smears and smears collected at three additional sites (Hervey Bay, Redcliffe, and Toowoomba; $n = 119$). Smears from the latter three sites ($n = 67$) were stained with DipQuick (JorVet Laboratories) and were also examined for haemoparasites. Smears previously stained in 2016 had faded in color and were therefore re-stained with DipQuick in 2022 to enable the

differentiation of leucocyte lineages. We calculated NL ratio as a measure of innate-to-adaptive immune function, which has been shown to be associated with stress in other wildlife species (Davis et al., 2008). Differential counts and additional haemoparasite screening were performed at Montana State University following Hansen et al. (in press). High numbers of smudge cells (lysed leucocytes) have been shown to skew differential counts (eClinPath, 2022); therefore, slides containing >15 smudge cells on average per differential were excluded from analysis.

Urine samples ($n = 39$) were shipped on cooler packs to the Australian Centre for Disease Preparedness (formerly, Australian Animal Health Laboratory). Samples were condensed into eight pools and screened for 11 paramyxoviruses (Cedar virus, Geelong paramyxovirus, Grove virus, Hendra virus, Hervey virus, Menangle virus, Nipah virus, Teviot virus, Tioman virus, Yarra Bend paramyxovirus, Yeeppoon virus) using a multiplex bead X-Tag assay for nucleic acid detection (Boyd et al., 2015).

2.3. Statistical analyses

All statistical analyses were performed in the R computing environment v 4.0.3 (R Core Team, 2019). One GHFF from Adelaide was captured twice; because the captures were a year apart (August 2016 and August 2017), metal concentrations from both captures were included in the dataset.

Table 2

Sample sizes for fur, blood smears, and urine, separated by sampling event. Fur samples are further separated by metal analyses performed, as mercury concentrations were measured separately from other metals.

State	Site	Capture date	Total bats captured	Fur samples analyzed			Blood smears examined	Urine samples analyzed	
				All metals	All metals except mercury	Mercury only			
New South Wales	Tamworth	July 2015	24	24	–	–	24	11	
	Woolgoolga	July 2015	23	23	–	–	23	12	
Queensland	Goldsborough	June 2015	11	9	2	–	–	3	
	Hervey Bay	July 2018	57	6	22	29	12	–	
		Redcliffe	May 2018	53	2	18	33	1	–
			July 2018	35	1	7	27	5	–
	Tolga	Sept. 2018	60	56	4	–	20	–	
		June 2015	34	32	2	–	34	13	
		Toowoomba	June 2018	31	–	7	24	7	–
	July 2018		49	1	16	32	7	–	
	Sept. 2018		57	14	32	11	15	–	
South Australia	Adelaide	Aug. 2016	49	48	1	–	–	–	
		Feb. 2017	57	53	4	–	–	–	
		Aug. 2017	48	42	5	1	–	–	
		Feb. 2018	53	51	2	–	–	–	
Total			641	362	122	157	148	39	

Recapture was not assessed for sites other than Adelaide; however, because flying fox recapture rates are generally very low (Boardman et al., 2020; Westcott et al., 2011) and only three sites were sampled more than once, we assume that nearly all bats captured here represent distinct individuals. In initial data exploration, six fur samples (4 male and 1 female BFF, 1 male GHFF; all adults captured in Hervey Bay) were found to have extremely low concentrations of all metals measured at Baylor University. Because these samples were processed consecutively in the laboratory, we considered it likely that a technical error had occurred and excluded those values from all analyses described below. To facilitate species comparisons for future studies, we calculated summary values (minimum, median, maximum) for metal concentrations for the three flying fox species. Due to the extreme range in values, metal concentrations were ln-transformed for model analyses described below.

We used linear mixed models (LMMs) to compare species differences in metal concentrations while controlling for site, sex, and age class. For each metal, we used the 'lmerTest' package (Kuznetsova et al., 2017) to run a LMM with species, sex, and age class as a fixed effect and site as a random effect. Given that later analyses indicated consistent seasonal differences in metal concentrations for fur samples (see Results), we only included samples from bats collected in winter ($n = 373$) in these models. Pairwise comparisons of species means were made with the 'multcomp' package (Hothorn et al., 2008) with a Holm adjustment for multiple comparisons.

To determine if bats captured at sites with greater human impact had higher metal concentrations, we first calculated the average human footprint score within a 20 km buffer (typical foraging range for flying foxes (Parsons et al., 2006; Tidemann, 1999)) around each site. Human footprint ranges from 0 to 50 and is a composite measure of human impacts including population density, and the proportion of land area assigned to agriculture, built environments, and transportation. We used the most recent (2013) human footprint dataset available (Williams et al., 2020a; Williams et al., 2020b). We next performed a principal component analysis on all metal concentrations in fur to build a composite picture of metal exposure. As above, we only included samples from bats captured in winter, and only included samples for which concentrations were measured for all 12 metals, leaving a final set of 258 samples for analysis. Metal concentrations were centered and scaled to have unit variance. Horn's parallel analysis supported retention of the first three principal components (PCs) (Dinno, 2018). We then used three LMMs to test whether site human footprint scores explained variation in PC1, PC2, or PC3. We included species as a fixed effect in each model (an attempt to include sex and age class caused model convergence issues) and treated site as a random effect.

For bats captured in Adelaide ($n = 203$ GHFF), we examined the effect of season, sex, body condition, and age class on metal concentrations in fur. We focused on Adelaide because 1) it was the only site at which bats were captured multiple times in separate seasons (two summer and two winter sampling periods), and 2) it allowed us to avoid possible confounding effects of species or site on relationships between metal concentrations and sex, body condition, and age. We used a separate linear model for each metal, with season, sex, body condition, and age class as predictor variables.

Finally, we performed analyses to examine associations between metal concentrations and three measures of health: ectoparasite burden, haemoparasite infection, and NL ratio. We used the 'glmmTMB' package (Brooks et al., 2017) to run a generalized linear mixed model (GLMM; Poisson distribution, log link) to model ectoparasite burden as a function of all metal concentrations (except mercury, to maximize the sample size available for the analysis: $n = 222$). We included species, sex, body condition, and age class as additional fixed effects and site as a random effect. We limited the analysis to flying foxes caught in Queensland (five sites), as 56 % of flying foxes from Queensland had ectoparasites, versus <1 % of flying foxes from New South Wales and South Australia (Table 3). We ran a second GLMM (binomial distribution, logit link) to model haemoparasite infection status ($n = 128$); predictors were the same as above, except species was excluded due to high collinearity with other predictors (Lüdtke et al., 2020). We lastly ran two linear models—one with PC1 (representing overall metal load; see Results) and species as predictor variables ($n = 76$), and one with mercury concentration and species as predictors ($n = 96$)—with NL ratio as the outcome of interest. We performed the mercury-only model because previous work in vampire bats (*Desmodus rotundus*) found a positive (though non-significant) association between NL ratio and mercury concentrations in fur (Becker et al., 2017). No models were run to assess predictors of viral infection because all samples were negative (see Results).

3. Results

3.1. Sample overview

Most fur samples used for metal analysis were from GHFF ($n = 315$; 49.1 %) and BFF ($n = 281$; 43.8 %); the remainder were from SFF ($n = 45$; 7.0 %). GHFF had the largest spatial distribution, with samples from across nearly ten degrees of latitude and six of eight sites (Fig. 1). Slightly more than half of all fur samples were from females ($n = 349$, 54.4 %). Nycteribiid ectoparasites were detected on 37 % of bats (231/619; ectoparasite data not recorded for 22 bats) (Table 3). Ectoparasite presence was high in bats captured in northern Queensland (Goldsborough: 100 %, Tolga: 94 %), moderate-to-low in bats from southeast Queensland (Hervey Bay: 74 %, Redcliffe: 72 %, Toowoomba: 36 %), and near zero in bats from New South Wales and South Australia (Woolgoolga: 9 %, Tamworth: 0 %, Adelaide: 0 %) (Table 3). Microscopic examination of blood smears (collected at all sites except Adelaide and Goldsborough) revealed that 33 % (49/148) of smears contained intraerythrocytic haemosporidian gametocytes (Landau et al., 2012) (Fig. S1). Haemoparasite prevalence varied between species, with higher prevalence in SFF (59 %, 20/34) and GHFF (50 %, 28/56) and near zero prevalence in BFF (2 %, 1/58) (Table 3). Within GHFF, prevalence also varied between sites (Tamworth: 19/24; Redcliffe: 1/2; Woolgoolga: 8/22; Hervey Bay: 0/8). Pooled urine samples ($n = 39$ samples in 8 pools; collected from SFF in Goldsborough and Tolga and GHFF in Tamworth and Woolgoolga) were negative for all 11 paramyxoviruses for which we screened.

Table 3

Summary of ectoparasite and haemoparasite infection by site and flying fox species. BFF: black flying fox, GHFF: grey-headed flying fox, SFF: spectacled flying fox.

State	Site	Ectoparasite presence			Haemoparasite presence (detected by blood smear microscope examination)		
		Species			Species		
		BFF	GHFF	SFF	BFF	GHFF	SFF
New South Wales	Tamworth	–	0/24	–	–	19/24	–
	Woolgoolga	0/1	2/22	–	0/1	8/22	–
Queensland	Goldsborough	–	–	11/11	–	–	–
	Hervey Bay	11/12	28/41	–	0/4	0/8	–
	Redcliffe	88/127	14/14	–	1/24	1/2	–
	Tolga	–	–	32/34	–	–	20/34
	Toowoomba	44/122	1/4	–	0/29	–	–
South Australia	Adelaide	–	0/207	–	–	–	–

Table 4

Fur metal concentrations (all in units of ng/g) for multiple *Pteropus* species measured in this study and other studies. Shaded cells indicate metals generally considered to be of highest concern to wildlife (Zukal et al., 2015). BFF: black flying fox, GHFF: grey-headed flying fox, SFF: spectacled flying fox, CIFF: Christmas Island flying fox.

Metal	Species	Statistic	This study ^a	Other studies
Antimony	BFF	Median	90.4	61.7 ^b
		Range	12.1–516	34.3–96.0 ^b
	GHFF	Median	83.0	20.3 ^b
		Range	14.1–1,294	3.93–96.0 ^b
Arsenic	SFF	Median	49.3	
		Range	19.3–189	
	BFF	Median	190	163 ^b
		Range	17.0–1,782	99.5–454 ^b
	GHFF	Median	212	110 ^b
		Range	28.4–3,463	42.4–247 ^b
Cadmium	SFF	Median	93.7	
		Range	32.1–596	
	BFF	Median	38.6	19.4 ^b
		Range	5.27–321	5.67–27.4 ^b
	GHFF	Median	31.6	8.88 ^b
		Range	6.31–510	2.23–44.4 ^b
Chromium	SFF	Median	84.0	
		Range	15.4–3,296	
	CIFF	Median		70 ^c
		Range		20–320 ^c
	BFF	Median	1,450	156 ^b
		Range	269–8,315	72.0–296 ^b
Cobalt	GHFF	Median	1,081	96.6 ^b
		Range	261–16,686	18.7–701 ^b
	SFF	Median	885	
		Range	530–8,859	
	BFF	Median	202	22.8 ^b
		Range	3.60–35,269	8.06–43.6 ^b
Copper	GHFF	Median	74.3	14.4 ^b
		Range	7.84–1,167	0.92–33.9 ^b
	SFF	Median	387	
		Range	55.6–3,424	
	BFF	Median	9,005	2,828 ^b
		Range	2,031–46,851	1,302–8,631 ^b
Lead	GHFF	Median	9,960	2,021 ^b
		Range	2,616–110,531	887–9,299 ^b
	SFF	Median	6,134	
		Range	2,665–22,043	
	CIFF	Median		1,610 ^c
		Range		1,170–11,400 ^c
Mercury	BFF	Median	1,255	1,610 ^b
		Range	172–9,958	27,310 ^d
	GHFF	Median	1,641	718–3,751 ^b
		Range	179–28,892	5,590–34,480 ^d
	SFF	Median	2,264	343 ^b
		Range	228–32,345	5,880 ^d
Nickel	CIFF	Median		87.0–1,349 ^b
		Range		0–42,070 ^d
	BFF	Median	20.1	580 ^c
		Range	4.84–416	0–8,910 ^c
	GHFF	Median	27.1	100 ^c
		Range	5.67–552	40–620 ^c
Strontium	SFF	Median	40.3	57.8 ^b
		Range	3.91–262	33.4–248 ^b
	CIFF	Median		120 ^b
		Range		25.9–442 ^b
	BFF	Median	718	40 ^c
		Range	120–19,711	20–580 ^c
	GHFF	Median	440	122 ^b
		Range	125–246,746	67.0–252 ^b
	SFF	Median	658	134 ^b
		Range	421–1,836	32.5–752 ^b
	BFF	Median	17,931	
		Range	2,646–118,067	
	GHFF	Median	3,506	
		Range	743–102,885	

Table 4 (continued)

Metal	Species	Statistic	This study ^a	Other studies
Tin	SFF	Median	3,390	
		Range	1,249–8,955	
	BFF	Median	5,167	58.1 ^b
		Range	1,271–27,597	20.9–191 ^b
Vanadium	GHFF	Median	5,420	43.4 ^b
		Range	1,151–53,901	15.8–240 ^b
	SFF	Median	4,022	
		Range	2,178–12,633	
	BFF	Median	460	
		Range	46.0–3,862	
	GHFF	Median	154	
		Range	16.9–1,570	
	SFF	Median	84.7	
		Range	28.6–210	

^a Sample sizes for all metals except mercury: BFF: $n = 162$; GHFF: $n = 277$; SFF: $n = 45$. Sample sizes for mercury: BFF: $n = 195$; GHFF: $n = 283$; SFF: $n = 41$.

^b Medians and ranges for free-living BFF and GHFF were calculated from raw data provided in (Phalen, 2020). Sample sizes: BFF: $n = 9$; GHFF: $n = 11$.

^c Medians and ranges for free-living CIFF are reported in (Pulscher et al., 2021). Sample sizes: cadmium: $n = 53$; copper: $n = 52$; lead: $n = 52$; mercury: $n = 51$.

^d Medians and ranges for urban BFF and GHFF submitted to the University of Queensland's Department of Veterinary Pathology were calculated from raw data reported in (Hariono, 1991). Sample sizes: BFF: $n = 4$; GHFF: $n = 33$.

^e Median and range for non-urban SFF were calculated from raw data reported in (Hariono, 1991). $n = 9$.

3.2. Metals concentrations in relation to species and human footprint

Minimum, median, and maximum values for concentrations of 12 metals measured in fur are presented in Table 4. Copper, tin, and strontium consistently had the highest median concentrations across species, while mercury, cadmium, and antimony had the lowest median concentrations (Fig. 2A). Controlling for sex, age class, and site, and including only samples collected in winter, we found statistically significant differences between bat species in mean concentrations of three metals (Fig. S2). SFF had significantly higher concentrations of cadmium and cobalt than BFF and GHFF, and BFF had significantly higher concentrations of vanadium than GHFF and SFF. Additionally, we found evidence for some sex and age class differences in metal concentrations. Adult bats had significantly higher concentrations of mercury ($\beta = 0.40$, $SE = 0.06$, $p = 6.7e-10$), and significantly lower concentrations of antimony ($\beta = -0.19$, $SE = 0.07$, $p = 0.01$), cobalt ($\beta = -0.23$, $SE = 0.1$, $p = 0.02$), copper ($\beta = -0.13$, $SE = 0.05$, $p = 0.005$), lead ($\beta = -0.29$, $SE = 0.09$, $p = 0.002$), and vanadium ($\beta = -0.36$, $SE = 0.08$, $p = 2.8e-6$) compared to non-adults. Female bats had significantly higher concentrations of arsenic ($\beta = -0.15$, $SE = 0.07$, $p = 0.04$) and mercury ($\beta = -0.22$, $SE = 0.06$, $p = 0.0002$) compared to males.

Principal component analysis showed support for three principal components for which adjusted eigenvalues were >1 . PC1, PC2, and PC3 explained 37.7 %, 15.5 %, and 12.8 % respectively, of the variation in metal concentrations in fur. Variable loadings are provided in Table S1; we considered loadings with an absolute value >0.326 as significant (Stevens, 2002). PC1 was loaded negatively by all metals except mercury, with five metals above the significance cutoff (antimony, chromium, copper, lead, tin); we therefore considered PC1 to represent overall metal load, with lower PC1 scores indicating higher metal load. PC2 had significant positive loadings of cobalt and nickel and significant negative loadings of arsenic. PC3 had significant positive loadings of strontium and vanadium and significant negative loadings of cadmium and lead.

Calculation of human footprint values across sampling locations showed that Adelaide and Redcliffe had higher human footprint scores (30.7 and 29.9 respectively), while the other sites had moderate to low scores (Toowoomba: 14.7, Hervey Bay: 12.1, Tamworth: 11.9, Woolgoolga: 10.0, Tolga: 9.6, Goldsborough: 7.2). LMM analyses of associations between metal composite values (PC1, 2, and 3) and human footprint showed a significant negative relationship between metal PC1 score and

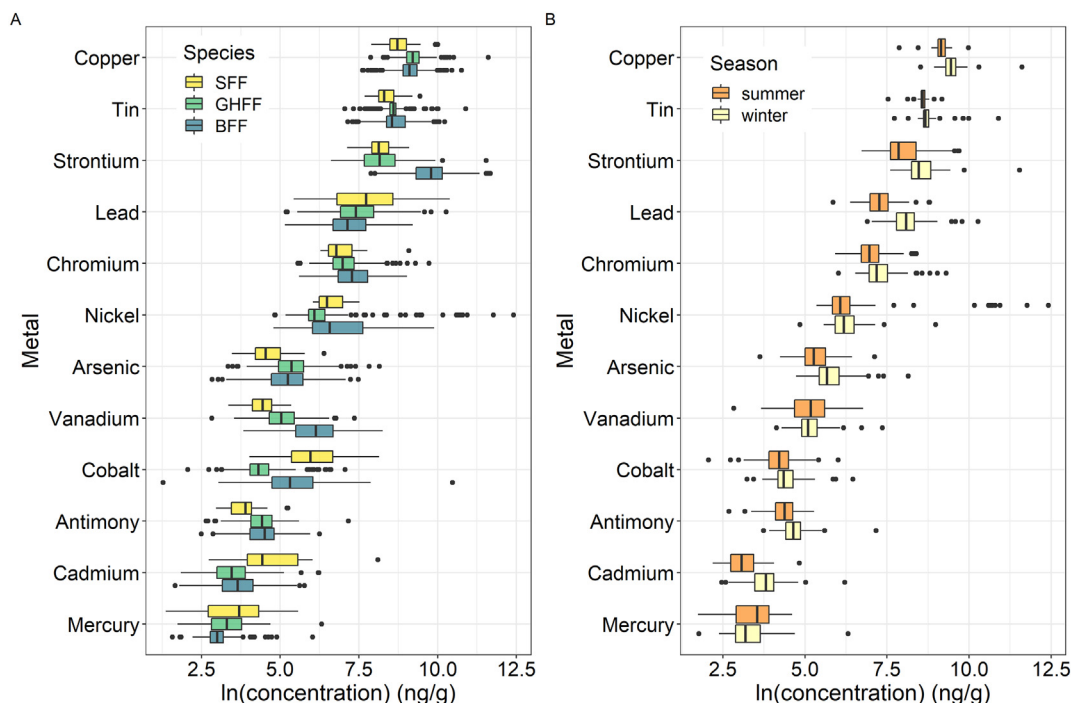


Fig. 2. Boxplots showing ln-transformed concentrations of 12 metals measured in flying fox fur, separated by A) species (all bats) and B) season (only bats from Adelaide). In each boxplot, the middle line represents the median value, box represents the interquartile range, the whiskers extend to 1.5 times the interquartile range, and any points beyond this range are plotted separately. BFF: black flying fox, GHFF: grey-headed flying fox, SFF: spectacled flying fox.

human footprint score ($\beta = -0.15$, $SE = 0.02$, $p = 0.02$; Fig. 3); bats captured at sites with higher human footprints had higher overall metal loads. A post-hoc comparison of species means showed that BFF and GHFF had significantly higher metal PC1 scores (lower overall metal

loads) than SFF (BFF-SFF = 1.53, $SE = 0.49$, $p = 0.004$; GHFF-SFF = 1.39, $SE = 0.42$, $p = 0.003$). There were no significant predictors of metal PC2 score, and there were significant differences in PC3 scores between all species comparisons, with SFF having the highest metal loads,

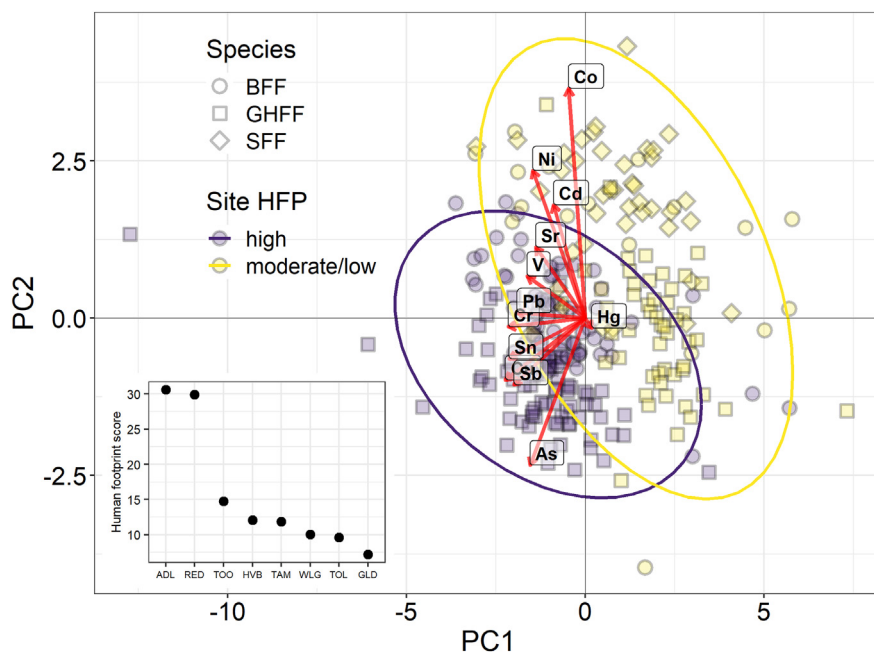


Fig. 3. Biplot of PC1 versus PC2 with loadings of 12 metals (As: arsenic, Cd: cadmium, Co: cobalt, Cr: chromium, Cu: copper, Hg: mercury, Ni: nickel, Pb: lead, Sb: antimony, Sn: tin, Sr: strontium, V: vanadium) measured in 258 flying fox fur samples. Variable loadings are described in Results text and Table S1. The shape of each point represents species (BFF: black flying fox, GHFF: grey-headed flying fox, SFF: spectacled flying fox). Each point is colored according to the human footprint (HFP) score of the capture site. Scores were condensed into two categories: high (Adelaide and Redcliffe) and moderate/low (Toowoomba, Hervey Bay, Tamworth, Woolgoolga, Tolga, and Goldsborough). Colored rings are 95 % data ellipses. The bottom-left inset shows HFP scores for sites.

followed by GHFF, then BFF (BFF-GHFF = 1.10, SE = 0.39, $p = 0.01$; BFF-SFF = 2.64, SE = 0.78, $p = 0.002$; GHFF-SFF = 1.54, SE = 0.72, $p = 0.03$; Fig. 3).

3.3. Seasonal and individual predictors of metal concentrations

Linear models demonstrated consistent seasonal differences in metal concentrations from GHFF captured in Adelaide (Fig. 2B, Table S2). Nine metal concentrations in fur (antimony, arsenic, cadmium, chromium, cobalt, copper, lead, strontium, and tin) were significantly lower in summer than in winter; concentrations of mercury and nickel were significantly higher in summer. Visual examination of metal concentrations separated by sampling session at all sites with multiple sampling events (Adelaide, Redcliffe, Toowoomba) suggested differences observed in Adelaide were due to a true seasonal effect (Fig. S3). There was also support for age class-associated differences in metal concentrations among Adelaide bats, with adults having higher concentrations of arsenic, chromium, strontium, tin, and vanadium (Table S2). There was limited evidence of sex and body condition differences. Female bats had significantly higher concentrations of mercury and strontium than males, while better body condition was significantly associated with higher concentrations of mercury and lower concentrations of tin and vanadium (Table S2).

3.4. Relationships between metals, parasites, and immune function

We found significant relationships between ectoparasite burden and three metals. In a GLMM, ectoparasite burden was positively correlated with chromium and nickel concentrations and negatively correlated with cobalt concentrations (Fig. 4, Table S3). Additionally, SFF had significantly more ectoparasites compared to BFF. In a separate GLMM, haemoparasite infection status was significantly negatively correlated with strontium concentrations (Table S4). Linear models showed no relationship between NL ratio and overall metal load (metal PC1 score), but a significant positive relationship between NL ratio and mercury concentration ($\beta = 1.03$, SE = 0.44, $p = 0.02$; Fig. 5). Additionally, BFF and SFF had significantly lower NL ratios than GHFF (BFF-GHFF = -2.04 , SE = 0.69, $p = 0.004$; SFF-GHFF = -2.66 , SE = 0.91, $p = 0.004$).

4. Discussion

4.1. Metals concentrations in relation to land use

Consistent with expectations that human-mediated landscape changes such as urbanization and industrial activities affect flying fox toxicant exposure, we found that bats captured at sites with higher human footprint values had higher overall metal loads. This result aligns with a previous report of higher lead concentrations in fur, bones, and tissues of Australian flying foxes from urban areas, where bats were likely exposed to

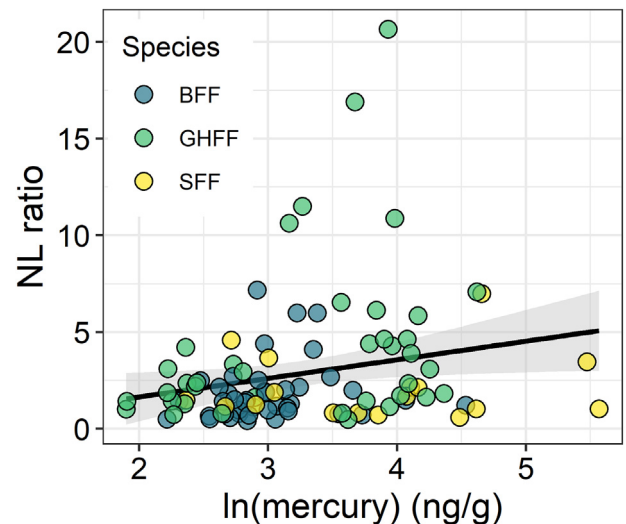


Fig. 5. Neutrophil:lymphocyte ratio plotted as a function of mercury concentration in flying fox fur. A best fit line from a linear model is plotted in black, with a 95 % confidence interval shaded in grey. BFF: black flying fox, GHFF: grey-headed flying fox, SFF: spectacled flying fox.

atmospheric deposition from car and industrial emissions (Hariono et al., 1993). In our study, Adelaide and Redcliffe had higher human footprint scores than the six other capture sites. Future work could explore metals sources at these sites to better determine potential exposure risks for flying foxes. For example, Adelaide has a history of mining; silver and lead ore were mined beginning in the mid-1800s, and a smelter was built to process ore (Both and Drew, 2008).

While our findings suggest that land use surrounding bat roosts can influence metal concentrations, flying foxes are highly mobile and can move long distances, both while foraging and between roosts (Meade et al., 2021; Roberts et al., 2012). Though we know the capture sites of the bats in our study, we have no data on the length of their stay at these sites, or whether they foraged in environments with a different land use type than that of the roost site (e.g. roosting in a non-urban area but foraging in an urban area) (Egert-Berg et al., 2021). Some studies have suggested that daily turnover at roosts can be between ~10–20 % for BFF and GHFF (Welbergen et al., 2020), while other work has found that GHFF exhibit higher fidelity to roosts located in more urban areas, and have shorter foraging distances when roosting in more urban sites (Meade et al., 2021). Foraging behavior can also differ depending on food availability, with flying foxes traveling long distances to feed on nectar and pollen when abundant, but congregating around resources when they are limited (Eby, 1991; Eby et al., 1999). Roost location also influences foraging; GHFF

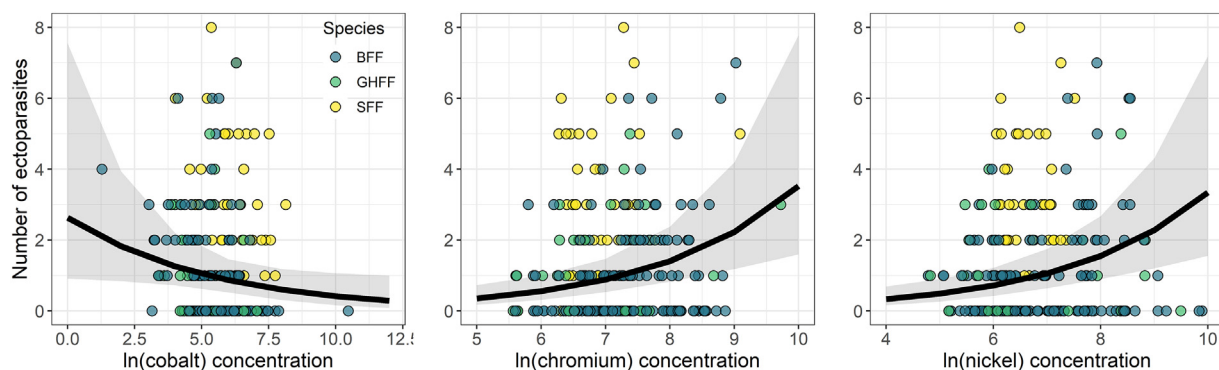


Fig. 4. Predicted counts of ectoparasites (bat flies, family Nycteribiidae) as a function of cobalt, chromium, and nickel concentrations in flying fox fur from each respective generalized linear mixed model. Raw data are overlaid as points. All metal concentrations are ln-transformed and in units of ng/g. BFF: black flying fox, GHFF: grey-headed flying fox, SFF: spectacled flying fox.

roosting at highly urban sites forage more in human-modified land than would be expected based on its availability in the landscape, while those roosting at less urban or non-urban sites forage less in human-modified land than expected (Yabsley et al., 2021). We attempted to control for flexibility in roosting and foraging by calculating average human footprint values within a 20 km buffer around capture sites, which is comparable to foraging distances estimated by some past studies of Australian flying foxes (Parsons et al., 2006; Tidemann, 1999). Longitudinal testing of fur from animals with a known movement history (e.g. obtained through GPS tracking) would likely be useful for inferring metal exposure due to site land use; however, repeated capture of flying foxes is very difficult.

4.2. Seasonal and individual differences in metal concentrations

We found generally lower concentrations of metals in Adelaide GHFF captured during summer (February) compared to those captured during winter (August). Two other sites that were sampled multiple times, but only during winter, did not show temporal changes in metal concentrations, lending support for a true seasonal difference in Adelaide. Although the timing of fur moult (i.e. seasonal shedding of old fur) has been found to influence metal concentrations in other animal species (Cossaboon et al., 2015; Fraser et al., 2013), we have not observed evidence of moulting in flying foxes (authors' personal observations and personal communication with K. Parry-Jones and J. Luly). Instead, seasonal differences in metal levels might be linked to seasonal changes in food availability. Blossoming of flowering species can be scarcer during winter (Birt, 2004; Eby and Law, 2008; Law et al., 2001), which could lead bats to forage more heavily on more available, reliable urban resources (Meade et al., 2021) and expose them to urban-associated contamination. For instance, a previous study of GHFF found that year-round food availability at a suburban site allowed occupation of the camp during winter, in comparison to other sites that were typically abandoned during winter (Parry-Jones and Augee, 2001). Adelaide was the only site where it was possible to make a seasonal comparison, as it was sampled in both summer and winter, while all other sites were sampled only in winter. Future work should aim to capture bats in multiple seasons to build a better picture of longitudinal patterns in metal levels and determine whether the seasonal differences observed in Adelaide occur at other sites. Adelaide is a geographic outlier in terms of GHFF roost sites, with the roost only formed in 2011 and located several hundred km northwest of the species' previously known range (Boardman et al., 2021; Sánchez, 2019), and it therefore may not be representative of other roost sites. A better understanding of flying fox fur replacement could provide insight into temporal changes in metal concentrations, and could be studied at flying fox rehabilitation centers, where bats can be observed over time.

Our findings that SFF and BFF typically had higher metal concentrations in fur than GHFF supports previous limited findings of differences between BFF and GHFF (Pulscher et al., 2020), and might reflect dietary differences between flying fox species. SFF rely heavily on rainforest fruit, sclerophyll (e.g. eucalypt) vegetation, and mangroves, orchards, and fruit trees in urban areas (Parsons et al., 2006; The State of Queensland, 2010). BFF and GHFF are considered dietary generalists, feeding on a variety of flowering and fruiting species (Hall and Richards, 2000; Palmer et al., 2000; Palmer and Woinarski, 1999; Schmeltzschek et al., 2009), though BFF may be more frugivorous compared to GHFF (Griffith et al., 2020). It is possible that greater reliance on fruit could lead to higher metal exposure for SFF and BFF if fruit are contaminated via pesticide spraying. Studies testing metal concentrations in the fruit, nectar, and pollen of common diet plants in different flying fox species across the extent of their ranges would therefore be valuable to understand possible dietary contributions in metal exposure. Data from Australia's *VegeSafe* program, which tests citizen-collected soil samples from gardens and yards for arsenic, cadmium, chromium, copper, manganese, nickel, lead, and zinc (Taylor et al., 2021), could be useful for estimating metal exposure risks for flying foxes that forage in people's yards (e.g. on fruit trees). Species differences in fur metal concentrations could also reflect differences in species distribution.

In particular, SFF have a limited geographic range in far North Queensland (Fig. 1) (Hall and Richards, 2000).

Mercury concentrations were associated with several individual-level predictors; across all sites, adults and females had higher concentrations, and in Adelaide, bats in better body condition had higher concentrations. These results echo previous findings of greater mercury concentrations in adult bats (Heiker et al., 2018; Yates et al., 2014) and bats with greater mass (Syaripuddin et al., 2014) (but see (Heiker et al., 2018)). Mercury is known to bioaccumulate in tissues, which could explain the higher levels found in older, heavier bats. Maternal transfer of metals during gestation and/or lactation (Lisón et al., 2017; Yates et al., 2014) would be expected to reduce mercury levels in female bats, but we observed the opposite pattern. Another study that also found higher mercury concentrations in female bats suggested that female bats may have foraged shorter distances while rearing pups, leaving them closer to point-source contaminated areas (Yates et al., 2014).

4.3. Consequences of metal exposure for flying fox health and parasitism

We collected biological samples from live, outwardly healthy bats, suggesting that the metal concentrations we detected were not sufficient to cause acute poisoning. However, exposure to metals can have cumulative, sub-lethal effects (Becker et al., 2017; Becker et al., 2021; Hernout et al., 2016a; Pulscher et al., 2021; Zukal et al., 2015). Comparison of our results to flying fox fur metal concentrations assessed in previous studies (Table 4) revealed potentially harmful levels of several metals. For instance, a study of Christmas Island flying foxes (CIFF; *P. natalis*) found evidence of chronic cadmium exposure, with 70 % of bats exhibiting urine cadmium concentrations greater than levels considered toxic in humans ($\geq 5 \mu\text{g/g}$ creatinine) (Keil et al., 2011; Pulscher et al., 2021). Additionally, two bats exhibited evidence of renal dysfunction, while another had bone lesions consistent with cadmium intoxication (Pulscher et al., 2021). Cadmium concentrations in SFF fur samples tested here were greater than those measured in CIFF fur samples (median of 84.0 ng/g for SFF versus 70 ng/g for CIFF; maximum of 3,296 ng/g for SFF versus 320 ng/g for CIFF), suggesting that SFF are also exposed to cadmium at toxic concentrations. In addition to cadmium exposure, SFF in our study also had higher fur concentrations of lead (median: 2,264 ng/g, maximum: 32,345 ng/g) than did SFF in the 1980s (median: 580 ng/g, maximum: 8,910 ng/g; (Hariono, 1991)), which is the opposite pattern than would be expected given discontinuation of leaded products (Kristensen, 2015; Pulscher et al., 2020). A possible cadmium and lead exposure source for SFF is recent water contamination events caused by the Baal Gammon copper mine (located ~22 km from the Tolga capture site and ~49 km from the Goldsborough capture site). This now-abandoned mine has been linked to toxic levels of multiple metals, including arsenic, cadmium, copper, and lead in nearby waterways, with residents advised to not use the water for drinking, recreation, or domestic purposes (Blucher and Willacy, 2019; Queensland Government, 2021; Thomas, 2019).

Beyond SFF, median concentrations of copper in BFF and GHFF were ~3–5 times higher than those previously reported (copper in BFF: median of 9,005 ng/g versus 2,828 ng/g; copper in GHFF: median of 9,960 ng/g versus 2,021 ng/g; (Pulscher et al., 2020)). Median concentrations of tin were ~90–125 times higher than previously reported (tin in BFF: median of 5,167 ng/g versus 58.1 ng/g; tin in GHFF: median of 5,420 ng/g versus 43.4 ng/g; (Pulscher et al., 2020)). Inductively coupled plasma mass spectrometry was used to measure concentrations in both studies, making it unlikely that these differences could be due to discrepancies in assay methods. Though strontium concentrations in our study were also high, no flying fox data exist for comparison. Research efforts should prioritize assessing health consequences of copper, tin, and strontium for flying foxes.

We found that higher concentrations of chromium and nickel in fur were associated with greater ectoparasite burden. In experimental studies, chromium and nickel have been demonstrated to have immunosuppressive effects in small mammals, sometimes causing increased susceptibility to

infection and mortality (Arfsten et al., 1998; Smialowicz, 1998). Thus, it is plausible that these metals might reduce flying fox immune defenses against ectoparasites. Higher metal concentrations could also cause lethargy in flying foxes, resulting in lower grooming rates and higher ectoparasite loads. Finally, instead of a direct link between metal concentrations and ectoparasites, particular bat roosts could independently have both higher metal concentrations and ectoparasites. In contrast to the positive correlation between ectoparasite burden and chromium and nickel concentrations, higher concentrations of cobalt were significantly associated with lower ectoparasite burden. Cobalt might be especially poisonous to ectoparasites, or ectoparasites could be acting as sinks for this metal (a phenomenon more typically observed with helminths (Sures et al., 2017)). Further work is needed to examine how combinations of metals collectively affect flying fox immunity and parasitism (Hernout et al., 2016a).

We found limited evidence that haemoparasite infection was associated with metal concentrations in fur; namely, higher strontium concentrations were significantly correlated with lower probability of haemoparasite infection. Our finding of few significant relationships between haemoparasite infection and metal concentrations could be due in part to study methodology. We determined infection status via microscopic examination of blood smears rather than the more sensitive PCR-based screening for detecting haemosporidian infections (Schaer et al., 2019; Schaer et al., 2018; Valkiūnas et al., 2008). Additionally, the blood smears were stained several months to years after they were collected, which can reduce blood smear quality (Valkiūnas et al., 2008). Alternatively, parasite infection risk could depend more strongly on factors that shape vector presence than on toxic effects of metals on host immunity. For instance, trypanosome infection prevalence in common fruit bats (*Artibeus jamaicensis*) was higher in bats captured in forest fragments compared to those in continuous forest, which was suggested to be due to greater exposure to arthropod vectors in fragmented habitats (Cottontail et al., 2009).

4.4. Future directions

Our work shows that nectarivorous and frugivorous bats, which have been understudied in wildlife toxicology research, are routinely exposed to many metals, and that at least some of these metals carry a physiological cost in the form of greater parasitism. Looking forward, there is a crucial need to understand how landscape variables and individual traits affect bat exposure to metal sources. Research is also needed on how toxicant exposure affects bat immune defense and susceptibility to infection. Early experimental work that involved repeatedly dosing flying foxes with lead acetate found that total and differential leucocyte counts fluctuated over time, but did not appear linked to lead toxicity (Hariono, 1991); however, only two flying foxes were involved in the study, making it difficult to generalize from the results. More recently, a study of 29 bat species in Belize found complex relationships between concentrations of mercury in fur, immune measures, and infection with two bacterial pathogens, with infection patterns mediated by bat taxonomy (Becker et al., 2021). Our study supported previous findings of a positive relationship between bat fur mercury concentrations and NL ratio (Becker et al., 2017). In other vertebrate taxa, high NL ratios are associated with greater stress (Davis et al., 2008), yet this assumption has not yet been validated in bats. Our study failed to detect paramyxovirus infection in a small set of urine samples, but studying antiviral gene expression in relation to toxicant exposure would be useful to explore (Schountz, 2014; Zhou et al., 2016). In Australia, flying foxes (in particular, BFF and SFF, but see (Wang et al., 2021)) can transmit deadly Hendra virus to horses and then to humans; transmission events typically occur in peri-urban areas, where bats feed on planted food resources (Plowright et al., 2015). If these urban areas are also where flying foxes face the most toxicant exposure, this could contribute to bat susceptibility to infection and potential for spillover.

Bats as a group face many stressors, including toxicant exposure, habitat loss, climate change and extreme weather events, disease, and hunting by humans (Jones et al., 2009; O'Shea et al., 2016; Zukal et al., 2015). In Australia, habitat loss and extreme heat events have exacerbated flying

fox population declines (Threatened Species Scientific Committee, 2019; Welbergen et al., 2008) and contributed to the listing of SFF and GHFF as Endangered and Vulnerable, respectively, under the IUCN Red List (Lunney et al., 2020; Roberts et al., 2020) and Australia's Environment Protection and Biodiversity Conservation Act (Threatened Species Scientific Committee, 2001; Threatened Species Scientific Committee, 2019). Given the critical role that flying foxes play in pollination and seed dispersal (Aziz et al., 2021; Fujita and Tuttle, 1991; Marshall, 1983), it is important to understand whether their use of urban habitats for roosting and foraging is further endangering these species through exposure to metals and other toxicants.

CRediT authorship contribution statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary material (methods, figures, and tables) to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2022.156699>. The data supporting this publication can be found online at <https://doi.org/10.5281/zenodo.6646941>.

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