1-1-2023

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10.1111/avj.13252
Hutchinson, D. J., Jones, E. M., Pay, J. M., Clarke, J. R., Lohr, M. T., & Hampton, J. O. (2023). Further investigation of lead exposure as a potential threatening process for a scavenging marsupial species. Australian Veterinary Journal, 101(8), 313-319. https://doi.org/10.1111/avj.13252

ORIGINAL ARTICLE

Further investigation of lead exposure as a potential threatening process for a scavenging marsupial species

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There is a growing recognition of the harmful effects of lead exposure on avian and mammalian scavengers. This can lead to both lethal and non-lethal effects which may negatively impact wildlife populations. Our objective was to assess medium-term lead exposure in wild Tasmanian devils (*Sarcophilus harrisii*). Frozen liver samples ($n = 41$), opportunistically collected in 2017–2022, were analysed using inductively coupled plasma mass spectrometry (ICP-MS) to determine liver lead concentrations. These results were then used to calculate the proportion of animals with elevated lead levels (>5 mg/kg dry weight) and examine the role of explanatory variables that may have influenced the results. The majority of samples analysed were from the south-east corner of Tasmania, within 50 km of Hobart. No Tasmanian devil samples were found to have elevated lead levels. The median liver lead concentration was 0.17 mg/kg (range 0.05–1.32 mg/kg). Female devils were found to have significantly higher liver lead concentrations than males ($P = 0.013$), which was likely related to lactation, but other variables (age, location, body mass) were not significant. These results suggest that wild Tasmanian devil populations currently show minimal medium-term evidence of exposure to lead pollution, although samples were concentrated in peri-urban areas. The results provide a baseline level which can be used to assess the impact of any future changes in lead use in Tasmania. Furthermore, these data can be used as a comparison for lead exposure studies in other mammalian scavengers, including other carnivorous marsupial species.

Keywords animal welfare; toxicology; trace elements; wildlife

Aust Vet J 2023;101:313–319 doi: 10.1111/avj.13252

Lead is a widely studied heavy metal with several well-known toxic effects. Exposure to lead is primarily detrimental to the nervous system, but lead adversely affects nearly every physiological system, and non-lethal exposure can manifest as neurological dysfunction, anaemia and loss of body condition.[1] Toxicoses are most often chronic and associated with cumulative low-dose exposures, and both acute and chronic toxicoses have the potential to prove fatal.[2] Contemporary research has implicated lead-based ammunition as one of the most significant sources of anthropogenic environmental lead around the world.[3] Studies analysing game meat (meat derived from wildlife) have demonstrated that the use of lead-based ammunition can contaminate carcasses.[4] This has led to concern regarding the numerous species of wildlife known to scavenge from wildlife shot with lead-based ammunition.[2]

In wildlife, the consequences of exposure to spent lead-based ammunition have been most extensively studied in avian species. Scavenging species such as the California condor (*Gymnogyps californianus*) have been comprehensively studied and shown to be negatively impacted by lead-based ammunition, this being the primary factor that nearly caused the extinction of the species.[5,6] In Australia, this effect has also been studied and demonstrated in wedge-tailed eagles (*Aquila audax*).[7–9]

While bird species have been the focus of most lead ecotoxicology research, there is growing recognition on the effects of lead exposure on wild mammalian scavengers.[10] The majority of studies in this field have assessed eutherian carnivores from the Northern Hemisphere, particularly brown bears (*Ursus arctos*),[11–14] as well as black bears (*Ursus americanus*),[13,15] grey wolves (*Canis lupus*),[13,16] and cougars (*Puma concolor*).[13] There is also concern for potential impacts on other species for which regular scavenging occurs but lead exposure levels are yet to be quantified, such as Iberian lynx (*Lynx pardinus*)[17] and dingoes (*Canis familiaris* / *Canis dingo*).[18] To our knowledge, lead exposure has only been reported in one mammalian scavenger species from the Southern Hemisphere, and only one marsupial species, the Tasmanian devil (*Sarcophilus harrisii*).[19]

The Tasmanian devil is a morphologically specialised scavenger (Figure 1).[20] As Tasmania's largest extant carnivore, they play important ecological roles such as influencing carrion persistence[21] and suppressing feral cats (*Felis catus*).[22] They are endangered due to the impacts of a transmissible cancer, devil facial tumour disease (DFTD), which has caused population declines by over 80% since 1996.[23] Devils are the focus of intense conservation efforts, including efforts to proactively identify any other threatening processes that may prevent or delay recovery of the species, including toxicological threats from anthropogenic activities such as lead derived from ammunition.[19] As such, a 2019 study investigated lead exposure in captive and wild devils and found that feeding captive devils carcasses of lead-shot animals was associated with raised lead levels,[19]

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A

B

Figure 1. Our study species, the Tasmanian devil (*Sarcophilus harrisii*), a nocturnal marsupial carnivore, displaying scavenging behaviour that makes them susceptible to elevated lead exposure from ammunition residues.

but the degree of lead exposure in wild devils was restricted to blood lead concentrations.

When analysing lead concentrations, different tissue types reflect varying timeframes of exposure. Because of the rapid uptake and redistribution of lead from the blood, blood lead is most representative of short-term lead exposure over 2–6 weeks.[24,25] In contrast, soft tissues, such as the liver, are the primary areas of initial lead redistribution from the blood and reflect medium-term lead exposure over 3–6 months.[16] Finally, bone and teeth store lead throughout the life of the animal and can be used as indicators of cumulative long-term lead exposure over an animal's entire lifetime.[15,26] In the aforementioned study on Tasmanian devils, only blood samples were used, and therefore only recent lead exposure was able to be evaluated.[19]

The potential for harmful lead exposure in threatened mammal species via the ammunition route is particularly concerning in Australia for three main reasons. First, several species of Australian scavenging mammals are endangered, and theoretically vulnerable to lead exposure via feeding on the carcasses of shot animals.[18] Australia has historically suffered from a reputation as having one of the highest mammalian extinction rates in the world,[27] with 38.8% (33/85) of the International Union for the Conservation of Nature (IUCN) Red List's recorded mammalian extinctions.[28] Second, shooting is widely used in wildlife management (culling, hunting, and commercial harvesting) in Australia, and relies almost exclusively on lead-based ammunition,[18] with very few exceptions.[29,30] Third, very few studies have been conducted on lead exposure in Australian mammals.[19,31,32] For Tasmanian devils in particular, their low population size and heavy reliance on scavenging makes the potential impact of lead contamination especially concerning.

Here, we attempted to provide a longer-term assessment of lead exposure in wild Tasmanian devils through opportunistic sampling of archived liver samples.

Materials and methods

Sample collection

Frozen liver samples from Tasmanian devils ($n = 41$) were obtained from archived tissue specimens in the Tasmanian Museum and Art Gallery (TMAG) and the University of Tasmania (UTAS). All liver samples were collected between April 2017 and February 2022 from across Tasmania but concentrated on urban areas surrounding Hobart (Figure 2). Whole frozen carcasses were thawed and then surgically dissected to allow collection of ~1 g of liver into a labelled and sterile polypropylene pot, and then re-frozen. Animal body mass (kg), age and sex were recorded during dissection using standard methods established for the species.[33] The Australian Bureau of Statistics (ABS) definition of a significant urban area (SUA) was used to define locations as 'urban', whilst all other locations were considered "rural" for the purpose of this study.[34]

Lead concentration measurement

The samples were analysed at Edith Cowan University Analytical Facility (Joondalup, WA, Australia) to determine their lead concentrations. The liver samples were homogenised, and a 0.4 g aliquot was transferred into inductively coupled plasma (ICP)-grade Teflon vessels which contained 5 mL of trace analysis-grade nitric acid 68% (Primer Plus™; Fisher Scientific), 0.5 mL of trace metal–grade hydrochloric acid 34%–37% (Fisher Scientific), and 3 mL of hydrogen peroxide 30% (Emsure ISO®; Merck). The samples were digested for 15 min in a Multiwave GO microwave digestion system (Anton Paar) which was set to 150°C. Samples were subsequently diluted with Milli-Q® reverse osmosis deionized water to a total volume of 50 mL before being transferred to polypropylene tubes. The lead concentration of these samples was determined via inductively coupled mass spectrometry (ICP-MS) using an iCAP Q ICP-MS (Thermo-Fisher Scientific) coupled to an ASX-520 AutoSampler (Agilent Technologies).

A standard curve was created with concentration ranges of iCAPQ element standards (Thermo- Fisher Scientific) and ICP-MS-68A solutions (High Purity Standards) to calibrate the instruments prior to analysis. Bovine Liver Certified Reference Material BCR-185R.

(Institute for Reference Materials and Measurements) was used as a positive control. The reference material was digested twice, and two

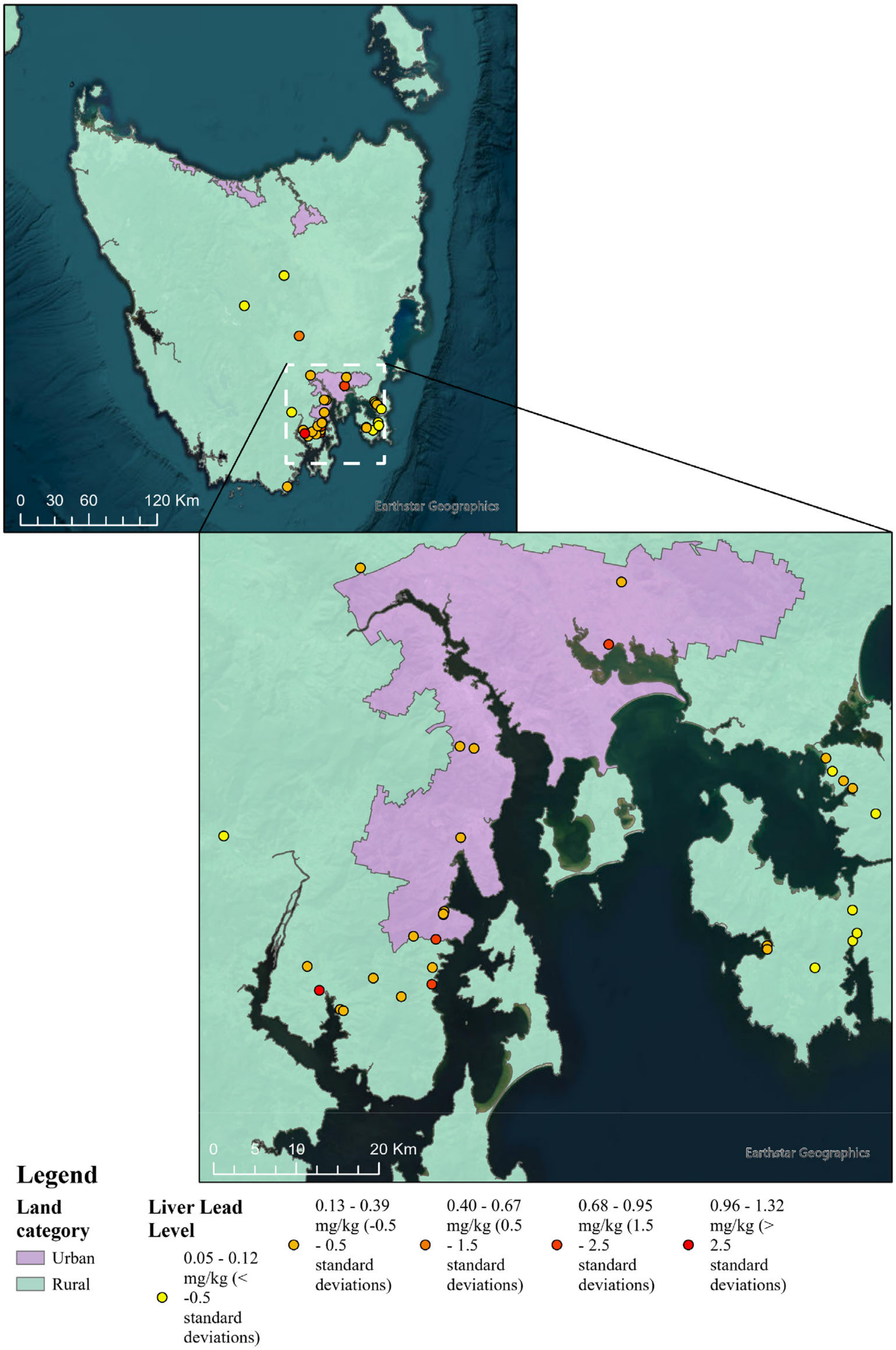

Figure 2. **Map of sample collection locations for 41 liver samples from Tasmanian devil (*Sarcophilus harrisii*) sampled opportunistically from 2017 to 2022. Land is categorised as either urban or rural. Samples are colour coded according to how many standard deviations lead concentrations lie away from the mean.**

ICP-MS readings were made on each digestion. The liver reference material had an average accuracy of 104.1% for the ICP-MS readings. Every fifth-sixth sample was re-analysed to create a duplicate read (average relative standard deviation [RSD] = 1.8%), and duplicate blind sample digestions were carried out on five randomly selected samples (average RSD = 1.3%). The lead concentrations were measured in mg/kg of dry weight. The limit of detection (LOD) was 0.0015 mg/kg and the limit of quantification was 0.005 mg/kg.

Data analysis

To answer each of our study questions, we conducted separate statistical analyses. The majority of the devil carcasses collected lacked specific metadata, such as the date and or body mass. We therefore chose separate analyses over multivariate models to maximise the data available to answer each of our questions. We used nonparametric tests in our analysis as the liver lead data did not meet assumptions for parametric univariate analyses even after log transformation. We used a significance level of $\alpha = 0.05$ in all analyses.

Devil carcasses were not collected in a spatially random manner. Therefore, to ensure that each sample could be considered independent for further analyses we first tested the lead concentrations data for spatial autocorrelation. We log transformed the liver lead concentrations and used Moran's Index (function 'Moran.I' in R package "ape")[35] to estimate the size of any spatial association in the lead concentrations between samples.[15]

We used Wilcoxon rank sum tests (function "wilcox.test")[36] to compare lead concentrations in liver between the sexes (male and female), between the age cohorts (adult and juvenile), and between devils founds in urban vs rural areas. As our liver lead level data were non-parametric, we used a Kendall-Theil Sen Siegel regression (function "mblm" in R package 'mblm')[37] to test the association of the liver lead concentrations with the mass of the devil and the date that the carcass was collected. Each variable was assessed in a separate model to maximise the data available.

To compare our lead concentrations with those in other studies, we used an exposure threshold of blood lead <5 mg/kg dry weight as indicative of low exposure,[38] and >5 mg/kg dry weight as indicative of elevated exposure.[16] However, we recognize that less work has gone into establishing clinical exposure thresholds for lead in mammals when compared with birds,[10] and we acknowledge that both physiological and behavioural effects have been reported in some wild animals at levels well below traditional thresholds for clinical effects.[39]

Table 1. **Summary statistics (range, median, geometric mean and standard deviation) of liver lead concentrations (mg/kg dry weight) for various groups of Tasmanian devils (*Sarcophilus harrisii*) sampled from 2017 to 2022**

	n	Min	Max	Median	GM (+SD)
All animals	41	0.05	1.32	0.17	0.18 ± 2.25
Male[a]	28	0.05	1.32	0.13	0.14 ± 1.93
Female[a]	12	0.05	0.91	0.26	0.29 ± 2.61
Adult	23	0.05	1.32	0.19	0.21 ± 2.45
Juvenile	16	0.05	0.81	0.14	0.14 ± 1.99
Urban	8	0.12	0.70	0.20	0.21 ± 1.69
Rural	27	0.05	1.32	0.13	0.17 ± 2.39

[a] Significant difference between these two groups for mean liver lead (P = 0.013).

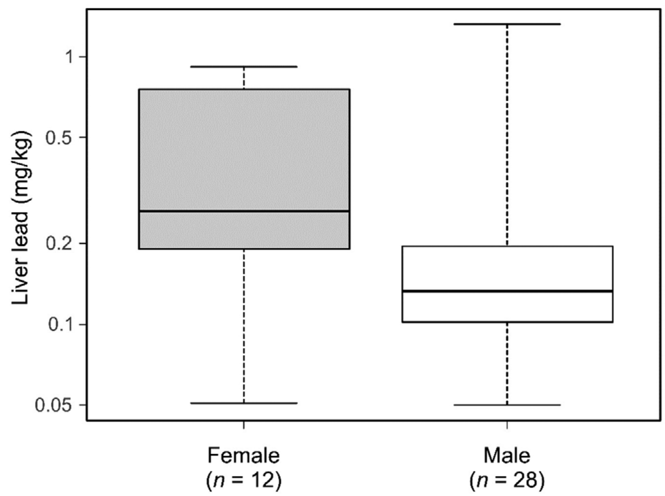

Figure 3. **Liver lead concentrations between sexes in Tasmanian devils (*Sarcophilus harrisii*) sampled opportunistically from 2017 to 2022. Box plot whiskers are extended to minimum and maximum values. The plot is presented on a log scale.**

Results

Lead concentrations were above the LOD of the ICP–MS in all Tasmanian devil liver samples analysed, but no samples were found to have elevated liver lead levels (>5 mg/kg), with a median concentration of 0.17 mg/kg (range 0.05–1.32 mg/kg, $n = 41$; Table 1). There was no evidence of spatial organisation in the lead concentrations (Moran's I: $I = 0.089$, P = 0.170, $n = 35$). Lead concentrations in livers were different between sexes ($W = 253$, P = 0.013, $n_{male} = 28$, $n_{female} = 12$), with females having higher lead concentrations (Figure 3). Lead concentrations in livers were similar in both age groups ($W = 230$, P = 0.194, $n_{adult} = 23$, $n_{juvenile} = 16$). Lead concentrations were not different between animals categorised as urban or rural ($W = 94$, P = 0.257, $n_{urban} = 8$, $n_{rural} = 32$). Liver lead concentrations were not associated with the body mass of the devils ($MAD = -0.003$, P = 0.393, $df = 14$).

Discussion

This study provides preliminary evidence to suggest that wild Tasmanian devils currently experience limited lead exposure, with no cases consistent with markedly elevated exposure discovered. This aligns with an earlier study on Tasmanian devils which found that 6.1% of sampled wild devils exceeded an uncommonly-used blood lead level threshold of 0.20 μmol/L (equivalent to ∼4.14 μg/dL).[19] However, it is worth noting that more recent studies have used a lower threshold for elevated blood lead concentrations in non-human mammals (1.2 μg/dL).[12,40] Interestingly, Hivert et al.[19] found that devils from the Forestier Peninsula had higher lead exposure levels than devils sampled in central Tasmania, but the authors did not provide a hypothesised mechanism for this difference.[19]

The only factor found to significantly affect liver lead in this study was sex, with female devils having higher mean liver lead levels. Similar findings have been made in liver lead levels in white-footed mice

(*Peromyscus leucopus*),[41] blood lead levels in brown bears[12] and tooth lead levels in black bears,[15] and were theorised to be a result of bone lead mobilisation during pregnancy and lactation. Studies in humans further corroborate this theory as they have demonstrated that both pregnancy and lactation lead to bone lead mobilisation in association with bone calcium mobilisation.[42,43]

Our results are comparable to a study assessing livers from grey wolves from the USA, which found only 0.7% (1/147) of animals had clinically elevated liver lead levels (>5 mg/kg dry weight), although they reported their results in wet weight.[16] A similar study in the USA's greater Yellowstone ecosystem found a much greater proportion of brown bears demonstrated clinically elevated lead levels, with 13.4% of analysed brown bears having blood lead concentrations greater than 10 μg/dL and a mean blood lead concentration of 5.5 μg/dL for all sampled brown bears.[13] None of the other species sampled in the greater Yellowstone ecosystem were found to have any individuals with clinically elevated lead levels for any of the sample types used.[13] It is worth noting that recreational hunting is not allowed within Yellowstone National Park.[13] Additional studies of brown bears in northern Europe have found blood lead levels ranging from 3.3–22.1 μg/dL with a mean concentration of 9.3 μg/dL.[11,12]

However, unlike the Northern Hemisphere eutherian carnivore species discussed above, the Tasmanian devil is morphologically specialised for scavenging (though they are also effective hunters), with robust teeth and jaw strength for processing bone.[20] In the absence of devils, other carnivores in Tasmania are unable to match their rate of carrion consumption.[21] Devils may therefore consume proportionally more carrion, and more of each carcass, than their North American counterparts, increasing their risk of ingesting lead fragments and explaining the relatively high proportion of devils showing detectable (although not elevated) lead exposure.

This study was limited by several factors. First, we achieved a relatively small sample size as a product of relying on opportunistic sampling ("active surveillance")[44] and a relatively short time window for these samples (5 years). Second, our samples were not randomly geographically distributed,[7] with a majority of samples derived from areas with relatively high human population density, close to Tasmania's largest city. Third, a lack of covariate data (e.g., age, sex, body mass, and cause of death) for the animals sampled constrained our ability to explore the role of explanatory variables in explaining the variation we observed. Fourth, tissues indicative of longer-term exposure (e.g., bone,[45] hair,[46] or tooth[15]) were not examined, so our results reflect only the dietary intake of sampled animals in the weeks–months leading up to their deaths.

Our findings do not necessarily suggest that no Tasmanian devils are at risk of harmful lead exposure from ammunition. Our results should be interpreted cautiously as we examined a relatively small geographical scale. It is likely that lead exposure in devils differs among landscape types.[15] Tasmanian devils commonly inhabit forestry plantation landscapes,[47] and forested areas fringing agricultural land.[48] They are known to scavenge on the carcasses of macropods (e.g. red-necked / Bennett's wallaby [*Notamacropus rufogriseus*]) shot during forestry or agricultural culls,[49] with one study showing that a quarter of marsupial browsers killed by a cull were scavenged.[50] Lead-based ammunition continues to be used for all such culling.[51] Devil populations in such sites are likely to be at higher risk of elevated lead exposure, even though this assessment has shown overall low exposure. Further investigation of the lead exposure status of devil populations living on or close to these land use types is warranted, as is analysis of tissue types were representative of long-term exposure, such as bone or tooth.

The results of this study also do not necessarily suggest that no scavenging mammals in Australia are at risk of elevated lead exposure from ammunition. We examined only a single species. There are other mammalian scavengers that may be at risk of elevated lead exposure from ammunition that we did not examine, for example, eastern quolls (*Dasyurus viverrinus*) in Tasmania,[52] and dingoes on mainland Australia.[53]

Conclusions

Our findings represent a preliminary assessment of medium-term lead exposure in Tasmanian devils and complement previous studies that have relied on blood sampling.[19] Our results can provide future studies with a baseline against which other mammalian scavengers can be compared and to assess the impact of future changes in wildlife management practices.

Acknowledgments

We acknowledge the Palawa people as the Traditional Owners of the lands on which this research was conducted. This study assessed tissues from dead animals and did not require Animal Ethics Committee (AEC) approval. Sample collection was performed under a permit to take threatened fauna for scientific purposes (#TFA 21284) from the Tasmanian Department of Natural Resources and Environment. Samples were shipped interstate for laboratory analysis under a Wildlife Export Permit (#TFA 21284) from the Tasmanian Department of Natural Resources and Environment. For access to samples, we acknowledge the following people: Bill Brown and Sarah Peck from the Department of Natural Resources and Environment Tasmania, and David Hocking and Nicole Zehntner from the Tasmanian Museum and Art Gallery (TMAG). For assistance with dissections, we thank Regi Broeren. For laboratory analysis, we thank Mark Bannister from Edith Cowan University. For fauna licences needed to import samples to Victoria, we thank Sue Hadden and Roberta Campbell from the Victorian Department of Environment, Land and Water. For the creation of the map (Figure 2) and assistance with the analysis of geospatial data, we thank Stephanie Daborn from Earthstar Geographics. Open access publishing facilitated by The University of Melbourne, as part of the Wiley - The University of Melbourne agreement via the Council of Australian University Librarians.

Conflict of interest and funding information

The authors declare no conflicts of interest. This study was funded by the University of Melbourne McKenzie Fellowship Program.

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(Accepted for publication 21 May 2023)