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The influence of faecal contamination on foraging behaviour in free-ranging eastern grey kangaroos (*Macropus giganteus*)

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Study site and species

Our study was conducted within the catchment of Yan Yean Reservoir (145°09'E, 37°32'S), 37 km north-east of Melbourne, Victoria, Australia. This 2285-ha catchment supported a high density of eastern grey kangaroos (*Macropus giganteus*), estimated at 2.2 ha^{-1} when last surveyed in 1997 [1]. Eastern grey kangaroos at Yan Yean were host to a rich assemblage (25 species) of parasitic nematodes, dominated by *Rugopharynx macropodis* (Strongyloidea) at 100% prevalence and mean abundance of 58,575 [2,3]. In sub-adult hosts, high mortality was correlated with heavy burdens of *Globocephaloides trifidospicularis* (Trichostrongyloidea); parasite load and mortality was markedly lower in adult kangaroos, suggesting that surviving hosts developed immunity to this species [2].

Experimental design

We conducted a grazing experiment on a gentle sloping clearing about 1 ha in area, which was typical of the habitat that eastern grey kangaroos favoured for foraging in the catchment [4]. The clearing was dominated by a range of native and exotic grass species including wallaby grasses (*Austrodanthonia* spp.), veined spear-grass (*Austrostipa rudis* ssp. *nervosa*), sweet vernal grass (*Anthoxanthum odoratum*), squirrel-tail fescue (*Vulpia bromoides*) and tussock grass (*Poa labillardierii*). Kangaroos emerged from the cover of surrounding woodland, consisting mainly of swamp gum (*Eucalyptus ovata*), river red gum (*E. camaldulensis*) and Monterey pines (*Pinus radiata*).

We established a chequerboard of 5 x 5-m cells, with 50 contaminated and 50 uncontaminated cells, in the centre of the clearing. In an enhancement of previous study designs [5,6] we randomised the position of each cell type in the chequerboard and added a buffer of randomised cells to prevent edge effects (Figure 1). We delineated the boundaries of the cells with a grid of taut string, and marked the intersections of the grid with small, colour-coded stakes to identify the ten rows and columns of the chequerboard during behaviour sampling. We also constructed a hide at the top of the slope, 20 m from the edge of the buffer.

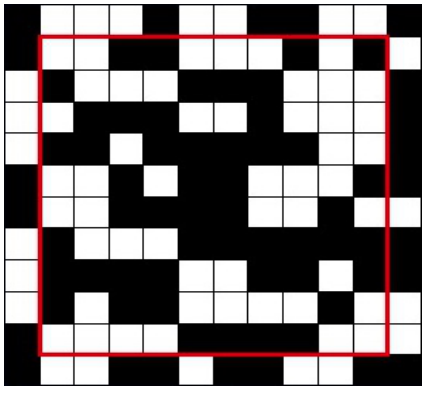

Figure 1. The randomised chequerboard design used at Yan Yean Reservoir. Each cell was 5 x 5 m in size. Shaded cells were contaminated with faecal pellets of eastern grey kangaroos (*Macropus giganteus*); unshaded cells had all pellets removed. The red line shows the boundary between the experimental array of 100 cells, where the behaviour of kangaroos was recorded as they foraged, and the outer buffer of cells.

In July 2003, we raked kangaroo faeces into piles within each cell, collected the pellets in buckets, hand-compressed them and measured the volume to the closest litre. We spread twice the average level of faeces evenly back onto only the contaminated cells, leaving the uncontaminated cells with close to zero pellets. We maintained the cells every three weeks to November 2003 by removing faeces only from uncontaminated cells and spreading them evenly over contaminated cells.

Behavioural observations

We entered the hide prior to each observation session in the two hours preceding dusk, corresponding to the activity peak for eastern grey kangaroos [7]. We used a grid-reference method to record any cell occupied by a kangaroo, minimising possible bias because the observer (VB) was essentially blind to the contamination type of each cell. The observer used 10 x 50 binoculars or a 20-60x Kowa spotting telescope mounted on a tripod as necessary, and described behavioural observations softly into a micro-cassette recorder. In total, we conducted 40 observation sessions from September to November, 12 of which were included in the analysis as these were the only times that kangaroos foraged on the chequerboard.

Scanning from left to right across the chequerboard (excluding the buffer), the observer recorded the grid reference of the cell occupied by each kangaroo (n = 108), its sex and age-class, and the time of observation. We distinguished sub-adults by their smaller size than adults, and classified adult females as having either a large or no visible pouch young. Whenever a kangaroo changed position, the observer recorded the time and grid reference, allowing us to determine the duration of time spent on a contamination type, then classify each as preferring (i.e. spending > 50% of time in) uncontaminated or contaminated cells. We were able to recognize individuals within an observation session. We assumed that individuals were not re-sampled over different sessions, which was supported five individuals that were recognizable by a distinctive feature (e.g. torn ear, blind eye) and were seen in only one session.

Results

Adult kangaroos (n = 88) showed only a weak and non-significant significant preference for either cell type (Fig. 2, $\chi^2 = 3.682$, $p = 0.055$). In contrast, 80% of sub-adults (n = 19) foraged more on uncontaminated cells (Fig. 2, $\chi^2 = 6.05$, $p < 0.025$), consistent with their greater vulnerability to parasite infection [2].

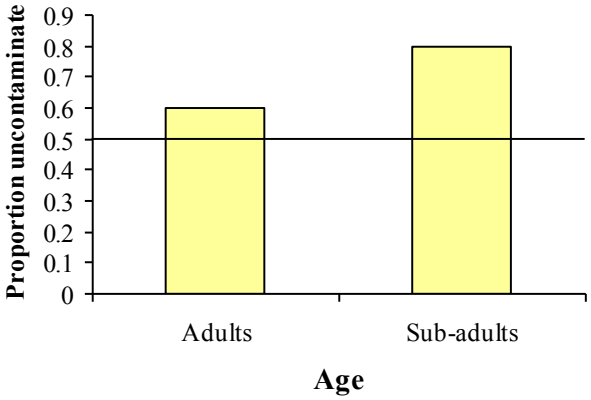

Figure 2. The proportion of adult and sub-adult eastern grey kangaroos preferinng uncontaminated cells at Yan Yean. Horizontal line indicates no preference.

There were also differences related to the costs of reproduction. Adult females with large pouch-young (n = 19) had heavy energetic demands to support lactation [8], and showed no clear preference for either cell type (Fig. 3, $\chi^2 = 0.211$, $p > 0.10$). In contrast, of adult females with no visible pouch-young (n = 41), hence negligible reproductive costs, 66% preferred uncontaminated cells (Fig. 3, $\chi^2 = 4.073$, $p < 0.05$).

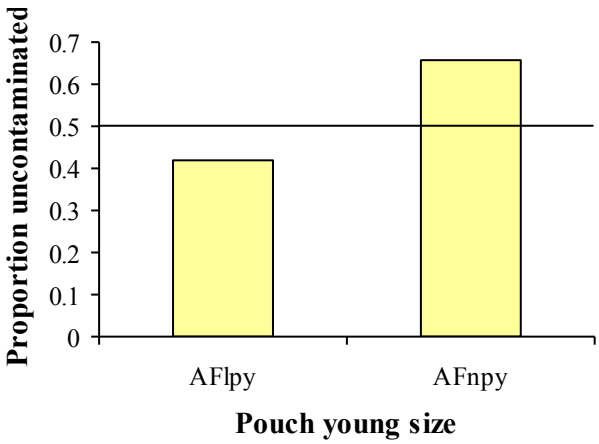

Figure 3. The proportion of adult female eastern grey kangaroos with large pouch young (AFlpy) and no visible pouch young (AFnpy) preferring uncontaminated cells at Yan Yean. Horizontal line indicates no preference.

References

1. Coulson G, Alviano P, Ramp D, Way S, McLean N, Yazgin V. 2000 The kangaroos of Yan Yean: Issues for a forested water catchment in a semi-rural matrix. In *Nature Conservation 5: Nature conservation on production environments: managing the matrix* (eds. JL Craig, N Mitchell, DA Saunders) pp. 146–156. Chipping Norton, Australia: Surrey Beatty & Sons.
2. Arundel JH, Dempster KJ, Harrigan KE, Black R. 1990 Epidemiological observations on the helminth parasites of Macropus giganteus Shaw in Victoria. Wildl. Res. 17, 39–51. (doi:10.1071/WR9900039)
3. Cripps J, Beveridge I, Martin JK, Borland D, Coulson G. 2015 Temporal dynamics of helminth infections in eastern grey kangaroos (Macropus giganteus) in Victoria. Aust. J. Zool. 63,163-174. (doi.org/10.1071/ZO15003)
4. Moore BD, Coulson G, Way S. 2002 Habitat selection by adult female eastern grey kangaroos. Wildl. Res. 29, 439–445. (doi:10.1071/WR01057)
5. Cooper J, Gordon IJ, Pike AW. 2000 Strategies for the avoidance of faeces by grazing sheep. Appl. Anim. Behav. Sci. 69, 15–33. (doi.org/10.1016/S0168-1591(00)00116-7)
6. Hutchings MR, Gordon IJ, Kyriazakis I, Jackson F. 2001 Sheep avoidance of faeces-contaminated patches leads to a trade-off between intake of forage and parasitism in subsequent foraging decisions. Anim. Behav. 62, 955–964. (doi.org/10.1006/anbe.2001.1837)
7. Southwell C. 1987. Activity pattern of the eastern grey kangaroo, *Macropus giganteus*. Mammalia 51, 211–223. (doi:10.1515/mamm.1987.51.2.211)
8. Cripps JK, Wilson ME, Elgar MA, Coulson G. 2011 Experimental manipulation of fertility reveals potential lactation costs in a free-ranging marsupial. Biol. Lett. 7, 859–862. (doi:10.1098/rsbl.2011.0526)