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Exploratory behaviour in northern brown bandicoots (*Isoodon macrourus*) in Tropical North Queensland, Australia

Exploration in bandicoots. Callaway et al.

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Abstract

Little is known of bandicoot and bilby (i.e. Peramelemorphia) exploratory behaviour (e.g. activity in an open field). In a pilot study, we assessed activity of 14 adult male northern brown bandicoots (*Isoodon macrourus*) in a modified open field over two nights. While we found no consistent intra-individual variation, males in poorer body condition were in better breeding condition, suggesting a possible trade-off between reproduction and maintenance. Older males with larger testes and in better breeding condition reduced activity from Day 1 to Day 2, possibly to minimise energetic expenditure. Our pilot study of the exploratory behaviour of northern brown bandicoots suggests interesting avenues for future research in Peramelemorphia behaviour generally.

Keywords: Inter-individual variation, Marsupial, Peramelemorphia, Repeatability, Trade-off

Introduction

Ecosystems are threatened by habitat loss and degradation (Chase *et al.* 2020), leading to population- and species-level extinctions. Consequently, organism persistence will be contingent on genetic variability (Pinto *et al.* 2024) and the ability of individuals to behaviourally adjust to novel conditions (Mazza and Šlipogor 2024).

Individuals vary in their levels of exploration of novel environments (Wat *et al.* 2020; Réale *et al.* 2007), which influences other behaviours (e.g. response to predators; Eccard *et al.* 2020). Exploration also affects fitness. For example, more exploratory individuals may be more competitive (Linnenbrink 2022) and more likely to forage under increased predation risk (Coomes *et al.* 2021).

"Fast explorers" can dominate spatially and temporally predictable resources due to faster, more superficial, exploration (Careau *et al.* 2009). However, fast explorers are slow reactors, which could result in increased likelihood of predation (Smith *et al.* 2009; Coomes *et al.* 2021). In contrast, "slow explorers", despite taking longer to explore the environment (Careau *et al.* 2009), are behaviourally flexible and faster learners, resulting in better coping with environmental changes (Mazza *et al.* 2018).

Behavioural studies of bandicoots and bilbies (order Peramelemorphia) are limited (Lyne 1981; Moloney 1982; Broughton and Dickman 1991; Fardell *et al.* 2022; Cornelsen

2023; Edwards *et al.* 2023; Randall *et al.* 2023; Tay *et al.* 2023; Gagnon and Bateman 2024; Waaleboer *et al.* 2024; McLean *et al.* In Press). Only two studies on exploration in northern brown bandicoots (*Isoodon macrourus*) have been conducted (Russell and Pearce 1971; Day *et al.* 1974). Therefore, in this pilot study, we investigated exploratory behaviour of adult male northern brown bandicoots in a modified open field.

Materials and Methods

Bandicoots (n = 14) were trapped (September-October 2015) on the James Cook University Nguma-bada Campus, Queensland, using baited cage traps (set by dusk and checked at first light). Bandicoots were marked (trimmed 1-2 cm of thigh hair) to prevent re-sampling. Bandicoots were transported to a quiet campus laboratory, transferred to a pet carrier (60 x 50 cm) with water and bedding (feed-quality hay), given food (mealworms, chopped apple, canned cat/kitten food), and left to adjust for approximately 12 hours.

We constructed a metal modified open field arena (2 m L x 2 m B x 1.8 m H) with vinyl floor (Fig. 1). Three novel objects (a clear class bowl, a blue mug and a white bowl, each approx. 5 cm at the longest length) were assigned randomly to a location, 30 cm from the wall (Fig. 1) to reduce wall-hugging (thigmotaxis) behaviour (Casarrubea *et al.* 2008). Locations remained consistent across all trials, but objects were rotated for each bandicoot to control for bias.

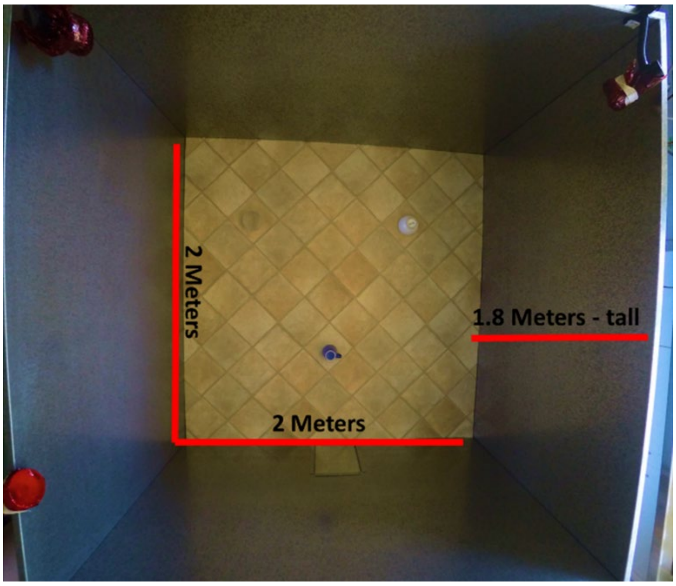

Fig. 1. Open field arena showing the placement of three novel objects. The placement of objects was consistent, but the type of object placed was random.

To assess short-term repeatability (i.e. consistency over time; Rowell and Rymer 2023), bandicoots were individually tested over two consecutive nights (under red light; Jud *et al.* 2005). We were restricted in the length of time we could hold the animals in captivity (permit requirements). At the test start (~ 20h00), a bandicoot was transferred to the arena in a cloth bag, gently removed after 10 minutes, and then had 15 minutes to explore the arena. As bandicoot activity sharply declined after the first 5 minutes (possibly due to stress), we did not feel it appropriate to conduct multiple trials in one night. Behaviours were video recorded from above (GoPro Hero4 camera) in the absence of observers. We measured duration of time spent active (wall-hugging, moving, investigating the novel objects) as a proportion of total time, which was our measure of exploration. Bandicoots were returned to the pet carrier until testing the next night. Equipment was cleaned and wiped down with 70% ethanol. Bandicoots were released at the site of capture after the second day of testing.

Statistical Analyses

Data were analysed using Rstudio (version 2022.07.2; https://www.rproject.org; R version 4.2.2, R Core Team 2020). Data were tested for normality (Shapiro-Wilks test) and transformed where necessary. Relative testis area (length x width) was standardized to relative body size (testis area/hind foot length). Condition was calculated based on residuals from a linear regression to denote breeding ([$\log_{10}$(testis area)] / [$\log_{10}$(body mass)]; Møller 1988) and body ([$\log_{10}$(hind foot length)] / [$\log_{10}$(body mass)]) condition (van der Marel *et al.* 2021).

We separated our sample into age cohorts based on mass relative to the sample median (1292.5 g): OLD (mean ± SE: 2037 ± 171.25 g) and YOUNG (mean ± SE: 882 ± 84.90 g). This largely follow's Gott's (1996) separation of bandicoot males into separate age cohorts, although our YOUNG males would have been separated into two age cohorts according to Gott (1996). We combined the lighter individuals into one cohort because of small sample size. Short-term repeatability of proportion of activity (Gaussian distribution) was assessed for each age cohort (rptR package, Stoffel *et al.* 2017). Confidence intervals were calculated by running 1000 bootstrapping samples on each model. Some species partition activity in an open field (e.g. more active at the start, Wilson *et al.* 1976). As bandicoots were generally more active in the first 5 minutes on both days, we repeated all repeatability analyses for this time bin.

We also used the z-score method to calculate exploratory scores for individuals:

$$z_i = \frac{x_i - \acute{x}}{s}$$

where x_i = individual's activity level, $\acute{x}$ = sample mean activity, and s = sample standard deviation (Massen and Koski 2013). Negative scores indicated an individual was less exploratory relative to others that were sampled. Repeatability was also calculated for exploration scores (as above).

We used correlations to determine whether a change in individual activity or exploration score was correlated with body mass, body condition and breeding condition. These give an indication of whether animals with particular characteristics, such as greater mass, respond differently over time to other individuals. Change in activity and exploration score was used as we found no repeatability (Supplementary Material). A negative change indicates the individual was less active or exploratory on the second day. All non-significant results are presented as Supplementary Material.

Results and Discussion

Limitations to the study

There were several limitations to this study that should be noted. Firstly, our sample size was limited by the number of unique individuals we were able to capture over the time period available for the project (2 months). While small sample sizes may require caution in the interpretation of results, studies with small sample sizes can still be important for providing the foundation for research moving forward (McLean *et al.* In Press).

Secondly, the study was conducted in captivity. The ability to record repeated measures of behaviour in the wild takes time, which again was limited here. While behaviour demonstrated in captivity may not be reflective of behaviour in the wild, some studies have found that personality in captivity does reflect personality in the wild (e.g. blue tits (*Cyanistes caeruleus*), Herborn *et al.* 2010). Thus, again, we argue that our study is important for providing a starting point from which future research can move forward.

Thirdly, activity of bandicoots in this study was generally low (range 2 – 24 % of the full time on Day 1; range 0 – 36% of the full time on Day 2; Supplementary Material), suggesting animals might have been stressed by capture and handling, which are known stressors for many Australian marsupials (Hing *et al.* 2014). How stress can be mitigated, and how it affects behaviour (Oswald *et al.* 2012), should be considered in future, particularly in field-based studies.

Body and breeding condition

OLD males were in relatively poorer body condition ($F_{1,12} = 4.76$, $R^2 = 0.22$, $p = 0.050$; Fig. 2), but apparently better breeding condition ($F_{1,12} = 4.76$, $R^2 = 0.48$, $p = 0.004$; Fig. 2), supporting a possible energetic trade-off between body maintenance and reproduction (Fisher 1930). It should, however, be noted that this is an indirect method of assessing breeding condition, and future studies are needed to assess whether sperm characteristics, such as ejaculate size and quality are directly related to this metric (Møller 1988).

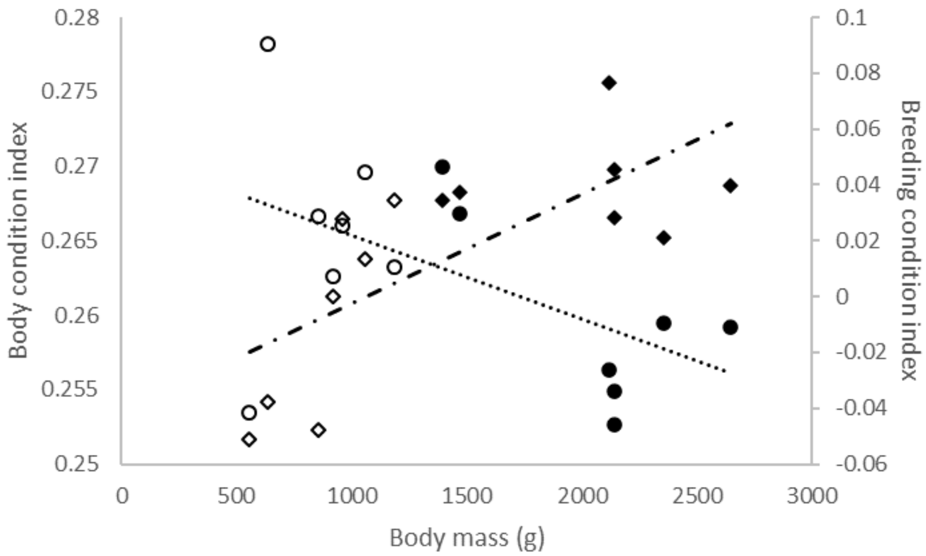

Fig. 2. Body (circles) and breeding (diamonds) condition indices of OLD (closed) and YOUNG (open) male northern brown bandicoots relative to body mass (g). Body condition index: dotted line; breeding condition index: dot-dash line.

Repeatability

Four individuals (3 OLD and 1 YOUNG) were more exploratory, while 10 individuals (4 OLD and 6 YOUNG) were less exploratory, on the second day of testing (Fig. 3). While repeatability for exploration in novel environments can be scale-dependent (Dammhahn 2012), behaviours are labile traits (Biro and Stamps 2015). We likely found no repeatability because of the short time period of observation and small sample size of the study.

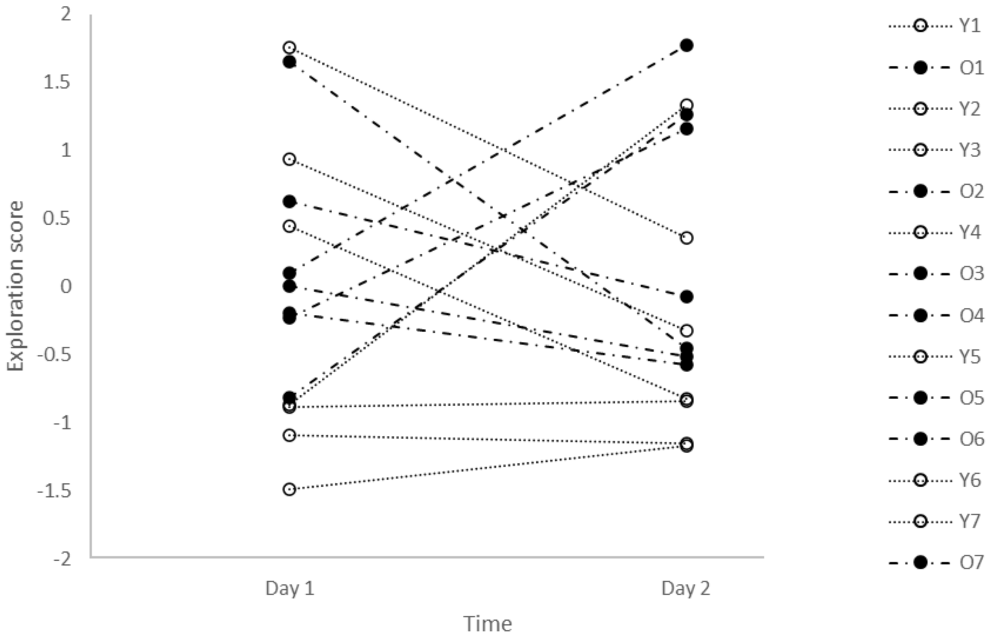

Fig. 3. Exploration of OLD (closed circles) and YOUNG (open circles) male northern brown bandicoots over two days

Inter-individual variation in the behaviour of bandicoots and bilbies is an avenue of future interest (Hayes and Jenkins 1997). Examining the success of alternative phenotypes (e.g. active or inactive) could enhance our understanding of mechanisms driving behaviour, and how selection acts on these mechanisms (Hayes and Jenkins 1997). Future studies exploring phenotypic correlations with other traits (Lande and Arnold 1983), and genotype-environment interactions, will provide more insights into the broader behavioural repertoires of this group.

Correlations

<u>Breeding Condition vs Activity and Exploration Score</u>

Breeding condition and a change in activity over time were significantly negatively correlated for OLD males (whole period: r = -0.75, df = 5, p= 0.050; first time bin: r = -0.84, df = 5, p = 0.017), with animals in better breeding condition becoming less exploratory over time (Fig. 4). Our results are consistent with Begall *et al.* (1999), who found that breeding coruros (*Spalacopus cyanus*) are less active than non-reproductives. Testosterone is energetically expensive to maintain (Wingfield *et al.* 2001), so older, larger bandicoots in better breeding condition may reduce activity over time to minimise energetic expenditure overall. Future studies assessing relative testosterone concentrations in males from different cohorts would provide insights into possible physiological and behavioural trade-offs. As 64% of the remaining Peramelemorphia species are currently at risk, studies that shed light on their exploratory behaviour broadly will provide a greater understanding on their potential dispersal ability and movements in their natural landscapes.

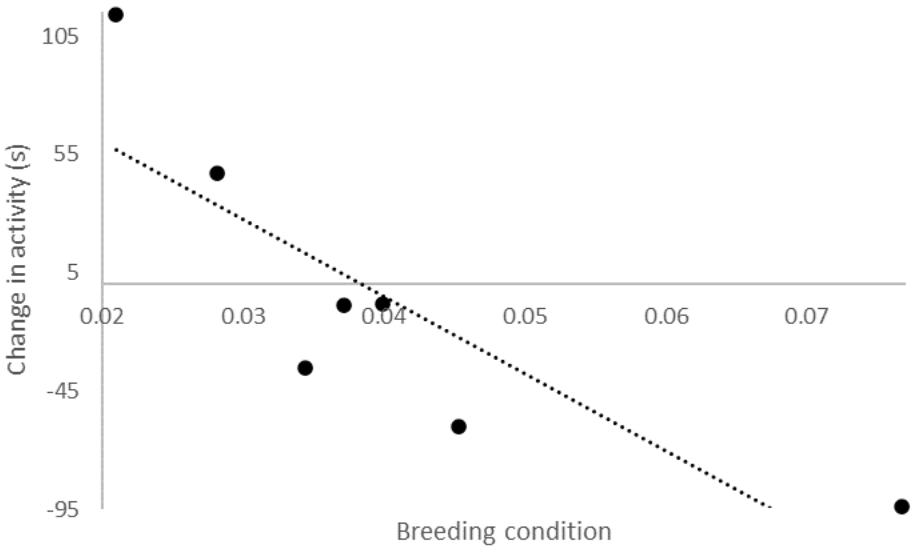

Fig. 4. Change in activity (s) in relation to breeding condition of OLD male northern brown bandicoots

Data Availability Statement

The data that support this study is included as supplementary material.

Conflict of Interest Statement

The authors declare no conflict of interest.

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