Improving reliability of scat counts for abundance and distribution estimations of Lumholtz's Tree-kangaroo (*Dendrolagus lumholtzi*) in its rainforest habitats

SIGRID R. HEISE-PAVLOV[1] and RHIANA D. MEADE[2]

Non-invasive methods are essential in the study of cryptic species. For estimations on abundances and distributions of arboreal folivores, such as Lumholtz's Tree-kangaroo (*Dendrolagus lumholtzi*) in Far North Queensland, Australia, scat counts seem to be the most promising ecological technique. However, the occurrence of Lumholtz's Tree-kangaroos in seasonal rainforests with dense understory, a high diversity of coprophagous invertebrates and with sympatric folivores increases the probability of invalidating results based on scat surveys.

This study investigates scat production and scat decomposition patterns to select diagnostic traits of Lumholtz's Tree-kangaroo scats that can, under varying environmental conditions, assist in distinguishing between fresh and old scats to reduce false positive and false negative errors in species presence due to non- or/and misidentification of scats. Scat production rates of six captive Lumholtz's Tree-kangaroos were highly variable resulting in different scat numbers and masses. Changes in scat size (mass and circumference), pH and the appearance of mould were monitored under different laboratory conditions and in forest trials. Under wet conditions scats gained mass until they reached an apparent plateau of 130% of their original mass. Scats under dry conditions lost up to 90% of their original mass. Changes in mass were accompanied by changes in circumference of scats. By Day 3 scats had developed signs of mould under laboratory conditions and showed an acidic pH. Field trials revealed a high loss of scats due primarily to their consumption by dung beetles *(Scarabaeoidea*).

For studying Lumholtz's Tree-kangaroos in their rainforest environment, scat surveys should be confined to dry periods to reduce the probability of false negative errors due to activity of coprophagous invertebrates. Additionally, only fresh scats of average size and with an acidic pH should be used to minimize the risk of misidentifying small sized scats from Red-legged Pademelons (*Thylogale stigmatica*) as tree-kangaroo scats. More studies on species-specific diagnostic traits of Lumholtz's Tree-kangaroo scats are necessary to validate false negative and false positive errors in scat counts for this species.

Key words: Non-invasive methods; scat counts; Lumholtz's Tree-kangaroo; *Dendrolagus lumholtzi*; Wet Tropics of Australia; scat production; decomposition pattern, scat detection

INTRODUCTION

THE conservation of mammals relies on data on their abundance, habitat utilization and movement. A lack of reliable data on these variables makes the accurate classification of the conservation status of a species and the planning of conservation efforts difficult (Rhodes *et al.* 2006; Robbirt *et al.* 2006).

A range of field based methods are now available for the acquisition of ecological data in various habitats (Sutherland 1996; Borchers *et al.* 2002). However, direct observations of mammals are often impractical, especially for species that are rare or cryptic. In response to these constraints, non-invasive methods have been developed (Kendall *et al.* 1992; Wilson and Delahay 2001) that rely on signs such as tracks and scats (Gompper *et al.* 2006).

The Wet Tropics of Australia is home to two endemic and cryptic species of tree-kangaroos (*Dendrolagus bennettianus* and *D. lumholtzi*) (Martin 2005). As folivores, they inhabit canopies of dense rainforests and are mainly active during the night and at dawn and dusk (Martin 2005). Studies of these species are therefore problematic and the consequent lack of ecological data limits the development of efficient conservation strategies for these species (Kanowski *et al.* 2001a, 2003).

Lumholtz's Tree-kangaroo (*D. lumholtzi*) prefers upland rainforest habitats such as complex notophyll vine forests (or type Mabi 5b forest) (Tracey 1982; Kanowski *et al.* 2001a, b) which are now mainly restricted to the Atherton Tablelands of the Wet Tropics. Due to the reduction of the original extent of this forest type (Latch 2008) and fragmentation of the remaining habitat, Lumholtz's Tree-kangaroos are now considered near threatened under the *Queensland Nature Conservation (Wildlife) Regulation Act* (2006).

The elusive life of Lumholtz's Tree-kangaroos in dense canopies contributes to the lack of studies on the ecology, abundance and distribution of this species (Procter-Gray 1985; Newell 1999a, b; Coombes 2005). Recently more attempts have been made to utilize non-invasive

[1]School for Field Studies, PO Box 141, Yungaburra 4884, Queensland, Australia. sheise-pavlov@fieldstudies.org
[2]Reed College, 3203 SE Woodstock Blvd. Portland, OR 97202, United States. rhianameade@gmail.com

PACIFIC CONSERVATION BIOLOGY Vol. **18:** 153–163. Surrey Beatty & Sons, Sydney. 2012.

methods to study this animal. Spotlights have been used to locate tree-kangaroos to enable estimates of population densities and habitat use (Coombes 2005; Laurance *et al*. 2008), but the effectiveness of this method is limited to forest edges. Scratch marks, left behind on tree trunks when tree-kangaroos descend from the canopies, are being explored to assess the distribution and habitat use of tree-kangaroos in rainforest fragments (Heise-Pavlov *et al*. 2011). Recently, scat counts have been applied to assess the spatial distribution and abundance of a Lumholtz's Tree-kangaroo population in a 5a upland rainforest fragment (Tracey 1982; Phillips pers. comm.). The results suggest that scat counts may be a useful ecological technique for this species.

The accuracy of scat counts for estimating abundance and spatial distribution of a species depends on reliable information on the production and the detection of scats (Laing *et al*. 2003; Murray *et al*. 2005).

Estimating scat production rates is problematic in wild populations. Animals may move over large areas (Blake *et al*. 2001; Coombes 2005; Buij *et al*. 2007), or/and have low digesta retention times (Clauss *et al*. 2007; DeGabriel *et al*. 2010). Many studies on production rates rely therefore on data collected from animals in zoos and wildlife sanctuaries. Fancourt (2009) used three captive Swamp Wallabies (*Wallabia bicolor*) to assess their scat production rate while Murray *et al*. (2005) recorded different scat production rates in captive wild Snowshoe Hares (*Lepus americanus*) depending on their age and degree of food digestibility (Murray 2003). Lumholtz's Tree-kangaroos utilize a wide variety of foliage from rainforest and introduced species (Coombes 2005; Martin 2005). It is likely that their food preferences change seasonally (Cianelli pers. comm.) resulting in changes of scat production rates (Coombes 2005). At present no information on scat production rates of Lumholtz's Tree-kangaroo has been published.

Detection of scats during surveys is influenced by various factors such as their detectability by the observer, the decay rate of scats and their misidentification as scats from sympatric species (Bulinski and McArthur 2000; MacKenzie *et al*. 2002; Triggs 2004; Rhodes *et al*. 2011)

The detectability of scats is limited in areas with dense vegetation cover (Buij *et al*. 2007). A previous study using scat counts of tree-kangaroos relied on the clearance of understory in rainforest plots (Coombes 2005).

Scat detection is affected by their decay rate (Rhodes *et al*. 2011). A rapid decay rate can result in a false negative error, negating the presence of a species in an area, while a slow decay rate results in a false positive error, assuming the presence of a species in an area (Laing *et al*. 2003; Royle and Link 2006; Rhodes *et al*. 2011). Numerous studies have therefore simulated scat decay rates as probabilities of their survival over a certain time and under various conditions (Hone and Martin 1998; Rhodes *et al*. 2011). Seasonal environmental conditions such as substrate moisture levels and temperature have been identified as critical for breakdown of scats (Putman 1984; Harestad and Bunnell 1987; White 1995). Lumholtz's Tree-kangaroos live in a seasonal environment with profound wet seasons and extended dry seasons. During the tropical dry season, scats of Lumholtz's Tree-kangaroo were reported to take up to two months to decay (Coombes 2005).

Besides climatic factors, scat survival can be affected by their composition, depending on the type of consumed food (Harestad and Bunnell 1987; Murray *et al*. 2005) and the activity of coprophagous invertebrates and fungi (Holter 1979; Dickinson *et al*. 1981; Herrick and Lal 1996; McGranaghan *et al*. 1999; Masunga *et al*. 2006). *Scarabaeoidea* beetles, earthworms and flies have been identified as main agents influencing decomposing processes of vertebrate dung (Holter 1979). Dung beetles are likely to play a major role in the decay of marsupial dung in the Wet Tropics of Australia (Vernes *et al*. 2005), as this area has probably the greatest dung beetle diversity in Australia (Zborowski *et al*. 1995). This may explain reports of rapid disappearance of scats from Bennett's Tree-kangaroo (*D. bennettianus*) in the Wet Tropics (Martin 2005).

Misidentifying scats of a target species with those from sympatric species can introduce false positive errors (Bulinski and McArthur 2000; Royle and Link 2006). Diagnostic traits of scats such as its shape and diameter can overlap considerably between species (Danner and Dodd 1982). Shape and size of scats are likely to change in the process of decay making them unrecognizable as scats from the target species (Kohn and Wayne 1997; Stuart and Stuart 1998; Triggs 2004). Changes in scat appearance can involve various traits simultaneously. Lumholtz's Tree-kangaroos share their habitat with Red-legged Pademelons (*Thylogale stigmatica*) (Van Dyck and Strahan 2008). Both utilize the same food resources resulting in comparatively similar compositions of their scats (Vernes *et al*. 1995; Flannery *et al*. 1996; Triggs 2004). Decay processes may therefore be affected by the same factors and to the same degree resulting in a similar appearance of scats from both species.

Problems in scat counting, arising from the various factors affecting detectability of scats (Prugh and Krebs 2004; Rhodes *et al*. 2011), can

be mitigated by including information on decomposition processes of scats, which may allow accurate ageing of detected scats in the field. If detected scats can be aged, the time at which a site was occupied by a species can be more accurately assessed and the probability of false positive errors based on the presence of old scats or/and misidentification of scats can be reduced (Royle and Link 2006; Rhodes *et al.* 2011). For example, Sullivan *et al.* (2002) classified Koala (*Phascolarctos cinereus*) scats (in their paper called pellets) into age classes by their level of Eucalyptus odour and their external colour and used only new pellets for estimating pellet densities. Rollins *et al.* (1984) used pH values to age scats (in their paper: pellet groups) and to identify species origin of pellets.

Our study aims at the improvement of scat counts for the cryptic arboreal Lumholtz's Tree-kangaroo living in seasonal rainforest environments with dense understory, a high diversity of coprophagous invertebrates and with sympatric folivores. Besides estimating scat production rates for this species, we investigate scat decomposition patterns under varying conditions to select diagnostic traits of Lumholtz's Tree-kangaroo scats that can assist in distinguishing between fresh and old scats and reducing the probability of misidentifying them as scats from Red-legged Pademelons. We hypothesize that the size of scats increases under moist conditions, while it decreases under dry conditions. Changes in pH and the appearance of mould are assessed as additional traits for ageing scats. A detailed description of changes of scat traits with age and under different environmental conditions will increase the accuracy with which results can be obtained from scat counts of this species.

METHODS

Defecation rates

Defecation rates were analyzed for six captive Lumholtz's Tree-kangaroos, all of which were held in North Queensland, Australia.

Captive animals included three adult females aged 48 months, 60 months and eight years, two adult males, aged 36 months and 28 months, and one juvenile male, aged 12 months. The 48 months old female lived in an enclosure of 80m² at a licensed welfare provider's home on the Atherton Tablelands. The 60 months old female, the eight year old female and the 28 months old male lived at the Wildlife Habitat in Port Douglas (licensed animal welfare provider) each in a 27m² sized enclosure. The 36 months old male and the 12 months old male lived in enclosures sized 15m² each at another licensed animal welfare provider on the Atherton Tableland.

All animals had daily access to freshly collected rainforest foliage from either type 5a rainforest (48 months old female), 1a rainforest (60 months old female, eight year old female and 28 months old male) or a type 1b rainforest (12 months and 36 months old males). Enclosures contained dirt or vegetation-covered floors at the two welfare providers on the Atherton Tablelands. At the Wildlife Habitat in Port Douglas, enclosures contained a concrete floor. Tree limbs were provided in all enclosures as climbing structures.

Enclosures were cleared of all scats at the commencement of the trial and those produced each 24 hours were collected over a four-day period for the three males, the 60 months and eight years old females, and over a three-day period for the 48 months old female. To ensure that all scats could be found, any vegetation in the enclosures was covered with cloth sheets staked down over the vegetated area.

Since the 48 months old female spent her nights inside the welfare provider's home, her scats were also collected at this location. From the 12 months old male, scats were only collected for eight hours during which he stayed in his enclosure, as he spent the remaining hours of a 24 hour period in a different location.

All collected scats were counted and massed with an electronic balance (Sartorius BL3100, Goettingen, Germany) to the nearest tenth of a gram.

Calculations

The approximate daily mass of a single scat was determined for each individual by dividing the total mass of all scats by the number of scats produced by this individual during each of the 24 hour collection periods. The average mass of a single scat was then obtained from the daily values over four (males and two females) and three (the 48 months old female) collection days. Average scat production was calculated by dividing the average total mass of scats produced by the body mass of the animal and expressed as average scat mass produced per gram of body mass.

Decomposition of Scats

The decomposition rate of scats was assessed in two ways: under controlled laboratory conditions and in a field trial.

1. Decomposition under controlled laboratory conditions

Scats were kept under four different sets of controlled climatic laboratory conditions (warm-

Table 1. Laboratory conditions under which scat decomposition was monitored.

Conditions	Warm-wet	Warm-dry	Cool-wet	Cool-dry
Average Temperature in °C	23.6°C ± 2.9°C	23.6°C ± 2.9°C	18.7°C ± 0.1°C	18.7°C ± 0.1°C
Average Relative Humidity in %	97% ± 2%	40% ± 5%	97% ± 2%	40% ± 5%

wet, warm-dry, cool-wet, cool-dry) to monitor their decomposition patterns. These conditions are summarized in Table 1.

Scats under wet conditions were sprayed with water each day to maintain a relative humidity in their vicinity between 95 and 99%. Dry conditions were simulated by placing scats on leaf litter containing 28% water. Water content of the leaf litter was determined by placing a sample of leaf litter of known mass overnight in a drying oven (type Memmert by Selby Bioloab) at 90 °C and calculating the water content by re-measuring the mass of the sample after drying.

Temperature and relative humidity of each climatic condition were monitored by a data logger (M&I Instruments, Inc., Mississauga, ON, Canada) over three 24 hour periods.

Test scats were collected fresh from either the 48 month old female or the 36 month old male. They were placed in open plastic containers measuring 980 cm^3. Each plastic container included a layer of 0.5 cm of soil covered by a leaf litter layer approximately 3 cm thick that originated from a notophyll vine forest (type 5a forest) located at 17°24'38"S, 145°31'21"E. Six scats were placed in each container. There were three containers for each described climatic laboratory condition.

Scats were examined daily on Days 2, 3 and 4, then on Day 6 and on Day 10. They were analyzed with respect to their mass, circumference, pH and appearance of mould. The mass of each scat was assessed daily with an electronic balance (Sartorius BL3100, Goettingen, Germany). Its circumference was determined by photographing the pellet and using the freehand tool in the software "Image J" (U. S. National Institutes of Health, Bethesda, MD). The pH of decomposing scats was tested on the day of their collection (prior to being allocated to one of the climatic conditions) followed by five successive days during which the scats were kept under different conditions. On testing days, five scats from each climatic laboratory condition were placed into clean test tubes. The samples were treated with barium sulfate powder and an indicator liquid from a soil pH testing kit (Rainbow by West Meters Ltd., Corwen, Denbighshire, UK). A colour chart included in the kit was used to determine the pH. Mould was measured by noting the first day it became visible on the surface of a scat to the naked eye.

Calculations

To assess within-condition variability, analyses of variances were performed on the masses of scats between the three containers per laboratory condition. Percentage mass change of scats was calculated for each laboratory condition, based on the difference between the measurement of the mass taken on the current day and the measurement of the mass taken on a previous day, over a period of ten days using successively larger time intervals. Measurements on Days 2, 3 and 4 were related to the previous day, while measurements on Day 6 were related to Day 4 and those taken on Day 10 were related to Day 6. Differences in the percentage mass change between the four laboratory conditions were tested by ANOVA performed in Microsoft Excel. Post-hoc Tukey tests were performed in PAST (Hammer *et al.* 2001).

A linear correlation between mass and circumference was run in Microsoft Excel to estimate for simultaneous changes in scat traits.

Measurements of pH were compared between the different climatic laboratory conditions on Days 2, 3, 4 and 5 using ANOVA in Microsoft Excel.

Days on which mould appearance was seen for the first time were compared between the different laboratory conditions using ANOVA in Microsoft Excel.

2. Decomposition under Forest Conditions

Freshly collected scats were placed in three different habitat sites within a 64 ha complex notophyll vine forest fragment of type 5a (Tracey 1982) on the Atherton Tablelands (17°24'38"S, 145°31'21"E). The habitat sites were located in a patch of sunny regrowth forest (thereafter called "sun"), a patch of shaded old growth forest (thereafter called "dark"), and a patch abutting a waterway (thereafter called "creek"). Temperature and relative humidity were measured at each location over a 24 hour period using a data logger (M&I Instruments, Inc., Mississauga, ON, Canada). This trial was undertaken over four days in November 2010.

On Day 1 at 4:00 PM six scats were placed in each location in separate wire mesh cages on the forest floor. Cages were used to ensure that scats would not be translocated due to gravity or rain, or removed by digging animals. Cages were either cylindrical with openings in the top and bottom, approximately 5 cm tall by 2–4 cm in

Table 2. Average scat production rate for six individual captive Lumholtz's Tree-kangaroos over four days (males, the 60 months and eight year old females) and three days (48 months old female), including an evaluation of the average scat mass per gram of body mass. As the 12 months old male's scats were collected for only eight hours daily, data for a 24 hour period was obtained by multiplying data collected over eight hours by three.

	Female 1	**Female 2**	**Female 3**	**Male 1**	**Male 2**	**Male 3**
Body weight (gram)	6250	7280	8380	5000	7290	3000
Age (months)	48	60	96	36	28	12
Mean number of scats produced over 24 hours (± standard error)	60.00 ± 11.00	127.70 ± 4.60	68.75 ± 7.23	75.20 ± 4.50	91.50 ± 8.99	70.50
Mean scat mass (gram) produced over 24 hours (± standard error)	67.50 ± 12.70	164.80 ± 12.90	129.77 ± 15.82	143.20 ± 8.40	168.72 ± 31.04	72.10
Mean mass of single scat (gram) (± standard error)	1.10 ± 0.00601	1.30 ± 0.06	1.87 ± 0.07	1.90 ± 0.23	1.81 ± 0.22	1.06 ± 0.09
Mean mass of produced scat material per gram body weight over 24 hours	0.01	0.02	0.02	0.03	0.02	0.02

diameter, or enclosed boxes measuring approximately 10 x 5 x 2 cm. Every day the mass of each scat was assessed with an electronic balance (Sartorius BL3100, Goettingen, Germany). The circumference of each scat was determined daily by photographing each scat and measuring its circumference using the freehand tool of the software "Image J" (U. S. National Institutes of Health, Bethesda, MD). Mass and circumference of the scats were compared between the three habitat sites for each day, using ANOVA in Microsoft Excel. The proportion of missing scats was recorded every day. Missing scats were replaced by fresh ones, which were measured as if starting from Day 1.

The assessment of insect abundances at the three habitat sites was conducted by using pitfall traps which were placed near each of the studied habitat sites, using a modified method adopted from the Queensland Dung Beetle Project Report (2001) over the last two of the four trial days. Traps were 20 cm long modified PVC tubes with endcaps and a diameter of 5cm with a cut-out-top section of 4.5 cm in diameter. Traps were baited with fresh tree-kangaroo scats and checked after each 24 hour period. Their contents were emptied and captured insects were separated from the soil. Dung beetles were counted and placed in 70% alcohol for future identification.

Table 3. Post-hoc Tukey p values for significant differences in percentage mass changes of scats under different laboratory conditions. ANOVA results provided for comparisons between two days (Loss = mass loss compared to percentage mass changes under condition in left column; Gain = mass gain compared to percentage mass changes under condition in left column, NS = not significant)

Day 1–2; ANOVA, $F_{3,68} = 8.54$, $p < 0.05$	**Warm-wet**	**Warm-dry**	**Cool-wet**	**Cool-dry**
Warm-wet	#	Loss; 0.0002	NS	Loss; 0.03
Warm-dry	NS	#	Gain; 0.003	NS
Cool-wet	NS	NS	#	NS
Cool-dry	NS	NS	NS	#
Day 2–3; ANOVA, $F_{3,68} = 17.6$, $p < 0.05$	**Warm-wet**	**Warm-dry**	**Cool-wet**	**Cool-dry**
Warm-wet	#	Loss; 0.0002	NS	Loss; 0.002
Warm-dry	NS	#	NS	NS
Cool-wet	NS	Loss; 0.0001	#	Loss; 0.0001
Cool-dry	NS	NS	NS	#
Day 3–4; ANOVA, $F_{3,68} = 35.9$, $p < 0.05$	**Warm-wet**	**Warm-dry**	**Cool-wet**	**Cool-dry**
Warm-wet	#	Loss; 0.0001	Gain; 0.02	Loss; 0.0001
Warm-dry	NS	#	NS	NS
Cool-wet	NS	Loss; 0.0002	#	Loss; 0.0002
Cool-dry	NS	NS	NS	#
Day 4–6; ANOVA, $F_{3,68} = 34.5$, $p < 0.05$	**Warm-wet**	**Warm-dry**	**Cool-wet**	**Cool-dry**
Warm-wet	#	Loss; 0.0001	NS	Loss; 0.0002
Warm-dry	NS	#	NS	NS
Cool-wet	Gain; 0.03	Loss; 0.0002	#	Loss; 0.0002
Cool-dry	NS	NS	NS	#

RESULTS

Scat production and size

The mean number of scats and the mean scat mass produced over 24 hours is summarized in Table 2. Mean mass for a single scat varied among the subjects from 1.06 to 1.9 gram. The subjects produced between 0.01 and 0.028 gram scat material per gram body weight in a 24 hour period (Table 2).

Decomposition under controlled Laboratory Conditions

There was no significant within-condition variance of the masses of scats between the three replicate containers per laboratory condition (ANOVA, warm-wet: $F_{2,15}$= 1.69, p=0.22 ; warm-dry: $F_{2,15}$=0.26, p=0.77 ; cool-wet: $F_{2,15}$=1.95 , p=0.18 ; cool-dry: $F_{2,15}$=0.60, p=0.56). Since there was homogeneity of variance across each laboratory condition, measurements for scats from containers in each laboratory condition were pooled for further analysis.

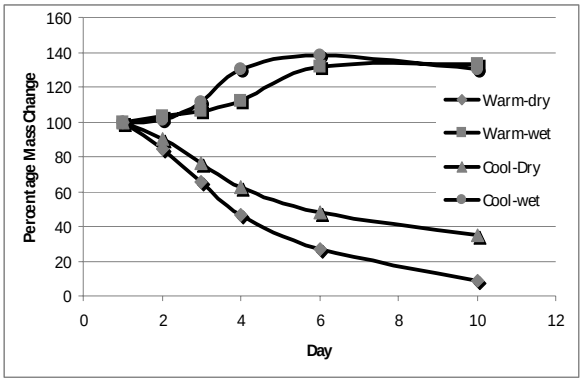

Fig. 1. Percentage mass change calculated as an average across all scats under each laboratory condition related to an initial value of 100. "Wet" scats gained mass until levelling out at around 130%, while "dry" scats lost mass as they dried. Cool-dry scats lost mass slower than warm-dry scats.

Change in mass of scats

Percentages of mass changes of scats, measured under different laboratory conditions, are shown in Figure 1. The results of ANOVA tests, comparing masses of scats between different days and different laboratory conditions, are summarized in Table 3. There was no significant difference of percentage mass changes of scats kept under different laboratory conditions between Days 6 and 10 (ANOVA, $F_{3,68}$=2.53, p=0.06).

Scats under wet conditions gained mass over a period of 10 days, reaching a plateau at around 130% of their original mass, while scats under dry conditions lost mass with up to 90% of their original mass.

Mass-circumference relationship

Mass versus circumference relationship was assessed for each of the four climatic laboratory conditions. All four were found to have significant positive correlations (Pearson's correlation coefficient: warm-wet: r = 0.6796; warm-dry: r = 0.6694; cool-wet: r = 0.7162; cool-dry: r = 0.7013).

pH

Freshly collected scats had a pH around 6.12 ± 0.65. On Day 2 scats under all four different laboratory conditions were found to have slightly more acid pH of around 5.38 ± 0.25. There was no significant difference in the pH values for sampled scats from the different laboratory conditions on Day 2 (ANOVA $F_{3;16}$ = 1.853, p = 0.178). By Day 3 and beyond, all scats tested at pH 7, the most basic point of the testing equipment available.

Appearance of mould

Mould appeared on all scats. The majority of scats had some mould by Day 3, but all scats had visible mould by Day 4. There was no statistically significant difference in the time that mould first appeared on scats kept under different laboratory conditions (ANOVA, $F_{3, 68}$ = 2.24; p = 0.091).

Decomposition under Forest Conditions

The "sun" site in the regrowth forest had an average temperature of 22.1 ± 6.11ºC , with a maximum temperature of 41°C and a minimum temperature of 16.5°C and 84.4% ± 16.7% relative humidity. The "dark" plot in a shaded old growth area of the forest had an average temperature of 19.5 ± 1.3ºC, with a maximum temperature of 23°C and a minimum temperature of 18°C and 98.3 ± 1.5 % relative humidity. The "creek" plot, that abutted a waterway, had an average temperature of 20.4 ± 1.0ºC, with a maximum temperature of 23°C and a minimum temperature of 19°C and relative humidity of 96.4 ± 2.1 %. Rain occurred sporadically at all field sites during the experiment.

In the "dark" plot, 100% of scats had disappeared within 24 hours over each of four days. In the "creek" plot, 100% of scats disappeared within 24 hours over each day. In the "sun" plot, 43% of scats had disappeared after 24 hours on Day 1, 78% on Day 2, 100% on Day 3, and 89% on Day 4. Due to the disappearance of scats statistical analyses of changes in mass and circumference of scat in the field trial could not be performed.

Pitfall Trap Results

Pitfalls at the "dark" site captured on average 50.5 dung beetles over two nights. An average

of 38 dung beetles was caught at the "creek" site and an average of 11.5 at the "sun" site.

DISCUSSION

Scat counts present one of the most reliable non-invasive method for estimating species abundances and distributions. They often provide more precise estimates than direct observations (Jachman 1991; Barnes 2002; Prugh and Krebs 2004).

In Australia, scat surveys have become an important tool for collecting ecological data from many of Australia's nocturnal mammals that inhabit large and often very remote areas (Johnson and Jarman 1987; Phillips *et al.* 2000; Murphy and Bowman 2007; Callaghan *et al.* 2011).

Recently scat counts have been applied in the study of cryptic species such as tree-kangaroos, which spend most of their time in dense canopies of rainforests (Martin 2005). However, reliable estimates on species abundance and distribution can only be obtained from scat counts when information on scat production and decay rates is available (Rhodes *et al.* 2011). This study focused on scat production and decomposition pattern of scats from Lumholtz's Tree-kangaroos under different climatic conditions to improve the accuracy of results derived from scat counts.

The number of scats produced within a 24 hour period varied between the sexes and ages of the studied captive Lumholtz's Tree-kangaroos. Scat production can be affected by the age of animals and their activity levels (Murray *et al.* 2005). Wild animals can defecate up to two times as much as those in captivity due to differences in their diet and activity levels (Johnson *et al.* 1987; Hume 1999; Coombes 2005). Johnson *et al.* (1987) noted a much higher scat production rate in wild populations of Eastern Grey Kangaroos (*Macropus giganteus*) and Red-necked Wallabies (*M. rufogriseus*) compared to captive animals. Animals used for our scat counts lived in enclosures sized between 15 and 80 m^2, while wild Lumholtz's Tree-kangaroos use home ranges between 0.7 to 2 ha (Newell 1999b; Coombes 2005).

Seasonal variations in food availability, digestibility and food demand due to reproductive activity also affect scat production rate and deposition (Rollins *et al.* 1984; Wiggins and Bowman 2011). For instance, alfalfa in diet of deer species causes a decrease in scat production (Rollins *et al.* 1984). Lumholtz's Tree-kangaroos feed on a large variety of rainforest plants and seasonal changes in food availability, as well as variations in food species preferences between individuals have been reported (Coombes 2005). Although two of the investigated females and two of the investigated males of our study received the same food, the results do not indicate consistent changes in either the number or the mass of produced scats. Scat production rates of additional animals of the same age need to be included to assess the impact of diet on scat production in this species. Limited food availability can also affect scat deposition as it may result in a dilution of scat distribution as animals move over larger areas (Bacigalupe *et al.* 2003). Therefore, seasonal changes in scat production and deposition pattern need to be considered for abundance estimations and habitat utilization of this species based on scat counts.

Lumholtz's Tree-kangaroo scats showed different decomposition patterns under different laboratory conditions. Environmental variables such as moisture level, temperature and topography have been shown to affect the persistence of scats (Lehmkuhl *et al.* 1994; Murray *et al.* 2005; Breuer and Hockemba 2007; Rhodes *et al.* 2011) and scat survival models have been developed for different seasons (Hone and Martin 1998; Rhodes *et al.* 2011). Increasing temperature and humidity result in a lower persistence of scats, with consequent negation of the presence of a species (false negative error) (Rhodes *et al.* 2011), while low temperature and humidity contribute to a long survival of scats, leading to the assumption that a species is using the habitat (false positive error) (Rhodes *et al.* 2011). Coombes (2005) reported that Lumholtz's Tree-kangaroo scats took up to two months to decay under dry conditions. Wiggins and Bowman (2011) found that more than 90% of scats from Tasmanian macropods can remain un-decomposed for more than five months.

While numerous studies describe the endurance of scats along environmental gradients, our approach focused on measurements of changes of scat diagnostic traits, as a reflection of specific decomposition patterns of scats under different environmental conditions. Knowledge of changes in diagnostic traits can allow the selection of those traits that can assist in ageing scats and in more reliable assessments of time at which a species has used the habitat (reduction of false positive errors, Royle and Link 2006). While Prugh and Krebs (2004) stressed that subjective criteria for age classification are highly inaccurate, Sullivan *et al.* (2002) emphasized the importance to reliably age scats (in their study: pellets). In their study on Koalas, Sullivan *et al.* (2002) used the intensity of Eucalyptus odour and the colour of the external patina of pellets to select new pellets (not older than 28 days), which they incorporated in an abundance model. As Lumholtz's Tree-kangaroos utilize a wide range

of rainforest plants odour and external appearance of their scats may vary. Instead we selected mass and circumference as measurements of scat size, and pH levels and the appearance of mould as traits to assess the age of scats, as those are commonly used variables in identifying scats (Nagy and Gilbert 1968; Rollins *et al.* 1984; Chame 2003).

Scats under dry laboratory conditions lost mass probably as a result of evaporation. Those under cool-dry conditions lost mass more slowly than those under warm-dry conditions. Given that warm temperatures aid in evaporation, this supports the water-loss hypothesis. Scats under wet laboratory conditions gained mass until they reached an apparent plateau at around 130% of their original mass after 10 days. The composition of the scats, may affect its absorption capacity (Chame 2003).

Our results reveal a positive correlation between mass and circumference, resulting in changes of the size of a scat in accordance to changes of its mass. Size variation of scats is more frequent among herbivores, because of the alteration in the quality and amount of food ingested in different seasons (Chame 2003). However, our results show clear trends in size changes of scats with progressing decomposition under different environmental conditions allowing the assessment of the age of a decaying scat. For future scat counts, the degree of scat enlargement or reduction should be modelled in relation to environmental variables in order to obtain scat size variability under different conditions. Triggs (2004) describes scats of Lumholtz's Tree-kangaroos as cylindrical scats with a width between one and two cm. Our results show that, under wet conditions, scats can be much larger while they can be smaller under dry conditions, which increase the likelihood of confusing them with scats from Red-legged Pademelons (Triggs 2004). The problem of misidentification of scats from sympatric species is often neglected (Bulinski and McArthur 2000), although it clearly can result in over- or underestimation of species occurrence.

Under all laboratory conditions scats developed mould. While most of the scats developed mould with long filaments around Day 4, several of the scats in warm-dry conditions only developed short fuzz around the entire scat. Although growth rates of mould were not measured in this study, it appeared that mould on scats under wet conditions grew the fastest, and that mould on scats under warm-wet conditions grew faster than mould on scats under cool-wet conditions. Future studies have to prove this observation, which may have been a result of the requirement of moisture by most types of mould (Pieckova and Zdenka 1999). Harestad and Bunnell (1987) showed a decrease in the persistence of scats (in their paper: pellets) under moist conditions due to fungi growing on their surface. Though in our study no scats placed in the forest developed mould, scats covered in mould were often detected in the enclosures of the 48 months old female and the adolescent male. Therefore, any scats encountered in the field that have mould on them could be assumed to be at least three or four days of age. However, weather conditions can affect the appearance of mould. Coombes (2005) did not record the development of mould on Lumholtz's Tree-kangaroo scats in her scat decay trials on the Atherton Tablelands. Trials were undertaken under very dry conditions, which have presumably suppressed the development of mould. Additionally, Coombes (2005) used defrosted scats. The pre-trial freezing of scats may have resulted in the extermination of viable mould spores, leading to a complete absence of mould.

Fresh scats generally had a pH of around five to six. By Day 3, there was a shift to a more neutral pH around seven. The observed pH shifts occurred around the same time that mould appeared, indicating a possible causal relationship between the two. Therefore, it can be assumed that scats in the field that show pH values under seven are likely to be less than three days old. Rollins *et al.* (1984) reported pH values between seven and 8.6 for deer scats (in their paper: feces) from five days to 170 days after excretion. This suggests that pH values are useful for identifying very fresh scats but also that more refined pH measurements are required. However, pH values should not be used in comparative studies of animals in different habitats as diet can affect the pH of scats (Rollins *et al.* 1984).

Scats in our field trial disappeared consistently within 24 hours making the monitoring of their decomposition rates impossible. The capture of dung beetles in pitfalls near our habitat sites indicates that dung beetles have consumed a high proportion of our sample scats. Australia has a unique dung beetle fauna that has adapted to use marsupial dung (Doube *et al.* 1991). Vernes *et al.* (2005) described high dung beetle species richness for wet sclerophyll habitats in the Wet Tropics, with dung preferences of some beetle species. Moist conditions support dung beetle activity (Hill 1993; Grimbacher *et al.* 2006) which may explain the low amount of scat loss in the "sun" site of our forest trial, which showed the highest variation in relative humidity. No scats were lost to beetles in Coombes' (2005) trials which were conducted under dry conditions. Martin (2005) reported that scats from the Matschie's Tree-kangaroo (*D. matschiei*) in New Guinea disappeared within

nine hours due to beetle activity. As our pitfall traps were set during the last two days of our forest trial only, the disappearance of scats was initially not influenced by additional beetle attractants. Future studies should investigate the relationship between beetle assemblages and scat consumption in greater detail.

The large impact of dung beetle communities on the persistence of folivore scats may be a rainforest-specific issue. Eastern Grey Kangaroos, studied in southern Queensland, have been successfully tracked via scat counts (Hill 1978) with only a negligible amount of scats lost. For studies on Eastern Grey Kangaroos and Red-necked Wallabies in dry sclerophyll forests at Wallaby Creek in the dry tropics, Johnson and Jarman (1987) recommended scat surveys to be done during winter when consumption of scats by insects such as *Scarabaeoidea* beetles and flies is negligible.

The observed changes in diagnostic traits of Lumholtz's Tree-kangaroo scats, as well as their rate of consumption by dung beetles, makes the assessment of the probability of false positive and false negative errors difficult (Royle and Link 2006; Rhodes *et al.* 2011). During the wet season we would expect a higher false negative error due to the higher consumption rate of scats by coprophagous invertebrates. However, size enlargement of scats due to wet conditions may minimize the risk of misidentifying them with scats from pademelons reducing false positive errors (Royle and Link 2006). During dry periods we can expect a lower consumption rate of scats by coprophagous invertebrates increasing the probability of false positive errors. Shrinking sizes of scats increases the risk of mistaking them for pademelon scats, thus increasing false positive errors (Royle and Link 2006). To minimize both false errors only fresh scats should be used in scat counts of studies on Lumholtz's Tree-kangaroos. Fresh scats should be identified on the basis of their pH and, under wet conditions, the lack of mould. Incorporation of older scats requires further studies on species-specific scat diagnostic traits and their changes.

In conclusion, our results show that scat counts of Lumholtz's Tree-kangaroos exhibit a large amount of variability with respect to the quantity of scats produced and the rate at which scats decompose. However, we believe that reliable data on the species' distribution and abundance can be obtained from scat counts in two possible ways. First, site and seasonal specific scat production rates (based on data on available food plants), scat decomposition pattern and activity of coprophagous invertebrates (Holter 1979) should be determined at the time of the survey. Appropriate protocols should be developed that outline site and seasonal specific scat sampling methods, based on pre-determined site specificities (Laing *et al.* 2003; Prugh and Krebs 2004; Sullivan *et al.* 2004). A second approach would be the development of models for site occupancy, based on the assessments of false negative and false positive error rates by incorporating variables such as food availability, climate and insect activity (Royle and Link 2006). Repeated visits to the study area are required for accurate estimates of probability of species detection (Tyre *et al.* 2003; Kendall and White 2009), encouraging more research on decomposition pattern of scats (Rhodes *et al.* 2011). With more non-invasive accurate methods at hand, more knowledge on the ecology of Lumholtz's Tree-kangaroos can be obtained to support conservation planning for this species (Kanowski *et al.* 2003).

ACKNOWLEDGEMENTS

We are thankful to Margit Cianelli and Dr. Karen Coombes on the Atherton Tablelands for giving us access to their animals for scat collection as well as organizing equipment. We are grateful for the help of Wildlife Managers Clare Andersen and Michelle Prince from the Wildlife Habitat who collected and weighed scats from several animals for us. Dr. John Kanowski gave valuable hints on this type of study. Dr. Peter Grimbacher helped with his knowledge on dung beetles of the Wet Tropics interpreting our results. The logistics of this study would not have been possible without the help of Ian Brennan. We thank Dr. Karen Coombes for her valuable comments on an earlier version of this manuscript and Prof. Adrian Bradley for text revision.

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