- Development of light-weight video-tracking technology for use in wildlife research: A
   case study on kangaroos
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#### 19 Abstract

20 There have been significant advances in the development of animal-borne sensor 21 technologies, or biologgers, in recent years. This has resulted in tremendous capacity for 22 wildlife researchers to remotely collect physiological, behavioural and social data from 23 wildlife in circumstances that were unthinkable just decades ago. While this technology can 24 provide us with a unique insight into the "secret lives" of wild animals, there is a need to 25 evaluate the utility of these new sensors versus traditional wildlife research methodologies, 26 and to critically evaluate the integrity of the data collected by ensuring that these devices 27 themselves do not alter the physiology or behaviour of the recipient animal. This paper 28 reports on the development of a light weight "animal borne video and environmental data 29 collection system" (AVED), which can be deployed on animals as small as 11 kg, whilst still 30 meeting the desired 3% body weight threshold. This AVED (referred to as the "Kangaroo-31 cam") simultaneously collects video footage and GPS location data for an average of 19 h. 32 Kangaroo-cams were deployed on seven kangaroos as a proof of concept of their potential 33 utility for the study of location specific behaviour and diet in a medium-sized terrestrial 34 herbivore. Following device recovery and data processing, we were able to successfully score 35 83 foraging events which allowed us to determine diet based on visual identification (to the 36 family level) of plants consumed. This approach could be further broadened to include a 37 comparison of plant species consumed versus plant species encountered to provide a novel 38 approach to diet selection analysis. When combined with GPS mapping of foraging locations, 39 this approach would allow researchers to address questions on diet selection at both fine 40 (within patch) and broad (habitat) spatial scales, overcoming some of the limitations of 41 traditional diet selection methodologies. However, animal capture and collar deployment 42 caused a significant elevation in stress hormone concentrations within the first 24 h post-43 capture, which highlighted the need to incorporate a time-delay capacity into these devices.

44 We conclude the paper by reviewing recent advances in the development of AVED

45 technology and providing suggestions for the improvement of this Kangaroo-cam device.

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Keywords: AVED, biologger, diet selection, GPS, macropod, movement ecology, telemetry,
wildlife

49

# 50 Introduction

51 Over the last decade, there have been significant advances in the development of animal-borne 52 sensor technology. These sensors, often termed *biologgers*, provide data about an animal's 53 movements, behaviour and/or physiology (Fehlmann and King 2016), and often facilitate the 54 collection of multiple forms of data simultaneously from wild animals. One particular type of 55 biologger that has seen significant technological advances recently is the "animal borne video 56 and environmental data collection system", or AVED (Moll *et al.* 2007).

57

AVEDs simultaneously record fine scale geolocations and continuous video footage of the environment from the perspective of the animal (Moll *et al.* 2007), thus facilitating the process of *video-tracking* (Bluff and Rutz 2008). This combination of time-referenced location and video images allow for a greater suite of ecological questions to be answered, including understanding how animals interact with the environment or conspecifics, and developing location and time-specific behavioural budgets (Moll *et al.* 2007).

Some of the greatest scientific impacts of animal-borne loggers have been in marine mammals
and birds, where direct observation is difficult or impossible (Machovsky-Capuska *et al.*2016a; Machovsky-Capuska *et al.* 2016b; Pearson *et al.* 2017). AVEDs in particular have been
deployed predominantly in large marine animals or birds, and this is partly related to the large

size of these units, which limits the size of animal upon which they can be deployed, or the short-term nature of deployments in birds. For example, Fehlmann and King (2016) recently reported that 90% of papers presented at the 5<sup>th</sup> bio-logging symposium in Strasbourg in 2014 involved birds or marine mammals. As such, the development of technology for use in terrestrial mammals has arguably fallen behind, despite many of the advantages of this technology still being highly relevant to this group of animals.

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75 Global positioning system (GPS) and other traditional telemetry technologies have been widely used to study the movement patterns of a broad range of terrestrial mammals. While telemetry 76 77 units have the capacity to tell where an animal has been, they do not provide detailed 78 information about what the animal was doing at each geographic location without the addition 79 of other sensors (Machovsky-Capuska 2016a). This gap can be partially filled by the use of traditional behavioural observations, but it is widely accepted that it is difficult, if not 80 81 impossible, to directly observe free-range behaviour of wildlife for extended periods of time 82 without affecting their behaviour (Beringer et al. 2004). Hence, AVEDs have the capacity to 83 provide an unbiased view of the complete repertoire of animal behaviour irrespective of the 84 location of an animal. As such, their potential utility is high, even for large, relatively 85 conspicuous, terrestrial mammal species.

86

In this paper, we report the development of the "Kangaroo-cam", a biologger that simultaneously collects video footage and the GPS location in time and space. Using the eastern grey kangaroo (*Macropus giganteus*; hereafter kangaroo) as a sample medium-large herbivorous, terrestrial mammal (females 17-42 kg, males 19-85 kg; Coulson 2008), we explore their fine scale behaviour and foraging ecology. We specifically aim to: 1. establish

92 the diel activity budgets and location-specific behaviours; and 2. identify the feeding locations 93 and diet. Further, we wanted to explore whether the "Kangaroo-cam" collected an unbiased 94 sample of animal behaviour, as it is important to ensure that the devices themselves do not have 95 a welfare or behavioural impact on the animal carrying the logger (Moll et al. 2009; Thomson 96 and Heithaus 2014). Hence, an additional aim of this study was to determine whether kangaroos 97 elicited a discernible stress response to capture, restraint and device fitting, as measured by 98 faecal glucocorticoid metabolite concentrations (FGMs), which are a proxy for circulating 99 stress hormone concentrations (Sheriff et al. 2011). A noticeable stress response is likely to 100 indicate that the animal's behaviour is altered by the deployment of the device and may not be 101 reflective of their "normal" behavioural repertoire, thereby influencing the integrity of the 102 results (Schulz et al. 2005).

103

### 104 Materials and methods

105 Study area

106 The study was conducted in February 2014 and 2015 at Nelson Bay Golf Course (NBGC), which is located 208 km north of Sydney, Australia (32°43'31"S, 152°8'44"E). The NBGC 107 108 has a population of 100-200, individually identifiable (via ear tags), free-range kangaroos with 109 a high level of site fidelity, making it an ideal site for testing new animal tracking technology. 110 The golf course itself is comprised of exotic, improved pastures, and is surrounded by Tomaree 111 National Park (TNP) to the south and east. Vegetation in the areas of TNP bordering on the 112 golf course is predominantly comprised of "Blackbutt-Apple Open Forest on Deeper Sands" 113 (open dry-sclerophyll forest dominated by Blackbutt, Eucalyptus pilularis; Sydney Red Gum, 114 Angophora costata; Red Bloodwood, Corymbia gummifera; and Old Man Banksia, Banksia serrata), with intermittent patches of "Nerong Open Forest" and "Wallum Scrub-Heath" (Bell 115 116 1997).

#### 118 Animal handling and collar deployment

119 Seven adult kangaroos (females (n=5; two with no young, one with a young-at-foot and two 120 with pouch young; and males (n=2)) were immobilised using Zoletil (Virbac, Milperra, NSW, 121 Australia) at a concentration of approximately 5 mg/kg body weight, delivered by either a CO<sub>2</sub> 122 powered projector (X-calibre, Pneu-dart, Williamsport, PA, USA using a 1 cc 3/4" dart) or a pole syringe (1 ml drug volume with 18 G <sup>1</sup>/<sub>2</sub>" needle). Each kangaroo was weighed (digital 123 hanging scale, WS603, 150 x 0.05 kg, Wedderburn, Ingleburn, NSW, Australia), sexed and ear 124 125 tagged (sheep button and/or mini tags, Allflex, Capalaba, Qld, Australia) for unique 126 identification. Additional samples, such as blood samples, were also collected as part of other 127 investigations on these animals. Capture, measurements, sampling and Kangaroo-cam 128 deployment took around 20 min. Kangaroos were then left in handling bags for approximately 129 two and a half hours to fully recover from anaesthesia prior to release. Collars were retrieved 130 by recapture approximately seven days post-release to facilitate GPS and video data download. 131 This study was conducted with the approval of the University of Sydney Animal Ethics 132 Committee (N00/7-2012/3/5791) and the NSW National Parks and Wildlife Service 133 (SL100961).

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# 135 Kangaroo-cam devices

We combined a miniaturised camera (previoulsy incorporated into other species-specific designs, see: Machovsky-Capuska *et al.* 2016b, Bombara *et al.* 2017, Pearson *et al.* 2017) with a GPS transmitter to develop video-tracking smart collars (Kangaroo-cam) (Fig. 1). The miniaturised-video-camera (U10 AU HD USB Flash Drive DVR Camera DV, Taiwan; see Machovsky-Capuska et al. 2016b for more details) and GPS logger (GT-730FL-S, Canmore, Taiwan) were powered by two 3400 mAh lithium polymer batteries (Table 1). Two 3D-printed

142 plastic cases covered with water-resistant paint were used to enclose the miniature camera and 143 GPS logger (L: 89 x W: 50 x H:37 mm) and the batteries (L: 83 x W: 48 x H:45 mm). Both cases were attached to a medium dog collar (Fig. 2) and secured to the neck of the kangaroos 144 145 (Nexaband liquid tissue adhesive) to reduce movement. The collars recorded approximately 146 20 h of continuous video footage with a 36° field of view at 30 frames per second (720 x 480 147 HD) and latitute and longitude data for up to two days (1 s intervals). The smart collars weighed 330 g, which was < 3% of the weight of the kangaroo adult body mass (mean  $\pm$  s.e.m. female 148 weight =  $27.5 \pm 1.5$  kg (n = 5, range 22.5 - 30.3 kg); male weights 46.7 kg and 61.9 kg). The 149 150 camera was mounted on the side of the collar (Fig. 2), which represented a compromise 151 between having a viewing angle which permitted us to determine when an animal was actively 152 chewing, versus a better camera placement for a wider angle of view, which may have made 153 it difficult to tell whether the animal was actively chewing.

154

### 155 Kangaroo behaviours

156 Kangaroo-cams enabled us to extract fine scale detailed behaviours. We determined the amount of time that animals undertook each of the following behaviours (to the nearest second): i) 157 resting: the animal was lying down and not feeding, sometimes sleeping; ii) feeding: the animal 158 159 had its head towards the ground and started nosing different foods until it raised its head again 160 (Garnick et al. 2010), including chewing and foraging at the same time; iii) grooming: the 161 animal was either scratching, self-cleaning, wetting forearms/inner thighs; iv) hopping: the 162 animal was in a bipedal motion; vi) standing: the animal was upright and stationary and not 163 actively feeding or chewing. These behavioural categories were mutually exclusive. Because 164 we were predominantly interested in exploring feeding behaviour, this category took 165 precedence over the other categories, and may include an animal that was simultaneously lying 166 or standing and feeding.

# 168 Feeding behaviour

169 Feeding events were identified from the videos as those where the animal could be seen to scan 170 available forage (usually depicted by the animal nosing different plants in the environment) 171 and select plant material, followed by short up-and-down head movements (discernible from the movement of the camera or in some cases the animals jaw could be seen moving) that were 172 173 defined as chewing. The combination of these behaviours was considered as a feeding event. 174 Feeding events separated by less than 1 min were treated as a single feeding event regardless 175 of the behaviour displayed in the intervening time to ensure that each feeding event was 176 independent and involved separate forage selection. For each feeding event, the plants that 177 were consumed were identified to Family based on visual characteristics. In some cases, 178 identification to species level was possible when the plant displayed unique characteristics or 179 displayed reproductive characters to confidently allow identification to that level. All 180 identifications were verified using PlantNet NSW Flora Online descriptions and distribution 181 data (National Herbarium of New South Wales).

182

183 *Video-tracking technique* 

184 The internal GPS clock and the camera clock were synchonised after recovery. The GPS clock 185 was set to Australian Eastern Daylight Savings time (AEDT) and the camera clock recorded 186 the time that had elapsed since it started recording video. As both devices were turned on 187 simultaneously, the starting time for both could be ascertained and "common times" recorded 188 for both as either AEDT or time (in seconds) relative to deployment. Once behavioural events 189 were identified by the video analysis, they were assigned to the GPS location with the same 190 common time within ArcGIS 3.2. When the behavioural event occurred at a time with no exact 191 coincident position, it was assigned to the position closest in time, within a tolerance range of 30 s. According to the average speed reported for these animals (6 km/h, Garnick *et al.* 2010),
this is a very conservative and accurate criterion to geographically locate behaviours.
Following this procedure, a total of 87 behavioural events were identified and classified as one
of three distinctive behavioural states (see below) and each assigned to a geographic location.
Behavioural states with "*common times*" greater than 30 s to the closest position were discarded
from further analysis.

198

Using the above-mentioned video-tracking technique, we established the spatio-temporal scale of three distinctive behavioural states: i) feeding, ii) resting and iii) moving. Kernel areas (50 (core), 60, 70, 80, 90 and 95%) were calculated for each animal using the adaptive Kernel method (Worton 1989) using the Home Range Tools extension in ArcGIS 9.8. Finally, these behavioural states were plotted on a map, along with movement tracks and Kernel areas to give a map of behavioural activities at different locations.

205

# 206 Faecal glucocorticoid metabolite assay

207 The physiological response to collar deployment was determined by measuring faecal 208 glucocorticoid metabolites (FGMs) in an additional subset of animals carrying collars that were 209 of similar weights to the devices used in the study, but minus the camera lens, as it was not 210 possible to collect samples at the time of the initial deployment. Stress hormone concentrations 211 were determined by measuring FGMs at 0, 24 and 48 h post-capture and collar deployment in 212 six animals (four females and two males), compared with the response to the same capture, 213 handling and release (without collar deployment) in eight control animals (four males and four 214 females). The females in the collar group had pouch young (PY) that were 10 d and 161 d, 215 while the remaining two had no PY. The control (capture only) females had PY that were 10 216 d, 62 d, 86 d and the remaining female had no PY. Circulating stress hormones (glucocorticoids, predominantly cortisol) are metabolised in the liver and secreted in faeces
following a lag time, which is equivalent to 24 h in this species (Fanson *et al.* 2017). Hence,
FGM concentrations at 0 h represent the baseline, pre-capture circulating stress hormone
concentration, with 24 h samples being indicative of the time of capture and 48 h samples
representing one day post capture and collar fitting.

222

223 Faecal samples were collected when voided at the time of capture and immobilisation and at 224 other times by searching the golf course for the collared or control individuals 24 and 48 h post 225 capture. All animals have a unique ear tag colour and number combination, which can be 226 readily discerned from distances in excess of 50 m with binoculars (Nikon, 10 x 50, Monarch 227 5, M511) or a spotting scope (Nikon, Prostaff 5, 20-60 x). Once a collared animal was 228 identified, its ear tag number was recorded and the animal was observed from a distance until 229 it defecated. Once defecation occurred, the faecal sample was visually located and collected in 230 a zip-lock bag, and stored on ice for up to 4 h before being placed in long-term storage at -20° C for subsequent enzyme immune-assay to determine FGM concentrations. 231

232

233 FGMs were extracted from 0.5 g ( $\pm$  0.01 g) thawed wet faeces with 5 ml of 80% methanol, 234 following the method described by Fanson et al. (2017). The EIA used an antibody raised in 235 rabbits against the FGM 3 $\beta$ ,5 $\alpha$ -tetrahydrocorticosterone (37e; Touma *et al.* 2003), and has 236 previously been validated in eastern grey kangaroos (Fanson et al. 2017) by demonstrating an 237 increase in FGM 24 h post adrenocorticotropic hormone (ACTH) challenge. The assay was run 238 as described in Fanson et al. (2017). Briefly, 0.05 ml of standard, diluted faecal extract, or 239 control were added to duplicate wells of a pre-coated 96-well plate, followed by 0.1 ml 240 biotinylated steroid (working dilution 1:15,000) and 0.1 ml of primary antibody (working dilution 1:15,000). Plates were incubated overnight at 4° C and then washed 3 times before 241

0.25 ml streptavidin-peroxidase was added to each well. After 45 min incubation at 4° C, plates were washed 6 times and 0.25 ml TMB substrate was added. The reaction was stopped with 0.05 ml H<sub>2</sub>SO<sub>4</sub> and optical density measured at 450 nm using a Dynex MRX Revelation plate reader (after Fanson *et al.* 2017). The intra-assay coefficient was calculated from repeated measures of 10 - 20 replicates of a single sample on one plate at 12.0%. Likewise, the interassay coefficients were calculated for low (7.7%) and high (12.9%) controls. The assay sensitivity was 0.02 ng/ml.

249

# 250 Data analyses

To assess the differences in food consumption in relation to geographic location and food type we used generalised linear models (GLMs). The first GLM was specified with a binary response denoting whether or not an observed feeding event occurred within the NBGC (0) or TNP (1). A second model depicted whether the kangaroos consumed native (0) vs non-native (1) plants. The third model tested whether foraging location (NBGC vs TNP) influenced the consumption of the different plant groups.

257

FGM concentrations were compared between collared and non-collared animals at 0, 24 and 48 h post-immobilisation, using the general linear model (repeated measures) function, the model being y = treatment, time, treatment × time, with time as the repeated subject. Results are presented as mean  $\pm$  s.e.m. All analyses were performed using the software SPSS (IBM, SPSS Statistics, version 24; Chicago, IL)

- 264 **Results**
- 265 *Camera deployments*

We collected 130 h of video footage from the seven kangaroos fitted with "Kangaroo-cams", with an average recording duration of  $18.6 \pm 1.6$  h per animal. This included periods of day and night for each animal (Table 2).

269

270 Kangaroo behaviour and diel activity patterns

For each kangaroo, an average of 99.9% of the post-release, day-time video footage was able

to be characterised into the different behavioural states, ranging from 97.5 - 100% (Table 2).

273 Overall, kangaroos spent the majority of their daytime hours standing or feeding (Fig. 3).

274

275 Feeding behaviour

A total of 83 feeding events were recorded and scored from the video footage ( $12 \pm 4$  per

animal). Of the total observed feeding events, 57% (n=47) occurred on the golf course

whereas 35 (n=36) were within the national park (Wald test, z = 12.18, df = 1, P < 0.0001).

279 Kangaroos consumed significantly more non-native (76%), than native plants (24%) (Wald

280 test, z = 41.10, df = 1, P < 0.0001).

281

A total of nine plant families were identified in foraging events (Fig. 4), but over 50% of their forage intake was from the Family Poaceae (grasses) and 22% from Cyperaceae (perennial or annual herbs) (Fig. 4). Consumption of plants in the family Poaceae and Haloragaceae was positively associated with foraging on the golf course rather than the national park (Poaceae: Wald test, z = 7.46, df = 1, P < 0.0001; Haloragaceae: Wald test, z = 6.48, df = 1, P < 0.01). However, no significant differences in foraging locations were observed for the other plant families.

289

# 290 Behaviour and habitat use

291 A total of 87 behavioural events identified by the cameras were assigned to a geographic 292 location, and the two most frequent behaviours (resting and feeding) were plotted onto maps 293 depicting habitat use areas (50-95% kernels) and movement trajectories for each individual. 294 Four examples are given in Fig. 6. The small sample-size means that statistical analyses were 295 not warranted, and the following account provides an exploratory, qualitative analysis only. 296 Fine scale movement showed by GPS tracks overlapped with behaviour locations revealed a 297 constant pattern for all animals, in which a large area of the golf course was explored with no 298 particular behaviour displayed other than moving. Only one smaller area was used for 299 feeding or resting by each kangaroo during the observation period (Fig 6a-c). The only 300 exception was animal K230 (Fig. 6d), who used three small areas for these behaviours, but 301 still reduced areas in comparison to total area visited and distance travelled.

302

303 Almost all feeding and resting behaviours were located within core areas (50% kernel), with 304 some of them located within 70-90% Kernel areas. No animal rested or fed beyond the 305 general use area (95% Kernel), with the exception of the animal K230 (Fig. 6d), who showed 306 three resting events outside of the 95% Kernel area. Despite this concentration of main 307 activities within core areas, all animals had at least one core area in which they did not feed 308 or rest during the recording period. Although activity was centred within the golf course, 309 most animals had some core-use areas outside of the golf course, as noted above for feeding 310 behaviour.

311

## 312 Stress response to capture and collar deployment

There was a significant difference in the stress response of control and collared animals (Fig. 7), with both the "time" and the "treatment x time" interaction being significant (P < 0.05 for both). FGMs were not significantly different between groups at the time of capture, but were

316 significantly elevated in collared animals compared to uncollared controls (collar =  $157 \pm 21$ 

317 ng/g; control = 91 ± 18 ng/g; p = 0.035) at time 24 h. By 48 h post capture, FGM

318 concentrations were indistinguishable between the two groups (Fig. 7). There was no

319 correlation between change in FGMs and reproductive status for females.

320

#### 321 Discussion

322 In this study we have successfully developed a biologging device for kangaroos (the so-called 323 "Kangaroo-cam") which can simultaneously log animal movements using GPS and capture 324 video footage from a "kangaroo's-eye-view". We have demonstrated the capacity of these 325 devices to collect continuous video footage for 19 h, and for that footage to be successfully 326 scored to identify location-specific animal behaviour, feeding locations and diet in this 327 grazing herbivore. Animal capture and collar deployment caused a significant elevation in 328 stress hormone concentrations within the first 24 h post-capture, coinciding with the time of 329 video-recording. As such, the behaviours reported here may be biased by stress-induced 330 behaviour in the time period immediately following collar deployment. Hence, the 331 significance of our research lies not so much in the biological findings, but rather as a 332 demonstration of the potential utility of this video-tracking technology in a medium-sized, 333 terrestrial mammalian herbivore, a group of animals that have previously been under-334 represented in the use of this type of technology.

335

Deployment of kangaroo-cam units on seven kangaroos resulted in the successful scoring of
83 foraging events (an average of 12 per animal), highlighting the potential strengths of
AVED technology for determining diet in mammalian herbivores. Furthermore, this
technology has the capacity to be utilised in diet selection studies. Diet selection, or
preference, is defined as an animal's choice of specific food(s) from those that are available,

341 and therefore requires a quantitative comparison of what is ingested by an animal versus what 342 is available to that animal at a given place and time (Norbury and Sanson 1992). As such, still 343 frames from the video footage of foraging events can be used to identify the plants 344 immediately available to an animal, versus those actively consumed, in a foraging event. This 345 has the capacity to overcome many of the current limitations with diet selection studies, which is the ability to look at diet selection at different temporal and spatial scales. At a 346 347 broader scale, GPS tracking data can be used to ascertain the broader habitat utilisation 348 choice through the analysis of home range location. At a finer scale, foraging locations within 349 a home range can be mapped by utilising the combined video and GPS data. At an even finer 350 scale again, preferred plants within those feeding areas can also be determined. Other 351 methodological approaches for measuring diet selection tend to focus on one or other of these 352 spatial and temporal scales (summarised in Table 3), thereby limiting the scale at which 353 statements about diet selection can be made and the ecological questions that can be 354 answered (Norbury and Sanson 1992). As such, one of the real advantages of incorporating 355 AVED technology into diet selection studies is the capacity to measure diet selection across a 356 range of spatial scales, using the one sampling approach to determine what foods the animals 357 encounter (i.e. availability) and what they ingest (i.e. select), regardless of where they eat it. This removes any potential location sampling bias, as animals are sampled irrespective of 358 359 their location rather than the researcher choosing where they sample. It also ensures there is 360 not a mismatch between the scale at which food availability and selection are assessed as 361 both can be measured simultaneously within video frames. It also allows both fine-scale, 362 within patch selection to be measured as well a broader-scale habitat selection within an 363 animal's home-range. For example, in the current study we could determine exactly where an 364 animal was foraging within its home range (Fig. 6), as well as what individual plants animals 365 were consuming or avoiding within patches (Fig. 5).

367 The potential utility of AVEDs for providing unbiased behavioural sampling is demonstrated 368 by the amount of time that kangaroos spent both on and off the golf course. While some 369 individual animals spent almost all of their time on the golf course, others spent little, if any, 370 time there (e.g. see Figs 6a and 6c for two extremes). Overall, kangaroos spent 43% of their 371 time foraging away from the course. Traditional behavioural observations of foraging would 372 have been limited to the golf course area, where the vegetation is open and the kangaroos are 373 highly habituated to people, allowing individual animals to be unobtrusively observed with 374 relative ease. However, the area surrounding the golf course is dominated by open dry-375 sclerophyll forest on sandy soils, an environment in which it is difficult to see animals, let 376 alone unobtrusively observe them. As such, traditional behavioural observation studies would 377 be biased towards the activities of animals in a limited proportion of their core area. This 378 would result in the loss of data relating to foraging activities in forested habitats, thereby 379 inflating the importance of some plant families, notably Poaceae (which was predominantly 380 associated with the Golf Course), at the expense of almost all other family groups. This again 381 highlights the importance of considering the spatial and temporal scale of diet selection 382 studies.

383

Whilst the discussion above has focussed predominantly on some of the advantages of this approach, a more detailed description of the advantages, disadvantages and inherent biases of different diet selection methodologies is provided in Table 3. The key disadvantage of using AVEDs to study diet selection lies in the laborious nature of scoring the videos, and the high cost of the units themselves, which limits sample size. It is clear from the comparisons in Table 3 that all of the different methods have some disadvantages and biases. What is important is that these limitations are recognised and that the most suitable methodology is

chosen to meet the objectives of any given diet selection study and the degree of accuracy
required (Norbury and Sanson 1992). It is our contention that the use of AVEDs, such as the
Kangaroo-cam, has the capacity to overcome some of the limitations of other approaches, but
that the added time and cost associated with AVED use may not be justified for some
research questions. They are merely another tool available to researchers interested in these
types of research questions.

397

398 This paper has deliberately focused on the potential utility of AVED technology for 399 behavioural investigations, with a focus on diet selection, rather than the biological outcomes 400 of the research for this species. This is for two important reasons. Firstly, this type of 401 technology has rarely been employed for the study of behaviour and diet in medium-sized, 402 terrestrial herbivores, with previous studies focusing on larger marine mammals or birds 403 (Fehlmann and King 2016). Hence, we wanted to demonstrate that advances in this 404 technology mean that it is now more accessible for a broader range of species, and is equally 405 amenable to the study of species with herbivorous diets. Even for species which are 406 seemingly easy to study in the field, such as kangaroos, AVEDs have the capacity to provide 407 additional insights into their behaviour in less accessible areas of their range. Secondly, the 408 outcomes of this study highlight the need to consider whether the device itself has the 409 capacity to change natural behaviours as a result of device- or capture- induced stress on the 410 recipient animal.

411

412 AVED's are not necessarily a new technology in wildlife investigations. The first iterations 413 date back to the use of the early National Geographic CRITTERCAM (Marshall 1998) on 414 loggerhead (*Caretta caretta*) and leatherback (*Dermochelys coriacea*) sea turtles, but these 415 devices were large, cumbersome and heavy (> 2 kg), and therefore not suited to many

416 animals (Bicknell et al. 2016). The Kangaroo-cam presented here is one example of how 417 such limitations are now being overcome. Table 4 compares the weight and technical 418 specifications of the Kangaroo-cam to a sample of historic and more recent innovative 419 AVEDs reported in the literature. This table highlights the dramatic reductions in weight of 420 devices, with seemingly simultaneous increases in recording times. As one example, the 421 Kangaroo-cam could potentially be deployed on animals as small as 11 kg, whilst still 422 meeting the desired 3% body weight threshold (and still obtaining approximately 19 h of 423 video footage).

424

425 The capture of kangaroos and fitting of Kangaroo-cam devices resulted in a transient increase 426 in FGM concentrations, which is indicative of a physiological stress response (Sheriff et al. 427 2011). This increase was not seen in control animals, which were captured and handled but 428 did not have collars fitted, suggesting that the collar itself is inducing a stress response, 429 independent of the capture process. These results are similar to those reported for white-tailed 430 deer (Odocoileus virginianus) fitted with AVEDs (Moll et al. 2009) and Dickcissels (Spiza 431 americana) fitted with radio-transmitters (Wells et al. 2003). Although Moll et al. (2009) 432 reported no difference between AVED and control deer over an extended period of time, 433 closer scrutiny of their data shows a transient increase in FGM in the acute period post collar 434 fitting. In all studies, this transient elevation in FGMs had diminished within 24 h. As such, it 435 is unlikely that this acute physiological response is detrimental to the welfare of the animal 436 (Wells et al. 2003). These findings are relevant, however, to the question of the integrity of 437 the data collected and point towards the need to exclude data collected during the first 1-2 d 438 after collar deployment, as it may not reflect the "normal" behaviour of the animals. In the 439 case of AVEDs, where battery life is so limited, this highlights the need to incorporate a

time-delay option for the commencement of recording, as has been incorporated into other
devices (e.g. Beringer *et al.* 2004; Bluff and Rutz 2008; Table 4).

442

443 The video-recording timeframe for the units developed in this study (approximately 19 h) 444 represents one of the longest recording timeframes reported (Table 4), and highlights the 445 recent advances in battery efficiency. However, the current study did not effectively utilise 446 this entire timeframe, as the camera was recording continuously from the time of deployment, 447 including anaesthetic recovery and night time when videos were un-scorable. As such, the 448 benefits of this enhanced battery life were not fully realised in this study. Further 449 modifications to the devices, such as addition of programable recording intervals (e.g. 450 Nifong et al. 2013; Nifong et al. 2014; Table 4) or a light-activated time-delay switch 451 (Beringer et al. 2004; Table 4), would ensure that the benefits of enhanced battery life are 452 fully realised in the future.

453

454 In this paper, we have discussed the advantages of this approach for diet selection studies in 455 kangaroos, and other terrestrial herbivores more generally (see Table 3 for a summary). 456 However, AVEDs have the capacity to study other aspects of the biology of wild animals, including social interactions. For example, a study employing a similar device on domestic 457 458 dogs was used to establish contact rates between con-specific animals (Bombara et al. 2017). 459 In the current study we were surprised by how few social interactions we observed between 460 conspecific kangaroos, especially since this species is highly gregarious and feeds in large 461 groups (or mobs) out in the open (Coulson 2008). This failure to observe close social 462 interactions is likely to be a result of the camera placement on the collar, rather than the 463 absence of such behavioural interactions. Mounting the camera on the side of the collar to 464 maximise our observations of feeding behaviour reduced the angle of view, which probably

465	accounts for the lack of social observations. Moreover, we found it difficult to find a robust
466	way of affixing the camera to the head of the animal (which would facilitate a broader view),
467	whilst still maintaining the cables between the battery unit and recording unit.
468	
469	In conclusion, this study has demonstrated the potential utility of AVEDs for studying diet
470	selection in a medium-sized, terrestrial herbivore. Whilst the technology is not without its
471	limitations, modifications to the existing "Kangaroo-cam" and the addition of other sensors,
472	has the capacity to further enhance the utility of this behavioural sampling approach.
473	
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481	
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574	
575	

576 <b>Table 1.</b> Video-tracking collar components, specifications, and approximate co	osts.
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Component	Dimensions	Model and manufacturer	Approximate	
	(LxWxH (mm),		unit cost (USD)	
	weight (g))			
Waterproof housing	89 x 37 x 50 (83o)	Custom-made, University of	\$40	
(camera/GPS)	0) X 3 7 X 30 (03 <b>G</b> )	Sydney	ψTO	
Waterproof housing	93 x 49 x 45 (72c)	Custom-made, University of	\$60	
(battery pack)	85 x 48 x 45 (72g)	Sydney	<b>\$00</b>	
x7' 1	100 07 07 ((0))	Custom-made, University of	¢1750	
Video camera	108 x 27 x 27 (68g)	Sydney	\$1750	
		GT-730FL-S, Canmore (Hsinchu	<b>* - •</b>	
GPS data logger	77 x 28 x 18 (15g)	County 30274, Taiwan)	\$50	

578	Table 2. Duration of simultaneous video recording and GPS data collection for each of seven
579	kangaroos in the study. The footage scored (%) reflects the percentage of post-recovery day-
580	time footage that was able to be categorised into the different behaviours. Reproductive status
581	of females: YAF, young-at-foot; PY, pouch young; NPY, no pouch young. The PY were ~75
582	and 124 days for 207 and 230 respectively.

Kangaraa		Reproductive	Foo	Footage		
ID	Sex sta (fem		Day	Day Night		scored (%)
003	F	YAF	9.7	0.3	10.0	100.0
022	М	-	10.6	5.9	16.5	100.0
001	F	NPY	6.8	13.7	20.5	97.5
031	М	-	5.8	12.2	18.0	98.3
207	F	РҮ	13.5	8.0	21.5	100.0
230	F	РҮ	14.8	8.2	23.0	100.0
261	F	NPY	12.3	8.2	20.5	100.0

- 585 **Table 3.** Summary of the characteristics of commonly used methods for measuring diet
- 586 selection in terrestrial, mammalian herbivores.
- 587 Information presented in the table is based in large part on an historic review by Norbury and
- 588 Sanson (1992), with the addition of new and emerging techniques, such as the use of AVEDs
- 589 (this paper) and the use of DNA barcoding of plant species in faeces (Guo *et al.* 2018).

Technique	Description	Temporal link between habitat/patch utilisation and diet selection?*	Lethal / Non- lethal	Advantages	Disadvantages	Spatio- temporal scale
Mouth contents	Animal shot and mouth contents identified	Yes	Lethal	Easy to identify ingested material. Quantification of different species possible.	Small sample of ingesta, over small timeframe, meaning large sample sizes needed. Limited spatial and temporal range. Biased towards sampling locations. Limited to common animals and ethical concerns associated with lethality.	Small
Stomach contents	Animals shot and stomach contents identified, usually by microscopic analysis of plant fragments in comparison to reference library	Yes	Lethal	Easier to identify ingested material than using faeces. Larger sample of ingested material than mouth contents. Quantification of different species possible.	Microscopic analysis of contents may be necessary. Limited to common animals and ethical concerns associated with lethality.	Small
Faecal contents - microscopic identification	Faecal samples collected and undigested plant fragments microscopically identified in comparison to a reference library	No	Non- lethal	Minimal disturbance to animals. Covers a broader spatial and temporal range. Quantification of different species possible. Not biased by sampling location.	<ul> <li>Biased by differential digestion of plant species.</li> <li>Difficult to compare food availability to food ingested due to lag between ingestion and excretion.</li> <li>Difficult to identify to genus and species.</li> <li>Significant time and expertise required.</li> </ul>	Broad
Observation	Observation of feeding animals and identification of plants ingested	Yes	Non- lethal	Minimal disturbance to animals. Easy to identify food if close enough. Quantification of different species possible.	Difficult for wild herbivores that are unapproachable or in vegetation types where observation is difficult. Quantification of species may be more difficult than for mouth, stomach or faecal contents.	Small (possibly broad depending on time invested and observability)

Technique	Description	Temporal link between habitat/patch utilisation and diet selection?*	Lethal / Non- lethal	Advantages	Disadvantages	Spatio- temporal scale
				Minimal preparation and equipment.	Biased towards sampling locations.	
Faecal contents – DNA barcoding	Faecal samples collected and molecular identification of undigested material (via sequencing) using universal plant primers and reference sequences	No	Non- lethal	Minimal disturbance to animals. Covers a broader spatial and temporal range. Potentially possible to identify to higher taxonomic level. Not biased by sampling location.	Difficult to compare food availability to food ingested due to lag between ingestion and excretion. Very high level of expertise and cost. Limited availability of reference sequences for some plants/regions	Broad
Observation using AVED	Identification of plants ingested based on video-recordings taken from devices mounted on the animals	Yes	Non- lethal	Minimal disturbance to animal (once acclimated to device). Easy to identify food. Quantification of different species possible. Combines fine and broad scale assessment of diet selection. Not biased by sampling location.	Quantification of species may be more difficult than for mouth, stomach or faecal contents. Expensive technology and time- consuming to analyse videos. Limited to medium-large animals with current technology.	Small-Broad (depending on recording time)

592 593 \* A spatio-temporal link between habitat/patch utilisation and food selection basically means they are sampled at the location they are foraging, thereby allowing for a direct measurement of food availability and selection at the same time (i.e. simultaneous sampling of available vs ingested food).

594 **Table 4.** Technical specifications of historic and recent AVEDs, highlighting differences in the size, weight and features offered by devices.

595 Note: this table is not an exhaustive list of AVEDs, but has been developed to highlight the changes in size over time and the taxonomic groups 596 studied, as well as other features that are desirable in AVED devices

Device	Species	Weight (% body weight)	Size (mm)	GPS (Y/N)	Time	Data storage/ retrieval	Video	Other features	Attachment	Reference
Crittercam	Harbour seal (Phoca vituline)	2000 g (1.8%)	?	N	3h (10 min bursts every 45 min)	Store on board (3 h video tape)		Water temperature and depth, salt water switch (to prevent recording out of water)	Epoxy attachment between shoulder blades	[1]
Crittercam	American alligator ( <i>Alligator</i> <i>mississippiensis</i> )	1000 g (~1.9%)	32x 10x 7.5	N?	6-8h*1	Store on board	1080 HD LED lights	Acceleration, depth, temperature sensors. Programmable recording intervals (time or sensor characteristics)	Harness	[2,3]
DCVS (Data- collecting video camera system)	White-tailed deer ( <i>Odocoileus</i> <i>virginianus</i> )	?	?	Ν	?	UHF wireless transmission		Light-activated time delay relay	Antler or collar	[4]
Terrestrial AVED	White-tailed deer ( <i>Odocoileus</i> virginianus)	1500 g (3-5%)	16.2 x 12.1 x 5.4	Y (1 min continuous every 5 min)	12.2, 12.3, 30.3, 41.6 h (4 animals)	Store on board	5 fps 176 x 144 pixels <sup>*2</sup>	Acceleration (2D), air pressure, temperature sensors. Remote collar release. Programmable recording intervals.	Collar	[5]
KittyCam	Domestic cat (Felis catus)	70 g (<3%)	75 x 50 x 25	Ν	10-12h	Store on board, VHF for retrieval	LED lights	Motion sensor activated	Break-away collar	[6]
(Custom)	New Caledonian crows <i>Corvus</i> moneduloides	13.6 g (4.3%)		Ν	Up to 94 min	Store on board, VHF for retrieval	640 × 480 pixels and 19·7 fps	Time-depth recorder. Programmable recording intervals.	Tail mounted with deflated rubber balloon	[7]

Device	Species	Weight (% body weight)	Size (mm)	GPS (Y/N)	Time	Data storage/ retrieval	Video	Other features	Attachment	Reference
C-VISS (cetacean-borne video camera and integrated sensor system)	Dusky dolphins (Lagenorhynchus obscurus)	342 g (~0.5%)	175 × 110 × 20	N (satellite transmitter)	67 min (9 – 284 min)	Store on board, VHF retrieval	30fps, 720 x 480HD		Suction cup mounted	[8]
(Custom)	Masked booby (Sula dactylatra tasmani)	70 g	60 × 60 × 15	Ν	?	Store on board, retrieved on return to nest	30fps, 720 x 480HD		Mounted on tail feathers	[9]
(Custom)	Domestic dog (Canis familiaris)	313 g (<3%)	90 x 30 x 20	Y	19 h	Store on board	30fps, 720 x 480HD		Collar mounted	[10]
Kangaroo-cam	Eastern grey kangaroo ( <i>Macropus</i> giganteus)	330 g (0.5- 1.4%)	8.9 x 5 x 3.7 and 8.3 x 4.8 x 4.5	Y	19 h	Store on board	30fps, 720 x 480HD		Collar mounted	[11]

References: [1] Bowen *et al.* 2002; [2] Nifong *et al.* 2013; [3] Nifong *et al.* 2014; [4] Beringer *et al.* 2004; [5] Moll *et al.* 2009; [6] Loyd *et al.* 2013; [7] Rutz and Bluff 2008; [8] Pearson *et al.* 2017; [9] Machovsky-Capuska *et al.* 2016; [10] Bombara *et al.* 2017; [11] This study 

600	Figure 1. Individual components of the kangaroo-cam units, which were incorporated into
601	one of two cases – the battery case or the component case (Shown as External lens in pod in
602	this figure). Details of the specific components, size, suppliers and cost are shown in Table 1.
603	
604	Figure 2. Eastern grey kangaroo (female) carrying the Kangaroo-cam device. The Kangaroo-
605	cam is oriented pointing forwards from its location, which means it is pointed directly
606	forward in this image .
607	
608	Figure 3. Proportion of time (as a percentage of total scorable recording time) that kangaroos
609	spent in each behavioural state during daylight hours, as determined by scoring videos
610	recorded by Kangaroo-cam units. Note that all states are mutually exclusive and that feeding
611	took precedence over other activities (see methods section).
612	
613	Figure 4. Proportion (as a percentage) of foraging events in which different plant families
614	were consumed by seven kangaroos.
615	
616	Figure 5. Still frames of images taken by the Kangaroo-cam units, showing the identification
617	of plants consumed versus those available during foraging events in kangaroos. This highlights
618	the potential utility of this approach for diet selection studies in herbivores.
619	
620	Figure 6. GPS movement tracks and core use areas, with behavioural categories superimposed
621	for four individuals: (a) animal 022 (male), (b) animal 261 (female); (c) animal 207 (female),
622	and (d) animal 230 (female). Squares indicate feeding sites and pentagons indicate resting sites,
623	while lines indicate movement trajectories. Shading represents the 50-95% kernel for core and

- 624 general animal use as follows: Red (50%), dark orange (60%), light orange (70%), yellow
  625 (80%), light green (90%) and dark green (95%).
- 626
- 627 **Figure 7.** Mean ± s.e.m. faecal glucocorticoid concentrations at 0, 24 and 48 h post capture in
- 628 GPS collared (black line, closed circles; n = 6) and non-collared animals (grey line, open
- 629 circles; n = 8) animals immobilised at time zero. Concentrations are significantly different
- 630 between groups at 24 h.
- 631



637 638	Fig. 1
639	8' -



**Fig. 2** 







# Selected plants



Ricinocarpus spp.

Platysace spp.

Lomatia spp.

Reed

# Non-selected plants



671 *Actinotus spp.* 

Banksia spp.

Bracken spp.

Hydrocotyle spp.

- 672 Fig. 5
- 673



**Fig. 6** 



**Fig. 7**