



## Koalas showed limited behavioural response and no physiological response to drones

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### ABSTRACT

Drones have become a popular conservation tool especially when monitoring cryptic species or species inhabiting locations difficult to access. We developed a non-invasive methodology to measure heart rate in koalas (*Phascolarctos cinereus*) using a Fitbit and investigated the behavioural (vigilance behaviour) and physiological (heart rate and breathing rate) responses of captive koalas to drones. We showed for the first time that heart rate values in koalas can be accurately obtained with a Fitbit. Koalas responded to a drone flight conducted 15 m above their heads with a short-term increase in vigilance, but no change in heart rate or breathing rate. Our results suggest that drones may not have long-term detrimental effects on koalas' fitness or energy demands and adds to the growing literature investigating animals' responses to drones to help develop best practices for drone monitoring.

### 1. Introduction

Assessing population trends is vital to implement effective conservation approaches (Ward et al., 2020). Yet evaluating population trends over large scales can be an ongoing challenge, especially when monitoring cryptic species or species living in complex environments (e.g., Hodgson et al., 2016; Hamilton et al., 2020; Colombelli-Négre, 2023). Drones (also referred to as Unmanned Aerial Vehicles) have become a popular conservation tool to overcome these problems, as they are cost effective, eliminate human bias, and can produce data over high spatial and temporal resolution, even for species that inhabit locations difficult to access (Chabot and Bird, 2015; Hodgson et al., 2017; Hodgson et al., 2018; Corcoran et al., 2021; Ivanova et al., 2022). However, using drones may have undesirable and unforeseen impacts on wildlife (e.g., Rümmler et al., 2016; Weimerskirch et al., 2018; Rebolo-Ifrán et al., 2019; Barr et al., 2020), which need to be properly assessed to develop best practice approaches (Hodgson and Koh, 2016).

Animal behavioural responses to drones vary across species and in relation to types of drones deployed and flight distances (Mulero-Pázmány et al., 2017; Weimerskirch et al., 2018; Barr et al.,

2020; Headland et al., 2021). A study on eleven sub-Antarctic seabirds, for example, showed that southern giant petrels (*Macronectes giganteus*) had a measurable behavioural reaction when a drone was 50 m above, whereas most other species showed strong behavioural reactions at 10 m (Weimerskirch et al., 2018). Similarly, eastern grey kangaroos (*Macropus giganteus*) only fled when overflown by a drone at 30 m (Brunton et al., 2019), while nocturnal southern hairy-nosed wombat (*Lasiorhinus latifrons*) retreated to their burrows when drones were at distances as high as 100 m above them (Headland et al., 2021). These studies clearly highlight that drone impacts on wildlife need to be further investigated across taxa, especially as many studies to date have focused primarily on birds (e.g., Rebolo-Ifrán et al., 2019; Barr et al., 2020; Egan et al., 2020; Weston et al., 2020), with fewer studies on terrestrial mammals (but see Ditmer et al., 2015; Bennitt et al., 2019; Brunton et al., 2019; Hartmann et al., 2021; Headland et al., 2021).

Animals may experience a physiological response to drones without any behavioural change (Ditmer et al., 2015; Weimerskirch et al., 2018). For example, a study on black bears (*Ursus americanus*) found that individuals showed minimal behavioural response to drone flights conducted 20 m above their location, but that they exhibited significant

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increase in heart rate during days of drone flights compared to days without drone flights (Ditmer et al., 2015). Another study showed that incubating king penguins (*Aptenodytes patagonicus*) showed minimal behavioural response to drones flying 10 m above their colony but their heart rates increased by 30% from baseline levels (Weimerskirch et al., 2018). Physiological monitoring has long been used in behavioural research to investigate potential stress responses to disturbance in animals (MacArthur et al., 1982; Von Borell et al., 2007; Ellenberg et al., 2013; Schaefer and Colombelli-Négrel, 2021), and there is increasing evidence that both behavioural and physiological responses to drones need to be considered.

Respiratory sinus arrhythmia, the synchrony between heart rate variability and respiration (whereby heart rate increases with a breath in and decreases with a breath out) is a physiological phenomenon observed in humans and some animals (Von Borell et al., 2007). The relationship between heart rate and breathing rate is widely studied in humans (Gaşior et al., 2016), but is less studied in other animals, with some studies showing a positive correlation between heart rate and breathing frequency in captive and domestic mammals (Piccione et al., 2019; Blawas et al., 2021; Grosso et al., 2021; Landeo-Yauri et al., 2021). Breathing rate could therefore be an important method, together with heart rate, to determine animal physiological response to stressors, one of the aims of this study.

Individuals may adjust their behavioural and/or physiological response to disturbance (such as drones) depending on their sex or habitat. Studies in birds for example have found that males showed more intense behavioural response to human disturbance, but less intense physiological response, than females (Ellenberg et al., 2009; Weimerskirch et al., 2002). In mammals, male guanacos (*Lama guanicoe*) have been found to spend more time in vigilance when scanning for predators than females (Marino and Baldi, 2008), while female polar bears (*Ursus maritimus*) with cubs tended to show more intense behavioural response to snowmobile disturbance than males (Andersen and Aars, 2008). Differences in habitat may also impact how much visibility individuals have to watch for predators and threats, which in turn could impact the time individuals spend in vigilance (Götmark et al., 1995; Borboroglu and Yorio, 2004). For example, a study on herring gulls (*Larus argentatus*) found that individuals responded differently to direct versus tangential approach to their nest but only in habitats with high visibility (Burger and Gochfeld, 1981).

Koalas (*Phascolarctos cinereus*) are arboreal Australian marsupials under threat from habitat disruption and anthropogenic disturbance (Lunney et al., 2007; McAlpine et al., 2015). Their conservation status has previously been controversial due to overabundance and over-browsing in South Australia and Victoria, where koala numbers historically reached unsustainable levels (McAlpine et al., 2015; Whisson and Ashman, 2020). Koala populations declined severely after the 2019/2020 bushfires, and, since then, their conservation status has been declared as “endangered” in Queensland, New South Wales, and the Australia Capital Territory under the Environmental Protection and Biodiversity Conservation Act. As a sedentary cryptic species, koala populations are generally spatially fragmented (White, 1999). Hence, drone based surveying has been a vital conservation tool in capturing the distribution and abundance of koalas (e.g., Beranek et al., 2020; Corcoran et al., 2021). Due to their dietary specialisation on *Eucalyptus* leaves (Seabrook et al., 2011), which are poor sources of proteins, koalas have developed several adaptations to conserve energy and can rest 20 h per day (Benesch et al., 2010). Therefore, any increase in energy demands in response to disturbance (whether behaviourally or physiologically) could negatively affect their fitness and compromise their energy expenditure (Ropert-Coudert et al., 2009; Larsen et al., 2014).

In this study, we investigated the behavioural (vigilance behaviour) and physiological (heart rate and breathing rate) responses of koalas to drones in captivity. We first developed a non-invasive and innovative methodology to measure heart rate in koalas using a Fitbit (Fitbit Inc, USA). We aimed to determine if heart rate correlated with breathing rate

response; in this way, a koala’s physiological response could be assessed visually therefore eliminating the need for capture. We then experimentally tested the response of captive koalas exposed to (1) a drone flight at 15 m or (2) a control (our experimental setup) and asked whether there was a significant change in the amount of time koalas spent in vigilance, as well as their breathing and heart rates during and following our experiments. We predicted that (1) the Fitbit would provide an accurate measurement of heart rates in koalas so that heart rate values obtained with a Fitbit would be not significantly different from those obtained with a stethoscope; (2) resting heart rate would positively correlate with resting breathing rate; (3) koalas would increase the amount of time they spent in vigilance, as well as their heart rate and breathing rate, when the drone was present but not during the control experiments, and (4) koalas would not recover (i.e., return to baseline) quickly if they perceived the drone as a threat.

## 2. Material and methods

### 2.1. Study site

We conducted all our experiments between May and September 2021 at Cleland Wildlife Park (thereafter referred to as “Cleland”;  $-34^{\circ}58'01.1''S$ ,  $138^{\circ}41'39.7''E$ ) between 0730 and 1000 (local time). The park is accessible every day to the general public between 0930 and 1700 (local time) and houses free-roaming and captive Australian animals.

The koala population at Cleland is composed of a mixture of females, unsterilised and sterilised males, and joeys; all of which are either wild born (rescued from Kangaroo Island and the Mt Lofty Ranges, both in South Australia) or captive born (born at Cleland). Cleland currently accommodates 82 captive koalas housed in four separate enclosures separated by a minimum of 100 m: (1) Kangaroo Island, housing 14 koalas: two juvenile males and 12 females; (2) Round Loft, housing 10 koalas of both sexes; (3) Bob Robins, separated into three adjoining pens and housing 31 adult koalas of both sexes, and (4) Eastern, which is the only enclosure with sexually active koalas and young joeys, and housing approximately 20 koalas of both sexes. In this study, we only tested koalas from the Kangaroo Island, Round Loft and Bob Robins enclosures as we were unable to conduct drone operations at the Eastern enclosure due to overhead powerline cables. All enclosures vary in their above visibility (which likely influenced the detection probability of the koalas from above and thus their ability to visually detect the drone): the Kangaroo Island enclosure has fewer trees and a more open canopy than the Round Loft and Bob Robins enclosures, while the Bob Robins enclosure has very limited above visibility due to a thick trellis roof covering most of the enclosure.

### 2.2. Aim 1: develop a new methodology to measure heart rate in koalas

We developed a new method (never used on koalas before) to measure heart rate in koalas using a Fitbit Inspire HR attached to their wrist. This method was chosen over other methods previously used on large mammals (e.g., RS800CX Polar Electro monitor; Essner et al., 2015; Coelho et al., 2019) or the use of a traditional stethoscope because the Fitbit device is small, non-invasive, and can be strapped to the koala’s wrist for continuous monitoring over extended periods of time. All individuals for this first phase were selected by Dr Hough, the veterinarian at Cleland, based on their potential to tolerate the Fitbit and being handled by Cleland staff. We ensured the accuracy of the Fitbit by comparing heart rate obtained from the Fitbit and a stethoscope on a single adult koala male under anaesthesia for castration. Measurements using both methods were conducted five times over 2-minute intervals (total 10 measurements). Once under anaesthesia, Dr Hough attached the Fitbit to the koala’s wrist, and heart rate was extracted via the “Fitbit: Health & Fitness” app on a phone while also being counted by Dr Hough using a stethoscope. We repeated our measurements on an active

(i.e., not under anaesthesia) castrated 15-year-old adult male in the Bob Robins enclosure as described above.

Once we identified that the Fitbit would be accurate (see results), we determined the maximum length of time that a koala tolerated a dummy Fitbit by using a black band of equivalent size and weight to a Fitbit. We chose an active castrated 15-year-old adult male in the Bob Robins enclosure to fit a Tulip extra wide rubber band (Tulip®, USA) mimicking a ‘dummy’ Fitbit on its right wrist. We then video monitored the koala continuously using a GoPro camera (GoPro Hero 7, GoPro Inc., San Mateo, CA, USA) attached to a branch 1 m from the koala for 1 h and 30 min (the length of our experiment; see aim 2). It was deemed that koalas would tolerate the Fitbit for the duration of our experiments as no changes in behaviour were observed, such as scratching, biting, or fleeing.

To assess if breathing rate correlated with heart rate, we measured heart rate and breathing rate for 1 h and 30 min in 16 koalas in the Round Loft enclosure (10 females and six males; all captive bred and non-sexually active adults between three and 12 years old). All individuals were video monitored with a GoPro and fitted with a Fitbit as previously described. Breathing rate was counted from the videos as the total number of movements of the thoracic cage per minute (breath per minute). Heart rate values (beats per min) could not be extracted from the app for so many koalas, so we extracted the values from the Fitbit website (<https://www.fitbit.com/global/au/home>) instead; heart rate data on the website were only available at five-minute intervals (the minimum interval setting).

### 2.3. Aim 2: Behavioural and physiological responses of koalas to drones in captivity

#### 2.3.1. Experimental set up

We tested the behavioural (vigilance) and physiological (heart rate and breathing rate) responses of 34 koalas (including 16 with a Fitbit) to (1) a drone flight ( $n = 16$  koalas including 10 with a Fitbit) or (2) a control, our monitoring set up with GoPro and Fitbit ( $n = 18$  koalas including six with a Fitbit). All experiments were conducted over four non-consecutive days. Koalas included in the control experiments were not included in the drone experiments (see [Table S1](#) for the details of the koalas tested).

To record their behaviour and breathing rate, we attached a GoPro camera 1 m opposite from each koala as described above. To measure their heart rate, Cleland staff attached a Fitbit to their wrist. Once the Fitbit was in place, we ensured that it was recording the individual’s heart rate using the Fitbit app. To ensure the experiments were uninterrupted, koalas were fed prior to our set up and the staff did not interact with the koalas during the experiments. Each koala was monitored for a total of 1 h and 30 min divided into three periods: (1) the first 20 min were used to determine baseline behaviour, heart rate and breathing rate (per-trial); this was (2) followed by 10 min of ‘drone flight’ or ‘control’ (trial), and (3) by another 1 h to assess individual recovery from the trial (post-trial).

To conduct the drone flight, we used a DJI Mavic 2 Pro (DJI Shenzhen, China) pre-programmed to launch 100 m east of either enclosure, to minimise potential initial take-off noise disturbance. We flew the drone above the enclosures and hovered it at 15 m above all the koalas (determined using the drones distance meter), moving horizontally and constantly back and forth from south to north (i.e., so it would not remain directly above a single koala but above the whole enclosure). After 10 min, we returned the drone to base.

#### 2.3.2. Behaviour and heart rate analyses

We analysed a total of ~128 h of video from 34 individual koalas using Solomon Coder beta 19.08.02 (<https://solomon.andraspeter.com/>). All koalas recovered within one minute of the trial (see results); therefore, we only analysed 30 min post trial behaviours. To avoid any bias in the scoring procedure, all recordings were scored by a single

researcher (IZS).

**Behaviours:** For each period (pre-trial, trial, post-trial), we recorded the percentage of time each koala spent in each behaviours (Comfort, Feeding, Resting, Socialising, Looking, Vigilant, Moving and Out of view) described in [Table 1](#). We measured ‘behavioural latency’ as the time taken in seconds for individuals to show signs of vigilance from the start of the trial period and ‘behavioural recovery’ as the total time in seconds taken by individuals to fully stop showings signs of vigilance once the trial started (e.g., [Schaefer and Colombelli-Négrel, 2021](#); [Iasiello and Colombelli-Négrel, 2023](#)). We created a new variable ‘vigilance intensity’ as the percentage of time spent in vigilance during the trial period minus the percentage of time spent in vigilance during the pre-trial period.

**Breathing rate:** To measure breathing rate, we visually counted on the videos the movements of the koala’s thoracic cage every minute for each period as described above. We measured ‘breathing rate latency’ as the time taken in seconds for individuals to deviate their breathing rate from baseline (by more than  $\pm$  one standard deviation) from the start of the trial period and ‘breathing rate recovery’ as the total time in seconds taken by individuals to return to baseline breathing rate once the trial started. We created a new variable ‘breathing rate intensity’ as the averaged breathing rate values during trial period minus pre-trial period.

**Heart rate:** Heart rate values every five minutes were extracted from the Fitbit website as described above. We measured ‘heart rate latency’ as the time taken in seconds for individuals to deviate their heart rate from baseline (by more than  $\pm$  one standard deviation) from the start of the trial period and ‘heart rate recovery’ as the total time in seconds taken by individuals to return to baseline heart rate once the trial started. We created a new variable ‘heart rate intensity’ as the averaged heart rate values during trial period minus pre-trial period.

### 2.4. Statistical analysis

We used SPSS version 28.0 for Windows (SPSS Inc., Chicago, IL, U.S.A) for all statistical analyses. Data are shown as mean  $\pm$  standard error unless otherwise stated. Outliers were capped to the nearest values.

#### 2.4.1. Aim 1: new methodology for measuring heart rate in koalas

We used Spearman’s correlations to determine (1) whether the Fitbit provided an accurate measurement of heart rate by comparing heart rate values obtained from the Fitbit and the stethoscope; and (2) whether resting heart rate values correlated with resting breathing rate values.

**Table 1**

Ethogram of the koala behaviours observed in this study.

Behaviour	Description	Reference (s)
Comfort	Yawning or scratching, biting, or licking any part of the body	I.Z. Sach pers. obs.
Feeding	Actively chewing or smelling leaves	<a href="#">(Larsen et al., 2014)</a>
Resting	Eyes open but sitting motionless or looking around at nothing in particular	<a href="#">Adam et al. (2021)</a>
Socialising	Approaching another koala or individual/object	<a href="#">(Larsen et al., 2014)</a>
Looking	Upright, head raised, eyes wide and ears pricked	<a href="#">(Larsen et al., 2014; I.Z. Sach pers. Obs.)</a>
Vigilant	Spine erects or whole body turned towards disturbance; ears forward, pricked or in movement while in a stationary position facing forward	<a href="#">Adam et al. (2021); I.Z. Sach and I. Hough pers. obs.</a>
Moving	Locomotion on the ground, jumping from perch to perch, or climbing	<a href="#">Adam et al. (2021)</a>
Out of view	Not visible on the video recordings	I.Z. Sach pers. obs.

## 2.4.2. Aim 2: behavioural and physiological responses of koalas to drones in captivity

**2.4.2.1. Behaviours.** We transformed ‘vigilance intensity’ as  $\log_{10}$  (‘vigilance intensity’ +10) to satisfy conditions of normality. As some individuals did not show any sign of vigilance (see below), we only obtained behavioural recovery data for 22 koalas (i.e. 12 koalas were dropped from the behavioural recovery analyses). We analysed ‘vigilance intensity’ ( $n = 34$  koalas) and ‘behavioural recovery’ ( $n = 22$  koalas) using ANOVAs with experiment type (drone, control), enclosure (our measure of habitat/visibility; Kangaroo Island, Bob Robins, Round Loft), sex (male, female) as fixed factors and Bonferroni post hoc pairwise comparisons. All koalas either showed signs of vigilance within less than 15 s or not at all during the experiments; hence, we categorised ‘behavioural latency’ into a binary variable such as: fast (< 15 s) or slow (> 15 s). We analysed ‘behavioural latency’ (fast, slow,  $n = 34$  koalas) using a binary regression with experiment type, enclosure, sex as independent variables.

**2.4.2.2. Breathing rate.** We obtained breathing rate data for 28 koalas (15 females and 13 males). As some individuals did not change their breathing rate during the trial and post-trial period compared to the pre-trial period, we only obtained breathing rate recovery data for 15 koalas. We analysed ‘breathing rate intensity’ and ‘breathing rate recovery’ using ANOVAs with experiment type, enclosure, sex as fixed factors and Bonferroni post hoc pairwise comparisons. All 15 koalas that changed their breathing rate during our experiment increased or decreased their breathing rate either immediately (within the first 10 s) or after more than 15 min during our experiments; hence, we categorised ‘breathing rate latency’ into a binary variable such as: fast (< 10 s) or slow (> 15 min). We analysed ‘breathing rate latency’ (fast, slow) using a binary regression with experiment type, enclosure, sex as independent variables.

**2.4.2.3. Heart rate.** We obtained heart rate data for 16 koalas (10 females and six males). We analysed ‘heart rate intensity’ and ‘heart rate recovery’ using ANOVAs with experiment type, enclosure, sex as fixed factors and Bonferroni post hoc pairwise comparisons. All koalas increased or decreased their heart rate either immediately (at the next heart rate values) or after more than 15 min during our experiments; hence, we categorised ‘heart rate latency’ into a binary variable such as: fast (< 5 min) or slow (> 15 min). We analysed ‘heart rate latency’ (fast, slow) using a binary regression with experiment type, enclosure, sex as independent variables.

## 3. Results

### 3.1. Aim 1: new methodology for measuring heart rate in koalas

We found that heart rate values obtained with the Fitbit and the stethoscope were significantly positively correlated ( $r_s = 0.89$ ,  $p < 0.0001$ ; Fig. 1a) and that heart rate values obtained with the Fitbit significantly correlated with breathing rate values ( $r_s = 0.97$ ,  $p < 0.0001$ ; Fig. 1b).

### 3.2. Aim 2: Behavioural and physiological responses of koalas to drones in captivity

#### 3.2.1. Behaviours

Fig. 2 presents the percentage of time koalas spent in each behaviour during the three periods (pre-trial, trial, and post-trial). During trial, koalas spent more time engaged in vigilance behaviour during the drone experiment ( $30.36 \pm 7.92\%$ ) compared to the control experiment ( $2.32 \pm 1.16\%$ ;  $F_{1, 33} = 25.03$ ,  $P < 0.001$ ; Fig. 3a). Koalas in the Round Loft enclosure tended to spend less time in vigilance behaviour than those in the other two enclosures (Table S2; Fig. 3b). Males and females did not differ in the time they spent in ‘vigilance intensity’ ( $F_{1, 33} = 0.39$ ,  $P = 0.54$ ; Fig. 3c). ‘Behavioural latency’ (experiment type Wald < 0.001,  $df = 1$ ,  $P = 1.00$ ; enclosure Wald = 0.05,  $df = 2$ ,  $P = 0.97$ ; sex Wald < 0.001,  $df = 1$ ,  $P = 0.98$ ) and ‘behavioural recovery’ (experiment type  $F_{1,21} = 1.10$ ,  $P = 0.31$ ; enclosure  $F_{2,21} = 1.38$ ,  $P = 0.28$ ; sex  $F_{1,21} = 1.00$ ,  $P = 0.33$ ) did not vary with experiment type, enclosure, or sex.

#### 3.2.2. Breathing rate

Resting breathing rate ranged from 8 to 17 ( $13 \pm 0.46$ ) breaths per minute. None of our breathing rate variables varied with experiment type, enclosure, or sex: ‘breathing rate intensity’ (experiment type  $F_{1,27} = 0.007$ ,  $P = 0.93$ ; enclosure  $F_{2,27} = 0.22$ ,  $P = 0.81$ ; sex  $F_{1,27} < 0.0001$ ,  $P = 1.00$ ), ‘breathing rate latency’ (experiment type Wald = 0.04,  $df = 1$ ,  $P = 0.84$ ; enclosure Wald < 0.001,  $df = 2$ ,  $P = 1.00$ ; sex Wald = 1.56,  $df = 1$ ,  $P = 0.21$ ), and ‘breathing rate recovery’ (experiment type  $F_{1,15} = 2.74$ ,  $P = 0.14$ ; enclosure  $F_{2,15} = 2.20$ ,  $P = 0.17$ ; sex  $F_{1,15} = 2.06$ ,  $P = 0.19$ ).

#### 3.2.3. Heart rate

Resting heart rate values ranged from 59 to 116 ( $86 \pm 3.87$ ) beats per minute. None of our heart rate variables varied with experiment type, enclosure, or sex: ‘heart rate intensity’ (experiment type  $F_{1,16} = 0.19$ ,  $P = 0.67$ ; enclosure  $F_{2,16} = 1.18$ ,  $P = 0.35$ ; sex  $F_{1,16} = 0.15$ ,  $P = 0.70$ ), ‘heart rate latency’ (experiment type Wald < 0.001,  $df = 1$ ,  $P = 1.00$ ; enclosure Wald = 0.04,  $df = 2$ ,  $P = 1.00$ ; sex Wald = 0.58,  $df = 1$ ,  $P = 1.00$ ).

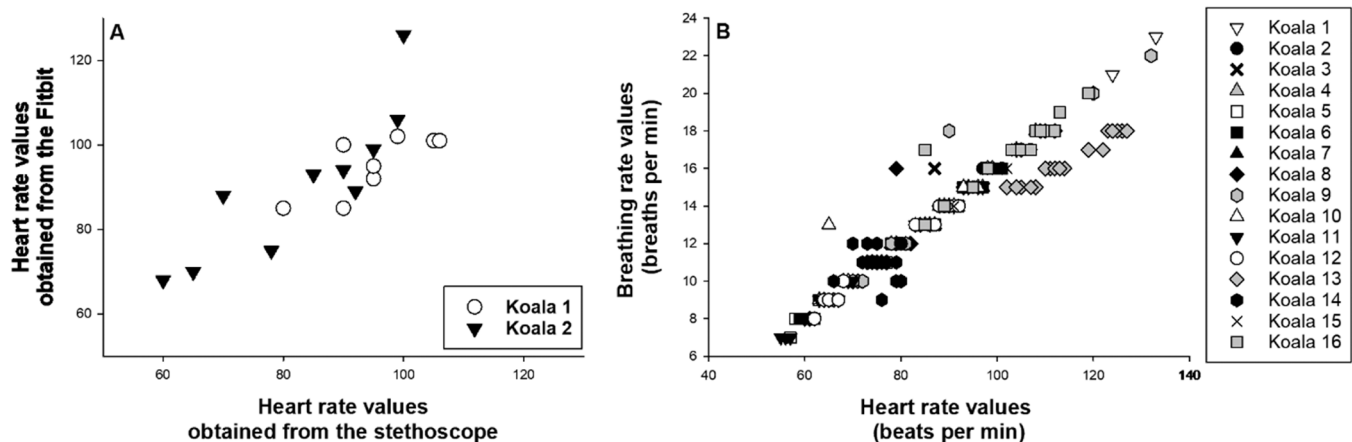
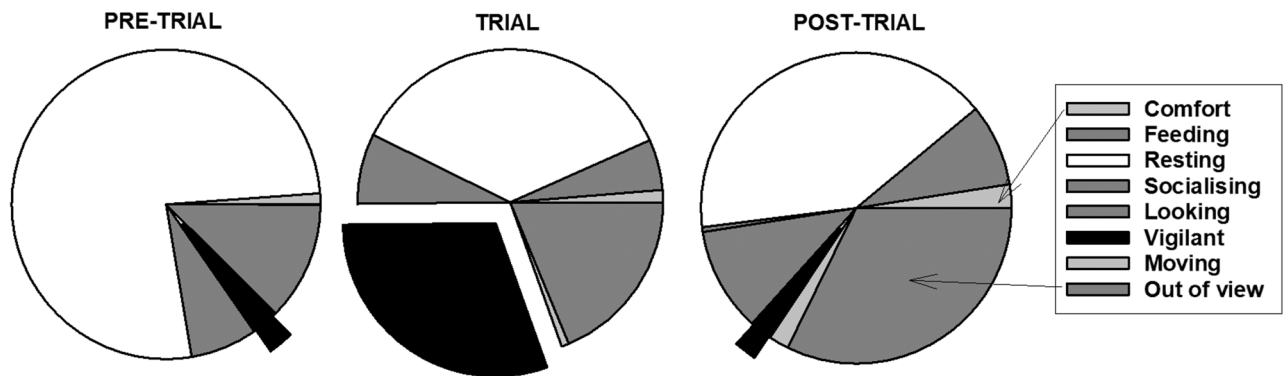


Fig. 1. Correlation between resting heart rate values (bpm) obtained with the Fitbit and (a) a stethoscope ( $n =$  two individuals) or (b) resting breathing rate ( $n = 16$  individuals).

(A) Drone Experiments



(B) Control Experiments

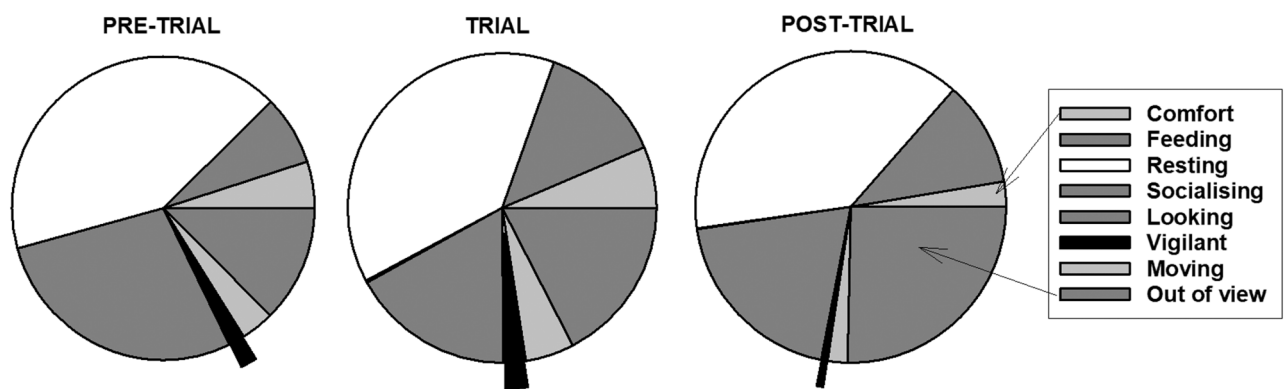


Fig. 2. Average percentage of time koalas spent in each behaviour during the three periods (pre-trial, trial, and post-trial). Data are presented for (a) the drone and (b) the control experiments (n = 34 individuals).

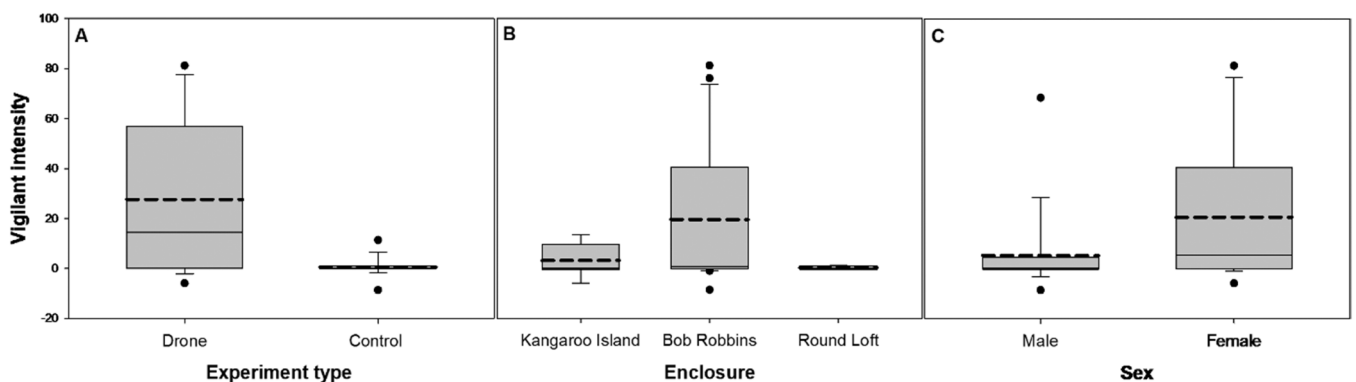


Fig. 3. Vigilant intensity (percentage of time spent in vigilance during the trial period minus pre-trial period) of captive koalas in relation to (a) experiment type, (b) enclosure, and (c) sex. Horizontal lines within the boxes represent means (dashed) and medians (straight). Whiskers represent  $\pm$  SE. Black circles indicate outliers.

= 1,  $P = 0.44$ ), and ‘heart rate recovery’ (experiment type  $F_{1,16} = 0.08$ ,  $P = 0.78$ ; enclosure  $F_{2,16} = 2.35$ ,  $P = 0.15$ ; sex  $F_{1,16} = 0.66$ ,  $P = 0.44$ ).

4. Discussion

Drones are increasingly used for wildlife conservation, including to monitor cryptic or declining species (Bogolin et al., 2021; Corcoran et al., 2021) or improve our knowledge of animal social structure (Maeda et al., 2021; Iwamoto et al., 2022). Yet our understanding of the potential impacts of using drones around wildlife remains limited or

data deficient. This study showed that during drone overflights at 15 m, captive koalas exhibited a short-term increase in vigilance, but no change in heart rate or breathing rate, suggesting that drone-facilitated monitoring of koala populations may not have long-term detrimental effects on individual fitness or energy demands. Our study adds to the growing literature investigating animals response to drones (e.g., Dittmer et al., 2015; Weimerskirch et al., 2018; Barr et al., 2020) to help develop best practices for drone monitoring (Hodgson and Koh, 2016).

This study showed for the first time that koala heart rates can be accurately measured with a Fitbit, which has important implications for

future monitoring in koalas. Our method differs from traditional methods used to measure heart rate in mammals, which often require capturing and shaving the individuals to either stitch a device to their skin or install electrodes subcutaneously (e.g., Weimerskirch et al., 2002; Ropert-Coudert et al., 2009; Essner et al., 2015; Chaise et al., 2017), thereby limiting their movements and thus behaviours. In our study, the Fitbit was attached to the wrist of the koalas within less than five minutes and without the need to shave the individuals. Due to the small size and weight of the Fitbit, heart rate of individuals could thus be obtained for extended periods, which could provide a unique advantage for long-term health monitoring in captivity (Benesch et al., 2010) or to better assess response to stressors (Larsen et al., 2014; Phillips, 2016).

Animal handling relies on highly trained researchers and is a labour-intensive process that can elicit a stress response in animals (Duka and Masters, 2005; Jewell, 2013). Developing non-invasive methods for individual monitoring should therefore be at the forefront of conservation research. Similar to previous studies in mammals (Piccione et al., 2019; Blawas et al., 2021; Grosso et al., 2021; Landeo-Yauri et al., 2021), we found a positive correlation between heart rate and breathing rate in koalas, which creates a window of opportunity to monitor physiological response of wild and captive populations without the need for capture. While we acknowledge that there could be limitations to this method, mainly due to variation in visual detectability of breathing, assessing physiological response (in addition to behavioural response) in wild populations will help better quantify the long-term impacts of anthropogenic disturbance (e.g., MacArthur et al., 1982; Ellenberg et al., 2013; Ditmer et al., 2015).

Contrary to other studies on birds (Ellenberg et al., 2009; Weimerskirch et al., 2002) and mammals (Andersen and Aars, 2008; Marino and Baldi, 2008), we found no sex-specific responses to our experiments. This aligns with previous work on captive koalas during which individuals increased their vigilance in response to tourist presence, regardless of their sex (Larsen et al., 2014). Similarly, we found overall no effect of enclosure type (a factor that covaried with visibility) on the time koalas spent in vigilance. Koalas in the Round Loft enclosure however tended to spend less time in vigilance behaviour than those in the other two enclosures. This may be due to the increased canopy cover in the Round Loft enclosure, as thicker and denser canopy cover could also offer added camouflage and hence a sense of protection. The effect of canopy cover on stress response may need to be further tested across states and populations as tree cover (and thus visibility) is expected to vary greatly between locations and may interact with threats experienced by the individuals. Other factors, such as previous experience with disturbance, reproductive status, or even individual personality (e.g., Ellenberg et al., 2009; Colombelli-Négrel and Katsis, 2021; Schaefer and Colombelli-Négrel, 2021), could also play a more important role than sex or visibility when responding to disturbances, which should be investigated in future studies.

Understanding what animals consider threats is important for conservation research (e.g., Colombelli-Négrel et al., 2010; Viblanc et al., 2012; Brunton et al., 2019). Koalas in our study spent more time engaged in vigilance during the drone experiment compared to the control experiment. It is unclear however whether koalas responded with an increase in vigilance due to the unfamiliar noise of the drone or the presence of an aerial object that may be perceived as a predator, as koalas can be predated upon by aerial predators such as wedge-tailed eagles (*Aquila audax*) (Melzer et al., 2003). Koalas are also known to have extremely sensitive hearing, especially considering their sexual communication rests mainly on acoustic communication (Charlton et al., 2011). A study on wild koalas for example found that noise generated by a music festival adversely affected their movement, whereby individuals moved up to 525 m away from the source of the noise (Phillips, 2016).

While repeated or ongoing exposure to stressors can lead to a reduction in individual fitness (Barnett and Hemsworth, 1990), stress response in wild animals is only critical at a population level when it

impacts survival or reproductive success (Gill et al., 2001). Without physiological analysis or baseline data from which to understand variance in response, it is thus difficult to determine the full effects of the disturbance. Here, we found that koalas did not exhibit any change in heart rate or breathing rate in response to our drone flight. This may be due to potential sensory adaptation to reoccurring noises in captivity as this study was completed on captive individuals, which are regularly exposed to other anthropogenic noises, such as lawn mowers, motor vehicles (including buggies driven through the park) or human voices. Yet a previous study in captive koalas found a notable change in their heart rate in relation to visitor noise and numbers (Ropert-Coudert et al., 2009). It is also possible that the noise they experienced during the drone flight in our study may not have been significant enough to be considered as a threat. This suggests that koalas may have assessed the drone as posing non-significant threat, which may have resulted in the lack of change in heart rate or breathing rate. Our results warrant further investigation into the effects of repeated noise disturbance on long-term fitness in koalas.

In conclusion, our study provides additional insights into the behavioural and physiological responses of a terrestrial species to drone surveys (Ditmer et al., 2015; Bennitt et al., 2019; Brunton et al., 2019; Headland et al., 2021). Current methods for monitoring koala populations are inconsistent across Australia – ranging from indirect surveys (such as faecal pellet surveys; McAlpine et al., 2006), diurnal or spotlight searches (Wilmott et al., 2019), physical tagging of individuals (Duka and Masters, 2005; Dexter et al., 2016, 2018) to drone monitoring (Beranek et al., 2020; Corcoran et al., 2021) - which can confound the management of the species (Adams-Hosking et al., 2016; Wilmott et al., 2019). It is therefore vital to implement a more consistent monitoring method across states. The limited behavioural response and the lack of physiological response found in our study suggest that drones may not have long-term detrimental effects on individual fitness or energy demands, which would validate their use for national wide koala population monitoring to ensure consistency between states. It is suggested that future studies incorporate both behavioural and physiological response to their study design to develop best practices for drone monitoring and reduce or alleviate any unwanted impacts on wildlife, especially for threatened and endangered species.

#### Ethics statement

This study was conducted under Flinders University Animal Ethics permit number BIOL4269. All drone flights were conducted by the same licenced pilot (IZS, RPA Remote Pilot Accreditation: 1131411) with appropriate permissions from the Civil Aviation Safety Authority and in accordance with local regulations.

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#### CRediT authorship contribution statement

All authors designed the research. IZS, DCN and IH conducted the experiments. IH handled the Koalas. DCN and IZS analysed data and wrote the first draft of the manuscript. All authors commented on the manuscript.

#### Declaration of Competing Interest

The authors declare no conflicts of interest.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.applanim.2023.105963.

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