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Influences of behaviour and physiology on body mass gain in the woylie (*Bettongia penicillata ogilbyi*) post-translocation

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Abstract

Context. Temperament can affect an individual's fitness and survival if it also influences behaviours associated with predator avoidance, interactions with conspecifics, refuge selection and/or foraging. Furthermore, temperament can determine an individual's response to novel stimuli and environmental challenges, such as those experienced through translocation. Increasing our understanding of the effect of temperament on post-translocation fitness is thus necessary for improving translocation outcomes.

Aims. The aim was to test whether differences in an individual's behaviour or physiology could help predict body mass changes post-translocation in the woylie (brush-tailed bettong, Bettongia penicillata ogilbyi). In the absence of predation (due to release into a predator-free exclosure), body mass was used as a proxy for an individual's success in securing resources in the new habitat, and therefore fitness.

Methods. Forty woylies were translocated from two predator-free exclosures to a larger exclosure, all in Western Australia. Behavioural and physiological measures were recorded during trapping, processing, holding, and release, and again at re-capture ~100 days post-release.

Key results. Translocated woylies generally increased in body mass post-translocation. This suggests that, in the absence of predation, the selected candidates were able to cope with the stress of translocation and possessed the behavioural plasticity to successfully find resources and adapt to a novel environment. The strongest predictors of body mass gain were sex, heart rate lability and escape behaviour when released (a convoluted escape path).

Conclusions. There was no significant difference in body mass between males and females pre-translocation but females showed greater mass gain post-translocation than did males, which could reflect greater investment in reproduction (all females had pouch young). Heart rate lability and escape behaviour are likely to reflect reactivity or fearfulness, a significant temperament trait in the context of translocation success.

Implications. Behavioural measures that can be easily incorporated into the translocation process – without increasing stress or affecting welfare of individuals – may hold promise for predicting the fate of translocated animals.

Additional keywords: activity, anti-predator response, corticosterone, escape behaviour, heart rate.

Introduction

Translocation is a strategy that is used to supplement declining populations or to re-establish locally extinct populations, both of which aim to secure populations of threatened fauna (Morris *et al.* 2015). Despite increasing use of translocations for the conservation of declining species, the success of such interventions is highly variable (Short *et al.* 1992; Short 2009). Although many potential causes for failure have been researched – including predator naïvety, habitat selection, demographics, handling trauma and environmental novelty (Armstrong *et al.* 2015) – only recently has individual temperament been investigated (e.g. Sinn *et al.* 2014; May *et al.* 2016; Germano *et al.* 2017).

Temperament is defined as individual differences in behaviour that are consistent over time and across situations (Réale *et al.* 2007). There are six key temperament traits recognised in animals: shyness–boldness, emotional reactivity or fearfulness, exploration–avoidance, activity, sociability and aggressiveness (Table 1). These traits affect how an individual interacts with conspecifics, finds resources, disperses and avoids predators; in turn, these traits can be linked to reproductive success, fitness and survival (e.g. Dingemanse and de Goede 2004). However, a temperament trait that is beneficial in an individual's current environment may not impart fitness advantages if that individual is moved to a new location (McDougall *et al.* 2006; Watters and Meehan 2007). It is therefore important to increase our understanding of the role of animal temperament on fitness, especially in the context of translocations.

Various methods of assessing temperament have been successfully used for animals that were born and raised in captivity, as well as for wild animals caught and held in captivity for a period of time before translocation (Bremner-Harrison *et al.* 2004; Sinn *et al.* 2014; Montagne 2016; Germano *et al.* 2017). However, prolonged holding is difficult to achieve during translocations of free-living animals, particularly if individuals are to be transported long distances on a tight schedule to ensure compliance with welfare requirements (Dickens *et al.* 2010). Furthermore, keeping free-living animals in temporary captivity generally increases stress, which can impact welfare and behaviour, thus potentially confounding the results of behavioural tests (Archard and Braithwaite 2010). If behavioural measures taken during temporary holding before translocation proved to be reliable indicators of temperament, they may be a useful measure of suitability for translocation, and thus an alternative to conducting behavioural tests in free-living individuals before translocation. For example, May *et al.* (2016) recorded a range of behavioural measures before and during translocation of common brushtail possums (*Trichosurus vulpecula*); these measures were compared with subsequent survival of individuals. The authors demonstrated that possums with higher reactivity to holding survived longer post-translocation than did less reactive possums. They also found that, post-translocation, bolder possums gained more body mass than did shy possums (May *et al.* 2016).

Body mass strongly ties into animals' fitness and survival, as well as reproduction. This is because energy is only used for reproductive needs once requirements are met for maintenance (i.e. survival) and growth or repair of injured tissue (e.g. Kooijman 2010). For example, some reptiles undergo follicular atresia when conditions are unfavourable (Guraya 1989). Lack of reproduction then impacts population establishment. Further, if animals have poor body condition, they are likely to be less resilient to environmental stress (Jakob *et al.* 1996). Energy allocation and use are also influenced by an animal's temperament. For example, many animals reduce or alter their foraging strategy in response to perceived increases in predation risk, which then lowers their growth rates. Bolder (e.g. Fraser *et al.* 2001) or more exploratory (e.g. Peterson *et al.* 2016) individuals would have an advantage through finding new resources and competing for these more successfully (Cote *et al.* 2010). However, activity, exploration and aggressiveness are also energetically costly, so temperament has a significant influence on metabolic rate and energy consumption (Careau *et al.* 2008). Temperament thus affects energy balance and body mass gain, and can be a useful indicator of post-translocation fitness (Molony *et al.* 2006).

The objective of the present study was to investigate the correlation between measures of individuals' temperament – as determined through measures of behaviour and physiology recorded during and after translocation – with change in body mass during a translocation in a threatened marsupial, the woylie (*Bettongia penicillata ogilbyi*; Fig. 1), also known as the brushtailed bettong. Historically, the species was found across 60% of mainland Australia (Yeatman and Groom 2012). Following European settlement, there were major reductions in abundance and distribution of woylies attributed to predation, changed fire regimes, and habitat clearance (Woinarski and Burbidge 2016). By the 1960s, only three populations persisted, all in south-western Western Australia (WA): Upper Warren; Tutanning Nature Reserve; and Dryandra Woodland (Wayne 2008). As a result of fox control and a series of translocations, the species made a substantial recovery by 1996 (Wayne *et al.* 2015); however, there was another decline from 1999 onwards (Wayne *et al.* 2013). Intensive research into the cause identified a complex interaction between disease, predation, direct human interference and food resources as contributing to the species' second decline (Wayne *et al.* 2015). Currently, only two remnant populations of woylies persist: those at Dryandra Woodland and in Upper Warren (Wayne *et al.* 2013). The species is listed as 'critically endangered' under WA legislation and 'endangered' under national legislation (Woinarski and Burbidge 2016).

Because the animals in the present study were released into a predator-free exclosure, predation risk by red foxes (*Vulpes vulpes*) and feral cats (*Felis catus*) was completely mitigated and a high survival rate was expected (Ruykys and Kanowski 2015). The study therefore presented an opportunity to investigate how temperament impacts body-mass gain, independent of the confounding factor of predation. Woylies make a good study species because they respond well to energy gain, i.e. they are continuous breeders and, in favourable conditions, can produce up to three young per year (Serventy 1970; Sampson 1984). We predicted that less fearful, bolder or more explorative individuals (based on behavioural and physiological measures) would be more successful in finding resources in their new environment and would thus gain more body mass post-translocation than would more reactive or fearful individuals. The aims of the present study were to determine:

(1) If temperament traits – activity, aggression and reactivity or fearfulness – could be assigned to individuals based on behavioural and physiological measures recorded during animal trapping, handling and release; and
(2) Whether there were relationships between behavioural and physiological measures and post-translocation body mass gain after accounting for effects of sex, source population and radio-collaring.

Methods

Study areas

Approximately 80% of translocations of Australian mammals have failed due to predation of the translocated individuals by introduced red foxes and feral cats (Woinarski *et al.* 2014). Such failures have resulted in a recent proliferation of predator-proof fences constructed to protect threatened mammals (Ringma *et al.* 2018). The present study followed individual woylies during two translocations: one from the Australian Wildlife Conservancy's (AWC) 'Karakamia Wildlife Sanctuary' (3 May 2016); and the second from the WA Government's Department of Biodiversity, Conservation and Attractions' 'Perup Sanctuary' (30 May–2 June 2016) into the newly constructed 7,832 ha fenced area at AWC's Mt Gibson Wildlife Sanctuary (Table 2). All three sites are in south-western WA: Perup is the southern-most site, ~260 km south-east of Perth; Karakamia is the central site, in the Perth Hills and ~50 km east of Perth; and Mt Gibson is the northern-most site, ~350 km north-east of Perth. These two translocations supplemented a previous translocation of 50 woylies from Karakamia to Mt Gibson that had occurred in August–September 2015, which showed a 100% survival rate of radio-collared woylies 4 months post-release (Ruykys *et al.* 2016).

Behavioural data collection

Behavioural data were collected at four stages of the translocation process (Table 3).

Stage 1: trapping for translocation

To capture animals for translocation from the two source sites, cage traps (0.22 m _ 0.22 m _ 0.58 m; Sheffield Wire Products, Welshpool, WA) were set at dusk along transects or within quadrats. Traps were baited with rolled oats and peanut butter, and had newspaper placed under them for scat collection (see 'Faecal sample extraction' section). At Karakamia, the first trap check occurred at ~2000 hours and then approximately every 2 h until 0300 hours. At Perup, traps were left open overnight and checked at 0400–0900 hours each morning. Animals' agitation inside and on exit from the trap was scored using the following scale:
(1) Low: Very little or no bouncing and enters the handling bag without hesitation;
(2) Medium: Some bouncing but settles quickly and enters the handling bag without much hesitation; and
(3) High: continuous 'bouncing' (i.e. banging into the cage walls in an attempt to escape); long latency or resistance to entering the handling bag, and/or injuries to the face or head from trap damage.

Owing to the nature of trapping for translocation, multiple people provided trap agitation scores; however, to try to instate consistency, all personnel involved were provided with a briefing by a single handler (KP) and queries were discussed.

Stage 2: processing

Both source sites had an indoor area that was used as a central location for animal processing and holding. The areas differed in layout and size, but each was large enough to hold multiple people and animals, plus had an adjoined but separate room in which woylies selected for translocation were held after processing. All trapped woylies were brought to the central site and held for varying amounts of time in their handling bags until processing of that individual could commence.

First, heart rate (beats per min; b.p.m.) was measured with a stethoscope for 1 min through the handling bag. Subsequent animal processing involved taking standard morphometric data that included body mass (g), pes length (mm), sex, reproductive status and pouch condition. At Karakamia, all processing was undertaken by a single, experienced handler (LR), with assistance in holding animals provided by other handlers. At Perup, all processing was undertaken by just two experienced handlers, one of whom was LR, with assistance in holding again provided by other handlers. Processing duration averaged 13.55 ± 4.18 min at Karakamia, and 30.03 ± 13.77 min at Perup. Animals' agitation during processing was assigned by the lead handler based on the behaviour that was observed during this time:

(1) Low: little or no resistance to handling; low reactivity to touch; no biting, kicking, vocalisation or resistance to pouch inspection;

(2) Medium: intermittent resistance to handling; moderately reactive to touch; some resistance to pouch inspection; no biting or vocalisation; and

(3) High: continual resistance to handling; biting, kicking or resisting pouch inspection; elevated respiration; highly reactive to touch; vocalisation.

Heart rate was then measured again immediately after processing. A single handler (KP) took all heart rate measurements.

Individuals were deemed suitable for translocation if they were adults, had no clear abnormalities and were within- or above-average ranges in terms of body mass (≥1.06 kg for animals from Karakamia; ≥0.98 kg for males and ≥0.75 kg for females from Perup). If females had a pouch young, the crown–rump length of the young needed to be <50 mm for females to be selected for translocation. Animals that were unsuitable for translocation were released at point of capture. A subset (n = 14) of suitable individuals >1.05 kg sourced from Perup were fitted with very high frequency (VHF) radio-collars weighing 35 g (Sirtrack Ltd, Havelock North, New Zealand).

Stage 3: post-processing holding and activity level measurement

After processing, animals were returned to their handling bags and placed into pet packs. The animals were then allowed to settle within a separate room of the central processing area for variable periods of time (average 1 h 5 min, range 9 min to 4 h 37 min across both source sites) while their activity levels were recorded. Due to the logistics of the translocations, it was not possible to have consistency in this timing. Tri-axial acceleration (m s–2) was recorded with an Apple iPhone accelerometer application (VibSensor, Now Instruments and Software, Inc., 2016; http://www.now-instruments.com/, accessed 29 May 2019). On each occasion, phones were placed on top of pet packs and set to record for 10 min after a 1 min delayed start. Activity during recording was logged on the phones' X, Y and Z axes. Up to three recordings were conducted per individual and phones were alternated between individuals. Animals' microchip numbers were used as unique identifiers for each record. To account for noise and disturbances that may have influenced activity levels, a video camera in the holding room was set to simultaneously record during the accelerometer recordings. To compare recording sensitivity between phones, two 10-min test runs were also conducted, for which all phones were placed on top of empty pet packs.

Stage 4: transport and release

Due to logistical constraints, there were unavoidable variations in the transport and release of woylies from the two source populations. Woylies sourced from Karakamia were transported ~350 km by vehicle, with the journey to Mt Gibson taking 4.5 h. Woylies sourced from Perup were transported by vehicle to Manjimup Airport (~1 h), where they were transferred to a small fixed-wing plane. The plane departed daily at ~1100 hours and landed at Mt Gibson at ~1300 hours. On arrival at Mt Gibson, all woylies were placed in the same dark, relatively quiet holding room. Post-travel accelerometer data using the methods described above were recorded at this stage. A video camera filmed for the duration of these recordings.

After dusk on each day of translocation, woylies were transported by vehicle to the release sites inside Mt Gibson Sanctuary's exclosure. Individuals were released at one of 15 release sites, each 200 m apart and located in Acacia shrubland and York gum (*Eucalyptus loxophleba*) woodland. On any one night, a maximum of two individual woylies were released per site, though some release sites were re-used on subsequent nights. Owing to the nature of translocations, releases were undertaken by, and related parameters measured by, varying personnel. However, all involved personnel were provided with a briefing by one person (KP) and all releases were led by a handler experienced with woylies. There was a maximum of four people present at the release of any one woylie, and all people present were cautioned to remain quiet and still. A maximum of one head torch was allowed to be used.

At the release site, the handling bag was rolled away from the back of the woylie towards the face until the eyes were exposed. At this point, a stopwatch was used to record the time that it took for the individual to exit the bag (latency to leave; s). The woylie's escape path (subjectively scored as straight or convoluted) and relative escape speed (subjectively scored as slow or fast) were also recorded.

Post-translocation recapture

In total, 40 woylies (*n* = 21 males, *n* = 19 females) were translocated to Mt Gibson from Karakamia and Perup Sanctuaries; 27 (n = 16 males, n = 11 females) of these individuals were re-sampled 4–6 months later (Table 4). Post-translocation recapture of animals at Mt Gibson for population monitoring purposes was carried out on 22–28 August 2016, at which time any radio-collars on recaptured individuals were also removed. Trapping for population monitoring purposes involved the setting of 80 cage traps throughout the exclosure. Animals that retained collars after this time were target trapped between 28 August and 13 October 2016. Target trapping involved radio tracking a collared individual during the day and, once located, placing 10 cage traps in an approximate circle around it. In all instances, traps were baited with peanut butter and oats before dusk and checked at or within 3 h of dawn the following morning. Faecal samples were again collected by placing newspaper under traps, and agitation during trapping and processing were both assessed using the above-described scale (numbers of animals in Table 4).

Faecal sample extraction

Faecal samples were collected from newspaper that had been placed underneath cage traps (newspaper was refreshed if it had become wet, or if it was used for collection of samples), transferred into snap-lock bags and temporarily frozen at –5°C. Thirty-eight faecal samples collected from woylies pre-translocation and 26 samples collected post-translocation were stored at –20°C for 9 months. Eight of the 26 samples collected post-translocation were initially frozen but were unintentionally left out of the freezer for ~4 days, then re-frozen; these samples were still processed. Following faecal steroid extraction (Appendix S1, available as Supplementary material for this paper), a commercial human corticosterone ELISA kit (K014-H1: Arbor Assays®, MI, USA) was used for the analysis of faecal corticosterone concentrations.

Statistical analyses

All statistical analyses were carried out using Statistica version 7.0 (StatSoft Inc., Tulsa, OK, USA) and Microsoft Office Excel 2007 (Microsoft Corporation, Redmond, WA, USA). All variables were checked for normality using the Shapiro–Wilk W (SW-W) test and homogeneity of variances was tested using Levene's test. An a level of P = 0.05 was used to determine significance. Latency to leave (s) and corticosterone concentration (pg mL–1) were log10-transformed to meet the assumptions of a normal distribution. Six individuals did not have data recorded for a behavioural variable, so these missing data were substituted with an average for these variables.

Behavioural data

Correlations between pairs of behavioural measures and heart rate were assessed using Spearman Rank Order correlations. Activity datasets were exported from iPhones as csv. files and saved as Excel files. Each file included a series of triaxial movement measures (acceleration in m s–2) with corresponding time stamps. Approximately 56 000 records were captured per 10 min of recording. For analyses, records were arranged into blocks of 400 (equivalent to 3–4 s of recording) and each block was numbered from 1 to 140. All records were converted to positive numbers by adding 1. The average, standard deviation and coefficient of variation (COV) of movement (m s–2) were calculated for each axis (X, Y and Z) of each block. The mean percentage COV ± 1 s.d. was then calculated from the COV of all three axes per group. Each phone was assigned a unique identification number (Phone ID); this allowed examination of variation in recordings between different phones. Data were analysed by mixed-model ANOVA with time point (pre- and post-transport) as a fixed factor, and individual animal ID and phone ID as random effects.

Physiological data

Heart rate data were normally distributed pre-processing (Shapiro-Wilk W = 0.976, P = 0.546) and post-processing (Shapiro-Wilk W = 0.983, P = 0.809); however, there were significant differences in variances in pre- and post-processing heart rates between source populations (Levene's test, $P = 0.002$) (Perup $n = 31$, Karakamia $n = 9$). Consequently, non-parametric Mann–Whitney U-tests were performed to test for site differences in these data. There were also significant differences in variances between the pre-processing heart rates of males and females (Levene's test, P = 0.01); consequently, Mann–Whitney U-tests comparing the sexes were conducted. This non-parametric analysis only allows a single factor to be tested; therefore, to compare differences in heart rates between sexes, data from both sources were pooled.

For faecal corticosterone concentration, Microplate Manager software (Bio-Rad, Benicia, CA, USA) was used to convert absorbance values into corticosterone concentration (pg mL^{-1}) by means of a four-parameter logistic curve fitted to the absorbance values of the standards. Corticosterone concentration was recorded for duplicate sub-samples for each sample (data were then averaged for the duplicate sub-samples). Only individuals from which both pre- and post-translocation samples had been collected were included in the analyses (males, n = 14; females, n = 11). Levene's test of log corticosterone concentration pre- and post-translocation was not significant, allowing use of a repeated-measures ANOVA followed by a *post hoc* Fisher's least significant difference (l.s.d.) test. Due to the small number of Karakamia-sourced individuals for which posttranslocation faecal samples were collected ($n = 4$), it was not possible to consider the effect of source population on corticosterone concentration as a separate factor in these analyses.

Correlations with change in body mass

The change in body mass for the 27 animals that were retrapped was calculated as a rate of change (i.e. g per days since release); these values are expressed as the rate of mass change for 100 days (the average length of time between release and recapture for the 27 individuals was 100 ± 18 days). For the 14 woylies that were radio-collared, radio-collar weights (35 g) were deducted from the post-translocation body mass.
We undertook multiple regression analyses to assess correlations between change in body mass and 12 independent variables: (1) source population (Karakamia or Perup); (2) sex (male or female); (3) presence of a radio-collar (yes or no); (4) pre-translocation faecal corticosterone concentration (Log10 pg mL–1); (5) pre-translocation records of trapping agitation level (low = 0, medium = 0.5, high = 1); (6) pre-processing heart rate (b.p.m.); (7) processing agitation level (low = 0, medium = 0.5, high = 1); (8) post-processing heart rate (b.p.m.); (9) heart rate lability (post-processing minus pre-processing heart rate; b.p.m.), (10) post-translocation latency to leave at release (log10 s); (11) escape speed (slow or fast); and (12) escape path (convoluted or straight). An information-theoretic approach using Akaike's information criterion corrected for small sample sizes (AICc) was used to identify correlations between rate of change in body mass and all combinations of the 12 independent variables. Akaike model weights (wi) were calculated for each model with DAICc <2, and model averaging was carried out calculating the model-averaged b values (wiB) (Burnham and Anderson 2003), using standardised b values calculated assuming that each variable input into a model had a mean of 0 and s.d. of 1 (Grueber *et al.* 2011).

There were no significant differences in variance in body mass between males and females (males, n= 16; females, n = 11) either pre- (Levene's test, P = 0.985) or post-translocation (Levene's test, P = 0.888). There was also no significant difference in variance in body mass between radio-collared woylies and nonradio-collared woylies pre- (Levene's test, P = 0.853) or post-translocation (Levene's test, P = 0.451). As such, change in body mass between sexes, and between collared and non-collared woylies, were assessed using repeated-measures ANOVA. *Post hoc* analyses were performed using Fisher's l.s.d. test. Values are presented as means ± 1 s.d. throughout.

Results

Behavioural measures

Personality theory suggests that there would be suites of measures that should show similar patterns between individuals; however, we recorded few correlations between behavioural measures (Table 5) and our physiological data were only recorded once for each animal due to logistical constraints of the translocation procedures. Pre- and postprocessing heart rate measures were correlated, and the difference in heart rate (heart rate lability) was correlated with post-processing heart rate. Processing agitation scores were negatively correlated with heart rate lability. Trap agitation on initial capture was negatively correlated with processing agitation on recapture, 5 months post-translocation. Other measures were not statistically significant.

Accelerometer data were reliably recorded from 31 individuals before and after transport; some animals were missed due to inadequate time as part of the translocation. The method successfully recorded activity levels; however, these were influenced by external stimuli such as the level of noise. These stimuli could not be kept consistent among individuals as recordings were made at different times. For example, a 10-min recording of tri-axial movement of a woylie at Karakamia during holding shows two peaks that occurred simultaneous to loud noises that were recorded on the video camera (Fig. 2). There was no significant difference (mixed-model ANOVA) in the variation in activity for individuals pre- and post-transport (P = 0.681), or for individuals (Animal ID random effect P = 0.416), and the majority of the variation in the data that we could account for was attributable to differences among phones (Phone ID random effect P = 0.060).

Source population and sex differences

Animals from Perup had significantly higher heart rates than did woylies from Karakamia, both pre-processing (Fig. 3a; Perup n = 31 and Karakamia n = 9; U = 27, Z = –3.64, P < 0.001) and post-processing (Fig. 3b; U= 28, Z = –3.61, P < 0.001). When the data from both populations were pooled, there was no significant sex difference in heart rates pre-processing (U= 197.5, Z= 0.054, P = 0.957) or post-processing (U = 193.0, Z = –0.18, P = 0.860).

There was a statistically significant sex x time interaction (F1, 23 = 6.64, P = 0.017) in faecal corticosterone concentrations (males n = 14, females n = 11) (Fig. 4a). There was a significant difference between males and females pre-translocation (*post hoc* analysis P = 0.035), with males' concentration levels high by 5%. Mean corticosterone concentrations for females increased pre- to post- translocation (3.57 ± 0.21 pg mL^{-1} to 3.77 ± 0.20 pg mL^{-1}), while males' levels decreased (3.76 ± 0.28 pg mL^{-1} to 3.62 ± 0.17 pg mL^{-1}).

Influence of behaviour, physiology and sex on change in body mass

Most woylies that were recaptured had gained mass post translocation, although five had experienced decreases and one was the same. Five models had a ΔAICc <2 and were therefore considered to have some support of being the best model to explain the rate of change in body mass of woylies (Table 6). Of the 12 variables in the AIC analyses, only three variables were included in these top models. The model averaged standardised β values for these three variables indicated that they had reasonably equal contribution to the description of change in body mass. Three of the top five models included sex as a variable (Fig. 5a), with females increasing in body mass (1.24 ± 0.15 kg pre-translocation cf. 1.40 ± 0.15 kg post-translocation; 12.4 ± 8.6% increase) at twice the rate of males (1.19 ± 0.16 kg pre-translocation cf. 1.27 ± 0.11 kg post-translocation; 5.7 ± 8.2% increase). This effect resulted in a statistically significant sex x time interaction for body mass (repeated-measures ANOVA: $F_{1,25} = 4.54$, $P = 0.043$; Fig. 4b). Change in heart rate during processing (Fig. 5c) was included in four of the top five models. The positive β values indicate that animals with greatest lability in heart rate also showed the greatest body mass gain. Escape path (Fig. 5b) was included in two of the top five models, with woylies that took a convoluted path showing 73% greater increase in body mass compared with those that took a straight path.

Nine variables included in the analyses did not appear in any of the top five models: (1) source population; (2) presence of a radio-collar; (3) pre-translocation faecal corticosterone concentration; (4) trapping agitation level; (5) heart rate pre-processing; (6) processing agitation level; (7) heart rate post-processing; (8) latency to leave on release; and (9) escape speed. There was no significant effect of radio-collaring on change in body mass over time (repeated-measures ANOVA: $F_{1,25} = 0.19$, $P = 0.668$; Fig. 4c). There was a bias in which animals were selected for collaring (repeated-measures ANOVA effect of collar presence: $F_{1,25} = 4.54$, $P = 0.043$), with radio-collared animals (initial capture: 1.32 ± 0.16 kg, $n = 13$) being heavier overall than non-collared woylies (initial capture: 1.21 ± 0.14 kg, n = 14). Both radio-collared and non-collared animals showed a significant increase in mass post-translocation (repeated-measures ANOVA effect of time: F1,25 = 24.16, $P < 0.001$) (Fig. 4c). Post-translocation, radio-collared animals (1.39 ± 0.15 kg) were heavier than non-radio-collared animals (1.27 ± 0.11 kg).

Discussion

The present study suggests that individual differences in behaviour and physiology – representative of differences in temperament – could be used to predict success (as measured by body mass gain) of translocated animals. Heart rate lability and escape behaviour, reflecting reactivity or fearfulness, show promise in predicting post-translocation mass gain in woylies. Individuals that had the greatest lability in heart rate, and those that took a convoluted escape path, showed the greatest mass gain between translocation and re-trapping ~100 days later. However, translocated individuals generally increased in body mass post-translocation, indicating that, in the absence of predation, most individuals coped with the stress of translocation and possessed both the behavioural and physiological plasticity to successfully find resources and adapt to a new environment.

Ideally, testing the effects of individual differences in behaviour and physiology would be conducted using treatments that maximise the opportunity to identify differences among individuals. Releasing animals into the wild (without fences and with predators) is therefore likely to generate the most valuable data in this respect. However, translocations into such environments are obviously riskier, and if conditions are too challenging (e.g. if all study subjects are rapidly preyed on), then this is also not informative. Therefore, while survival is often used as a measure of translocation success, it is useful to consider other parameters that identify differences in fitness of individuals that are translocated to predator-free destinations. Change in body mass is presumably a reliable metric of an individual's foraging success and physiological energy balance and is therefore a useful indicator of survival prospects. In the present study, change in body mass appeared to be a useful measure of fitness.

Due to the low woylie population density and high availability of food resources at Mt Gibson, an increase in body mass of translocated woylies post-release was both expected (Wayne 2008; Wayne *et al.* 2015) and observed. The result also suggests that food resources – and thus capacity for body mass gain – were limited at both source sites. Also, the translocations were conducted in May and June (Australian autumn and winter), when hypogeal fungi – the primary food source of woylies – is at its peak (Zosky 2011; Yeatman and Groom 2012). Animals translocated to Mt Gibson may therefore have been afforded both greater abundance and diversity of fungi and decreased competition for the same. Similarly, body condition and fecundity of Tasmanian bettongs (*Bettongia gaimardi*) increases during periods of peak fungi availability (Johnson 1994; Robinson *et al.* 2007).

In the present study, both sexes generally increased in body mass when translocated to a low-density population at Mt Gibson, but females increased in body mass at twice the rate of males (12.4% compared with 5.7% increase). Part of this difference between the sexes would be due to growth of pouch young; however, we found females gained in mass even when we subtracted the estimated mass of their pouch young (data not presented; to do this, pouch young were measured for crown–rump or head length (mm) and their body mass estimated (Thompson *et al.* 2015)). There were no significant differences between males and females at Mt Gibson in either dispersal distance or average home range (Ruykys *et al.* 2016), but we cannot exclude the possibility that females spent more time foraging for and consuming food in order to meet energetic demands for reproduction (Wade and Schneider 1992). May *et al.* (2016) also established that all translocated common brushtail possums gained body mass 5 months post-release, but that bolder animals (that had greater dispersal distance and therefore access to resources) and females showed greater gain in body mass than did shyer and male animals (May *et al.* 2016). Both sexes are required for a successful translocation, but recognising that there are sex differences in body mass responses post-release is an important factor to account for when considering translocation success criteria.

Many species, including woylies (de Tores and Start 2008), zig-zag or exhibit frequent directional changes when being chased ('protean' behaviour), allowing them to increase unpredictability and evade a pursuing predator (Domenici *et al.* 2011). Prey that choose the optimal escape response for a given situation can gain fitness advantages by not only surviving an attack, but also through using less energy during escape. The choice of escape path is a reflection of perception of risk and can be influenced by distance from threat (generally, short distances between predator and prey induces a zig-zag escape path, while greater distances induce a straight escape path; Hodges *et al.* 2014). An individual's escape response is also influenced by their temperament (López *et al.* 2005). Escape path was the only behavioural measure that was correlated with change in body mass, where woylies that took a convoluted path had 73% greater increase in body mass compared with those that took a straight path. Unfortunately, we only recorded escape behaviour once for each individual in the present study (on release at the translocation site). Repeated measures would have allowed analysis of whether escape path was a repeatable individual trait (i.e. influenced by temperament) or was a reflection of perceived threat at the time of release and/or the novel environment into which individuals were being released. Escape behaviour is also not a particularly useful predictor for selection of individuals for a translocation, but could be if there was a way to record escape behaviour as part of pre-selection trap and release (e.g. through trapping as part of annual monitoring of the source populations).

An increase in heart rate is mainly caused by an increase in sympathetic control of cardiac contraction. Heart rate variability therefore reflects reactivity and emotional responses to challenge and has been used as a valuable measure of welfare in livestock (reviewed by von Borell *et al.* 2007). Individual woylies with the greatest lability in heart rate showed the greatest mass gain. We recorded our initial heart rate measurements through the handling bag with minimal disturbance to the animal, while our second measurement was taken also through the handling bag but after the animal had been processed, which involved reasonably invasive measures. Most animals showed an increase in heart rate between these two time points. Only a few individuals showed a decrease, which probably suggests that they already had an elevated initial heart rate (pre-processing); these animals showed the least gain in body mass post-translocation. Additional data comparing temperament and heart rate lability in other species would be informative in determining whether heart rate lability can be more generally used as a measure of wildlife welfare.Wealso found differences in heart rate between our two source populations, with Perup animals having higher heart rates than did Karakamia-sourced woylies. This could be due to higher levels of habituation to human disturbance at Karakamia, and more noise during processing at Perup, where there were more people, more woylies captured, and longer processing times.

We recorded an increase in faecal corticosterone concentrations for female woylies post-translocation, but a decrease for males. At the time of translocation, males had significantly higher faecal corticosterone concentrations than did females, which could reflect that breeding males engage in energetically expensive activities such as territorial aggression, male–male competition and dominance interactions (Reeder and Kramer 2005; Lane 2006). For example, at Perup Sanctuary, woylies' home ranges overlap by 77%, even at low population densities (Yeatman and Wayne 2015). Agonistic interactions would be more likely at Karakamia and Perup Sanctuaries than in MtGibson's exclosure, which is 28 times larger than Karakamia and 18 times larger than Perup, and has a lower population density than either of the two source populations. Contrary to our results, Hing *et al.* (2017) found that both male and female woylies translocated from Perup Sanctuary to two unfenced destination sites (both within the Greater Kingston National Park) had significantly elevated faecal corticosterone concentrations 6 months after translocation compared with base measures before or at translocation. Both the current and Hing *et al.*'s (2017) study were carried out at a similar time of year, but differences between them could reflect differing conditions in the destinations.

Methodological considerations

There was no significant effect of radio-collars on change in body mass of woylies post-translocation, although we note that the largest individuals were intentionally selected for radio-collaring. Similarly, Golabek *et al.* (2008) found no negative effects on foraging efficiency and percentage weight change in radio-collared meerkats (Suricata suricatta) after 3 months. Radio tracking is likely to be important for obtaining repeated measures on individuals, and thus for determining the repeatability of temperament measures. It is therefore valuable to recognise that there was minimal effect of the presence of the radio-collars on woylies in the present study.

One of the restrictions in working with animals as part of a translocation process is that there is limited opportunity to re-test the same individuals. We did not find strong correlations among our various behavioural and physiological measures; repeated testing, or analysis of a wider sample size, may contribute to addressing this issue. Another issue with testing the effects of individual differences in temperament is the selection or inclusion of traits that are a useful reflection of responses. Many of the behavioural measures we tested were not significantly correlated between the two time-points (pre- and post-translocation) for individuals. This raises the questions of whether measures of temperament are sufficiently accurate or precise, and whether responses are transferrable among species. Developing measures of temperament is also dependent on the conditions under which measures can be carried out. For example, temperament measures that are to be used during translocation must have minimal impact on animals that are already challenged with trapping, handling, transport and exposure to a novel environment. Furthermore, the number of observers recording behavioural measures should be minimised and trapping and processing conditions should be standardised where possible.

We tried using an empirical measure of animal movement (accelerometers in phones) but found no significant relationships between individuals' level of activity before and after transport. This suggests that woylies' level of reactivity or fearfulness did not differ pre- and post-transport. Alternatively, as several models of iPhone were used, variation in recording sensitivities may have obscured any true variation in individuals' activity levels. Recommended improvements to methodology include a more robust calibration method to standardise between units, use of purpose-built accelerometers, and attachment of accelerometers to the animals to improve accuracy.

Faecal corticosteroid concentration was not useful as a predictor of body mass gain. There are numerous limitations associated with faecal corticosterone sampling and interpretation of results. An important consideration is the accumulation of glucocorticoids in faeces over time (Touma and Palme 2005; Hing *et al.* 2017), and defecation rate does not necessarily reflect rates of accumulation. The lag time for excretion in faeces following exposure to a stressor is estimated to be ~5 h in small mammals (Bosson *et al.* 2009), though this is known to be longer in some Australian mammals (e.g. up to 27 h in the long-nosed bandicoot (*Perameles nasuta*); Moyle *et al.* 1995). Furthermore, sympathetic nervous stimulation associated with a fight-or-flight response reduces gut activity and may decrease the rate of defecation (Boissy 1995). Taking these factors into account, it is difficult to ascertain whether the changes in corticosterone levels observed in the present study were due to a stress response from confinement in a trap (ranging from ~2–16 h), or a response to stressors experienced in the period leading up to the pre- and post-trapping events. Further research into lag time in woylies would assist with interpretation and ensuring that measures accurately reflect the physiological events of interest (Narayan *et al.* 2012).

Conclusions and conservation implications

Physiological measures used in the present study were effective for answering questions related to short- and medium-term stress responses in woylies during and after translocation. It may be useful to incorporate similar measures into other translocations, particularly into locations where survival may be more challenging (e.g. to areas with introduced predators); this, in turn, could also increase practitioners' understanding of the impacts of trapping, processing, transport and release on animals' stress levels and fitness (Letty *et al.* 2000; Hing *et al.* 2014).

Although few of the behaviours measured in the present study explained variation in post-translocation body mass gain, the results may have been vastly different if animals had been translocated to unfenced areas, particularly if they had been sourced from captive populations and/or were predator naïve (Banks *et al.* 2002; Moseby *et al.* 2011). In such scenarios, it may be beneficial to select individuals that exhibit temperaments known to improve survival. However, ultimately, post-release survival is likely to be a function of the interplay between temperament, release strategy, site conditions, predation pressure and life history traits (Moseby *et al.* 2014). There is no optimal combination of temperament traits that unequivocally leads to the highest fitness in all conditions (Boon *et al.* 2007), meaning that animals across a spectrum of individual temperaments should be selected for translocation.

Conflicts of interest

The authors declare no conflicts of interest.

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Table 1. Six temperament traits recognised in animals and their influence on fitness and survival

Temperament trait	Definition	Examples of links to fitness and survival
Activity	The general level of activity of an individual (Réale *et al.* 2007).	Quinn and Cresswell (2005)
Aggressiveness	An individual's agonistic reaction towards conspecifics (Réale *et al.* 2007).	Boon *et al.* (2007); Höjesjö *et al.* (2004)
Exploration – avoidance (continuum)	An individual's reaction to a novel object or situation (Réale *et al.* 2007).	Boon *et al.* (2007); Dingemanse *et al.* (2004)
Reactivity or fearfulness	Reactivity – the capacity to perceive and react to potentially anxiogenic situations (Boissy 1995). Fearfulness – the general susceptibility of an individual to react to a variety of potentially threatening situations (Boissy 1995).	May *et al.* (2016); Riechert and Hedrick (1993)
Boldness – shyness (continuum)	An individual's reaction to any risky situation, but not new situations (Réale *et al.* 2007).	Fraser *et al.* (2001); Godin and Dugatkin (1996); Réale and Festa-Bianchet (2003); Réale *et al.* (2000)
Sociability	An individual's reaction to the presence or absence of conspecifics (excluding aggression) (Réale *et al.* 2007).	Armitage (1986); Cote *et al.* (2008)

Table 2. Overview of Karakamia and Perup Sanctuaries

Sanctuary; exclosure completion date; site manager[A]	Location	Direct distance to Mt Gibson (km)	Size of exclosure (ha)	Climate	Mean annual rainfall (mm)[B]	Max. annual temp (°C)[B]	Dominant vegetation types	Woylie population; body mass	Average home range (ha)
Karakamia (source population); 1994; AWC	Perth Hills, ~50 km north-east of Perth. 31°49′15.35′S, 116°14′45.29′E	270	275	Mediterranean	877	16–30	Jarrah (*Eucalyptus marginata*), marri (*Corymbia calophylla*), wandoo (*E. wandoo*), banksia woodlands.	249 ± 259 individuals (95% CI: 211–291) (AWC, unpubl. data); ~1.1 kg	~14.6 (Yeatman and Wayne 2015)
Perup (source population); 2010; DBCA	South-west WA, ~260 km south-east of Perth. 34°10′30.44′S, 116°35′4.51′E	520	423	Mediterranean	994	15–26	Jarrah (*E. marginata* subsp. *marginata*), marri (*C. calophylla*), *E. patens* and *E. rudis* in valleys, and wandoo (*E. wandoo*) on flats	250; males: 1.0–1.9 kg, females: 0.8–1.8 kg	~65 (Yeatman and Wayne 2015)
Mt Gibson (destination population); 2014; AWC	WA Wheatbelt, ~350 km north-east of Perth. 29°36′29.49′S, 117°24′40.92′E	–	7832	Semiarid Mediterranean	320	18–37	*Acacia* shrublands and salmon gum (*E. salmonophloia*), York gum (*E. loxophleba supralaevis*) and gimlet (*E. salubris*) woodlands	Population is still being established, so a population estimate is not yet available	~246 (Ruykys *et al*. 2016)

[A]Sanctuaries managed by: AWC = Australian Wildlife Conservancy; DBCA = Western Australian Department of Biodiversity, Conservation and Attractions.

[B]Climate data from Australian Government Bureau of Meteorology (http://www.bom.gov.au/, verified May 2019).

Table 3. Behavioural and physiological measures recorded during translocations of woylies

Stage of translocation	Behavioural and physiological measures	Justification	References (examples)
Trapping	Agitation level	Associated with aggression. Woylies readily enter cage traps but can become highly agitated when inside the trap, which can result in females ejecting pouch young, physical injury and/or capture myopathy.	Armstrong *et al.* (2015); de Tores and Start (2008); May *et al.* (2016)
	Faecal glucocorticoid concentration	The secretion of glucocorticoids is an endocrine response to stress. Animals typically mount a glucocorticoid response immediately upon capture.	Dickens *et al.* (2010); Sapolsky *et al.* (2000)
Processing	Heart rate	Can be used to assess stress level during handling and has also been associated with temperament traits of emotional reactivity and exploration.	Ferrari (2010); Montiglio *et al.* (2012)
	Agitation level	Agitation level during handling ('processing') can influence body mass gain post-translocation and can be correlated with heart rate post-handling.	May *et al.* (2016)
Holding	Activity (tri-axial acceleration m s^{-2})	Animals exposed to adverse stimuli may either decrease or increase their activity according to either a conservation-withdrawal response or fight or flight response. Physical activity can be recorded with tri-axial accelerometers and can be used to infer an individual's level of emotional reactivity.	López-López (2016); Wilson and McMahon (2006)
Release	Agitation level, latency to leave (s), escape path and escape speed	Tonic immobility is an anti-predator response adopted to cause the predator to lose interest. Correlations between tonic immobility durations and fearfulness. Inter-individual variability in escape behaviour has been shown to be consistent and repeatable, and correlated with the temperament traits of activity and exploration.	Cooper and Blumstein (2015)

Table 4. Summary of data collected pre- and post-translocation and number of individuals from which they were collected

Numbers in brackets indicate radio-collared individuals

Summary of data collected	Karakamia-sourced		Perup-sourced		Total
	Male	Female	Male	Female	
No. individuals translocated to Mt Gibson	5 (0)	4 (0)	16 (6)	15 (8)	40 (14)
Post-translocation trapping	4 (0)	0 (0)	12 (5)	11 (8)	27 (13)

Table 5. Spearman Rank Order correlation coefficients (R_s) for pairs of behavioural and physiological measures recorded before translocation

Statistical significance is in bold and indicated as: *P < 0.05, ** P < 0.01

Behavioural and physiological measures	Trap agitation (low, medium, high)	Heart rate pre-processing	Processing agitation	Heart rate post-processing	Heart rate lability	Latency to leave	Escape path	Escape speed
Trap agitation (low, medium, high)								
Heart rate pre-processing (bpm)	0.166							
Processing agitation (low, medium, high)	0.214	0.144						
Heart rate post-processing (bpm)	0.048	**0.569****	0.308					
Heart rate lability (bpm)	–-0.002	–-0.261	**0.390***	**0.532****				
Latency to leave (s)	–-0.096	–-0.208	0.027	–0.373	–0.111			
Escape path (convoluted or straight)	0.225	0.068	–-0.118	–0.155	–0.339	0.194		
Escape speed (slow or fast)	–-0.120	0.049	0.231	0.286	0.179	–0.165	–0.227	
Processing agitation (recapture 5 months post-translocation)	**–-0.546****	0.007	–-0.092	0.125	0.182	–0.087	–0.203	–0.203

Table 6. Top five models with corresponding ΔAICc (Akaike Information Criterion corrected for small sample sizes) values and model weights (w_i)

The non-standardised model-weighted β values (w_i β) show the overall influence of each variable on body mass and the standardised β values (w_i β) illustrate the relative increase in body mass attributable to each variable. Ticks indicate variables that were included in a model. 12 variables were included in the analyses: (1) source population; (2) sex; (3) presence of a radio-collar; (4) pre-translocation faecal corticosterone concentration (log pg mL^{-1}); (5) trapping agitation level (low, medium, high); (6) pre-processing heart rate (bpm); (7) processing agitation level (low, medium, high); (8) post-processing heart rate (bpm); (9) heart rate lability (post-processing minus pre-processing heart rate); (10) latency to leave (log s); (11) escape speed (slow or fast); and (12) escape path (convoluted or straight).

Model	Sex (female = 0 or male = 1)	Heart rate lability (change in heart rate)	Escape path (convoluted = 1, straight = 0)	d.f.	ΔAICc	w_i	R^2	Test for goodness of fit
1		✓	✓	2	0.000	0.33	0.28	$F_{2,24} = 4.75$, $P < 0.018$
2	✓	✓	✓	3	0.944	0.20	0.35	$F_{3,23} = 4.08$, $P < 0.018$
3	✓			1	1.131	0.18	0.17	$F_{1,25} = 5.12$, $P < 0.032$
4	✓	✓		2	1.417	0.16	0.24	$F_{2,24} = 3.89$, $P < 0.034$
5		✓		1	1.884	0.13	0.15	$F_{1,25} = 4.29$, $P < 0.049$
No. occurrences	3	4	2					
Standardised (w_i β)	–0.335	0.414	0.369					
Non-standardised (w_i β)	–0.76	2.27	0.84					

Fig. 1. The woylie (*Bettongia penicillata ogilbyi*) is a small, nocturnal potoroid marsupial with a mass of ~1.0–1.9 kg (males), and ~0.8–1.50 kg (females) (Van Dyck and Strahan 2008). Photograph by Kimberley Page.

Fig. 2. Example recording of tri-axial movement of a woylie (*Bettongia penicillata ogilbyi*) over 10 min post-processing, showing (*a*) overall data and (*b*) the variation in magnitude of movement over time (arbitrary units).

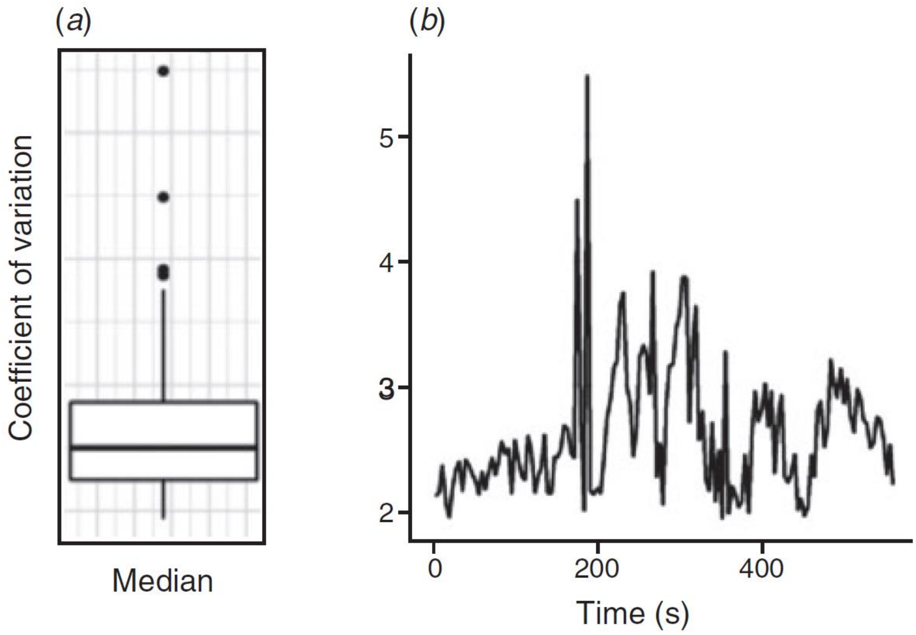


Fig. 3. (*a*) Pre-processing and (*b*) post-processing heart rate for woylies (*Bettongia penicillata ogilbyi*) from Karakamia and Perup Sanctuaries.

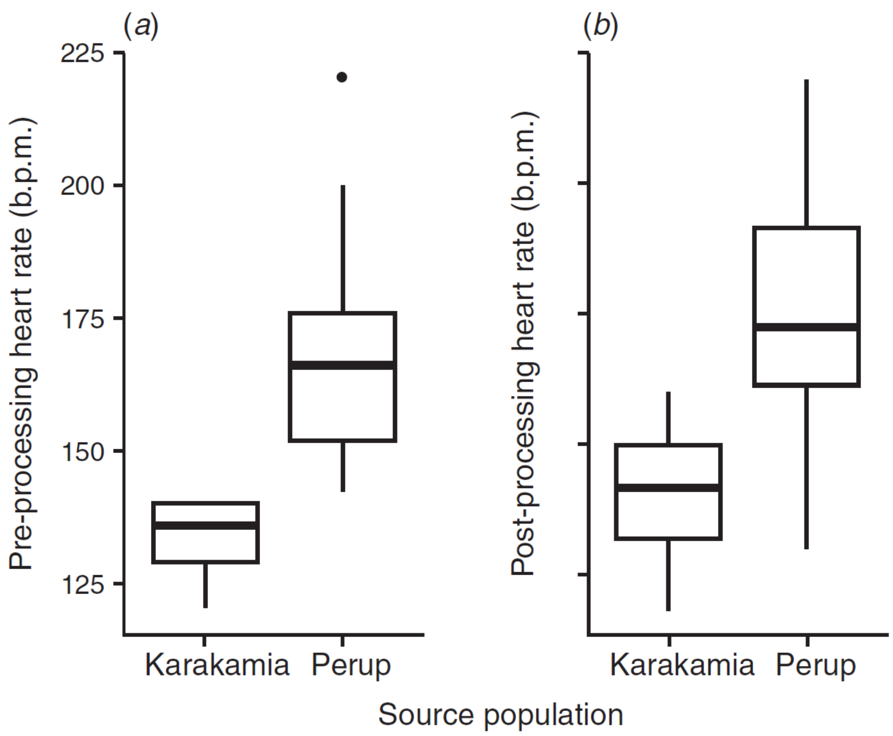


Fig. 4. (*a*) Mean faecal corticosterone concentration and (*b*) body mass, shown for male and female woylies (*Bettongia penicillata ogilbyi*) when initially trapped ('at source') and then 100 ± 18 days later when re-trapped post-translocation at Mt Gibson Sanctuary ('translocated'). (*c*) Mean body mass of both radio-collared and non-radio-collared woylies increased over time. Letters link data that were not significantly different (*post hoc* Fisher's least significant difference analysis).

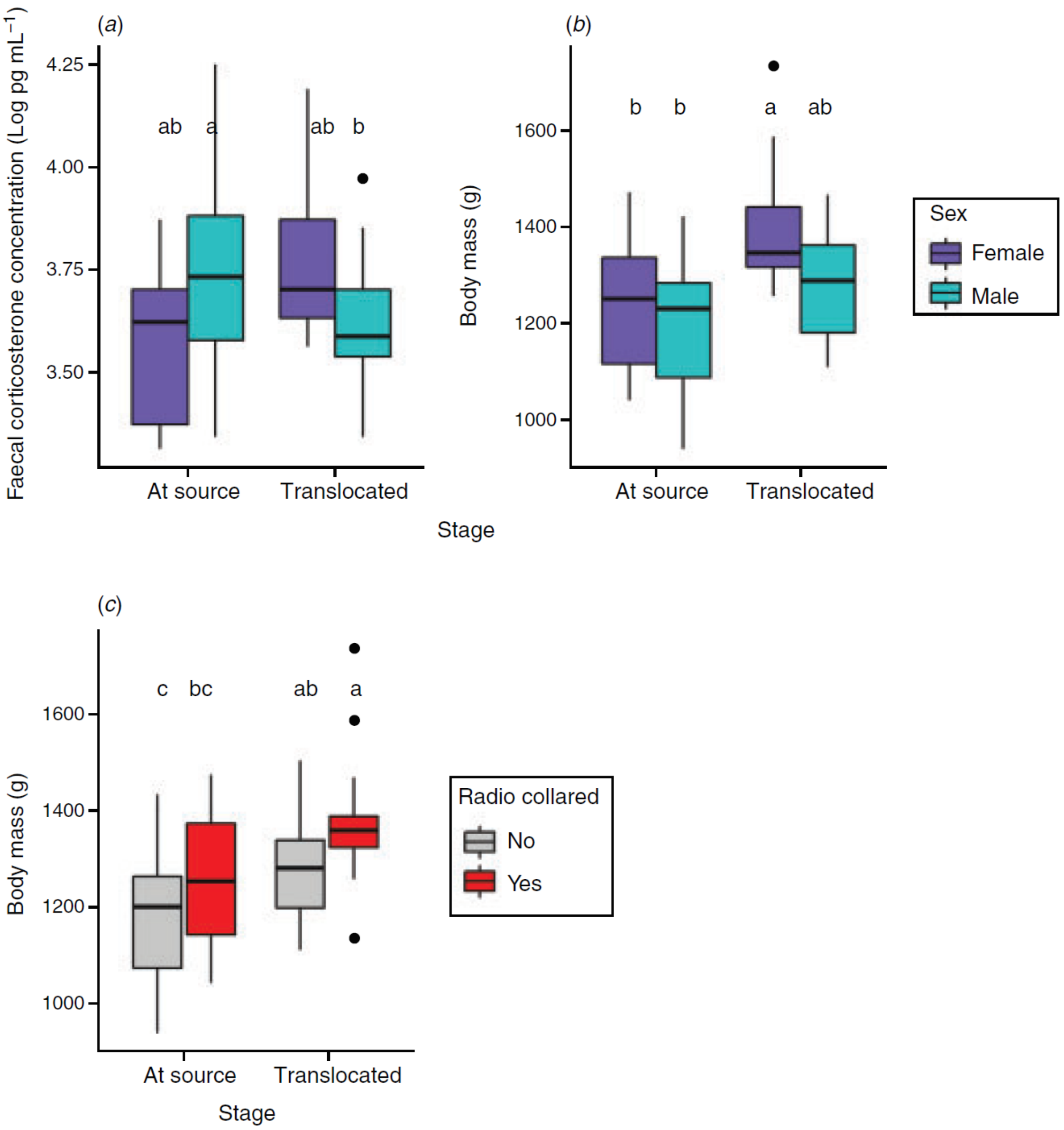
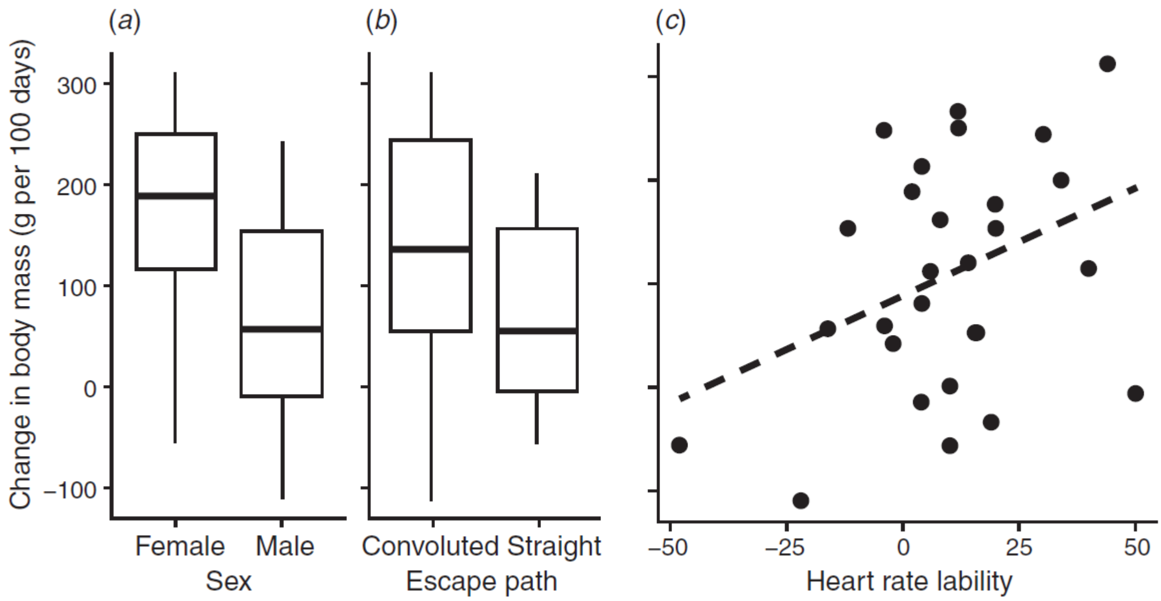


Fig. 5. Comparison between the rate of change in body mass (g per 100 days since release) for woylies (*Bettongia penicillata ogilbyi*) with the factors included in the top models: (*a*) sex; whether the animal was (*b*) radio-collared or (*c*) took a straight or convoluted escape path; (*d*) heart rate lability (bpm) (post-processing heart rate minus pre-processing heart rate); and (*e*) difference in glucocorticoid concentration from faeces collected at pre- and post-translocation(log pg mL^{-1}).



Supplementary material

Enzyme Immunoassay process

A commercial human corticosterone ELISA kit (K014-H1: Arbor Assays®, Michigan, USA) was used for the analysis of faecal corticosterone concentrations following faecal steroid extraction. Corticosterone is identical across all species (Hill *et al.* 1991) so it was expected that the human kit would be able to measure corticosterone from woylies; however, to confirm this, validation for woylie faecal samples was carried out. The validation process involved examination of the parallelism and fitted regression between the human corticosterone standard curve and a serially-diluted woylie faecal extract curve (Fig. S1). There was no significant difference in the faecal extract curve between the two species ($p > 0.05$). The sensitivity of the assay was 18.6 pg/mL, the limit of detection was 16.9 pg/mL and the intra-assay precision was 3.9 % (COV).

The faecal steroid extraction was based on the DetectX ™ Steroid Solid Extraction Protocol. Each animal's total faecal sample was pre-blended to obtain an homogenous 0.2 g sub-sample. Each sub-sample was mixed with 1 ml of 80 % methanol per 0.1 g of faecal solid. The samples were then placed in an overhead shaker for 30 min before centrifugation at 3,000 rpm for 15 min. The supernatant (2 ml) was evaporated to dryness under nitrogen, and then dissolved with 100 µL of 100 % ethanol and 400 µL of the assay buffer provided in the kit. An additional 500 µL of assay buffer was then added to each tube to ensure that the ethanol content was below 5 %. Samples were then covered with parafilm and refrigerated overnight.

Before starting the assay, kits and extracted samples were removed from the refrigerator for 30 min to bring them up to room temperature. The DetectX ™ Assay Protocol was then followed. Firstly, the wash buffer concentrate was diluted to 1:20 by adding 30 mL of the concentrate to 570 mL of distilled water. For standard preparation, 450 µL of assay buffer was pipetted into tube number 1 and 250 µL into tubes numbered 2 to 8. Then 50 µL of the corticosterone solution was added to tube no. 1 and the mixture was vortexed. A total of 250 µL of the corticosterone solution was pipetted from tube no. 1 and added to tube no. 2 and vortexed. This was repeated for tubes numbered 3 to 8. Then 50 µL of samples or standards were pipetted into each of the 96 wells in each plate. Plate 1 had eight wells of standards, a 5-stage serial dilution and 64 wells with faecal extract samples, all with duplicates. Plate 2 had eight wells of standards, 68 wells for samples, four wells for maximum binding, four wells for non-specific binding, plus duplicates. To start the assay, 75 µL of assay buffer was pipetted into the non-specific binding wells and 50 µL was pipetted into the maximum binding (zero standard) wells. Then 25 µL of the corticosterone conjugate was added to each well (except for the non-specific binding wells), followed by 25 µL of the corticosterone antibody. Plates were then placed in a mini-plate shaker for 1 hour, aspirated, then washed four times with 300 µL of wash buffer. A total of 100 µL of tetramethylbenzidine substrate was then added to each well and plates were incubated at room temperature for 30 min. Stop Solution (50 µL) was then added to each well and the optical density was read at 450 nm on a Bio-Rad iMark Microplate Reader. The corticosterone concentration (pg/mL) was then calculated using the Microplate Manager software package (BioRad, California, USA).

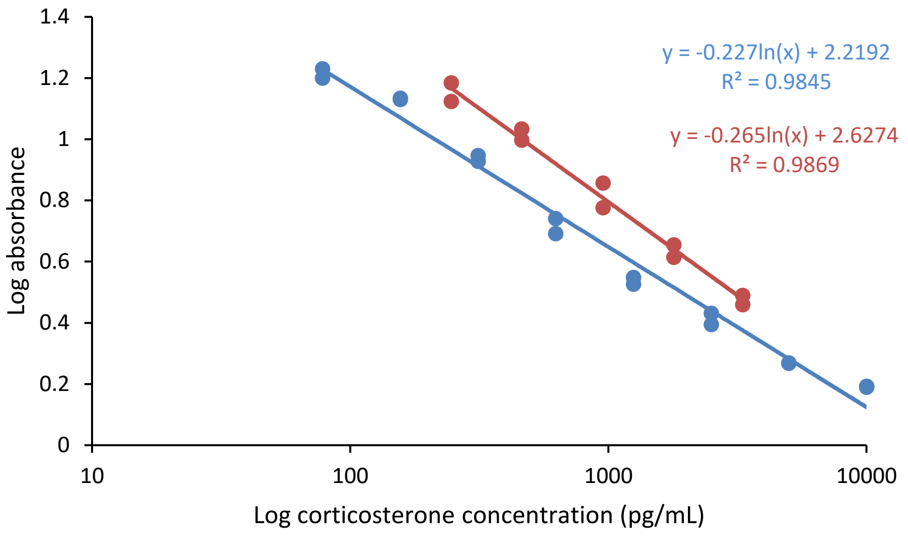


Fig. S1 Corticosterone parallelism validation curve for the human standard curve (lower line) and the woylie faecal extract curve (upper line).

Hill, R. A., Makin, H., Kirk, D., and Murphy, G. (1991) 'Dictionary of steroids.' (CRC Press, London.)