Does my bum look big in this exclosure? Associations of behaviour and physiology on body mass gain in the woylie (*Bettongia penicillata ogilbyi*) post-translocation.

by

**Kimberley Dale Page**
Bachelor of Science in Conservation and Wildlife Biology (Honours)

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Author’s Declaration

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

Kimberley Dale Page

Abstract

An individual’s temperament can affect its fitness and survival by influencing behaviours associated with predator avoidance, interactions with conspecifics, refuge selection and 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 fitness post-translocation is thus necessary for improving translocation outcomes. This study focused on the woylie (Bettongia penicillata ogilbyi) and, specifically, investigated the influence of individuals’ temperament – determined through measures of behaviour and physiology – on change in body mass post-translocation. Forty woylies were translocated from two predator-free exclosures to a larger exclosure at Mt Gibson Wildlife Sanctuary, near Wubin, Western Australia. Behavioural and physiological measures were recorded during trapping, processing, holding and release, and again ~5 months post-release. In the absence of predation, 100% survivorship of translocated individuals, and extremely high fecundity, body mass was used as a proxy for survival rate. On initial capture (‘pre-translocation’), males had significantly higher faecal corticosterone levels than did females; however, at post-release recapture (‘post-translocation’), there was no significant difference. There was no significant difference in body mass between males and females pre-translocation; however, post-translocation, females had gained significantly more body mass than had males. Combined analyses of behavioural and physiological variables showed that the strongest predictors of body mass gain were sex, heart rate lability and a measure of escape behaviour when released (a convoluted escape path).
Most behavioural measures were not consistent or repeatable and hence, individual temperament could not be determined. However, it is also possible that the translocation was not sufficiently challenging for individuals to be able to test differences in temperament. Future research could involve similar trials on animals that are released into unfenced settings. More robust behavioural measures that can be easily incorporated into the translocation process without increasing stress or affecting welfare of individuals should also be trialled. On average, all woylies recaptured had increased in body mass, suggesting that, in the absence of predation, the selected candidates were able to cope with the stress of translocation, and also possessed the behavioural plasticity to successfully find resources and adapt to a novel environment.

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Chapter 1 – General Introduction

1.1 Individual temperament traits in animals and their effects on fitness

Temperament has been defined as individual differences in behaviour that are consistent over time and across situations (McDougall et al. 2006; Réale et al. 2007). An individual’s temperament is shaped by its genetic predisposition and developmental experiences; this, in turn, influences its behavioural and physiological responses to novel stimuli and environmental challenges (Boissy 1995; Réale et al. 2000; Watters & Meehan 2007). There are six key temperament traits recognised in animals: shyness-boldness, emotional reactivity/fearfulness, exploration-avoidance, activity, sociability, and aggressiveness (Table 1) (Boissy 1995; McDougall et al. 2006; Réale et al. 2007). These traits affect how an individual interacts with conspecifics, finds resources, disperses and avoids predators; all of which are linked to their reproductive success, fitness and survival (Réale et al. 2000; Dingemanse & de Goede 2004; Réale et al. 2007).

To date, there have been relatively few studies on temperament in free-living animals. Thus, the effects of temperament on fitness and survival in wild populations are not well-understood (Réale et al. 2000; Dingemanse & Réale 2005; Boon et al. 2007; Archard & Braithwaite 2010). This can have consequences for the outcomes of conservation interventions such as translocations. For instance, a beneficial temperament trait in an animal’s current environment may not impart fitness advantages in their new environment (McDougall et al. 2006; Watters & Meehan 2007; May et al. 2016). It is therefore important to increase our understanding of the role of temperament on fitness in the context of translocation or re-introduction (McDougall et al. 2006; Réale et al. 2007; Sinn et al. 2014). Further research is required to determine whether selecting translocation candidates with particular temperament traits can lead to increased post-translocation fitness and survival and, in turn, improve translocation success (Bremner-Harrison et al. 2004; McDougall et al. 2006; Watters & Meehan 2007).
Table 1. Six temperament traits recognised in animals and their influence on fitness/survival.

<table>
<thead>
<tr>
<th>Temperament trait</th>
<th>Definition</th>
<th>Examples of links to fitness/survival</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activity</td>
<td>The general level of activity of an individual (Reale et al. 2007).</td>
<td>Hypoactive chaffinches (<em>Fringilla coelebs</em>) were more likely to freeze than flee in response to a model predator and took longer to resume activity than hyperactive individuals. Hypoactive chaffinches also showed greater behavioural plasticity in response to high and low predation risk treatments than did hyperactive chaffinches. This behaviour was considered optimal for predator avoidance and therefore may improve survival in hypoactive individuals.</td>
<td>Quinn &amp; Cresswell 2005</td>
</tr>
<tr>
<td>Aggressiveness</td>
<td>An individual’s agonistic reaction towards conspecifics (Reale et al. 2007).</td>
<td>In black rhinoceros (<em>Diceros bicornis</em>), the less aggressive males had greater breeding success than those that were assessed as more aggressive than their female partner. Due to resource and territory defence, aggressive brown trout (<em>Salmo trutta</em>) had lower growth rates and therefore reduced fitness than non-aggressive trout in complex habitats. Less aggressive female North American red squirrels (<em>Tamiasciurus hudsonicus</em>) had higher survival of juvenile offspring than did aggressive females.</td>
<td>Carlstead et al. 1999, Hojesjo et al. 2004, Boon et al. 2007</td>
</tr>
<tr>
<td>Exploration – avoidance (continuum)</td>
<td>An individual's reaction to a novel object or situation (Reale et al. 2007).</td>
<td>Breeding pairs of great tits (<em>Parus major</em>) exhibiting the same level of exploratory behaviour (either fast or slow) had a higher number of offspring than did pairs exhibiting opposing levels of exploratory behaviour. In years with high food availability, the most exploratory female North American red squirrels (<em>Tamiasciurus hudsonicus</em>) had faster growing offspring than did less exploratory females.</td>
<td>Dingemanse et al. 2004, Both et al. 2005, Boon et al. 2007</td>
</tr>
<tr>
<td>Reactivity/ fearfulness</td>
<td>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).</td>
<td>Funnel-web spider (<em>Agelenopsis aperta</em>) showing less fear towards predators were also more likely to win a territorial dispute with a conspecific.</td>
<td>Riechert &amp; Hendrik 1993</td>
</tr>
<tr>
<td>Sociability</td>
<td>An individual's reaction to the presence or absence of conspecifics (excluding aggression) (Reale et al. 2007).</td>
<td>Highly sociable female yellow bellied-marmots (<em>Marmota flaviventris</em>) had higher reproductive success compared to less sociable females. More social female common lizards (<em>Lacerta vivipara</em>) had higher reproductive success than did asocial females.</td>
<td>Armitage 1986, Cote et al. 2008</td>
</tr>
</tbody>
</table>
1.2 Assessing temperament and physiology in the context of translocation

Translocation is an important conservation tool that is used to supplement declining fauna populations or to re-establish locally extinct populations (Dickens et al. 2009; Tarszisz et al. 2014; Morris et al. 2015). Despite increasing use of translocations and re-introductions for conservation of declining species, the success of such interventions is highly variable (Moseby et al. 2014; Germano et al. 2017). Many potential causes for failure have been investigated, including predator naivety, habitat selection, demographics and release strategies (Letty et al. 2000; de Azevedo & Young 2006; Cabezas & Moreno 2007; Frair et al. 2007; King et al. 2012; Goudarzi et al. 2015). Only recently has individual temperament been investigated as a potential influence on post-translocation fitness and survival among individuals (Sinn et al. 2014; May et al. 2016; Germano et al. 2017).

Another key factor influencing survival and fitness post-translocation is stress, which can be defined as the functional response of an organism to an external stressor (Selye & Fortier 1950). Each phase of translocation – capture, processing, holding, transport and release to a new area – is a stressful event and not all individuals respond to these stressors in the same way. The stress response is comprised of both the physiological processes and behavioural responses experienced by an organism in the face of a challenge (Selye & Fortier 1950; Bolig et al. 1992; Romero 2004; Reeder & Kramer 2005). Short-term, stress can alter physiological processes, assisting an animal to cope with a physical challenge or environmental change, such as that experienced through translocation. However, a prolonged stress response can lead to immunosuppression, impaired growth and condition, and reduced survival and fitness (Goymann et al. 1999; Reeder & Kramer 2005; Baker et al. 2013). Chronic stress can also suppress feeding behaviour, leading to a decrease in body weight (Reeder & Kramer 2005; Marin et al. 2007; Dickens et al. 2010). These responses can therefore, have significant consequences for the health and well-being of individuals and, collectively, the persistence of a translocated population (Harrington et al. 2013). Both the physiological processes and behavioural responses to a stressor can
be observed and measured using hormonal, physiological and behavioural indices and, together, can be used to infer underlying temperament (Boissy 1995; McDougall et al. 2006; Réale et al. 2007).

1.2.1 Methodological approaches and measures for assessing temperament and physiology

Temperament

One of the most common methods of measuring behaviour is through direct observation and video recordings (Ropert-Coudert & Wilson 2005; Carter et al. 2013). However, the challenge with observational data has been to establish a means to quantify observed behaviours and reduce subjectivity (Bolig et al. 1992). This has been achieved through the development of standardised inventories or ethograms that are used to quantify species-specific behaviours exhibited by an individual. Researchers use ethograms to identify and then quantify behaviours by recording the duration and/or frequency of each behaviour observed (Carlstead et al. 1999). Furthermore, many researchers use an ethogram in conjunction with behavioural tests, whereby the context of the situation is manipulated and the individual’s response recorded (Bremner-Harrison et al. 2004; Smith & Blumstein 2008; Sinn et al. 2014; May et al. 2016; Montagne 2016). For example, boldness and exploration were assessed in swift fox (Vulpes velox) and Mojave Desert tortoise (Gopherus agassizi) using novel object and novel environment tests (Bremner-Harrison et al. 2004; Germano et al. 2017). Here, animals were exposed to a novel object or environment and their response – such as latency to emerge from cover, distance of approach to the object, and investigation such as touching or sniffing – were recorded and scored. These scores were then summed to provide overall scores of boldness and exploration for each individual (Bremner-Harrison et al. 2004; Germano et al. 2017).

This approach has been successfully used for captive animals and for animals held in captivity for a period of time prior to translocation (Bremner-Harrison et al. 2004; Sinn et al. 2014; Montagne 2016; Germano et al. 2017). However, this may be difficult to achieve during translocations of free-living animals, particularly if animals are to be transported long distances on a tight schedule to ensure compliance with ethical and welfare requirements (Waas et al. 1999; Dickens et al. 2010; Armstrong
et al. 2015). Furthermore, keeping free-living animals in temporary captivity increases stress, which can impact welfare and behaviour; thus, potentially confounding results of behavioural tests (Waas et al. 1999; Archard & Braithwaite 2010; Moseby et al. 2014). As an alternative to conducting behavioural tests prior to release, May et al. (2016) recorded a range of behavioural measures during and post-translocation of brushtail possum (*Trichosurus vulpecula*). As with other studies, observed behaviours were then scored and summed to obtain overall scores for temperament traits including reactivity/fearfulness, boldness, exploration, aggression and sociability (May et al. 2016). Using this method, 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). However, some measures – such as time taken to remove the animal from the trap – were not found to be informative for assessing post-release survival (May et al. 2016). Thus, the methodology and some of the measures used in the study require further testing and development. If this approach can be refined and proves to be reliable, it may be a suitable alternative to conducting behavioural tests prior to translocation in free-living animals.

**Physiology**

Many studies assessing temperament have combined measures of behaviour with physiological parameters (Montiglio et al. 2012; Sinn et al. 2014; May et al. 2016; Montagne 2016). These parameters can be measured quantitatively during an individual’s response to a stressor via heart rate, blood pressure, respiration rate, temperature, and release of glucocorticoid hormones (Sapolsky et al. 2000; Palme et al. 2005; Teixeira et al. 2007; Dickens et al. 2010; Sinn et al. 2014).

Heart rate is commonly used to assess stress level during handling and has also been associated with the temperament traits of emotional reactivity and exploration (Waas et al. 1999; Montiglio et al. 2012; Ferrari 2013; Montagne 2016). For example, in wild Eastern chipmunk (*Tamias striatus*), there were correlations between exploration – assessed through activity level in a novel environment – and
heart rate during a restraint test (Montiglio et al. 2012). Specifically, chipmunks with higher exploratory tendencies showed a greater increase in heart rate during restraint than did those with less exploratory tendencies (Montiglio et al. 2012).

Another frequently used measure of the stress response is that of glucocorticoid levels (Waas et al. 1999; Sapolsky et al. 2000; Tarszisz et al. 2014; Hing et al. 2017). When an animal encounters a stressor, there are two acute stress responses: the immediate fight or flight response and the slower glucocorticoid response (Dickens et al. 2010). These responses modulate normal behavioural and physiological processes throughout the body to focus on immediate needs for survival (Romero 2004). Following the fight or flight response, the hypothalamic-pituitary-adrenal (HPA) axis stimulates the hypothalamus, which signals the pituitary to secrete adrenocorticotropic hormone (ACTH). ACTH then stimulates the adrenal gland to release glucocorticoids; either cortisol or corticosterone depending on the species. For most mammal species the predominant glucocorticoid released is cortisol (Sapolsky et al. 2000; Dickens et al. 2010). Glucocorticoids are detectable in blood, urine, saliva or faeces and can be extracted and quantified using a radio-immunoassay or enzyme-immunoassay kit (Sheriff et al. 2011).

However, some sampling techniques for glucocorticoids, although informative, can be invasive as they may involve temporary captivity, sedation, catheterisation and repeated restraint (Waas et al. 1999; Sheriff et al. 2011). Using such invasive techniques to collect samples can be counterproductive as they can alter the levels of stress hormones being measured and influence animals’ behaviour (Sheriff et al. 2011). The disturbance associated with collecting biological samples using these techniques may therefore, introduce bias in the measurements (Waas et al. 1999; Tarszisz et al. 2014).

A less invasive technique of measuring glucocorticoid levels is through the collection and analysis of glucocorticoid metabolites in faecal samples (Sheriff et al. 2011). Glucocorticoids are metabolised by the liver prior to excretion through both urine and faeces. Faecal samples can then be collected with minimal disturbance to the animal if in captivity, or during trapping of free-living animals (Palme et
al. 2005; Narayan et al. 2012; Dowle et al. 2013). For example, Hing et al. (2017) measured change in faecal cortisol metabolite (FCM) concentrations in both resident and translocated woylies. Changes in FCM concentration were successfully detected over the sampling period, with the highest levels observed in woylies 6 months post-release. However, given this elevation was observed in both the translocated and non-translocated woylies, the authors suggested that seasonal conditions or exposure to predators – as opposed to a response to the translocation – may have been the cause (Hing et al. 2017).

Both heart rate and glucocorticoid metabolites from faecal samples are easy to collect and relatively non-invasive, making them ideal for use in free-living animals (Sheriff et al. 2011). Additionally, they are both reliable indicators of stress and may be correlated with particular temperament traits (Waas et al. 1999; Montiglio et al. 2012). Thus, physiological measures are valuable in assessments of temperament and may also be useful to predict post-translocation fitness. Furthermore, given that physiological measures are quantitative and objective, they contribute a robust dataset to complement more subjective behavioural measures.

1.2.2 Radio-collaring

A particular aspect of translocations that has seemingly been overlooked in assessments of temperament and physiology is the impacts of radio-tracking devices. Wildlife research through the use of radio-tracking (via implants, transmitter units or collars) is a very common means of monitoring animals post-translocation (Banks et al. 2002; Bremner-Harrison et al. 2004; Sinn et al. 2014; May et al. 2016; Montagne 2016; Germano et al. 2017). However, radio-tracking devices can increase stress levels of the animals fitted with them (Wilson & McMahon 2006; Munerato et al. 2015). Furthermore, these devices may make animals more vulnerable to predation and/or interfere with activities such as mating and foraging (Wilson & McMahon 2006; Golabek et al. 2008; Tarszisz et al. 2014). For example, Tuyttens et al. (2002) found wild European badger (Meles meles) that had been radio-collared for up to 100 days had lower body condition scores compared with non-collared
badgers. Negative effects were also found in American mink (*Mustela vison*), with five out of eight mink that wore a collar for more than 2 months having neck wounds and ulcerations (Zschille et al. 2008). As such, it is important that in future studies, the impacts of radio-collaring on post-translocation fitness and survival is assessed.

### 1.2.3 Concluding remarks

To conduct informative studies on temperament and physiology in translocated animals, the methodology and measures should be tailored to the study species and its source (i.e. captive or wild). Furthermore, to obtain accurate, reliable and repeatable data, both behavioural and physiological measures should be incorporated into the study design. To avoid increasing stress, altering behaviour, and potentially confounding results, the selected sampling methods should also be non-invasive. A final consideration is that if radio-tracking is used for post-release monitoring, the effects of collars on behaviour and physiology should be examined.

### 1.3 Context of the project

Translocations aim to reconstruct faunal ecosystems and improve the conservation status of threatened species. In Western Australia, such translocations have been conducted since the 1970s (Morris et al. 2015). However, approximately 80% of translocations fail due to predation of the translocated species by introduced European red foxes (*Vulpes vulpes*) and feral cats (*Felis catus*) (Burrows et al. 2003; Moseby et al. 2011; Moseby et al. 2014; Woinarski et al. 2014). One strategy for managing this issue is to construct fences that are impermeable to cats and foxes, with introduced predators also eradicated from the area within the fence. The first such exclusion fence was constructed at Warrawong in South Australia in 1975 (Dickman 2012). Since then, government and private conservation organisations have been conducting translocations of threatened species into these exclosures Australia-wide (Moseby & Read 2006; Morris et al. 2015). Currently, there are 16 large-scale functional exclosures across the country, although the number grows steadily (Legge et al. 2017). These fenced areas
eliminate or reduce predation, allowing the translocated species to establish in their new environment. Ideally, this then results in a secure insurance population; a key outcome of translocations and re-introductions into exclosures (Moseby & O'Donnell 2003; Moseby et al. 2011; Wayne et al. 2011; Tarszisz et al. 2014; Morris et al. 2015).

The objective of this study is to investigate the influence of individuals’ temperament – determined through measures of behaviour and physiology recorded during and post-translocation – on change in post-translocation body mass in an endangered marsupial, the woylie (*Bettongia penicillata ogilbyi*). Translocations were carried out by the Australian Wildlife Conservancy (AWC); a not-for-profit organisation implementing on-ground activities to conserve Australia’s biodiversity (Australian Wildlife Conservancy 2016). The organisation manages 26 properties for conservation, several of which contain predator-free fenced exclosures that protect threatened fauna populations (Australian Wildlife Conservancy 2016). In September 2015, AWC conducted the first translocation of 50 woylies to a newly-fenced exclosure at Mt Gibson Wildlife Sanctuary near Wubin, Western Australia. A further two translocations were carried out in early 2016, during which time woylies from AWC’s Karakamia Wildlife Sanctuary and the Department of Biodiversity, Conservation and Attraction’s (DBCA) Perup Sanctuary were moved to Mt Gibson (Ruykys & Kanowski 2015). The current study was conducted during these two subsequent translocations.

As the animals examined in this study were released into a predator-free exclosure, predation risk was largely mitigated and hence, a high survival rate was expected. This assumption was also based on the 100% survival rate of radio-collared woylies 4 months after the first translocation in 2015 (Ruykys et al. 2016). Body mass gain was therefore selected as an alternative measure of post-translocation fitness to survival rate. It was predicted that less reactive/fearful individuals (based on behavioural and physiological measures) would be more successful in finding resources in their new environment than more reactive/fearful individuals and would thus gain more body mass post-translocation.
The aims of this study were specific to translocations of woylies into a predator-free exclosure (Mt Gibson) and were to determine:

1) if behavioural and physiological measures recorded during and post-translocation could be used accurately and consistently to assign individuals with temperament traits – activity, aggression and reactivity/ fearfulness; and

2) whether there were associations between behavioural and physiological measures and post-translocation body mass gain in woylies (accounting for effects of sex, source population and radio-collaring).

Chapter 2 – General Methods

This project was approved by Murdoch University’s Animal Ethics Committee (RW2837/16). It was also conducted under a translocation proposal (Ruykys & Kanowski 2015) approved by the DBCA’s Animal Ethics Committee (approval no 2015-10). AWC planned and executed the translocations in collaboration with the DBCA. Staff, interns and volunteers from both organisations assisted with the collection of data for this study.

2.1 Study species - The woylie

2.1.1 Biology of the woylie

The woylie is a small potoroid marsupial that weighs ~980–1850 g (males) and ~750–1500 g (females). Woylies have strong, clawed fore-feet for digging and a long prehensile tail that is used for carrying nesting material (de Tores & Start 2008; Yeatman & Groom 2012). Woylies are nocturnal and spend the night foraging for their primary food source – hypogaeal fungi – as well as for arthropods, roots, bulbs, seeds and tubers (Christensen 1980; Nelson et al. 1992). One individual can complete up to 100 diggings per night, displacing on average ~4.8 tonnes of soil per woylie per year (Garkaklis et al. 2004; Zosky et al. 2010; Yeatman & Groom 2012). Woylies therefore, provide vital ecosystem services through redistributing nutrients between trophic levels, improving water
penetration, assisting seed and fungal spore dispersal, and increasing seedling recruitment (Garkaklis et al. 2004; Murphy et al. 2005; Fleming et al. 2014).

Woylies are generally solitary, although nest sharing has been recorded, particularly between mothers and their young-at-heel (Sampson 1971). In favourable conditions, woylies breed continuously throughout the year, with up to three young being produced annually. Females display embryonic diapause, with parturition after only ~17 days (de Tores & Start 2008; Yeatman & Groom 2012; Thompson et al. 2015).

Woylies have distinct home ranges that comprise separate feeding and territorial nesting areas with males maintaining greater spatial separation from their neighbours (~110 m) than females (87 m) (Christensen & Leftwich 1980; Nelson et al. 1992; Start et al. 1995). Home range varies depending on the site, habitat type and population density. At high density, home ranges can be ~4 ha but in lower density populations can average ~65 ha (Nelson et al. 1992; Groom 2010; Yeatman & Wayne 2015). Nests of grasses, strips of bark and leaves are assembled and well-concealed under dense understorey vegetation, logs, Macrozamia fronds or Xanthorrhoea skirts (Sampson 1971; Christensen & Leftwich 1980; de Tores & Start 2008). Woylies take refuge in these nests during daylight hours, with a single individual constructing and utilising three to four nests in different locations within its home range (Christensen & Leftwich 1980; de Tores & Start 2008).

2.1.2 Distribution, conservation status and causes of decline

Historically, the woylie was found across approximately 60% of mainland Australia, including the arid and semi-arid zones of Western Australia (WA), the Northern Territory, South Australia, New South Wales and Victoria (Fig. 2.1) (Sampson 1971; Nelson et al. 1992; Yeatman & Groom 2012).
Following European settlement, woylie populations experienced major reductions in size and distribution. These declines were attributed to predation, changed fire regimes and habitat clearance (Sampson 1971; Burbidge & McKenzie 1989; Groom 2010). By the 1960s, only three populations persisted in south-west WA: Upper Warren, Tutanning and Dryandra Woodland (Wayne 2008). As a result of fox control and a series of translocations, the species had made a substantial recovery by 1996 (Wayne et al. 2015). However, there was another decline from 1999 onwards, which resulted in a > 90% reduction from a peak of c. 250,000 individuals (Woinarski & Burbidge 2016). This prompted intensive research into the cause (Wayne et al. 2011; Woinarski & Burbidge 2016). To date, this research has identified disease and predation as key contributing factors to the species’ second decline (Wayne et al. 2015).

Currently, only two remnant populations of woylies persist: those at Dryandra Woodland and the Upper Warren Region encompassing Perup Nature Reserve, large areas of state forest and Greater Kingston National Park in south-west WA (Wayne et al. 2013). Hence, the woylie is ranked as
‘critically endangered’ under the Western Australian *Wildlife Conservation Act 1950* and ‘endangered’ under the national *Environmental Protection and Biodiversity Conservation Act 1999* (Woinarski & Burbidge 2016). One of the recovery actions recommended to enhance the species’ conservation status was to undertake targeted translocations (Start et al. 1995; Yeatman & Groom 2012). Thus, between 1977 and 2006, over 3,400 woylies were translocated across Australia (Groom 2010). Destinations included more than 61 different reserves, introduced predator-proof exclosures and offshore islands, making the woylie the most translocated marsupial for the purpose of conservation (Groom 2010; Pacioni et al. 2013; Morris et al. 2015; Hing et al. 2017).

### 2.2 Study sites

#### 2.2.1 Source population 1 – Karakamia Sanctuary

Karakamia Wildlife Sanctuary is a 275 ha predator-proof exclosure in Chidlow owned and managed by AWC since 1991. It has a number of paddocks and areas of grazed bushland, as well as areas of relatively undisturbed jarrah (*Eucalyptus marginata*) and marri (*Corymbia calophylla*) woodland (Figure 2.2). The site’s climate is Mediterranean, with hot, dry summers and cool, wet winters. Average annual precipitation is 871 mm at Mt Helena (< 10 km away), with the majority falling in winter (Ruykys & Kanowski 2015). The founding population of woylies was 31 individuals that were released in 1994, and subsequently supplemented by a small number of orphaned individuals. A population of ~400-500 woylies exists in the sanctuary and is believed to fluctuate around the site’s carrying capacity (Wayne 2008; Wayne et al. 2015). Average home range is ~14.6 ha (Yeatman & Wayne 2015). This population has a seasonal breeding cycle and individuals generally have a below-average body mass (~1.057 kg) when compared to that of other populations (Ruykys & Kanowski 2015; Wayne et al. 2015). This is the only population to have remained stable during the major declines observed in this species across south-west WA (Wayne 2008; Wayne et al. 2013).
2.2.2 Source population 2 – Perup Sanctuary

This 423 ha exclosure is located within Tone-Perup Nature Reserve, ~50 km east of Manjimup, WA. The sanctuary was established in 2010 and is managed by the DBCA (Yeatman & Wayne 2015). Mean annual rainfall in this locality is 700–900 mm (Department of Environment and Conservation 2012), with an average maximum temperature of 15°C in winter and 26°C in summer (Bureau of Meteorology 2017). Three main vegetation types occur within the site; open forest and woodland of jarrah (*Eucalyptus marginata* subsp. *marginata*) and marri (*Corymbia callophylla*) (Figure 2.3), *Eucalyptus patens* and *Eucalyptus rudis* in valleys, and wandoo (*Eucalyptus wandoo*) woodland with melaleuca thickets on flats (Wayne et al. 2013; Yeatman & Wayne 2015).

The woylie population was founded by 41 individuals from free-living populations in the Upper Warren Region. Woylies were also sourced from Dryandra in 2013 and from Tutanning between 2013 - 2016 (Wayne et al. 2013). Average home range is ~ 65 ha (Yeatman & Wayne 2015) and body mass of woylies in this population ranges from 980–1850 g for males and 750–1800 g for females.

2.2.3 Destination – Mt Gibson Wildlife Sanctuary

Mt Gibson Sanctuary is located ~350 km north east of Perth and was acquired by AWC in 2001, with completion of the 7832 ha exclosure in 2014 (Ruykys & Carter *in prep*). The sanctuary is situated in a transition zone between the mulga-dominated arid interior and the eucalypt-dominated south-west (Australian Wildlife Conservancy 2017). Woylies were the first species to be released into the exclosure in September 2015 (Australian Wildlife Conservancy 2017). Habitats identified as being suitable for woylies were those of shrubland and woodland sites with high floristic diversity (Figure 2.4), high sedge and grass ground cover and moderate to high shrub cover (Ruykys & Kanowski 2015). Post-translocation home ranges (using 95% kernel) of woylies averaged ~293 ha (±SD 259 ha) in this sanctuary (Ruykys et al. 2016).
Figure 2.2 Jarrah (*Eucalyptus marginata*) and marri (*Corymbia calophylla*) woodland within Karakamia Sanctuary.

Figure 2.3 Jarrah (*Eucalyptus marginata* subsp. *marginata*) and marri woodland (*Corymbia calophylla*) within Perup Sanctuary.
Figure 2.4 York Gum (Eucalyptus loxophleba spp. supralaevis) woodland within Mt Gibson Sanctuary.
2.3 Translocations

2.3.1 Research design

For both translocations, a series of physiological and behavioural measures were recorded during trapping, processing, holding (both pre- and post-transport) and at release (Table 2.1). These measures were selected to identify individual temperament traits of activity, aggression, and reactivity/fearfulness, and to monitor stress levels during and post-translocation.
Table 2.1 Behavioural and physiological measures recorded during the translocations.

<table>
<thead>
<tr>
<th>Stage of translocation</th>
<th>Behavioural &amp; physiological measures</th>
<th>Justification</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trapping</td>
<td>Agitation level, faecal corticosterone concentration</td>
<td>Agitation level is 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. The secretion of glucocorticoids is an endocrine response to stress. Animals typically mount a glucocorticoid response immediately upon capture.</td>
<td>May et al. 2016, de Tores &amp; Start 2008, Armstrong et al. 2015 Sapolsky et al. 2000, Dickens et al. 2010</td>
</tr>
<tr>
<td>Processing</td>
<td>Heart rate, agitation level</td>
<td>Heart rate can be used to assess stress level during handling and has also been associated with temperament traits of emotional reactivity and exploration. Agitation level during handling can influence body mass gain post-translocation and can be correlated with heart rate post-handling.</td>
<td>Montiglio et al. 2012, Ferrari 2013 May et al. 2016</td>
</tr>
<tr>
<td>Holding</td>
<td>Activity (tri-axial acceleration m/s²)</td>
<td>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 (see Appendix 1 for review) and be can used to infer an individual’s level of emotional reactivity.</td>
<td>Moberg 1985, Durnin et al. 2004, MacKay et al. 2014, Wilson et al. 2014, López-López 2016</td>
</tr>
<tr>
<td>Release</td>
<td>Escape time(s), path and speed</td>
<td>Tonic immobility is an anti-predator response adopted to cause the predator to lose interest. Correlations have been found between tonic immobility durations and fearfulness. Inter-individual variability in escape behaviour has been shown to be consistent and repeatable, and correlated to the temperament traits of activity and exploration.</td>
<td>Jones et al. 1991, Cooper &amp; Blumstein 2015</td>
</tr>
</tbody>
</table>

2.3.2 Trapping

To capture animals for translocation from the two source sites, cage traps (0.22 x 0.22 x 0.58 m; Sheffield Wire Products, Welshpool, WA) were baited with rolled oats and peanut butter and set at dusk along transects or within quadrats. To collect faecal samples, sheets of newspaper were laid underneath each trap. The same process was followed for post-release trapping (Table 2.2)
Table 2.2 Details of trapping for translocations and post-release monitoring.

<table>
<thead>
<tr>
<th>Trapping site</th>
<th>Date(s)</th>
<th>Number of traps set/night</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Karakamia Sanctuary</td>
<td>3 May 2016</td>
<td>49</td>
</tr>
<tr>
<td>2. Perup Sanctuary</td>
<td>30 May – 2 June 2016</td>
<td>81</td>
</tr>
<tr>
<td>3. Mt Gibson (post-release)</td>
<td>22-28 August 2016</td>
<td>80</td>
</tr>
</tbody>
</table>

At Karakamia, the first trap check occurred at ~8 pm then approximately every two hours until 3 am.

At Perup, traps were left open overnight and checked between 4 - 9 am each morning. Agitation level during trapping was assigned using predetermined scores based on behaviour that was observed whilst animals were inside and exiting the trap (Table 2.3). Faecal samples were collected from newspaper underneath cage traps, placed in snap-lock bags and frozen at -5°C.

Table 2.3 Behaviour used to assign trap agitation level.

<table>
<thead>
<tr>
<th>Agitation level</th>
<th>Trap behaviour</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>Frozen, or continuous bouncing and latency to enter the handling bag, or injuries to the face or head from trap damage.</td>
</tr>
<tr>
<td>Medium</td>
<td>Some bouncing but settles quickly and enters the handling bag without much hesitation.</td>
</tr>
<tr>
<td>Low</td>
<td>Little or no bouncing and enters the handling bag without hesitation.</td>
</tr>
</tbody>
</table>

Criteria for translocation candidates were as follows: for woylies sourced from Karakamia, an adult with body mass of ≥1,057 g; for males sourced from Perup, 980-1850 g and for females sourced from Perup, 750-1800 g. If females had a pouch young, the crown-rump length of the young needed to be <50 mm. If woylies met these criteria they were transported a short distance by vehicle to a central laboratory for processing.

2.3.3 Processing

At the laboratory, heart rate (beats per min; bpm) was taken with a stethoscope for 1 min before processing. Processing involved taking standard morphometric data including body mass (g), pes length (mm), sex, reproductive status and pouch condition. A processing agitation level was assigned based on the behaviour that was observed during this time (Table 2.4). Heart rate was then taken again immediately after processing.
### Table 2.4 Behaviour used to assign processing agitation level.

<table>
<thead>
<tr>
<th>Agitation level</th>
<th>Processing behaviour</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>Frozen or continual resistance to handling; biting, kicking or resisting pouch inspection; elevated respiration; highly reactive to touch; vocalisation</td>
</tr>
<tr>
<td>Medium</td>
<td>Intermittent resistance to handling; moderately reactive to touch; some resistance to pouch inspection; no biting or vocalisation</td>
</tr>
<tr>
<td>Low</td>
<td>Little or no resistance to handling; low reactivity to touch; no biting, kicking, vocalisation or resistance to pouch inspection</td>
</tr>
</tbody>
</table>

Candidates were deemed suitable for translocation if they were healthy, had no clear abnormalities and did not have an unusually high parasite load. At Perup, a subset of suitable individuals >1050 g were fitted with radio-collars. Collars weighed 35 g, which was < 5% of the lightest collared woylie’s body mass.

#### 2.3.4 Holding

After processing, animals were allowed to settle for variable periods of time (minutes to hours) prior to activity levels being recorded. Tri-axial acceleration (m/s²) was recorded with an accelerometer application (VibSensor, Now Instruments and Software, Inc., 2016) on Apple iPhones. 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. 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 accelerometer recordings. Two 10-minute test runs were also conducted, with all phones placed on top of empty pet packs that were located directly next to each other to allow comparisons in recording sensitivity between phones.

#### 2.3.5 Transport

Woylies from Karakamia were transported ~400 km to Mt Gibson by vehicle, with the journey taking approximately 4 hours. Woylies sourced from Perup were transported by vehicle to Manjimup Airport
(~1 hour), where they were transferred to a small fixed-wing plane. The plane departed daily at ~11:00 am and landed at Mt Gibson at ~1:00 pm. Upon arrival, woylies were placed in a dark, relatively quiet holding room at Mt Gibson’s Field Research Station. Post-travel accelerometer data was recorded at this stage. A video camera was filming for the duration of the recordings.

2.3.6 Release

At dusk, woylies were transported by vehicle to the release site inside Mt Gibson Sanctuary’s exclosure. Five release sites that were 200 m apart had been pre-selected along a transect in *Acacia* shrubland and York gum woodland. On any one night, a maximum of two individuals were released per site, though sites were re-used on subsequent nights.

At the release site, the handling bag was rolled 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 woylie to exit the bag (latency to leave). The woylie’s escape path (straight or convoluted) and escape speed (slow, medium or fast) were also recorded.

Due to the nature of the translocation (i.e. not experimental), it was not possible to completely control for a large number of variables that may have influenced animals’ behaviour. For example, time of release differed for individuals, as did the number of observers (though there were never any more than five people at an individual’s release), level of noise (although minimised as much as practicable) and level of lighting (also minimised to one head-torch).

2.3.7 Post-release trapping

From 22-28 August 2016, AWC staff and volunteers conducted trapping at Mt Gibson Sanctuary’s exclosure, with the aims of removing radio-collars, re-recording agitation levels and morphometric data, and collecting faecal samples. As not all radio-collared animals were successfully caught during this time, additional targeted trapping at Mt Gibson was conducted from 7 September - 13 October 2016. Collared individuals were radio-tracked during the day and once located, ten traps were set in an
approximate circle around the individual. Each day, traps were baited prior to dusk and checked at, or within 3 h of dawn the following morning.

2.4 Faecal sample extraction

Thirty-eight faecal samples that had been collected from translocated woylies pre-translocation and 26 samples collected post-release were stored at -20 °C for nine months. Eight of the 26 faecal samples that had been collected post-release were initially frozen but were unintentionally left out of the freezer for approximately 4 days, then re-frozen; these samples were still processed.

A commercial human corticosterone ELISA kit (K014-H1: Arbor Assays®, Michigan, USA) was used for 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 (Figure 2.6). 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).

![Figure 2.6 Corticosterone parallelism validation curve for the human standard curve (lower line) and the woylie faecal extract curve (upper line).](image)
For steroid extraction, the DetectX™ Steroid Solid Extraction Protocol was followed. As part of this, each 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 (1 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.

2.5 Enzyme Immunoassay process

Before starting the assay, kits and extracted samples were removed from the refrigerator for 30 min. 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 an S.E.M Vor-Mix was used to vortex the mixture. 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 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).

2.6 Statistical analyses

All statistical analyses were carried out using Statistica version 7.0 (StatSoft Inc) and Microsoft Office Excel© 2007 (Microsoft, 2017).

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 alpha level of \( p = 0.05 \) was used to determine significance. Escape time (s) and corticosterone concentration (pg/mL) were log transformed to meet the assumption of a normal distribution. Six individuals did not have data recorded for a single variable so these missing data were substituted with an average for that variable.

2.6.1 Body mass data

At translocation, 11 of 19 pouch young had their crown-rump or head length (mm) measured. Based on these measures, their body masses and age (days post-partum) were estimated using linear regression. Pouch young estimated to be 0 - 30 days old were assigned a mass of 0 g (Thompson et al. 2015). The estimated body mass of the pouch young was then deducted from their mother’s pre-translocation body mass. The post-translocation body masses of four females were adjusted using the same method. For the remaining females with no pouch young (either ejected prior to weighing or elongated teat indicating a young-at-heel), change in body mass was calculated by deducting pre-translocation body mass from post-translocation body mass.

Fourteen woylies were radio-collared; however, one individual’s collar fell off, so post-translocation body mass analyses were completed on 13 individuals. Radio-collar weights (35 g) were deducted from the post-translocation body mass of the collared individuals.
Due to the small number \((n=4)\) of Karakamia-sourced woylies (all males) that were trapped post-translocation, the effect of source population on post-translocation body mass could not be considered as a separate factor in these analyses.

There were no significant differences in variances in body mass between males and females \((n=16, \text{females } n=11)\) either pre- (Levene’s test, \(p = 0.985\)) or post-translocation (Levene’s test, \(p = 0.888\)). There was also no significant difference in variances in body mass between radio-collared woylies and non-radio-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 least significant difference (LSD).

### 2.6.2 Correlations with change in body mass

An information-theoretic approach was used to identify correlations between rate of change in body mass and all independent variables. This was then used to develop an average model to explain the dataset. Results of the analyses were interpreted using Akaike’s Information Criterion (AIC) (Burnham & Anderson 2003). Due to small sample size \((n=27)\), the model likelihood was corrected for the number of variables in each model; this was done by calculating AICc values from AIC values. The model with the lowest AICc value contained the most support for the data set. The Akaike weight \((w_i)\) was then calculated for each model with a \(\Delta\text{AICc} < 2\). For each combination of variables in each of the models, the standardised beta regression coefficients were calculated using multiple regression. To calculate model-weighted beta values, beta values were corrected using the corresponding model weight \((w_i)\). These were then summed so as to obtain the model-averaged beta values \((w_iB)\) for the top five models (Burnham & Anderson 2003). These values represent the relative contribution of each variable to the change in woylie body mass.
2.6.3 Behavioural data

Agitation level, escape speed, escape path, escape time, and heart rate

Relationships between pairs of behavioural measures and heart rate recorded pre-translocation were assessed using Spearman Rank Order correlations.

Activity recordings

Datasets were exported from phones as csv. files and saved as Excel files. Each file included a series of tri-axial movement measures (acceleration in m/s²) with corresponding time stamps (s).

Approximately 56,000 records were captured per 10 min 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/s²) were calculated for each axis (X, Y and Z) for each block. The mean percentage COV ±1 standard deviation (SD) was then calculated from the COV of all three axes per group. To examine variation in recordings between different phones during test runs, and in movement between individuals pre- and post-transport, each phone was assigned a unique identification number (Phone ID). Microchip numbers were used as unique identifiers for individual woylies (Animal ID).

2.6.4 Physiological data

Heart rate

Heart rate data between source populations were normally distributed pre-processing (Shapiro-Wilk W = 0.976, p = 0.546) and post-processing (Shapiro-Wilk W = 0.983, p = 0.809). A t-test (t = -0.74, P = 0.46, P variances = 0.002) showed that there were significant differences between source populations in P variances in woylies’ heart rates both pre- and post-processing (Perup n=31, Karakamia n=9). Consequently, Mann-Whitney U tests were performed.
To compare differences in heart rates between sexes, data from both sources were pooled. There was a significant difference in variances between the pre-processing heart rates of male and female woylies (Levene’s test, \( p = 0.01 \)); consequently, Mann-Whitney U tests were conducted.

*Faecal corticosterone concentration*

Microplate Manager® software (Bio-Rad, California, USA) was used to convert absorbance values into picograms per millilitre. To obtain corticosterone concentration (pg/mL), the mean of the sample and its duplicate were calculated. This value was then multiplied by a dilution factor of two to obtain neat sample values.

Due to the small number of Karakamia-sourced individuals \((n=4)\) from which post-translocation faecal samples were collected, it was not possible to consider the effect of source population on corticosterone concentration as a separate factor in these analyses. 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 (pre- \( p = 0.501 \), post- \( p = 0.422 \)) so a repeated measures ANOVA was performed.
Chapter 3 – Results

A total of 40 woylies (n=21 males, n=19 females) were translocated to Mt Gibson from Karakamia and Perup Sanctuaries (Table 3.1).

Table 3.1 Summary of the number of woylies involved in this study according to source and the faecal data collected pre- and post-translocation.

<table>
<thead>
<tr>
<th>Summary of data collected</th>
<th>Karakamia-sourced</th>
<th>Perup-sourced</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Individuals translocated to Mt Gibson</td>
<td>5 (0)</td>
<td>4 (0)*</td>
<td>16 (6)*</td>
</tr>
<tr>
<td>Post-translocation trapping</td>
<td>4 (0)</td>
<td>0 (0)</td>
<td>12 (5)**</td>
</tr>
</tbody>
</table>

Numbers in brackets were radio-collared individuals. No faecal sample collected for one (*) or two (**) individuals.

3.1 Relationships among behavioural and heart rate measures

There were no significant correlations between any of the behavioural and physiological measures (Table 3.2).
3.2 Activity data - Accelerometer recordings

Accelerometer data were recorded from 31 individuals before and directly after transport. The method used successfully recorded woylies’ activity however activity levels were influenced by external stimuli such as the level of noise. For example, a 10-min recording of tri-axial movement of a woylie at Karakamia during holding, shows two peaks which occurred simultaneously with loud noises recorded on video camera at the same time (Figure 3.1).
Mixed-measures ANOVA revealed no significant differences in the variation in activity for individuals pre-transport and post-transport, between individuals, or between phones (pre/post-transport fixed effect $p = 0.681$, Animal ID random effect $p = 0.416$, Phone ID random effect $p = 0.060$).

### 3.3 Physiology

#### 3.3.1 Heart rate

Pre-processing heart rates (bpm) (Figure 3.2[a]) were significantly different between woylies from Perup ($n=31$) and Karakamia ($n=9$; Mann-Whitney: $U = 27, Z = -3.644, p < 0.001$). There were also significant differences in post-processing heart rates in woylies from the two sources (Mann-Whitney: $U = 28, Z = -3.611, p < 0.001$) (Figure 3.2[b]). When the data from both populations were examined, due to the increased variability in the data and disproportionate sample sizes, there was no significant sex difference in heart rates pre-processing (Mann-Whitney: $U = 197.5, Z = 0.054, p = 0.957$) or post-processing (Mann-Whitney: $U = 193.0, Z = -0.176, p = 0.860$).

Figure 3.1 Recording of tri-axial movement of a woylie over 10 minutes post-processing.
3.3.2 Faecal corticosterone concentration

Repeated-measures ANOVA comparing pre- and post-translocation faecal corticosterone concentrations between sexes (males n=14, females n=11) indicated a statistically significant sex \times time interaction ($F_{1, 23} = 6.641, p = 0.017$) (Figure 3.3). A post-hoc analysis (LSD) showed that there was a significant difference between males and females’ corticosterone concentrations pre-translocation, with males’ concentration levels higher by 5% ($p=0.035$). Mean corticosterone concentrations for females increased pre- to post- translocation (3.57 ± 0.21 pg/mL to 3.77 ± 0.20 pg/mL), while males’ levels decreased (3.76 ± 0.28 pg/mL to 3.62 ± 0.17 pg/mL).

Figure 3.2 Woylies from Perup had significantly higher heart rates than woylies from Karakamia, both pre-processing (a) and post-processing (b).
Figure 3.3 Mean corticosterone concentration for male and female woylies over time. Letters identify differences between and among groups (a), (b), (ab), (post-hoc LSD analysis).

Table 3.3. Post-hoc analysis of corticosterone concentration between males and females over time, with the significant p value indicated (bold).

<table>
<thead>
<tr>
<th>Sex</th>
<th>Time</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Males</td>
<td>Log corticosterone pre-translocation (pg/mL)</td>
<td>0.126</td>
<td>0.035</td>
<td>0.883</td>
<td></td>
</tr>
<tr>
<td>2 Males</td>
<td>Log corticosterone post-translocation (pg/mL)</td>
<td>0.572</td>
<td>0.086</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Females</td>
<td>Log corticosterone pre-translocation (pg/mL)</td>
<td>0.053</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 Females</td>
<td>Log corticosterone post-translocation (pg/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.4 Change in body mass

There was a statistically significant sex x time interaction for change in body mass ($F_{1,25} = 4.806, p = 0.038$; Figure 3.4) with females showing a greater increase in body mass post-translocation (pre-translocation: 1227 ± 147 g, post-translocation: 1402 ± 151 g; increase 14%) than males (pre-translocation: 1194 ± 155 g, post-translocation: 1273 ± 116 g; increase 7%) (Post-hoc analysis LSD, $p=0.027$) (Table 3.4).
Figure 3.4 Mean body mass of male and female woylies pre- and post-translocation, with differences between and among groups identified (a), (b), (ab), (post-hoc LSD analysis).

Table 3.4 Change in mean body mass over time between males and females. Significant p values are indicated (bold).

<table>
<thead>
<tr>
<th>Sex</th>
<th>Time</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Males</td>
<td>Body mass pre-translocation</td>
<td>0.010</td>
<td>0.561</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>2 Males</td>
<td>Body mass post-translocation</td>
<td></td>
<td>0.421</td>
<td>0.027</td>
<td></td>
</tr>
<tr>
<td>3 Females</td>
<td>Body mass pre-translocation</td>
<td>0.421</td>
<td></td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>4 Females</td>
<td>Body mass post-translocation</td>
<td></td>
<td></td>
<td></td>
<td>0.027</td>
</tr>
</tbody>
</table>

There was no significant effect of radio-collaring on change in body mass over time ($F_{1, 25} = 1.385, p = 0.250$; Figure 3.5). Pre-translocation, radio-collared woylies had greater mean body mass ($n=13$, $1255 \pm 147$ g) than did non-collared woylies ($n=14$, $1163 \pm 144$ g). Post-translocation, body masses showed the same pattern (radio-collared: $1401 \pm 155$ g; non-radio-collared: $1255 \pm 91$ g).
Figure 3.5 Mean body mass of both radio-collared and non-radio-collared woylies increased over time. Letters identify differences between and among groups (a), (b), (ab), (post-hoc LSD analysis).

### 3.5 Associations amongst behaviour, physiology, sex and body mass

Five models had an ΔAICc < 2 and were therefore considered to have equal support of being the best model to explain the rate of change in body mass of woylies pre- to post-translocation (Table 3.5). Eleven variables were included in the analyses: source population, sex, presence of a radio-collar, log pre-translocation faecal corticosterone concentration (pg/mL), trapping agitation level (low, medium, high), heart rate (bpm) pre-processing, heart rate (bpm) post-processing, change in heart rate, processing agitation level (low, medium, high), escape time (log-s), escape speed (slow or fast), and escape path (convoluted or straight). Of these, only three were included in the top five models. The standardised beta values for these three variables indicated that they had reasonably equal contribution to the description of change in body mass.

Sex occurred in three of the top models, with females increasing in body mass (12.4±8.6% increase: pre-translocation: 1.24±0.15 kg cf. post-translocation: 1.40±0.15 kg) at twice the rate of males (5.7±8.2% increase: pre-translocation: 1.19±0.16 kg cf. post-translocation: 1.27±0.11 kg) (Table 3.5).
This effect resulted in a statistically significant sex \times time interaction for body mass (repeated-measures ANOVA: \( F_{1, 25} = 4.54, p = 0.043 \)). Change in heart rate during processing was included in four of the top models. The positive beta values indicate that animals with the greatest lability in heart rate also showed the greatest mass gain (Table 3.5). Escape path was included in two of the top models, with woylies that took a convoluted path showing 73\% greater increase in body mass compared with those that took a straight path (Table 3.5).

Table 3.5 Top five models with corresponding ΔAICc values and model weights (\( w_i \)). The non-standardised model-averaged beta values (\( w_i \beta \)) show the overall influence of each variable on body mass while the standardised beta values (\( w_i \beta \)) values illustrate the relative increase in body mass attributable to each variable.

<table>
<thead>
<tr>
<th>Model no.</th>
<th>Sex (female=0 or male=1)</th>
<th>Change in heart rate (bpm)</th>
<th>Escape path (convoluted=1, straight=0)</th>
<th>df</th>
<th>Δ AICc</th>
<th>( w_i )</th>
<th>( R^2 )</th>
<th>Test for goodness of fit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>0.000</td>
<td>0.33</td>
<td>0.28</td>
<td>( F_{2,24}=4.75, p&lt;0.018 )</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>0.944</td>
<td>0.20</td>
<td>0.35</td>
<td>( F_{3,23}=4.08, p&lt;0.018 )</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>1.131</td>
<td>0.18</td>
<td>0.17</td>
<td>( F_{1,25}=5.12, p&lt;0.032 )</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>1.417</td>
<td>0.16</td>
<td>0.24</td>
<td>( F_{2,24}=3.89, p&lt;0.034 )</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>1.884</td>
<td>0.13</td>
<td>0.15</td>
<td>( F_{1,25}=4.29, p&lt;0.049 )</td>
</tr>
</tbody>
</table>

No. occurrences 3 4 2

Standardised (\( w_i \beta \)) -0.335 0.414 0.369

Non-standardised (\( w_i \beta \)) -0.76 2.27 0.84
Chapter 4 – General Discussion

This study investigated whether individual differences in behaviour and physiology recorded during and post-translocation were associated with post-translocation body mass gain in the woylie. Behavioural and physiological measures representative of temperament traits including activity, aggression and reactivity/fearfulness were collected during two translocations and again ~5 months post-release. The behavioural measures were not found to be consistent or repeatable for individuals, hence it was not possible to identify individual temperament traits from the data collected (Réale et al. 2007). However, measures of heart rate lability and escape behaviour showed potential in predicting post-translocation body mass gain in woylies. Individuals that had the greatest lability in heart rate, and those that took a convoluted escape path at release, showed the greatest mass gain between translocation and post-release recapture. 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. This study suggests that stress responses can be both sex-specific (faecal corticosterone level) and source-specific (heart rate), and that some measures of behaviour (escape path) and physiology (heart rate lability) may be useful to predict body mass gain of translocated animals.

4.1 Associations amongst behaviour, physiology, sex and body mass

The strongest associations with body mass gain post-translocation were those of escape path at release, heart rate lability and sex.

4.1.1 Escape behaviour

The only behavioural measure that was correlated with change in body mass was escape path, whereby woylies that took a convoluted path at release had a 73% greater increase in body mass compared with those that took a straight path. Many species, including woylies, zigzag or exhibit frequent directional changes when being chased (‘Protean’ behaviour) to allow them to increase
unpredictability and evade a predator in pursuit (Furuichi 2002; de Tores & Start 2008; Domenici et al. 2011; Cooper & Blumstein 2015). Prey can however, alter their escape response in order to minimise the costs of the escape (Ydenberg & Dill 1986). The escape trajectory selected by a fleeing animal can be influenced by the individual’s distance from the threat, with a short distance between predator and prey inducing a zig-zag escape path, whilst greater distances induce a straight escape path (Hodges et al. 2015). Hence, prey that choose the optimal escape response for the given situation may have fitness advantages by not only surviving an attack, but also using less energy during escape which can then be utilised for foraging, mating or defending territory (Cooper 2000; López et al. 2005; Cooper & Blumstein 2015).

An individual’s escape response can also be influenced by temperament (López et al. 2005). In a study of anti-predator behaviour in Iberian rock lizard (Lacerta monticola), bolder lizards that spent a shorter amount of time hiding following predatory approaches, had better health and body condition than did shy lizards that took longer to emerge from refuges. These results indicate that the relative boldness of an individual can influence its anti-predator behaviour which, in turn, may afford fitness advantages (López et al. 2005). Hence, individual differences in boldness between woylies in this study may have influenced escape path at release, with bolder woylies taking a convoluted path and shyer woylies taking a straight path (López et al. 2005; Réale et al. 2007). In the absence of predation at Mt Gibson, bolder woylies may have been more efficient in finding resources, leading to greater body mass gain when compared to those individuals that were potentially shyer. This has been observed in brushtail possums whereby boldness has been associated with greater dispersal and access to resources, leading to greater gain in body mass post-translocation (May et al. 2016). However, in this study escape behaviours were only recorded once for each individual, so it was not possible to determine whether escape path was influenced by temperament, perceived threat at the time of release, and/or the novel environment into which they were being released. Further research is required to examine if variability in escape path is repeatable in different individuals under similar conditions, and if so, to ascertain whether this behaviour is correlated with specific temperament traits such as boldness (López et al. 2005).
4.1.2 Heart rate lability

Woylies with the greatest lability in heart rate showed the greatest body mass gain. An increase in heart rate is caused by an increase in sympathetic control of cardiac contraction (Von Borell et al. 2007). Therefore, heart rate variability reflects reactivity and an emotional response to a challenge (Montiglio et al. 2012). The greater body mass gain observed in more reactive individuals is intriguing as it was expected that bolder (and presumably less reactive) individuals would be more successful in finding resources than more reactive/fearful individuals and thus gain more body mass post-translocation. Further investigation into associations between temperament, heart rate lability and body mass in other species would be informative for interpreting this finding.

4.1.3 Sex

By ~3-5 months post-translocation, all woylies had gained body mass on average, although females gained significantly more than males. May et al. (2016) established similar results, with all brushtail possums translocated from Karakamia to Paruna Sanctuary having gained body mass 5 months post-translocation, but females had gained significantly more body mass than males.

The overall mass increase seen in both sexes may have been due to increased resource availability. Translocations were conducted in autumn and winter during which time hypogean fungi - the primary food source of woylies - is in its peak (Zosky 2011; Yeatman & Groom 2012). Similarly, body condition and fecundity of Tasmanian bettongs (Bettongia gaimardi) also increased during periods of peak fungi availability (Johnson 1994; Robinson et al. 2007). Hence, the woylies in this study may have had access to more resources, while the size of the exclosure may also have reduced competition for those resources (Wayne 2008; Ruykys & Kanowski 2015; Wayne et al. 2015).

It is possible that females gained more body mass than males as they spent more time foraging for and consuming food, given that reproduction is continuous in woylies and is one of the most energetically demanding activities that a female mammal can undertake (Bronson 1985; Wade & Schneider 1992;
Bronson 2009; Montagne 2016). For example, an assessment of the energy requirements of lactation in tammar wallaby (Macropus eugenii) found that female tammars increased their food intake in days 150-218 of lactation when reproductive energy demand was at its highest (Cork 1991). Moreover, female tammars have been shown to gain body mass over the course of lactation (Schwanz & Robert 2012). Hence, increased energy intake for the purposes of reproduction may explain why the females had a greater increase in post-translocation body mass than did males; however, this would need to be experimentally tested (i.e. by having controls).

There was no significant effect of radio-collars on change in body mass of woylies post-translocation. Correspondingly, Golabek et al. (2008) also found no negative effects on foraging efficiency and percentage weight change in radio-collared meerkat (Suricata suricatta) after 3 months (Golabek et al. 2008). Future studies assessing impacts of radio-collaring on body mass should standardise the body mass of collared and non-collared animals so more accurate comparisons can be made between the two groups.

4.3 Physiological measures

4.3.1 Heart rate

Woylies from Karakamia had significantly lower pre- and post-processing heart rates than did woylies from Perup. There may be two possible explanations for this. The first is the level of disturbance experienced by woylies within their respective sanctuaries. Karakamia Sanctuary is a small exclosure and numerous monitoring and management activities are regularly conducted within it on an annual basis (Australian Wildlife Conservancy 2016). There is also regular vehicle traffic (day and night) associated with the staff that live and work within the sanctuary. In comparison, woylies within Perup Sanctuary are subject to similar activities but less regularly. Perup is also closed to the public, is larger in size and supports a smaller population of woylies.
Animals living close to humans can be disturbed by human activities and if exposure is prolonged, this can eventually lead to reduced reactivity and habituation (Boissy 1995; Bremner-Harrison et al. 2004; Archard & Braithwaite 2010). For instance, in a colony of king penguin (Aptenodytes patagonicus) that had been exposed to humans for over 50 years, individuals’ heart rates were attenuated in response to stressors such as noise and human approach when compared with the heart rates of penguins in undisturbed colonies. The authors proposed that over time, the exposed penguins adjusted to human disturbance and habituated to associated low-level stressors (Viblanc et al. 2012). Similarly, the lower pre- and post-processing heart rates seen in Karakamia-sourced woylies may have indicated that they were less reactive to human presence, noise and handling due to their level of exposure to these disturbances.

The second potential explanation is that of the differing conditions during sampling. Karakamia-sourced woylies were processed at night but Perup-sourced woylies were processed in the morning. The Perup woylies may also have been impacted by more noise during processing as there were more people, more woylies captured, and longer processing times there than at Karakamia. Factors such as noise, timing, and duration of handling should be standardised in future studies. This will ensure that responses observed can be attributed to handling, as opposed to other external factors (Réale et al. 2007; Herborn et al. 2010).

4.3.2 Faecal corticosterone concentration

At the time of translocation, males had significantly higher faecal corticosterone concentrations than did females. Reasons for this may include the fact that breeding males may engage in energetically expensive activities, such as territorial aggression, male–male competition and dominance interactions (Nelson et al. 1992; Reeder & Kramer 2005; Lane 2006). In the context of this study, such interactions may have been occurring more frequently at Karakamia and Perup Sanctuaries than at Mt Gibson Sanctuary’s exclosure, which is 28 times larger than Karakamia and 18 times larger than Perup. At Perup Sanctuary, woylies’ home ranges overlap by 77% even at low population densities (Yeatman & Wayne 2015). In male anubis baboon (Papio hamadryas anubis), elevated faecal glucocorticoid levels
were recorded in all males in a troop during periods of social instability, when male–male aggressive interactions were most frequent (Bergman et al. 2005). For male woylies, antagonistic interactions and competition for territory may have reduced at Mt Gibson, which may explain the decrease in corticosterone levels observed ~5 months post-release.

At post-translocation re-capture, corticosterone levels for females had increased while males had decreased, although the differences were not significant. Sex is one of the most widely recognised factors influencing variation in glucocorticoids (Narayan et al. 2013). This variation has been observed in several other Australian marsupials, including southern brown bandicoot (*Isoodon obesulus*), bilby (*Macrotis lagotis*) and koala (*Phascolarctos cinereus*), with females having higher mean faecal glucocorticoid levels than males in response to a range of stressors (Narayan et al. 2012; Dowle et al. 2013; Narayan et al. 2013). These sex differences have been attributed to steroid metabolism, reproductive status and pituitary responsiveness (Lane 2006; Goymann 2012; Narayan et al. 2013). In woylies, the presence or absence of pouch young was not found to have an effect on females’ glucocorticoid levels (Hing et al. 2016). Thus, in contrast with other marsupial species, reproductive status was an unlikely cause of the increase in corticosterone levels observed in female woylies post-translocation.

A more viable explanation for the increase seen in females is the effect of hormones and steroids (Reeder & Kramer 2005). Due to the actions of oestrogen on the hypothalamic-pituitary-adrenal (HPA) function, females generally exhibit a greater response to stress than males. Opposingly in males, androgen inhibits the HPA axis which counteracts the stress response (Handa et al. 1994; Ježová et al. 1996). For instance, Hing et al. (2017) assessed stress associated with translocation in woylies, by comparing faecal glucocorticoid levels at the time of translocation and 6 months post-translocation. Males and females had similar levels at translocation; however, 6 months after translocation, there was a significant difference between sexes with females having higher glucocorticoid levels than males (Hing et al. 2017). These results were attributed to inherent endocrine differences between the sexes in response to translocation, as well as differing responses to varying
population density, or site attributes at the destination site (e.g. predators, resources) (Handa et al. 1994; Ježová et al. 1996; Hing et al. 2017).

A significant factor that should be considered when assessing changes in faecal glucocorticoid levels is that accumulation of glucocorticoids in faeces occurs over time (Touma & Palme 2005; Hing et al. 2017). The lag time for excretion in faeces following exposure to a stressor is estimated to be ~5 hours in small mammals (Bosson et al. 2009). In bilby (*Macrotis lagotis*) however, lag time is 24 hours, and can be up to 27 hours in long-nose bandicoot (*Perameles nasuta*) (Moyle et al. 1995; Narayan et al. 2012). Furthermore, sympathetic nervous stimulation associated with a fight or flight response also 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 this study were due to a stress response from confinement in a trap (ranging from ~2-16 hrs), 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 be valuable to assist in interpreting observed changes in faecal glucocorticoids and ensuring measures obtained, accurately reflect the physiological events of interest (Narayan et al. 2012).

There were a number of limitations associated with faecal corticosterone sampling and interpretation of results. In an attempt to keep the sampling non-invasive by collecting faecal samples during trapping events, the data only represented two time-points in the translocation process. Future studies should endeavour to obtain a baseline level of faecal glucocorticoids prior to translocation (Fletcher & Boonstra 2006). Moreover, the quantities of faecal samples collected differed due to the amount of time that the individual was in the trap, and samples were also collected at different times. Circulating glucocorticoid concentrations in mammals are known to fluctuate diurnally and nocturnally (Touma & Palme 2005). Consequently, the time of sample collection and duration of capture should be standardised where possible, particularly for repeated measurements or for comparisons between populations or individuals (Touma & Palme 2005). In this study, male and female woylies had contrasting levels of stress at the time of translocation when compared to ~3-5 months post-
translocation. Whether these responses were directly related to the translocation or to being trapped is difficult to determine, given the multitude of factors that influence faecal corticosterone levels (Touma & Palme 2005). Standardising the methodology of faecal sample collection and increasing knowledge of factors that influence stress responses, are vital considerations for future studies on stress physiology. Furthermore, having control populations (i.e. non-translocated cohorts) in both the source and destination sites would facilitate discrimination of translocation effects from those that were due to natural seasonal changes or other factors.

4.4 Behavioural measures

Many of the behavioural measures were not found to be consistent or repeatable for individuals. As such, it was not possible to distinguish individual temperament traits from these measures. In this study, behavioural observations were recorded by up to twelve different observers. In future, the number of observers recording behavioural measures should be kept to a minimum and trapping and processing conditions (e.g. duration, noise, time) should be standardised where possible.

There were no significant relationships between individuals’ level of activity before and after transport, as measured through mobile phones and an accelerometer application. This suggests that woylies’ level of reactivity (measured via activity levels [m/s]) did not differ pre- and post-transport. Alternatively, as several models of iPhone were used, there may have been variation in recording sensitivities that obscured any true variation in individuals’ activity levels. Recommended improvements to methodology include use of purpose-built accelerometers to reduce variation associated with different device models, and attachment of accelerometers to the animals to improve accuracy of the data (Carrier et al. 2012; Williams et al. 2016). Alternatively, recording devices (such as iPhones) should be firmly secured to the pet packs so as to negate any slippage and/or dampening of movement between the pet pack and the recording device.
4.5 Conservation implications

Physiological measures used in this study were effective for answering questions related to short (heart rate) and longer-term (faecal corticosterone) stress responses in woylies during and post-translocation. It may therefore be useful to incorporate these measures into translocation protocols; which in turn, could 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). Developing processes that reduce these impacts could improve the welfare of individuals during and post-translocation and enhance conservation outcomes (McDougall et al. 2006; Harrington et al. 2013; González et al. 2015). Additionally, these methods may also be valid in assessing stress responses in other threatened bettong and/or macropod species.

Although there was generally little association between body mass gain and the behaviours that were measured, the results of the current study may have been vastly different if animals had been translocated to unfenced areas, particularly if they had been sourced from captive populations and were predator naive (Banks et al. 2002; Moseby et al. 2011). In this scenario, if temperament is known among captive individuals, it may be beneficial to select those that exhibit temperaments known to improve survival and translocation success (Bremner-Harrison et al. 2004; McDougall et al. 2006; Watters & Meehan 2007). Ultimately though, post-release survival is likely due to the interplay between temperament, release strategy, site conditions, predation pressure, and life history traits (Moseby et al. 2014). Thus, there is no optimal combination of temperament traits that unequivocally leads to the highest fitness in all conditions (Boon et al. 2007). As such, when selecting translocation candidates, it is important to maintain variation in temperaments to ensure the survival and fitness of the translocated population in a fluctuating environment (Sih et al. 2003; Dingemanse et al. 2004; Watters & Meehan 2007; Archard & Braithwaite 2010).
In this study, change in body mass post-translocation was most strongly influenced by sex, heart rate lability and escape path. However, on average, all woylies increased in body mass post-translocation, which indicated that they were capable of dealing with the stress associated with translocation, and were successful in finding resources in a novel environment. Moreover, both the climate and habitat at Mt Gibson are different to that of the source populations suggesting that woylies are adaptable, and can prosper if resource limitation and predation are managed (Start et al. 1995). This outcome also emphasises the value of predator-free exclosures for the protection of threatened species that are vulnerable to predation, such as the woylie.

**Conclusion**

This study demonstrated that woylies’ responses to translocation can be sex- and source-specific. Furthermore, specific measures of heart rate lability and escape behaviour may be useful for predicting post-translocation body mass gain in this species. Behavioural measures were less informative as they were not repeatable and hence, could not be used to infer individual temperament traits.

Collecting physiological and behavioural measures during translocation can reduce time in captivity for free-living animals, although ensuring consistency in measures, particularly in different locations, is challenging. If the recommended improvements to the methodology are implemented the collection of data can remain non-invasive whilst providing more reliable measures on behaviour and physiology. Refinement of the methodology may also facilitate its application during translocations of other similar species of conservation concern.

Further research is necessary to gain a clearer understanding of the causes of the opposing faecal corticosterone stress responses observed in males and females. Investigations should involve more frequent sampling, attainment of baseline corticosterone levels prior to stressful events, and incorporation of control/reference cohorts within an appropriate scientific design. A parallel
assessment of hormonal differences between males and females would also be beneficial, given that this can be a cause of variation in stress responses between sexes.

This study established that, regardless of their temperament, woylies were able to cope with the stressors imposed during translocation. Irrespective of their source location, woylies were able to successfully adapt to the climate and habitat in their new location, find resources, gain weight and reproduce. This is an encouraging outcome given that translocation is likely to continue to be an essential management intervention for the conservation of this critically endangered species.
References


Montagne J-P. 2016. The effect of personality on movement and survival following translocation of California ground squirrels (*Otospermophilus beecheyi*). Thesis. San Diego State University, USA.


Appendix 1 – Applications of accelerometry in animal behaviour research: past, present and future (literature review assessed independently).

Abstract

Technologies in accelerometry have advanced greatly in recent years, leading to an increase in their use in animal behavioural research. A review of the literature revealed that these devices have applicability for use in many disciplines to address a range of questions relating to both captive and free-ranging species. These devices have the ability to measure a plethora of physical, physiological and emotional variables; however, there is some debate over their accuracy and their influence on the behaviour of study subjects. A common shortfall of many studies was the absence of a reference to protocols for fitting these devices; in turn, this has implications for animal ethics and data accuracy. A notable trend was that numerous studies utilised additional methods such as visual observation or other sensors to collect a more extensive dataset of both quantitative and qualitative measures. Also discussed, is a unique approach using in-built accelerometers in smartphones and an innovative method to apply them to captive scenarios. Overall, there is a knowledge gap in the use of accelerometers in species conservation. This topic is examined in terms of using accelerometry to monitor health, welfare and behaviour of threatened species during captive breeding or translocation. Exploring novel approaches and new methodologies in accelerometry may provide an opportunity to expand knowledge in the field of animal behavioural research, and pave the way for new advances in threatened species conservation.

**Keywords:** accelerometer, animal behaviour, physical activity, emotional reactivity, physiology.

Glossary

| **Accelerometer** | an electronic device that measures acceleration over time and records data in multiple axes, generally X, Y and Z (López-López 2016). |
| **Bio-logger** | a miniaturised animal-attached tag used for recording and/or relaying data about an animals’ movements, behaviour, physiology and/or their environment (López-López 2016). |
| **Inertial Measurement Unit** | a self-contained system that measures linear and angular motion usually with a triad of gyroscopes and a triad of accelerometers (Andriamandroso et al. 2014). |
Introduction

An animal’s behaviour is a response to its physiological condition and surrounding environment. It can therefore be used as a measure of an individual’s health and well-being, which can affect its ability to obtain resources, survive and reproduce (McDougall et al. 2006; Shepard et al. 2008).

As such, measuring animal behaviour can facilitate more informed management decisions to enhance animal performance and to improve animal welfare, environmental and conservation outcomes (González et al. 2015). Research on animal behaviour spans many disciplines and has broad applications including: advancement in sustainable practices for recreational fisheries (Brownscombe et al. 2013), identification of links between neurological function and behaviour (Venkatraman et al. 2010), improving management of production animals (Rushen et al. 2012; MacKay 2013), and expanding knowledge of threatened species (Ismail et al. 2012; Wilson et al. 2013).

One of the most commonly used methods to study animal behaviour is visual observation (Ropert-Coudert & Wilson 2005). However, this method has limitations as the observer is seldom undetectable; thus, their presence is likely to affect the behaviour of the animal being observed, even in subtle ways (Brown et al. 2013). In addition, it can be time intensive (Rushen et al. 2012) and hindered by distance, or environmental elements that obscure the observer’s view (Ropert-Coudert & Wilson 2005; Gervasi et al. 2006). Despite these shortfalls visual observation is still frequently used in behavioural research, although now, it is commonly used in conjunction with other methods (Ropert-Coudert & Wilson 2005).

Historically, jiggle cages (Smith & Smith 1973), pressure transducers (Webber & O’dor 1986) and omnidirectional mercury switches (Mitchell et al. 1997) were devices utilised to collect behavioural data. They each provided a measure of activity; however, they were large, complex and invasive (Müller & Schrader 2003; Mann et al. 2005). Typically, these devices were used on captive animals so their behaviour was likely influenced by the artificial environment in which the studies were conducted or by the devices themselves affecting natural movement (Müller & Schrader 2003). These issues were addressed with the development of bio-loggers - small animal-attached devices used for recording data on movement, behaviour and physiology (López-López 2016). Bio-loggers have become a popular option for researchers as they can remotely collect quantitative data from relatively undisturbed subjects in both captivity and in the wild (Müller & Schrader 2003; Rutz & Hays 2009; Brown et al. 2013). They can be singular or multi-functional devices which can include satellite or radio transmitters (Brown et al. 2012; Bouten et al. 2013), accelerometers (Robert et al. 2009; Brownscombe et al. 2013), gyroscopes (Wilson et al. 2013; Noda et al. 2014), light sensors (Laich et al. 2008) or temperature gauges (Bouten et al. 2013), to name a few.

Accelerometers in particular, have received widespread attention as they can measure an animal’s acceleration over time in multiple axes (López-López 2016). These data can in turn be used to quantify physical activity, movement patterns, body micro-movements and body posture (Shepard et al. 2008; Wilson et al. 2014; López-López 2016). An animal’s internal state, emotional reactivity, energy expenditure and health can then be deduced (Rushen et al. 2012; MacKay 2013; Wilson et al. 2014; Jeanniard-du-Dot et al. 2016).

Ongoing advances and developments in technology have resulted in accelerometers reducing in size and weight (Williams et al. 2016). This has facilitated their use on a greater range of species, further
increasing their appeal to researchers (Wilson et al. 2014). Several types of accelerometers are used in animal behaviour studies including piezoelectric (Müller & Schrader 2003), mono-axial, bi-axial and tri-axial (Shepard et al. 2008). Mono-axial accelerometers were used in research on Alpine swifts (Tachymarptis melba) to monitor their activity patterns during breeding, non-breeding and migration (Liechti et al. 2013). Body pitch angle and activity levels from the accelerometers were used to distinguish between flying and resting behaviours. Results showed that flight behaviour changed markedly between the different periods. Swifts remained airborne for more than 6 months and slept during flight in the non-breeding period (Liechti et al. 2013).

These findings have implications for energetic costs and locomotive control of flight in swifts (Liechti et al. 2013). Okuyama et al. (2010) used bi-axial accelerometers attached to the lower beaks of captive loggerhead turtles (Caretta caretta) to monitor feeding and breathing behaviours. Accelerometer data allowed differentiation between feeding and breathing based on head pitch, and provided an indication of feeding duration and biting force used for different food items. This study provides a basis for further investigation into the respiratory frequency and feeding strategies of loggerhead turtles (Okuyama et al. 2010). Mono and bi-axial accelerometers measure acceleration in one or two axes and have proved to be useful in identifying different behaviours; however, as movement typically occurs in three dimensions, quantification of activity and thus behaviour is considered to be more accurate with tri-axial accelerometers (Laich et al. 2008; Shepard et al. 2008).

This review focuses on behavioural research conducted on animals using accelerometers, with a particular focus on bi-axial and tri-axial accelerometers. Topics addressed include how accelerometers have been applied in behavioural research across different disciplines; what they have been used to measure; the species on which they have been deployed; and different methods of use, including attachment types, and other complementary methods and sensor types. Also discussed are common trends identified in the literature, methodological conflicts, gaps in knowledge, new approaches in this field of research.

Methods

Data for this review was sourced from peer-reviewed literature identified by conducting searches in Google Scholar (http://scholar.google.com). The following is an outline of the keywords used for each search. All searches excluded the word “human”.

1. accelerometer, animal behaviour, physiology, activity and bio-logging;
2. accelerometer, animal behaviour, physiology, activity, stress, terrestrial and emotional state;
3. accelerometer, animal behaviour, physiology and stress;
4. accelerometer, animal behaviour, physiology and reactivity;
5. tri-axial accelerometer, reactivity and animal behaviour;
6. bio-logging; and
7. iPhone accelerometer and animal behaviour.

For each search abstracts of articles from the first five pages of results were examined. Studies were included if they met the following criteria:

1. studies were conducted on animals;
2. accelerometers were used as a method of measuring one or more variables of interest; and
3. variables measured included physical activity, behaviour, health, emotional state and/or physiology.
The selected studies were read, key findings highlighted and details of each paper were entered into an excel spreadsheet for analysis. The list of literature was expanded by examining the reference lists of the selected studies. A further search for ‘grey’ literature using the same keywords in Google revealed one relevant book chapter. A total of 61 journal articles, consisting of 25 reviews, 36 primary studies and one book chapter were deemed relevant to this literature review. Key findings and conclusions have been drawn from the 36 primary studies.

**Results**

The 36 studies analysed spanned 28 scientific journals. There was a fairly even spread across each, with the highest proportion from *Applied Animal Behaviour Science* (8%). The year of publication ranged from 1999 to 2016, with the majority of studies (25%) published in 2014.

**Accelerometry Applications**

Accelerometry has been applied to a broad range of topics to answer a diverse range of questions across many disciplines (Table 1).

**Table 1.** A sample of topics and questions being addressed by the use of accelerometers (adapted from Wilmers et al. 2015).

<table>
<thead>
<tr>
<th>Topic</th>
<th>Questions</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environmental</td>
<td>How do patterns of variation in individual activity levels of woodland caribou change in relation to environmental parameters?</td>
<td>Mosser et al. 2014</td>
</tr>
<tr>
<td>Welfare</td>
<td>Is it possible to remotely characterise certain elements of cattle behaviour on a daily basis to monitor individual health and welfare?</td>
<td>MacKay 2013, Rushen et al. 2012</td>
</tr>
<tr>
<td>Physical activity/behaviour</td>
<td>Can different behaviours of cryptic species in their natural habitat be classified by accelerometers?</td>
<td>Wang et al. 2015</td>
</tr>
<tr>
<td></td>
<td>How do movement strategies in free-ranging Malayan colugos differ between males and females?</td>
<td>Byrnes et al. 2011</td>
</tr>
<tr>
<td>Threatened species management</td>
<td>Can accelerometers be used to measure activity of threatened species to improve understanding of their biology and assist with conservation efforts?</td>
<td>Ismail et al. 2012, Okuyama et al. 2010, Wilson et al. 2013</td>
</tr>
<tr>
<td>Neurology</td>
<td>Can accelerometers be used to determine how behaviour emerges from the activity of the nervous system in rodents?</td>
<td>Venkatraman et al. 2010</td>
</tr>
</tbody>
</table>

Examples of studies from different disciplines demonstrate the varied aspects of behaviour covered. The highest number of studies were published in *Applied Animal Behaviour Science* and were generally focussed on behaviour and activity of dairy cattle. Accelerometers were attached to cows to
record their daily activity, which was then used to identify behavioural traits such as fearfulness and sociability, lameness and evidence of disease or illness (Martiskainen et al. 2009; MacKay 2013; MacKay et al. 2014). Accelerometers were capable of recording repeatable activity traits related to behaviour and are therefore useful as a welfare assessment tool (MacKay 2013). As such, these devices can improve the management of herds by assisting farmers to identify and address welfare issues and increase production (MacKay 2013). In the field of marine biology, Noda et al. (2014) fitted accelerometers to Japanese amberjacks (*Seriola quinqueradiata*) to measure escape behaviour. Escape movements were separated into single-bend and double-bend mechanical types and results showed significant differences in movement between the two (Noda et al. 2014). This data provided insights into the relationships between movement performance, fitness, and survival strategies of fish (Noda et al. 2014). In contrast, Roupert-Coudert et al. (2006) studied pursuit of prey by Little penguins (*Eudyptula minor*) using accelerometers to measure flipper beat frequency. Data allowed the penguins’ underwater activity to be separated into pursuit dives and cruising dives. Pursuit dives showed increased acceleration and indicated a prey encounter (Roupert-Coudert et al. 2006). Analysis of such data provided the opportunity to infer predator-prey interactions and assess the foraging efficiency of individuals (Roupert-Coudert et al. 2006). In a study on a threatened species, Wilson et al. (2013) attached accelerometers to wild cheetah (*Acinonyx jubatus*) to record their acceleration during hunting events, consequently obtaining some of the highest measured values of acceleration for any terrestrial mammal. These data contributed to limited knowledge of wild cheetah locomotion dynamics, which could also be measured for other cursorial species using the same method (Wilson et al. 2013).

The value of accelerometers is evidenced by their application to a range of subjects and research questions. These devices have been used to collect diverse data on animal behaviours and activities, which have made important contributions to behavioural research. Hence, their continued use in this field is imperative to progress our understanding of their capabilities and provide new insights into their application.

Variables Measured

Variables measured were grouped into seven categories (Fig. 1). Physical activity was the most common variable (78 %) measured using accelerometers, followed by emotional behaviour (5 %), and both physical and emotional behaviour (5 %) (Fig. 1). The four other categories covered physical behaviour plus health, physiological, neurological or emotional behaviours each at 3 %.
Variables measured were grouped into seven different behavioural categories. 78% of studies (n=36) measured physical activity to infer aspects of behaviour. Accelerometers have been used to measure an array of different physical, emotional and physiological variables (Fig. 1). The most commonly measured variables are physical ones, including fine-scale swimming behaviour using the frequency and acceleration of tail or fin strokes in elasmobranchs (Carrier et al. 2012), hunting in puma based on stride frequency, length, and speed (Williams et al. 2014), eating based on beak movements of turtles (Okuyama et al. 2010), diving activity established using body orientation and decent speed in seals (Mitani et al. 2009) and gliding length and duration of Malayan colugos using horizontal velocity (Byrnes et al. 2011). Many studies stated that accelerometers were capable of recording both accurate and specific behavioural data (Shepard et al. 2008; MacKay 2013; Jeanniard-du-Dot et al. 2016). Laich et al. (2008) demonstrated this in a study on Imperial cormorants (Phalacrocorax atriceps). Authors used tri-axial accelerometers to measure surge, sway and heave orientations, plus pitch angle of cormorants’ bodies. Using these data, they were able to identify when cormorants were standing, sitting, flying, diving and walking. A schematic flow-diagram was then able to be developed to assist in differentiating between each behaviour. Characterising individual behaviours in such a way can assist in future research to quantify the energetic costs of these activities, which has important implications for fitness and survival (Laich et al. 2008; Brownscombe et al. 2014).

Emotional behaviours were less commonly studied using these tools and were typically inferred from both physical and physiological measures. For example, MacKay et al. (2014) used tri-axial accelerometers on dairy cows to record home pen behaviour, including lying, standing and locomotion, over several weeks. This behaviour was then used to predict responses of the same individuals to a novel object test to assess fearfulness (MacKay et al. 2014). Results showed a significant association between ‘neophobia’ (fear of novelty) and home pen behaviour (MacKay et al. 2014). This has management implications in terms of identifying fearful individuals and modifying handling and treatment of those individuals to improve productivity and breeding success (MacKay et al. 2014).

Although many studies maintain that accelerometers can record accurate behavioural data, there is some debate as to the accuracy and level of activity the devices are capable of measuring. Williams et al. (2015) found that accelerometry alone was not capable of distinguishing between different flight
behaviours of vultures (*Vultur gryphus* and *Gyps fulvus*), and that additional sensors such as magnetometers which showed changes in altitude and heading, were required to categorise the dominant flight types of these birds. Wang et al. (2015) concluded that non-locomotive behaviours of puma (*Puma concolor*), such as feeding, could only be accurately identified with additional methods such as visual observation. This allowed identification of the accelerometer data sequences that corresponded with feeding events; in turn, these sequences could then be used to classify future events (Wang et al. 2015). Other studies have also expressed that non-locomotor activities such as sleeping, feeding and grooming have been difficult to identify based on accelerometry alone (Sellers & Crompton 2004; Gervasi et al. 2006; Laich et al. 2008). A lack of sensitivity to detect these activities may be due to methodological flaws including small sample sizes of only a few individuals (Sellers & Crompton 2004; Gervasi et al. 2006), inadequate number of accelerometers deployed per animal (Gervasi et al. 2006) and the method of data analysis selected (Laich et al. 2008).

Ultimately, the accuracy of the data and inferences made from the data obtained from accelerometers depends upon the variables measured, species studied, whether subjects are in captivity or free-living, the number and type of accelerometers attached to the animal, device placement and whether other complementary methods have been employed.

Species Studied

Of the 34 species studied using accelerometers, (listed in Supplementary material) most were mammals (58 %), followed by birds (33 %), fish (6 %) and reptiles (3 %) (Fig. 2).

![Figure 2](image_url) Percentage of species across all studies grouped by taxon (n=36).

This bias towards mammals may be attributed to the fact that birds and fish may be harder to locate, capture and fit with accelerometers (Carrier et al. 2012; López-López 2016), and that reptile behaviour with the exception of turtles, is more commonly assessed using physiological parameters such as thermoregulation and heart rate (Cooke et al. 2004). Species such as rats and mice are a conventional choice as they are easily bred and studied in controlled conditions (Venkatraman et al. 2010), and production animals are likely prominent due to their high economic value (MacKay 2013).
The majority of studies (53% of the 36 studies) were carried out on captive animals - which included domestic animals, wild animals captured and held and laboratory animals (Fig. 3.). A total of 31% were carried out on wild or free-ranging species while 17% of studies included both wild and captive animals (Fig. 3.).

Figure 3. Percentages of wild, free-ranging animals, captive animals, or both wild and captive animals in accelerometer studies (n=36).

There are numerous benefits to studying animals in captivity, including ease of observation (Gervasi et al. 2006; Ismail et al. 2012; Mosser et al. 2014), ability to fit and then retrieve accelerometers from study subjects (Okuyama et al. 2010; Carrier et al. 2012), the presence of a controlled environment (Venkatraman et al. 2010), and the ability to rectify issues such as device displacement or faults (Mann et al. 2005; Ismail et al. 2012).

Methods of Use

Attachment

External attachment of accelerometers was the most commonly utilised method in studies reviewed. This was achieved via harness or collar (Gervasi et al. 2006; Martiskainen et al. 2009; Moreau et al. 2009; Liechti et al. 2013; Wilson et al. 2013; Duriez et al. 2014; González et al. 2015; Wang et al. 2015); epoxy directly to skin, quills, or fur (Mitani et al. 2009; Okuyama et al. 2010; Jeanniard-du-Dot et al. 2016); sutures (Browncombe et al. 2013; Noda et al. 2014); Tesa tape (Ropert-Coudert et al. 2006; Laich et al. 2008); leg straps (MacKay 2013; MacKay et al. 2014); and glue (Byrnes et al. 2011; Ismail et al. 2012). Numerous studies specified that orientation, position and fit of the accelerometer were vital to obtaining accurate data (Martiskainen et al. 2009; Gleiss et al. 2011; Carrier et al. 2012). Carrier et al. (2012) stated that the devices axes should be positioned on the animal’s body latitudinally and longitudinally to ensure data recorded was reflective of the animal’s posture and movement (Carrier et al. 2012). For example, Whitney et al. (2010) successfully captured mating events using tri-axial accelerometers in nurse sharks (Ginglymostoma cirratum). The accelerometers were attached to the base of the second dorsal fin and secured at both ends to ensure the device was
longitudinally aligned with the sharks’ bodies (Whitney et al. 2010; Carrier et al. 2012). Sharks display unique body movements during mating and these were detected from all three axes of the accelerometers. Mating behaviour was identified by changes in body roll or pitch of 30 degrees or more, with erratic tailbeats and periods of quiescence (Whitney et al. 2010). The body movements recorded allowed identification and interpretation of 26 individual mating events which were differentiated from other typical behaviours exhibited during the study period (Whitney et al. 2010).

Accelerometer attachment was a topic of conflict across studies, with some authors suggesting that the devices affected the behaviour of study subjects (Rushen et al. 2012; MacKay 2013), and others claiming the contrary (Mann et al. 2005; Robert et al. 2009). Wilson & Vandenabeele (2012) state that device mass and size can be problematic for seabirds, and that unacceptable physical and behavioural effects caused by devices act as a limitation to their use (Wilson & Vandenabeele 2012). Alternatively, Robert et al. (2009) found that accelerometers provided an effective and non-invasive way of measuring activity of cattle.

Adverse effects of accelerometers can be reduced by ensuring the weight of the device is no greater than 3-5 % of the body mass of the animal (Williams et al. 2015; López-López 2016). The choice of attachment method should suit the animal’s body shape, covering, and size, as well as its surrounding environment (Williams et al. 2016). Another important consideration is placement on the animal’s body, as are the fit of the device and how it affects movement (Ismail et al. 2012; González et al. 2015). If a device is not fitted correctly to the animal it can compromise their ability to perform natural behaviours, which can be detrimental, particularly to free-living animals (Wilson & McMahon 2006; MacKay 2013). An ill-fitted device may also affect the accuracy of the data being collected (Carrier et al. 2012). McKay (2013) recommended a 48-hour adjustment period for cattle following attachment of accelerometers so as to allow them to acclimatise to wearing the device (MacKay 2013). This is also likely to be beneficial for other species, as it provides the opportunity to observe behaviour post-attachment and make an assessment of the effects of the device on study subjects (Mann et al. 2005; Sikes & Gannon 2011; Ismail et al. 2012; Noda et al. 2014). Above all, it is vital that ethical standards are followed and precautions taken to reduce risk of injury and minimise the impacts to individuals fitted with these devices (Wilmers et al. 2015).

A gap in knowledge occurs in the general absence of detail on how devices were fitted and which standards were followed so as to ensure that the most appropriate technique was selected. Williams et al. (2016) refer to a series of taxon-specific guidelines for use of bio-loggers prepared by different societies. However, on inspection the guidelines discuss general methods of attachment and impacts of radio-telemetry devices, with only one mentioning bio-logging devices and none covering specific techniques for appropriately fitting the devices (Gaunt et al. 1997; Care & Committee 2004; Nickum et al. 2004; Sikes & Gannon 2011). Such guidelines have been produced to reduce the negative impacts of these devices on animals but they lack the detail and currency required to ensure the devices are fitted correctly. The best source of information for any particular species undoubtedly lies with specific researchers who have relevant experience in this area (Care & Committee 2004; Hawkins 2004; Wilson & McMahon 2006). It would be valuable to draw from this experience to establish protocols for fitting accelerometers for each taxon and device configuration which are widely accepted and implemented in behavioural research (Wilson & McMahon 2006; Ropert-Coudert et al. 2012). By doing so, device impacts may be reduced and data accuracy can be enhanced.
Additional Methods and Sensor Types

Many of the studies utilised additional sensor types or methods to supplement accelerometer data (Fig. 4.). The most popular complementary method was visual observation (19 %) followed by GPS (14 %) and video cameras (11 %) (Fig. 4). Most studies utilised several methods or deployed multi-functional sensors; consequently, each study may be represented in more than one category.

Figure 4. Additional sensors or methods used to collect data in conjunction with accelerometers across studies (n=35). One study did not use any additional methods or sensors.

Visual observation and remote cameras allow researchers to observe animals engaging in different behaviours, and many authors used these methods to verify and interpret their accelerometer data (Gervasi et al. 2006; Okuyama et al. 2010; Ismail et al. 2012; Brownscombe et al. 2014; Wang et al. 2015). Visual observations or video recordings during study periods also allowed researchers to take into account external stimuli such as noise, light, temperature, and presence of conspecifics or predators in the environment, all of which can influence level of movement and activity of study subjects (Shepard et al. 2008).

Other devices or sensors used in addition to accelerometers included Global Positioning Systems (GPS), radio-transmitters, temperature and depth gauges and gyroscopes (Okuyama et al. 2010; Brown et al. 2012; Bouten et al. 2013). In some instances accelerometers and GPSs were incorporated into multi-functional devices that recorded several variables simultaneously, allowing vast amounts of data to be collected over a short timeframe (Bouten et al. 2013; Wilson et al. 2013). For instance, Mosser et al. (2014) deployed GPS and accelerometer devices on 131 free-ranging caribou to assess the variation of individual activity levels associated with environmental parameters. In summer, levels of vegetation abundance were higher and caribou were less active. In winter when vegetation abundance was lower foraging activity increased (Mosser et al. 2014). There was also a positive relationship between activity level and snow depth as caribou foraged in the snow for food and used more effort travelling through it (Mosser et al. 2014). Results showed that activity in free-living caribou varied significantly with spatial and temporal variation in environmental conditions (Mosser et al. 2014). This data can be used to infer impacts of habitat loss, climate change and anthropogenic disturbances on wildlife (Gervasi et al. 2006; Brown et al. 2012; Mosser et al. 2014).
Used in combination, visual observation, video recordings, GPSs and accelerometers provide quantitative, qualitative and spatial data that can inform on important aspects of animal behaviour, dispersal patterns, habitat preference and use, and energy expenditure (Brown et al. 2013; Wilson et al. 2013; Brownscombe et al. 2014; MacKay et al. 2014; Mosser et al. 2014).

New Approaches

In order to surmount potential impacts from accelerometers on animals’ natural behaviour, accelerometers could be used remotely (i.e. without being attached to the study subject). This method would be appropriate for captive animals that are confined to a particular area such as laboratory or zoo animals, or wild animals held in cages. Provided the cage was immobile, an accelerometer attached to one of its surfaces may be able to record movements of the animal inside it. Such data could provide information on how animals cope with being confined, how they behave prior to, or after handling, or after being transported to a new location. This is potentially a less invasive option that could mitigate the effects of attached devices and may also address the restriction on device weight and size (Williams et al. 2015). Without this restriction, battery size can be increased allowing for longer recording periods and improved storage capability (Cooke et al. 2004; Ropert-Coudert et al. 2006; Rushen et al. 2012; Liechti et al. 2013).

New tools

Smartphones are sold with inertial measurement units (IMU) inside them which contain 3D gyroscopes and tri-axial accelerometers. In a novel approach, Andriamandroso et al. (2014) attached one iPhone and one iPod to two dairy cows’ necks and recorded their activity while on pasture and in a stable. The authors incorporated video recordings for validation of data from the IMU’s and found that the IMU signals were capable of accurately detecting the behaviours of grazing and rumination (Andriamandroso et al. 2014).

Further investigation into the capabilities of in-built, tri-axial accelerometers in smartphones is required. Most smartphones have an in-built accelerometer which re-orient the phones screen to match the phones orientation (MacKay 2013). Given that accelerometers are so readily available in smartphones, they may be a cheaper alternative to more expensive, purpose-built accelerometers (Wilson et al. 2015). Smartphones can also be used in conjunction with accelerometer applications; these allow data to be easily downloaded to a computer for analysis and storage (Andriamandroso et al. 2014; González et al. 2015). Reducing costs and improving accessibility by using smartphone accelerometers may also allow researchers to increase samples sizes; a limitation identified in several previous studies (Ropert-Coudert et al. 2006; Jansen et al. 2009; Duriez et al. 2014). Logistically, smartphones would be challenging to attach to smaller animals, so their use may be restricted to larger animals or in circumstances whereby attachment can be avoided.

New Applications of Accelerometry

Despite an increasing number of commercially available accelerometers and their vast application potential in animal behavioural research, there are limited examples of their application to conservation (Wilson et al. 2015). There are many opportunities to utilise accelerometers for research on threatened species to monitor activity, health and emotional state of individuals, particularly during conservation practices such as translocations or captive breeding (Ismail et al. 2012). Physical activity can be used to infer emotional reactivity of individuals (MacKay et al. 2014). Hence, this technique may be applicable to threatened species to quantify stress or agitation during the translocation process.
or captive breeding. In turn, this presents an opportunity for researchers and keepers to identify and mitigate stressors and may also provide an indication of how individuals will cope once released. Improving ethics and welfare during these practices may increase breeding or translocation success, thereby improving management outcomes (Teixeira et al. 2007; Hing et al. 2014).

Accelerometry data can be used in conjunction with other physiological measures such as heart or respiration rate, temperature, or faecal glucocorticoid concentration (Cooke et al. 2004; Payne et al. 2014; Fanson et al. 2015; Wilson et al. 2015). For example, Jansen et al. (2009) used accelerometry and heart rate monitors on horses to measure their emotional response to novel objects. Heart rate was decomposed into physical and emotional parameters, and an emotional response was detectable in 33 of 40 trials. This type of analysis may assist in quantifying how a horse is coping with the emotional stress of a race, or training exercises (Jansen et al. 2009). Similarly, the combination of heart rate measures with accelerometer data may be a valid way to monitor emotional stress in threatened species.

Combining accelerometry with other techniques can produce an informative dataset of physical and physiological measures, providing a comprehensive representation of an individual’s behaviour. Such data allow for an integrative approach to understanding how physiology influences behaviour, performance, and fitness and can improve our understanding of the biology and behaviour of species of conservation concern (Evans et al. 2013; Williams et al. 2016).

Conclusion

Technological advances in accelerometry have led to a surge in their application in animal behavioural research in recent years (Williams et al. 2016). Accelerometers have applicability across many disciplines and have been used effectively to study a range of captive and free-living species. Contrasting views on device accuracy and attachment affecting behaviour may be addressed through improved methodology and the creation of specific protocols for fitting devices appropriately. A new approach involving the use of in-built smartphone accelerometers for recording behaviour may be a cheaper alternative to current options which also addresses the limitations associated with battery life and storage capability. Accelerometry in conservation research has potential for assessing the health, welfare and behavioural responses of threatened species to management actions. Extended application in this field may contribute to improving conservation outcomes. Accelerometers have already improved our knowledge of animal behaviour. However, there is still scope for their wider application if they can be utilised in novel ways by modifying current methodologies and capitalising on more accessible and economic options. Delving further into accelerometer technology increases our ability to collect an accurate and extensive dataset, gain further knowledge on animal behaviour, and realise the full potential of these informative devices.
Literature cited


