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Wildlife in the line of fire: evaluating the stress physiology of a critically endangered Australian marsupial after bushfire

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Abstract

Australian native fauna are thought to be well-adapted to fire-prone landscapes, but bushfires may still pose considerable challenges or stressors to wildlife. We investigated the impact of bushfire on the stress physiology of the woylie (brush-tailed bettong, *Bettongia penicillata*) a critically endangered Australian marsupial, and assessed whether fitness indices (body condition and parasite load) influenced stress physiology before and after the fire. We hypothesised that there would be a significant change in stress physiology indicators (in the form of faecal cortisol metabolites; FCM) following the fire, compared to the months previous. We trapped woylies (n=19) at Whiteman Park Reserve in Perth, Western Australia two days after a major bushfire and measured FCM concentration by enzyme immunoassay. Population level comparisons of FCM were made between these samples and those collected in previous months (n=58). While mean FCM varied by month of sample collection, it was not higher after the fire. We suggest that woylies may be able to maintain homeostasis through change (allostasis), at least in the period immediately after the fire. This is supported by our finding that FCM did not relate significantly to body condition or parasite load. Our results potentially highlight the physiological and behavioural adaptations of woylies to fire which could be further explored in future studies.

Introduction

Bushfires are a globally significant abiotic factor influencing wildlife populations (Payne *et al.*, 2014) and are ubiquitous in the Australian landscape where they have influenced the evolutionary history of native fauna and flora (Doherty *et al.*, 2015). In addition to direct mortality, fire may entail several proximate stressors including extreme heat and smoke, the necessity to flee (Christensen, 1980), changes to food availability, diet quality and composition (Vernes, Castellano & Johnson, 2001), disruption of social networks (Banks *et al.*, 2012), destruction of shelter, and an influx of predators (Torre & Díaz, 2004). Stressors such as these may increase activation of the hypothalamic pituitary adrenal (HPA) axis (Moberg & Mench, 2000). The stress response to fire may vary among taxa, species or populations (Santos *et al.*, 2014). In humans, encountering a single fire emergency can result in a considerable stress response (Proulx, 1993) and post-traumatic stress disorder with related neuropsychological and physiological changes (Chen *et al.*, 2006). Adult female African elephants (*Loxodonta africana*) have also been found to have higher faecal glucocorticoid metabolites (downstream end-products of HPA axis activation) after a major fire event compared to three months before the fire occurred (Woolley *et al.*, 2008). However, there are no studies evaluating the physiological stress response of Australian wildlife to bushfire.

As unexpected and severe stressors can cause immunosuppression and exacerbate infectious disease (Biondi & Zannino, 1997), it is important to understand the physiological stress response of wildlife to fire and the potential ramifications for their health. This is especially relevant for small populations of endangered species that are vulnerable to stochastic events. They are particularly vulnerable when confined to limited areas (Kaplan Smith, 2000; Legge *et al.*, 2008) where the impact of fire is predicted to intensify with climate change (Lunney, Lunney & Recher, 2008).

Woylies (syn. brush-tailed bettong, *Bettongia penicillata*) are a critically endangered native Australian marsupial that have undergone a dramatic and continuing population decline since 2002 (Wayne *et al.*, 2013; Yeatman *et al.*, 2016). Remnant native populations are now only found in the south-west of Western Australia, and insurance populations are housed in several predator-proof reserves (Wayne *et al.*, 2013). While Australian marsupials are thought to be well adapted to fire-prone landscapes, woylies have been observed after fires to demonstrate behavioural responses consistent with shock such as standing still and staring into space (Christensen, 1980). Intense and large-scale fires have also had devastating effects on populations of marsupials including quokka (*Setonix brachysurus*) (Bain *et al.*, 2016a; Bain *et al.*, 2016b) and western ringtail possums (*Pseudocheirus occidentalis*) in south-west Western Australia, common ringtail possums (*Pseudocheirus peregrinus*) in New South Wales (Russell, Smith & Augee, 2003) and koalas (*Phascolarctos cinereus*) in south-west Victoria (Wallis, 2013). Since remaining populations of woylies are small and isolated, and thus vulnerable to stochastic events within a fire-prone landscape (Bryant, 2008), it is pertinent to investigate their response to fire. In addition, it has been suggested that, among other potential drivers, stress-related changes in immune function and exacerbation of infectious disease may play a role in the woylie's decline (Botero *et al.*, 2013; Hing *et al.*, 2016). Hence, understanding the impacts of fire on woylie stress physiology and population health will aid future conservation management.

An insurance population of woylies were faced by a bushfire in December 2014. The fire ignited on December 14th, 2014 in Whiteman Park and quickly entered its Woodland Park Reserve, a 200ha nature park surrounded by a predator-proof fence in Perth, Western Australia. The high intensity ground and canopy fire burned out-of-control for up to thirty six hours, destroying undergrowth vegetation including woylie nest sites. Due to concerns that the fauna within the enclosure would be trapped, emergency gates were opened allowing escape from the fire. By the time the fire was extinguished on December 16th 2014, it had burned approximately 90% of the total area of the reserve (Figure 1).

To investigate the stress hormone response of woylies to the fire, we measured faecal cortisol metabolites (FCM) before and immediately after the event. Faecal glucocorticoid metabolites (of either cortisol or corticosterone) are commonly used as stress physiology metrics in wildlife and can be measured using minimally invasive methods (Keay *et al.*, 2006). We hypothesised that there would be a significant increase in FCM immediately following the fire compared to the months preceding the fire. Recommendations have been made to assess fitness indices (body condition and parasite load) in the context of bushfires (Sutherland & Dickman, 1999). Thus, we also assessed the relationship between the selected fitness indices and stress physiology. We predicted a population-level increase in FCM after the fire particularly if body condition was low and parasite load was high.

Materials and methods

Trapping and sample collection

We studied a population of woylies at Whiteman Park Woodland Reserve in Perth, Western Australia. Woylies were trapped in June 2014 (n=35) and October 2014 (n=23) as part of an existing study. On December 16th 2014, two days after the bushfire had started and after it had been extinguished, we trapped 19 woylies during the emergency post-fire monitoring response. Galvanized wire Sheffield traps (220 x 220 x 550mm, Sheffield Wire Products, Western Australia), baited with whole peanuts, were set and checked during the evening (maximum total duration in the trap was not more than two hours). Woylies were individually identified by unique ear tag and microchip code. Animals were weighed, females were checked for pouch young (pouch status), and the size of pouch young (mm) was estimated by palpation of the pouch.

Faecal cortisol metabolite (FCM) enzyme immunoassay (EIA)

Faecal samples were collected from newspaper laid underneath the trap, and (sample volume permitting) two grams was preserved in 10 mL of formalin for faecal flotation, with the remainder stored frozen at -20 °C for FCM assays. All assays could not be completed for every sample (e.g., due to insufficient sample volume), but a total of 77 faecal samples were assayed for FCM, of which 51 were also analysed for parasites.

FCM concentration was measured using an enzyme immunoassay (EIA) previously used for woylies (Hing *et al.*, 2016). In summary, faecal samples (0.2 g dry weight) were lyophilised (freeze-dried), and extraction was carried out using 90% ethanol and heat treatment (80 °C for 10 min). Extracts were assayed for FCM by EIA using a polyclonal anti-cortisol antiserum R4866 protocol (Narayan *et al.*, 2012; Hing *et al.*, 2016). Results were expressed as FCM concentration (pg/g) on a dry weight basis.

Faecal flotation for parasite egg counts

To detect gastrointestinal parasites (nematodes and protozoans), one gram wet weight of faeces was floated for 10 minutes using a concentrated sodium nitrate ($NaNO_3$) solution with centrifugation, after washing (Dryden *et al.*, 2005). The area under the coverslip was observed systematically under a BX51 microscope (Olympus, Japan) at 10x objective and eggs were classified as oxyurid, strongyle or *Strongyloides*-like nematodes (as these were the three major groups observed). Data was recorded as total egg count per gram wet weight per individual woylie.

Statistical analyses

We used linear mixed effect models to make population level comparisons of FCM, comparing a total of 58 samples before the fire to a total of 19 immediately after the fire. Fitness indices (body condition and parasite load) were also included to investigate how a potential stress response to fire may relate to these variables. To fulfil model assumptions of data conforming to a normal distribution, FCM (the dependent variable) was log-transformed.

Fixed effects included in our model were: month (June/October/December), sex (male/female), body condition index, and oxyurid, strongyle and *Strongyloides*-like egg counts. All two-way interactions between these effects were also included. We checked for collinearity between covariates and all Variance Inflation Factors (VIF) were below 2.5. Body condition index was derived from the residuals of a regression of hindfoot (pes) length to weight, calculated separately for males (p=0.06, co-efficient=18.15, R^2=0.10) and females (p<0.0001, co-efficient=17.55, R^2=0.37). In the calculation of body condition index, we adjusted for pouch young size in females by including it as a covariate. Woylie ID was included as a random effect in all models to account for repeated measures from the same individuals.

To determine the minimal adequate models, we undertook model simplification by stepwise reduction, removing non-significant terms from the maximal model until further model reductions resulted in significant changes in model deviances (Crawley, 2007). Significance ($p \leq 0.05$) was tested in a likelihood ratio test (χ^2). Models were run using R 3.1.0 with the packages 'lme4' (Bates *et al.*, 2015) and 'car' (Fox & Weisberg, 2011).

Results

Month was the only fixed effect remaining in the minimum model. The month of collection had a significant effect on FCM (co-efficient = -0.40, SE=0.25, df=1, χ^2=6.93. *P*=0.03).

However, mean FCM was not higher two days after the fire compared to the preceding months of October and June (Figure 2). Mean FCM concentration before the fire was 6.58±1.17 pg/g in June and 14.60±2.78 pg/g in October. After the fire, the mean FCM concentration was 9.75±1.95 pg/g. There were no significant interactions between the fixed effects considered. Differences in FCM did not relate significantly to sex or body condition (P>0.05).

Strongyloides-like eggs most commonly detected (41/52). Strongyle eggs were also detected commonly (33/52) but oxyurid eggs were only detected in a small number of faecal samples (8/52). For individual woylies, egg counts per gram (epg) of faeces followed a similar pattern with a mean *Strongyloides*-like egg count of 5.7 epg. Mean strongyle egg count was 2.46 epg and mean oxyurid egg count was low at 0.6 epg. However, we did not find evidence for the predicted population-level increase in FCM after the fire nor a relationship between FCM and parasite load.

Discussion

An acute stress response to fire was predicted given that the woylies would have experienced significant stressors during the bushfire, including extreme temperatures, smoke, loss of habitat, and possibly increased exposure to predators. However, we did not find a temporal association between the fire and an increase in FCM. Given the severity of the fire, within two days of the event woylies may have been stressed to a point of allostatic overload, that is, a state where the HPA axis can no longer maintain homeostasis through change (allostasis) (McEwen, 2005). Allostatic overload is associated with HPA axis dysregulation resulting in either hypo- or hyper-activity of the HPA axis in response to stressors (Dickens, Delehanty & Romero, 2010). However, the absence of peak FCM after the fire is less likely to indicate allostatic overload and HPA axis dysfunction in woylies after fire. We found no significant relationship between FCM and individual fitness indices (body condition and parasite load) that may be aberrant in allostatic overload. For example, a permanent reduction in body weight was observed in rats exposed to an experimental stressor (forced restraint) for three days (Harris *et al.*, 1998). The woylies were also examined by veterinarians after the fire and found to be in good general health (S. Hing and K. Jones, personal observation, December 16th, 2014).

In this study, the lack of apparent acute effects of fire on FCM and fitness indices is more likely to be associated with adaptations of woylies to fire-prone landscapes (Christensen, 1980). Studies in woylies (Christensen, 1980) and northern bettongs (*B. tropica*), a closely related species, have suggested that bettongs are flexible in their response to fire events (Vernes *et al.*, 2001). Woylies in our study displayed previously identified behavioural responses to fire including seeking out unburnt refugia (K. Jones, observation, December 16th 2014; Christensen, 1980). Bettongs are also known to increase foraging activity for a short period immediately following a fire due to increased productivity of fire-attenuated species of mycorrhizal fungi, a major food source (Johnson, 1995; Vernes, Johnson & Castellano, 2004). Johnson (1995) noted that fresh *B. gaimardi* diggings appeared as early as two days after an experimental fire, suggesting that bettongs commence modified foraging behaviour to take advantage of an available resources “almost as soon as the fires had gone out”. These behaviours may have helped woylies respond to altered conditions after the fire.

In addition, efforts by reserve staff to manage woylies in the period immediately following the fire may have prevented stress from reaching levels where it may compromise their health. Woylies were provided with supplementary feed of herbivore pellets, fruits, hay and vegetables immediately after the fire was extinguished. Invasive predator control was also instigated almost immediately after the fire to minimise the possible impact of predation by cats and foxes on surviving woylies, due to the gates having been opened during the fire (G. Deegan, *pers.comm.*). Indeed, the potential impact of fire may be greater on remnant wild woylie populations exposed to the ongoing threat of feral predators because native Australian fauna facing multiple potential stressors are likely to be more vulnerable to population decline (Narayan, 2015; Narayan & Williams, 2016).

Another possible explanation for an absence of peak FCM after the fire is that a single session of opportunistic sampling was insufficient to capture an acute stress response. This explanation may be less likely given the severity of the fire and the immediacy of the sampling afterwards. However, delays of up to three days between the adminstration of exogenous adrenocorticotropic hormone (ACTH) and a peak in faecal glucocorticoid metabolite concentration have been noted in other marsupial species (Narayan, Evans & Hero, 2014). In woylies, samples were collected three times a day for several days in a zoo enclosure in order to detect a peak within four days after ACTH administration (Fanson *et al.*, 2015). While such intense sampling is not feasible in free-ranging woylie populations, sampling woylies later that two days after fire may improve our ability to detect a bush-fire response if it is present. There are also limitations to interpreting ‘snapshot’ measures of FCM in a small sample size because various factors can influence FCM including season (Hing et al., 2016). A final alternate explanation is that the animals sampled may not have been those individuals most severely affected by the fire. However, this is less likely considering the pervasiveness of the heat, smoke and influx of vehicles and firefighters (K. Jones, observation, December 16th 2014).

Our study was not able to gauge chronic effects of the fire on woylies and we would recommend long-term FCM monitoring when any trap work is being undertaken so that baseline patterns can be established to enable more effective interpretation of deviations following a disturbance. A closely related species, the Tasmanian bettong (*B.gaimardi*), has been found to return to pre-fire behavioural activity by four months after fire (Johnson, 1995) and other marsupials such as koalas are also known to recover rapidly after fire (Matthews *et al.*, 2016).

Bushfires are an ever present concern to wildlife researchers and managers in Australia, and managing fire is an important consideration in the conservation of woylies and their habitat (Taylor, 1991). However, we found little evidence to suggest stress-related changes in the physiology of an insurance population of woylies two days after a major bushfire in their reserve. Our results suggest that woylies may be able to maintain allostasis, at least in the period immediately after a fire, provided that they display the appropriate behavioural responses and are well protected from concurrent stressors during this time.

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Figures

Figure 1. Photographs showing **a)** unburnt and **b)** burnt areas of Whiteman Park Woodland Reserve, Perth, Western Australia taken on December 16th, 2014.

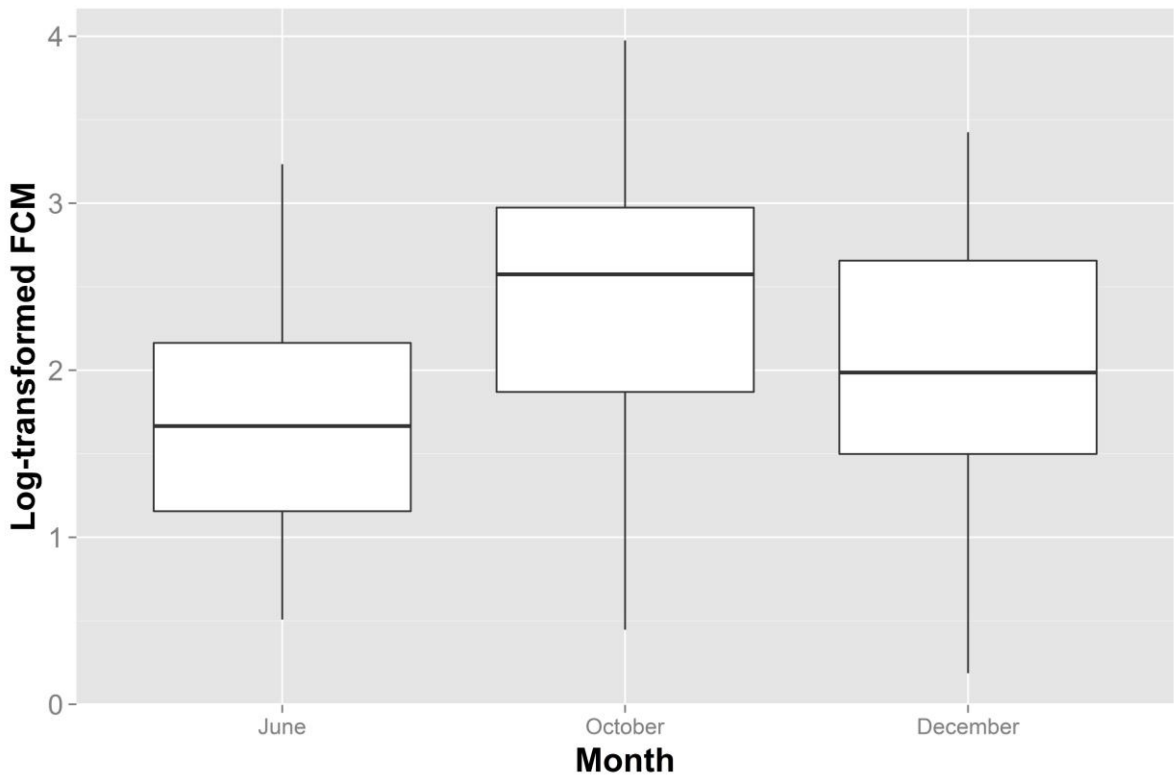

Figure 2. Log-transformed faecal cortisol metabolite (FCM) concentration by month. The box marks the lower (25%) and upper (75%) quartiles, with vertical lines indicating total range of values. The bar in the middle of the box represents the median.

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