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# Physiological stress in rescued wild koalas (*Phascolarctos cinereus*) being held in a rehabilitation sanctuary: a pilot study

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**Simple Summary:** Koalas (*Phascolarctos cinereus*) are one of Australia's most charismatic native small marsupial species, however they continue to face increasing pressure from a changing ecosystem. Negative stimulants in the environment can elicit stress responses through activation of the hypothalamic-pituitary-adrenal (HPA) axis. This response can be either acute or chronic and is shown through the activation of the neuroendocrine stress system and the release of glucocorticoids (e.g., cortisol). Wild koalas entering clinical care face novel physical and psychological stressors that can be out of a wildlife carer's control. In this pilot study, we monitored physiological stress in wild koalas at a wildlife rehabilitation centre in NSW, Australia. Acute and chronic stress was indexed non-invasively, with faecal samples taken to evaluate acute stress, and fur samples taken to evaluate chronic stress. Results attempt to understand the stress response of koalas to negative stimulants in the environment, and to our knowledge, this is the first evidence of stress tracking of wild rescued koalas in a sanctuary. Further monitoring of baseline, acute and chronic stress will be needed to better understand how koalas respond to negative stimulants associated with clinical care.

**Abstract:** Koalas (*Phascolarctos cinereus*) are one of Australia's most charismatic native small marsupial species. Unfortunately, populations of koalas are rapidly declining throughout Australia and they continue to face increasing pressure from a changing ecosystem. Negative stimulants in the environment can elicit stress responses through activation of the hypothalamic-pituitary-adrenal (HPA) axis. Depending on the duration of the negative stimulant, the stress response can lead to either acute or chronic side effects, and is shown through the activation of the neuroendocrine stress system and the release of glucocorticoids (e.g., cortisol). Wild koalas entering clinical care face novel stressors that can be out of a wildlife carer's control. In this pilot study, we monitored physiological stress in three wild koalas at a wildlife rehabilitation centre in New South Wales, Australia. Acute and chronic stress was indexed non-invasively, with faecal samples taken to evaluate acute stress, and fur samples taken to evaluate chronic stress. Sampling occurred sporadically over four months, from the start of September 2018 to the end of December 2018. Results attempt to understand the stress response of koalas to negative stimulants in the environment by comparing faecal glucocorticoids on days where a known stressor was recorded with days where no known stressor was recorded. Furthermore, variations in faecal and fur glucocorticoids were compared between the three koalas in this study. To our knowledge, this is the first evidence of stress tracking of wild rescued koalas in a sanctuary. We suggest that further monitoring of baseline, acute and chronic stress will be needed to better understand how koalas respond to negative stimulants associated with clinical care.

**Keywords:** Faeces, Glucocorticoids; Hair; HPA-axis; Koalas; Rehabilitation Sanctuary; Stress.

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## Introduction

The koala (*Phascolarctos cinereus*) is one of the most charismatic native small marsupial species iconic to Australian identity (Hundloe et al., 1997). Unfortunately, populations of koalas are rapidly declining throughout Australia, particularly in Queensland and New South Wales (Charalambous & Narayan, 2020; Gonzalez-Astudillo et al., 2017). Threats faced by koalas are varied, but predominantly include trauma from vehicle collision, being attacked by animals (e.g., cats, dogs, cattle), and succumbing to disease (Charalambous & Narayan, 2020; Gonzalez-Astudillo et al., 2017). A previous study of wild koalas admitted into clinical care in New South Wales indicate that between 1989 and 2018, 9.7% of koalas were struck by a motor vehicle, 4.4% sustained an injury from another animal, and 34.4% were diagnosed with a disease (Charalambous & Narayan, 2020). Similarly, in Queensland between 1997 and 2013, 15.5% of koalas were struck by a motor vehicle, 5.2% sustained injury from another animal, and 55.6% were diagnosed with a disease (Gonzalez-Astudillo et al., 2017). Of those injured koalas in New South Wales, 20.7% were released back into the wild (Charalambous & Narayan, 2020), whereas 17.2% of injured koalas were released back into the wild in Queensland (Gonzalez-Astudillo et al., 2017).

Stress is described as an unpredictable and/or uncontrollable stimulus which elicits a physiological response (Beehner & Bergman, 2017). This response starts with activation of the hypothalamus-pituitary adrenal (HPA) axis which signals the hypothalamus to synthesise corticotrophin-releasing factor (CRF) neuropeptides to stimulate the pituitary gland to release adrenocorticotropic hormone (ACTH) (Denver, 2009). The result of such is a secretion of glucocorticoids which aid in the production of sugars aimed at providing energy to either fight or flee from said stressor (Denver, 2009). Once the stimulus causing stress has ceased, the process of homeostasis acts to return the body to a pre-stress state optimal to maintain life, and the animal is said to have experienced an acute stressor (Romero et al., 2009). In the event that the stressor does not cease and the body can no longer maintain homeostasis, the animal is said to be experiencing chronic stress (O'Connor et al., 2000). Stimuli causing stress can stem from external factors (e.g., a loud noise) or internal factors (e.g., dehydration), meaning a single event can impact individuals differently (Selye, 1955).

It is well documented that chronic stress can have deleterious effects on physiological health and often leads to a greater susceptibility to disease (Sapolsky et al., 2000). This is because stress is adaptive for an animal over the short term as present energy use is prioritised over future energy storage (Wingfield & Sapolsky, 2003). During activation of the HPA-axis and the production of glucocorticoids, the function of immunological processes are altered, with changes immune gene expression on target tissues, having complex effects on both innate and acquired immunity (Hing et al., 2016). For example, glucocorticoids reduce the trafficking of leukocytes and accessory immune cells (cells which are responsible for fighting infection), as well as supressing the secretion of proinflammatory cytokines (regulators of inflammation as a response to infection to heal and repair) (Chrousos, 2009). The pathogenesis of chronic stress related disorders can be explained by sustained, excessive secretion and effects of the major mediators of stress and sickness syndromes, which influence the activities of multiple homeostatic systems (Chrousos, 1992; Karalis et al., 1991). These disorders thus represent chronic, maladaptive effects of two physiological processes whose mediators are meant to be secreted in a quantity-limited and time-limited fashion but have gone awry (Chrousos, 2009). Koalas exposed to chronic stress are at risk of immune cell related disorders including but not

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limited to, inflamed tissues, systemic infection, and organ dysfunction (Grogan et al., 2018). In New South Wales between 1989 and 2018, 34% of koalas admitted into clinical care were diagnosed with a disease (Charalambous & Narayan, 2020), whereas in Queensland between 1997 and 2013, 55% of koalas admitted into clinical care were diagnosed with a disease (Gonzalez-Astudillo et al., 2017).

Faeces are a popular biological sample used to obtain readings of glucocorticoids (Keay et al., 2006). The popularity of faecal sampling is due to the fact that it is almost a completely non-invasive procedure that is able to be performed by untrained personnel (Sheriff et al., 2011). Faeces are able to be collected fairly easily from animals within the field after careful observation of recent defecation (Narayan et al., 2012). The only concern however, is that unlike other biological samples such as fur, right after collection faeces need to be stored at -20˚C (Narayan & Vanderneut, 2019). Following collection and appropriate storage, a faecal glucocorticoid metabolite (FCM) enzyme-immunoassay can be used to index glucocorticoid hormones (Narayan & Vanderneut, 2019). A previous study discovered excretory lag-times of FCM between koala sexes with 24 hours for females, and 48 hours for males (Narayan et al., 2013). This is due to the species excessively long gut system, as well as natural fluctuations in reproductive hormones leading to increased metabolic demands (Cork, 1996; Touma & Palme, 2005).

Like faeces, fur too is a popular biological sample used to obtain readings of glucocorticoids (Burnard et al., 2017). Fur collection is also an almost completely non-invasive procedure, and can be collected from animals without capturing them, such as through the use of hair traps (Woods et al., 1999). Alternatively, fur can be shaved when an animal is undergoing routine medical checks, removing the need to add further stress through additional capture and handling (Charalambous & Narayan, 2019). Furthermore, fur is easy to store as it can be sealed in paper envelopes or aluminium foil, and kept at ambient temperature away from direct sunlight (Bortolotti et al., 2008; Macbeth et al., 2010). Following collection and appropriate storage, a cortisol based enzyme-immunoassay can be used to index glucocorticoid hormones (Charalambous & Narayan, 2019). Measurements of glucocorticoids in fur indicate an average cortisol concentration over a period of weeks to months, as the predictable rate of hair growth is ~1cm per month, and blood-borne hormones such as glucocorticoids are known to be incorporated into fur during the active growth phase (Burnard et al., 2017; Macbeth et al., 2010; Wennig, 2000).

The International Wildlife Rehabilitation Council defines wildlife rehabilitation as the "managed process whereby a displaced, sick, injured or orphaned wild animal regains the health and skills required to function normally and live self-sufficiently". However, of those rehabilitated individuals who have a chance to be released into the wild, there is a low chance of survival post admission into clinical care, (Molony et al., 2006). Referring to that earlier in New South Wales between 1989 and 2018, 20% of koalas admitted into clinical care were released into the wild (Charalambous & Narayan, 2020), whereas in Queensland between 1997 and 2013, only 17% of koalas admitted into clinical care were released into the wild (Gonzalez-Astudillo et al., 2017). Factors that could affect survival in those individuals who have been released into the wild include handling stress (Monnett, 2000), pre-release conditioning such as identifying food and predators (Suarez et al., 2001), and the suitability of the release location (Monnett, 2000). However, there are few studies that monitor post-release success in wildlife (Molony et al., 2006).

 doi:10.20944/preprints202108.0407.v1

The aim of this study is to understand the stress response of koalas to negative stimulants that are out of a wildlife carer's control through faecal and fur glucocorticoids. It is hypothesised that there will be a larger variation in faecal glucocorticoids from koalas on days where a known stressor was recorded, compared to days where no known stressor was recorded. It is also hypothesised that that the average of fur glucocorticoids between the three rescued koalas will not vary significantly.

## Methods

Research was performed in accordance with relevant guidelines and regulations. Formal approval was granted by the Western Sydney University Animal Care and Ethics (ACEC) Committee (approval number: A12373).

### *Study Site*

This research was performed in collaboration with Port Stephens Koalas, who are located at 562 Gan Gan Road, One Mile (GPS Coordinates: -32.763792, 152.115904). Koalas are frequently admitted into the care of Port Stephens Koalas as they operate a fully functional rehabilitation sanctuary for injured and orphaned koalas within the New South Wales region. Faecal samples were collected almost daily during routine cleaning procedures by staff from the enclosures of three koalas (Maree, Tai & Solstice) who stayed in the care of Port Stephens Koalas for a period of four months (from the start of September 2018 to the end of December 2018) undergoing rehabilitation for their injuries. Fur samples were collected opportunistically from the same three koalas during their time in clinical care. Once each sample was collected, it was stored in a labelled resealable bag (name of koala & date collected) and stored in a freezer (-18˚C) before being transported on ice to the laboratory for analysis. During delivery and analysis of the samples, the faeces were kept frozen to minimise effects of deterioration.

### *Glucocorticoid Extraction*

Once removed from the freezer, each sample was dehydrated in a freeze dryer until they were completely dry. Each sample was then individually ground into a fine powder using a mortar and pestle, which was cleaned between samples using 10% ethanol. Each sample was then sifted through a fine mesh strainer to remove any course particles. Two grams (g) of the ground and sifted sample was placed in a labelled test tube with 2 millilitres (mL) of 90% ethanol solution. On medium-high speed, the test tubes were vortexed in an Eppendorf Mini-spin centrifuge for 30 seconds in order to mix the solution, and then placed in an 80°C water bath for 10 minutes. While in the water bath, the test tubes were gently shaken to ensure the samples remained submerged in the ethanol. The contents of the test tubes were then poured into labelled Eppendorf tubes, closed, then centrifuged at 10,000 revolutions per minute (RPM) for 5 minutes. At this stage, the liquid residue should have separated from the hormones dissolved in the ethanol, and 0.6 mL of solution was aliquoted into a new and clean labelled Eppendorf tube. Left open, the tubes were stored in a laminar flow chamber for 24 hours, ensuring enough time for the ethanol to completely evaporate, then 1 mL of assay buffer was added. The tubes were vortexed at medium-high speed in an Eppendorf Mini-spin centrifuge for 30 seconds, and then centrifuged at 10,000 RPM for 10 minutes. Following this, 850 microlitres (µL) of supernatant was pipetted into a new and clean labelled Eppendorf tube, ensuring any of the

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solid section of the solution was avoided. Note: if the sample looked cloudy, tubes were re-centrifuged for 10 minutes and pipetted into a new and clean labelled Eppendorf tube.

*Hormone Analysis*

Glucocorticoid concentrations were determined using a polyclonal anti-cortisol antiserum diluted to 1:15,000, horseradish peroxidase (HRP) conjugated cortisol 1:80,000 and cortisol standards (1.56-400 pg well $^{-1}$). Sample extracts were then assayed in duplicate on Nunc Maxisorp™ plates (96 wells). Plates were coated with diluted cortisol antibody and left to stand and incubate for a minimum 12 hours in a fridge at 4°C. The plates were washed using an automated plate washer (ELx50, BioTek™). The dilution factor for the glucocorticoids in koala hair and fur samples were based on the concentration of pooled samples that resulted in 50% binding on the parallelism curve (as seen in (Narayan et al., 2013)). For each assay, 50 µL of cortisol standard, control and diluted faecal extract was added to each well-based on the plate map, immediately following 50 µL of HRP. Plates were covered and incubated at room temperature for 2 hours, following a wash and 50 µL of substrate buffer to generate a colour change. Colour reaction was halted after 15 minutes using 50 µL of stop solution, and the plates were read at 450 nanometres (nm) on an ELx800 (BioTek™) microplate reader.

*Statistical Analysis*

Statistical analysis was performed in Microsoft Excel 2021©. Hormone analysis yielded glucocorticoid results represented as cortisol nanogram per gram (ng/g). Faecal glucocorticoid results over the period of data collection were graphed in three separate box and whisker plots, one for each koala, and after factoring in faecal cortisol metabolite lag times, the results were displayed by "stressor recorded" and "no stressor recorded". Faecal glucocorticoid results over the period of data collection for all three koalas were then graphed in a single box and whisker plot, and the same was performed for fur glucocorticoids.

**Results**

During their time at Port Stephens Koalas during September 2018 to the end of December 2018, koalas Maree, Tai and Solstice encountered a number of negative stimulants of which were out of the wildlife carer's control (*Table 1*).

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**Table 1:** Negative stimulants experienced by koalas in care.

| Negative Stimulants | |
|---|---|
| **Bellowing** | Refers to hearing one or more other koalas either in the sanctuary or in the wild bellowing |
| **Maintenance/Construction** | Refers to maintenance happening around the sanctuary, or construction happening on the outskirts of the sanctuary |
| **Visitors** | Refers to tourists visiting the sanctuary |
| **Moved Cages** | Refers to the koala being placed in a new enclosure |
| **Weather** | Refers to severe weather events such as storms or hail |
| **Fire Crackers** | Refers to hearing fire crackers being released on the outskirts of the sanctuary |
| **Campers** | Refers to people staying at the campsite which neighbours the sanctuary |
| **Vet** | Refers to the koala being transported to a veterinary clinic for treatment or a check up |

The average faecal cortisol result for Maree on days where no stressor was recorded was 23 ng/g, whereas the average faecal cortisol result on days where a stressor was recorded was 53 ng/g (*Figure 1*). On days where no stressor was recorded, the bottom whisker measured 9 ng/g, and the top whisker measured 82 ng/g (*Figure 1*). However, on days where a stressor was recorded, the bottom whisker measured 4 ng/g, and the top whisker measured 160 ng/g (*Figure 1*). This demonstrates that for Maree, there was a larger variation of faecal cortisol when a stressor was recorded, compared to when no stressor was recorded.

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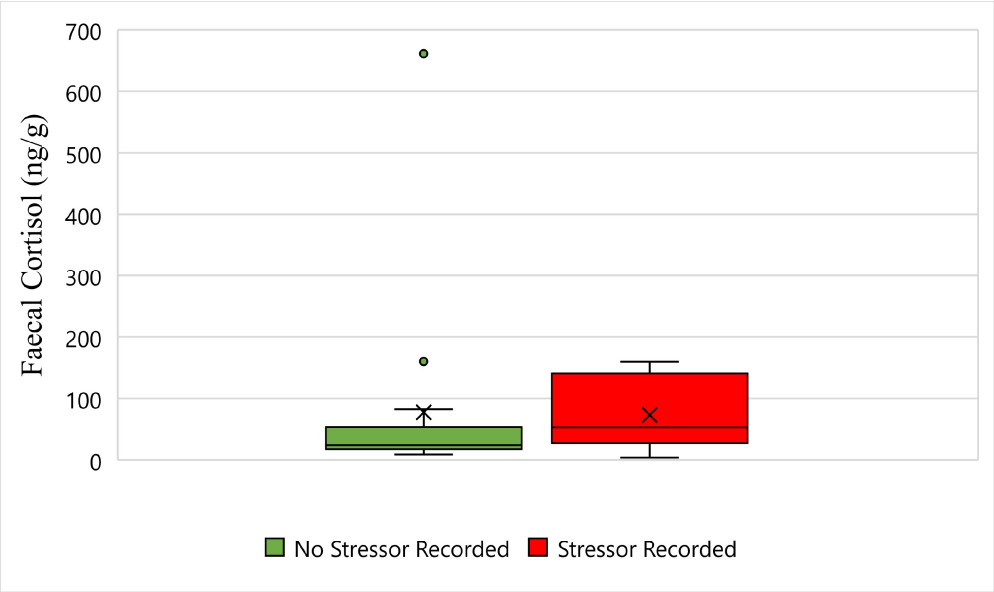


**Figure 1:** This figure displays the variance of faecal cortisol for Maree from the start of September 2018 to the end of December 2018, with results grouped by "no stressor recorded" and stressor recorded".

The average faecal cortisol result for Tai on days where no stressor was recorded and on days where a stressor was recorded was 53 ng/g (*Figure 2*). On days where no stressor was recorded, the bottom whisker measured 5 ng/g, and the top whisker measured 160 ng/g (*Figure 2*). Furthermore, on days where a stressor was recorded, the bottom whisker measured 15 ng/g, and although there was no top whisker recorded, the maximum value was the same as the third quartile for Tai, and measured 160 ng/g (*Figure 2*). This demonstrates that for Tai, there was almost no variation of faecal cortisol when a stressor was recorded, compared to when no stressor was recorded.

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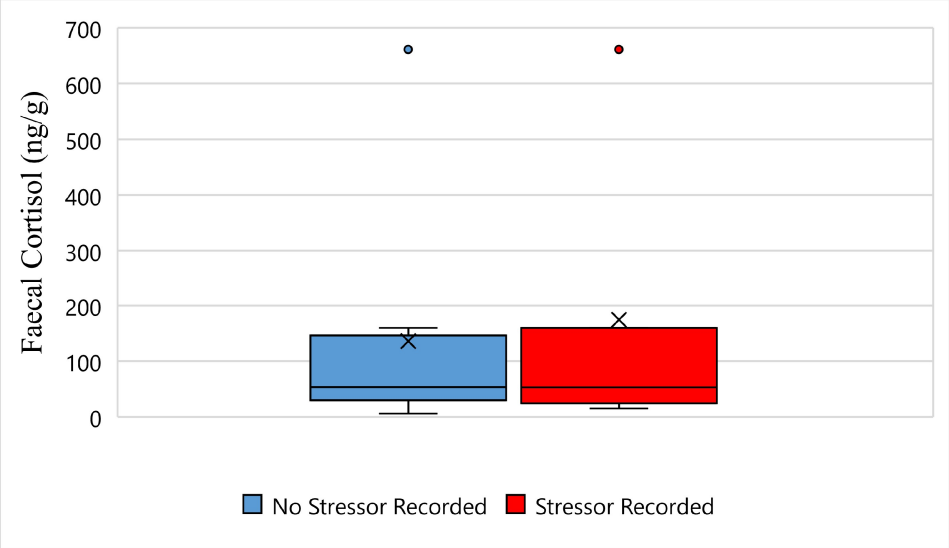


**Figure 2:** This figure displays the variance of faecal cortisol for Tai from the start of September 2018 to the end of December 2018, with results grouped by "no stressor recorded" and stressor recorded".

The average faecal cortisol result for Solstice on days where no stressor was recorded was 223 ng/g, whereas the average faecal cortisol result on days where a stressor was recorded was 215 ng/g (*Figure 3*). On days where no stressor was recorded, the bottom whisker measured 198 ng/g, and the top whisker measured 241 ng/g (*Figure 3*). However, on days where a stressor was recorded, the bottom whisker measured 197 ng/g, and the top whisker measured 238 ng/g (*Figure 3*). This demonstrates that for Maree, there was a larger variation of faecal cortisol when no stressor was recorded, compared to when a stressor was recorded.

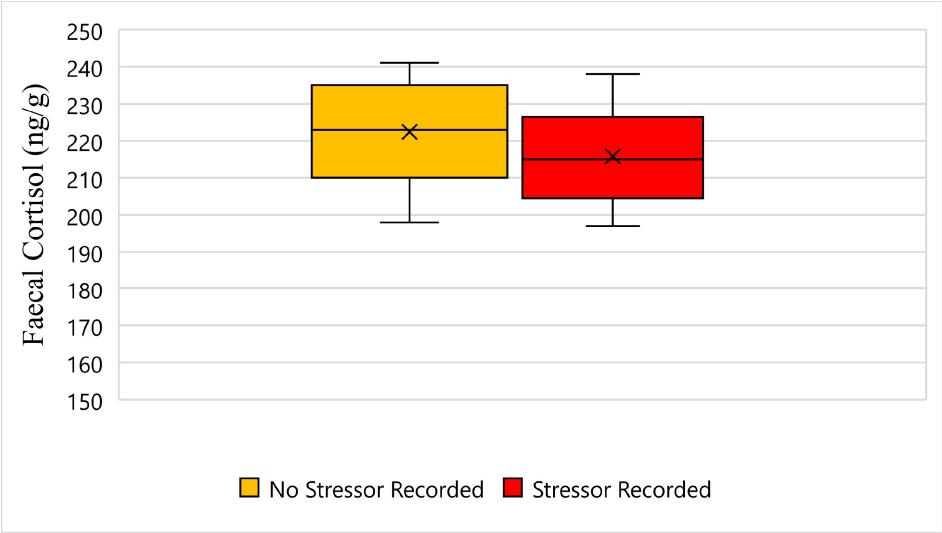

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**Figure 3:** This figure displays the variance of faecal cortisol for Solstice from the start of September 2018 to the end of December 2018, with results grouped by "no stressor recorded" and stressor recorded".

Overall faecal cortisol results that included the days with no stressor recorded, and the days when a stressor was recorded between Maree, Tai and Solstice varied significantly (*Figure 4*). Although the average faecal cortisol result was similar between Maree and Tai, 38 ng/g and 53 ng/g respectively, the average for Solstice was 218 ng/g (*Figure 4*). The bottom whiskers for Maree and Tai measured 4 ng/g and 5 ng/g respectively, whereas the bottom whisker for Solstice measured 197 ng/g (*Figure 4*). Furthermore, the top whisker for Maree measured 160 ng/g, and although there was no top whisker recorded, the maximum value was the same as the third quartile for Tai, and also measured 160 ng/g (*Figure 4*). This is contrary to Solstice, where the top whisker measured 241 ng/g (*Figure 4*). This demonstrates that between Maree and Tai, there was limited variation of faecal cortisol, whereas variation was larger between results shown by Maree and Tai, with Solstice (*Figure 4*).

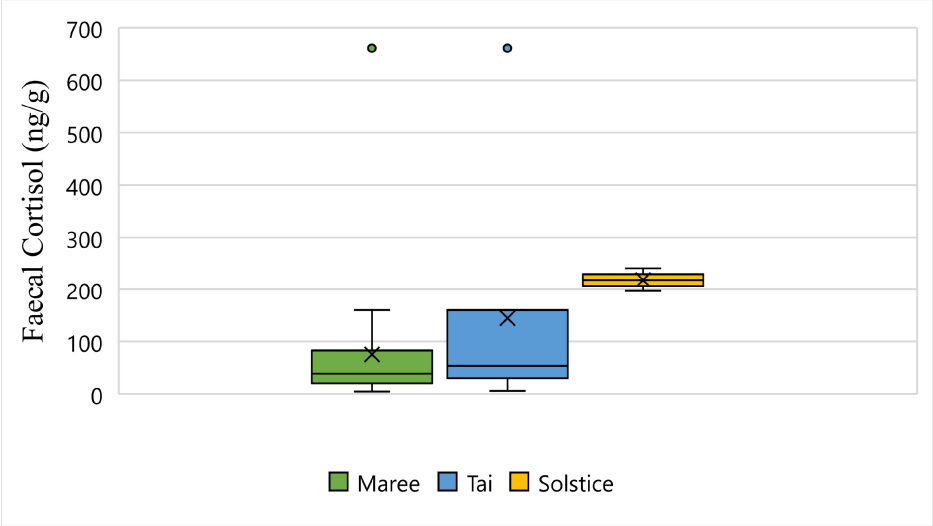


**Figure 4:** This figure displays the variance of faecal cortisol for Maree, Tai and Solstice from the start of September 2018 to the end of December 2018.

Overall fur cortisol results between Maree, Tai and Solstice varied significantly (*Figure 5*). The average fur cortisol result for Maree was 0.57 ng/g, the average for Tai was 0.39 ng/g, and the average for Solstice was 0.63 ng/g (*Figure 5*). The bottom whisker for Maree measured 0.10 ng/g, the bottom whisker for Solstice measured 0.49 ng/g, and although there was no bottom whisker recorded, the minimum value was the same as the first quartile for Tai, and measured 0.27 ng/g (*Figure 5*). Furthermore, the top whisker for Maree measured 1.75 ng/g, the top whisker for Solstice measured 0.80 ng/g, and although there was no top whisker recorded, the maximum value was the same as the third quartile for Tai, and measured 0.52

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ng/g (*Figure 5*). This demonstrates that there was a large variation in fur cortisol between Maree, Tai and Solstice (*Figure 5*).

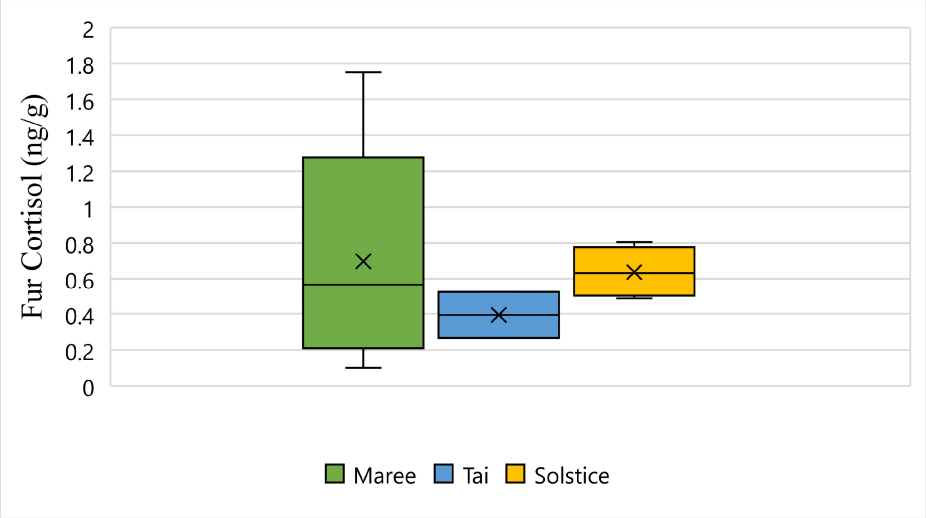


**Figure 5:** This figure displays the variance of fur cortisol for Maree, Tai and Solstice from the start of September 2018 to the end of December 2018.

## Discussion

The aim of this study is to understand the stress response of koalas to negative stimulants that are out of a wildlife carer's control through faecal and fur glucocorticoids. It is hypothesised that there will be a larger variation in faecal glucocorticoids from koalas on days where a stressor was recorded, compared to days where no stressor was recorded. It is also hypothesised that the average of fur glucocorticoids between the three koalas will not vary significantly.

Maree was brought into the care of Port Stephens Koalas in late 2017 after being struck by a motor vehicle. On assessment, it was discovered that the force of the collision impaired her vision, rendering her permanently blind and unable to be released into the wild. As a result, Maree resides permanently in the care of Port Stephens Koalas. Just before data collection between September 2018 to the end of December 2018, Maree was moved to a new enclosure within the facility. Furthermore, Tai was brought into the care of Port Stephens Koalas in late 2017 after being found sitting on the ground in a place that was not his natural habitat. On assessment, it was discovered that he had bilateral cataracts and nystagmus, meaning he required an extensive period of veterinary treatment before he could be released into the wild. As a result, Tai remained in the care of Port Stephens Koalas for more than 12 months. Finally, Solstice was brought into the care of Port Stephens Koalas in mid 2018 after being struck by a motor vehicle. On assessment, it was discovered that the force of the collision fractured his elbow, eye socket and jaw, meaning he required an extensive period of veterinary treatment before he could be released into the wild. As a result, Solstice remained in the care of Port Stephens Koalas for 6 months.

 doi:10.20944/preprints202108.0407.v1

Between Maree, Tai and Solstice, the highest recorded faecal cortisol result was 661 ng/g, and the lowest recorded faecal cortisol result was 4 ng/g (*Figure 4*). On the contrary, the highest recorded fur cortisol result was 1.75 ng/g, and the lowest recorded fur cortisol result was 0.10 ng/g (*Figure 5*). The use of biological samples such as faeces and hair to obtain readings of glucocorticoids is a method of measuring absolute levels of physiological stress that is still evolving for koalas. This means that there is no current glucocorticoid baseline to compare the results of this study with. However, measuring the variation of glucocorticoids in koalas in response to negative stimulants is an important factor in understanding how this species responds to stress while undergoing rehabilitation.

The hypothesis that there will be a larger variation in faecal glucocorticoids from koalas on days where a stressor was recorded, compared to days where no stressor was recorded, was only true for Maree. The hypothesis was not true for Tai or Solstice, as for Tai, there was almost no variation between days where a stressor was recorded and when no stressor was recorded, and for Solstice, there was a larger variation when no stressor was recorded, compared to when a stressor was recorded. The hypothesis that the average of fur glucocorticoids between the three koalas will not vary significantly was not true, as there was a large variation in fur cortisol between Maree, Tai and Solstice. It is well known that rehabilitation sanctuaries are inherently stressful for all animals, as it is an unfamiliar environment where the animal cannot predict or control what will happen to them (Fischer & Romero, 2019; Lloyd, 2017). This experience is even more challenging for wild animals than it is for domestic animals, as it is entirely unnatural for wild animals to be around humans (Fischer & Romero, 2019). Furthermore, rehabilitation sanctuaries are riddled with negative stimulants (Fischer & Romero, 2019), and this is a likely explanation as to why there is such a large variation in faecal glucocorticoids and fur glucocorticoids between Maree, Tai and Solstice.

Some of the negative stimulants experienced by the koalas in this study include bellowing, maintenance/construction, visitors, moved cages, weather, fire crackers, campers, vet (*Table 1*). The most common occurring of these negative stimulants were bellowing, maintenance/construction, and visitors.

Animal vocalisations occur in a variety of contexts, and usually bellowing in koalas occurs as a sexual advertisement call of males to females (Ellis et al., 2011). It is not unusual for male koalas to have been bellowing at the time of data collection in this study, as September to the end of December coincides with the koala breeding season. However, the full context of bellowing is unknown, and research suggests that environmental factors such as weather events can also trigger male koala bellowing (Ellis et al., 2011).

Research shows that wildlife being held in captivity can adapt to noises they hear on a regular basis, however the noise of maintenance/construction can be particularly stressful (Jakob-Hoff et al., 2019). This is because the noise of maintenance/construction can be intense and often occurs unpredictably (Jakob-Hoff et al., 2019). Several studies have been published that describe aversive responses of wildlife to maintenance/construction when being held in captivity, with examples including studies performed in snow leopards (*Panthera uncia*) (Sulser et al., 2008), giant pandas (*Ailuropoda melanoleuca*) (Powell et al., 2006), and Hawaiian honeycreepers (*Drepanidinae* spp.) (Shepherdson et al., 2004).

It has been well documented that visitors can elicit a stress response from wildlife being held in captivity (Fernandez et al., 2009), and this is especially so for koalas (Webster et al., 2017). Some studies suggest that animals can habituate to visitors after a period of time (Margulis et al., 2003), however most studies suggest that visitors in-fact result in increased stress in animals (Fernandez et al., 2009). However, this does depend on the temperament of the species or an individual, and the behaviour of the visitors themselves (Hosey, 2000).

*Summary*

The hypothesis that there will be a larger variation in faecal glucocorticoids from koalas on days where a stressor was recorded, compared to days where no stressor was recorded, was only true for Maree. The hypothesis was not true for Tai or Solstice, as for Tai, there was almost no variation between days where a stressor was recorded and when no stressor was recorded, and for Solstice, there was a larger variation when no stressor was recorded, compared to when a stressor was recorded. The hypothesis that the average of fur glucocorticoids between the three koalas will not vary significantly was not true, as there was a large variation in fur cortisol between Maree, Tai and Solstice. In summary, rehabilitation sanctuaries are riddled with negative stimulants, and this is a likely explanation as to why there is such a large variation in faecal glucocorticoids and fur glucocorticoids between Maree, Tai and Solstice. As previously mentioned, the use of biological samples such as faeces and hair to obtain readings of glucocorticoids is a method of measuring physiological stress that is still evolving for koalas. This means that there is no current glucocorticoid baseline to compare the results of this study with. However, measuring the variation of glucocorticoids in koalas in response to negative stimulants is an important factor in understanding how this species responds to stress while undergoing rehabilitation.

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