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Differing mortality rates in two concurrently radio-tracked populations of koala (*Phascolarctos cinereus*)

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Abstract. Radio-tracking studies enable insights into factors that contribute to koala mortality. Two radio-tracking studies investigating the impacts of disturbance events on koalas were undertaken in different areas over the same period. Both studies employed similar techniques for koala capture, processing and monitoring. In one study, none of nine koalas died during a 5-month monitoring program following their translocation into a new habitat area, while in the second study 6 of 11 koalas died over the same period during an *in situ* impact-monitoring study. The two populations differed morphologically and genetically: that with the higher mortality rate notable for a smaller head and neck circumference and lower genetic diversity. Differing outcomes from the two studies lend support to a hypothesis that inbreeding and the loss of genetic information may predispose some individuals and/or populations of koalas to an elevated stress response and/or increased susceptibility to disease, the expression of which may become exacerbated in the presence of ongoing disturbance or novel stressors that can include research activities. If this is the case, the endocrinology and genetic structure of free-ranging koala populations needs to be afforded greater consideration in terms of predicting a given population's immunological response to potential isolation and/or disturbance events.

Additional keywords: population fitness, genetic diversity, koala stress syndrome.

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Introduction

Radio-tracking studies have contributed much to our understanding of koala ecology, conservation and management (e.g. Melzer 1995; Lunney *et al*. 2002; Dique *et al*. 2003; Kavanagh *et al*. 2007; Janssen 2012; Goldingay and Dobner 2014; Matthews *et al*. 2016). Amongst outcomes from some studies are observations of koala deaths, aspects of which provide insights into factors influencing population dynamics and viability. Of 23 known koala mortalities over a nine-year study at Iluka on the north coast of New South Wales (NSW) by Lunney *et al*. (2002) and where the cause of death was known, vehicle-strike ($n = 10$) and disease ($n = 11$) were considered to collectively account for nearly 87% of known mortalities. In a study of 40 mostly subadult dispersing koalas in south-eastern Queensland, Dique *et al*. (2003) reported on 10 mortalities, half of which were due to vehicle-strike, three to dog attack and two to other causes. Cristescu *et al*. (2012) similarly reported vehicle-strike ($n = 35$), disease ($n = 21$) and dog attack ($n = 16$) as the main causes of koala mortality on Stradbroke Island, Queensland. From such data, and especially in peri-urban environments, it is clear that koala mortalities due to anthropogenic factors such as vehicle-strike have the potential to be key drivers of population decline.

Beyond the peri-urban landscape, factors contributing to koala mortality appear to be different. Of the eight of 30 koalas that died during the course of a study in central western NSW undertaken by Kavanagh *et al*. (2007), necropsy results indicated death from septicemia associated with infections caused by spines of the introduced tiger pear (*Opuntia aurantiaca*) ($n = 2$), predation ($n = 2$), heat stress ($n = 2$) and disease in the form of pneumonia/lung congestion ($n = 2$). Mortalities due to dehydration and heat stress were also identified as a primary cause of the death of 16 of 34 individuals during the course of a two-and-a-half-year study of koalas in the subhumid woodlands of central Queensland, an outcome that appeared to be exacerbated by age and/or disease (Melzer 1995). Gordon *et al*. (1990) considered drought to be a primary contributor to koala mortalities in central Queensland, while Lunney *et al*. (2012) also attributed drought-exacerbated heat stress/dehydration to be responsible for the deaths of four of 15 koalas in a localised study on the Liverpool Plains near Gunnedah in central western NSW.

The importance of understanding factors that contribute to koala mortality at local and metapopulation levels of resolution becomes more apparent as techniques such as Population Viability Analysis are increasingly applied to the task of investigating reasons for population decline and/or modelling the responses of populations to specific management actions or circumstances. Regardless of the reason for undertaking Population Viability Analysis, it remains important for factors that have been, or are, contributing to koala mortality to be accurately identified (Penn *et al*. 2000). The purpose of this paper is to examine the context and results of two concurrently undertaken but otherwise independent koala radio-tracking studies that were originally undertaken to investigate the responses of koalas to specific disturbance events. While koala mortalities were a possibility in both studies, numbers were expected to be few. This was not the case, however, with disproportionately high numbers of mortalities occurring in one of the study populations. While sample sizes were small in both populations, the contrasting results warrant consideration, if for no other reason than to stimulate further discussion on factors that can contribute to koala mortality that may need to be taken into account in future studies.

Methods

Study populations

Study populations were located in northern New South Wales, the more southerly at Thrumster (31.27°S, 152.50°E) near Port Macquarie on the mid-north coast ~375 km north of Sydney, the second at Tyagarah (28.35°S, 153.33°E) near Byron Bay on the far-north coast ~765 km north of Sydney.

Koalas comprising the Thrumster study population were the subject of a translocation program arising from displacement and habitat loss associated with a highway upgrade, while koalas comprising the Tyagarah population were the focus of an *in situ* study investigating potential impacts of a music festival. The habitat being utilised by both populations was similar, that at Thrumster comprising a combination of forest types that contained the preferred food tree species tallowwood (*Eucalyptus microcorys*), forest red gum *E. tereticornis*), grey gum (*E. propinqua*) and swamp mahogany (*E. robusta*), whereas forest communities occupied by the Tyagarah population comprised forest types commonly containing swamp mahogany and forest red gum, with occasional tallowwood and grey gum.

In both studies koalas were captured by flag and pole or by trapping (Bali and Delaney 1996; Phillips 2011) and fitted with Micro-lite VHF radio-collars (Titley Electronics). Data collected either at the time of capture or during subsequent processing included sex and body weight, the tooth-wear class of Gordon (1991), disease status (presence/absence of clinical expression of Chlamydiosis), body condition score (*sensu* Ellis and Carrick 1992) and the reproductive status of females (presence of pouch/back young/recent signs of lactation). Captured koalas from both populations were also ear-tagged, a small hole being made in the ear using leather-punch pliers and through which an individually numbered ear-tag was inserted. The resulting punchlet tissue samples obtained by this process were retained and stored in 70% ethanol to assist genetic and/or disease studies being undertaken elsewhere. These procedures were undertaken on anaesthetised animals only (see below).

Koalas comprising the Thrumster study population were required to be translocated to similar, but otherwise unoccupied, habitat located ~7.5 km to the south of the highway upgrade. Because of the proximity of the new habitat to that from which they were being removed, once captured, animals from this population were transported to interim holding facilities at the Port Macquarie Koala Hospital where they were processed under anaesthetic (intramuscular injection of 2.5–3.5 mg kg^{-1} Zoletil within 12 h of capture and thereafter maintained in captivity for 7–10 days prior to being transported and introduced into the new habitat where they were temporarily confined to individual food trees before being afforded full liberty. This temporary confinement was achieved by way of a fence constructed from a series of 1200 mm × 1200 mm × 5 mm corflute panels erected to fully enclose an area ~2 m out from the base of each release tree. In contrast, koalas comprising the Tyagarah study population were processed in the field using Alfaxan (intramuscular injection of 0.3 mg kg^{-1} alfaxalone), fitted with radio-collars and released at the point of capture. This task usually took no more than 45–60 min, this being the period required to allow anaesthetic uptake, processing and recovery to a stage where the animal could be released back into the tree from which it had been captured. The use of different anaesthetic agents between the two populations reflected different koala-processing protocols utilised by the Port Macquarie Koala Hospital staff and author, respectively.

Radio-tracking of animals in both populations was undertaken on a daily basis for 5–7 days following release and thereafter reduced to 2–3 times weekly. Both programs required radio-tracking programs of ~6 months duration to be undertaken to enable any impacts to be identified.

Results

Captures of koalas comprising the two populations commenced in January–February 2010. Nine koalas (two males, four females, one subadult male, two subadult females) comprised the translocated population at Thrumster. The mean weight of adult koalas comprising the Thrumster study population was 5.68 kg ± 0.54 kg (s.d.). Two of the females that were captured for translocation purposes were also suckling small (<200 g) unfurred pouch-young. The ages of captured koalas ranged between Tooth-wear Classes 2 and 6 and were approximately normally distributed. At capture, clinical expression of disease (Chlamydiosis) was noted in one of the four female koalas. Given a poor prognosis upon initial capture and consequently placed on a six-week course of antibiotic therapy, this animal eventually required euthanasia following development of a gastrointestinal stasis during the initial phase of treatment.

Eleven koalas (four males, six females, one subadult male) comprised the monitored population at Tyagarah. The mean body weight of these adult koalas was 5.77 kg ± 1.54 kg (s.d.). One of the adult females was suckling a small (<200 g) pouch young, and another appearing to be sharing her home range with a small (2.5 kg) subadult male, which was presumed to be her young from the previous year. In common with the Thrumster animals, the ages of captured koalas ranged between Tooth-wear Classes 2 and 6 and were approximately normally distributed. At capture, clinical expression of disease (Chlamydiosis) was noted in four koalas (two males, two females); the extent of clinical expression in each instance not considered severe enough to warrant the animals being taken for treatment and/or placed into care.

Noticeable morphological differences were apparent between the two populations, those at Tyagarah being typified by smaller heads and neck circumferences that necessitated modification (shortening and reholing) of the collar component of the radio-

Table 1. Demographic, morphological and mortality data for six koalas comprising part of a radio-tracked population at Tyagarah near Byron Bay on the far north coast of NSW

BCS, body condition score; TWC, tooth wear class of Gordon (1991)

Koala ID	Sex	Weight (kg)	BCS	TWC	Date of capture	Date of death
9874	F	5.05	7	P4fM1 g	19 Jan. 2010	29 Jan. 2010
Lil' Fi	F	3.8	7	P4e	13 Jan. 2010	05 Mar. 2010
Renee	F	–	6	P4D	12 Jan. 2010	17 May 2010
Emmy-lou	F	4.25	5	P4D	03 Apr. 2010	28 Jun. 2010
Sonny Boy	M	7.55	5	P4e	13 Jan. 2010	16 Apr. 2010
Junior	M	2.5	7	P4b	19 Jan. 2010	01 Mar. 2010

tracking assembly in order for it to be fitted to the animal, while those at Thrumster did not. This anomaly might have gone unnoticed were it not for the fact that the collar component of all radio-transmitters to be used in both studies had been renewed before the commencement of the capture program (thus all collars were the same length).

Mortalities

No mortalities were recorded during the radio-tracking of the koalas that were translocated from the Thrumster site. In contrast, the first mortality in the Tyagarah population occurred during the establishment phase of the project when a female koala ('9874') was found dead on the ground ~10 days after her capture on 19 January 2010. A further five deaths (three adult females, one adult male and one subadult male) occurred over the following 16 weeks, the last being recorded on 28 June 2010 (Table 1). Four of the six mortalities were of koalas initially assessed as potentially compromised by disease (i.e. clinical expression of Chlamydiosis in the form of 'wet-bottom' or 'dirty-tail', conjunctival scarring and/or opaqueness of one or more eyes). Aside from confirming underlying disease status, necropsy results on one of the adult females and the adult male were uninformative. Necropsy was not possible in the remaining instances because of the time that had elapsed between last sighting and location of the dead animal.

Koalas were recaptured and radio-collars progressively removed from study animals in both populations during July and August 2010.

Discussion

The contrasting results from these two studies raise questions regarding the causes of koala mortality that can occur within free-ranging populations. While the concurrent undertaking of the two radio-tracking programs described herein was coincidental, the differing results arising from the two studies warrant discussion if some benefit is to be gained, especially given that the number of mortalities observed in the Tyagarah study exceed that accrued over nearly 30 years of radio-tracking/research experience collectively involving many hundreds of koala captures/recaptures (author's unpubl. data). In this circumstance such discussion is made possible because several aspects relating to the habitat, capture and handling of animals in both populations were similar, thus enabling consideration of those factors that *were* different – anaesthetic, population/management/research context – to be examined in more detail.

Anaesthetic

The management of free-ranging koalas captured for research purposes has progressed from that where at one time most, if not all, procedures such as ear-tagging, blood sampling, ocular and uro-genital swabbing were accomplished with the animal under physical restraint, to nowadays the use of more refined approaches reliant upon various forms of sedation. To this latter end the use of tiletamine/zolazepam (Zolatil®) and alfaxalone (Alfaxan®) as sedation/anaesthetic agents for free-ranging koalas has been a component of best-practice koala research for many years with no adverse effects thus far being reported (Bush *et al*. 1990; Lynch and Martin 2003; Unwin 2004; Markey *et al*. 2007; West *et al*. 2014; author's unpubl. data). For these reasons it is considered unlikely that the anaesthetic agent used in the Tyagarah study was a contributing factor to the mortalities that occurred, especially given that the period over which they occurred exceeded that reasonably likely to have been associated with either misadministration or sensitivity issues.

Population context

High mortality rates can be associated with ageing populations where senility can be considered to have some bearing on observed mortality rates and/or the susceptibility of individuals (Melzer 1995). However, while sample sizes in the two study populations were small, both appeared to be at, or close to, demographic equilibrium (i.e. age classes of captured animals were approximately normally distributed) at project commencement, neither were mortalities restricted to older cohorts, thus ruling out senility as a possible contributing factor.

Concerns about the mortalities that were observed, motivated in part by the aforementioned morphological anomalies, prompted a *post hoc* genetic profiling of the Tyagarah population using the tissue samples collected during processing. The results of these analyses suggested a koala population with relatively low genetic diversity of 2–5 alleles $locus^{-1}$ (Kristen Lee, University of Queensland, pers. comm.). More recent but unrelated work, also using samples obtained from other koalas in this population, has reinforced this earlier assessment, analysis by Neaves *et al*. (2015) returning a value of 4.3 ± 0.3 (s.e.) alleles $locus^{-1}$ while those from the Port Macquarie area returned a value of 6.1 ± 0.5 (s.e.) alleles $locus^{-1}$ (averaged across 15 loci in both instances). The two populations were further determined to have inbreeding coefficients (F) of 0.063 and 0.027 respectively (Neaves *et al*. 2015). It is noteworthy that results for the Tyagarah population are similar to the 3.8 alleles $locus^{-1}$ reported by Cristescu *et al*. (2009) for koalas from French Island in Victoria, all of which originated from a small founder population of 2 or 3 koalas (Taylor *et al*. 1997).

Management context

In both populations it is unarguable that the koalas being monitored were not subjected to varying measures of disturbance/stress. Possible stressors ranged from the capture and screening process (both populations) to that of temporary confinement and associated translocation into new habitat (Thrumster) while an increase in development-related activity and exposure to novel stimuli (development works and loud music) occurred at Tyagarah. While some radio-tracking studies have reported mortality rates as high as 38% for translocated koalas (e.g. Whisson *et al*. 2012), no mortalities were recorded while the koalas translocated from Thrumster were being radio-tracked. Moreover, two of the translocated females had unfurred pouch young at capture, both of which had survived to back-young stage at the time of collar removal. Factors contributing to the low mortality rate of the Thrumster koala translocation program were considered to have been the selection of an unoccupied but otherwise suitable release site containing the same preferred food tree species as those found in the area to be cleared and the associated 'soft-release' protocols that were employed as part of the translocation procedure. The absence of mortalities implies that either the translocation procedure was not an overly stressful event for these animals, or if it was, that they were physiologically capable of dealing with the event.

Stress susceptibility may have been an issue affecting koalas in the Tyagarah study population for several reasons that are perhaps best typified by the response of koala '9874'. By any measure the capture of this koala was effected efficiently and was uncomplicated (1–2 min from a 3-m tree using the flag-pole method), yet immediately following processing and release back into the capture tree an acute stress response in the form of laboured breathing and vocalisations extended over a period of at least 3 h. A mature female, '9874' had been assessed as being in good physical condition (body condition score of 7/10) with no clinical signs of disease. Last sighted alive five days after capture, '9874' died shortly thereafter; when last sighted she was located outside of the immediate study area ~600 m from her point of capture, her position thereafter triangulated because of dense understorey vegetation. Radio-tracking had transitioned to every 2–3 days and it was the absence of any apparent movement on Day 7 after capture that resulted in a more detailed inspection of the area on Day 9 after capture; when found, the body was in a state of partial decomposition. While there may be other reasons, what could be interpreted as a mortality that occurred as a direct consequence of an acute stress response by this individual may have been averted if the animal was not required to be captured in the first instance.

Given that it was an inaugural event, development works at the Tyagarah site were both novel (from a koala's perspective) and ongoing during the period leading up to the festival event (in contrast to subsequent years when it was restricted to just set-up periods) so it is possible that such activities may have contributed to elevated stress levels. Some support for this notion arises from knowledge that four of the six mortalities occurred in or around the immediate periphery of the festival site where most development activity was taking place, thus raising the possibility that an increase in stressors such as noise levels, the presence of many people and the movements of machinery may have contributed to acute stress responses. Phillips (2016) described aversive behaviour in koalas associated with this population, two of which ('Renee' and 'Sonny Boy') are amongst the mortalities included in this work.

Conclusion

The impacts of stressors on both individuals and populations of free-ranging koalas have long been of interest to researchers but little progress would appear to have been made in terms of understanding the physiological basis and diagnostic pathology of the stress response in koalas since early investigations by Butler (1978). Indeed, and in the broader context of wildlife generally, the stress response in most instances is recognised as a syndrome with a pathology that is both poorly defined and understood (Reeder and Kramer 2005).

Research-related mortalities that might occur during the course of koala radio-tracking studies are rarely reported. In the Tyagarah study at least one of the recorded mortalities was arguably attributable to researcher activities, specifically the capture of female '9874', which, despite the text-book capture, anaesthetic and best intentions of the research team, responded to the situation with what can only be described as a chronic stress response. Interestingly, 'koala stress syndrome' for the want of a better name, shares many behavioural similarities with the phenomenon of capture myopathy (see Cole *et al*. 1994) including (in wild koalas during the capture process) a reluctance to move and/or laboured breathing, often accompanied by a bleating-like vocalisation. The bleating response appears synonymous with that described as a 'staccato' call by Mitchell (1990), who additionally reported the almost exclusive association of this call with the process of koala capture. While any association between a capture-induced stress response that involves bleating and a subsequent mortality within a relatively small time frame after capture must remain speculative, similar associations are occasionally documented – sometimes inadvertently – during the course of other koala research programs or radio-tracking studies (e.g. Phillips 1999 (capture data on koala 'M701A' refers); Endeavour Veterinary Ecology 2013*a* (capture data on koala 'Xoryan' refers), 2013*b* (capture data on koala 'Norm' refers), 2013*c* (capture data on koala 'Tamara' refers). If a link between capture stress and shortly ensuing mortality can be proven, then capture-related koala mortalities that are inadvertently attributed to other causes such as disease or predation may be more common than has previously been acknowledged.

The genetic data and the observed morphological anomalies insofar as the Tyagarah population is concerned additionally raise the possibility of inbreeding as a contributing factor to the mortalities that were observed, especially given that known consequences of inbreeding can be the compromising of immunological processes and increased susceptibility to disease and stress as well as reduced fitness of offspring (O'Brien and Evermann 1988; Hedrick and Kalinowski 2000; Sherwin *et al*. 2000; Reed and Frankham 2003). In terms of the Tyagarah population it is plausible that in the loss of genetic information through inbreeding has resulted in a population with an elevated stress response that is manifested in a greater sensitivity to disturbance when compared with other populations. If this is the case then there is a need to accommodate this consideration into the design of research programs, aspects of which might include the genetic profiling of populations as a precursor to more detailed investigations if there is some evidence of likely isolation and/or susceptibility. This also means that the issue of inbreeding generally may be of greater relevance to koala conservation and management throughout the species' remaining range, but especially in those areas where small founder populations are likely. While the extent to which this circumstance might explain mortalities in the Tyagarah population remains unknown at this time, it remains an issue that warrants consideration. Along with the need for more detailed investigations into endocrinology of the koala stress response, such considerations introduce new elements into longer-term koala management and research programs.

Conflicts of interest

The author declares no conflicts of interest.

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