Living with fire: Ecology and genetics of the dasyurid mammal

*Dasykaluta rosamondae*

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Bachelor of Science (Wildlife Management) Hons

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School of Biological Sciences

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Thesis Declaration

I, Genevieve Louise Tavani Hayes, certify that:

This thesis has been substantially accomplished during enrolment in the degree.

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Summary

The dasyurids are a group of Australian mammals with relatively few threatened species. This is rare for mammals in Australia, where over 30% of the world’s terrestrial mammal extinctions have occurred since 1600 AD, and may be a consequence of their unusual and ‘fast’ life histories. The kaluta, *Dasykaluta rosamondeae*, is a small dasyurid, common in the broader Pilbara region of northwestern Australia. In my study, I examined the ecology of kalutas to determine whether their current conservation status is appropriate and whether management actions are required to ensure the persistence of this species. Specifically, I assessed life history characteristics, patterns of paternity, responses to fire, diet, and population structure at broad and fine scales. My study was conducted in Millstream Chichester National Park (MCNP) between July 2013 and December 2014. In total, I captured 131 individual kalutas (54 males, 77 females) at a trap success rate of 3.8%. Sex ratios varied over the year, with an equal sex ratio in winter compared with only females in summer following male die-off. Adult males were significantly larger than adult females and were also in better condition. Kalutas were cathemeral, with activity times ranging from crepuscular in summer to more diurnal in winter. I confirmed that males were obligately semelparous and that females were polyandrous, with litters sired by up to three males. Intraspecific positive testis allometry was demonstrated in adult males, consistent with sperm competition occurring in this species. Populations of kalutas were larger on sites that had not been burnt for a long time and had higher spinifex (*Triodia*) cover than they were on sites that had been recently burnt and had less spinifex cover. Capture success of kalutas was significantly influenced by spinifex cover, but not time since fire. Spinifex hummocks were significantly smaller on recently burnt sites than the other sites, but no difference was found in spinifex size and cover between intermediate aged and long unburnt sites, suggesting that there was a threshold for the effect of time since fire on spinifex size. These findings suggest that the frequency of fire is not as important for kaluta persistence as the occurrence of spinifex of sufficient size. I used DNA barcoding to study the diet of the kaluta; this new technology is revolutionising the diet analysis of cryptic species. My data revealed that kaluta diet consisted predominantly of termites, as well as another small dasyurid species (*Pseudantechinus* sp.), spiders and centipedes. The diet analysis did not identify any differences in diet between the sexes, sites of differing fire age or between seasons. Lastly, I used microsatellite genotyping to study the population structure of kalutas across their range as well as on a fine scale within MCNP. I found at the broad scale no genetic structuring was evident in kalutas, while at the local scale both males and females exhibit clear population structure and individuals are more closely related than by chance up to distances of 8 km. These genetic results indicate different dispersal patterns, and highlight the importance of assessing structure at both broad and local scales in conservation genetic studies.
Together, the results of my comprehensive study of the demography, ecology, mating system, diet and genetics of the kaluta will inform future management actions for a species inhabiting a region likely to experience substantial changes as the global climate warms. My findings suggest fire management that encourages less frequent, less intense fires would support this species, with patches of long unburnt habitat a vital niche for their long-term survival. My results suggest that the current conservation status of kalutas is appropriate. I advise caution, however, since the kaluta has the potential to quickly become threatened, particularly through the negative impact of fire frequency, which is likely to increase under a warming climate. My data are consistent with the pattern of dasyurid species with fast life histories being more robust to the threats that have driven other small mammal species to extinction.
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I dedicate this thesis to Bronson.
Authorship Declaration: Co-authored Publications

This thesis contains work that has been prepared for publication.

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I, Joseph Tomkins, certify that the student statements regarding their contribution to each of the works listed above are correct

**Coordinating supervisor signature:**

Date: 18/08/2017

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Each chapter has been written with the intention of publication upon completion. Therefore, the chapters have been written as stand-alone papers.
Prologue
MAMMAL DECLINES IN AUSTRALIA

Australia contains many unique species, with 86% of the terrestrial mammal fauna endemic to the continent and surrounding islands (Woinarski et al., 2014). However, this distinctive fauna is declining. Approximately 30% of the world’s terrestrial mammal extinctions since 1600 AD have occurred in Australia, despite only 6% of the world’s terrestrial mammal fauna inhabiting the continent (McKenzie et al., 2007). Nearly 30 terrestrial mammal species have become extinct in Australia since European settlement (Woinarski et al., 2014). Most declines on the mainland have occurred in the arid and semi-arid regions of central Australia, especially southern Australia, and have predominantly affected non-volant, ground-dwelling mammal species in what has been described as the ‘critical weight range’ of 35 – 5500 g (Burbidge and McKenzie, 1989; Woinarski et al., 2014). Numerous threatening processes have been cited as the primary causes of these declines; however, they are most likely attributable to predation by introduced mammals, altered fire regimes, and habitat modification caused by introduced herbivores (Andersen et al., 2005; Legge et al., 2008; Pardon et al., 2003). Taxa in northern tropical Australia, which appeared resilient to these declines until recently, are now also experiencing significant population declines (Legge et al., 2011a; Woinarski et al., 2010, 2011, 2015). Current debate exists around the causes of both waves of extinctions, in particular whether the historic and current extinctions share a common cause. However, excessive fire frequency and intensity appear to be important common features of regions with many mammal declines, in both southern and northern Australia, because burnt landscapes favour feral cats and decrease mammal survival (e.g. Fisher et al., 2014a, 2014b; Murphy and Davies, 2014; Woinarski, 2014).

DASYURIDS AND THEIR LIFE HISTORY STRATEGIES

The family Dasyuridae forms part of the carnivorous marsupial order Dasyuromorphia. Species are divided into four subfamilies, including the Dasyurinae (Tasmanian devil, quolls, mulgars, kalutas, and false antechinuses) and Phascogalinae (phascogales and antechinuses). Species within the Phascogalinae are similar in body form, and rear litters of six to 12 pouch young, depending on species. These predominantly mesic forest-dwelling taxa are relatively resilient. Of the species-rich groups in Australia, they have the equal lowest proportion of threatened taxa, with only 20% currently threatened (Woinarski et al., 2014).

All species of phascogales and antechinuses exhibit a highly unusual life history strategy for mammals – male semelparity – in which males die following the reproductive season (also known as male die-off). Obligate male semelparity has been reported in 15 species of Antechinus, and three
species of *Phascogale*. It has also been reported in the Dasyurinae *Dasykaluta*, a monotypic genus. Under obligate semelparity, males cease producing sperm before the mating period, and rely on a finite supply of stored sperm for fertilisations. They die after a highly-synchronised, brief mating period, as a result of elevated levels of free corticosteroid hormones causing immune suppression, disintegration of testes, haemorrhaging, infections and death (Braithwaite and Lee, 1979). Incomplete die-off, or facultative semelparity, occurs when some males are capable of surviving to a second breeding season if sufficient resources are available, but the physiological syndrome that destroys the reproductive system does not occur, allowing males to breed again. This strategy has been documented in two Dasyurine species, *Dasyurus hallucatus* and *Parantechinus apicalis* (Dickman and Braithwaite, 1992; Mills and Bencini, 2000). Other Dasyurines are iteroparous: males and females can breed more than once. Recently, comparative tests suggested that obligate male semelparity evolved due to intense sexual selection, because males that forego future reproduction by investing heavily in sperm competition in their first breeding season have higher fitness, although they are infertile and do not survive after one season. Intense male competition is promoted by very short mating seasons, timed so that young are weaned during the brief annual peaks in food abundance in the strongly seasonal and predictable environments where Phascogalines live (Fisher *et al.*, 2013). These species live in areas with stable, dense, old growth vegetation and do not occur in frequently burnt or early successional habitat (Fisher *et al.*, 2013).

*Antechinus* species are a highly-studied genus of dasyurids and most species are of little conservation concern (Woinarski *et al.*, 2014). This may be due in part to their mesic coastal habitat and their ‘fast’ life history strategy, because Australian forest mammal species and those with small body size and ‘fast’ life histories have largely escaped declines (Burbidge and McKenzie, 1989; Cardillo, 2003; Cardillo and Bromham, 2001; Johnson and Isaac, 2009). In marsupials, fast life histories are characteristic of small-bodied didelphids and dasyurids, with large litter sizes (typically 10-16 pouch young) and life-spans of a year or less (Fisher *et al.*, 2001). Indeed, a number of studies support the view that life histories are generally the most important predictors of survival when species are faced with global threats, such as invasive predators and loss of habitat (Fisher and Blomberg, 2011b; Fisher and Owens, 2004).

**PILBARA REGION**

The Pilbara is a biogeographic region of north-western Australia with an ancient, weathered landscape (McKenzie *et al.*, 2009; Thackway and Cresswell, 1995). It is located in the Australian arid zone, which covers more than half the continent; however, the western Pilbara does not have the
unpredictable rainfall of much of central Australia, as it experiences wet summer seasons and dry winter seasons. Most annual rainfall occurs in the wet season and comes from thunderstorms and occasional cyclones (McKenzie et al., 2009). The region is a well-known centre of biological endemism, and the evolution of the biota reflects long-term geological stability and the historical transition from mesic climates in the Miocene to current arid climates (Byrne et al., 2008; Pepper et al., 2006, 2011). The endemism and niche specialisation that has evolved through this ancient stability combined make the Pilbara a highly significant region with regard to conserving Australia’s biodiversity. Despite this, much of the region’s biodiversity remains poorly studied (Pepper et al., 2008).

The Pilbara has experienced considerable change in the past century, resulting from altered fire regimes, and pastoral and mining activity (McKenzie et al., 2009). Significantly, a combination of over-grazing and frequent fires, and to a lesser extent habitat clearing, has reduced vegetation cover, or altered its structure across the Pilbara. Many species have been introduced to the area, including 12 mammal species, notably feral cats (*Felis catus*), foxes (*Vulpes vulpes*) and cattle, along with numerous weed species. Consequently, 15% of the mammal fauna from pre-European settlement is now extinct (McKenzie et al., 2009). In addition to these historic threats, fauna in the region will be faced with wide-ranging impacts from future climate change. Projected increases in annual average temperatures, intensity and duration of hot spells (days over 33°C), and evaporation potential may alter fire patterns, especially by increasing extreme fire behaviour. These changes will be dependent on rainfall events, which are projected to increase in intensity, decrease in frequency, and generally continue to be driven by natural variability (Loechal et al., 2011; Sudmeyer, 2016).

**THE KALUTA**

The kaluta, *Dasykaluta rosamondae*, is a small, carnivorous marsupial, endemic to the broader Pilbara region of Western Australia. This little-known dasyurid is brilliantly rufous in colour and was described scientifically by Ride (1964) only 53 years ago, from specimens collected on Woodstock Station and surrounds in the Pilbara. According to Woolley, Ride named the species *Antechinus rosamondae* as a tribute to Lady Rosamund, King Henry II’s red-headed mistress whom he is rumoured to have concealed in his palace at Woodstock, Oxfordshire. Archer (1982), in a review of dasyurid phylogeny, reclassified the kaluta to the newly-created monotypic genus *Dasykaluta* in 1982. This generic name was derived from the Aboriginal Nyamal language name for the species, “kaluta”.

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The kaluta is currently classified as ‘Least Concern’ by the International Union for Conservation of Nature (IUCN); however, there have been only limited studies on the reproduction and physiology of this species, and opportunistic studies as part of environmental surveys. No comprehensive studies have been conducted to provide evidence to support the IUCN classification, or indeed, to assess the value of the kaluta to the Pilbara region. My study addressed questions on the ecology, life history and population genetics of the kaluta. It is the first study to thoroughly examine life history characteristics, patterns of paternity, impacts of fire, diet and population ecology of this species. My overall aim was to examine the ecology of wild kalutas to assess whether their current conservation status is appropriate and what management actions, especially related to fire, are necessary to safeguard the persistence of this species.

The major results of this thesis are presented in five data chapters, summarised below.

**Life history.** Understanding the reproductive biology and sex-specific life history strategy of a species is key to predicting its long-term persistence, particularly in relation to the predictability of its environment. In kalutas, if males are semelparous as expected, we expect females to have a short life span (1-2 years), high litter size and promiscuous mating system. In Chapters 1 and 2, I explore the life history of males and females, and the mating system of kalutas, to:

- describe basic demographic patterns – sex-dependent survival and recruitment rates;
- confirm obligate semelparity, as expected from analyses of life history patterns in other dasyurids;
- confirm multiple paternities within litters (polyandry); and
- assess testis allometry to determine whether the relationship is positive, as is consistent with a mating system driven by sperm competition.

**Fire.** Inappropriate fire regimes are a major threat to small mammals in Australia. Understanding the effects of frequent fire on species is integral to predicting their persistence in the face of a changing climate, where fires are likely to be more frequent. Given the life history and body size of kalutas, they are expected to rely on old growth vegetation of long unburnt spinifex habitat. In Chapter 3, I describe:

- the impact of fire on kaluta distribution and abundance; and
- habitat use by kalutas.
**Diet.** Dietary studies can inform a species’ susceptibility to changes in its environment. Specialists are more at risk from environmental change than generalists. Most dasyurids have a generalist insectivorous or omnivorous diet. In Chapter 4, I examine the diet of kalutas by comparing two techniques:

- a novel, molecular approach (DNA fragment profiles from faecal material); and
- a traditional approach (scat analyses quantifying hard-parts left undigested).

**Population structure.** Knowledge of a species’ genetic diversity and population structure provides information on dispersal patterns. The kaluta is expected to have the ability to disperse between areas of suitable (less recently burnt) vegetation, given the high wildfire frequency of the Pilbara. More broadly, genetic diversity and population mixing can inform us about the ability of a species to respond to changes in its environment. In Chapter 5, using microsatellite genotypes, I assess the genetic structure of kalutas at two scales:

- broad scale structuring across the whole of their Pilbara range, using Bayesian assignment analyses; and
- fine scale structuring in both sexes in MCNP, using spatial autocorrelation.

I conclude with an Epilogue, summarising the findings of this study and suggest future research directions for the kaluta.
Chapter 1
Life history and activity schedules of a small dasyurid *Dasykaluta rosamondae*
in the Pilbara region of Western Australia
1.1 Abstract

The kaluta, *Dasykaluta rosamondae*, is a small, carnivorous marsupial from the semi-arid Pilbara region of Western Australia. As with the well-known genus *Antechinus*, which has characteristic annual patterns reflecting its unusual reproductive strategies, the kaluta is presumed to have a life history strategy of obligate semelparity in males and a one to two-year lifespan in females. I evaluated schedules of reproduction, activity and body mass changes in the wild in male and female kalutas between July 2013 to December 2014, to find if these are consistent with the patterns characteristic of *Antechinus*. I recorded 131 kalutas from 311 captures at a success rate of 3.8%, with success rates by trapping period increasing over the length of the study. Sex ratios varied over the year, culminating in 100% females in November/December. I found sexual size dimorphism in kalutas, not only in body weight but also in condition, measured as relative weight and relative tail width. This sexual dimorphism in condition suggests that males are investing in muscle bulk or in body fat stores, as well as in tail fat stores, in the lead-up to the frenetic breeding season. I also documented increased diurnal activity of kalutas in winter. Adult sex ratio and body mass variation over the year were similar to that found in *Antechinus* species. Diel activity differed from *Antechinus* in that kalutas of both sexes were cathemeral, and most activity during the year was crepuscular. This is the first major study of kaluta life history and activity in the wild.
1.2 Introduction

The kaluta, *Dasykaluta rosamondae* (Ride, 1964), is a small (20-40 g) dasyurid endemic to the broader Pilbara region of Western Australia. Its range extends south into the Carnarvon Basin, and east into the Little Sandy Desert (Burbidge, 2016; Withers and Cooper, 2009; Woolley, 2008). The kaluta inhabits spinifex (*Triodia* spp.) hummock grasslands on sand plains and dunes, favouring mazes formed by dense tussocks of spinifex (Chapter 3; Burbidge, 2016; Withers and Cooper, 2009; Woolley, 2008). There has been no previous study of demography of the kaluta in the wild. In a conservation context, it is vital to know the life history traits of the organism under study. Life history traits are those describing the schedules of growth, reproduction and survival, which influence population growth rates and, therefore, the ability of a population to persist. These include age at sexual maturity, the number of offspring produced per reproductive event, inter-birth interval, and lifespan. Species with “fast” life histories (short life-spans, large litters; e.g. Fisher *et al.*, 2001; Promislow and Harvey, 1990) can experience large fluctuations in abundance, and this may make them vulnerable to extinction from stochastic events during times of small population size (Saether *et al.*, 2002).

Carnivorous marsupials have large variation in life history strategies between genera and species (Fisher *et al.*, 2013). Species in the genera *Antechinus* and *Phascogale* have male semelparity or ‘male die-off’: a reproductive strategy in which all males die after their first breeding season, as a result of elevated levels of free corticosteroid hormones causing immune suppression, haemorrhaging, infections and death (Braithwaite and Lee, 1979). Reproductive studies in captivity suggest that male kalutas are obligately semelparous, i.e. regardless of resource availability or breeding success, all adult males become reproductively senescent and die after a single, highly-synchronised mating period (Woolley, 1991). How and Cooper (2002), Thompson and Thompson (2008) and Woolley (1991) found an absence of males from wild populations during trapping at the period expected for male die-off. Females in captivity can have one litter of up to eight young in two annual breeding seasons, although Woolley (1991) suggests that it is likely that most only reproduce once in their lifetime. Pouch young sex ratios have not been reported for wild kalutas, although a captive study reported an equal sex ratio among 40 pouch young in the pouches of five females (Woolley, 1991). Females live for one to two years, mate during a two-week period in September, and give birth in November. Young are weaned in February and March, at three to four months of age, and participate in the following breeding season, indicating that they sexually mature by 10 months of age (Woolley, 1991).
Körtner et al. (2010) and Pavey et al. (2016) have recently reported activity times and home ranges of kalutas. Woolley (2008) described kalutas as mainly nocturnal or crepuscular. However, in a field study following the movements of seven males and four females between June and July, Körtner et al. (2010) determined that kalutas were predominantly diurnal during the winter months, that individuals of both sexes entered daily torpor (although torpor patterns differed between the sexes), and that home ranges, calculated as minimum convex polygons, of males (3.1 ± 1.0 ha) were greater than that of females (1.0 ± 0.4 ha). The authors predicted that kalutas might shift to bimodal activity patterns during summer (i.e. the crepuscular activity described previously). In the same study, Pavey et al. (2016) suggested that the most likely reason for a shift to diurnal activity was to avoid prolonged temporal overlap with the predatory brush-tailed mulgara (Dasycercus blythi), which is nocturnal in winter. Other possible reasons for a temporal switch include males increasing their activity time to mate with as many females as possible during the short rut, when mating is prolonged (e.g. Lazenby-Cohen and Cockburn, 1988), or may be related to heat avoidance in summer (e.g. Withers and Cooper, 2009).

Antechinus species are largely nocturnal; however, several species, including A. flavipes and A. subtropicus, are also diurnal in both sexes, not just in winter (Parra-Faundes, 2014; Van Dyck and Strahan, 2008). These species live in cool, mesic areas; therefore, their thermoregulation likely accounts for their ability to be diurnal in seasons other than winter. The males of nearly all Antechinus species are constantly active during the mating period, during winter or early spring (e.g. Lazenby-Cohen and Cockburn, 1988).

Trap success rates for kalutas appear to vary with site and year, especially increasing at the time juveniles enter the population (How and Cooper, 2002). Three studies have reported trap success rates for kalutas. Two were conducted at Abydos and Woodstock Stations, approximately 150 km south of Port Hedland, approximately 15 years apart, one between August 1975 and November 1976 and the other between March 1988 and November 1990 (How and Cooper, 2002; Woolley, 1991). The third study, conducted approximately 125km south-southeast of the other two, between January and June 2007, also reported densities of a kaluta population (Thompson and Thompson, 2008). The latter study, a relocation project for a mining company, reported a density of at least 1.88 individuals per hectare; however, the authors suggested that, given not all individuals were captured and large numbers were still being captured on the final night of trapping, the density reported probably underestimated the true density of kalutas in the area. Individuals appeared to be evenly distributed across the study site (Thompson and Thompson, 2008).
The overall goal of this study was to assess male and female strategies in the wild to test if they are consistent with the kaluta having a similar life history to *Antechinus* species. Specifically, I assessed, by sex and season: capture rates; sex ratios; body condition and sexual dimorphism; and activity times, of a population of kalutas in Millstream Chichester National Park.

1.3 Methods

Study area

I conducted my study in Millstream Chichester National Park (MCNP), in the west of the Pilbara region of Western Australia (Figure 1.1a), between July 2013 and December 2014. MCNP, a 238,000 ha park, is characterised by snappy gum (*Eucalyptus leucoxphloia*) and bloodwood (*Corymbia hamersleyana*) tree steppe over spinifex (*Triodia* spp.) with shallow, porous, loamy soils. The climate in the area is semi-arid/tropical, with a wet season from November to March and a dry season from April to October. Average temperatures ranged from 28.2°C in the winter/dry months to 32°C in the summer/wet months and mean annual rainfall in the area was approximately 200 to 350 mm (Traditional Owners Millstream Park Council, 2011). During the study years, large rainfall events were recorded on individual days in January (62 mm), June (64 mm), and December 2013 (103 mm), and May 2014 (90 mm; Millstream weather station: no. 005012, Bureau of Meteorology, 2017). MCNP had permanent water sources fed by underground aquifers filled indirectly by the Fortescue River. Both wild (lightning-strike) and deliberately-lit fires (prescribed burns, arson, traditional burning) were common during the study period.

**Figure 1.1 (a)** Map of the location of Millstream Chichester National Park (MCNP) in Western Australia. Insert: MCNP with location of sampling area in square. **(b)** Satellite image of sites trapped within MCNP (white circles, site names).

Sites

I established 13 trapping grids in the far southwest corner of MCNP, in an area 10 km x 5 km (Figure 1.1a and 1.1b). Grids were selected based on their proximity to the Ranger’s Station (MCNP
Headquarters) to allow for easy monitoring of the sites, and because the area was considered to have the greatest diversity of fire ages within a small area. Three sites (Stargazers fire trail 1 and 2; and Opposite Biological Survey) were burnt by a lightning-strike fire in November 2013. Trapping was discontinued on two of these sites (Stargazers fire trail 1 and 2) and a replacement site (Stargazers) was established between them. Trapping continued as normal on ‘Opposite Biological Survey’ (Table 1.1). Therefore, most of the trapping occurred on 11 grids. The placement of grids sampled habitats of varying fire age and spinifex cover. For example, the ‘Cliff-Burnt’ site was last burnt in September 2012 and had spinifex cover of 13%, while the ‘Cliff’ site was last burnt prior to 1988 and had spinifex cover of 46% (Table 1.1). Fire age of sites was confirmed by fire records kept by the Western Australian Department of Parks and Wildlife. Vegetation and soil/substrate composition on sites varied, but not demonstrably (see Chapter 3). Sites were typically ‘paired’, but were considered to be independent.

**Sampling techniques**

I sampled during five trapping sessions, at intervals of 2-5 months. Traps were arranged in 1 ha grids (100 m x 100 m), with 36 traps per grid. Each grid consisted of six transects spaced 20 m apart, with six trapping locations at 20 m intervals along each transect. I recorded the location of each trap site using a hand-held GPS unit (Garmin GPS 60). Trapping was conducted using Elliott (9 cm x 10 cm x 33 cm) collapsible, metal box-traps baited with peanut butter, rolled oats and sardines. Bait was removed during the day, while traps were closed, and replaced after any captures. Coopex ant sand (Bayer) was lightly spread around traps to protect captured animals against ants. During summer, soaked sponges were placed in traps to provide water; while in winter, fleece bedding inside traps and plastic bags around traps were provided for warmth and insulation. Traps were placed within vegetation when available, or weighed down with rocks to minimise movement when vegetation was not available. Traps were set for five nights per site, barring exceptional circumstances such as during wildfire. In total, sampling occurred over 8100 live-trap nights.

Motion sensor cameras (Reconyx PC850 and HC550), baited using the same bait as for live-trapping, were also placed on the sites. Cameras were active for between one and six 24-hour periods, with between three and 15 cameras per site, depending on availability, for a total of 1105 camera-trap nights. Each camera was set to take three or five photos in burst sequence when the motion sensor was triggered. No delay existed before the next sequence commenced. Cameras were set on the ground with bait approximately 50 cm in front of the lens and were positioned in various arrangements, most often aimed at or near Elliott traps. The cameras were used to increase
detection rates of kalutas, particularly for sites where few to no individuals were trapped in the Elliott traps, as well as to record their activity patterns.

Table 1.1. Sampling site details for 13 sites surveyed in Millstream Chichester National Park between July 2013 and December 2014. 1 sites burnt in a fire in November 2013. * sites were discontinued after the fire and were replaced by site ‘Stargazers’. Superscript letters refer to fire-age categories: A) recently-burnt sites (fire-age <5 years); B) intermediate-aged sites (fire-age 6-16 years); and C) long-unburnt sites (fire-age >17 years).

<table>
<thead>
<tr>
<th>Site</th>
<th>Code</th>
<th>Fire-age in 2013 (years)</th>
<th>Average spinifex cover (%)</th>
<th>Latitude (S)</th>
<th>Longitude (E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opposite Biol. Survey</td>
<td>BO^A</td>
<td>&gt;25/0</td>
<td>4</td>
<td>21°36'7.49&quot;</td>
<td>117°4'38.89&quot;</td>
</tr>
<tr>
<td>Stargazers Fire trail</td>
<td>SF^A</td>
<td>12/0</td>
<td>-</td>
<td>21°36'5.4&quot;</td>
<td>117°5'45.78&quot;</td>
</tr>
<tr>
<td>Stargazers Fire trail</td>
<td>ST^A</td>
<td>12/0</td>
<td>-</td>
<td>21°36'12.24&quot;</td>
<td>117°5'40.78&quot;</td>
</tr>
<tr>
<td>Stargazers^1</td>
<td>SG^A</td>
<td>12/0</td>
<td>5</td>
<td>21°36'6.16&quot;</td>
<td>117°5'44.77&quot;</td>
</tr>
<tr>
<td>Cliff-Burnt</td>
<td>CB^B</td>
<td>1</td>
<td>13</td>
<td>21°35'13.09&quot;</td>
<td>117°5'23.06&quot;</td>
</tr>
<tr>
<td>Water Corp-C</td>
<td>WC^B</td>
<td>12</td>
<td>44</td>
<td>21°37'8.65&quot;</td>
<td>117°5'46.14&quot;</td>
</tr>
<tr>
<td>Pannawonica</td>
<td>PA^B</td>
<td>13</td>
<td>51</td>
<td>21°37'46.34&quot;</td>
<td>117°9'14.15&quot;</td>
</tr>
<tr>
<td>Snappy Gum</td>
<td>GU^B</td>
<td>13</td>
<td>57</td>
<td>21°36'34.49&quot;</td>
<td>117°7'6.64&quot;</td>
</tr>
<tr>
<td>Water Corp-B</td>
<td>WB^B</td>
<td>16</td>
<td>36</td>
<td>21°37'21.47&quot;</td>
<td>117°5'50.1&quot;</td>
</tr>
<tr>
<td>Airstrip 2</td>
<td>A2^C</td>
<td>17</td>
<td>36</td>
<td>21°37'29.71&quot;</td>
<td>117°4'54.05&quot;</td>
</tr>
<tr>
<td>Airstrip 1</td>
<td>A1^C</td>
<td>24</td>
<td>40</td>
<td>21°37'17.87&quot;</td>
<td>117°4'29.64&quot;</td>
</tr>
<tr>
<td>Biological Survey</td>
<td>BS^C</td>
<td>&gt;25</td>
<td>51</td>
<td>21°36'15.59&quot;</td>
<td>117°4'37.6&quot;</td>
</tr>
<tr>
<td>Cliff</td>
<td>CL^C</td>
<td>&gt;25</td>
<td>46</td>
<td>21°35'12.3&quot;</td>
<td>117°5'24.29&quot;</td>
</tr>
</tbody>
</table>

In addition to the fixed trapping schedules above, I trapped ‘Pannawonica’, ‘Snappy Gum’ and ‘Airstrip 2’ sites during September 2014 for one to two nights each. The breeding season of kalutas is very brief (two weeks) and changes slightly each year; hence, I aimed to coincide this sampling with the breeding season and to sample as efficiently as possible during this time. The sites were selected based on their high capture success during the July-August 2014 trapping session. In total, sampling in September occurred over 180 live-trap nights and 140 camera-trap nights.

I recorded short pes length (length of hind foot pad, from the base of the heel; to nearest 0.1 mm), tail length and width (to nearest 0.1 mm), and weight (to nearest 1 g) for each individual trapped. Many dasyurids store fat in their tail, allowing comparisons of tail width to a measure of size (e.g. pes length) to assess body condition. All captured individuals were sexed. The reproductive status of each individual was assessed by inspecting the pouch of females for the presence of pouch young (number) and to determine the condition of the pouch and nipples; or measuring the length and width (to nearest 0.1 mm) of the testes of males. Any individual above 20 g was micro-chipped.
with a *Trovan* nano passive transponder (7 mm x 1.25 mm) inserted subcutaneously between the shoulder blades. Ear notches or hair samples were collected for use in a genetic study.

**Statistical analyses**

I tested for sexual dimorphism of body size and condition using measures of pes length, tail width and body weight. First, I performed a t-test on pes length to assess body size. I then analysed the effects of sex, pes length (covariate to control for body size) and their interaction on body mass and tail width using linear models performed in R (R Core Team, 2016). I performed Fisher’s exact tests to test for significant differences from parity in sex ratios between season.

**1.4 Results**

In total, I recorded 610 captures of small vertebrates in 8100 trap nights (7.5% capture success), including 131 independent kalutas (54 male, 77 female) from 311 captures (3.8% capture success). Capture rates of individual kalutas were similar over trapping periods, while total captures of kalutas increased over the trapping study (Figure 1.2). The remaining captures were: *Pseudomys desertor* (197 captures), *Ps. hermannsburgensis* and *Ps. chapmani* (difficult to differentiate in the field; reported as a combined capture of 70), *Sminthopsis macroura* (22), *Planigale sp.* (3), *Ningaui sp.* (2), *Tiliqua multifasciata* (3) and unknown Scincidae (2).

![Figure 1.2. Percentage capture success rates of individual (grey bars) and total captures (black bars) of kalutas (*Dasykaluta rosamondae*) in different sampling periods.](image)

Capture ratios of females to males differed between seasons, with a significantly greater proportion of females captured relatively soon after young were weaned, and a ratio not significantly different to parity in the lead-up to the breeding season (Table 1.2). No males were captured after the mating season (Table 1.2).
Table 1.2. Sex ratios of male and female *Dasykaluta rosamondae* in different seasons in Millstream Chichester National Park. P-values, calculated using Fisher’s exact test, indicate whether ratios differ significantly from parity (* significant at α=0.05, ** significant at α=0.01).

<table>
<thead>
<tr>
<th>Season</th>
<th>Life event</th>
<th>Females</th>
<th>Males</th>
<th>Ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>April</td>
<td>Post-dispersal</td>
<td>25</td>
<td>10</td>
<td>1:0.4</td>
<td>0.017*</td>
</tr>
<tr>
<td>July-August</td>
<td>Pre-breeding</td>
<td>15</td>
<td>22</td>
<td>1:1.47</td>
<td>0.324</td>
</tr>
<tr>
<td>November-December</td>
<td>Pouch young; post-die-off</td>
<td>23</td>
<td>0</td>
<td>1:0</td>
<td>&lt;0.0001**</td>
</tr>
</tbody>
</table>

Body mass of captured kalutas ranged from 16 to 50 g. Adult females, excluding those carrying pouch young, weighed significantly less than adult males according to a two-sample t-test (t = -5.78, df = 58, p < 0.0001; Table 1.3). Mean body mass increased over the year, and male mass was greatest in September (the mating period). Mean female mass was greatest in November and December, when females were carrying pouch young (Table 1.3).

Table 1.3. Body weights of kalutas (*Dasykaluta rosamondae*) at different life stages: mean weight (g) ± SE with sampling size (individuals) in parentheses. Individuals are independent (not carrying pouch young), except for females in the breeding category.

<table>
<thead>
<tr>
<th>Life stage</th>
<th>Females</th>
<th>Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>Juveniles</td>
<td>25.1 ± 1.3 (22)</td>
<td>27.3 ± 0.8 (9)</td>
</tr>
<tr>
<td>Adults</td>
<td>25.4 ± 1.1 (31)</td>
<td>33.0 ± 0.8 (43)</td>
</tr>
<tr>
<td>Breeding</td>
<td>36.7 ± 0.9 (40)</td>
<td>35.6 ± 1.5 (4)</td>
</tr>
<tr>
<td>ALL</td>
<td>25.5 ± 0.8 (53)</td>
<td>32.3 ± 0.7 (53)</td>
</tr>
</tbody>
</table>

Pes lengths of nonbreeding adult males (13.48 ± 0.10, mean ± SE) were significantly larger than nonbreeding adult females (12.70 ± 0.11, t = 5.26, df = 1, n = 74, p < 0.0001). Adult males (nonbreeding and breeding) were also significantly heavier than nonbreeding females (i.e. without pouch young; Figure 1.3a) and had significantly larger tails (Figure 1.3b) for a given body size (Table 1.4), demonstrating that they were in better condition with more tail fat. The relationships between pes length and weight, and pes length and tail width were not different between males and females (Table 1.4).
Figure 1.3. Relationship between pes length and weight (a) and pes length and tail width (b) for male (black) and female (grey) adult kalutas, *Dasykaluta rosamondae*.

Table 1.4. Effect of pes length, sex, and their interaction on the body weight and tail width of breeding and nonbreeding adult male, and nonbreeding adult female kalutas, *Dasykaluta rosamondae*.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Source</th>
<th>df</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>Pes</td>
<td>1</td>
<td>22.05</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Sex</td>
<td>1</td>
<td>18.15</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Pes x Sex</td>
<td>1</td>
<td>0.00</td>
<td>0.983</td>
</tr>
<tr>
<td></td>
<td>Residual</td>
<td>74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tail width</td>
<td>Pes</td>
<td>1</td>
<td>11.59</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Sex</td>
<td>1</td>
<td>5.95</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>Pes x Sex</td>
<td>1</td>
<td>0.02</td>
<td>0.887</td>
</tr>
<tr>
<td></td>
<td>Residual</td>
<td>74</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Camera traps captured 322 independent recordings of kalutas from 1245 trap nights over the trapping period. Generally, sexes could not be determined from the images; therefore, capture reports are for all individuals recorded on camera traps. Kalutas were largely crepuscular; their activity was concentrated around dusk and dawn but varied seasonally. In summer (November/December), most activity occurred in the early morning, just after sunrise, although they were active again in the evening, just before and after sunset (Figure 1.4). Conversely, in winter (July/August), activity times were broader and more activity occurred in the afternoon. They were somewhat active in the early to mid-morning, but were predominantly active in the early to late afternoon until approximately sunset, or just after sunset (Figure 1.4). At the start of the dry/winter season (April/May), as in the summer months, individuals were most active in the morning, from sunrise and for most of the early to mid-morning. They were active again in the afternoon, from mid-
afternoon until just after sunset (Figure 1.4). In the breeding season (September, end of the dry winter season), kalutas were mostly bimodal in their activity, which was evenly split between morning and afternoon periods. Activity periods in the morning were more prolonged, and ranged from approximately sunrise until mid-morning, with activity resuming just before sunset and ceasing just after sunset (Figure 1.4).

![Figure 1.4. Activity times of kalutas Dasykaluta rosamondae in Millstream Chichester National Park during (a) April; (b) July/August; (c) September; and (d) November/December. Red lines indicate sunrise and sunset times during the sampling period.](image)

1.5 Discussion

My results confirm that kalutas share similar life history patterns with Antechinus species. I recorded trap capture rates that were similar or higher than other studies have reported for kalutas. Two studies conducted in the same location approximately 15 years apart reported different trap success. Woolley (1991) captured 30 individuals from 1156 trap nights, equating to a success rate of 2.6%, while How and Cooper (2002) reported a much lower success rate of 38 individuals from 8131 trap nights at 0.5% success. A third study by Thompson and Thompson (2008) reported the highest success rate of 4.0% from 9900 trap nights and 395 individuals, a rate more comparable to my overall rate of 3.8%. My high capture rate may be reflective of good quality habitat able to support a large population of kalutas.
Capture rates vary greatly between *Antechinus* species and habitats. For example, Banks and Dickman (2000) recorded success rates of 5.6% for *A. stuartii* in the Blue Mountains in south-eastern Australia; Leung (1999) recorded 3.1% and 4.8% using different trapping designs for *A. leo* in north Queensland; Magnusdottir *et al.* (2008) recorded 4.8% for *A. minimus* in the Otway Ranges in south-eastern Australia; and Marchesan and Carthew (2004) recorded 7.1% for *A. flavipes* in the Mt Lofty Ranges of South Australia. These high rates were all recorded in productive forest environments. Conversely, in arid Australia, Masters and Dickman (2012) recorded three-fold variation in success rates (1.5% to 0.5%) for brush-tailed mulgaras over three years in Uluru Kata Tjuta National Park and the Tanami Desert, respectively.

How and Cooper (2002) found that capture rates of kalutas increase in late summer at the time when juveniles enter the population. This timing is typical of *Antechinus* species. For example, Dickman (1989) captured high numbers of *A. agilis* in March/April after an influx of juveniles, as well as during the breeding season, likely from males searching for mates. He found lowest captures after the males had died off, between September and December. Surprisingly, I found capture success rates were greatest in August 2014 in the lead-up to the breeding season, and December 2014 after male die-off, when pouch young were approximately one month old, and captured most new individuals in August 2014. The higher rate of new individuals captured in August 2014 was likely reflective of the high trapping effort during that trapping event. However, the high success rates, which factored in trapping effort, in August and December 2014 were potentially due to increased activity from males in preparation for the breeding season, and high activity from females searching for food in order to care for their young.

Sex ratios of both adult (captures) and pouch young in *Antechinus* species have frequently been studied with a view to understanding male die-off. For example, Fisher *et al.* (2006a), in their study of *A. agilis*, found a pouch sex ratio that was not significantly different from parity (1.2 males: females). Similarly, Recher *et al.* (2009), in a long-term study running from 1975 to 2005, recorded sex ratios of captures that were close to parity for both *A. agilis* (0.91 males: females) and *A. swainsonii* (0.97 males: females). Cockburn *et al.* (1985) studied three species of *Antechinus* and found sex ratios in pouches varied between the species. *A. stuartii* had female-biased ratios, *A. flavipes* had ratios close to parity and *A. swainsonii* had male-biased ratios. Cockburn *et al.* (1985) also found sex ratios were positively correlated with the degree of iteroparity in females in the population: there were more male-biased litters in populations with more females breeding in their second year. In my study, I found sex ratios of independent (i.e. free-living, non-pouch young)
kalutas varied seasonally, switching from a significant female bias after juveniles were weaned, to a non-significant male bias in the lead-up to the breeding season, and then to solely females after the breeding season, confirming the death of all adult males. Hence my study documents the seasonal changes in sex ratio expected under male semelparity.

Male kalutas were significantly larger than females in pes length and body mass, and were also in significantly better condition. Masters and Dickman (2012) reported condition in brush-tailed mulgaras over different seasons and noted similar trends between the two sexes. In a review of dasyurid reproductive biology, Taggart et al. (2002) found that males had higher body mass in analyses based on 35 dasyurid species, with females heavier in only one species (*Planigale ingrami*) and three others displaying no sexual dimorphism in body mass (*Pl. gilesi*, *Sminthopsis crassicaudata*, and *S. macroura*). The greatest dimorphism found was in *A. leo*, where males were nearly twice as large as females. The review by Taggart et al. (2002) reported male kalutas were 1.3 times as heavy as female kalutas. My results reflect this finding of male-biased sexual size dimorphism but suggest that the dimorphism for pes length (1.1) is less than that for body weight (1.3). This is borne out by the greater relative weight of the males (Figure 1.3a), which seems to reflect increased muscle bulk or body fat stores, alongside the increased fat storage in the tail of males evident in Figure 1.3b.

Kalutas were cathemeral, but their peak activity times changed seasonally. While the peak activity time differed, the kaluta’s ability to change activity times depending on season was similar to most *Antechinus* species. In general, kalutas were largely crepuscular, although during winter this switched to being more diurnal than at other times of the year. Pavey et al. (2016) found a clear shift to diurnal activity during winter in kalutas, and suggested that the change was to avoid temporal overlap with brush-tailed mulgaras, a known predator. As mulgaras are absent from my study area, this hypothesis does not explain the kalutas’ change in activity times. The switch may be made to avoid other nocturnal predators, such as owls, and possibly bats and bush stone-curlews. However, as Pavey et al. (2016) highlighted, kalutas are unlikely to temporally avoid avian species, as these predators are unable to extract prey from the tightly-packed spinifex hummocks that kalutas inhabit. Furthermore, a switch to avoid nocturnal avian species would increase temporal overlap with diurnal avian species. The temporal switch may be a consequence of evolving in the presence of quolls and mulgaras, even if these predators no longer spatially overlap across the kalutas’ entire range. However, it is more likely that a switch in activity time was associated with thermoregulation, as suggested by Fisher et al. (2011) for *Antechinus* species.
My results reveal sex differences in condition, as well as confirm the sexual dimorphism in weight that was already known, and also reveal seasonal variability in the activity of this species. By and large, these results suggest that kalutas have many life history traits in common with *Antechinus* species. Kalutas also resemble other arid-zone dasyurids in their physiological characteristics, including a thermolabile body temperature, low basal metabolic rate, low evaporative water loss, and use of daily torpor (Withers and Cooper, 2009). Masters and Dickman (2012) suggested that brush-tailed mulgaras, a slightly larger marsupial predator with a broad range across inland Australia, were able to persist in central Australia due to their flexible diet, use of torpor and ability to tolerate substantial changes in body condition, and use of deep burrows. Kalutas may owe their success in a changing environment to similar traits.
Chapter 2

Multiple paternity and male semelparity confirmed in an arid-zone dasyurid, *Dasykaluta rosamondae*
2.1 Abstract

Approximately 20% of dasyurids exhibit male semelparity (die-off) – where males die after their first breeding season but females may breed in more than one season. Both sexes in some semelparous dasyurid species have been shown to be highly promiscuous, and this may be linked to the evolution of semelparity through sperm competition, which is indicated by testes size. We assessed whether the kaluta (*Dasykaluta rosamondae*), a small dasyurid in the Pilbara region of Western Australia, has obligate male semelparity, multiple paternity within litters and positive testis allometry. We used mark-recapture methods and morphometric measurements to assess semelparity and testis allometry in wild-captured males. We used microsatellite markers to estimate paternity of wild-captured individuals based on ear clips from eight different mothers, and small biopsies from the tip of the tail of each of their seven or eight pouch-young. We documented complete die-off of males. Multiple paternity was confirmed in seven of eight pouches, where either two or three fathers sired young. The seven young in the remaining pouch appeared to be sired by one male. Possible fathers could only be confidently assigned to two full pouches. Intraspecific positive testis allometry was demonstrated in adult males. Our data indicate a significant role for sperm competition in the mating system of kalutas.
2.2 Introduction

Obligate male semelparity, or physiological die-off, is a phenomenon reported in the dasyurid genera *Antechinus* (15 species), *Phascogale* (three species) and *Dasykaluta* (one species). The syndrome associated with semelparity results from elevated levels of corticosteroid hormones that cause immune system suppression, which in turn result in demodectic mange, haemorrhaging and infections, and eventually death (Braithwaite and Lee, 1979; Woolley, 1966; Fisher et al., 2013; Lazenby-Cohen and Cockburn, 1988; Woolley, 1991). Incomplete die-off, or facultative semelparity, has been documented in two species, *Dasyurus hallucatus* and *Parantechinus apicalis*, where males are capable of surviving to a second breeding season if sufficient resources are available. The remaining species exhibit obligate semelparity, with all males in the population dying after each breeding season (Fisher et al., 2013; Krajewski et al., 2000). A recent hypothesis for the evolution of semelparity is intense sexual selection that arises from female multiple mating (polyandry; Fisher et al., 2013).

Polyandry (females mating with multiple males during one reproductive cycle) occurs in a wide range of mammal species, and often results in litters sired by more than one male (Beasley et al., 2010; Glen et al., 2009; Holleley et al., 2006; Kraaijeveld-Smit et al., 2002c, 2003; Parrott et al., 2005). It can provide several advantages to both the female and her offspring. These include reducing the risk of incompatibility between mates (such as minimising inbreeding), increasing genetic diversity within litters, or ensuring ‘good/sexy sperm’ fertilise the eggs (Fisher et al., 2006a, 2006b; Glen et al., 2009; Holleley et al., 2006; Kraaijeveld-Smit et al., 2002b; Parrott et al., 2005; Simmons, 2005). When females mate polyandrously, males will be subject to intense postcopulatory sexual selection due to sperm competition (Parker, 1970; Birkhead and Möller, 1998). It is this intense sperm competition, where males invest intensive effort during the short mating season, that is thought to give rise to semelparity (Fisher et al., 2013).

Precopulatory sexual selection is expected to generate the common pattern in marsupials (and mammals more generally) of sexual size dimorphism, in which males are the larger sex (Fisher and Cockburn, 2005; Holleley et al., 2006; Kraaijeveld-Smit et al., 2002b, 2002c, 2003; Miller et al., 2010; Parrott et al., 2005; Shimmin et al., 2000; Taggart et al. 2003). Postcopulatory competition among males is expected to place selection on male traits such as sperm production and testes mass (Parker, 2016). It has been shown previously among mammals, including marsupials, that postcopulatory sexual selection is associated with large testes relative to body size (Harcourt et al., 1995; Kenagy and Trombulak, 1986; Short, 1979; Soulsbury, 2010). There has also been a
longstanding interest in the within-species allometric scaling of traits subject to sexual selection (Bonduriansky, 2007; Bonduriansky and Day, 2003; Fromhage and Kokko, 2014; Huxley, 1932; Petrie, 1988; Simmons and Tomkins, 1996). These studies suggest that condition-dependent expression of costly traits that increase male reproductive success can often evolve to be positively allometric, the phenomenon where males in better condition (reflected as larger size) invest relatively more in the trait than males in poorer condition (Fromhage and Kokko, 2014). However, while the allometry of secondary sexual traits has been frequently investigated, relatively little is known about the intraspecific allometry of testis size (Hosken et al., 2005; Simmons and Emlen, 2006; Woodall and Johnstone, 1988).

In this study, we examined evidence of sperm competition in the mating system and life history of the kaluta (Dasykaluta rosamondae). The kaluta is a small (20-40 g), sexually dimorphic marsupial common in the Pilbara region of Western Australia. Male kalutas, the larger sex, are hypothesised to be obligately semelparous and die after a short, frenzied breeding season (Woolley, 1991). Males kept in a laboratory become reproductively senescent after the breeding season and most die before birth of the young. There is complete mortality of wild males expected prior to weaning of the young (Woolley, 1991). Females are monoestrous, capable of breeding in up to two breeding seasons, and produce litters of up to eight young per year (Woolley, 1991). It has been hypothesised that, like antechinus species, female kaluta may be capable of storing spermatozoa for several days prior to fertilisation (Woolley, 1991), suggesting potential for sperm competition. In this study, we had three aims: i) to confirm the presence of obligate semelparity in a wild population of kalutas using mark-recapture methods; ii) to confirm multiple paternity within litters, by sampling mothers and their pouch young, and assigning paternity using microsatellite markers; and iii) to assess testis allometry in adult males. We expected: i) that males would be obligately semelparous in the wild; ii) litters would be sired by multiple males; and iii) if males are semelparous and females polyandrous, we predicted strong selection on sperm production to have generated positive allometric scaling of testis mass with body mass, whereby larger males invest disproportionately more in their testes.

2.3 Methods

Sampling techniques

Our study was conducted in Millstream Chichester National Park (MCNP; 21°35’28.93” S 117°40’21.11” E; Figure 2.1), in the west of the Pilbara region of Western Australia (WA), approximately 100 km SE of Karratha. Trapping grids were established in the far southwest corner of MCNP. We established 11 grids in a 10 km x 5 km area. Two of these sites were sampled for the
paternity study (sites A and B, Figure 2.1b). Traps were arranged in 1 ha grids (100 m x 100 m), with 36 traps per grid spaced 20 m apart. Trapping was conducted using Elliott (9 cm x 10 cm x 33 cm) collapsible, box-metal traps baited with universal bait (peanut butter, rolled oats and sardines). Sampling was carried out during five trapping sessions, at intervals of typically 2-3 months, from July 2013 to December 2014. Traps were set for five nights at each site, barring exceptional circumstances such as wildfires. Tissues of pouch-young used in the paternity study were collected during December 2014.

Figure 2.1. (a) Google map of the location of Millstream Chichester National Park (MCNP) in Australia. Insert: Google image of MCNP with location of sampling area in square. (b) Google Earth image of sites trapped in MCNP (circles), including sites used for paternity study (triangles, with site identifiers “A” and “B”).

Morphometric data were recorded for each individual, including body mass (to nearest 1 g). All captured individuals were aged and sexed. The reproductive status of each individual was assessed by inspecting the pouch of females for the presence of pouch young (number and crown-rump length measurements, to nearest 0.1 mm using calipers) and to determine the condition of the pouch and nipples (which was also used to age individuals and assess previous breeding events); or by measuring the length and width (to nearest 0.1 mm using calipers) of the testes of males. Any individual above 20 g was microchipped with nano (7 mm x 1.25 mm) passive integrated transponders (Trovan) inserted subcutaneously along the dorsal midline between the shoulder blades.

We sampled small ear notches or hair samples from juveniles and adults. Males were assessed as being adults from July onwards (8 months old). For the paternity component, small biopsies from the tip of the tail of each pouch young from eight known mothers were sampled. Males on the sites were sampled opportunistically prior to the breeding season, in an attempt to identify as many potential fathers as possible. Tissue samples were stored in 80% ethanol for genetic analysis.
Genetic analysis

DNA was extracted from tissue samples using a salting-out procedure similar to Sunnucks and Hales (1996), with minor adjustments. These included adding 10µL (not 100µg/mL) Proteinase K to the sample tubes containing TNES; incubating samples overnight at 56°C to digest tissue (rather than 37°C for 3-18 hours); vortexing samples and then iring them for 5 minutes after the 5M NaCl had been added to the digested tissue samples; centrifuging samples to pellet DNA and transferring the supernatant to a new tube twice rather than once; and resuspending the DNA in 20-100µL sterile water (rather than 20-50µL). Once extracted, DNA quality was tested using a NanoDrop ND-1000 spectrophotometer (ThermoFisher Scientific).

Genotypes were scored at 12 microsatellite loci (Table 2.1), using fluorescently-labelled primers cross-amplified from closely related species (Antechinus agilis – Banks et al., 2005; Dasyurus geoffroii – Spencer et al., 2007; Dasyurus spp. – Firestone, 1999; Parantechinus apicalis – Mills and Spencer, 2003; Sarcophilus laniarius – Jones et al., 2003). PCR conditions for seven of the 12 loci were as follows: 15 mins at 95°C, 35 cycles of 30 secs at 94°C, 1.5 mins at 52°C and 1 min of 72°C, followed by 30 mins at 60°C. PCR conditions for one of the loci differed only in the annealing temperature, where it was 56°C instead of 52°C. PCR conditions for the final four loci differed in the number of repeats of the original cycle (40 cycles instead of 35). PCR products were run on a 3730XL capillary sequencer (Applied Biosystems), against the size standard GeneScan-500 LIZ. Allelic sizes were scored using a GS filter set and analysed using GeneMarker, version 1.91 (Soft Genetics).

Statistical analyses

We used the eight loci in Hardy-Weinberg Equilibrium to determine paternity. The number of sires per litter was assigned using the program GERUD 2.0 (Jones, 2005). Due to computational limitations, we selected 5-7 loci for each progeny array to ascertain the likely number of sires. Collective expected exclusion probabilities for the loci were estimated to be 0.9988 for seven loci (Table 2.1), 0.9984 for six loci (minus pDG1A1) and 0.9963 for five loci (minus pDG1A1 and pPa9D2; Table 2.1). Simulations were carried out in GERUDsim2.0 to determine the probability that the number of sires assigned to a progeny was the actual number of sires. We used the program Cervus (Kalinowski et al., 2007) to assign candidate fathers for each offspring, using all eight loci.

Scrotal volume, assuming an oblate spheroid shape of the testes, and testes mass were estimated using the formulas described by Schwab (2000). We analysed the log-log relationship between body mass and testes mass using reduced major axis (RMA) regression with the program
SMATR (Warton et al., 2006). This approach allows the comparison of groups similar to an ANCOVA using model II regression.

Table 2.1. Characteristics of 12 microsatellite loci genotyped for kalutas Dasykaluta rosamondae in Millstream Chichester National Park. Characteristics included number of alleles identified at each locus, observed heterozygosity ($H_0$), Bonferroni-corrected significance values for Hardy-Weinberg Equilibrium (HWE, where $p$-values of $<0.004$ were significant), frequency of null alleles, expected exclusion probabilities and species for which each microsatellite marker was originally designed.

<table>
<thead>
<tr>
<th>Locus</th>
<th>No. alleles</th>
<th>$H_0$</th>
<th>HWE</th>
<th>Frequency null alleles</th>
<th>Expected exclusion</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aa4a</td>
<td>9</td>
<td>0.701</td>
<td>0.0159</td>
<td>0.0582</td>
<td>0.5791</td>
<td>A. agilis</td>
</tr>
<tr>
<td>pPa9D2</td>
<td>10</td>
<td>0.696</td>
<td>0.0770</td>
<td>0.0548</td>
<td>0.5680</td>
<td>P. apicalis</td>
</tr>
<tr>
<td>pPa4B3</td>
<td>9</td>
<td>0.858</td>
<td>0.5804</td>
<td>-0.0191</td>
<td>0.6620</td>
<td>P. apicalis</td>
</tr>
<tr>
<td>pPa2B10</td>
<td>10</td>
<td>0.755</td>
<td>0.2396</td>
<td>0.0403</td>
<td>0.6333</td>
<td>P. apicalis</td>
</tr>
<tr>
<td>3.1.2</td>
<td>16</td>
<td>0.808</td>
<td>0.8380</td>
<td>0.0315</td>
<td>0.7352</td>
<td>Dasyurus spp.</td>
</tr>
<tr>
<td>pDG1A1</td>
<td>5</td>
<td>0.458</td>
<td>0.8591</td>
<td>-0.0137</td>
<td>0.2211</td>
<td>D. geoffroii</td>
</tr>
<tr>
<td>Sh3o</td>
<td>15</td>
<td>0.850</td>
<td>0.5109</td>
<td>0.0087</td>
<td>0.7346</td>
<td>S. laniarius</td>
</tr>
<tr>
<td>pDG1H3</td>
<td>9</td>
<td>0.735</td>
<td>0.7692</td>
<td>0.0338</td>
<td>-</td>
<td>D. geoffroii</td>
</tr>
<tr>
<td>pDG5G4</td>
<td>2</td>
<td>0.015</td>
<td>-</td>
<td>-0.0005</td>
<td>-</td>
<td>D. geoffroii</td>
</tr>
<tr>
<td>3.3.1</td>
<td>12</td>
<td>0.722</td>
<td>0.0003**</td>
<td>0.0592</td>
<td>-</td>
<td>Dasyurus spp.</td>
</tr>
<tr>
<td>3.3.2</td>
<td>16</td>
<td>0.368</td>
<td>$&lt;0.0001^{***}$</td>
<td>0.4001</td>
<td>-</td>
<td>Dasyurus spp.</td>
</tr>
<tr>
<td>pDG7F3</td>
<td>12</td>
<td>0.422</td>
<td>$&lt;0.0001^{***}$</td>
<td>0.3324</td>
<td>-</td>
<td>D. geoffroii</td>
</tr>
</tbody>
</table>

2.4 Results

No male kalutas survived between the mating season (September) and the birth of young two months later (November) in 2013 or 2014 (Table 2.2). Nearly a quarter of females (22%) survived after the breeding season and parturition of young, until the following mating season 12 months later (Table 2.2). Individual females were captured in all five trapping events between July 2013 and December 2014, but males were not captured in the November 2013 or December 2014 trapping events, after birth of young, in 2376 trap nights (Figure 2.2). Recaptures of males were only recorded in the August 2014 trapping event, after males re-entered the population in February or March and were marked in April 2014. Three females were recorded breeding at 2 years of age, with two of those confirmed to have given birth the previous year (i.e. breeding in two consecutive years). Of the 46 females captured during November and December, only three were recorded without pouch young and two of those likely had not yet given birth (i.e. sampled prior to giving birth).
Table 2.2. Survival of male and female Dasykaluta rosamondae between years from a mark-recapture study conducted in Millstream Chichester National Park between July 2013 and December 2014.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Number individuals captured</th>
<th>Number recaptures</th>
<th>% survival post-breeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>77</td>
<td>17</td>
<td>22%</td>
</tr>
<tr>
<td>Male</td>
<td>49</td>
<td>0</td>
<td>0%</td>
</tr>
</tbody>
</table>

From site A, we sampled tissue for genetic analysis from two pouches: one pouch contained seven young and the other contained eight young. Five males were trapped on this site throughout the year from post-weaning until the pre-breeding season, and were therefore considered candidate fathers. Of these five candidate fathers, three were confidently assigned as sires in the two litters, and the Cervus program successfully assigned a father for every young in these pouches. From site B we sampled six pouches, three with seven young and three with eight young. We identified nine candidate males in this site prior to the paternity study, and two males were confidently assigned as fathers (four out of 45 young; Table 2.3). One male sired the majority of the young in a pouch, and the remainder of the young (generally one or two individuals) were sired by a second or third male (Table 2.3). The results of the simulations suggested that we could be confident when assigning two or three males as sires of each young (98% and 70-81% chance, respectively, of correctly estimating the true number of sires), but we could not be confident when assigning four males as sires (5-19% chance of correctly estimating the true number of sires; Table 2.4).

Figure 2.2. Number of captures and recaptures of male and female Dasykaluta rosamondae per trapping event between July 2013 and December 2014 in Millstream Chichester National Park.
Table 2.3. The number of pouch young sired by different male kalutas *Dasykaluta rosamondae* from two sites in Millstream Chichester National Park. (A) and (B) refer to the two sites sampled.

<table>
<thead>
<tr>
<th>Pouch (site)</th>
<th>Number of pouch young</th>
<th>Number of sires</th>
<th>Number of offspring sired by each male</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Male 1</td>
<td>Male 2</td>
</tr>
<tr>
<td>P19 (B)</td>
<td>7</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>P18 (B)</td>
<td>7</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>3F (A)</td>
<td>7</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>6F (A)</td>
<td>8</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>P14 (B)</td>
<td>8</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>P25 (B)</td>
<td>8</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>P35 (B)</td>
<td>7</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>P29 (B)</td>
<td>8</td>
<td>3</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 2.4. Probability of correctly estimating the true number of sires for each litter. Offspring is the number of young in the litter, Loci is number of loci used to estimate probability, Sires is the number of sires per litter, Iterations is the number of iterations used for the simulation, Sires correct is the number of iterations when the correct number of sires were assigned, and Estimated sires < real is the number of iterations where the estimated number of sires was less than the real number.

<table>
<thead>
<tr>
<th>Offspring</th>
<th>Loci</th>
<th>Sires</th>
<th>Iterations</th>
<th>Sires correct</th>
<th>Estimated sires &lt; real</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>5</td>
<td>2</td>
<td>1000</td>
<td>98.3%</td>
<td>0.6%</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>3</td>
<td>1000</td>
<td>70.4%</td>
<td>27.2%</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>4</td>
<td>1000</td>
<td>4.8%</td>
<td>93.2%</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>2</td>
<td>1000</td>
<td>97.9%</td>
<td>0.5%</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>3</td>
<td>1000</td>
<td>81.1%</td>
<td>17.2%</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>4</td>
<td>1000</td>
<td>18.9%</td>
<td>78.9%</td>
</tr>
</tbody>
</table>

The slope of regression line of body mass versus testes mass for adult male kalutas was 1.45 (1.178/1.780; 95% CI), $r^2 = 0.61$, $p < 0.001$, $n = 39$ (Figure 2.3). The slope was significantly greater than one ($F = 13.7$, df = 38, $p = 0.001$; Figure 2.3).
Figure 2.3. Log body mass versus log estimated testes mass (g) in adult male kalutas *Dasykaluta rosamondae*. Known sires are black, other males are grey. The dotted line is the regression line.

2.5 Discussion

Our results confirm that male kalutas are obligately semelparous in the wild. In a laboratory study, Woolley (1991) noted that most males died soon after the mating period in September, before females gave birth two months later, and predicted that wild populations would also have complete male die-off prior to weaning of the young. We observed complete male die-off before birth of the young, suggesting males died sometime between the end of September and the start of November. This is identical to the schedule of male mortality in species of *Antechinus* and *Phascogale* (Braithwaite and Lee, 1979; Woolley, 1966). Fisher and Blomberg (2011a) suggested that in smaller *Antechinus* species females also tend towards semelparity. For example, in *A. stuartii* and *A. agilis* that weigh 18 to 20 g, nine to 13% of females survive to breed a second time. In the larger species *A. subtropicus*, *A. swainsonii* and *A. flavipes* that weigh 30 to 40 g, 17 to 26% of females survive to breed a second time. Consistent with this pattern, we found that female kalutas, which weigh around 30 g on average, had 22% post-first breeding season survival rate, and were able to reproduce in at least two breeding seasons.

Our results also support the expectation that kalutas would be polyandrous, like other semelparous and sexually-dimorphic dasyurid species and other animals generally (Holleley *et al.*, 2006; Kraaijeveld-Smit *et al.*, 2002c; Lazenby-Cohen and Cockburn, 1988; Parrott *et al.*, 2005; Shimmin *et al.*, 2000; Wooller *et al.*, 2000). Seven of eight pouches were characterised by multiple paternity. As in previous studies of paternity success in dasyurids with die-off, we observed a skew in the number of offspring sired by each male in a pouch, where one of the sires dominated paternity...
of the litter (e.g. Holleley et al., 2006). In previous research, biased paternity success has been due to both last-male precedence, and individual differences in male sperm competitiveness. In captive *A. agilis*, mating order played an important role in paternity success, because last-males sired 70% of offspring in a litter (Kraaijerveld-Smit et al., 2002b; Shimmin et al., 2000). In an experiment using *A. stuartii*, the proportion of offspring sired by a male in competition was correlated with the survival of that male’s offspring when he was the only mate of another female, showing that male quality and competitiveness under sperm competition was the main source of paternity bias (Fisher et al., 2013). This highlights the importance of postcopulatory sexual selection to male reproductive success.

Sperm competition that results from polyandry is expected to impose strong selection on testis size and structure (Harcourt et al., 1981, 1995; Kenagy and Trombulak, 1986; Parker, 1970, 2016; Ramm and Schärer, 2014; Short, 1979). Our results demonstrate that the testes of male kalutas show positive allometry. This would be the expected pattern if testis tissue is costly and only males in better condition can afford these costs (Fromhage and Kokko, 2014). If testis size reliably reflects sperm production, larger male kalutas may have a significant advantage in sperm competition as well as in other forms of male-male competition. In interspecific studies, a pattern of larger testes relative to body mass in multiply-mating species compared to monandrous species has been demonstrated in numerous mammal groups (Harcourt et al., 1995; Soulsbury, 2010), including marsupials (Rose et al., 1997), and dasyurids specifically (Taggart et al., 2003). However, our results provide evidence for intraspecific positive allometry. This appears to be an unusual case in adult mammals (Oosthuizen and Miller, 2000; Woodall and Johnstone, 1988; Yurkowski et al., 2011) suggesting that the extreme mating system has exerted strong condition dependent sexual selection on testis size through sperm competition (Hosken and Ward, 2001; Simmons and Emlen, 2006).

We were unable to confidently assign male sires to all offspring, particularly from one of the sites. This may reflect insufficient trapping effort to sample all potential sires prior to the breeding season, despite 432 trap nights per site between April (post-weaning) and September (just prior to the breeding season). However, it may also reflect high mobility in males, which appear to travel to new sites during the breeding season to mate. Previous studies, such as those conducted by Lazenby-Cohen and Cockburn (1988) and Fisher et al. (2011), found males of several species of *Antechinus* were also highly mobile during the mating season. Fisher et al. (2011) suggested these species probably employ the mating strategy ‘scramble polygyny’. If kalutas employ a similar strategy, it may help to explain why we were unable to sample the appropriate males as potential
sires prior to their post-mating mortality. The ability to move long distances may relate to the availability of continuous suitable habitat, with site B seemingly having more suitable surrounding habitat than site A. This may help to explain why more sires were sampled on site A than site B.

In conclusion, we have demonstrated that kalutas exhibit (i) obligate male semelparity; (ii) multiple paternity within litters (polyandry) and (iii) an unusual positive intra-specific testis allometry. Collectively these finding strongly support the hypothesis that polyandry and sperm competition are strong drivers of the evolution of the kaluta mating system.
Chapter 3

Fire and decreased *Triodia* cover negatively affect the small, Pilbara region endemic dasyurid *Dasykaluta rosamondae*
3.1 Abstract

Excessive fire frequency is a key threatening process for many small mammals in northern Australia. We assessed the impact of fire frequency on a small dasyurid marsupial, the kaluta (*Dasykaluta rosamondae*), in the semi-arid Pilbara of Western Australia by trapping 11 grids of differing fire age and following the occupancy of sites burnt during a two-year field study. We assessed the cover of spinifex (*Triodia* spp.), the preferred habitat of kalutas, in relation to fire age over three years of sampling. Sites of the same fire age did not necessarily exhibit similar percentages of spinifex cover. Kalutas are specialists of long unburnt habitat. They had larger populations on sites not burnt for at least ten years, and that had spinifex cover higher than 25%. This knowledge will help managers to plan burning to retain patches of spinifex for small mammal conservation, as this region has frequent natural and deliberately-lit fires, and may experience more frequent and more intense fires with a warming, drying climate.
3.2 Introduction

Frequent fire is a key threatening process in many environments worldwide (Pastro et al., 2011; Recher et al., 2009). High frequency and intensity of fire, and early dry season timing of fires are known to negatively impact biodiversity, including birds, reptiles and mammals (Chia et al., 2016; Kelly et al., 2015). The mechanisms include destruction of vegetation, loss of seasonal refuge habitat, food and shelter, and increase of competition and risk of predation, especially from introduced predators (Chia et al., 2016; Kelly et al., 2010; Nimmo et al., 2014; Pastro et al., 2011). For example, feral cats (Felis catus) have been shown to preferentially select recently burnt habitat, known to support high abundance of small mammals, for hunting (McGregor et al., 2014); while, hot, late dry season fires have been shown to remove cover and increase the vulnerability of small mammals to predators (Leahy et al., 2015). Fire affects many small mammals, with species reacting differently to post-fire changes in their environments (e.g. How and Cooper, 2002; Recher et al., 2009). In Australia, inappropriate fire regimes have been identified as the second most common threat to terrestrial mammals, after feral cats (Woinarski et al., 2014).

Fire is often used as a management tool globally (Kelly et al., 2010, 2012, 2015). In Australia, deliberate burning to create mosaics of heterogeneous fire age patches is the favoured strategy in the arid zone and tropical savannas (Kelly et al., 2011, 2012, 2015; Law and Dickman, 1998; Letnic and Dickman, 2005). Patchy mosaics may allow individuals to acquire different resources from patches of differing fire age (Allan and Southgate, 2002; Chia et al., 2016; Letnic and Dickman, 2005). Fire experiments across the fire-prone north of Australia have collectively shown that hot, late dry season fires are particularly detrimental for the persistence of small mammals, with no small mammals benefiting from frequent fires (e.g. Pardon et al., 2003; Legge et al., 2011b). These experiments show that patchy mosaics of varying fire age increase small mammal survival because the small-scale, cool fires lower the risk of large-scale, intense wildfires late in the dry season by providing a decreased fuel load and natural fire breaks (Burrows and Christensen, 1990; Greenville et al., 2009; Legge et al., 2011b; Letnic and Dickman, 2005). This highlights the importance of determining the timing and extent of fire regimes to maintain habitat for small mammal conservation in the Australian arid zone (Greenville et al., 2009; Kortner et al. 2007).

As well as spatial distribution of habitat patches, climate is also a key determinant of species composition, richness and abundance of small mammal assemblages (Kelly et al., 2011, 2012). Letnic and Dickman (2005) suggested that rainfall is more influential in determining mammal assemblages than fire. Rainfall affects the capture rates of many small mammals (e.g. Letnic and Dickman, 2010;
Letnic et al., 2005, 2011). Rainfall affects primary productivity, in turn affecting availability of food such as seeds and invertebrates (Kelly et al., 2011, 2012), often leading to boom and bust population fluctuations in mammals in arid zones (Letnic et al., 2005, 2011; Letnic and Dickman, 2010).

In arid Australia, most fires are ignited by lightning strikes, and are rarely controlled due to the remoteness or inaccessibility of the landscape (Burrows et al., 1991, 2009; Greenville et al., 2009). This results in large areas being burnt, which contrasts with historical Aboriginal practices of smaller-scale burning (Burrows et al., 2009; Greenville et al., 2009; Letnic and Dickman, 2005; Bird et al., 2013). Consequently, recent regimes have seen a trend towards pyric homogeneity rather than the fine-scale mosaics characteristic of indigenous burning regimes (Greenville et al., 2009).

Spinifex (Triodia spp.) grasslands cover over 1.3 million km$^2$ in Australia, including ~43% of Western Australia’s land surface area (Burrows et al., 2009; Department of the Environment and Energy, 2007). Spinifex grasslands are highly flammable and fires can spread rapidly across large areas. Fuel loads increase after large rainfall events (Greenville et al., 2009), and with increasing time since fire, until maximum load is reached at approximately 20 years (Burrows et al., 2009; Haslem et al., 2011; Kelly et al., 2010). Old growth vegetation is thought to be beneficial for small mammals inhabiting spinifex grasslands. Dense spinifex provides shelter and protection from predation for small mammals and is a key habitat requirement for many small mammals inhabiting semi-arid environments (Lawes et al., 2015; Letnic and Dickman, 2005; Letnic et al., 2005; Oakwood, 2000).

We determined the effect of fire age on the persistence of the kaluta (Dasykaluta rosamondae) in spinifex grasslands in the Pilbara region of Western Australia. Kalutas are small (20-40g), cathemeral dasyurids, with obligate male semelparity. They are widespread and abundant throughout the broader Pilbara region of Western Australia (Burbidge, 2016), and are restricted to spinifex grasslands. Several authors have suggested that they prefer long unburnt habitat, as they have captured kalutas in areas with large spinifex hummocks (How and Cooper, 2002; Withers and Cooper, 2009; Woolley, 1991). In a review of the impact of fire regime on small vertebrates in temperate Australia, Friend (1993) argued that species that are highly specialised in their life histories, particularly those that rely on particular habitat requirements and have seasonal, highly synchronised breeding seasons, are likely to be particularly negatively affected by intense fires. We aimed to quantify the association between time since the last fire and spinifex cover in the habitat used by kalutas at Millstream Chichester National Park, to quantify the association between spinifex cover and the abundance and survival of kalutas, and to determine what fire age is needed for local
persistence of kalutas at a site. Given that kalutas are a seasonally breeding species dependent on spinifex grasslands, we expected that:

i) spinifex cover would reflect time since fire;

ii) kaluta abundance would increase with spinifex cover; and

iii) kalutas would have lower abundance in recently burnt sites compared with longer unburnt sites.

3.3 Methods

Study area

Millstream Chichester National Park (MCNP), in the west Pilbara region of Western Australia, is a 238,497 ha national park, characterised by Eucalyptus leucophloia and Corymbia hamersleyana tree steppe over spinifex (Triodia spp.) on shallow, porous, loamy soils (Figure 3.1a). The climate is semi-arid-tropical, with a wet season from November to March and a dry season from April to October. Average temperatures ranged from 28.2°C in the winter/dry months to 32°C in the summer/wet months and mean annual rainfall in the area was 200 to 350 mm (Traditional Owners Millstream Park Council, 2011). MCNP has permanent water sources fed by underground aquifers filled indirectly by the Fortescue River. Both wild (lightning-strike) and deliberately-lit fires (prescribed burns, arson, traditional burning) were common during the study period, burning approximately 60% of the park and 50% of our study sites during the study period.

Grids were established in the far southwest corner of MCNP, in an area 10 km x 5 km (Figure 3.1a and 3.1b). Thirteen grids were trapped during the study period. Time since the last fire on each grid ranged from 1.3 to greater than 28 years, based on records of the Western Australian Department of Parks and Wildlife. Due to wildfire, eleven grids formed the main component of the trapping (Table 3.1; for further details, see Chapter 1).

Figure 3.1 (a) Map of the location of Millstream Chichester National Park (MCNP). Insert: MCNP with location of sampling area in square. (b) Satellite image of sites trapped (white circles, site names).
Table 3.1. Sampling site fire history at 13 sites surveyed in Millstream Chichester National Park between July 2013 and November 2016. >25/0, 12/0 indicate sites that burnt during this study. * sites burnt during the study and became site SG. Superscript numbers refer to fire age categories: 1) recently burnt sites (0-5 years); 2) intermediate age sites (6-16 years); and 3) long unburnt sites (≥17 years). Subscript letters refer to Triodia cover categories: A) low cover sites (≤35%); B) intermediate cover sites (36-45%); C) high cover sites (≥46%).

<table>
<thead>
<tr>
<th>Site</th>
<th>Code</th>
<th>Initial fire age (years)</th>
<th>Fire during study</th>
<th>Final age (years)</th>
<th>Most recent fire year (previous fire year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opposite Biol. Survey</td>
<td>BO\textsuperscript{1\textsubscript{a}}</td>
<td>&gt;25/0</td>
<td>Nov. 2013</td>
<td>3</td>
<td>2013 (pre-1988)</td>
</tr>
<tr>
<td>Stargazers Fire trial 1</td>
<td>SF*</td>
<td>12/0</td>
<td>Nov. 2013</td>
<td>3</td>
<td>2013 (2001)</td>
</tr>
<tr>
<td>Stargazers Fire trial 2</td>
<td>ST*</td>
<td>12/0</td>
<td>Nov. 2013</td>
<td>3</td>
<td>2013 (2001)</td>
</tr>
<tr>
<td>Stargazers</td>
<td>SG\textsuperscript{1\textsubscript{a}}</td>
<td>12/0</td>
<td>Nov. 2013</td>
<td>3</td>
<td>2013 (2001)</td>
</tr>
<tr>
<td>Cliff-Burnt</td>
<td>CB\textsuperscript{1\textsubscript{b}}</td>
<td>1</td>
<td>-</td>
<td>4</td>
<td>2012 (pre-1988)</td>
</tr>
<tr>
<td>Water Corp-C</td>
<td>WC\textsuperscript{1\textsubscript{a}}</td>
<td>12</td>
<td>-</td>
<td>15</td>
<td>2001 (pre-1988)</td>
</tr>
<tr>
<td>Pannawonica</td>
<td>PA\textsuperscript{2\textsubscript{c}}</td>
<td>13</td>
<td>Jan. 2015</td>
<td>1.8</td>
<td>2015 (2000)</td>
</tr>
<tr>
<td>Snappy Gum</td>
<td>GU\textsuperscript{2\textsubscript{c}}</td>
<td>13</td>
<td>Jan. 2015</td>
<td>1.8</td>
<td>2015 (2000)</td>
</tr>
<tr>
<td>Water Corp-B</td>
<td>WB\textsuperscript{1\textsubscript{b}}</td>
<td>16</td>
<td>-</td>
<td>19</td>
<td>1997 (1989)</td>
</tr>
<tr>
<td>Airstrip 2</td>
<td>A2\textsuperscript{1\textsubscript{b}}</td>
<td>17</td>
<td>-</td>
<td>20</td>
<td>1996 (pre-1988)</td>
</tr>
<tr>
<td>Airstrip 1</td>
<td>A1\textsuperscript{3\textsubscript{b}}</td>
<td>24</td>
<td>-</td>
<td>27</td>
<td>1989 (pre-1988)</td>
</tr>
<tr>
<td>Biological Survey</td>
<td>BS\textsuperscript{2\textsubscript{c}}</td>
<td>&gt;25</td>
<td>Jul. 2015</td>
<td>1.3</td>
<td>2015 (pre-1988)</td>
</tr>
<tr>
<td>Cliff</td>
<td>CL\textsuperscript{3\textsubscript{c}}</td>
<td>&gt;25</td>
<td>-</td>
<td>&gt;28</td>
<td>Pre-1988</td>
</tr>
</tbody>
</table>

**Trapping**

We conducted five trapping sessions, at intervals of 2-5 months, from July 2013 to December 2014. Traps were set in late afternoon and checked in early morning. We used Elliott (9 cm x 10 cm x 33 cm) collapsible, box-metal traps with a mix of peanut butter, rolled oats and sardines as bait. Traps were arranged in approximately 1 ha grids (100 m x 100 m), with six lines of six traps in a square array, with traps within lines and trap lines separated by 20 m intervals. We recorded the location of each trap site using a hand-held GPS unit (Garmin GPS 60). Traps were set for five nights at each site. Motion sensor cameras (Reconyx PC850 and HC550), baited using the same bait as for the live-trapping, were also placed on the sites for a total of 1105 trap nights. Cameras were active for between one and six 24-hour periods, with between three and 15 cameras per site, depending on availability. Each camera was set to take three or five photos in burst sequence when the motion sensor was triggered. No delay existed before the next sequence would commence. They were positioned near Elliott traps and were set on the ground with bait approximately 50 cm in front of the lens. The cameras were used to increase detection rates of kalutas, particularly for sites where few or no individuals were trapped in the Elliott traps.
Morphometric data were recorded for each individual. Captured individuals were sexed and aged. Individuals above 20 g were micro-chipped with Trovan nano passive transponders (7 mm x 1.25 mm) inserted subcutaneously between the shoulder blades, for individual identification.

Vegetation survey

Vegetation surveys were conducted at seven sites in November 2013 (after SG and BO were burnt) and at all 11 sites in April 2014. After the trapping study had concluded, all sites were sampled in August 2015 (seven months after a deliberately-lit fire burnt PA and GU) and again in November 2016 (one month after a prescribed fire burnt BS; Table 3.1). Point-intercept and line-intercept methods, as described by Clarke (2009), were used to estimate cover and species composition, as well as bare ground cover. A 100 m transect was established through the centre of each trapping grid (between the third and fourth trapping rows), with transects permanently marked to ensure subsequent surveys monitored the same sites.

Statistical analyses

Apparent survival and recapture probabilities of kalutas between trapping periods were assessed using a Live Capture (Cormack Jolly Seber, CJS) Model, and population sizes were estimated using a POPAN (CJS) Model, in the Program MARK (White and Burnham, 1999). The models assume equal catchability of both marked and unmarked individuals, retention (and correct reading) of tags during the study, instantaneous sampling during the study, equal survival probabilities for marked and unmarked individuals, and a constant study area. All assumptions were met in this study. As the species exhibits male semelparity, sample size and sampling events were limited for males. Therefore, survival and population estimates were analysed for females only. The results of the modelling in Program MARK are presented in Appendix 1 (Figure 3.7 and 3.8). The POPAN model was unable to accurately estimate population sizes. Therefore, minimum known to be alive (KTBA) estimates for each trapping event were used as a proxy for population size and were analysed for both sexes. KTBA estimates are determined by the number of individuals that are captured in a trapping event, plus the number of previously captured individuals that are captured in subsequent trapping events. These estimates underestimate population size when captures frequently occur on the edge of the sampling area (Masters and Dickman, 2012). For the analyses, the sites were grouped into three categories indicative of time since fire: 1) recently burnt, where sites were 0-5 years since fire (three sites); 2) intermediate fire age, where sites were 6-16 years since fire (four sites); and 3) long unburnt, where sites were 17 years or greater since fire (four sites). For the second set of analyses, the sites were grouped according to spinifex cover on the site: 1) low cover,
where sites had less than 35% cover (three sites); 2) intermediate cover, where sites had between 36% and 45% cover (four sites); and 3) high cover, where sites had 46% or greater cover (four sites).

We analysed the effects of fire age (years since fire), spinifex cover (%), area (m²), and elevation (m) (all fixed effects) on the arcsin square root transformed proportion of capture success using linear models (i.e. site was our unit of replication and, for each site, success was scored as Ns/Nt, where Nt is the total number of trap nights per site and Ns is the total number of successes for that site). Proportions were weighted by Nt for each site. To identify which variables to include in the model, we ran a forward stepwise regression analysis. The final model included all four terms. P values were obtained by sequentially dropping effects and comparing models with the full model using F tests (Table 3.2). Area (m²) of sites was included in the linear models as not all sites measured exactly 1 ha as intended.

Table 3.2. Number of captures of kalutas, and sampling site variables used in the forward stepwise regression analysis of 13 sites surveyed in Millstream Chichester National Park between July 2013 and November 2016.

<table>
<thead>
<tr>
<th>Site Code</th>
<th>Captures</th>
<th>Trap nights</th>
<th>Fire age (years)</th>
<th>Triodia cover (%)</th>
<th>Area (m²)</th>
<th>Site elevation (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BO</td>
<td>0</td>
<td>360</td>
<td>0</td>
<td>4</td>
<td>9651.2</td>
<td>308</td>
</tr>
<tr>
<td>SG</td>
<td>0</td>
<td>360</td>
<td>1</td>
<td>5</td>
<td>9473.6</td>
<td>296</td>
</tr>
<tr>
<td>CB</td>
<td>0</td>
<td>540</td>
<td>12</td>
<td>11</td>
<td>13564.9</td>
<td>306</td>
</tr>
<tr>
<td>WC</td>
<td>15</td>
<td>468</td>
<td>13</td>
<td>44</td>
<td>10721.2</td>
<td>309</td>
</tr>
<tr>
<td>PA</td>
<td>99</td>
<td>936</td>
<td>13</td>
<td>51</td>
<td>14681.7</td>
<td>317</td>
</tr>
<tr>
<td>GU</td>
<td>39</td>
<td>864</td>
<td>13</td>
<td>57</td>
<td>9677.4</td>
<td>300</td>
</tr>
<tr>
<td>WB</td>
<td>6</td>
<td>252</td>
<td>16</td>
<td>36</td>
<td>10142.3</td>
<td>304</td>
</tr>
<tr>
<td>A2</td>
<td>23</td>
<td>900</td>
<td>17</td>
<td>36</td>
<td>10154.0</td>
<td>313</td>
</tr>
<tr>
<td>A1</td>
<td>15</td>
<td>900</td>
<td>24</td>
<td>40</td>
<td>9864.7</td>
<td>308</td>
</tr>
<tr>
<td>BS</td>
<td>18</td>
<td>900</td>
<td>25</td>
<td>51</td>
<td>9731.5</td>
<td>308</td>
</tr>
<tr>
<td>CL</td>
<td>37</td>
<td>864</td>
<td>25</td>
<td>46</td>
<td>15220.5</td>
<td>308</td>
</tr>
</tbody>
</table>

To determine the effect of fire age on spinifex size, we (i) compared spinifex size for sites that were recently (0-5 years), intermediate (6-16 years), and long unburnt (≥17 years), and (ii) compared spinifex size across consecutive survey events for sites that burnt at the start of the study period. We first performed a principle component analysis (PCA) on spinifex cover (%), height (mm) and diameter (cm). All three measures were highly positively correlated, and PC1 explained 97% and 82% of the variation for (i) and (ii), respectively. Therefore, we used PC1 as our measure of spinifex size. We then analysed the effect of fire age (i) and time since fire (ii) on PC1 using a linear model (i) and a linear mixed effect model (ii). For the former, we performed post hoc Tukey comparisons between groups. For the latter, we applied a repeated measures design (repeated sampling across survey
events) with site included as a random factor. Linear models were performed in R (R Core Team, 2016), and a linear mixed effects model was analysed using LME4 (Bates et al., 2015).

### 3.4 Results

Spinifex was present on all 11 sites, except immediately post-fire (i.e. in the first weeks or months after fire). Sites that were recently burnt (0-5 years) had lower percentage spinifex cover than sites that were longer unburnt. Long unburnt (16+ years) sites reached an asymptote of spinifex cover of approximately 50% (Figure 3.2a). Maximum recorded spinifex cover was 70% at GU in November 2013 (Figure 3.2a). Sites that were burnt prior to the commencement of the sampling period (SG, BO and CB) showed a significant increase ($\chi^2 = 5.62$, df = 1, $p = 0.017$) in spinifex cover over the sampling period. The site BS, after burning in July 2015, remained at less than 1% spinifex cover for the remainder of the sampling period (Figure 3.2a).

Spinifex hummocks were taller and had larger diameter on longer unburnt sites compared with recently burnt sites (Figure 3.2). Prior to their burning, spinifex was tallest on the intermediate aged PA (approx. 33 cm) and GU (approx. 41.5 cm), and long unburnt BS (approximately 40 cm) and CL (approx. 32.5 cm; Figure 3.2b). Spinifex was on average taller on SG (approx. 10 cm) than on the contemporaneous BO (approx. 7 cm; Figure 3.2b). As with height, hummocks were longest on the intermediate aged PA (approx. 70 cm) and GU (approx. 90 cm), and long unburnt CL (approx. 80 cm) and BS (approx. 90 cm; Figure 3.2c). The three recently burnt sites (SG, BO and CB) all had a mean hummock diameter of approximately 15 cm (Figure 3.2c). Spinifex hummocks were significantly affected by time since fire ($F = 14.30$, df = 2, $p = 0.002$), with recently burnt sites having significantly smaller hummocks than long unburnt (t = 4.81, df = 1, $p = 0.003$) and intermediate fire age sites (t = 3.17, df = 1, $p = 0.004$; Figure 3.2). However, the latter two categories were not significantly different (t = 0.10, df = 1, $p = 0.987$; Figure 3.2) suggesting that time since fire only affects spinifex size for a few years post-fire.

Percentage of bare ground was significantly greater in recently burnt sites and declined with time since fire (t = 5.76, df = 4, $p = 0.005$; two-sample t-test based on April 2014 data; Figure 3.3). Recently burnt sites SG (on average, approx. 85%), BO (on average, approx. 80%) and CB (on average, approx. 70%) had the highest percentage bare ground, while long unburnt sites BS (on average, approx. 25% prior to burning) and CL (on average, approx. 25%) had the lowest percentage bare ground (Figure 3.3). Percentage bare ground increased on sites after they were burnt (PA, GU and BS; Figure 3.3).
Figure 3.2. (a) Percentage cover; (b) mean height (cm, SE bars); and (c) mean diameter (cm, SE bars) of spinifex (Triodia) at four survey times between November 2013 and November 2016 at 11 sites in Millstream Chichester National Park. Superscript numbers refer to: 1) recently burnt sites (f≤5); 2) intermediate aged sites (6≤f≤16); and 3) long unburnt sites (f≥17). * sites burnt during the survey period. † sites burnt immediately prior to the survey period.

Figure 3.3. Percentage bare ground at 11 sites in Millstream Chichester National Park at four survey times between November 2013 and November 2016. Superscript numbers refer to: 1) recently burnt sites (f≤5); 2) intermediate aged sites (6≤f≤16); and 3) long unburnt sites (f≥17). * sites burnt during the survey period. † sites burnt immediately prior to the survey period.

No individuals were captured on recently burnt sites (f≤5) or low cover sites (c≤35). However, BO and SG (recently burnt, low cover sites) had established populations pre-fire (Figure 3.4). Females
were most abundant on intermediate fire age sites (max. 20 individuals) compared to recently (0 individuals) and long unburnt sites (max. 12 individuals; Figure 3.4a). Similarly, estimates were greatest for males on long unburnt sites (max. 14 individuals), compared to recently (0 individuals) and intermediate aged sites (max. 13 individuals). Estimates of males for intermediate and long unburnt sites were similar, and followed the same trends (Figure 3.4b). Estimates for females were greatest on high cover sites (max. 24 individuals), compared to intermediate (max. 10 individuals) and low cover sites (mean 0 individuals; Figure 3.4c). This was reflected in males, where estimates were also greatest on high cover sites (max. 15 individuals), compared with intermediate (max. 12 individuals) and low cover sites (0 individuals; Figure 3.4d). Again, estimates of males for intermediate and high cover sites were largely similar, and followed the same trends (Figure 3.4d). KTBA estimates were consistently lower for males compared with females (Figure 3.4).

![Figure 3.4](image-url)

**Figure 3.4.** Known to be alive estimates of kalutas (*Dasykaluta rosamondae*) in Millstream Chichester National Park, between July 2013 and December 2014. Estimates of (a) females and (b) males inhabiting sites burnt by fires 0-5 years ago (triangles), 6-16 years ago (squares), or ≥17 years ago (circles). Estimates of (c) females and (d) males inhabiting sites with ≤35% *Triodia* spinifex cover (triangles), 36-45% cover (squares), or ≥46% cover (circles). Dashed grey lines indicate captures on sites prior to sites burning (transitioned from intermediate fire age and intermediate *Triodia* cover, to recently burnt and low cover).

Captures were linearly correlated with spinifex cover (F = 32.95, df = 1, p = 0.001; Figure 3.5b) but not with fire age (F = 3.11, df = 1, p = 0.128; Figure 3.5a), site area (F = 3.57, df = 1, p = 0.108;
Figure 3.5c) or site elevation ($F = 3.80$, $df = 1$, $p = 0.099$, Figure 3.5d). A dichotomy existed between sites of less than five years and sites greater than 10 years since fire.

![Graphs showing relationship between capture success and various factors](image)

**Figure 3.5.** Capture success of kalutas *Dasykaluta rosamondae* as predicted by (a) fire age (years), (b) *Triodia* cover (%), (c) area ($m^2$), and (d) elevation (m) of sites. Dotted regression lines indicate non-significant relationships, solid regression line indicates significant relationship. Regression equations are estimates from the linear model.

Of the 1105 camera-trap nights across the 11 sites, we recorded significantly fewer kalutas on recently burnt sites (2 captures) compared with intermediate and long unburnt sites (271 captures; $X^2 = 85.1$, $df = 1$, $p < 0.0001$; Figure 3.6). We tested for significance using a $\chi^2 2 \times 2$ contingency table where each camera on each night was scored as success or failure.
Figure 3.6. Camera trap effort (dark grey) and success (light grey) of capturing kalutas *Dasykaluta rosamondae* by site in Millstream Chichester National Park over five survey events between July 2013 and December 2014.

3.5 Discussion

We found that kalutas were negatively affected by recent fire. They did not occupy habitat that was recently burnt, with kalutas not recorded in sites five years or less since fire. There was a gap in our data of sites of fire ages between five and 10 years since fire. Our results suggest that kalutas occupy habitat burnt more than ten years previously, however, as no sites covered this age bracket, the threshold may be between five and 10 years since fire. Population estimates for males were similar on intermediate aged and long unburnt sites, while estimates for females were higher on intermediate aged sites compared with long unburnt sites. This suggests that there is a threshold of around ten years, after which the fire age of sites is not important in determining the size of kaluta populations.

Previous studies have found that small mammals are generally negatively affected by frequent or intense fire. For example, Kelly *et al.* (2010) examined small mammal populations in semiarid mallee shrublands in southern Australia and found spinifex-dependent species were particularly susceptible to frequent fire, while Kelly *et al.* (2015) suggested that long unburnt vegetation was disproportionately more important than more recently burnt habitat for several vertebrate groups, including small mammals. Masters (1993) also showed a reduction in abundance of most small mammal species studied in recently burnt compared with long unburnt spinifex habitat. More specifically, Körtner *et al.* (2007) found that brush-tailed mulgara (*Dasycercus blythi*) population sizes in Uluru-Kata Tjuta National Park in northern Australia were greater on longer unburnt sites, compared with more recently burnt sites, and several studies found that the desert mouse (*Pseudomys desertor*) shows a strong preference for long unburnt habitat and is uncommon in recently burnt habitat (e.g. Letnic and Dickman, 2005; Letnic *et al*., 2005). Desert mice were
frequently captured in our study and were closely associated with kaluta populations – when one species was present/abundant or apparently absent on a site, so too was the other species.

Immediate survival of kalutas on two sites burnt during the study was nil, with live and camera trapping showing no evidence of recolonisation during the remainder of the study (a further 13 months). Small, remnant pockets of spinifex were left unburnt on the sites and other small mammals, including *Pseudomys chapmani*, *Ps. hermannsburgensis*, *Sminthopsis macroura*, and *Planigale sp.*, were subsequently captured in this unburnt spinifex immediately post-fire. This later recolonisation by kalutas post-fire is consistent with other small dasyurids, including species of *Antechinus*, which have also been shown to delay colonisation (e.g. Fox, 1983; 1990; Wilson and Friend, 2003).

Kalutas appeared to take several years after fire to recolonise sites. One site (CB) that burnt 10 months prior to the commencement of trapping (2.25 years fire age at the cessation of trapping), did not contain any patches of unburnt spinifex and showed little sign of spinifex recovery. The site was not recolonised by kalutas during the trapping period and only one kaluta was captured using the site (two capture events on the same night from 125 camera-trap nights). We witnessed several kalutas crossing a dirt road from the site into a neighbouring long unburnt site (>25 years fire age); hence, it is possible that kalutas were sheltering in the nearby long unburnt site and occasionally foraging in or traversing the recently burnt site. This exploitation of ecotones has been reported previously in other small mammal species (e.g. Chia *et al.*, 2016; Law and Dickman, 1998; Letnic and Dickman, 2005).

Spinifex was present and dominant on all survey sites, except immediately post fire. As expected, sites that were longer unburnt typically had greater spinifex cover and larger hummock size than sites that were more recently burnt. The mechanism of the fire age threshold is the growth of vegetation cover – kalutas are unable to persist with very low cover, and cover needs to reach approximately 25% before they recolonise and establish. Sites with more cover were able to support larger kaluta populations than sites with less cover. Other studies have also suggested that vegetation cover and the length of time for recovery of vegetation after fire are the most important determinants of habitat suitability for small mammals, rather than other effects of fire age. For example, Kelly *et al.* (2012) suggested that the extent of certain vegetation types in suitable habitat were sometimes more influential on small mammal abundances than the actual fire age of the site. Letnic *et al.* (2005) showed that vegetation cover was more important than fire age for desert mice.
Reduced spinifex cover is detrimental to small mammal populations, as it reduces or completely removes cover, exposing individuals to increased predation risk because less shelter is available. Several predator species, including feral cats and foxes (Vulpes vulpes), have previously been shown to target recently burnt habitat during hunting (e.g. Leahy et al., 2015; McGregor et al., 2014). Both cats, which are abundant across the distribution of kalutas, and foxes, which are rare or absent in the study area (Traditional Owners Millstream Park Council, 2011), are likely to pose significant risk to kalutas in burnt areas and across their broader range (Fisher et al., 2014a; Woinarski et al., 2011).

Spinifex cover is typically positively correlated with time since fire. However, other fire characteristics may also be important. Severe fires can completely remove spinifex from a site or slow its recovery, meaning that affected sites will take longer to achieve cover sufficient for mammal species to colonise. Conversely, cooler fires may leave patches of unburnt habitat in a fire area. These unburnt patches are important for the persistence of small mammal species, as they provide habitat that supports recolonisation of burnt areas (Kelly et al., 2012).

Our results suggest that elevation was not important in determining kaluta populations, and the number of captures did not depend on the area of the grid. However, other variables that were not tested were possibly important. Previous studies have suggested that factors other than fire age per se are as, or more, important for small mammal abundances (e.g. Kelly et al., 2010, 2015). Rainfall is considered important because it affects primary productivity, which affects availability of food (Kelly et al., 2012). This has been shown previously in arid Australia (Letnic et al., 2005, 2011; Letnic and Dickman, 2010). Higher capture rates after high rainfall are likely indicative of both increases in local abundance of populations, increased food availability, and cooler temperatures and higher humidity that may allow longer or more frequent activity periods leading to higher ‘trappability’ (Kelly et al., 2012).

Two intermediate fire age sites (GU and PA) had spinifex cover that was similar to long unburnt sites and disproportionally higher than other intermediate aged sites. This may be reflective of differences in soil characteristics between the sites. Soils on the study site were generally skeletal, while other areas of the park have loamy or clay soils (Traditional Owners Millstream Park Council, 2011). The GU and PA sites were located further away from the other nine sites, on the other side of the Fortescue River, and possibly exhibited different site characteristics, including less skeletal soils. They may also have been subject to minor variations in climate, such as rainfall. Previous studies have suggested that spinifex recovery is highly variable and dependent on rainfall events after
burning (Burrows et al., 2009; Greenville et al., 2009). This may account for their cover being greater than expected.

Conversely, some of the study sites exhibited a slower than expected recovery of spinifex, particularly the CB site. Spinifex species develop from both seedlings and rhizomes, and reach their maximum development after 20-30 years (Department of the Environment and Energy, 2007). Sites that have more spinifex species with rhizomes than with seedlings will recover more quickly.

At the conclusion of the study, over 50% of the sites conformed to the recently burnt age category of 0-5 years since fire. Three sites were burnt in deliberately-lit fires, two by lightning strike fires and one by prescribed burning. As the study sites were close to infrastructure and easily accessible, both prescribed and deliberately-lit fires were more common than expected in arid areas of WA (Burrows et al., 1991, 2009). Further, in the first two years of surveying, over 60% of the park burnt, mostly as a result of deliberately-lit fires that could not be controlled. This is contrary to findings in other areas of arid WA, where unmanaged lightning strike fires are the most common form of major fire (Greenville et al., 2009). In the subsequent two years, prescribed burns were conducted by the Western Australian Department of Parks and Wildlife, in an attempt to achieve a patchy mosaic of habitat ages that would protect the remainder of the longer unburnt landscape. This has become common practice in arid-zone management, in an attempt to lessen the trend towards greater fire patch size and an homogeneous landscape (Greenville et al., 2009; Haslem et al., 2011).

We found considerable variation in fire ages at the start of the study, including some sites which were of unknown fire age, because they had not burnt since fire recordings began in 1988. This suggests that sites are able to exist for long periods without burning. Following several fires in the study period, the environment was far more homogeneous by its conclusion, highlighting how quickly the environment can change and how slowly sites recover after these changes. Each year approximately one million hectares of the Pilbara region of Western Australia is burnt in bushfires. Most fires occur between August and December, when they are also most intense. More intense and frequent wildfires are projected for much of Australia, potentially including the Pilbara, in response to climate change (Letnic et al., 2011; Letnic and Dickman, 2010; Pastro et al., 2011). Given our results, this will be an important consideration in the future for management of kalutas, and other small mammal species. We recommend a patch mosaic fire management strategy, with large patches of habitat at least 10 years since fire, to ensure the persistence of the kaluta in the future.
Appendix 3.1.

Models predicting survival and recapture probabilities differed depending on the categories being assessed. The best model for fire age classes was $\phi(t)p(.)$, suggesting that survival probabilities ($\phi$) varied by time (different trapping event) while recapture probabilities ($p$) were constant across time and groups (age classes; Figure 3.7). The best model for Triodia cover classes was $\phi(.)p(.)$, suggesting that both survival and recapture probabilities were constant across time and groups (Figure 3.7). Neither model included groups (i.e. categories) as an important variable in determining fit.

![Figure 3.7](image-url)

**Figure 3.7.** Survival (black) and recapture (grey) probabilities ($\pm$ SE) between trapping periods of female kalutas (*Dosykaluta rosamondae*) in Millstream Chichester National Park. (a) Probabilities when captures are grouped by fire age of sites: intermediate aged ($6<f<16$) and long unburnt ($f>17$). (b) Probabilities when captures are grouped by Triodia spinifex cover of sites: intermediate cover ($36<c<45$), and high cover ($c>46$).

Population sizes were estimated using a POPAN model, without inclusion of the recently burnt and low cover categories, as no captures were made on these sites. The most appropriate model for predicting population estimates of female kalutas when arranged by both fire age classes and Triodia cover classes was $\phi(.)p(.)b(.)$, suggesting that survival ($\phi$) and capture ($p$) probabilities, and the probability of entrance from the superpopulation ($b$) were constant across time and groups. Estimates of females were significantly greater on intermediate fire age sites ($83.5 \pm 16.7$, SE) compared with long unburnt sites ($47.5 \pm 10.3$, SE; Figure 3.8). While, estimates of females were significantly greater for high cover sites ($85.2 \pm 16.5$, SE), compared with intermediate cover sites ($28.1 \pm 6.5$, SE; Figure 3.8).
Figure 3.8. POPAN model population estimates (± SE) of female kalutas (*Dasykaluta rosamondae*) in Millstream Chichester National Park, between July 2013 and December 2014. (a) Estimates for kalutas inhabiting sites burnt by fires between 6 and 16 years ago (6≤f≤16) or greater than 17 years ago (f≥17). (b) Estimates for kalutas inhabiting sites with between 36% and 45% (36≤c≤45), or greater than 46% spinifex cover (c≥46).
Appendix 3.2.

Figure 3.9. Comparisons of burnt sites BO (1; burnt November 2013); GU (2; burnt January 2015, originally burnt 2001) and BS (3; burnt July 2015, originally burnt pre-1988) in Millstream Chichester National Park from vegetation surveys in (a) April 2014, (b) August 2015 and (c) November 2016. Photographs: Genevieve Hayes.
Figure 3.10. Comparisons of unburnt sites CL (1; last burnt pre-1988) and WC (2; last burnt 2001) in Millstream Chichester National Park from vegetation surveys in (a) April 2014, (b) August 2015 and (c) November 2016. Photographs: Genevieve Hayes.
Chapter 4

Diet of the small, arid dasyurid *Dasykaluta rosamondae* using DNA barcoding of prey in scats
4.1 Abstract

The diet of the kaluta, *Dasykaluta rosamondae*, in the western Pilbara region of Western Australia, was examined through molecular analysis of scats collected between 2013 and 2014. In total, 28 food categories were identified. The major prey item was *Microcerotermes* termites (49.1% of scats), followed by the dasyurid *Pseudantechinus* sp. (16.4%), Linyphiidae spiders (14.6%) and Scolopendridae centipedes (12.7%). No observable differences in prey choice were identified between males and females, sites of differing fire ages or seasons. Molecular methods largely reflected the results of a previous study based on scat contents, although the DNA study had less taxonomic resolution. The results presented are the first molecular analyses of diet in dasyurids.
4.2 Introduction

Determining trophic relationships or feeding preferences is important to ecological studies (Deagle et al., 2005, 2007, 2009; Peters et al., 2015; Zeale et al., 2011). There are various ways to analyse diet, including observational methods, analysis of stable isotopes, and identification of hard-parts in stomach or scat contents (Deagle et al., 2009; Peters et al., 2015). Dietary studies of mammals have often focused on analysis of scat contents (e.g. Gilfillan, 2001; Pavey et al., 2009; Trites and Joy, 2005), as observational methods often prove difficult, and obtaining stomach samples or samples for stable isotopes can be invasive or lethal, and less accurate (Deagle et al., 2005). Scat studies rely on the indigestible material that is excreted to be identifiable; material such as bones, feathers, hair or scales of vertebrates, and the exoskeleton of invertebrates (Deagle et al., 2005; Zeale et al., 2011). However, not all faecal samples contain such material, and those that do likely over-represent species with hard-parts (Deagle et al., 2005, 2007; Dickman and Huang, 1988). Furthermore, related invertebrates often have low morphological disparity, and prey items can be finely masticated during consumption, or highly digested, rendering them difficult to identify (Green, 1989; Peters et al., 2015; Zeale et al., 2011). This creates problems for accurately assessing diet, particularly to a low taxonomic rank (Zeale et al., 2011).

The advent of DNA techniques to examine diet has provided an alternative to the traditional hard-part analyses (Deagle et al., 2009; Zeale et al., 2011). This recent technique has been applied to various species in both marine and terrestrial environments, ranging from invertebrates to mammals. Several studies on mammalian predators have shown that it is a viable method that is both non-invasive and potentially comprehensive, especially as a complementary method (Deagle et al., 2005; Peters et al., 2015; Zeale et al., 2011). In marsupials, DNA extracted from faecal samples has been used as a non-invasive method to identify individuals of rare species or to assess population sizes (Lachish et al., 2011; Ruibal et al., 2009); however, few studies have used faecal DNA to assess diet.

The kaluta (*Dasykaluta rosamondae*), a small (20-40g), cathemeral marsupial, is endemic to the Pilbara region and exhibits male obligate semelparity. Like the other dasyurids, it is an insectivorous-carnivorous marsupial. Withers and Cooper (2009), when assessing the physiology of the kaluta, suggested it may be the most carnivorous of the small dasyurids, based on its morphology and behaviour. A recent study by Pavey et al. (2016) analysed the diet of kalutas using scat contents, and found a diet dominated by Coleoptera (26.7% volume) and Formicidae (25% volume). In my study, I aimed to: (i) assess the diet of the kaluta using DNA techniques and compare my results to those of

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Pavey et al. (2016); and (ii) compare differences in the diet between sexes, sites of differing fire ages and seasons. Based on the findings of Pavey et al. (2016), I expected (i) kalutas to consume mostly invertebrate species, supplemented with vertebrate species, and for the diets as assessed by the two techniques to largely reflect one another, with potentially greater taxonomic accuracy in the molecular method. I expected (ii) no observable differences between sexes, sites of differing fire ages, or seasons.

4.3 Methods

Kalutas were trapped in Millstream Chichester National Park (MCNP), in the west of the Pilbara region of Western Australia (Figure 4.1a), between July 2013 and December 2014. The area was characterised by snappy gum (Eucalyptus leucophloia) and bloodwood (Corymbia hamersleyana) tree steppe over spinifex (Triodia spp.). I established ten, 1 ha trapping grids in the far southwest corner of MCNP, in an area covering 10 km x 5 km (Figure 4.1a and 4.1b). The grids were of differing fire ages, and were grouped together in two categories: intermediate age and long unburnt (Table 4.1). I trapped using Elliott (9 cm x 10 cm x 33 cm) collapsible, box-metal traps baited with universal bait (peanut butter, rolled oats and sardines). Scats were collected from the traps of captured kalutas and traps were cleaned after each capture, to avoid confusing scats of different individuals. Scats were frozen for storage. All captured kalutas were sexed and any individual above 20 g was micro-chipped with nano (7 mm x 1.25 mm) passive transponders (Trovan) inserted subcutaneously between the shoulder blades, for future identification.

Table 4.1. Sampling site details for 10 sites trapped in Millstream Chichester National Park between July 2013 and December 2014. Site names, codes, fire age of the sites sampled, average spinifex cover on each site, and latitude and longitude co-ordinates. Superscript numbers refer to fire age categories: 1) intermediate age sites; and 2) long unburnt sites.

<table>
<thead>
<tr>
<th>Site name</th>
<th>Code</th>
<th>Fire age</th>
<th>Spinifex (%)</th>
<th>Latitude (S)</th>
<th>Longitude (E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stargazers Fire trail 1</td>
<td>SF¹</td>
<td>12</td>
<td>-</td>
<td>21°36′5.4″</td>
<td>117°5′45.78″</td>
</tr>
<tr>
<td>Water Corp-C</td>
<td>WC¹</td>
<td>12</td>
<td>45</td>
<td>21°37′8.65″</td>
<td>117°5′46.14″</td>
</tr>
<tr>
<td>Pannawonica</td>
<td>PA¹</td>
<td>13</td>
<td>52</td>
<td>21°37′16.43″</td>
<td>117°9′14.15″</td>
</tr>
<tr>
<td>Snappy Gum</td>
<td>GU¹</td>
<td>13</td>
<td>64</td>
<td>21°36′34.49″</td>
<td>117°7′6.64″</td>
</tr>
<tr>
<td>Water Corp-B</td>
<td>WB¹</td>
<td>16</td>
<td>43</td>
<td>21°37′21.47″</td>
<td>117°5′50.1″</td>
</tr>
<tr>
<td>Airstrip 2</td>
<td>A2²</td>
<td>17</td>
<td>39</td>
<td>21°37′29.71″</td>
<td>117°4′54.05″</td>
</tr>
<tr>
<td>Airstrip 1</td>
<td>A1²</td>
<td>24</td>
<td>41</td>
<td>21°37′17.87″</td>
<td>117°4′29.64″</td>
</tr>
<tr>
<td>Biological Survey</td>
<td>BS²</td>
<td>&gt;25</td>
<td>47</td>
<td>21°36′15.59″</td>
<td>117°4′37.6″</td>
</tr>
<tr>
<td>Opposite Biol. Survey</td>
<td>BO²</td>
<td>&gt;25</td>
<td>12</td>
<td>21°36′7.49″</td>
<td>117°4′38.89″</td>
</tr>
<tr>
<td>Cliff</td>
<td>CL²</td>
<td>&gt;25</td>
<td>45</td>
<td>21°35′12.3″</td>
<td>117°5′24.29″</td>
</tr>
</tbody>
</table>
I assessed the diet of kalutas from 120 scat samples collected from 99 individual kalutas (60 female, 39 male). DNA extraction, sequencing and profiling were carried out by the Australian Genome Research Facility (AGRF) and generally followed the methods outlined by Zeale et al. (2011). For each sample, DNA was extracted from 250 mg of scat. Negative controls were performed with extractions to verify absence of contamination. I used COI universal minibarcode primers, adapted from Meusnier et al., 2008 (Table 4.2). Additional, overhang sequences were added to the minibarcodes ("Uni-MinibarF1_TS") to make them compatible with the Nextera indexing and Illumina sequencing used by AGRF. Three additional forward and reverse primers were used to add “N” nucleotides (one, two or three) between the overhang sequence and the original minibarcode primer ("Uni-MinibarF1_TS_N1-3"). The PCR procedure involved two stages. All four forward and reverse primers were mixed for the primary PCR, to increase diversity in the initial read and, in turn, improve read quality. A secondary PCR was performed using Nextera barcode adapters, to add a unique ID to each sample prior to multiplexing. Based on previous studies using the minibarcodes, amplicon length was expected to be 130 bp. However, after initial screening, amplicons were found to be 164 bp (a total of 300 bp, including 136 bp of the Nextera adaptor sequence). Sequencing runs were performed using the Illumina MiSeq platform (Paired End Chemistry) of 150 bp paired end reads. After diversity profiling, I measured consumption of food items by frequency of occurrence (proportion of scats containing particular prey items). Taxa were identified by aligning sequences to sequences contained in the Barcode of Life Database (BOLD) and GenBank’s BLAST database. I tested for significant differences in occurrence of prey items between sexes, sites and seasons using chi-squared tests of independence.
Table 4.2. Forward and reverse minibarcodes used to amplify prey DNA in the diet of kalutas *Dasykaluta rosamondae*. Minibarcodes were adapted from: Meusnier *et al.*, 2008.

<table>
<thead>
<tr>
<th>Forward</th>
<th>Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uni-MinibarF1_TS</td>
<td>TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTCCTAATCACAARGATATTGGTAC</td>
</tr>
<tr>
<td>Uni-MinibarF1_TS_N1</td>
<td>TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNTCCCTAATCACAARGATATTGGTAC</td>
</tr>
<tr>
<td>Uni-MinibarF1_TS_N2</td>
<td>TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNTCCCTAATCACAARGATATTGGTAC</td>
</tr>
<tr>
<td>Uni-MinibarF1_TS_N3</td>
<td>TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNTCCCTAATCACAARGATATTGGTAC</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reverse</th>
<th>Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uni-MinibarR1_TS</td>
<td>GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAAGAATCATAATGAAGGCATGAGC</td>
</tr>
<tr>
<td>Uni-MinibarR1_TS_N1</td>
<td>GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAAGAATCATAATGAAGGCATGAGC</td>
</tr>
<tr>
<td>Uni-MinibarR1_TS_N2</td>
<td>GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAAGAATCATAATGAAGGCATGAGC</td>
</tr>
<tr>
<td>Uni-MinibarR1_TS_N3</td>
<td>GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAAGAATCATAATGAAGGCATGAGC</td>
</tr>
</tbody>
</table>

4.4 Results

Of the 120 samples analysed, DNA was amplified in 104 samples. Large quantities of bacterial DNA were amplified in eight samples, while human DNA was amplified in six samples, in very low quantities. Dietary items were only included in analyses if 20 or more sequences were amplified for that item. Fifty-five samples (41 female, 14 male) from 50 individuals (36 female, 14 male) were used in the dietary analyses. I identified 28 dietary items, to various classification levels (Table 4.3). Scats contained between one and seven prey items, with an average of 1.7 items per scat. The most frequently occurring prey items were *Microcerotermes* termites (49.1% of scats), the dasyurid *Pseudantechinus* sp. (16.4%), spiders in the family Linyphiidae (14.6%) and centipedes in the family Scolopendridae (12.7%; Table 4.3). A taxon accumulation curve confirmed that I identified everything possible to identify using my methods, with an asymptote reached after approximately 35 scat samples (Figure 4.2).

![Figure 4.2](image-url) Cumulative number of prey items in the scats of *Dasykaluta rosamondae* in Millstream Chichester National Park, plotted against the number of scats analysed.
Table 4.3. Dietary items of *Dasykaluta rosamondae* in Millstream Chichester National Park between July 2013 and December 2014.

<table>
<thead>
<tr>
<th>Higher classification (Class, Sub-class, Order, Sub-order, Super-family, Family, Sub-family, Genus)</th>
<th>Number of scats with DNA present</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entognatha</td>
<td></td>
</tr>
<tr>
<td>Collembola</td>
<td>3</td>
</tr>
<tr>
<td>Insecta</td>
<td>1</td>
</tr>
<tr>
<td>Pterygotes</td>
<td>1</td>
</tr>
<tr>
<td>Blattodea</td>
<td></td>
</tr>
<tr>
<td>Blaberoidae</td>
<td>1</td>
</tr>
<tr>
<td>Blaberidae</td>
<td></td>
</tr>
<tr>
<td><em>Laxta sp.</em></td>
<td>1</td>
</tr>
<tr>
<td>Blattoidea</td>
<td></td>
</tr>
<tr>
<td>Termitoidae (<em>Higher taxon</em>)</td>
<td>1</td>
</tr>
<tr>
<td>Blattidae</td>
<td>2</td>
</tr>
<tr>
<td>Kalotermitidae</td>
<td></td>
</tr>
<tr>
<td><em>Neotermes sp.</em></td>
<td>1</td>
</tr>
<tr>
<td>Termitidae</td>
<td>2</td>
</tr>
<tr>
<td><em>Microcerotermes sp.</em></td>
<td>27</td>
</tr>
<tr>
<td>Coleoptera</td>
<td>1</td>
</tr>
<tr>
<td>Adephaga</td>
<td></td>
</tr>
<tr>
<td>Carabidae</td>
<td>1</td>
</tr>
<tr>
<td>Polyphaga</td>
<td></td>
</tr>
<tr>
<td>Staphylinidae</td>
<td>1</td>
</tr>
<tr>
<td>Diptera</td>
<td></td>
</tr>
<tr>
<td>Chrinomidae</td>
<td>3</td>
</tr>
<tr>
<td>Hemiptera</td>
<td>1</td>
</tr>
<tr>
<td>Heteroptera</td>
<td></td>
</tr>
<tr>
<td>Orthoptera</td>
<td></td>
</tr>
<tr>
<td>Gryllidae</td>
<td>5</td>
</tr>
<tr>
<td>Eneopterinae</td>
<td></td>
</tr>
<tr>
<td>Lebinthini (<em>Tribe</em>)</td>
<td>4</td>
</tr>
<tr>
<td>Acrididae</td>
<td>1</td>
</tr>
<tr>
<td>Chilopoda</td>
<td></td>
</tr>
<tr>
<td>Pleurostigmorpha</td>
<td></td>
</tr>
<tr>
<td>Lithobiomorpha</td>
<td>6</td>
</tr>
<tr>
<td>Geophilomorpha</td>
<td></td>
</tr>
<tr>
<td>Oryidae</td>
<td>1</td>
</tr>
<tr>
<td>Scolopendromorpha</td>
<td></td>
</tr>
<tr>
<td>Scolopendridae</td>
<td>7</td>
</tr>
<tr>
<td>Diplopoda</td>
<td></td>
</tr>
<tr>
<td>Chilognatha</td>
<td></td>
</tr>
<tr>
<td>Polydesmida</td>
<td></td>
</tr>
<tr>
<td>Paradoxosomitidae</td>
<td></td>
</tr>
</tbody>
</table>
Diet of females and males was not significantly different ($\chi^2 = 3.07$, df = 4, $p = 0.55$). The most frequently occurring dietary items in the female diet were Termitoidae (43.6%), Orthoptera: Gryllidae (17.1%) and Dasyuridae (17.1%), compared to the male diet of Termitoidae (35.7%), Araneae (28.6%), Diptera (14.3%) and Dasyuridae (14.3%; Figure 4.3). Some prey items were found in the diet of one sex only; however, most were recorded in one scat only. The exceptions were in the diet of females: Lithobiomorpha centipedes (6 scats), Blattidae roaches (2 scats), and Polydesmida millipedes (2 scats) were all recorded multiple times (Figure 4.3).

**Figure 4.3.** Proportion of scats with different prey items in the diet of females (dark grey) and male (light grey) kalutas *Dasykaluta rosamondae* in Millstream Chichester National Park. Prey items are to order, except for the infra-order Blattodea: Termitoidae, and the generalised “other insects” in the class Insecta.
Diet did not differ significantly between intermediate aged and long unburnt sites ($\chi^2 = 3.90$, df = 5, $p = 0.56$). The major food categories were consumed in similar proportions on the different sites and prey diversity was similar between the two site categories (Figure 4.4).

![Figure 4.4. Proportion of scats with different prey items in the diet of kalutas Dasykaluta rosamondae foraging on intermediate aged (dark grey) and long unburnt sites (light grey) in Millstream Chichester National Park. Prey items are identified to order, except for the generalised “other insects” in the class: Insecta](image)

The most frequently occurring dietary item in every season was termites (Blattodea: Termitoidae). Diet did not differ significantly between seasons ($\chi^2 = 9.32$, df = 10, $p = 0.50$), although the pre-breeding season (July-September) seemingly had lower diversity than other times of the year (Figure 4.5).

![Figure 4.5. Diversity of prey items in kaluta Dasykaluta rosamondae diet at different times of the year in Millstream Chichester National Park.](image)

4.5 Discussion

Invertebrates formed the main component of the kaluta diet, with termites the most frequently occurring prey item. My results largely support the findings of Pavey et al. (2016), who also found
termites to be the most important component of the kaluta diet, by occurrence. This was not surprising as Morton et al. (1983) identified termites as an important food source for Smynthopsis macroura, a species sympatric with kalutas. Given the abundance of termites in the study area and the known high energy content of termites (Pavey et al., 2009), it is unsurprising that termites formed a major component of kaluta diet. Pavey et al. (2016) identified Coleoptera (beetles), closely followed by Formicidae (ants), as the most important prey items by volume, with these prey items also important by occurrence. I found no indication of ants in the diet of kalutas in my study. The lack of ants may have been a result of differences in analysis (see below), a reflection of differences in diet based on location, or seasonal and temporal differences in sampling. Pavey et al. (2016) sampled in the dry season (late-May to mid-July), likely avoiding major periods of surface activity of termites after summer rains. Kalutas were previously predicted to have a notable small vertebrate, likely mouse, component to the diet, based on their morphology, behaviour and anecdotal observations of feeding (Withers and Cooper, 2009). While I did not find a large proportion of scats with rodent DNA, I did find substantial consumption of a small dasyurid, identified as a Pseudantechinus sp. Two Pseudantechinus species (P. roryi and P. woolleyae) have been recorded in MCNP (Gibson and McKenzie, 2009), although neither were recorded during my study. Several other small mammals (e.g. Pseudomys delicatulus, Ningau) spp.) known to occur in the park also (largely) failed to be detected in my study, but were captured during a single night of pitfall trapping on one site. Potentially, Pseudantechinus individuals may also have been present during my study despite being undetected. Both species are similarly-sized or larger than kalutas, and were likely predated as juveniles or scavenged.

There was no observable difference in diet between males and females, nor between sites or seasons. Very few studies have examined diet in dasyurids, and those that have typically compare between sympatric species rather than compare within species (e.g. Fisher and Dickman, 1993; Fox and Archer, 1984; Warnecke et al., 2012). Scarff et al. (1998; Phascogale tapeatafa) and Chen et al. (1998; Dasy cercus cristicauda) both identified minor differences in diet between seasons, while Fox and Archer (1984; Smynthopsis murina and Antechinus stuartii) and Gilfillan (2001; Pseudantechinus macdonnellensis) found no differences in the diet of their study species between seasons.

The limited taxonomic clarification in my study was surprising, given other studies have found molecular methods useful for identifying prey to lower taxonomic rank than other methods (Zeale et al., 2011). However, my methods had clear restrictions that likely accounted for this. Firstly, molecular methods rely on prey DNA surviving through the digestive process (Peters et al., 2015).
Although some rare DNA may not have been preserved in the kaluta scats, it is unlikely that major prey items were missed, due to my use of short, but informative minibarcodes (Zeale et al., 2011). This is contrary to microscopy techniques, which are known to identify only hard-part prey items and miss potential major soft-bodied prey items (Dickman and Huang, 1988). Secondly, molecular methods rely on primer sequences that are suitable for the expected prey items and for reference sequences to exist for those species (Zeale et al., 2011). COI barcode libraries are ever increasing (e.g. BOLD). Future studies seeking more detailed taxonomic inference would be well served by ensuring that potential prey in the study area have been sequenced for popular markers, such as barcodes.

Although common in marine studies, DNA methods of assessing diet are rare in terrestrial mammals. Only a few other papers have assessed diet using DNA in scats (Egeter et al., 2015; Iversen, 2011). More frequently, studies have used DNA from scats to identify the species that excreted the scat, and then applied traditional, morphological methods to assess diet (e.g. Caryl et al., 2012; Foster et al., 2010; McVey et al., 2013). My results show the potential for application of this method to terrestrial mammals, particularly when used as a complementary method to the traditional microscopy methods. I recommend careful consideration of the study species, particularly how well sampled (genetically) potential prey items are in the study area and which primers are most appropriate for the study.
Chapter 5

Local, but no broad scale genetic structure in the arid-zone dasyurid

*Dasykaluta rosamondae*
5.1 Abstract
The Pilbara region of Western Australia is an ancient, geologically-stable landscape, with high rates of endemism and many poorly-studied species. One such species is the kaluta, *Dasykaluta rosamondae*, an obligately male-semelparous dasyurid that is endemic to the broader Pilbara region. Few studies have assessed genetic structuring of mammal populations within the Pilbara, yet understanding population genetics is important for conservation, as it informs knowledge of dispersal and recruitment capabilities. This may be particularly pertinent in the Pilbara, given that the region is exploited by mining practices and is likely to undergo substantial climate change. In this study, we assessed population structure of kalutas using genetic samples collected from 222 individuals using 12 microsatellite loci that were cross-amplified from closely related species. Broad scale structure was examined for 112 kalutas across the Pilbara from 1988 to 2012, while fine scale structure was examined for 110 kalutas sampled in a 10 km x 5 km area of Millstream Chichester National Park between 2013 and 2014. We found no evidence of broad scale genetic structuring in kalutas across the Pilbara; however, we did find evidence of local genetic structuring within the Millstream Chichester National Park samples. These results suggest potential movement of some individuals over large distances, and may also be reflective of differences in historical and contemporary dispersal patterns. Our study provides an interesting perspective for the kalutas’ ability to respond to future changes in their environment.
5.2 Introduction

Knowledge of a species’ genetic diversity and spatial structure is increasingly recognised as necessary information for the conservation of fauna (Frankham, 2003; Jones et al., 2004; Lada et al., 2008). Habitat fragmentation, from natural (e.g. geographic barriers) and anthropogenic causes (e.g. habitat destruction), limits dispersal and consequently is often detrimental to the persistence of small populations (Banks et al., 2005; Kraaijeveld-Smit et al., 2002b; Lada and Mac Nally, 2008; Lada et al., 2008). This is because populations confined to small patches of habitat tend to experience demographic and genetic stochasticity, increasing their probability of extinction (Banks et al., 2005; Eldridge et al., 1999). These populations also tend to lose genetic variation through genetic drift, making them less likely to recover from declines and adapt to changing conditions (Banks et al., 2005; Jones et al., 2004; Lada and Mac Nally, 2008). Reduced gene flow also increases the insularity of populations resulting in increased inbreeding, and burdens the population with the negative effects of inbreeding depression (Frankham, 2003; Jones et al., 2004). Therefore, the loss of genetic variation in fragmented populations is heavily dependent upon the degree of isolation, the effective population size \( (Ne) \) and the number of generations since fragmentation occurred (Banks et al., 2005; Jones et al., 2004; Lada et al., 2008). Knowledge of a species’ life history characteristics and dispersal capabilities can inform knowledge of gene flow and population genetic structuring, and vice versa (Kraaijeveld-Smit et al., 2002a, 2007). Species capable of dispersing over large distances should theoretically show minimal genetic structuring (Jones et al., 2004). However, natural and artificial barriers limit dispersal capabilities, thus reducing gene flow and the ability of populations to persist (Cockburn et al., 1985; Kraaijeveld-Smit et al., 2002b; Kraaijeveld-Smit et al., 2007; Lada and Mac Nally, 2008). Thus, understanding the genetic structuring of natural populations has important implications for their conservation.

The Pilbara, a biogeographic region of Western Australia, is an ancient, weathered landscape associated with the Pilbara Craton (McKenzie et al., 2009; Pepper et al., 2006, 2008; Thackway and Cresswell, 1995). The main geographic features include the Hamersley and Chichester Ranges, as well as several major river systems, particularly the Fortescue River (Pepper et al., 2008). As with over half of the continent, the Pilbara is situated in the arid zone of Australia, experiencing most annual rainfall from thunderstorms and occasional cyclones in the summer, wet season, when maximum temperatures reach averages of 37°C (McKenzie et al., 2009). The long-term geological stability, coupled with a transition from mesic to arid climates initiated in the middle Miocene, have likely affected the evolution of the region’s biota (Byrne et al., 2008; Pepper et al., 2006, 2011). Indeed, the region is a zone of high biological endemism and therefore of importance for the
conservation of Australia’s biodiversity. Despite a number of broad surveys of the Pilbara, most of the species unique to the region remain poorly studied (Pepper et al., 2008).

In the past century, the Pilbara has undergone considerable change through altered fire regimes, as well as pastoral and mining activities (McKenzie et al., 2009). In particular, vegetation cover has been reduced through over-grazing and frequent fires. Numerous feral species have been introduced to the area, including 12 mammal species, such as the predatory feral cat (*Felis catus*) and red fox (*Vulpes vulpes*), and many weed species. The consequence of this rapid and dramatic change in the environment and biota has meant that 15% of the pre-European Pilbara mammal fauna is now extinct (McKenzie et al., 2009). Furthermore, future climate change is anticipated to have a wide range of impacts on the remaining fauna, likely altering distributions and abundances (McKenzie et al., 2009). Therefore, the Pilbara offers an interesting platform in which to study and compare historical, broad scale population structure – where dispersal may only be limited by a few large geographical barriers – and fine scale, contemporary population structure – where forces such as fire may influence gene flow. Few studies have focussed on genetic structuring of terrestrial vertebrate populations within the Pilbara. Most work relates to invertebrates (Pilbara snails; e.g. Johnson et al., 2004, 2006) or structuring across species’ broader ranges, which include the Pilbara (e.g. ghost bat *Macroderma gigas*, Wilmer et al., 1999; Australian magpie *Gymnorhina tibicen dorsalis*, Toon et al., 2003).

In this study, we assessed both broad scale historical, and fine scale contemporary, population structure of the kaluta (*Dasykaluta rosamondae*; Ride, 1964). The kaluta is a small (20-40 g), carnivorous marsupial common across its range. Commonly described as endemic to the broader Pilbara region, its range extends south into the Carnarvon Basin, and east into the Little Sandy Desert (Burbidge, 2016; Withers and Cooper, 2009; Woolley, 2008). Male kalutas are obligately semelparous and die after a short, frenzied breeding season, while females are capable of breeding in up to two breeding seasons (Chapter 2; Woolley, 1991). Like many species in the region, the kaluta is poorly studied. Given its short, discrete generation time, healthy population size and broad range, this species is particularly interesting for studying the population structure (indicative of dispersal patterns) of a small mammal in an ancient, geologically-stable landscape. At a broad scale, large geographic barriers may limit dispersal, isolating populations and causing strong population structure. At a fine scale, such population structuring may be absent or caused by patches of burnt vegetation or roads/rivers. Moreover, most mammal species exhibit male-biased dispersal (Greenwood, 1980). The males of *Antechinus* and other dasyurid species are known to travel large
distances when dispersing, relative to females; therefore, we might expect to see different patterns of population structure for males and females at the fine scale.

5.3 Methods

**Sampling techniques**

Our sampling was conducted in two ways. First, to assess fine scale population structure, we trapped kalutas at 11 sites within Millstream Chichester National Park (MCNP; 10 km x 5 km) between July 2013 and December 2014 (see Chapter 1 for full details of sites and trapping routines; Figure 5.1). We collected small ear notches or hair samples from 110 adults (45 males, 65 females) across the 11 sites. Males were assessed as being adults from July onwards, while female age was estimated based on time-of-year and the condition of their pouch (see also Chapter 1). Tissue samples were stored in 80% ethanol for genetic analysis.

![Figure 5.1](image-url) **Figure 5.1.** (a) Google map of the location of Millstream Chichester National Park (MCNP) in Australia. Insert: Google image of MCNP with location of trapping area in square. (b) Google Earth image of sites trapped within MCNP (white circles, site names).

Second, to assess broad scale population structure, we collected samples from 112 individuals (56 males, 56 females) stored in the Western Australian Museum. These samples were collected from across the broader Pilbara region over a period of approximately 30 years, from 1988 to 2012 (Figure 5.2). Samples were preserved as “wet-tissues”, either with ethanol in -20°C freezers or pure in -75°C freezers, and consisted of hearts, kidneys, livers, tails, or ears.

**DNA extraction and genotyping**

DNA was extracted from tissue samples using a salting-out procedure similar to Sunnucks and Hales (1996), with minor adjustments. These included adding 10μL Proteinase K to the sample tubes containing TNES; incubating samples overnight at 56°C to digest tissue; vortexing samples and then icing them for five minutes after the SM NaCl had been added to the digested tissue samples;
centrifuging samples to pellet DNA and transferring the supernatant to a new tube twice; and resuspending the DNA in 20-100μL sterile water. Once extracted, DNA quality and concentration was tested using a NanoDrop ND-1000 spectrophotometer (ThermoFisher Scientific). Samples were stored at -20°C until they were genotyped.

Figure 5.2. (a) Map of the Interim Biogeographical Regionalisation for Australia bioregions in Australia [adapted from Thackway and Cresswell (1995), IBRA version 7 (2012)]. (b) Google Earth image of north-western Australia with locality records from the Western Australian Museum for Dasykaluta rosamondae (white circles). Black squares indicate Pilbara and parts of surrounding bioregions.

Genotypes were scored at 12 microsatellite loci, using fluorescently-labelled primers cross-amplified from closely related species (Table 5.1). PCR conditions for seven of the 12 loci were as follows: 15 mins at 95°C, 35 cycles of 30 secs at 94°C, 1.5 mins at 52°C and 1 min of 72°C, followed by 30 mins at 60°C. PCR conditions for one of the loci differed only in the annealing temperature, where it was 56°C instead of 52°C. PCR conditions for the final four loci differed in the number of repeats of the original cycle (40 cycles instead of 35). PCR products were run on a 3730XL capillary sequencer (Applied Biosystems), against the size standard GeneScan-500 LIZ. Allelic sizes were scored using a GS filter set and analysed using GENEMARKER, version 1.91 (SoftGenetics).

Table 5.1. Names and associated species of 12 microsatellite loci used to genotype kalutas, Dasykaluta rosamondae.

<table>
<thead>
<tr>
<th>Microsatellite loci</th>
<th>Species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aa4a</td>
<td>Antechinus agilis</td>
<td>Banks et al., 2005</td>
</tr>
<tr>
<td>pDG1A1, pDG1H3, pDG5G4, pDG7F3</td>
<td>Dasyurus geoffroii</td>
<td>Spencer et al., 2007</td>
</tr>
<tr>
<td>3.1.2, 3.3.1, 3.3.2</td>
<td>Dasyurus spp.</td>
<td>Firestone, 1999</td>
</tr>
<tr>
<td>Sh3o</td>
<td>Sarcophilus laniarius</td>
<td>Jones et al., 2003</td>
</tr>
</tbody>
</table>
Data analyses

We tested for the presence of null alleles for each locus and population using the program MICROCHECKER version 2.2.3 (Van Oosterhout et al., 2004). FSTAT was used to assess linkage disequilibrium (LD) between each pair of loci. Loci with null alleles and in LD were removed accordingly. For the 11 MCNP sites sampled in 2013-2014, we assessed allelic richness (AR, the number of alleles per locus independent of sample size) and gene diversity (H) using FSTAT version 2.9.3 (Goudet, 1995). This program was also used to test for deviations from random mating within each sample, with the results expressed using the $F_{IS}$ statistic (inbreeding coefficient), where positive values indicate a deficit of heterozygotes and, hence, higher levels of inbreeding than expected by chance. Tests for differences in allelic richness, gene diversity and $F_{IS}$ values across sites were performed using a Friedman’s ANOVA in R (R Core Team, 2016). We then tested for genotypic differentiation among sites ($F_{ST}$; Weir and Cockerham, 1984).

To assess broad scale population structure, we performed Bayesian assignment analyses in the program STRUCTURE (Pritchard et al., 2000), with no a priori knowledge of number or composition of populations. These analyses were used to assess spatial and temporal genetic structure, using clustering of genotypes where LD and departure from Hardy-Weinberg Equilibrium (HWE) are minimised. Probabilities of the data fitting $K$ number of populations, where $K$ ranged from 1-20, were calculated using the admixture model, with a burn-in of 10 000 iterations and a Markov Chain Monte Carlo (MCMC) of 100 000 iterations. The most likely value of $K$ was determined using the $\Delta K$ method (Evanno et al., 2005) in STRUCTURE HARVESTER version 0.6.94 (Earl et al., 2012). As described by Evanno et al. (2005), log likelihood $L(K)$ probabilities for a given $K$ are calculated a posteriori as half the variance of the average log likelihood value per MCMC step subtracted to the mean. $\Delta K$ values are calculated in a series of steps to determine the mean absolute value of the second order rate of change of the likelihood function with respect to $K$, divided by the standard deviation of the log likelihood. First, $L'(K)$, the rate of change of the likelihood function with respect to $K$, is calculated as $L'(K) = L(K) - L(K-1)$. Second, $|L''(K)|$, the absolute value of the second order rate of change of the likelihood function with respect to $K$, is calculated as $|L''(K)| = |L'(K+1) - L'(K)|$. Last, $\Delta K$, is calculated as $\Delta K = \text{mean}(|L''(K)|) / \text{SD}(L(K))$.

To assess fine scale spatial genetic structure, we used spatial autocorrelation (SA). We applied these analyses solely to the samples collected in MCNP (i.e. fine scale and contemporary) because these samples were collected at a single time point with accurate GPS locations, contrary to the museum samples. We tested for a relationship between genetic relatedness and geographic distance.
matrices between all pairs of individuals within a population in the program GENALEX version 6.5 (Peakall and Smouse, 2006, 2012). This produced a spatial autocorrelation coefficient \( r \) for all genotypes within a specified geographical distance. The results are presented as multiple distance class (MDC) plots, and autocorrelogram plots are presented in Appendix 5.1. Significant positive spatial structure is present until \( r \) does not differ significantly from zero. This is assessed by tests for statistical significance using random permutations and calculating the bootstrap 95% confidence limits (CL) or \( r \) for 1000 replicates. A two-dimensional local SA plot is presented in Appendix 5.2. This analysis involves comparing an individual with its \( n \) nearest neighbours (in this case, \( n = 5 \)) to assess local patterns of SA within the 2D landscape. Statistical significance was again determined using permutation tests.

5.4 Results

We removed a total of five loci due to the presence of null alleles (pDG7F3 and 3.3.2) or to account for LD (Sh3o, 3.1.2, pPa9D2). We found no evidence for broad-scale, historical population structuring for kalutas across their range (Figure 5.3). That is, our STRUCTURE analysis suggested that, across the broader Pilbara region, samples were best represented as one genetic cluster. This is evident in Figure 5.3, where the relatively stable log likelihood estimates \([L(K)]\) and fluctuating \(\Delta K\) values indicate the probable number of clusters was one. Log likelihood values should plateau or gradually continue to rise, while \(\Delta K\) values should peak, at the true value of \(K\). As neither of these conditions were met, it suggests that the best estimate of genetic clusters \((K)\) was one.

**Figure 5.3.** Mean estimates of the log probability \((L(K), \text{black circles, SD bars})\) and \(\Delta K\) (open diamonds) for \(K\) 1-20 genetic clusters determined by the STRUCTURE analysis of 222 adult kalutas *Dasykaluta rosamondae* sampled across the broader Pilbara region of Western Australia.
We then assessed fine-scale population structure. No significant genetic differentiation was detected among sites within MCNP ($F_{ST} = 0.067, p = 0.305$). There were no significant differences in allelic richness ($\chi^2 = 13.31, \text{df} = 8, p = 0.101$), gene diversity ($\chi^2 = 12.65, \text{df} = 8, p = 0.124$) or $F_{IS}$ values ($\chi^2 = 6.53, \text{df} = 8, p = 0.588$) between sites (Table 5.2). No $F_{IS}$ values were significantly different from zero, suggesting kaluta populations were in HWE. However, we identified spatial structure at the local scale for both sexes. For females, the MDC analysis indicated that genetic correlation coefficient ($r$) values were significantly positive for distances up to 8 km, before becoming non-significant at 9 km (Figure 5.4a). A similar pattern was evident for males (Figure 5.4b).

**Table 5.2.** Genetic variation among kalutas *Dasykaluta rosamondae* within Millstream Chichester National Park. Allelic richness (AR) and gene diversity ($H$) are mean values with SE in parentheses. * SG site includes samples from the sites SF and ST.

<table>
<thead>
<tr>
<th>Site</th>
<th>N</th>
<th>AR</th>
<th>$H$</th>
<th>$F_{IS}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>7</td>
<td>3.3 (0.5)</td>
<td>0.67 (0.12)</td>
<td>0.056</td>
</tr>
<tr>
<td>A2</td>
<td>11</td>
<td>3.2 (0.4)</td>
<td>0.65 (0.11)</td>
<td>-0.056</td>
</tr>
<tr>
<td>BS</td>
<td>6</td>
<td>2.8 (0.4)</td>
<td>0.55 (0.12)</td>
<td>0.013</td>
</tr>
<tr>
<td>BO</td>
<td>6</td>
<td>2.5 (0.4)</td>
<td>0.48 (0.12)</td>
<td>-0.034</td>
</tr>
<tr>
<td>CL</td>
<td>9</td>
<td>3.2 (0.5)</td>
<td>0.63 (0.14)</td>
<td>0.224</td>
</tr>
<tr>
<td>SG*</td>
<td>9</td>
<td>3.3 (0.5)</td>
<td>0.64 (0.13)</td>
<td>0.032</td>
</tr>
<tr>
<td>WC</td>
<td>6</td>
<td>2.7 (0.4)</td>
<td>0.53 (0.11)</td>
<td>-0.175</td>
</tr>
<tr>
<td>GU</td>
<td>18</td>
<td>3.0 (0.4)</td>
<td>0.62 (0.11)</td>
<td>-0.074</td>
</tr>
<tr>
<td>PA</td>
<td>35</td>
<td>2.9 (0.4)</td>
<td>0.60 (0.11)</td>
<td>0.004</td>
</tr>
</tbody>
</table>
5.4. Multiple distance class plots showing the decline in relatedness with increasing distance for (a) 58 female and (b) 37 male kalutas Dasykaluta rosamondae in Millstream Chichester National Park. Dotted lines represent upper and lower 95% confidence limits.

5.5 Discussion

Broad scale spatial structure for kaluta populations across the Pilbara was not evident in our study, demonstrating that the populations that we have sampled were not historically isolated. This broad scale result is reflected in similar findings from a study of the northern quoll (Dasyurus hallucatus), another dasyurid in the Pilbara. Results in an unpublished technical report suggest that northern quolls do not exhibit any genetic clustering across their Pilbara range, but do form clusters across the whole of their northern Australian range. This study of the northern quoll was based on over 500 genetic samples from across Australia, including over 200 samples from the Pilbara (Spencer et al., 2013). Preliminary work on other small mammals in the Pilbara (species from the genera Ningaui, Planigale, and Pseudomys) also suggests similar patterns are evident (pers. comm. K. Ottewell 2017). This finding is not consistent for all taxa in the Pilbara, undoubtedly because the species studied exhibit a wide-range of life history strategies, ecological specialisations and dispersal capabilities. For example, Pepper et al. (2008) found geckos formed genetic clusters aligned with geological features in the region, while Johnson et al. (2006) found gastropods on Barrow Island in the Pilbara showed distinct populations, but with no detectable geographic pattern. Unfortunately, it remains unclear whether or not our results indicate, for example, high dispersal capabilities of the kaluta (historical or contemporary), or a lack of structure due to limited statistical power. In this study, we analysed museum samples of only 112 adults over a temporal scale of approximately 30
years and a spatial scale covering approximately 900 km x 400 km. For a small species with nearly-discrete generation times of one year, using so few individuals to sample such large spatial and temporal scales may have resulted in our falsely accepting the null hypothesis. Therefore, identifying whether or not there is contemporary, broad scale population structure warrants further investigation, particularly when comparing to fine scale structure.

In contrast to the finding of no broad scale structure, we detected fine scale population structure in both sexes of kalutas, suggesting that not all individuals were dispersing widely. Females and males were not discernibly different in their structuring, which was inconsistent with our hypothesis of male-biased dispersal. This is surprising as many other semelparous dasyurids are reported to have male-biased dispersal, and are known to exhibit strategies to avoid inbreeding. For example, Banks et al. (2014) suggested that male Antechinus agilis were more likely to settle in patches with highly unrelated females. Similarly, Fisher (2005) reported that juvenile male A. stuartii dispersed more frequently and significantly further than females (1230 m vs 270 m), and were also likely to disperse further if they were raised in areas with low population density. Fisher (2005) also found that in cases where the mother died, normal home-range establishment was affected and often resulted in philopatry of sons and dispersal of some daughters. Conversely, Sale et al. (2009) found a high degree of philopatry for both sexes in another antechinus A. minimus and noted an apparent lack of inbreeding avoidance strategies, despite finding no evidence of inbreeding. Like ours, this is one of few reports of non-male-biased dispersal in mammals.

The southwest corner of MCNP, where our sampling sites were located, is dominated by human infrastructure, with offices for both the WA Department of Parks and Wildlife and Water Corporation of WA located in the area, as well as tourist camps and attractions. Frequent prescribed burns, to protect the infrastructure, are used as insurance against large, difficult-to-control wildfires. Additionally, neighbouring, pastoral-lease property is heavily-grazed by cattle. Together, this has created a highly-modified landscape in a confined area, with a mosaic of habitat highly fragmented by roads, clearing, frequent fires and over-grazing. Potentially, the fine-scale structure (limited dispersal) is a response to this highly-modified landscape. These results may differ in other areas of the kaluta’s range, highlighting a need for more genetic studies, focusing on the local genetic structure of kalutas in areas not influenced by humans. Comprehensive analyses of population structure (i.e. analyses of contemporary broad scale structure and fine scale structure in unperturbed habitat) may shed light on the consequences of these aforementioned activities for the interconnectivity of kaluta populations.
The impacts of climate change are predicted to be wide-ranging in the Pilbara and include increased intensity of cyclonic events, as well as changes in fire regimes (Loechal et al., 2011; Sudmeyer, 2016). The detrimental impact of fire on kalutas was highlighted in Chapter 3. These changes, together with increased mining and human activity in the region, are likely to impact dispersal capabilities of kalutas. Our broad scale results suggest that in the past no impediments to dispersal existed (i.e. populations were not historically isolated). However, these results may instil a false sense of security, given their limitations and the results of the fine scale structure where limited dispersal patterns were suggested. Therefore, this suggests that future studies should be careful in interpreting a lack of broad scale structure as an indication that no barriers exist to dispersal. Furthermore, a more powerful broad scale study would help to better understand what is truly happening across the range of the kaluta. This study should incorporate a greater number of samples from more localities within a shorter temporal scale. Finally, we encourage a comparison of the population structure of kalutas in undisturbed versus disturbed habitat. This may be fundamental for identifying whether or not dispersal is typically male-biased (or simply an artefact of the disturbed environment), as well as evaluating the consequences of human modifications of the landscape shaping the population structure of kalutas.
Appendix 5.1.

For females, the SA analysis was significantly positive at a distance of 1km and then oscillated between non-significance and negatively significant for the remaining distances (Figure 5.5a). Similarly, for males, \( r \) was significantly positive at a distance of 1km and then became non-significant for the remaining distances, until becoming negatively significant at 9km (Figure 5.5b).

![Correlogram plots](image)

**Figure 5.5.** Correlogram plots for (a) 58 female and (b) 37 male kalutas *Dasykaluta rosamondeae* in Millstream Chichester National Park. Dotted lines represent upper and lower 95% confidence intervals.
Appendix 5.2.

A two-dimensional plot of local spatial autocorrelation indicated that the greater-than-expected relatedness between individuals up to 8 km was not driven by one family group. This was a consistent finding across the study area, with over 35% of individuals showing significantly greater-than-expected relatedness to their five nearest neighbours (Figure 5.6).

![Figure 5.6](image_url)

**Figure 5.6.** Plot of two-dimensional spatial autocorrelation analyses of kalutas *Dasykaluta rosamondae* in Millstream Chichester National Park. Black stars represent sites where individuals were significantly positively-related, based on five nearest neighbours. Open circles indicate other sites sampled (non-significant).
Epilogue
In my thesis, I examined the ecological characteristics of kalutas to assess which management actions are required to ensure the persistence of this species in an environment increasingly modified by mining, with associated infrastructure developments (road and rail systems); pastoral practices; and changes to Aboriginal land management, especially fire. Specifically, I studied the life history characteristics, breeding biology and overall mating system, responses to fire, diet, and population genetics of this species.

In Chapter 1, I assessed the life history characteristics of kalutas in a field study. In this chapter, I documented a trapping success rate of 3.8% between July 2013 and December 2014. I found that sex ratios of independent kalutas varied over the year, ranging from near-parity in winter, to 100% females in summer after male die-off was complete. Adult male kalutas were significantly larger than females, in both body weight and pes length, and were also in better condition, according to two measures of condition – tail width and body weight regressions with pes length. My results suggest that kalutas are cathemeral, with their peak and range of activity times changing depending on the season. My study is the first comprehensive focus on the kaluta. The resilience of this species, which correlates with success in other dasyurids, contrasts with declines in many Australian small mammals and makes a valuable contribution to discussion on broad geographic patterns in, and causes of, Australian mammal declines.

In Chapter 2, I confirmed that male kalutas are obligately semelparous. It was widely assumed that kalutas would exhibit this trait given it is shared by approximately 20% of dasyurids. This confirms that the strategy of obligate male semelparity has evolved at least twice (once in the Phascogalines and once in the Dasyurines). I confirmed the existence of multiple paternity within litters, indicating that female kalutas are polyandrous. Last, I showed that male kalutas exhibit positive testis allometry. Together, these results suggest that there is a significant role for sperm competition in the mating system of kalutas, and also confirms that kalutas conform to a similar life history strategy of other, resilient dasyurids (Phascogalines) that are polyandrous and show male semelparity.

In Chapter 3, I assessed the impact of fire on the distribution and abundance of kalutas and found that, as expected given their life history and body size, kaluta populations were larger on longer unburnt sites with high spinifex cover, compared with recently burnt sites with low spinifex cover. I also found that capture success of kalutas was significantly influenced by spinifex cover, while fire age, site elevation and site area were not significant predictors of capture success. My
results suggest that less frequent, less intense fires are beneficial for kalutas, as this will ensure sufficient habitat with high spinifex cover. These data can inform future management actions for a species inhabiting a region likely to experience more frequent fires as a result of a warming climate. Inappropriate fire regimes are a key threatening process for many small mammals in Australia. Patchy burning to achieve mosaics of differing fire ages is currently preferred by managers in the arid zone. As with many mammal species, this regime is likely to best support kalutas in the long-term.

In Chapter 4, I used a relatively novel DNA approach to assess the diet of kalutas and compared the results with a previous study using traditional, hard-part analyses of scats. I identified 28 food categories in the diet of kalutas using DNA based techniques. The major prey items were termites, a dasyurid species (*Pseudantechinus* sp.), spiders and centipedes, indicating that kalutas are insectivorous-carnivorous generalists. I did not identify any difference in prey choice between males and females, across sites of differing fire age, or between seasons. My results largely reflect those produced using traditional methods, suggesting that DNA analysis of scats is a valuable, efficient tool for future dietary studies of Australian mammals. Together with traditional methods, this novel technique provides greater opportunity for studying cryptic species because of its easy application across different terrestrial taxa.

In Chapter 5, I assessed the spatial genetic structure of kalutas across their range, using Bayesian assignment analyses, as well as at the finer scale, through spatial autocorrelation. I found no genetic structuring across the kaluta’s range, suggesting that there is one continuous, genetic population. These results also suggest that in their recent history no major barriers to dispersal have existed in the kaluta’s range. However, the results of the spatial autocorrelation suggest that fine scale structure exists for both males and females, with individuals of both sexes more closely related than expected by chance up to distances of 8 km. This fine scale structure suggests limited dispersal at the home range scale. These results provide hope that species such as the kaluta will be able to respond to changes in climate through movement, although that might be constrained by availability of spinifex and local fire history.

Together, the results in my first two chapters suggest this species shares common life history attributes with other, successful dasyurids, which may account for their current low conservation concern. My third chapter highlights the importance of understanding fire regimes and applying that knowledge to ensure appropriate fire intervals for fauna management. It also highlights the need to
monitor fire management across the Pilbara, to assess the risk of fire to kalutas at a landscape scale. The results in my fourth chapter suggest that termites are an important component of kaluta diet. Given many termite species rely on spinifex as a food source, it is clear that kaluta abundance and persistence is strongly tied to the health of spinifex in the landscape. Finally, the results of my population genetics study seemingly reflect a species able to adapt to changes in their environment, with caution advised. I conclude that the kaluta is currently persisting well, despite all adversities, and may have its strange life history to thank.

FUTURE DIRECTIONS

Future focus should be directed to identifying other possible reasons why the kaluta is succeeding, with a view to applying that knowledge to the management of species currently in decline. With climate change likely to substantially influence the habitat of the kaluta’s restricted range, there is also potential for this species to become threatened in the near-future. Fire regimes in the Pilbara are predicted to change, along with increased cyclonic intensity, and invasion from native and exotic species. During this time, cane toads may invade and present new threats to small mammals in the region. Therefore, studies aimed at identifying whether the cane toad poses a threat to kalutas should be undertaken. Studies on the diet of both feral cats and foxes in the Pilbara should be conducted to determine whether these species pose major predatory threats to the kaluta. Given the current success of kalutas, cats and foxes may not predate heavily on this species. If so, it would be interesting to learn if this is behavioural-avoidance on the kalutas’ behalf, a preference for other food items on the predators’ behalf, or other reasons.

Inappropriate fire regimes are considered a major threat to Australian terrestrial mammals. Management plans should be implemented to reflect the fire needs identified here. I recommend long-term studies be conducted to experimentally determine the impact of time since fire on kalutas and other sympatric, small vertebrates. These studies should be paired (burnt and not burnt sites) and be conducted across the broader Pilbara, to incorporate areas of varying substrate, vegetation and climate. The predicted change to future fire regimes in the Pilbara may involve an increase in both the frequency and intensity of fires. Therefore, modelling studies could be conducted that include data from my thesis (particularly the fire impacts and dispersal patterns), as well as the proposed fire experiment, to assess whether we can predict the likely impact of climate change on kalutas.
Future research for kalutas should also incorporate better, more comprehensive genetic studies. NextGeneration sequencing is advancing rapidly and provides an exciting opportunity for application to conservation genetics. For example, this technique could be used for a more in-depth look at the broad scale population structuring of kalutas. My study used microsatellites cross-amplified from closely-related species. While this is fast becoming an outdated method, it proved cost-effective and informative. Potentially, with techniques/markers designed for the species, it may be possible to identify barriers to dispersal. Furthermore, pairwise studies around human interference (e.g. mine sites, roads, rail) could be conducted to assess fine scale population genetics to determine whether the findings presented in this thesis are consistent across the kaluta’s range or whether anthropogenic impacts are impeding dispersal capabilities for this species. In addition to DNA techniques for population genetics, I also advocate establishing a reference library and designing improved primers for use in a dietary study to enhance results. Other DNA studies could identify which genes are under selection as a result of male-semelparity, and whether there is a selective consequence of this phenomenon. These findings could be coupled with a dedicated study of testis allometry within a range of dasyurids with different mating systems, to compare testis allometry in semelparous and non-semelparous, species.

Much research is still required to ensure the persistence of this species into the future. However, the current success of the kaluta provides a good-news story in a time when we are faced with an anthropogenic extinction crisis.
References


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