The Identification, Presence, Population Status and Ecology of Monjon and Nabarlek in the Kimberley based on Faecal DNA and Remote Sensor Camera Data

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Declaration

I certify that the substance of this thesis has not already been submitted for any degree and is not currently being submitted for any other degree or qualification. I certify that any help received in preparing this thesis, and all sources used, have been acknowledged in this thesis. All photographs in the thesis are my own unless otherwise stated.

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Abstract

The monjon (*Petrogale burbidgei*) and nabarlek (*Petrogale concinna*) are two small, cryptic and poorly known species of rock-wallaby that occur in remote areas of the North Kimberley, northwestern Australia. Both species are within the 'critical weight range' which predicts them to have an increased risk of extinction. The IUCN Red List lists *P. burbidgei* as 'near threatened', and *P. concinna* as 'data deficient'. Recent alarming declines in other mammal fauna across northern Australia, coupled with poor knowledge of the current abundance and distribution of *P. burbidgei* and *P. concinna* mean that more work on these sympatric species is urgently needed. However, research on *P. burbidgei* and *P. concinna* is challenging because despite them being morphologically and genetically distinct, the two species are almost indistinguishable to identify by sight, and are often confused even when 'in hand'. To add to the confusion, the monjon possibly contains an additional cryptic species yet to be described. Such issues have led to substantial confusion, with misidentification in the field having previously occurred. For these reasons, even basic information about distribution and abundance is lacking for both species.

My study aimed to overcome some of the difficulties with surveying these species by developing robust survey techniques while simultaneously building on the scientific knowledge of the population status, distribution and ecology of both species. I used sensor cameras as a non-invasive tool that were effective at detecting the presence of these trap-shy nocturnal wallabies. Site occupancy was modeled from the detection data provided by the sensor cameras in order to investigate which habitat variables might be important for the species' presence and to estimate occupancy accurately by modeling imperfect detection. However, in order to verify which rock-wallaby species occurred on cameras, I extracted genomic DNA from faecal pellets that I collected at each site. I then tested primers for several short fragments of mitochondrial DNA from the gene *cytochrome b*, and selected the one that yielded the most DNA from polymerase chain reactions. Sequences of the amplified fragment were used to assign species to pellets, thereby identifying which species were at which sites. Only short fragments were used as the DNA in faecal pellets is often highly degraded and more complex genetic relationships were beyond the scope of this

study. Additionally, faecal pellets were measured prior to extraction to determine if species could be correctly assigned to a pellet by morphometric measurement.

Petrogale burbidgei was detected by cameras and analysis of faecal DNA at six of ten potential study sites. Occupancy was estimated to be 0.644±0.075, however when three sites potentially outside its distribution range were excluded occupancy was at least 0.865. Complex rocky habitat (i.e. numerous caves and crevices) had a significant positive influence on site occupancy. In addition, the results of this study doubled the published extant distribution range of this species and this in combination with high occupancy demonstrated that *P. burbidgei* populations are likely to be currently stable.

Petrogale concinna was not detected by camera traps or by faecal DNA at any of the 29 possible mainland study sites. It was, however detected on Augustus Island. It is highly likely that this species has declined due to threatening processes on the mainland that are absent on nearby offshore islands. Possible threats include frequent fire, feral cats, habitat or food resource alteration by livestock, or less likely, disease. My study suggests differential resilience of these two similar sympatric species.

It is clear that urgent work is needed to locate remaining mainland Kimberley populations of *P. concinna*, assess the impact of threats, and implement strategies to reverse the decline. This work should also extend to Northern Territory populations which may have similarly declined. Further research should also include a re-assessment of all museum specimens to clarify correct labeling, potentially with the aid of molecular genetics, in order to obtain the best knowledge of the former distribution of *P. concinna*.

In addition to achieving the study objectives, this study also recorded the Kimberley endemic scaly-tailed possum (*Wyulda squamicaudata*) for the first time on Augustus Island. This highlights the merit of using sensor cameras in ecological surveys as cameras collect data on a wide range of species without any additional survey effort.

Based on the results of this study I recommend the use of sensor cameras or faecal pellet counts in conjunction with molecular genetics for monitoring Kimberley rock-wallabies. Occupancy modeling and potentially abundance estimation based on either of these methods of detection is practicable, however special attention should be paid to the measurement of covariates and the number of sites should be maximised. The fragment of mitochondrial DNA used in this study can reliably be sequenced from DNA obtained non-invasively from faecal pellets and unambiguously distinguishes these species. No survey method should be used without genetic (or skull morphology) confirmation as the risk of misidentification is considerable.

Acknowledgements

Firstly I would like to thank my supervisors Karl Vernes (UNE) and Mark Eldridge (Australian Museum) for their outstanding wisdom, excellent support and advice. Karl thanks for always being helpful, positive and encouraging no matter what the circumstance, and Mark thanks for leading the ways with the genetic analysis and for sharing your wealth of rock-wallaby knowledge.

No less important to this project were the Broome WWF-Australia team Tanya Vernes and Jess Koleck, and Greta Frankham from the Australian Museum. This project was essentially an extension of a WWF survey of the three species of rare and poorly studied Kimberley rock-wallabies, the subjects of this study and *Petrogale lateralis* (black-footed rock-wallaby). Jess led the field work and was largely responsible for the success of this project. Tanya coordinated the logistics of the project and, like her brother, was always positive. Many an occasion she transformed Jess' and my mood after a stressful conversation about things that had gone wrong in the field. Thanks WWF for giving me such an amazing honours project.

Greta Frankham was endlessly patient as she guided me through the extraction, amplification and sequencing of DNA in the Australian Museum Wildlife Genomics laboratories. Greta was responsible for the trial of two possible methods for processing the faecal pellets in order to determine which yielded the most PCR product, as well instructing me in detail on standard operating procedures, and any specific alterations I should make to the extraction process and PCR setup in order to achieve successful outcomes. Thanks Greta for your generosity, time and knowledge. I enjoyed working with you.

A big thank you to the Dambimangari, Wunambal Gaambera and Balanggarra Aboriginal Corporations who hold Native Title Rights for their lands and generously granted us access to their country for this study. Thankyou especially to the Dambimangari, Uunguu and Balanggarra Rangers, facilitated by the Kimberley Land Council, who assisted us and shared with us some of their amazing ecological knowledge. In particular I would like to thank Augie and his family for working tirelessly with us throughout our entire field work on Balanggarra

country. Augie seemed to be able to magically produce rock-wallaby scats from sites where we could not find any.

I would like to thank Chris Malam who kindly volunteered to assist us on the Kalumburu field trip and who put up with us along the way. I would like to especially thank him for climbing all the way down the cliff and back up again to bring another sensor camera when the one we had brought failed.

I was very fortunate to have access to the Australian Museum Wildlife Genomics laboratories to conduct my genetic analysis and identification of scats. I would like to thank the museum and its staff for their contribution to this project. I was lucky to be able to use state of the art equipment and have access to an office desk at the museum for the duration of my research. The genetic analysis was an integral part of the project and without it the field work would have far less significance.

I was honoured to meet Andrew Burbidge at Kalumburu and he has since contributed greatly to the project. In particular, his help with identifying mammals from camera images was invaluable. I do not believe anybody knows more about Kimberley rock-wallaby ecology.

I would also like to thank Natasha Crook for selflessly helping me solve my many frustrations with PRESRENCE and occupancy modelling, when no one else could. Thanks also for the loan of the Occupancy Estimation and Modelling book.

The majority of this project was funded by the World Wide Fund for Nature- Australia, which amongst other things enabled the field work to happen, negotiated access with Traditional Owners, facilitated involvement of Indigenous Rangers and allowed access to very remote areas of habitat in helicopters. I was very fortunate to have such great resources available for an honours project. UNE provided funding for the purchase of 15 of the camera traps used in this study, other miscellaneous field equipment, the three stool extraction kits, and covered the DNA sequencing costs. The Australian Museum assumed the cost of the PCR reagents, primers and equipment such as tubes, pipette tips, plates and gels.

Thanks to all who read and commented on various drafts of this thesis (Karl, Mark, Tanya, Greta, Jess, my sister Jemma Todd and my dad Colin Todd). I believe it has been greatly improved by your additions. Also thanks to my husband Roi Dadi who was responsible for creating the maps in this thesis.

I feel like I have spent the last year being part of something meaningful and important. It has been hard work but was definitely worth it. I will never forget the day Karl asked me 'would you like to go to the Kimberley to study two species of small, rare rock-wallabies?'

Chapter 1: Introduction and Literature Review

Rock-wallabies are an endemic genus of almost exclusively rock dwelling macropodid. They have a shy nature, are amazingly agile on vertical cliff faces and are often ornamented with attractive markings. Yet their choice of habitat makes them difficult to study and their rapid and recent evolution has led to structurally complex inter and intra-specific relationships. These factors have both intrigued and frustrated biologists attempting to understand them.

Monjon (*Petrogale burbidgei*) and Nabarlek (*Petrogale concinna*) are two small, cryptic and poorly known species of rock-wallaby. They occur in remote, rugged and difficult to access areas of the Kimberley region of north-eastern Australia, hence little is known of their biology and ecology. *Petrogale concinna* is more widely distributed, occurring also in the Northern Territory. Their similarity in appearance and inaccessibility of their habitat resulted in *P. burbidgei* being described only relatively recently, in 1978 (Kitchener and Sanson).

Petrogale concinna and *P. burbidgei* feature in a body of literature that discusses the evolutionary and biogeographic origin of *Petrogale* and the remarkable rapid diversification of its members (Briscoe *et al.* 1982; Campeau-Peloquin *et al.* 2001; Eldridge and Close 1993; Eldridge and Close 1997; Potter *et al.* 2012a; Sharman *et al.* 1990). The majority of early studies are investigations into phylogenetic relationships and biogeographic patterns based on the knowledge of karyotypes (i.e. chromosomes) established by Sharman et al. (1990) and Eldridge et al. (1992). More recently, molecular genetic evidence has shed some light on these relationships and challenged conclusions drawn by cytogenetic studies (Campeau-Peloquin et al. 2001; Potter et al. 2012a; Potter et al. 2012c). Scientific knowledge of evolution in *Petrogale* and the placement of *P. concinna* and *P. burbidgei* within macropodid phylogeny is sound, if not fully complete and it is clear that *P. concinna* and *P. burbidgei* are genetically distinct despite being morphologically similar.

By contrast, there has been little to no research on the ecology, biology and population size of *P. concinna* and *P. burbidgei* in the Kimberley. This is mostly due to the remote inaccessibility of habitat and the fact that it is inherently difficult to distinguish *P. burbidgei* and *P. concinna* in the field. There is however some understanding of the biology and ecology of the Northern Territory subspecies *P. concinna canescens* in terms of reproduction, diet and habitat preferences.

One study addressed the distribution and habitat preferences of *P. c. canescens* (Churchill 1997). One study investigated the reproduction of *P. c. canescens* in captivity (Nelson and Goldstone 1986), two studies included insights into the diet of *P. c. canescens* (Sanson et al. 1985; Telfer and Bowman 2006), one study attempted to distinguish the faecal pellets of *P. c. canescens* from sympatric species based on morphology (Telfer et al. 2006), and one recorded indigenous knowledge of rock-dwelling kangaroos, including *P. c. canescens*, in the Northern Territory (Telfer and Garde 2006). Three papers reviewed the conservation of rock-wallabies in general (Eldridge 1997; Lundie-Jenkins and Findlay 1997; Pearson and Kinnear 1997). In the literature there are also descriptions of morphology from collected specimens of *P. burbidgei* and *P. concinna* and incidental records of behaviour of both species (Kitchener and Sanson 1978; Lochman 1987; Sanson et al. 1985). However, there has been limited geographical coverage in the Kimberley, limited intensity, and little temporal replication of surveys.

Information from such a sparse body of literature must be gleaned with care as there is often no further research to support or refute claims made by a single, sometimes imperfect study and it is not known how much can be inferred from one subspecies to another. There have been no studies conducted on the habitat preferences, population status, reproduction, or diet of *P. burbidgei* or the Kimberley subspecies of *P. concinna, P. c. monastria*. Additionally, with the exception of some recent studies on phylogeny and genetics (e.g. Potter et al. 2012a; Potter et al. 2012c), most studies are more than seven and often more than 15 years old. There is a lack of detailed information on the ecology of these two species and there are no population estimates for either *P. burbidgei* or *P. concinna*.

Both *P. burbidgei* and *P. concinna* are potentially declining, and have been identified as conservation priority species by the World Wide Fund For Nature (WWF) ’s ‘Action Plan for Threatened Macropods’ (Roache 2011) and the West Australian Department of Environment and Conservation (DEC)'s 'Recovery Plan For Five Species of Rock Wallabies' (Pearson 2012). The status of these species is of particular concern because there have been dramatic recent declines in similar sized mammals in the Top End, Northern Territory (Woinarski *et al.* 2010). Furthermore, approximately 30 years ago *P. concinna* had already disappeared from many places where it was collected 100 years ago (Churchill 1997; Lundie-Jenkins and Findlay 1997; Sanson *et al.* 1985) and *P. burbidgei* occupies a restricted range (Burbidge *et al.* 2008b).

There have been no attempts to assess possible threats to these species; however the potential impact of some threats can be deduced indirectly by examining causes of decline in the Top End, declines in other *Petrogale* species, and the effects of landscape, habitat and species composition changes in the Kimberley. The most significant threats are likely to be fire and the impact of introduced herbivores and carnivores, although mining and climate change may become more important in the future. *Petrogale burbidgei* and *P. concinna* are Critical Weight Range (CWR) mammals and live in a highly seasonal environment; hence they may be inherently sensitive to changes in the productivity of the system. Furthermore the absence of these two macropodids from the state and federal endangered species lists may say more about our lack of knowledge than the actual status of these populations. It is vitally important to establish a sound estimate of their population size and distribution to ensure the best management decisions are made.

1.1 Biology and Ecology

1.1.1 Evolution and Phylogeny

There are 16 currently recognised species of *Petrogale* and 10 genetically distinct subspecies and races, making them the most species rich group of extant macropodids (Van Dyck and Strahan 2008). Four main lineages or groups have been identified within *Petrogale* (Figure 1.1; Potter *et al.* 2012a). It is widely recognised that *Petrogale* diversified recently and rapidly, throughout the late Miocene, Pliocene and Pleistocene (Campeau-Peloquin *et al.* 2001; Eldridge and Close 1993; Potter *et al.* 2012a). The high rate of speciation has been attributed to geographic isolation, restriction to rocky outcrops and contraction into refugia during periods of aridity (Eldridge and Close 1993; Potter et al. 2012c).

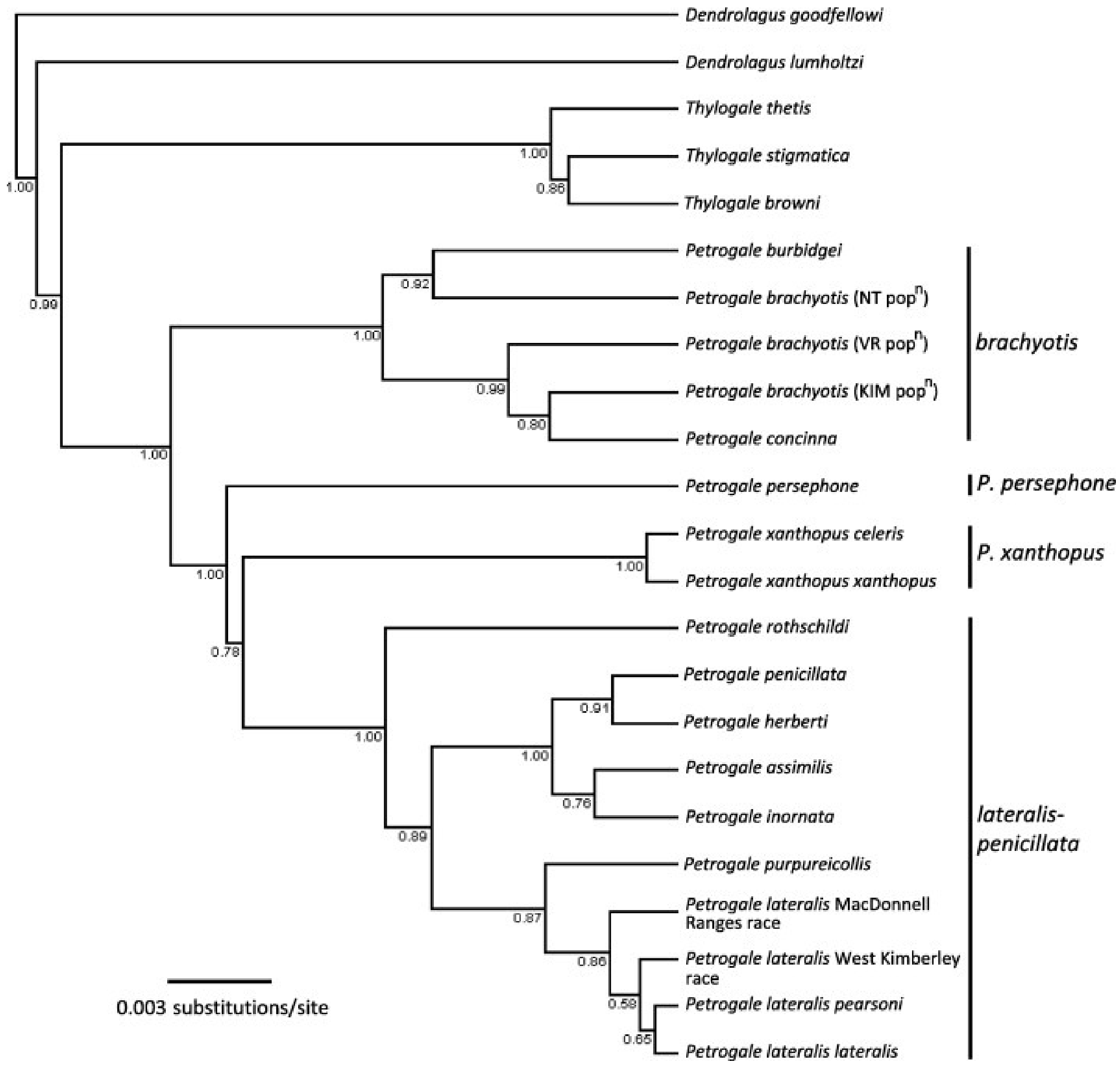
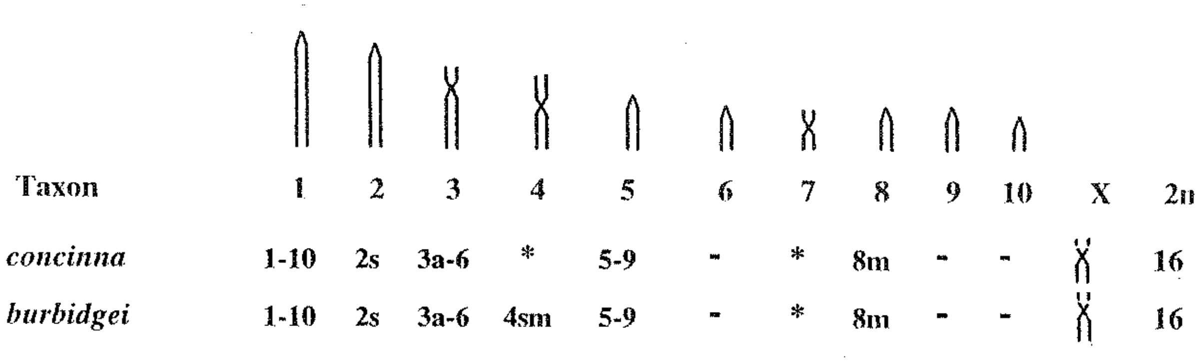


Figure 1.1. Phylogenetic tree of *Petrogale* showing the four main lineages, constructed from molecular DNA data using BEST analysis. From Potter, Cooper et al. (2012a)

There is consensus in the current literature that *P. burbidgei*, *P. brachyotis* and *P. concinna* form a distinct monophyletic linage (Figure 1.1), despite *P. concinna* mistakenly being placed in the monotypic genus *Peradorcas* in 1904 (Campeau-Peloquin et al. 2001; Eldridge and Close 1993; Eldridge et al. 1992; Potter et al. 2012a; Sharman et al. 1990; Thomas 1904).

Cytogenetic studies by Sharman et al. (1990) and Eldridge et al. (1992) revealed that the chromosomes of *P. concinna* and *P. burbidgei* have undergone several centric fusions, inversions and centric shifts (Table 1.1). This led to a reduced number of chromosomes compared with what is believed to be the ancestral karyotype, 2n=22 (Eldridge and Close 1997). The main difference between the two species is that chromosome four of *P. burbidgei* has undergone a shift to submetacentric, while that of *P. concinna* has remained metacentric. This suggests that *P. burbidgei* is the more derived of the two; however such conclusions cannot be drawn without further research.

Table 1.1. Ancestral karyotype of *Petrogale* (top) and the respective changes in the chromosomes of *P. concinna* and *P. burbidgei*. s and sm = change to submetacentric, m = change to metacentric, a= change to acrocentric, - = fusion, and * = no change. Adapted from Eldridge and Close (1997)



Taxon	1	2	3	4	5	6	7	8	9	10	X	2n
concinna	1-10	2s	3a-6	*	5-9	-	*	8m	-	-		16
burbidgei	1-10	2s	3a-6	4sm	5-9	-	*	8m	-	-		16

In addition to knowledge of chromosome structure, several regions of mitochondrial and nuclear DNA have now been sequenced for *P. burbidgei* (O'Neill et al. 1997; Potter et al. 2012a; Potter et al. 2012c) and *P. concinna* (Potter et al. 2012a; Potter et al. 2012c) (Table 1.2). These studies assessed the genetic distance between different *Petrogale* species, including between *P. burbidgei* and *P. concinna*. The results clearly confirm that they are genetically distinct species despite their morphological similarity. From the phylogenies constructed from sequence data, it appears that *P. concinna* is more closely related to *P. brachyotis* than to *P. burbidgei*, however only one *P. concinna* specimen was used in these analyses (Potter et al. 2012a; Potter et al. 2012c). More work is needed with additional sequences and preferably additional specimens to resolve the complex intra and inter-specific relationships of these species (Potter et al. 2012a).

Table 1.2 Regions of genomic DNA that have been sequenced for *P. concinna* and *P. burbidgei*.

gDNA type	Region	Length	*P. concinna* or *P. burbidgei*	Source
Mitochondrial	Control Region (CR)	599bp	Both	(Potter et al. 2012c)
Mitochondrial	NDAH dehydrogenase subunit 2 (ND2)	586bp	Both	(Potter et al. 2012c)
Nuclear	Breast and ovarian cancer susceptibility gene (BRCA1)	1044bp	Both	(Potter et al. 2012c)
Nuclear	Noncoding nuclear ω-globin	537bp	Both	(Potter et al. 2012c)
Nuclear	Two random, anonymous loci	Approx 700bp each	Both	(Potter et al. 2012c)
Mitochondrial	Cytochrome oxidase c subunit 1 (CO1)	1315bp	Both	(Potter et al. 2012a)
Mitochondrial	Cytochrome b (cytb)	1140bp	Both	(Potter et al. 2012a)
Mitochondrial	NDAH dehydrogenase subunit 2 (ND2)	569bp	Both	(Potter et al. 2012a)
Nuclear	ω-globin intron	739bp	Both	(Potter et al. 2012a)
Nuclear	Breast and ovarian cancer susceptibility gene (BRCA1)	1048bp	Both	(Potter et al. 2012a)
Nuclear	Male sex determining gene SRY	Approx 600bp	*P. burbidgei*	(O'Neill et al. 1997)

1.1.2 Distribution and Habitat

Petrogale concinna

Petrogale concinna has a disjunct distribution in the Northern Territory and Western Australia (Figure 1.2; Churchill 1997; Lundie-Jenkins and Findlay 1997). The subspecies *P. c. monastria* occurs in Western Australia and the subspecies *P. c. canescens* occurs in the Northern Territory. The third subspecies, *P.* c. *concinna*, is from Victoria River and is only known from the original specimen collected in 1839 from which the species is described (Sanson and Churchill 2008). However, the status of these three subspecies has not been verified (Sanson and Churchill 2008). Localities where *P. concinna* has been recorded in the Kimberley, both in the past and more recently, are shown in Figure 1.3.

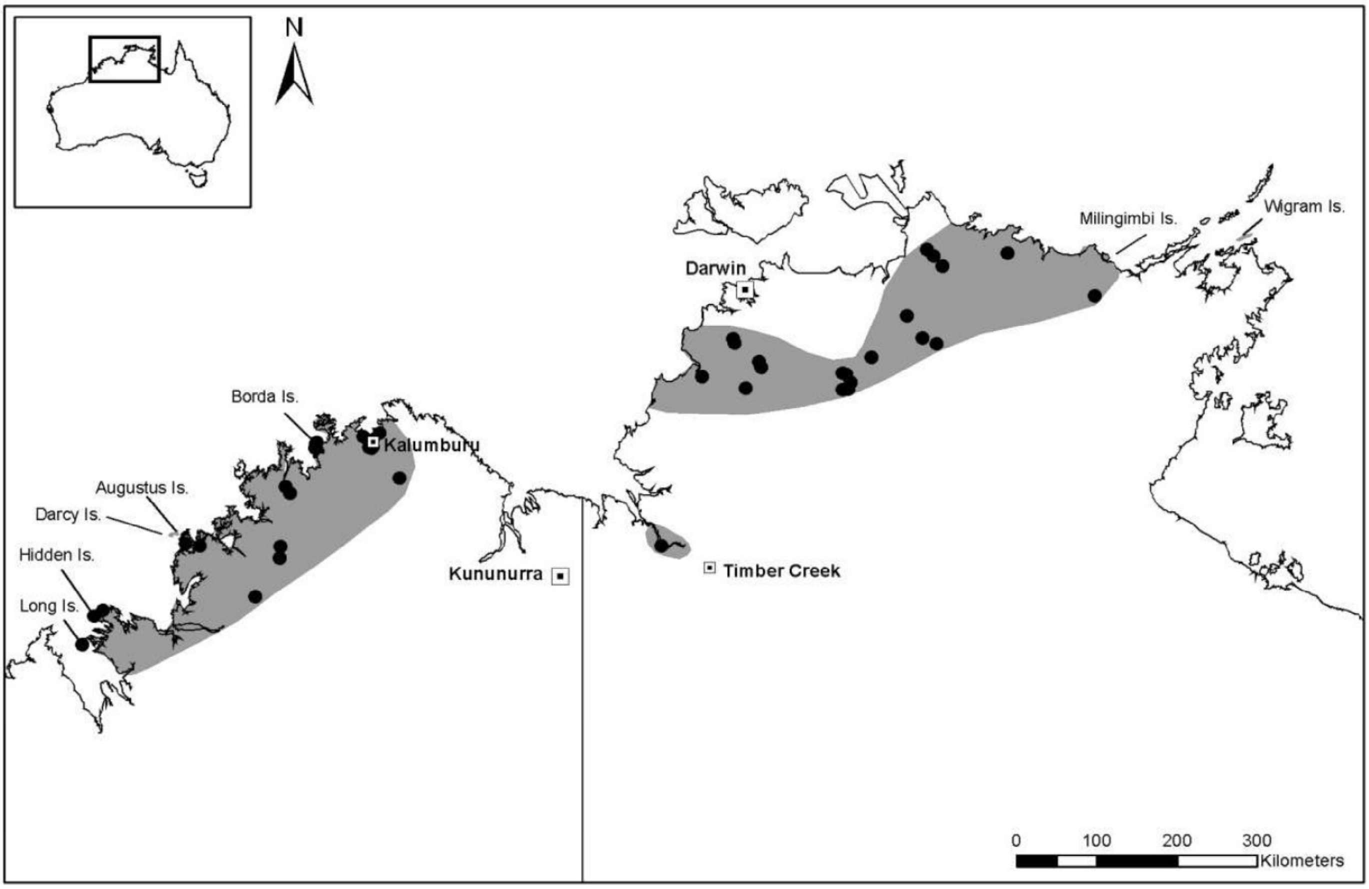


Figure 1.2. Distribution of *Petrogale concinna* across northern Australia. From Pearson (2012)

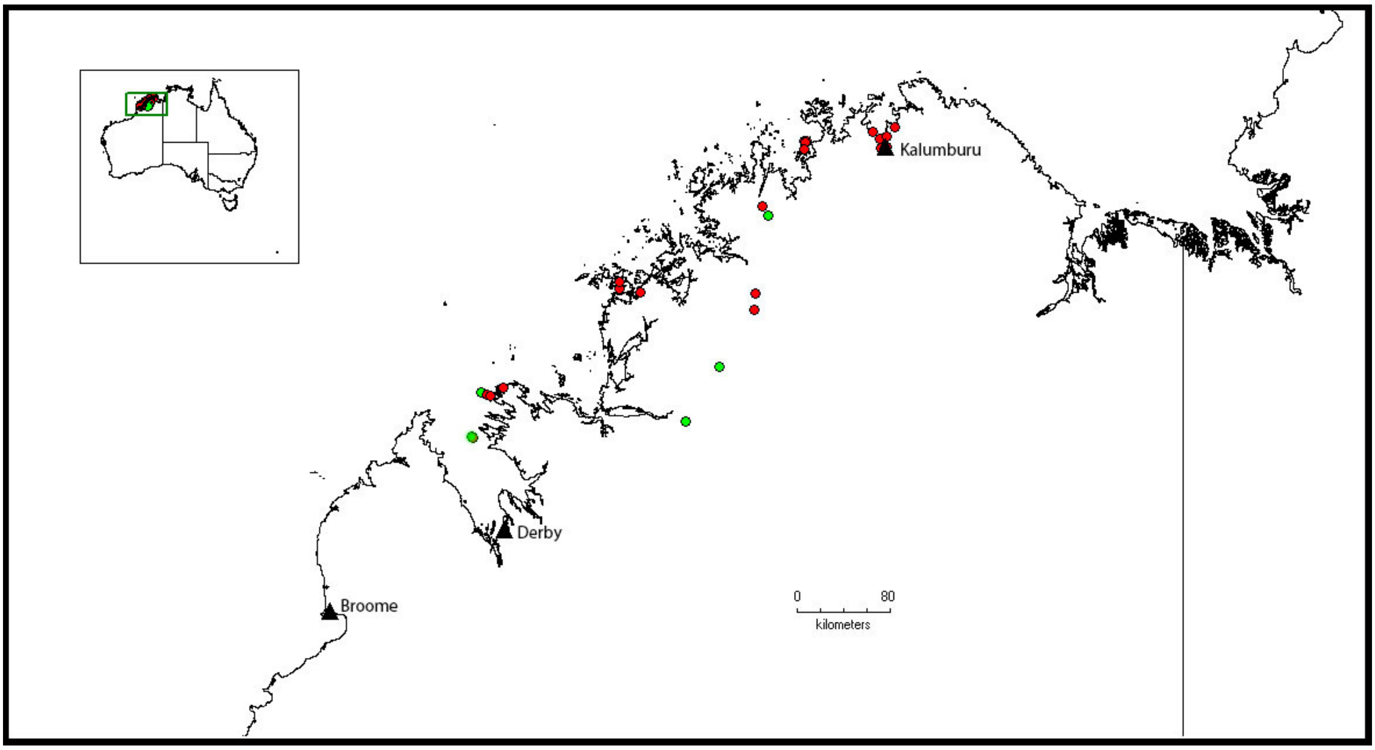


Figure 1.3. Localities where *P. concinna* has been recorded in Western Australia. Red points= records > 20 years old; green points= records <20 years old. Adapted from NatureMap (DEC 2013)

The presence of *P. concinna* in the NT is correlated with the presence of steep slopes, large numbers of caves and crevices, a high percentage shrub cover, the presence of tall shrubs and large areas of broken cliff (Churchill 1997). Yet it exists in a range of habitats and have been found in areas of low granite boulders as well as steep sandstone cliffs (Churchill 1997). Indigenous knowledge confirms that *P. concinna* exists in remote rocky country, and that the presence of caves is important as it shelters in them during the day (Telfer and Garde 2006).

Churchill (1997) did not find any significant correlation between the abundance of *P. concinna* and environmental variables. However, the measurement of abundance in this study was potentially biased. Individuals were counted as they were sighted along transects using active and passive spotlighting, and during the day in some instances. The number of individuals sighted was taken as a proxy for abundance for that location without any attempt to address issues of detectability, or any attempt to avoid counting the same individual multiple times.

In the same study by Churchill (1997), a significant difference in abundance of *P. concinna* faecal pellet counts between floristic groups was found, yet no post-hoc pair-wise comparisons were made to determine where the differences lay. The floristic group comprising of *Buchanania obovata, Eucalyptus miniata, Livistona sp., Owenia vernicosa,* *Planchonia careya, Plectrachne pungens, Sorgum sp.* and *Terminalia ferdinandiana* had the most pellets and the floristic group comprising of *Eucalyptus polycarpa, E. miniata, Pandanus spiralis, Sorgum sp., T. ferdinandiana* had the least pellets. It has been verified for a number of species, including several species of macropods, that pellet counts reflect true density (e.g. Hill 1981; Johnson and Jarman 1987; Karels *et al.* 2004; Vernes 1999; Walker *et al.* 2000) therefore it can be concluded that *P. concinna* was more common in the former habitat than in the latter habitat.

Petrogale burbidgei

Petrogale burbidgei is endemic to the North Kimberley, Western Australia (Pearson *et al.* 2008). It is known to occur on three islands in the region: Bigge, Boongaree and Katers, with possible occurrence on a fourth; Wollaston (Abbott and Burbidge 1995; Gibson and McKenzie 2012). On the mainland this species' published extant distribution range is between (and including) Prince Regent Nature Reserve and Mitchell Plateau (Figure 1.4; Pearson *et al.* 2008), although more recently it has been recorded further south west in the Artesian Range by Australian Wildlife Conservancy (AWC) staff (Figure 1.4; unpublished data). Subfossil evidence of *P. burbidgei* was found by Start *et al.* (2012) 220km outside its published distribution, extending its historic distribution range into the more arid central Kimberley (Figure 1.4). It is not clear when the range contraction occurred, although it was likely to have been prior to or around the same time as European settlement (Start *et al.* 2012). Sites where *P. burbidgei* have been recorded or collected are shown in Figure 1.4.

Favourable habitat appears to be rugged King Leopold sandstone boulders, scree, slopes and outcrops with a high proportion of caves and crevices (Kitchener and Sanson 1978; Pearson et al. 2008). Specimens have been collected in low open woodland dominated by *Owenia vernicosa* and *Eucalyptus spp*., with and understory of low open scrub and hummock grass (*Plectrachne sp.*) (Kitchener and Sanson 1978). Additionally, *P. burbidgei* appears to be limited by rainfall, as it currently only occurs in the higher rainfall areas of the northern Kimberley. As no studies have been carried out on habitat requirements, detailed habitat preferences are not known.

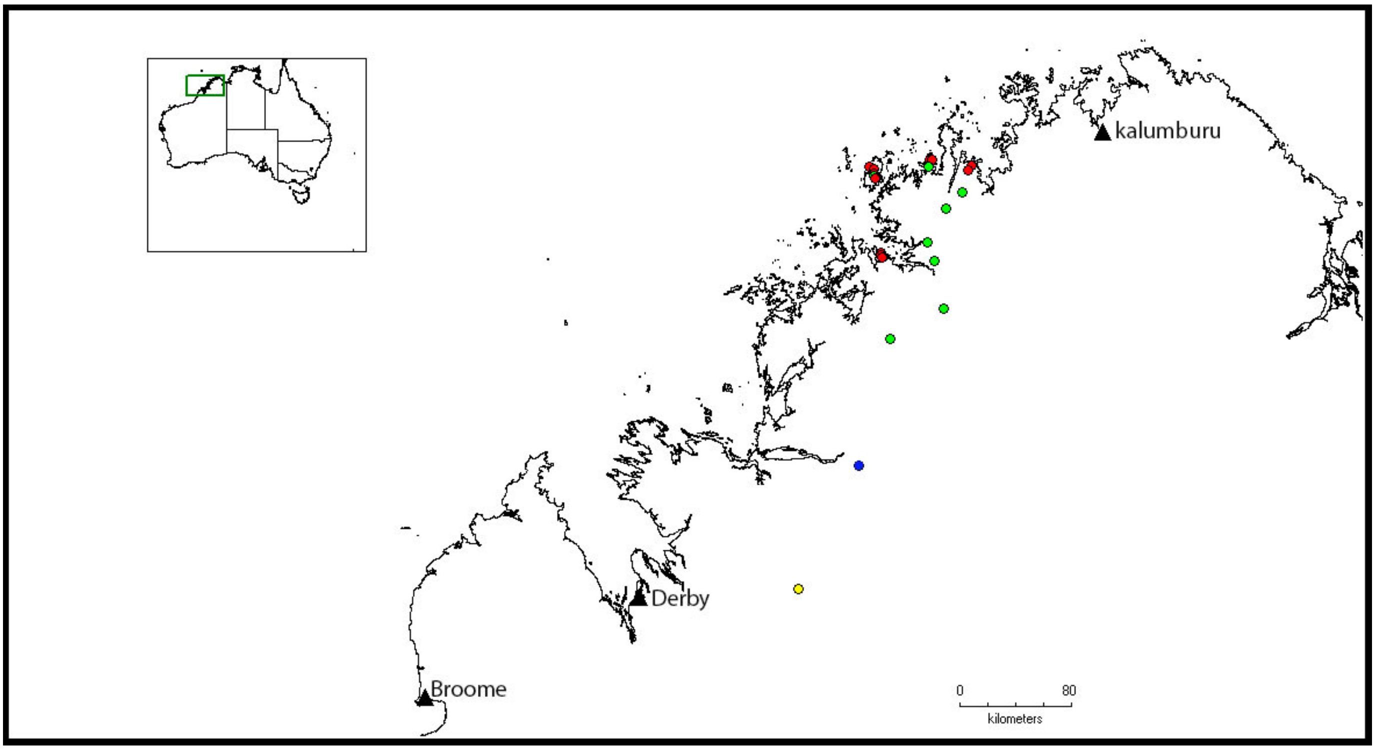


Figure 1.4. Locations where *Petrogale burbidgei* has been recorded. Red points = records >20 years old, green points = records <20 years old, yellow point= historic subfossil record, blue point= unpublished record by AWC. Adapted from DEC (2013)and Start *et al.* (2012)

1.1.3 Morphology and Identification

Petrogale burbidgei and *P. concinna* are the two smallest species of rock wallaby weighing 960-1430g and 1050-1700g respectively (Kitchener and Sanson 1978; Pearson et al. 2008; Sanson and Churchill 2008). They are very similar in appearance, however *P. burbidgei* has smaller ears (less than 35mm) and a shorter hind foot (less than 93mm) (Kitchener and Sanson 1978). Additionally, there are some subtle pelage differences and a number of differences in skull morphology and dentition (Kitchener and Sanson 1978; Menkhorst and Knight 2011). The main difference in dentition is that *P. concinna* has continually erupting molars, a feature for which it is unique among all marsupials (Sanson and Churchill 2008). *Petrogale burbidgei* is limited to four molars and has permanent premolars which *P. concinna* does not. For detailed descriptions on dentition, skull morphology and colouration see Kitchener and Sanson (1978).

Distinguishing *P. burbidgei* and *P. concinna* in the field without taking morphometric measurements is difficult. The pelage of both species can vary greatly, especially between populations, and there is substantial overlap in coloration (Mark Eldridge, pers. comm. 2013).

Further, both can be confused with juvenile *P. brachyotis*, which is similarly coloured. In general *P. burbidgei* often has more rufous colouring around the eye and on the head, blackish tip to the tail and more mottled dorsal pelage compared with *P. c. monastria*, while *P.* c. *monastria* usually has a stronger dark crescent under the elbow, and a shorter more brushy tail with lighter hairs on the tip (Mark Eldridge, pers. comm. 2013; Kitchener and Sanson 1978). The general body colour of *P. burbidgei* is a deep olive-buff whereas the dorsal pelage of *P. concinna* is grayish-olive (Kitchener and Sanson 1978), although these characteristics can be ambiguous (Mark Eldridge, pers. comm. 2013).

Petrogale concinna has been observed holding its tail over its head while hopping at high speeds, with its body mostly horizontal to the ground and its tail fluffed out (Churchill 1997). This characteristic has been used to distinguish it from sympatric *P. brachyotis* in the Northern Territory (Churchill 1997). It is not known if this is a useful feature in distinguishing *P. concinna* from *P. burbidgei*.

1.1.4 Reproduction

Petrogale concinna

In captive individuals, the mean oestrous cycle length was found to be 33.73± 1.65 days and gestation was 30 days (Nelson and Goldstone 1986). This is similar to oestrus and gestation length in other rock wallabies (see Johnson 1979; Johnson and Delean 1999; Johnson and Delean 2002; Poole *et al.* 1985).

Like many other macropods, including all other members of *Petrogale* with available data on reproduction (Johnson 1979; Johnson and Delean 1999; Johnson and Delean 2002; Poole et al. 1985; Taggart et al. 2005), *P. concinna* exhibits post partum oestrus and embryonic diapause (Nelson and Goldstone 1986). When pouch young were removed by Nelson and Goldstone (1986) lactation anoestrus ceased and females gave birth 14-18 days later which is significantly shorter than in other rock wallabies, for which this is usually around 30 days (Johnson 1979; Johnson and Delean 1999; Johnson and Delean 2002; Poole et al. 1985; Taggart et al. 2005).

Growth is rapid and maturity reached early in *P. concinna*, compared with other rock-wallabies (see Johnson 1979; Johnson and Delean 1999; Johnson and Delean 2002; Poole et al. 1985; Taggart et al. 2005). Pouch young first open their eyes at about 110 days; are furred at 125 days, when they weigh approximately 90 grams; and leave the pouch at 160 days old, weighing about 200 grams. Weaning is abrupt and young at foot are independent within two weeks after leaving the pouch due to aggression from the female. This is apparently unique among *Petrogale*, where weaning is usually drawn out three to six months post permanent pouch emergence (Johnson 1979; Johnson and Delean 1999; Johnson and Delean 2002; Poole et al. 1985; Taggart et al. 2005). Maturity is reached at around 1000g and 400 days of age (Nelson and Goldstone 1986).

In a captive environment female *P. concinna* formed a dominance hierarchy, where dominant individuals had shorter oestrous cycles (32 days) and subordinate individuals had longer cycles (35 days; Nelson and Goldstone 1986). Intersexual aggression was also found to occur in captivity, with females attacking and even killing males after mating (Goldstone and Nelson 1986). It is possible that these social-reproductive interactions also occur in the wild, however the confinement of captivity may place additional pressure on individuals and alter behaviour (Nelson and Goldstone 1986).

Petrogale burbidgei

No studies have been conducted on the reproduction of *P. burbidgei*, however records from collected specimens revealed that pouch young are unfurred at 51 grams and furred by 77 grams (Kitchener and Sanson 1978). Thus development of fur in *P. burbidgei* occurs at much smaller size (and presumably younger age) than for the marginally larger adult *P. concinna* (Nelson and Goldstone 1986). Additionally, the presence of similar sized pouch young (51-85g) in late spring suggests that there is a distinct breeding season that times pouch emergence with the onset of the wet season (Pearson et al. 2008).

1.1.5 Behaviour

Both *P. concinna* and *P. burbidgei* are mostly nocturnal (Pearson et al. 2008; Sanson and Churchill 2008; Sanson et al. 1985). *Petrogale concinna* reportedly rests during the day in cliffs, but has been observed to move several hundred metres away from rocks at night to graze (Sanson et al. 1985). *Petrogale concinna* is more active during the wet season than the dry, often emerging from caves before dusk (Sanson et al. 1985).

Petrogale burbidgei has been observed 'foot thumping'; a common phenomena in wallabies to warn others of danger (Lochman 1987; Rose et al. 2006). Such alarm signalling potentially indicates that *P. burbidgei* occurs in social groups, however foot thumping may also be used to signal to the predator (see Rose *et al.* 2006 for a discussion of these hypotheses). Foot thumping was also reported for *P. concinna* by Indigenous Custodians (Telfer and Garde 2006).

1.1.6 Diet

The diet of *P. concinna* varies spatially, temporally and intraspecifically (Sanson et al. 1985; Telfer and Bowman 2006). It is therefore likely that *P. concinna* is able to browse opportunistically on a wide variety of plants, depending on seasonal and spatial availability. Sanson et al. (1985) found the diet of *P. concinna* at Mt. Borridale was very different between the wet and dry seasons. At this location, their wet season diet comprised of *Cyperus cuspidatus*, *Eriachus* sp., *Triodia* sp., *Marsilea crenata*, *Cartonema* sp. and small amounts of other browse (Sanson et al. 1985). In the dry season their diet was comprised almost exclusively of the fern *M. crenata* but also a small amount of *Triodia* grass. The sample size in this study was small (n=4 for the dry season and n=2 for the wet season) and individuals were only from one locality, possibly leading to misrepresentation of the diet if dietary preferences of this species vary across its range.

Sanson et al. (1985) proposed that the high silica content, and therefore abrasive nature, of the main dry season dietary item (*M. crenata*) was the reason that *P. concinna* evolved continually erupting and progressing molars. It fits neatly that unlimited number of molars is an adaptation to a diet of tough plants in the dry season. A trait that allowed increased feeding in the dry season would have a strong selection advantage as rock wallabies in northern Australia have significantly reduced body weight and poor body condition during this period (Sanson and Churchill 2008; Telfer and Garde 2006). However, *P. concinna* also occurs in areas where *M. crenata* and other high silica plants do not currently exist (Churchill 1997).

Indigenous knowledge suggests that the diet of *P. concinna* is the same as, or very similar to, other rock-wallabies such as *P. brachyotis* (Telfer and Garde 2006). A total of 10 species of plant were identified by various Indigenous Peoples as being eaten by *P. concinna* in West

Arnhem Land (Table 1.3), and all of them were also identified as being eaten by either wallaroos (common wallaroo, *Macropus robustus*, Black wallaroo, *M. bernardus*) or *P. brachyotis*. Similarly, Telfer and Bowman (2006) found no significant difference in the dominant plant group composition of faecal pellets of *P. concinna* (at Robin Falls Apostles) and *P. brachyotis* (at a range of sites across the NT). Both species consumed mostly browse and forbs, with grass playing a relatively small part of their diet. However as there is some overlap in faecal pellet size between the two species, this information should be treated with caution.

It is possible that the continually erupting molars that characterise this species are a vestige from when the species evolved. *Petrogale concinna* may have moved more recently into areas where a less abrasive diet is possible due to high food availability and therefore reduced competition (Telfer and Bowman 2006).

There have been no studies on the diet of *P. burbidgei*, and none on the diet of *P. concinna* in Western Australia. What is known of the diet of *P. concinna* comes from studies conducted in the Northern Territory. Since there is unlikely to be the same vegetation composition and structure across northern Australia, and different subspecies of *P. concinna* occur in the Kimberley and Northern Territory, the information on diet of this species should be interpreted with caution.

Table 1.3. Plants eaten by *P. concinna* in Western Arnhem Land according to Indigenous Knowledge. Adapted from Telfer and Garde (2006)

Species	Growth Form	Part Eaten	Number of Indigenous people that identified it as being eaten by *P. concinna*
Blepharocarya depauperata	Tree	Leaves and fruit	>1
Buchanania obovata	Tree	Fallen fruit	>1
Decaschistia byrnesii	Shrub	Leaves and sometimes tubers dug up and eaten	>1
Ficus platypoda	Tree	Leaves and fruit	1
Gardenia fucata	Tree	Leaves	1
Ipomoea abrupt	Climber	Roots (dug up and eaten)	1
Melodorum rupestre	Climber	Leaves	>1
Memecyton arnhemensis	Shrub	Leaves	One, but identified as not being eaten by another
Sterculia quadrifida	Tree	Fruits/seeds (in the wet season)	1
Terminalia carpentariae	Tree	Fruit/seeds	>1
Triodia plectrachnoides	Grass	Young and old leaves	1
Triodia microstachya	Grass	Shoots, seeds and seedheads	>1

1.2 Conservation Issues

1.2.1 Conservation Status

Both *P. burbidgei* and *P. concinna* are potentially declining, and have been identified as conservation priority species. The International Union for the Conservation of Nature (IUCN) currently (IUCN Red List version 2013.2) lists *P. burbidgei* as ‘near threatened’ while *P. concinna* is listed as ‘data deficient’, meaning there is little current information on population trends (Burbidge et al. 2008b; Woinarski et al. 2008). Both exotic predators and changes in fire regime have been identified as possible threats to these two species, although their effects are yet to be studied (Pearson 2012; Roache 2011). Neither species appear on the EPBC Act List of Threatened Species (national) or the Western Australian Threatened Species List (state) (Jacob 2012; Threatened Species Scientific Committee 2009).

1.2.2 Critical Weight Range (CWR)

Australia has the highest recent mammal extinction rate in the world, with 22 out of 305 non-marine mammals extinct in the last 200 years and many more declining (Burbidge et al. 2008a; McKenzie and Burbidge 2002). Modelling by McKenzie and colleagues (1989; 2002; 2007) looked at factors that contributed to the continent-wide attrition of mammals. It was discovered that virtually all extinctions (except the thylacine, *Thylacinus cynocephalus*) have been in the weight range 35-5500g. This was termed the ‘Critical Weight Range’ (CWR) by Burbidge and McKenzie (1989), and is now generally accepted (McKenzie *et al.* 2007; Van Dyck and Strahan 2008; Woinarski *et al.* 2011) despite some contention on statistical grounds (Cardillo and Bromham 2001; Johnson and Isaac 2009).

It is believed that these small-to medium sized mammals are particularly prone to declines because they have limited mobility compared to larger mammals, coupled with high absolute metabolic requirements compared to very small mammals. This has important consequences when landscape changes reduce productivity and energy availability (Burbidge and McKenzie 1989). Moreover, CWR mammals are the appropriate prey size for the decimating introduced predators; cats and foxes (Short and Turner 1994). Both *P. burbidgei* (1-1.5kg) and *P. concinna* (1-1.7kg) fall within this range, potentially increasing their risk of decline.

1.2.3 Seasonality of Rainfall

According to McKenzie et al. (2007), Burbidge and McKenzie (1989) and McKenzie and Burbidge (2002), the biggest explanatory variable of Australian mammal attrition was rainfall; an assumed surrogate for productivity. Mammals were most likely to have declined or become extinct from low rainfall arid areas, and least likely from high rainfall mesic areas (Figure 1.6).

The North Kimberley has reportedly experienced the lowest level of mammal species attrition in all of Australia (Figure 1.6; Burbidge *et al.* 2008a; McKenzie and Burbidge 2002). The North Kimberley region experiences high annual rainfall, meaning more resources are available which could provide some resilience to change in this ecosystem (McKenzie et al. 2007). However, McKenzie and Burbidge (2002) cautioned that the North Kimberley may not be the refuge that it is purported to be by other authors, as there have been declines in this region as well. Further, because the region is relatively poorly studied on account of its remote inaccessibility, it may be that few declines have been reported partially due to lack of documentation of change rather than persistence of species.

Rainfall in the Kimberley is highly seasonal, with less than 6mm falling between the months of June and September (Bureau of Meterology 2011). This is less than the mean rainfall for that period in the driest areas of Australia (Bureau of Meterology 2011). It is possible that the dry season survival rate largely determines population survival (Sanson and Churchill 2008; Telfer and Garde 2006). Animals that are just managing to survive during the dry season could easily perish with a small change in resource availability. The ‘aridification’ effect of land degradation could have a significant impact on animals in highly seasonal environments (McKenzie et al. 2007).

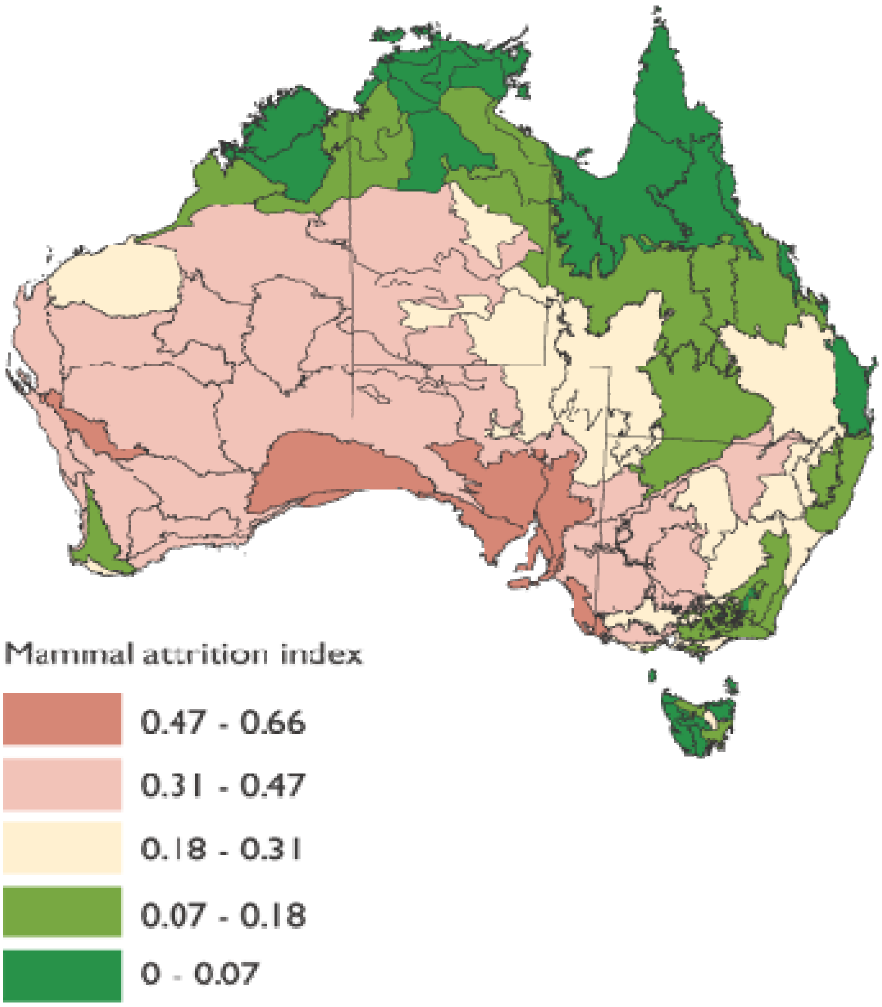


Figure 1.6. Biogeographical pattern of mammal decline in Australia. From McKenzie and Burbidge (2002)

1.2.4 Fire

There have been dramatic changes in the extent, severity and frequency of burning in the Kimberley since the arrival of Europeans (Russell-Smith et al. 2003). Pre-colonisation, the land was generally burnt early in the dry season in small areas creating a patchwork of vegetation at different stages of recovery. This was believed to create a high diversity of forage, that was advantageous for many herbivores (Parr and Anderson 2006; Russell-Smith *et al.* 2003). The modern pattern of fire is that of more frequent, late dry season, intense fires (Fisher *et al.* 2003; Russell-Smith *et al.* 2003; Vigilante 2001; Yates *et al.* 2008).

It is commonly stated that early mosaic burning is better than large late dry season fires for most species (Parr and Anderson 2006). Modern fire patterns may negatively impact populations of *P. burbidgei* and *P. concinna* in a number of ways. Firstly, these species may require vegetation at a certain stage of post-fire recovery, and therefore would have reduced quality feed most of the time under modern fire regimes. Secondly there may be a population crash after a large fire due to no food being within foraging distance, or an increase in predation as wallabies have to forage far from shelter. Thirdly, mortality is likely to be greater in a large, hot fire, especially for rock-wallabies occupying sub-optimal habitat.

Experimental evidence shows that for mammals and other fire sensitive species fire frequency is more important than fire scale or timing (Anderson *et al.* 2005; Williams *et al.* 2003). However, unlike most mammal species recorded by Anderson *et al.* (2005) to have declined under frequent fire treatments, *P. burbidgei* and *P. concinna* use rock caves and crevices for shelter, which may mitigate some of the direct negative impacts. It is therefore most likely that if frequent fire is negatively impacting populations of these species, then it is through the changes in vegetation structure and composition and therefore resource availability. For example, invasive buffel grass (*Cenchrus ciliaris* L.) has been shown to increase post fire in arid and semi-arid Australia (Miller et al. 2010) and some native obligate seeder species have collapsed under frequent fires (Fisher *et al.* 2003). Further, buffel grass, and probably other invasive grasses, create a positive fire invasion feedback cycle, with invasions increasing the frequency and intensity of fire, thereby further altering the vegetation community (Miller et al. 2010). It is not clear if and how fire may affect *P. burbidgei* and *P. concinna*, and without specific studies it is only possible to speculate what may have been the impacts of an altered fire regime.

1.2.5 Predation

Kinnear *et al.* (2010) demonstrated that foxes (*Vulpes vulpes*) had a significant impact on black-footed rock-wallabies *P. lateralis lateralis*. Predator baiting experiments over 20 years showed greatly improved wallaby numbers in response to fox control. Susceptibility to predation is dependent on the security of the habitat, with only the deepest crevices providing protection against predation (Kinnear et al. 2010). In addition to reducing numbers, fox predation dramatically altered the foraging behaviour of *P. lateralis lateralis*. Fox predation was so detrimental that it had the ability to drive a population to extinction (Kinnear et al. 2010). Although foxes do not occur in the north Kimberley (Burbidge et al. 2008a), this demonstrates the potential threat of introduced predators to rock-wallabies.

Cats (*Felis catus*) occur throughout Kimberley region and are likely to be a significant threat to *P. burbidgei* and *P. concinna* (Pearson 2012; Roache 2011). Direct evidence of cat predation on rock-wallabies was demonstrated by Spencer (1990). A single individual was believed to have eaten 45.5% of young at foot, 14.2% of subadult and 4.6% of adult individuals in a population of allied rock-wallabies (*P. assimilis*) during a span of nine months. The minimum number of individuals known to be alive decreased from 83 to 26 individuals, and very few juvenile wallabies were observed even though 87% of females had pouch young. Such predation rates could easily decimate an isolated population of *P. concinna* or *P. burbidgei*, particularly as smaller animals are most at risk.

Cats were shown to predate mostly on rabbits in central eastern NSW (Molsher et al. 1999). *P. burbidgei* and *P. concinna* are similar sizes to rabbits (the latter being 1-2.4kg; Molsher et al. 1999) and therefore may act as a prey alternative for cats in northern Kimberley where rabbits do not occur. Rock-wallabies may escape predation by hiding in caves and crevices, but rabbits similarly construct warrens deep underground to avoid predation (Molsher et al. 1999). Research on the impact of feline predation is an essential step toward the management and understanding of these two species.

1.2.6 Landscape Changes, Mining and Introduced Herbivores

Other factors that McKenzie et al. (2007), Burbidge and McKenzie (1989) and McKenzie and Burbidge (2002) found helped explain mammal declines were presence and time since arrival of introduced herbivores and carnivores, environmental change, and whether species were-ground-dwelling or arboreal.

Feral cattle (*Bos indicus, B. taurus*) and other large herbivores have become ubiquitous throughout the Kimberley (Legge et al. 2011). Significant recovery of mammal populations occurred in the years following the removal of large herbivores from an area in Mornington sanctuary, central Kimberley, demonstrating that introduced herbivores do have a negative impact on native mammals (Legge et al. 2011). Introduced herbivores alter the ground vegetation, decreasing the grass and forb cover which may decrease food availability and increase predation of native mammals (Legge et al. 2011). It was also suggested by Legge et al. (2011) that feral cattle and horses (*Equus ferus*) have the potential to alter patterns in fire and change floristic composition.

Despite the presence of large herbivores, there has been relatively little change to the landscape in north-western Australia compared with the more populated east coast and south-west corner (Burbidge et al. 2008a). Further, members of the genus *Petrogale* appear to be less affected by changes and have largely avoided the decline of other similar weight mammals due to their rock dwelling habits (McKenzie et al. 2007). Rocks and caves provide protection from predators, weather extremes and fire, and rocky habitats are less likely to be altered by grazing of livestock or clearing of land (Pearson 2012).

No extinction of a rock-wallaby species has occurred, yet there still have been reported declines. *Petrogale* species that are listed by the IUCN Red List (2013.2) as threatened or near threatened include *P. persephone* (Proserpine rock-wallaby), *P. xanthopus* (yellow-footed rock-wallaby), *P. penicillata* (brush-tailed rock-wallaby), *P. coenensis* (Cape York rock-wallaby), *P. burbidgei* and *P. lateralis* (IUCN 2013). Factors contributing to these declines are complex and not always obvious, however most declines have been linked to habitat alteration by humans or predation (Kinnear *et al.* 2010). Additionally, restriction to small areas of rocky outcrops may predispose *Petrogale* to be sensitive to fragmentation (Potter et al. 2012b).

The Kimberley is rich in valuable minerals including bauxite, iron ore, copper, uranium and diamonds (Geoscience Australia 2012). There are six proposed mines across the range of *P. concinna* and *P. burbidgei* (Australian Conservation Foundation). In particular Cape Bouganville and Mitchell Plateau contain rich deposits of Bauxite which occur in primary rock-wallaby habitat (Britt and Towner 2012). Currently it is uneconomical to develop these deposits (Britt and Towner 2012), however, as other mines exhaust their deposits and the resource becomes more valuable it is likely such deposits will become more economical.

Direct impacts of mining such as habitat destruction are often dramatic but localised (Pearson 2012). However, there are many indirect impacts associated with the development of mines and settlement of mining communities such as building of infrastructure, increased traffic, increased fire frequency, pollution and invasion of feral predators (Pearson 2012).

1.2.7 Declines in Northern Australian Mammal Fauna

Recent accounts of the current precipitous decline in northern Australian mammal fauna are disturbing (Fitzsimmons et al. 2010; Woinarski et al. 2010; Woinarski et al. 2011; Woinarski et al. 2001). Mostly this decline is evidenced by comprehensive, systematic mammal surveys in Kakadu National Park, Northern Territory by Woinarski *et al.* (2010; 2001) between the years 1986-2009. In these studies, the most dramatic declines occurred in the last decade (Woinarski et al. 2010). There is less documentation of declines in the Kimberley, perhaps because declines have been less extreme (some species persist in this region that have disappeared from the Northern Territory) or perhaps simply because there has been no systematic monitoring equivalent in the Kimberley (Woinarski *et al.* 2011). None the less there have been notable disappearances from the lower rainfall south east and south west Kimberley (McKenzie 1981; Woinarski *et al.* 2011).

These declines are particularly worrying because northern Australia remains largely an unaltered natural landscape, with low human population density, minimal intensive development and land clearing, and large areas protected in a conservation reserve network (Fitzsimmons *et al.* 2010; Woinarski *et al.* 2011; Woinarski *et al.* 2007), thus there is no obvious single driving cause of these declines. Furthermore, the northern tropics were noted previously for their lack of mammal extinctions compared to the notorious declines in the arid interior and populated south and east coast (Burbidge and McKenzie 1989; McKenzie *et al.* 2007; Woinarski *et al.* 2011).

1.2.8 Climate Change

The fact that the global climate is changing has now become unequivocal (IPCC 2013). According to the International Panel on Climate Change it is very likely that this change is caused by anthropogenic emission of greenhouse gasses, particularly carbon dioxide (IPCC 2013). Australian average annual temperatures are predicted to rise 1.0^{o}C by 2030, and an increase of between 1.8 ^{o}C and 3.4 ^{o}C is expected by 2070, depending on the emissions scenario (CSIRO 2007). Warming will be greater in inland areas and in the north of Australia (CSIRO 2007). Changes in rainfall are harder to predict, with more seasonal variation in rainfall and a greater frequency of extreme weather events likely (CSIRO 2007; Hughes 2003; IPCC 2013). It is a great concern if the Kimberley becomes hotter and seasonally drier as range contraction of both *P. burbidgei* and *P. concinna* is likely. Both species have a small ‘climatic envelope’ and may be unable to move south to cooler areas. In particular, *P. burbidgei* may be at higher risk as it is endemic to a small area of the wetter north Kimberley (Roache 2011).

Increased levels of atmospheric carbon dioxide increase plant growth. However if soil nutrients such as nitrogen are limited, foliage grown under increased CO_2 is of poorer quality (Hunter 2001). This may cause nutritional deficiencies for folivorous mammals such as *P. concinna* and *P. burbidgei* because total foliage intake is limited by physiology and ecology. Additionally, increased plant growth leads to greater fuel loads, especially since reduction in foliage nitrogen reduces decomposition rate (Hughes 2003). Increasing the potential for intense frequent fires is likely to be detrimental for these two species (Anderson *et al.* 2005).

Greater evaporation rate associated with increased temperatures leads to environmental water stress (Hughes 2003). More severe droughts are predicted for the north west of Australia (CSIRO 2007). This could also reduce food availability for *P. concinna* and *P. burbidgei*, particularly in the dry season. Furthermore, the impacts of climate change and increased atmospheric CO_2 are likely to interact with existing threats. For example fire patterns will be influenced by temperature, fuel load and rainfall; and grazing of introduced herbivores influenced by vegetation structure.

1.3 Study Objectives

The aims of this study were to:

A) Develop and integrate novel techniques for studying cryptic Kimberley rock-wallabies. This was achieved by addressing the following questions:
  - Are camera traps a suitable survey tool for these species; specifically, is it possible to identify these species from camera images?
  - Is modelling the detection-non detection from camera images statistically suitable for estimating the occupancy and indicating important habitat variables for these species?
  - Can these species be unambiguously identified from fragment/s of DNA that can be successfully extracted and amplified from faecal material? Criteria for a suitable fragment are: sufficient nucleotide differences between species of interest, high PCR success, and low cross-contamination
  - Can species be correctly assigned to faecal pellets by morphological measurements?

B) Gain a better understanding of the distribution, population status and habitat requirements of *P. burbidgei* and *P. concinna* in Western Australia. This was achieved by:
  - Identifying if these species are still present at locations previously recorded
  - Identifying if there are locations where these species are present but have not been recorded from previously, including localities outside their currently published distribution
  - Estimating occupancy by modelling camera trap data
  - Modelling site occupancy with factors that may influence distribution and occurrence including geomorphology, vegetation composition, potential interspecific interactions and availability of fresh water

Chapter 2: Methods

2.1 Study Site

2.1.1 The Kimberley

The Kimberley is a vast expanse (423,517km^2) of largely intact wilderness in Australia's north-west. The region is sparsely populated with only 40, 000 people, 14 436 of which in Broome (ABS 2010). Traditional Owners hold Native Title rights over areas covering approximately 75% of the Kimberley (KLC 2013). These areas are managed by 12 Indigenous Ranger Groups and contain eight Indigenous Protected Areas (Figure 2.1; KLC 2013) which form an integral part of Australia's National Reserve System (Australian Government 2013). The Kimberley Region also supports a variety of other land uses, including military training areas (Yampi Sound Training Area), pastoral land (mostly cattle stations, e.g. Carson Station near Kalumburu), Public Conservation Reserves (e.g. Prince Regent River Nature Reserve, Mitchell River National Park, Drysdale River National Park), and private sanctuaries (e.g. the AWC managed Mornington and Artesian Range Sanctuaries). This study was conducted solely on traditionally managed land, with the participation and assistance of Indigenous Rangers.

The North Kimberley is one of Australia's 85 recognised bioregions first defined by Thackway and Cresswell (1995), the boundary of which corresponds closely to that of Beard's (1979) Gardner phytogeographic district. The exception being that the perimeter of the Gardner district lies just south of Walcott Inlet, while the North Kimberley bioregion extends further south-west to include the Yampi Peninsula (Environment Australia 2000; Rye et al. 1992). The Yampi Peninsula is classified as part of the Fitzgerald phytogeographic district (Rye et al. 1992). The bioregion will henceforth be referred to as North Kimberley, while the more general northern Kimberley will be referred to as such.

Geologically, the North Kimberley is a dissected plateau of Proterozoic sandstone tablelands and rugged ranges (Environment Australia 2000). It is bordered to the south west by the King Leopold Range, and to the south-east by the Durack Range, both consisting of highly folded and faulted quartzite and sandstone (Rye et al. 1992).

Vegetation in the region is generally savanna woodland, with Eucalyptus species forming the tree layer (Rye et al. 1992). On shallow sand soils from Proterozoic siliceous sandstone the savanna woodland is dominated by Darwin Woolybutt (*Eucalyptus miniata*) and Darwin stringy bark (*E. tetrodonta*) with tussock grass *Sorghum* and hummock grass *Plectrachne schinzi* (Spinifex) forming the grassy understory (Environment Australia 2000). On laterite soils surrounding Proterozoic basalt outcrops the savanna woodland is dominated by *E. tectifica* (Darwin box) and *Corymbia grandifolia* (large leaf cabbage gum) with tall *Sorghum* grass as an understory. In higher rainfall laterite areas species of *Lycas*, *Livingstona*, *Terminalia* and *Gardenia* also form the tree layer along with *Eucalyptus* (Rye et al. 1992). Basic soils have a higher diversity of grasses from the genera *Sehima*, *Chrysopogon*, *Themeda*, *Heteropogon* as well as *Sorghum* and *Plectrachne* (Rye et al. 1992). Riparian or drainage line vegetation is usually closed and features *Terminalia*, *Ficus*, *Melaleuca*, *Acacia*, *Eucalyptus* and *Pandanus* species (Environment Australia 2000; Rye et al. 1992). Deciduous monsoon rainforest occurs in small pockets across the north of the region with high species richness and endemism. Extensive areas of mangroves grow on the flat sheltered coastal plains (Environment Australia 2000).

The ecological system is ruled largely by the highly seasonal monsoon rainfall. There is a distinct wet and dry season with the months November to May receiving 95% of the annual rainfall (Bureau of Meterology 2011). Temperature remains high throughout the year, with minimum temperatures above 20°C for seven months of the year, and maximum temperatures above 30°C all year round (Bureau of Meterology 2011).

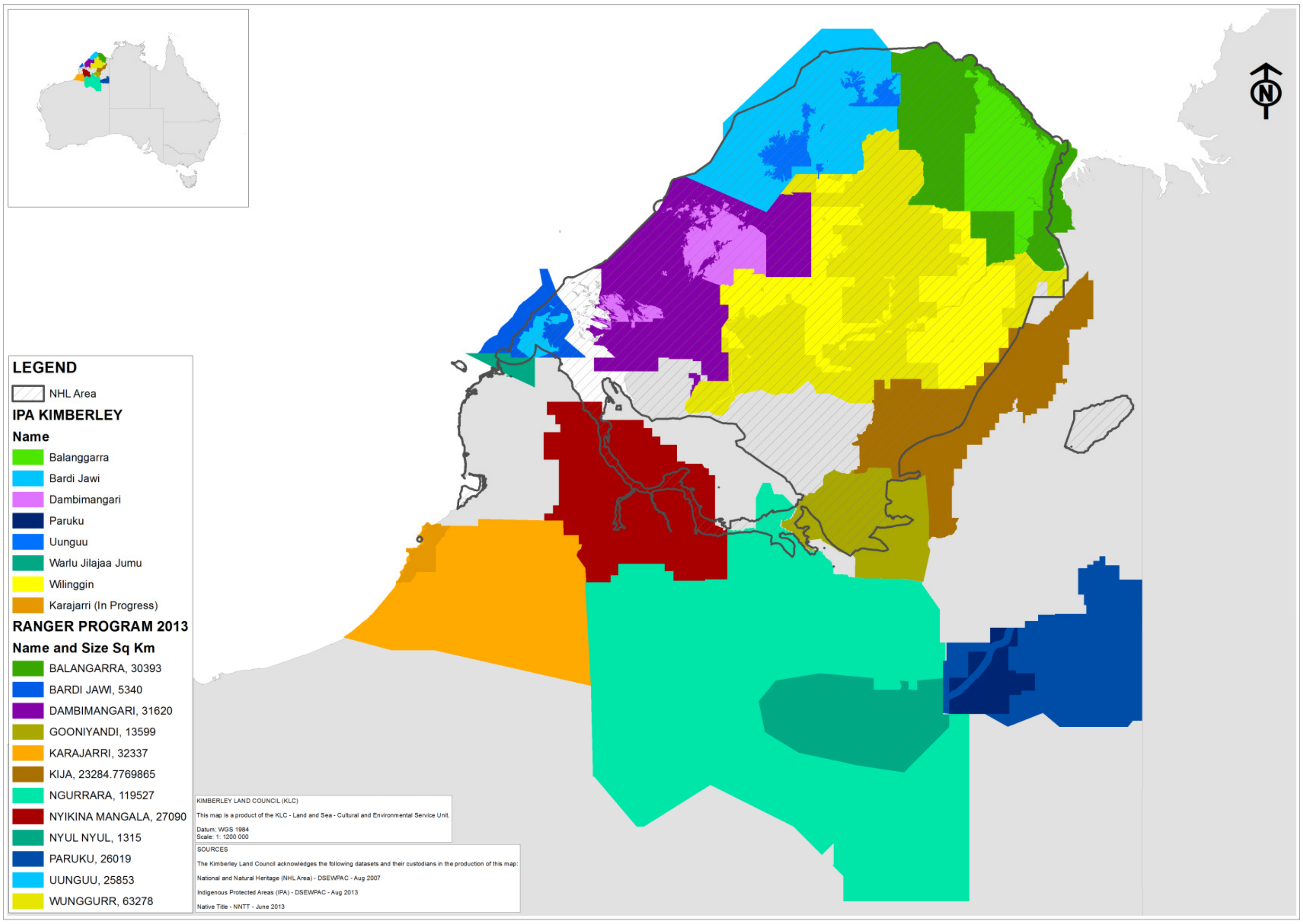


Figure 2.1. Kimberley Ranger Network and Kimberley Indigenous Protected Areas. From KLC (2013), data from: National and Natural Heritage (NHL) Area (DSEWPAC 2007); Indigenous Protected Areas (IPA) (DSEWPAC 2013); Native Title (NNTT 2013).

2.1.2 This Study

This study was conducted in two areas of the North Kimberley in May, June and July 2013. One study area was in the north-west Kimberley on Dambimangari country (see Figure 2.1), and was approximately 8147km^2 in area. The other study area was in the northern most part of the Kimberley, on Balanggarra country (Figure 2.1) near Kalumburu, and was approximately 237km^2 in area. The minimum distance between the two study areas was 184km.

Sites were selected based on the NatureMap® data base of *P. burbidgei* and *P. concinna* records (DEC 2013) and by looking for suitable habitat on Google Earth® and Landgate®. Where possible, we visited sites where the target species had been recorded ≥ 20 years ago in order to determine if the species were still present. In general, rugged King Leopold Sandstone cliffs with complex rocky habitat were targeted as well as some basalt outcrops. In total 30 sites were searched for signs of rock-wallaby presence.

A Bell Jet Ranger® helicopter was hired (Frontier Helicopters, Derby, WA) on the 30th and 31st of May, and again on the 4th July, 2013 to access sites between the Yampi Peninsula and Prince Regent Nature Reserve (Figure 2.2a). Eleven sites were visited on Dambimangari country in an area boarded by the Yampi Sound Training Area to the south-west, the ocean to the north and the Prince Regent Nature Reserve to the north-east. Hereafter the sites visited in May are called 'Derby' sites, and sites visited in July are called 'Dambi' sites. See Appendix A for a full table of site names and localities.

Additionally, a three week trip by four wheel drive vehicle was taken to Kalumburu, where there are historic records of *P. concinna* (DEC 2013). We visited a total of 19 sites on Balanggarra country around Kalumburu, from the south side of the Carson River to Napier Broome Bay (Figure 2.2b). Access to sites was restricted by the presence and condition of roads and height of rivers, and some sites near the old Mission at Pago with historic records and some sites across the river were inaccessible at the time of the study.

Surveys were conducted as part of a WWF-Australia program to survey the three rarer species of Kimberley Rock-Wallabies (*P. lateralis*, *P. concinna* and *P. burbidgei*) while simultaneously providing training for Indigenous Rangers in the use of sensor cameras for monitoring wildlife. All research was agreed upon and access granted from the Kimberley Land Council, Traditional Owners and relevant Aboriginal Corporations prior to the commencement of field work. The field team consisted of a WWF staff member (Jessica Koleck), local Indigenous Rangers, myself, and a volunteer (Kalumburu trip).

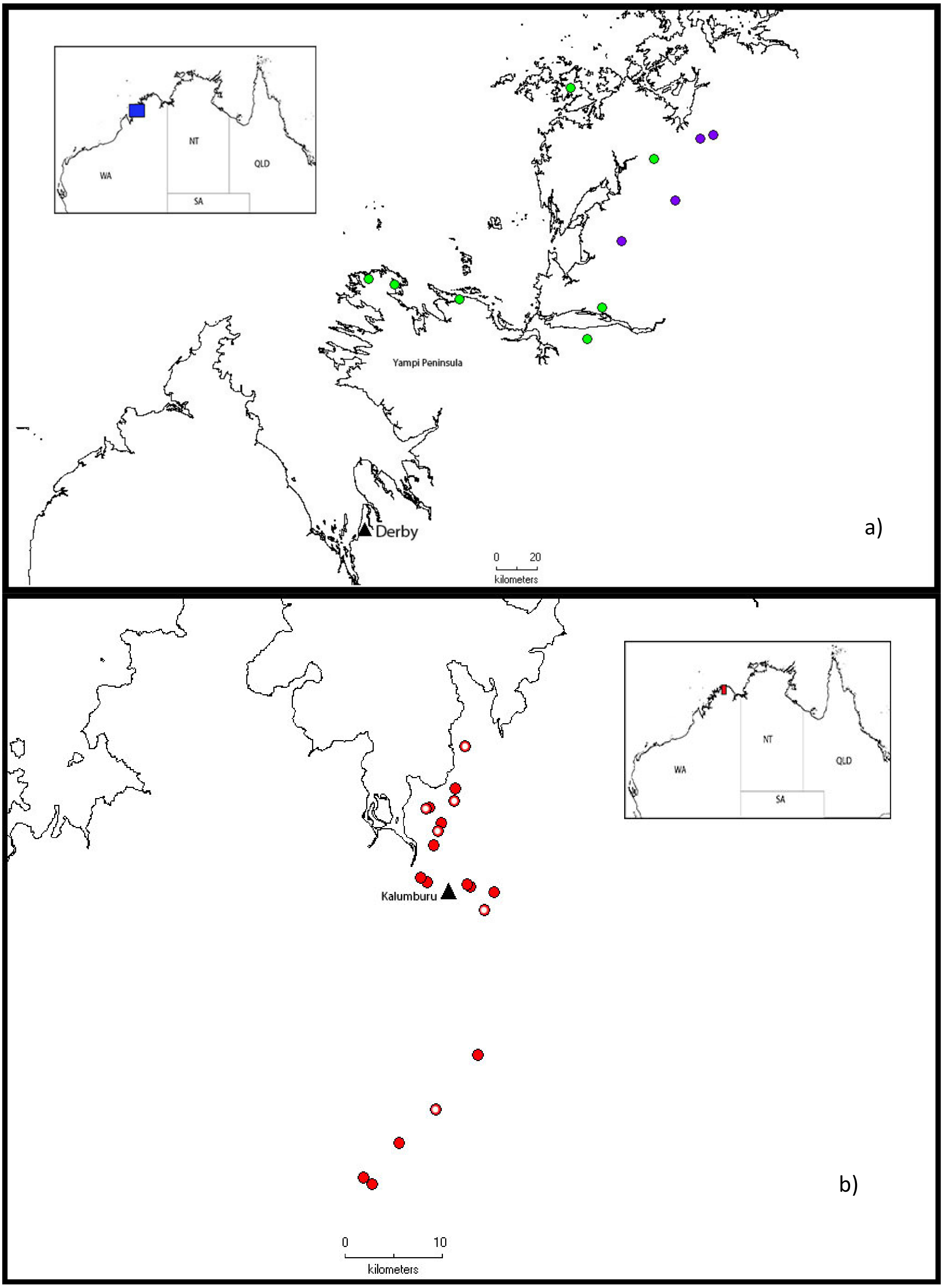


Figure 2.2.Study sites visited by helicopter from Derby (a) and by vehicle from Kalumburu (b). Green= Derby sites (first helicopter trip), purple= Dambi sites (second helicopter trip), red= Kalumburu sites. Filled circles= sites with cameras, unfilled circles= sites searched for signs of presence only.

2.2 Sensor Cameras

Twenty-six Scout Guard white flash sensor cameras (SG860C; Trail Cameras Australia) and six UoVision infra-red flash sensor cameras (UV535; Trail Cameras Australia) were set up at 24 locations, where there were apparent signs of rock-wallabies (scats, used caves). Sensor cameras, sometimes referred to as camera-traps, are remotely triggered cameras that automatically capture images of wildlife (Meek *et al.* 2012; Rovero *et al.* 2010). Both camera types used passive infrared (PIR) motion sensors to detect the heat and motion of animals and trigger the camera to photograph. Cameras were baited with a mixture of peanut butter, oats and truffle oil contained in spherical tea infusers which were attached to a rock surface in view of the camera. Cameras were affixed to large trees or rocks and angled so they pointed 25-60cm off the ground or rock surface at the focal point (Figure 2.3; Appendix B). Cameras were placed where they would be likely to detect wallabies, clear of any interfering vegetation and where they would be stable. Wooden door stoppers (Bunnings Warehouse, Broome) were used when necessary to wedge cameras into position (Figure 2.3). See Appendix B for more examples of camera setup.

Figure 2.3. Setup of sensor cameras in the field. Pictured: Jessica Koleck

Cameras were set to take three photographs per trigger with no delay between triggers. Sensitivity was set according to camera position; cameras with unavoidable vegetation in the field of view were set to low sensitivity, otherwise all other cameras were set to medium sensitivity. The display screen was used to adjust the position of the cameras during setup (see Appendix B). Once the camera was in place a photograph was taken of a metre ruler to give the field of view scale. Depending on the depth of view, some of the flash was blacked out with tape, as these cameras are designed to illuminate large game at distances in North America and tend to wash out small animals at close range.

Based on the dry season home range size of *P. brachyotis* (Telfer and Griffiths 2006) I determined that cameras needed to be a minimum of 483 meters apart to ensure independence which was needed to meet the assumptions for occupancy modelling (Rovero et al. 2010). Two cameras (Kalumburu G and Kalumburu S) were unintentionally positioned less than this distance (410m) apart. However, because I calculated minimum distance from work on a larger species, presumably which has a larger home range than the substantially smaller *P. concinna* and *P. burbidgei*, data from these cameras were still included, and considered valid. Additionally, all sites were each on distinct rock outcrops separated by open, flat areas so it is unlikely that the same individuals would be captured at more than one site. All of the helicopter (Yampi Peninsula) sites were over 5km apart and often more than 10km apart.

All 14 cameras at the 11 Derby and Dambi (helicopter) sites were collected on the 31st of July 2013 by Jessica Koleck and Dambimangari Rangers and all Kalumburu cameras were collected on the 16th of August by Balanggarra Rangers.

2.3 Habitat Variables

At each site five ordinal categorical variables were documented. These were recorded as low, medium or high (scale of 1-3), and are listed in Table 2.1. Although these are subjective variables they were independently rated by two observers and then compared and a consensus was reached, so there were no observer differences. Examples of sites with different grades of habitat variables are shown in Appendix C.

Table 2.1. Ordinal categorical habitat variables recorded at each study site.

Variable	Category 1	Category 2	Category 3
height	low rocks/no cliff	moderately high rocks/cliff	high cliff
steepness	flat/nearly flat	sloped about 45°	vertical
structure	few caves or crevices	moderate amount of caves or fracture lines	many caves and crevices
vegetation	low quality vegetation with poor nutrition such as spinifex grass and dry sorghum grass	mixture of tough grass with some more nutritional plants	high quality and diversity wet vegetation with lots of high nutrition plants such as rock figs
water	no fresh water present	fresh water several hundred meters away or likely to dry up	fresh water abundant and at the base of the cliff

Previous research has suggested that these variables are important factors influencing the distribution of *P. burbidgei* and *P. concinna* (Churchill 1997; Sanson et al. 1985; Telfer and Bowman 2006). It was assumed that high, steep cliffs with complex structure, high nutrition vegetation and nearby water would be favourable. Therefore, each variable was considered when grading the overall habitat quality out of five (Appendix A).

An additional variable was added to the analysis after the camera images had been sorted; if an introduced predator (i.e. feral cat or wild dog) was detected at the site, it was recorded as a dummy (indicator) variable.

2.4 Collection and Sub-sampling of Faecal Pellets

Paper envelopes were used to collect faecal pellets (scats) at each site. One envelope was used for each sample; which was usually a single pellet unless it was obvious from grouping that several pellets came from the same individual. Scats from the '*P. concinna*' at Perth Zoo were used as reference examples when searching habitat. Additionally it was reported by

Telfer et al. (2006) that *P. concinna* scats could be distinguished from *P. brachyotis* by their size; *P. concinna* scats were less than 12mm long (Telfer et al. 2006).

Latex examination gloves or vegetation were used to transfer the scats into envelopes to avoid the risk of sample contamination. As many samples as possible were taken from each site. Scats destined for use in genetic analysis needed to be fresh, therefore we only collected scats that were dark in colour and exposed to the elements (Piggott and Taylor 2003). We avoided collecting scats from caves and other enclosed sites as they can remain dark and fresh looking for much longer periods (Georgeanna Story, pers. comm. 2013).

Samples were refrigerated in an Engel® refrigerator while in the field, and then express posted to the Australian Museum, Sydney, for storage in a -20°C freezer at the first available opportunity.

Budget and time constraints meant that it was not possible to extract DNA from every sample collected. In order to maximise the chance of detecting *P. concinna* and/or *P. burbidgei*, systematic sub-sampling was undertaken, which is tabulated in Appendix D. Firstly, all samples were visually examined and a decision was made about which pellets were worth extracting based on freshness and size. Pellets that were too large were most likely to be *P. brachyotis* and pellets that were too old were unlikely to yield enough intact DNA. This resulted in a subset of samples for each site, ranging from 2-39 pellet samples. As it was important to detect both *P. concinna* and *P. burbidgei* if both were present, more samples collected from a site warranted more extractions. If the number of samples for a site was less than five, all samples were extracted. If the number of samples was between five and 20, five were randomly selected for extraction. If the number of samples was greater than 20, 10 were randomly selected for extraction. Additionally, sites where no cameras were placed because it appeared no *P. concinna*/*P. burbidgei* were present were excluded. This resulted in 141 samples across all sites being selected for extraction (Appendix D). Not all extractions were successful, so where possible another sample from the same site was randomly selected for extraction. However as there was a limit of 150 extractions, some sites could not have a full complement of sequences.

2.5 Measurement and Analysis of Faecal Pellet Size between Species

Prior to extraction, each pellet was measured using callipers to the nearest 0.1 millimetre (Figure 2.4). Maximum width, length and length plus squeeze point tip were recorded. If the pellet had been squashed during transport, mean of the maximum and minimum with was used.

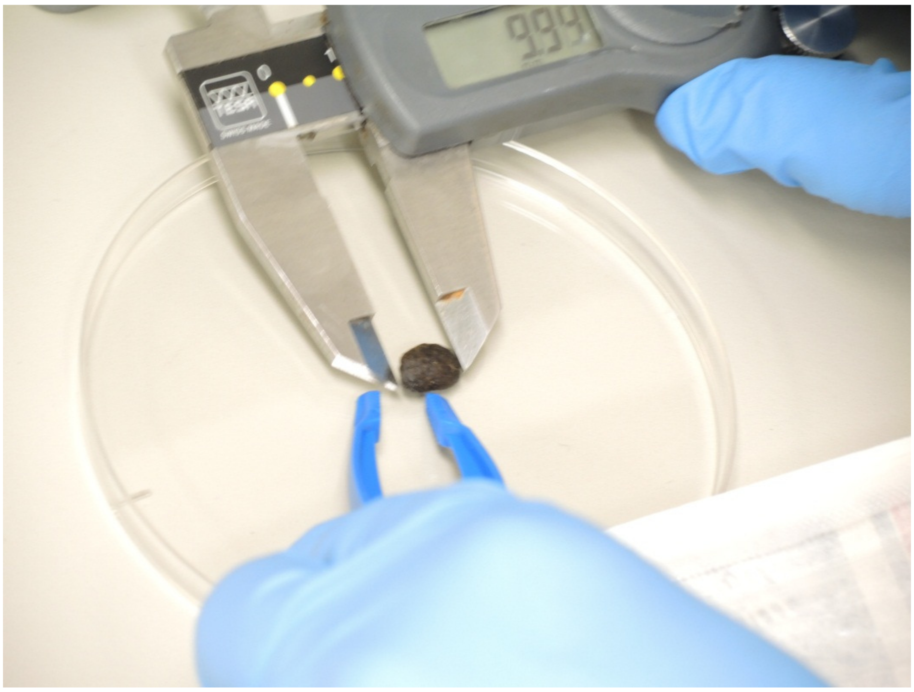


Figure 2.4. Measurement of faecal pellets to the nearest 0.1mm

Following genetic assignment to each faecal pellet (see section 2.6), I used multiple linear regression analysis (R Version 3.0.2; R Foundation for Statistical Computing, Vienna, Austria) to determine if a species could be predicted from the length and/or width of faecal pellets. Stepwise regression (forward and backward) was used with AIC model selection. As the model with both length and width as factors had the lowest AIC score (-144.89) neither variable was removed from the analysis (both length and width were used). Significance of the regression was determined at the $p \leq 0.05$ level.

Additionally, a two way ANOVA with interactions was performed to determine if there was a significant difference ($p \leq 0.05$) in length and/or width between species. A post-hoc pairwise t-test with the Bonferroni adjustment to p-values was performed to determine where differences lay between species.

2.6 DNA Analysis

2.6.1 Extraction of Genomic DNA

DNA extraction was undertaken at the Australian Museum (Figure 2.5). Genomic DNA (gDNA) was extracted from frozen faecal pellet samples using a QIAGEN QIAamp stool mini kit (QIAGEN GmbH, Hilden, Germany). Extraction was conducted according to the 'Protocol: Isolation of DNA from Stool for Human DNA Analysis' in the QIAamp® DNA Stool Handbook. However, in the final step DNA was eluted from the spin column with 100µL Buffer AE instead of 200µL to increase the concentration of DNA.

Two methods were trialled for obtaining DNA from the pellet sample; a) slicing the end off the sample, and b) washing the sample. For the first method, ~4mm of the end was sliced off the pellet using a sterile scalpel blade and broken up with a pestle in 160µL buffer AL. For the second method, the remaining sample was 'washed' by pouring 160µL buffer AL in two stages over the pellet in a Petri dish and then pipetting the buffer out into the tube and discarding the pellet. Slicing the end off and digesting the pellet resulted in more DNA being extracted than washing, so this method was used for the remaining extractions. When possible, the squeeze point end (the end that comes out of the animal last) was used.

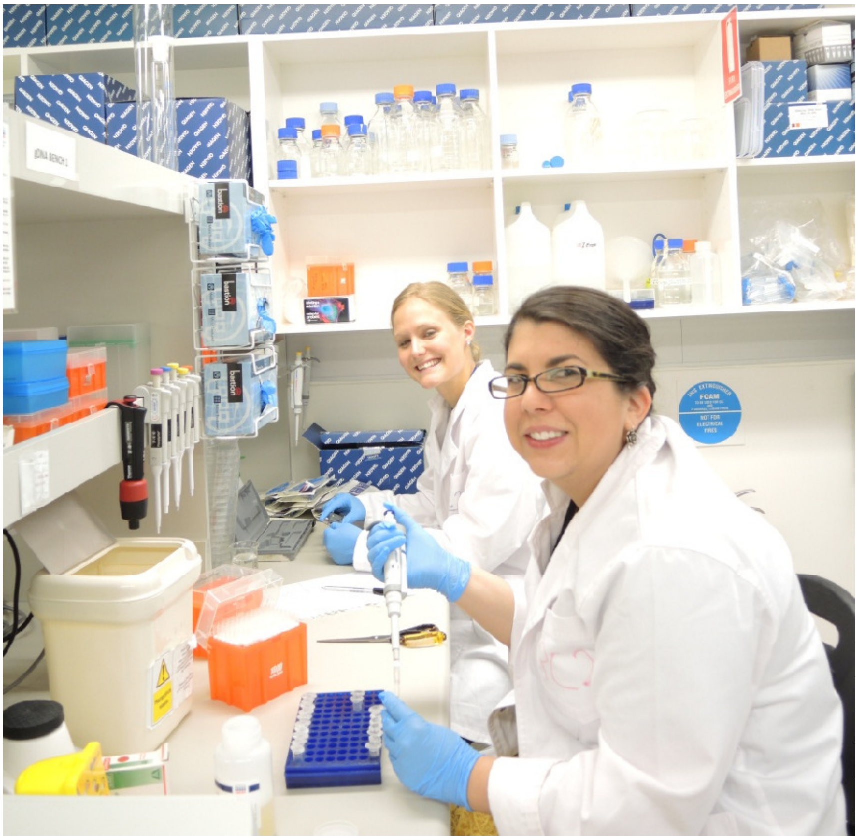


Figure 2.5. Extraction of faecal genomic DNA at the Australian Museum. Greta Frankham (foreground) and Stephanie Todd (background). (Photo: Mark Eldridge)

2.6.2 Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) was used to amplify mitochondrial DNA (mtDNA) fragments. Initially, three short fragments (approximately 200bp) from the gene *cytochrome b* were trialled (primers are listed in Table 2.3). These were suitable choices of primer sets as they are marsupial specific and therefore avoid possible contamination from human DNA (Spencer *et al.* 2010). Additionally *cytochrome b* has been identified as a suitable gene for distinguishing species of macropodid (Alacs et al. 2003; Alacs et al. 2010). The original macropod *cytochrome b* primers, including *Macyt1* and *Macyt3*, were designed by Spencer et al. (2010). However, *Macyt 10F/R2* were developed more recently at the Australian Museum based on the original *Macyt* primers and are as yet unpublished. During trials, the *Macyt 10F/R2* primers produced the most reliable PCR amplification results and the amplified fragment was sufficient for distinguishing species, thus it was used to amplify all remaining samples

Table 2.2. Sequences for three *Macyt* primer sets trialled

Priner Name	**Sequence (5'-3')**	**Length of Fragment**
Macyt 10F	TATGGACTTTAACCATAACCTATG	223bp
Macyt 10R2	TRTGGGCKACKGARGAGAARGC	
Macyt 1F	CTGCCTAATCATCCAAATCYT	164pb
Macyt 1R	GGCATATAAAGAATATGGATGCT	
Macyt 3F	GTGGATTCTCCGTAGACAAAG	202bp
Macyt 3R	GTCTTTGATTGTATAGTAKGGATGG	

Quantities of PCR reagents used are listed in Table 2.4, giving a reaction volume of 25µL. To ensure consistency when dealing with extremely small quantities a ‘master mix’ was made up containing the reagents (except sample DNA) for all of the samples, and then pipetted into individual tubes. In cases where extraction resulted in only a small amount of DNA, the amount of water was decreased to 7.9µL per tube and the amount of template DNA added was increased to 4µL to improve productivity of PCR reactions. Negative controls were included in each reaction.

Table 2.3. Quantities of reagents used in the PCR reactions.

Solution	**Concentration**	**Units**	**Amount**	**µL per tube**
H_20	-	-	-	9.9
PCR Reaction Buffer	10	x	1	2.5
$MgCl_2$ (QIAGEN)	25	mM	3	3
dNTPs	10	mM	200	1
Primer-Forward (macyt 10F)	10	µM	200	0.5
Primer- Reverse (macyt 10R2)	10	µM	1	0.5
QIAGEN Q Soln	5	X	1	5
Bovine serum albumin (New England Biolabs)	100	X		0.5
Taq polymerase (QIAGEN)	5	units/µM	2.5	0.13
Template DNA	-	-	-	2

Thermocycling was conducted in an Eppendorf Mastercycler EpS (Eppendorf, Hamburg, Germany) according to the following conditions: Initial denaturation at 94˚C for three minutes, followed by 38 cycles of the following; denaturation at 94°C for 30s, annealing at 50°C for 40s, extension at 72°C for 40 seconds. Final extension was at 72°C for five minutes, and the holding temperature was 11˚C.

Gel Electrophoresis was conducted in a standard 1% Agarose Gel at 80 or 85 volts for 35 or 30 minutes respectively. A 100 base pair ladder was used to compare sample bands with. Gels were visualised using the BioRad GelDoc XR System (Bio-Rad Laboratories Incorporated). All samples for which extraction and PCR had been successful (i.e. visible bands of PCR products were obtained) were purified by the addition of 1.5µL USB ExoSAP-IT©. Using an Eppendorf Mastercycler EpS, samples were then incubated for 15 minutes at 37^{o}C followed by enzyme inactivation at 80^{o}C for 15 minutes. Purified samples were prepared for sequencing by combining 10µL DNA and 1µL primer in a 96-well plate. Sequencing was done by the Australian Genome Research Facility, Sydney.

2.6.3 Sequence Analysis and Species Identification

Sequences were viewed and edited in SEQUENCHER version 4.10.1 (Gene Codes Corporation, Ann Arbor, MI, USA) with reference to chromatograms. Where possible, a consensus of forward and reverse sequences was used to maximise sequence accuracy. Where only a forward or reverse sequence had sequenced adequately any ambiguous base pair calls were cropped and/or compared to existing rock-wallaby sequences. It was fortunate that all ambiguous base-pairs from poor sequencing corresponded to consistent bases across all possible species of rock-wallaby.

Sequences were aligned using MEGA version 5.0 (Tamura et al. 2011) by CLUSTAL W (Thompson et al. 1994). Previously analysed *cytochrome b* sequences from known *P. burbidgei*, *P. concinna* and *P. brachyotis* specimens were compared to current sequences. MEGA was used to identify and construct the most parsimonious neighbour joining tree, with 2000 bootstrap replications. Species identification was determined by obvious, clear groupings with known specimens.

2.7 Camera Data Analysis and Occupancy Modelling

All species detected by the cameras were identified by both myself and Jessica Koleck from WWF, using *a priori* knowledge or field guides (Menkhorst and Knight 2011; Slater *et al.* 1986; Van Dyck *et al.* 2013). In addition, any images that were difficult to identify were passed on to experts at the Australian Museum (Drs Mark Eldridge, Ross Sadlier and Sandy Ingleby) or Western Australian mammal expert, Dr Andrew Burbidge.

Attempts were made to identify *P. burbidgei* and *P. concinna* using known morphological differences outlined in Section 1.1.3; however as discussed previously one of the main issues with recording the presence of *P. concinna* and *P. burbidgei* is that there is believed to be no definitive way to distinguish them from images (Mark Eldridge, pers. comm. 2013). As suspected, we could not distinguish them with certainty. Therefore, species assignments from the genetic analyses were used to confirm which species occurred at which sites.

A detection history for *P. burbidgei* was developed for each camera (site) within its potential range. Detection histories involve a matrix of binomial events (1=detected, 0=not detected) across the camera trapping period at each site. To increase detection probability, three camera trap days were considered one event i.e. if the species was detected in any one of those three days it was detected for that event. This was possible because most cameras were running for sufficient time to return a reasonable number of events (or ‘surveys’).

Detection histories were analysed using the program PRESENCE version 6.2 (Hines 2006), which uses the Maximum Likelihood method to estimate model parameters from detection/non-detection data. Single-species single-season analyses were considered the most appropriate type of model for the data collected. Model complexity was limited by the amount of data, and the survey was conducted within a single season. The parameters estimated by a single-season single-species model are occupancy probability (ψ) and detection probability (p). Such models have an advantage over simply using detection-non detection data to determine area occupancy as they account for imperfect detection (see MacKenzie et al. 2002) that would otherwise lead to an underestimate of true occupancy.

Single-season models assume that occupancy of the study sites does not change over the duration of the study; that detection of the study species is independent between sites and surveys; and that there is no unexplained heterogeneity in detection or occupancy. The latter was possibly not met and is discussed in Section 4.3.2. In all occupancy studies that use sensor cameras, there will be some association between detection of an animal one day and its detection the next. This cannot be helped and is not considered to significantly violate the assumptions. Detections between sites are considered independent, as explained in Section 2.2, and it is believed that no changes in occupancy occurred within the study period as the study was conducted within a three month window in one season and these species are not known to be migrational.

A total of 28 models were fitted to the data, with various combinations of the six site covariates ranging from the global model, ψ(all covariates + interactions), to the most basic (null) model, ψ(.). Detection probability was initially allowed to vary according to site p(site), but this produced models with poor precision and low model rank. For all other models, detection probability was held constant across sites, p(.). Models with considerable support are listed in Table 2.5.

As the sample size was small (n=10), corrected Akaike's Information Criterion (AICc; Equation 2.1) values were used instead of AIC values to rank models, with the lowest AICc value having the highest weight and rank (Table 2.5). Using AICc values reduces the probability of selecting models that have too many parameters i.e. that have been over-fitted (Burnham and Anderson 2002; MacKenzie *et al.* 2006). The number of sites was chosen to represent the effective sample size (n) as this is produces the most conservative estimate, i.e. the biggest penalty for too many parameters (k) (Burnham and Anderson 2002). One consequence of using AICc is that there cannot be more parameters than sites, because this results in a negative correction which falsely increases the model rank.

$$AICc = AIC + \frac{2k(k+1)}{n-k-1}$$ ………………………………**[Equation 2.1]**

Where k is the number of model parameters and n is the effective sample size and AIC = -2*natural log Likelihood.

Table 2.4. The top 19 models fitted to the detection history data of *P. burbidgei* with ΔAICc values ≤ 7 and model weights (wi) ≥ 0.01. ψ= occupancy probability, p = detection probability

Model	**AICc**	**ΔAICc**	***wi***	***k***
ψ(structure),p(.)	114.36	0	0.3244	3
ψ(height),p(.)	116.30	1.94	0.1230	3
ψ(water),p(.)	116.79	2.43	0.0963	3
ψ(.),p(.)	116.96	2.60	0.0884	2
ψ(vegetation),p(.)	117.97	3.61	0.0534	3
ψ(steepness),p(.)	117.97	3.61	0.0534	3
ψ(structure + vegetation),p(.)	118.41	4.05	0.0428	4
ψ(structure + steepness),p(.)	118.41	4.05	0.0428	4
ψ(structure + height),p(.)	118.41	4.05	0.0428	4
ψ(structure + water),p(.)	119.87	5.51	0.0206	4
ψ(height + vegetation),p(.)	119.96	5.60	0.0197	4
ψ(structure + predator),p(.)	120.36	6.00	0.0162	4
ψ(water + height),p(.)	120.60	6.24	0.0143	4
ψ(water + steepness),p(.)	120.60	6.24	0.0143	4
ψ(water + veg),p(.)	120.60	6.24	0.0143	4
ψ(predator),p(.)	120.98	6.62	0.0118	3
ψ(structure*predator),p(.)	121.17	6.81	0.0108	3

Chapter 3: Results

3.1 Key Findings

- *Petrogale burbidgei* was detected at six sites with the sensor cameras, and the same six sites with faecal DNA (Figure 3.1)
- *Petrogale concinna* was detected at only one site, Augustus Island, with both the sensor camera and faecal DNA (Figure 3.1)
- *Petrogale burbidgei* was recorded outside its published extant distribution (115km further south-west)
- *Petrogale concinna* was not detected anywhere on the mainland using either method, despite being previously recorded at sites visited around Kalumburu.
- The mean probability that *P. concinna* was present at a site but not detected was 0.275, although this has limited applicability
- The occupancy of *P. burbidgei* at the mainland helicopter sites (Dambi and Derby) was estimated to be 0.644±0.075
- The scats of *P. brachyotis* were significantly wider than the scats of *P. burbidgei*, although there is limited power to assign a species to a scat based on its measurements

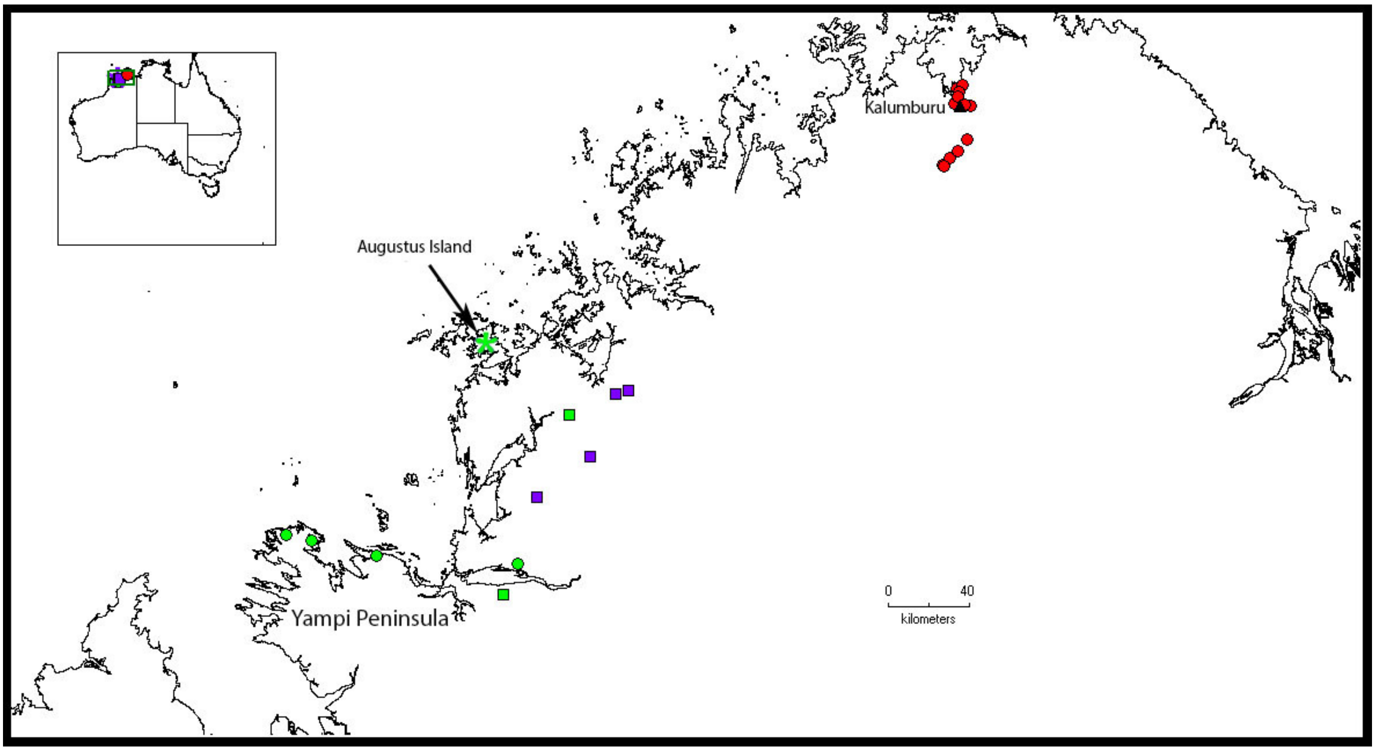


Figure 3.1. Sites where *P. burbidgei* (squares), *P. concinna* (star) or only *P. brachyotis* (circles) were detected using both cameras and faecal pellet DNA. Red = Kalumburu sites, green = Derby sites, Purple = Dambi sites.

3.2 Faecal Pellet Morphology

There was a significant linear relationship of faecal pellet length and width between species ($F_{2,123}$= 5.38, p= 0.0057). Both length and width had p-values ≤0.05 indicating that they were both significant factors explaining which species a pellet came from (Table 3.1). However the multiple r^2 value for the regression was low (r^2= 0.08) indicating that there was a lot of scatter of data and the model had poor predictive power.

Table 3.1. Results of the multiple linear regression analysis showing the estimates of beta coefficient values for faecal pellet length and width, and their associated p-values

Variable	**Estimate**	**SE**	**t-value**	**P(>|t|)**
Intercept	1.91	0.45	4.24	$4.28\text{x}10^{-5}$
Length	0.07	0.03	2.39	0.018
Width	-0.16	0.06	-2.81	0.006

The two-way ANOVA revealed that there was no significant difference in pellet length between species ($F_{1,122}$=2.82, p=0.096) but there was a significant difference in pellet width between species ($F_{1,122}$=7.86, p=0.006) (Table 3.2). No interaction between the two was found. The post-hoc pairwise t-tests showed that this result was due to a significant difference in width between the pellets of *P. brachyotis* and *P. burbidgei*. The lack of significant difference in pellet size between *P. concinna* and the other two species was likely due to the small sample size for this species (n=5).

The maximum length of a *P. burbidgei*/*P. concinna* pellet was 16.0mm, and the maximum width was 9.5mm. However, usually the pellets of these species were smaller thus the median pellet size was calculated to be 11.5mm in length and 7.1mm in width.

Table 3.2. Two-way ANOVA results for faecal pellet length and width between the three species of rock-wallaby.

	DF	Sum Sq	Mean Sq	F-value	P(>F)
length	1	0.09	0.88	2.82	0.096
width	1	2.45	2.45	7.86	$5.89\text{x}10^{-3}$
length*width	1	$5.0\text{x}10^{-3}$	$5.3\text{x}10^{-3}$	0.02	0.897
residuals	122	38.04	0.31		

3.3 DNA Analysis

The 223 base pair region of mtDNA, amplified in this study contained sufficient nucleotide differences to distinguish each of the three sympatric species of North Kimberley rock-wallaby. One hundred and eighty-two base pairs of this fragment overlap with the 5'end of the gene *cytochrome b* which enables the use of previously published sequences of this gene for comparison. For this study, sequences were compared to *P. brachyotis* ABTC99375, *P. concinna* WA S2706, *P. burbidgei* S2832 and *P. burbidgei* S982 *cytochrome b* sequences. Polymorphisms were contained within the centre part of the fragment which is not usually affected by poor sequencing.

Within this fragment, there were six sites where *P. burbidgei* was different from *P. brachyotis* and *P. concinna*, three sites where *P. concinna* and *P. burbidgei* were different from *P.*

brachyotis and one site where *P. concinna* was different from *P. burbidgei* and *P. brachyotis*. The sequences identified to be *P. burbidgei* are similar (1bp difference) to *P. burbidgei* S2832 from Prince Regent River yet are very different (9bp difference) to *P. burbidgei* S982 from Mertens Creek, Mitchell Plateau. The *P. concinna* sequences are the same as *P. concinna* S2706 from Augustus Island, and the *P. brachyotis* sequences are very similar (1bp difference) to *P. brachyotis* ABTC99375 from Mitchell River, Doongan Station.

Of the 148 faecal pellet samples that were extracted, 134 (90.5%) were successfully amplified and sequenced for the target mtDNA fragment. Considering that the DNA contained in faecal pellets is often degraded and poor quality, this is a high success rate. Likely reasons why the 14 samples did not produce results are sample age, exposure to environmental conditions, other factors prior to collection, or the sample being from a non-target species.

Of the 134 faecal pellet samples sequenced, 131 were rock-wallaby, two were *Macropus robustus* (euro), and one was likely to be *Wyulda squamicaudata* (scaly-tailed possum). *P. burbidgei* was detected at six study sites (49 samples), *P. concinna* at one site (Augustus Island; 5 samples), and *P. brachyotis* at 17 sites (77 samples). However as *P. brachyotis* was not the target species this does not reflect its true presence at study sites. Figure 3.2 demonstrates the clear grouping of sequences with the reference *P. burbidgei*, *P. concinna* and *P. brachyotis* specimens, despite low bootstrap percentages at nodes. A full list of samples that were successfully sequenced, and their identification based on a neighbour joining tree (MEGA, version 5) with 2000 bootstrap replications is given in Appendix F.

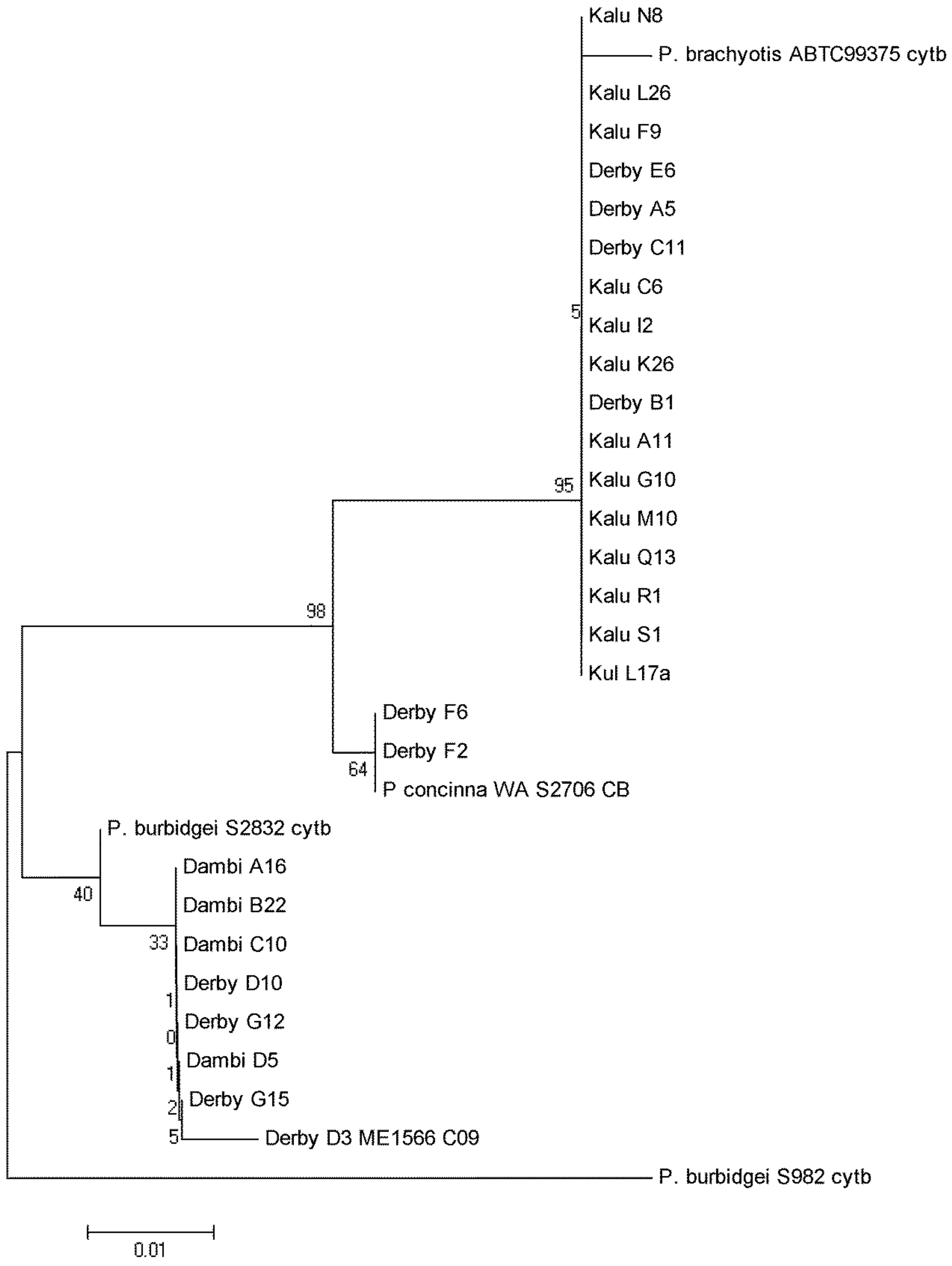


Figure 3.2. Neighbour Joining Tree containing one 180 bp sequence from each site, or if more than one haplotype at a site, one representative of each haplotype. As Derby site F was the only site with *P. concinna*, two samples were included. Bootstrap values (percentages) are shown at the nodes based on 2000 bootstrap replications.

3.4 Sensor Cameras

A total of 30 species were detected across 1294 camera days (Table 3.3), examples of which are given in Appendix I. Capture frequencies of the commonly detected species are given in Table 3.4. The most commonly detected species overall was *P. brachyotis*, which was detected more frequently at the Kalumburu sites than at the Dambi and Derby sites (Table 3.4). Other commonly detected species were northern quolls (*Dasyurus hallucatus*), white-quilled rock-pigeons (*Petrophass albipennis*) and scaly-tailed possums (*Wyulda squamicaudata*) (Table 3.4). Rock Ringtail possums were found at two of the Kalumburu sites (28 detections), but not at any of the Dambi and Derby sites.

Scaly-tailed possums were detected on Augustus Island (site Derby F), where they have not been recorded previously (Gibson and McKenzie 2012). Introduced predators (cats and dogs), were detected at seven of the 24 study sites, although detection frequency was low. There was a greater detection frequency of predators around Kalumburu (cats detected 0.7% of camera days, dogs detected 1.0% of camera days) than the Dambi and Derby sites (cats 0.2% camera days, dogs 0.2% camera days).

Table 3.3. Species detected across the 24 study sites in 1294 camera days

Area	Common name	Scientific name
Helicopter sites- Dambi and Derby (Dambimangari country)	Short-eared rock-wallaby	*Petrogale brachyotis*
	Nabarlek	*Petrogale concinna*
	Monjon	*Petrogale burbidgei*
	Delicate mouse	*Pseudomys delicatulus*
	Golden-backed tree rat	*Mesembriomys macrurus*
	Common rock rat	*Zyzomys argurus*
	Kimberley rock rat	*Zyzomys woodwardi*
	Northern quoll	*Dasyurus hallucatus*
	Scaly-tailed possum	*Wyulda squamicaudata*
	Feral cat	*Felis catus*
	Dingo/dog	*Canis lupus*
	Bar-shouldered dove	*Geopelia humeralis*
	White-quilled rock pigeon	*Petrophassa albipennis*
	Variegated fairy wren	*Malurus lamberrti rogersi*
	Great bower bird	*Ptilonorhyncus nuchalis*
	Sandstone shrike-thrush	*Colluricincla woodwardi*
	Pied Butcherbird	*Cracticus nigrogularis*
	Black-palmed monitor	*Varanus glebopalma*
	Kimberley rock monitor	*Varanus glauerti*
	Magnificent tree frog	*Litoria splendida*
	Gecko sp.	unknown
	Other rodent (central pebble mouse?)	Potentially *Pseudomys johnsoni*

Area	Common name	Scientific name
Kalumburu sites (Balanggarra country)	Short-eared rock-wallaby	*Petrogale brachyotis*
	Kimberley rock-rat	*Zyzomys woodwardi*
	Rock ringtail possum	*Petropseudes dahli*
	Scaly-tailed possum	*Wyulda squamicaudata*
	Euro	*Macropus robustus*
	Feral cat	*Felis catus*
	Dingo/dog	*Canis lupus*
	Echidna	*Tachyglossus aculeatus*
	White-quilled rock pigeon	*Petrophassa albipennis*
	Pheasant coucal	*Centropus phasianinus*
	Great bower bird	*Ptilonorhyncus nuchalis*
	Sandstone shrike-thrush	*Colluricincla woodwardi*
	Pheasant coucal	*Centropus phasianinus*
	Torresian crow	*Corvus orru*
	Willie wagtail	*Rhipidura leucophrys*
	Boobook owl	*Ninox novaeseelandiae*
	Singing honeyeater	*Lichenostomus virescens*
	Black-palmed monitor	*Varanus glebopalma*
	Kimberley rock monitor	*Varanus glauerti*
	Geko sp.	*Gehyra xenopus* or *Oedura gracilis*
	Children’s python	*Antaresia childreni*

Table 3.4. Detection frequency expressed as no. of detections per 100 camera days (% camera days) of the four most commonly detected species at the two study areas in north-western Australia.

Species	No. Detections	No. Sites	Frequency (total)	Frequency Heli sites	Frequency Kalumburu
P. brachyotis	592	20	45.7	11.3	72.0
D. hallucatus	48	5	3.7	8.6	0
P. albipennis	30	7	2.3	3.9	1.1
W. squamicaudata	94	9	7.3	15.6	1.0

Petrogale burbidgei was detected at six of the possible ten sites within its potential range (Table 3.5). Trapping frequency across these sites was 6.8% of trapping days. *Petrogale concinna* was detected at only one site, Augustus Island (Derby F; Table 3.5), out of the possible 24 sites. At this site it was only detected twice in 62 trap days. The naive occupancy (without accounting for imperfect detection) of *P. burbidgei* was 0.6 (60%), and of *P. concinna* was 0.04 (4.2%).

Table 3.5. Detection frequency (% camera days) of *Petrogale burbidgei* and *Petrogale concinna* across the 11 Derby and Dambi study sites. Detection frequency of both species at Kalumburu was zero.

Area Name	Site	Frequency	Species
Dambi	A	11.5	*P. burbidgei*
	B	14.3	*P. burbidgei*
	C	3.6	*P. burbidgei*
	D	28.6	*P. burbidgei*
Derby	A	0	neither
	B	0	neither
	C	0	neither
	D	4.0	*P. burbidgei*
	E	0	neither
	F	3.2	*P. concinna*
	G	17.7	*P. burbidgei*

3.5 Occupancy Modelling

3.5.1 *Petrogale burbidgei*

The top ranked model was ψ(structure),p(.) with an AICc value of 114.36 and a model weight of 0.32 (Table 3.6). As no one model had a model weight ≥0.9 (90% chance or greater of being the best model), the top six most parsimonious models with ΔAICc values ≤4 and model weights ≥0.01 were averaged to produce the occupancy estimates. According to Donovan and Hines (2007c) there is strong support for models with delta AICc values ≤2 and considerable support for models with delta AICc values between two and seven. Individual model estimates, along with the average occupancy and detection probabilities estimated by this study are given in Table 3.6.

The occupancy of *P. burbidgei* across the study sites, given the observed detection history, as estimated by the top six models was 0.653 (SE=0.034). This was greater than the naive occupancy estimate of 0.60, indicating that without taking detection into account occupancy was underestimated by 5.28%.

Table 3.6. Estimated mean occupancy (ψ) and detection probability (p) of the six top ranked models for *P. burbidgei* at mainland Dambi and Derby sites, Western Australia. Struct=structure, steep=steepness, veg=vegetation. Variables refer to those defined in Table 2.1

Model	AICc	ΔAICc	wi	K	ψ(±S.E.)	p(±S.E)	Model Precision (%)
ψ(struct),p(.)	114.36	0.00	0.439	3	0.664(0.052)	0.331(0.053)	7.76
ψ(height),p(.)	116.30	1.94	0.167	3	0.629(0.057)	0.332(0.053)	8.98
ψ(water),p(.)	116.79	2.43	0.130	3	0.690(0.061)	0.330(0.053)	8.83
ψ(.),p(.)	116.96	2.60	0.120	2	0.656(0.159)	0.331(0.053)	24.26
ψ(steep),p(.)	117.97	3.61	0.072	3	0.639(0.055)	0.332(0.053)	8.61
ψ(veg),p(.)	117.97	3.61	0.072	3	0.587(0.059)	0.332(0.053)	10.08
Model averaged					0.644(0.075)	0.331(0.053)	11.70

Not all sites have the same predicted occupancy probability. Occupancy probability per site, averaged across the top models, is given in Table 3.7. These values represent the probability of *P. burbidgei* occurring at a particular site as predicted by the model given the values of covariates for that site. Thus, the null model ψ(.),p(.) only estimates one parameter for all sites as the model states that there are no variables influencing occupancy.

Table 3.7 Estimated occupancy probability of *P. burbidgei* for each site, averaged across the top models.

Site	ψ	S.E.
Derby A	0.584	0.263
Derby B	0.264	0.287
Derby C	0.264	0.287
Derby D	0.839	0.212
Derby E	0.653	0.282
Derby G	0.884	0.184
Dambi A	0.818	0.255
Dambi B	0.584	0.263
Dambi C	0.761	0.255
Dambi D	0.921	0.137

Bootstrapping (1000 replications) of the top model revealed there was no evidence for lack of fit ($p=0.49$, $\hat{c} = 0.70$) of the model to the data. The top ranked models also had precision <25% and detection probability >0.15 (Table 3.6) indicating that they were valid models.

The covariate ‘structure’ had a significant positive influence on occupancy of *P. burbidgei* (the β coefficient was positive and had a 95% CI that did not include zero). This covariate also had the highest summed weight across all models containing it (50.04%; Table 3.8). The covariates ‘height,’ ‘vegetation’ and ‘steepness’ were also significant, although they were negatively related to site occupancy. The model ψ(water),p(.) was ranked third, however the covariate ‘water’ was a non-significant predictor of *P. burbidgei* occupancy, i.e. the 95% confidence intervals of the untransformed β coefficient for water included zero. Similarly, the presence of predators did not appear to influence the occupancy of *P. burbidgei,* as it was a non-significant covariate and had a low summed model weight (Table 3.8).

Table 3.8. Summed AICc weights of models that included each covariate.

Covariate	Weight
Structure	50.04%
Height	21.05%
Water	15.98%
Vegetation	13.02%
Steepness	11.05%
Predator	4.95%

3.5.2 *Petrogale concinna*

There was not enough detection data for *P. concinna* to model its occupancy or detection. Instead, limited information about the probability that it was present but not detected (false absence) was gathered from the one site that it was detected (Augustus Island). *Petrogale concinna* was detected twice in 62 camera days, giving it a detection probability (proportion of days detected) of 0.032 at that site. Assuming that detection probability was constant across sites, the probability of not detecting *P. concinna* if it was present can be given by Equation 3.1:

$$P_i = (1 - p)^{T_i} \qquad \text{..[Equation 3.1]}$$

Where P_i is the probability of not detecting *P. concinna* if it was present at site *i*; *p* is the detection probability (0.032) of *P. concinna*; and T_i is the number of trap days at site *i*. Values for P_i across the study sites are given in Table 3.9.

The mean probability that it was not detected at a site but present was 0.275. Nearly 30% probability of a false absence is high, although interpretation of this value is limited by the fact that the detection probability is calculated from just one site with two detections, and has no measure of precision or accuracy.

Table 3.9. Probability (P_i) that *P. concinna* was present at each site (i) but not detected, given the number of trap days (T_i).

Site (i)	**No. Trap days (T_i)**	**P_i**
Derby A	122	0.018
Derby B	63	0.127
Derby C	63	0.127
Derby D	75	0.086
Derby E	2	0.937
Derby G	62	0.131
Dambi A	26	0.426
Dambi B	56	0.159
Dambi C	28	0.399
Dambi D	28	0.399
Kalu A	20	0.519
Kalu B	7	0.795
Kalu C	121	0.019
Kalu F	23	0.470
KaluG	70	0.101
Kalu I	18	0.554
Kalu K	61	0.135
Kalu L	61	0.135
Kalu M	118	0.021
Kalu N	37	0.297
Kalu O	58	0.149
Kalu Q	58	0.149
Kalu S	55	0.165
MEAN		**0.275**

Chapter 4: Discussion

The ecology and population status of *P. concinna* and *P. burbidgei* in the Kimberley are poorly understood, and there are concerns that these species could be in decline. Scientific knowledge of these species has been limited by the remote inaccessibility of their habitat, their shy nature and similarity in appearance. This study addressed these issues by simultaneously recording presence with remote sensor cameras and faecal pellet DNA. The significance of the results and the potential causes of the observed decline in *P. concinna* will be discussed below. The limitations to the study design will also be discussed.

4.1 Population Status and Ecology of *Petrogale burbidgei*

The sensor camera survey in conjunction with the analysis of faecal DNA revealed that *P. burbidgei* occurs 115km further west than recorded in the general literature (Figure 4.1; Burbidge *et al.* 2008b; Pearson 2012; Pearson *et al.* 2008; Roache 2011). Live trapping by AWC recorded *P. burbidgei* at the Artesian Range, near the most western site where this species was detected in this study (Figure 4.1). The AWC record was not published in a peer reviewed journal but appears on NatureMap, a multi-database mapping site produced by the Western Australian Government Department of Environment and Conservation (DEC 2013). The range extension recorded by AWC and in this study is significant because the extension is a large proportion of the original distribution (Figure 4.1).

The occupancy modelling confirmed that rock structure was an important habitat attribute for this species. A positive beta value for rock structure indicated that the probability of occupancy increased with an increasing amount of caves, crevices and other interstices. This is as expected because it is established that members of the genus *Petrogale* use caves and crevices for protection from environmental extremes and predators (e.g. Churchill 1997; Sanson et al. 1985; Short 1982).

The negative correlation of steepness, height and vegetation quality with occupancy does not necessarily mean that *P. burbidgei* prefers low, flat areas with poor quality vegetation.

The observed negative relationships are most likely simply an artefact of these variables being associated with other unmeasured, but important, habitat variables. For example, three of the four sites where *P. burbidgei* was not detected were high, steep and had good vegetation quality and diversity, but were part of a different phytogeographic region (see Section 2.1.1; Rye *et al.* 1992). If *P. burbidgei* is restricted to the Gardener Phytogeographic district, or limited in distribution range for any other reason, then this would explain the observed negative association of these variables. Furthermore, all variables were measured as categorical variables, mostly ordinal, thus limiting the precision and sensitivity of the models to detect real relationships. Of particular interest would be to measure habitat variables using a mapping database or satellite imagery. Thus it is not clear from this study how these variables influence occupancy of *P. burbidgei*.

Petrogale burbidgei was recorded by cameras and faecal DNA at six out of 10 potential sites, and had an occupancy probability at these sites of 0.644, accounting for imperfect detection. Occupancy across the whole study area cannot be estimated accurately with the available data, because a) the habitat variables used in this modelling are not known for other sites and b) rock-wallabies inhabit discrete rock outcrops and are therefore are not evenly distributed across the landscape. None the less, occupancy of surveyed suitable habitat greater than 60% is high and coupled with the large range extension indicates that the status of *P. burbidgei* is secure. Furthermore, this is likely to be an underestimate of the true occupancy across its range.

The three study sites on the Yampi Peninsula (Figure 3.1) included in this analysis may be outside its distribution range. The furthest west that this species has been recorded was just below Walcott Inlet (Artesian Range and our site Derby G; Figures 1.5 & 3.1), probably approximating its current true western-most limit, although subfossil remains of *P. burbidgei* have been recorded further south-west by Start *et al.* (2012) (Figure 1.5). The three Yampi peninsula sites were still included in the occupancy analysis to increase sample size and thus power of the analysis; it is not known for sure if *P. burbidgei* is absent from this area as this area has never been surveyed previously. However if the Yampi Peninsula sites are excluded, then the occupancy of *P. burbidgei* is exceptionally high (85.7%).

The one site within the assumed range where *P. burbidgei* was not detected was site 'Derby E' (just north of Walcott Inlet). There was no sign of *P. burbidgei* at this specific location (no small rock-wallaby pellets), however the area surveyed was near an extensive, high and rugged cliff line with a rainforest patch in the valley below. Rainforest patches may be important habitat features for *P. burbidgei* evidenced by approximately 20% of captures on islands of this species being in rainforest (Gibson and McKenzie 2012). The camera at this site malfunctioned and only took images for the first two days so its presence at this site could have been missed. Furthermore, the team was unable to search the best habitat in the area because of the danger posed by the steep cliff, meaning that *P. burbidgei* could have been present nearby but remained undetected.

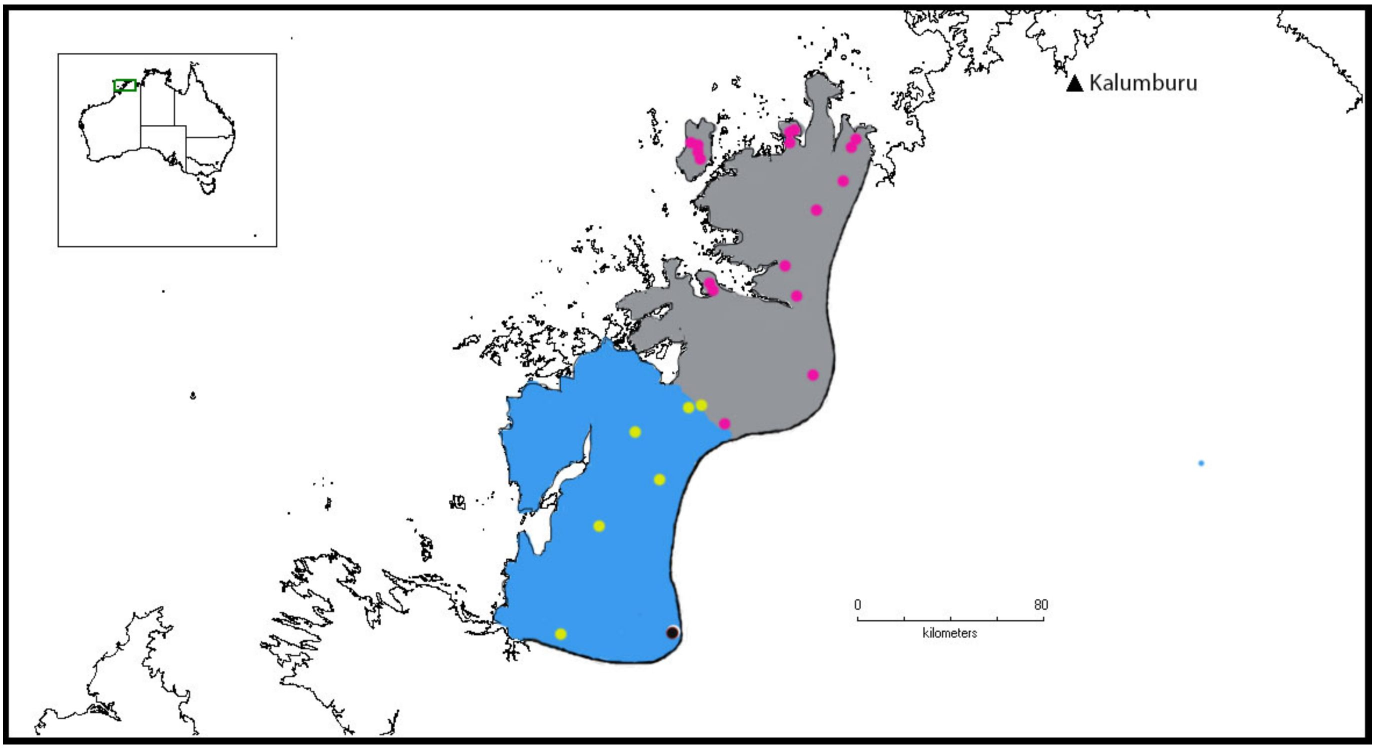


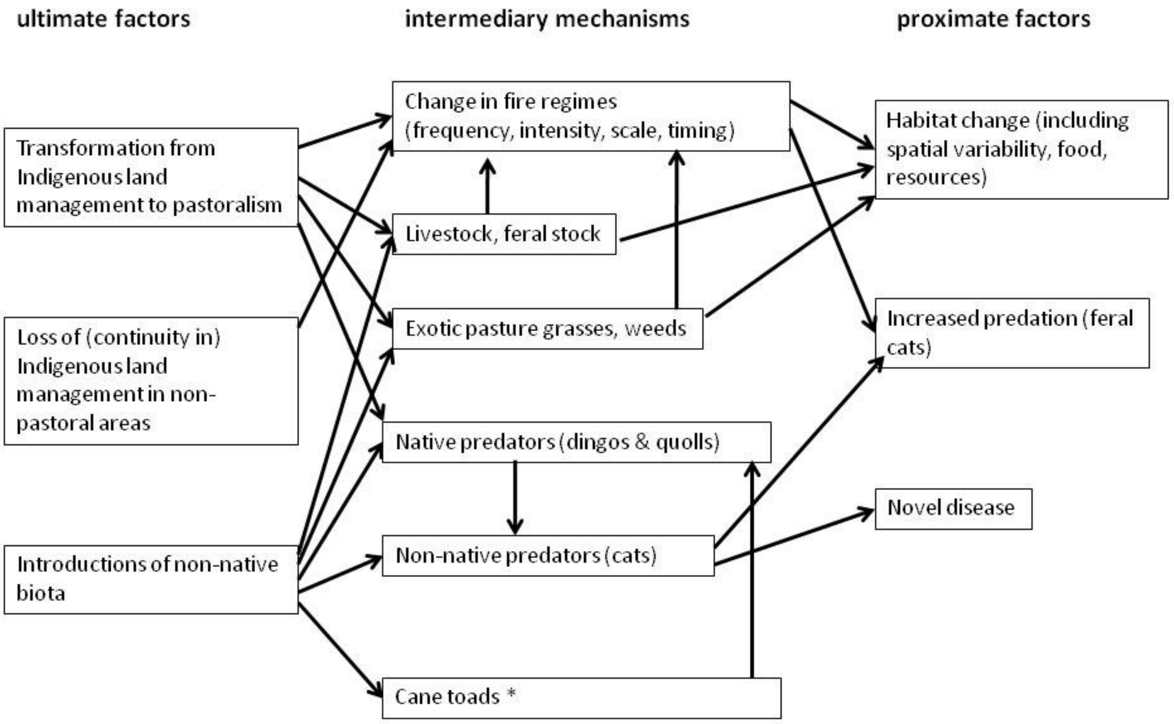
Figure 4.1. Published extant distribution range (grey area; pink points) of *P. burbidgei* and the range extension (blue area) resulting from both records in this study (yellow points) and the unpublished 2011 record by AWC at the Artesian Range (black point).

4.2 Population Status and Decline of *Petrogale concinna*

Petrogale concinna appears to have severely declined on the mainland to the extent that it was not detected by the remote sensor cameras or faecal DNA. A total of 30 sites were searched for signs of *P. concinna*, and 24 of these had sensor cameras deployed. Additionally, 148 scat samples were analysed for *P. concinna* mtDNA. Yet this species was detected at only one site; Augustus Island.

The average probability that it was present at a site but that it was missed with the cameras was high, almost 30%. However this value is highly likely to be biased because it was calculated using only two detections at one site, which produced a probably false low detection probability. Additionally, this value is different from the probability of a false absence at all sites if at least one site in the area was occupied. The probability of not finding *P. concinna* at 24 sites if present in the study areas is likely to be much lower. Thus this measure is not ecologically significant, and it should simply be considered unlikely, but possible, that *P. concinna* was missed.

Possible causes of mammal decline in northern Australia outlined by Woinarski et al. (2011) are discussed here for *P. concinna*. These include inappropriate fire regimes, predation by cats, habitat alteration by livestock, and novel disease. Figure 4.1 is a schematic representation of interactions between these factors.



*Cane toads have as of yet only reached the eastern Kimberley, however their progression to the western Kimberley is imminent. They are unlikely to directly influence *P. concinna*, but may indirectly through their impact on predators.

Figure 4.2. Interactions between ultimate factors, intermediary mechanisms and proximate factors that could be negatively influencing *P. concinna* populations. Adapted from Woinarski et al. (2011)

The key questions to consider when attempting to determine the cause or causes of a species' decline are:

1) What are the similarities between the decline of this species and declines of other species in comparable areas?
2) What is different between related species that persist and the species that has declined?
3) What is different between areas still occupied and areas unoccupied?
4) When did the decline start? and then;
5) What changed around this time?

These are addressed each in turn below, in terms of the threats listed above. References are made to the recent mammal declines in northern Australia noted by Woinarski et al. (2010) (see Section 1.3.2) because perhaps the disappearance of *P. concinna* is part of a much bigger picture.

4.2.1 Similarities between the Mammal Declines in Northern Australia and *P. concinna*

Puzzlingly, *P. concinna* does not fit the bill of mammals determined to be most susceptible to declines in the Top End, or declines in the rest of Australia. Small to medium sized (CWR) mammals that are ground dwelling and occur in arid to semi-arid areas are most susceptible to extinction across Australia, with an overrepresentation of bandicoots, larger rodents, small macropods and large dasyurids in the endangered lists (Burbidge and McKenzie 1989; McKenzie et al. 2007; Woinarski et al. 2011). This is true also for the recent declines observed in northern Australia, except that declines are also occurring in higher rainfall areas (Woinarski et al. 2010). *Petrogale concinna*, like all *Petrogale* species, is rock- and cave-dwelling, an attribute that is supposed to protect it from agents of decline (Burbidge and McKenzie 1989; McKenzie et al. 2007). Rocky areas are less likely to be altered by grazing of introduced herbivores, are protected from fire and offer shelter from predators irrespective of vegetative cover (McKenzie et al. 2007). Additionally *P. concinna* occurs in the higher rainfall north Kimberley where most species that have declined elsewhere persist (McKenzie and Burbidge 2002). Despite this, this study provides evidence *P. concinna* has declined and even perhaps disappeared from areas previously recorded within the North Kimberley.

The main similarity between *P. concinna* and species that have declined, both on a continental scale and in northern Australia, is size. Adult *P. concinna* weighs 1050-1700g (Sanson and Churchill 2008), which is well within the CWR first coined by Burbidge and McKenzie (1989) and now generally accepted as a major risk factor for Australian mammals (McKenzie *et al.* 2007; Van Dyck and Strahan 2008). Johnson and Isaac (2009) demonstrated that extinction risk was statistically only strongly related to body size for ground dwelling mammals in lower rainfall areas. However the recent mammal declines in high rainfall areas of the Northern Territory (Woinarski et al. 2010) may add strength to the already weak relationship between body size and extinction for mammals in higher rainfall areas (Johnson and Isaac 2009). The CWR is believed to be a significant risk factor because size relates to ecological attributes that increase the impact of threats on a species (discussed in section 1.3.3). In the context of declines in northern Australia examples include small to medium size species being more susceptible to predation by feral cats, more susceptible to changes in productivity or food availability by disturbance from feral herbivores or fire, and lower fecundity resulting in slower rebound after population reductions (Burbidge and McKenzie 1989; Cardillo 2003; Johnson and Isaac 2009). Despite the fact that some of these factors may be mitigated for rock-dwelling *P. concinna*, this medium-small species may still be more susceptible to declines that very small or large species.

4.2.2 Declines of *P. concinna canescens* (Northern Territory)

Northern Territory populations of *P. concinna* may have similarly declined (Lundie-Jenkins and Findlay 1997). Sanson *et al.* (1985) reported that in 1976 and 1977 they searched for *P. concinna* around Mount Gardiner, Pine Creek, Nabarlek Range, McCalium Creek on Table Top Range, and Nellie Creek where it had been recorded in the late 19th and early 20th century by Dahl and Tunney or later by E.R. Pethericky. Yet despite thorough searching no *P. concinna* was found at these locations. Conversely Churchill (1997) concluded that although it had disappeared from some sites the status of *P. concinna* was secure as they were recorded at eight sites in her study. Some of these records may have been misidentifications, particularly at Hayes Creek where *P. concinna* was identified only from faecal pellets and from and Jim Jim Falls campground where the *P. concinna* was noted as interacting amongst a group of *P. brachyotis*. Given the difficulty differentiating the faecal pellets of *P. concinna* from those of young *P. brachyotis*, and the difficulty telling juvenile *P. brachyotis* and *P. concinna* apart, these records should be interpreted with caution.

No further research has been done on *P. concinna* in the Northern Territory since Churchill (1997). This species was not mentioned by Woinarski et al. (2010) despite occurring in Kakadu National Park (Lundie-Jenkins and Findlay 1997). Therefore there is no evidence for or against this species declining in a similar fashion to the other mammal fauna of the Top End in the last decade (Woinarski et al. 2010). It is most likely that *P. concinna* has continued to decline since it had already disappeared from some sites by the 1970's.

4.2.3 What is the Difference between *P. burbidgei* and *P. concinna*?

While *P. concinna* has evidently declined, *P. burbidgei* is still locally common. Superficially, these two species appear no different from each other. Both are similar sized, look similar, inhabit similar habitat in high rainfall areas of the North Kimberley (although *P. concinna* has also been recorded more broadly), presumably both have similar diet, both use caves and crevices in rocks for shelter, and together with *P. brachyotis* share a common ancestor (Pearson et al. 2008; Potter et al. 2012a; Sanson and Churchill 2008). However, my data suggests there must be an ecological or biological difference in their ability to withstand threatening processes.

One potential difference between these species that could have led to the decline of only *P. concinna* comes from limited information on reproduction. Species with higher fecundity have higher population growth and are less prone to extinction (Cardillo 2003). Based on information from records of collected specimens it can be deduced that *P. burbidgei* pouch young develop fur at a smaller size (between 51-77g; Kitchener and Sanson 1978), and presumably younger age, than *P. concinna* (90g; Nelson and Goldstone 1986). If *P. burbidgei* young are able to develop quicker, then this species would have a higher reproductive output which may have made it more resilient to decline.

The persistence of *P. burbidgei* provides circumstantial evidence that novel disease is unlikely to be the cause of decline. If a disease was spread by introduced species (rats of cats) then it should have spreads to both species (Woinarski et al. 2011). Further understanding of each species’ ecology and reproduction is required in order to postulate why one has declined and the other survived.

4.2.4 Why has *P. concinna* Persisted on Islands?

The persistence of *P. concinna* on Augustus Island, and other offshore islands, may offer insight into potential threats. Islands have provided refuge for number of species that have declined and even disappeared from the mainland (Burbidge et al. 2008a; Conservation Comission 2010). Augustus Island is not occupied by cats or feral herbivores, is isolated from potential diseases, and has infrequent fire as it is uninhabited by people (Conservation Comission 2010; Fitzsimmons et al. 2010; Gibson and McKenzie 2012). Any one of these factors could be causing the differential survival of *P. concinna* between island and mainland sites. Fitzsimmons et al. (2010) postulated that the persistence of mammal species on islands indicated the role of feral cats or disease in decline, although didn’t elaborate why this was so over the role of introduced herbivores or fire.

Interestingly, the habitat on Augustus Island where *P. concinna* was observed was one of the poorest of all the sites visited (Appendices A and C). It was virtually flat with minimal scattered boulder scree, no fresh water and the vegetation was almost uniformly Spinifex (*Plectrachne sp.*) without any *Ficus* or other more nutritious plants. Considering this it appears most likely that predation by cats is the main driver of decline. Fox baiting experiments in the Western Australian Central Wheatbelt demonstrated that the removal of foxes caused a niche expansion of *P. lateralis lateralis* into areas lacking deep fractures, crevices and caves (Kinnear et al. 2010). Thus it fits that if predation pressure of cats is great, then their absence on Augustus Island enabled *P. concinna* to occupy habitat without safe refuges, but their presence on the mainland caused *P. concinna* to decline even from rugged, steep, structured habitat. Cats are able predators in rocky environments and even a single individual can have a devastating impact on rock-wallaby populations (Spencer 1990). Further, around Kalumburu some sites with previous records of *P. concinna* (from the 1960's) were re-visited, and the habitat was similarly poor. These sites had no evidence of *P. concinna* and even very little evidence of the more common *P. brachyotis*. However, if cats are the main driver of decline then it is puzzling why the smaller species, *P. burbidgei,* has not similarly declined.

Dingoes have been recorded on Augustus Island (Abbott and Burbidge 1995), likely brought there by Aboriginal People prior to European settlement (Start et al. 2007). It is therefore unlikely that the high numbers of dingoes observed around Kalumburu are linked to the absence of *P. concinna*. In fact, it has been suggested that the presence of dingoes may be beneficial to rock-wallaby populations through mesopredator (cat) suppression (Johnson et al. 2007; Kennedy et al. 2012).

At a superficial level it appears that *P. concinna* is able to survive on poor quality vegetation. This is evidenced by its ability to continually replace molars (Sanson et al. 1985), and by existence at a site dominated by Spinifex grass. Large feral herbivores can negatively impact mammal species through altering fire patterns, destroying habitat or reducing food resources (Figure 4.1; Legge et al. 2011; Woinarski et al. 2011). It is unlikely then that herbivores would impact *P. concinna* to the extent observed as in addition to not requiring high quality forage *P. concinna* relies on rocks rather than vegetation for shelter. However, this is perhaps over simplifying the impact of herbivores which may have disturbed the delicate ecological balance that was in place, thereby causing *P. concinna* to disappear.

Fire intensity, pattern and frequency can greatly impact on mammal populations (Anderson et al. 2005; Legge et al. 2008; Woinarski et al. 2004). Changes in fire patterns since the shift away from traditional burning may have impacted negatively on *P. concinna*. If this is the case, it is likely declines are the result of frequent fire, evidenced by the species persistence on uninhabited islands which have infrequent fire. This is also substantiated the by the findings of Anderson et al. (2005) who conducted a large scale five year study on biodiversity response to experimental burning of tropical savanna at Kapalga in Kakadu National Park. They found that small mammals were the most severely affected faunal group, with almost all species significantly more abundant in unburnt sites compared with two fire treatments (early and late burn). Obligate seeder species of grass in sandstone areas are suppressed and even displaced by frequent fire, and may be an important food resource for *P. concinna* (Fisher et al. 2003). Fire management in the northern tropical savannas of Australia has focused on early dry season fires to prevent large scale, high intensity fires late in the dry season which create uniform landscapes and impact negatively on biota (Anderson et al. 2005; Yates et al. 2008). However, Anderson et al. (2005) found limited evidence of the benefit of early dry season, low intensity fire over high intensity, late dry season fire on small mammals. It appears that for conservation of *P. concinna* fire suppression may be beneficial.

4.2.5 When did *P. concinna* Begin to Decline?

A major difficulty in deducing the cause of decline is the fact that there has been no previous research into the population status of *P. concinna* in the Kimberley, and records are tainted with misidentifications. This limits our ability to determine the timing of decline, and thus investigate what threats appeared contemporaneously. *Petrogale burbidgei* was only described in the late 1970s (Kitchener and Sanson), so all *P. burbidgei* specimens collected prior to this were labelled *P. concinna,* although at least some of these have been corrected (A. Burbidge, pers. com 2013). Additionally the 2003/4 Kimberley mammal surveys reported by Start *et al.* (2007) contained a misidentified '*P. concinna'* from Forest Creek in Drysdale River National Park (DRNP), which later molecular genetic analysis revealed to in fact be *P. brachyotis* (Potter *et al.* 2012c). Similarly, live-trapped animals in Mitchell Plateau were identified in-hand as *P. concinna* but were later identified as *P. burbidgei* by molecular genetics (Mark Eldridge, pers. comm. 2013). Until late 2013 it was believed that an orphaned joey from the Kimberley on display at Perth Zoo was a *P. concinna*. However this animal, formerly known as 'Norbet the Nabarlek' is now double the weight of an adult *P. concinna*, about the size of a normal *P. brachyotis*.

This study highlights how difficult it is to distinguish *P. concinna* from sympatric *P. burbidgei* and *P. brachyotis*. All records, unless confirmed by skull morphology or DNA should be treated with caution. The most recent mainland records of *P. concinna* are at Mitchell Plateau (1998), the Artesian Range (2000), and Mt Elizabeth Station below Prince Regent Nature Reserve (2001) (see Figure 1.3; DEC 2013). Any specimens associated with these records should be re-examined, and the sites re-surveyed to determine if *P. concinna* is still present.

It is likely that *P. concinna* started declining sometime between the 1960s, when most of the specimens were collected, and now. This lends the argument away from cats or herbivores as being the primary cause of decline as they have been present in the region for at least 100 years (Abbott 2002; Freeland 1990), although both have likely become more numerous in recent years. Without concrete knowledge of the timing of decline it is only possible to speculate about its causes.

4.2.6 Is the Disappearance of *P. concinna* Part of a More General Mammal Decline in the Northern Kimberley?

Fortunately, from the evidence presented here it appears that Kimberley mammals are faring better than mammals in the Top End. Several species that were reported to have significantly declined in the last decade (Woinarski et al. 2010) were detected by the camera traps. These include; *D. hallucatus, Z. argurus* and *P. delicatulus*. Further, *M. macrurus* was detected in this study frequently even though it has become extinct in the Northern Territory (Menkhorst and Knight 2011). Other species that are Kimberley endemics but of a similar CWR size also persist and were frequently detected (*P. burbidgei*, *W. squamicaudata* and *Z. woodwardi*).

However, this study was biased by examining only rock pile habitat and not surveying suitably for small mammals (dasyurids and rodents) which are not usually identifiable from camera images. Declines of non rock-dwelling fauna in the region may be more perceptible. Indeed, Start et al. (2007) noticed a lack of small granivorous rodents and Legge *et al.* (2011) recorded a rapid significant increase in ground dwelling mammal fauna in a de-stocking experiment. Furthermore the disappearance of *P. concinna* is unlikely to supervene in isolation; especially given the precipitous, prevalent decline of mammals in similar ecosystems with similar threats in the Northern Territory.

4. 3 Techniques for Surveying *P. concinna* and *P. burbidgei* in the Kimberley

4.3.1 Using Cameras to Detect these Species

This study was the first to use remote sensor cameras to survey the Kimberley rock-wallabies. The camera trapping frequencies given in Table 3.5 were comparable to or higher than those reported in the literature for other species (e.g. Rovero and Marshall 2009; Sarmento et al. 2011; Tobler et al. 2009) indicating that sensor cameras are a suitable survey tool for these species. In particular, the white flash cameras (Scout Guard SG860C) produced colour images aiding in some species identification without apparently disturbing animals.

Cameras were usually set to moderate sensitivity with three photos per trigger. Moderate sensitivity was suitable in some situations (i.e. in caves); however in most instances the sensitivity was too high causing numerous false triggers, even when vegetation had been cleared from the field of view. This model of white flash camera (SG860C) seemed to be particularly sensitive to false triggers. In eight cases the camera failed to take images for the full survey period, and this was usually because the battery was exhausted by rapidly fired false-triggered images. At two sites the cameras malfunctioned (despite passing testing at setup) and either did not take any pictures, or only took pictures for two days. Thus the study design could be improved by either setting the sensitivity to low (and risk missing animals) or by using higher quality (albeit more expensive) cameras such as Rerconyx PC850 white flash cameras that are less prone to false triggering (Karl Vernes, pers. comm. 2013). Three photos per trigger was deemed to be optimal as this increased the likelihood that a subject could be correctly identified.

Interestingly, in this study both *P. concinna* and *P. burbidgei* appeared totally disinterested in the peanut butter, oat and truffle oil bait ball that was used as an attractant. Their lack of interest was not due to low aromaticity of the bait, evidenced by *P. brachyotis*, *D. hallucatus, C. lupus*, *W. squamicaudata* and other species being highly attracted to it. Moreover, this bait is commonly used with high success (e.g. Kitchener et al. 1981; Start et al. 2007; the author's personal experience). In camera-trapping bait is non-essential and was used only to improve detectability. *Petrogale concinna* and *P. burbidgei* are known to be very cage-trap shy (Kitchener and Sanson 1978; Nelson and Goldstone 1986) which is probably at least partly explained by their disinterest in commonly used baits. Consequently, sensor cameras were determined to be a more suitable survey technique than cage trapping as sufficient detection of the target species occurred regardless of bait.

Previous records of these species in the Kimberley have mainly been obtained by spotlight shooting and opportunistic sightings (Churchill 1997; Kitchener *et al.* 1981; Kitchener and Sanson 1978; Start *et al.* 2007). Spotlight shooting is effective at producing objective records but has several disadvantages compared with camera trapping. Firstly, there are ethical concerns associated with this method, particularly if the study species is potentially declining or threatened. Secondly, although precise information about the specimen’s morphology and reproduction status is obtained, little information can be gathered about the study species behaviour, habitat preferences or abundance. Finally shooting may be more logistically difficult or expensive; constraints include experience with and licensing firearms, and the likelihood that fewer sites could be visited (probably one per night of shooting) which would increase field costs. In my study, the research team were able to visit up to four sites and set five cameras in a day when travelling by helicopter, and two sites a day when travelling by vehicle and on foot. Collection of cameras at the end of the survey period was also efficient with all cameras only taking one day for each area. Camera traps (remote sensor cameras) were found to be a useful, efficient tool that allowed the researchers to gather objective data on these shy species with minimal disturbance and ethical concerns (Rovero *et al.* 2010).

Additionally, cameras provide useful information about non-target species without any additional effort. This can be useful if interspecies interactions are of interest, or if the purpose is to study all species of a particular group (e.g. mammals). In my study, the scaly-tailed possum was recorded for the first time on Augustus Island by the sensor cameras (see Gibson and McKenzie 2012), which may have significant management implications for this Kimberley endemic. The three species of rock-wallaby were frequently recorded active during the middle day, despite being reported in the literature as mostly nocturnal (Van Dyck and Strahan 2008). All species records are valuable as most sites with cameras were previously unsurveyed and therefore provide useful distribution and population information.

The main issue with using cameras to survey *P. concinna* and *P. burbidgei* is that these species could not be distinguished from images alone. Several people simultaneously attempted to key out images of small *Petrogale* s*p.* as being *P. concinna*, *P. burbidgei* or juvenile *P. brachyotis* based on morphology and size however it was not possible to independently confirm the species. The angle of the photograph influenced the appearance of ear size (*P. burbidgei* has smaller ears than *P. concinna*; Kitchener and Sanson 1978), and the light or time of day influenced the pelage colour (see Appendix G and Appendix H). Additionally, *P. burbidgei* from this region did not have dark tail-tips as noted for the more northern (Mitchell Plateau) *P. burbidgei* (Mark Eldridge, pers. comm. 2013), making identification even more difficult. Moreover, there was a significant amount of intraspecific variation in *P. burbidgei* within the study area and even within sites. This was particularly so at site Dambi B where two individuals had completely different pelage and facial morphology (see Appendix G). It is possible that one of these individuals was *P. concinna*, but it is unlikely because neither had the 'defining characteristics' of *P. concinna*; and DNA was extracted from a total of 10 faecal pellets from this site, all yielding the same *cytochrome b* sequence, identified to be *P. burbidgei*.

It can be concluded that remote sensor cameras are an excellent tool for surveying the small *Petrogale* species of the Kimberley, however it is essential that species are confirmed with other, unambiguous means, such as DNA.

4.3.2 Occupancy Modelling for Camera Data Analysis

This section deals with *P. burbidgei* only as the detection of *P. concinna* was too low to model occupancy. In this study single-season type occupancy models were used to model the occupancy of *P. burbidgei* across 10 study sites. This type of analysis produced statistically acceptable results sites, indicated by precision of less than 25%, a detection probability of >0.15, and bootstrap Chi^2 and ĉ values for the top model indicating no evidence for lack of model fit (Donovan and Hines 2007b; Linkie *et al.* 2007; MacKenzie and Bailey 2004). However, statistical acceptability is not the same as biologically applicability. The biological/ecological inference about the model results was limited because of two main reasons:

1) number of sites and;
2) measurement of covariates;

Single-season single-species type models with occupancy covariates (MacKenzie et al. 2002) were used to model the occupancy of *P. burbidgei* because the number of sites surveyed (N=10) was sufficient to produce valid results; however modelling could be improved with additional sites. A greater number of sites would have allowed the use of a more biologically appropriate, albeit more complex, type of model (Dorazio 2007). The Royle-Nichols (R-N) model (Royle and Nichols 2003) uses detection non-detection data to estimate abundance, and indirectly calculate occupancy (Donovan *et al.* 2007; Royle and Nichols 2003). This method is based on the concept that heterogeneous abundance among sites will produce heterogeneous detection probabilities. As the number of individuals increases, the chance of detecting at least one individual (and thus demonstrating that the site is occupied) also increases according to Equation 4.1:

$$p_i = 1 - (1 - r)^{N_i} \quad \text{.......................................}\textbf{[Equation 4.1]}$$

Where p_i is detection probability at site *i*; *r* is the individual detection probability (the probability of detecting a single animal of that species); and N_i is the abundance at site *i*. Essentially, unless abundance across sites is uniform, this model will produce a more accurate occupancy estimate than simple single-season analyses (Royle and Nichols 2003). Dorazio (2007) demonstrated that this was particularly the case when the study species was cryptic or rare and had a consequently low detection probability. The R-N model has the added advantage of estimating absolute abundance, which is usually viewed as a superior population statistic despite occupancy being a suitable surrogate for abundance and for monitoring population change (Joseph et al. 2006)

Furthermore, having data from few sites limited the number of covariates able to be modelled (MacKenzie et al. 2006). In general, the more covariates (parameters) incorporated into a model the better the model will fit the data, regardless of the biological or ecological significance of those covariates (Burnham and Anderson 2002). Thus, when ranking models a penalty is applied for the number of parameters in the model. The penalty is greater when sample size is small and AICc is used as it is more likely that differences between sites are due chance rather than covariates, and hence there is a greater risk of over-parameterising (Burnham and Anderson 2002). Consequently, in this study models containing ecologically important covariates for *P. burbidgei* detection and occupancy may have been ranked lower than simpler models because of the small sample size.

Although models were designed to contain the variables believed to be the most important, there is the potential that the assumption of no unexplained heterogeneity in detection or occupancy was not met. Single-season single-species type models assume that differences in detection probability are explained by covariates (MacKenzie et al. 2002; Royle and Nichols 2003). Unless differences in detection probability between sites are explained, they would not produce valid occupancy estimates. For example, it is likely that all sites (cameras) had different detection probabilities simply because of where the camera was positioned with respect to fine-scale rock-wallaby habitat use. Yet when detection probabilities were allowed vary between sites, i.e. p(site), the resulting model was ranked very low.

The study design could be greatly improved with better quantification of variables, especially with GIS mapping. In this study all five habitat covariates were ordinal categorical variables, although some such as cliff height, slope and distance to water could have been measured quantitatively if maps were used. All variables, except one, were poor at explaining occupancy, or most likely falsely explained it. It is unclear whether this was an artefact of the way the variables were measured, which variables were used, small sample size, or a true reflection of the habitat generality of *P. burbidgei*. Quantitative variables offer a more precise, higher resolution way of examining the relationships between a species occurrence and ecological factors (Burnham and Anderson 2002). Vegetation was not identified to the species level and a more detailed vegetation description at sites may reveal a significant positive relationship between vegetation and species occurrence. Moreover, with GIS technology variables can be recorded for sites without having to visit them. This is useful as occupancy probability can be extrapolated to broader areas than just the study sites and be used in producing species distribution maps (MacKenzie 2012).

Ideally, sites should be laid out in a regular grid and include a range of habitat types if estimates are to represent a larger area (Rovero et al. 2010). Rock wallabies are not spread evenly over the landscape, occurring in discrete patches associated with rock outcrops (Van Dyck and Strahan 2008). As it was not practical in this small-scale study to study habitat in an un-biased fashion, even with the use of GIS technology inference about the occupancy of *P. burbidgei* across its whole range is limited, and occupancy could only be predicted for similar rocky outcrops within the area studied.

If the objective of a survey is to produce useful estimates of abundance or occupancy and determine important covariates across a large area then occupancy modelling is suitable, provided the number of sites is large, the study sites are representative, and covariates are measured precisely (preferably with GIS). Unfortunately, these requirements are impractical in the Kimberley for all but the most resourced studies as sites are mostly inaccessible except by helicopter.

Conversely if the objective of a survey is only to determine occupancy of *P. burbidgei* in a restricted area, rather than model it with covariates, then it may be unnecessary to model occupancy if cameras are deployed for greater than a month. The occupancy probability produced by modelling imperfect detection was not much higher than the naive occupancy estimate. This is because, although the detection probability per camera day or ‘survey event’ (three camera days) was not high (0.36), the cameras were in the field for a sufficient period of time such that probability of missing *P. burbidgei* if present at a site was low. However, not all cameras recorded images for the whole survey period, thus increasing the probability of a false absence at some sites.

4.3.3 Faecal Pellets

Faecal pellet counts are popular tool for surveying shy, cryptic mammal species or species of mammal otherwise difficult to detect. It has been verified for a number of species, including several species of macropods, that pellet counts reflect true density (e.g. Hill 1981; Johnson and Jarman 1987; Karels et al. 2004; Vernes 1999; Walker et al. 2000). Estimating absolute density of a species requires additional knowledge of deposition rate but often it is suffice to simply estimate relative density (Jarman and Capararo 1997). According to Jarman and Capararo (1997), faecal pellets can be used to monitor the presence, habitat use, relative density and possibly (but less practically) absolute density of rock-wallabies. These types of surveys are simple and economical to conduct yet are an effective way of monitoring populations. However, this method does not produce reliable results if the pellets of the target species cannot be distinguished from those of other sympatric species (Bowkett et al. 2013).

The length and width of faecal pellets from sympatric *P. brachyotis*, *P. burbidgei* and *P. concinna* (species confirmed by DNA) were compared to asses if faecal pellet counts can be used as survey tool for these species. Although the scats of the larger species, *P. brachyotis*, were significantly larger than the scats of *P. concinna* and *P. burbidgei*, there was no significant difference between the size of *P. burbidgei* and *P. concinna* scats. Further, the species from which a pellet came cannot be predicted from its length and width alone. Although *P. brachyotis* is almost twice the body weight of the two smaller species, the ability of an observer to distinguish a pellet from this species is confounded by the scats of juveniles. Considering the ubiquity of *P. brachyotis* at rocky sites across the Kimberley and the relative scarcity of *P. concinna* and *P. burbidgei*, faecal pellets of *P. concinna* and *P. burbidgei* should not be identified on size alone.

It is possible that faecal pellets of these species differ in other morphological aspects other than length and width. For example, subtle differences in colour, regularity, or in the length of squeeze point may occur between the species, which could be used to assign species to pellets (Telfer et al. 2006). Furthermore, only five samples of *P. concinna* pellets were used in this analysis potentially leading to a type two error. However, information such as colour or shape is relatively difficult to define and even more difficult for potentially inexperienced observers in the field to measure. To the somewhat experienced observers in this study, *P. concinna*, *P. burbidgei*, and small *P. brachyotis* pellets appeared very similar, so unfortunately faecal pellet counts based on pellet morphology alone are not considered a suitable survey method for these species.

4.3.4 DNA

This study was successful at identifying a single fragment of DNA that can be used to unambiguously identify the three species of sympatric Kimberley rock-wallaby from faecal pellet samples. Criteria for a suitable fragment were: sufficient nucleotide differences between species of interest, high PCR success, and low contamination. All three criteria were met for the 223bp mtDNA fragment of the gene *cytochrome b* that is amplified by *Macyt 10F/R2* primers. Suitable PCR conditions were also developed for optimal results.

Identification of species from faecal DNA is an invaluable tool for surveying *P. concinna* and *P. burbidgei* because they are morphologically similar species but genetically distinct. There have been multiple misidentifications of these species (see Section 4.2.5 for details) even when examined in-hand. This highlights the necessity of using unambiguous methods such as molecular genetics to distinguish between *P. burbidgei*, *P. concinna* and juvenile *P. brachyotis*.

The ability to obtain meaningful DNA sequences from faecal pellets is particularly advantageous as pellets can be collected non-invasively (Piggott and Taylor 2003). This has a three-fold advantage over using DNA from animal tissue. Firstly, there far are less ethical (and potentially ecological) concerns over collecting DNA non-invasively as opposed to obtaining it through live trapping or shooting (Piggott and Taylor 2003). Secondly, *P. burbidgei* and *P. concinna* are very cage trap-shy (Churchill 1997; Kitchener and Sanson 1978; Nelson and Goldstone 1986) making it difficult to obtain accurate estimates of population abundance with traditional trapping techniques. Thirdly, collecting faecal pellets is a resource efficient way to gather population information such as distribution or abundance, thus enabling a greater area and/or more sites to be surveyed. These have significant management and implications for such poorly understood species (Piggott and Taylor 2003).

The cost of using faecal pellets for surveying is its biggest drawback, however cost efficiency could be greatly improved if methods are developed that reduce the cost of extracting DNA. In this study, each QIAGEN QIAamp Stool Mini Kit (3 kits used in total) was used to extract gDNA from 50 samples according to the instructions provided by QIAGEN. However, in the kit far more quantity of reagents were provided than were necessary to complete the 50 extractions, with the exception of the INHIBEX (inhibitor) tablets of which there were only 50. To decrease the cost per sample for future studies it may be possible to use half a tablet and still obtain acceptable results. Thus one kit ($500) could be used to extract 100 samples. This would be relatively easy to test by dividing several pellets into two subsamples and extracting DNA from one half using a full tablet and extracting DNA from the other half using half a tablet.

Conclusions

My work suggests that the north-west Kimberley *P. burbidgei* populations are stable. I demonstrated that *P. burbidgei* occurs further west than previously published and occupies over 60% of rocky sites within its range. Additionally, I confirmed that rock structure was an important habitat attribute for this species. Other habitat variables were poor predictors of *P. burbidgei* occupancy, or were misleading, although this was most likely an artefact of their measurement.

Conversely, I provided the first evidence that *P. concinna* has declined on the mainland of Western Australia. In this study *P. concinna* was not found at the 29 possible study sites on the mainland, either because it was truly absent or because its population density was so low it eluded detection. It can be deduced from comparative evidence that the most likely causes of decline are frequent fire and predation by cats, although without experimental evidence this is only speculation. The disappearance of *P. concinna* is significant because it may reflect more pervasive mammal declines across northern Australia.

My work demonstrated that the three different species of North Kimberley rock-wallaby can be successfully and unambiguously identified from faecal DNA. I demonstrated for the first time that sensor cameras are a useful tool for gathering data on these shy and cryptic species. However, sensor cameras are unsuitable as a sole tool for surveying these wallabies due to the difficulties distinguishing the three species from images. Similarly, I demonstrated that faecal pellet counts based on pellet morphology to detect species is unsuitable for monitoring as pellets from the three sympatric species could not be distinguished by measurements. Thus species identification by faecal DNA analysis should be an essential part of any non-invasive monitoring program used in the future for these species.

Further Research

It is clear that urgent work is needed, firstly to locate remaining Kimberley populations of *P. burbidgei* on the mainland, but also to assess the impact of the possible threats outlined above, and implement strategies to reverse the decline. Based on the results of this study I recommend the use of sensor cameras or faecal pellet counts in conjunction with molecular genetics for monitoring Kimberley rock-wallabies. It is important that any future research gains approval for access and that research is undertaken with the relevant Native Title groups.

The exact methodology for achieving these outcomes would depend on a number of factors. Cameras would be useful to look for small *Petrogale sp.* simultaneously across many locations, and can be moved around after a predefined period of time to cover a large area. Sites with small *Petrogale sp.* detected by the cameras could then be searched for faecal pellets in order to identify the species.

If faecal deposition (and decomposition) rate can be determined for these species, then perhaps a good technique would be to use scat plots to estimate density. This would be suitable if there are few sites but are relatively accessible as reliable estimates of site density can be achieved over repeated visits. If sites are less accessible then a simple pellet count at sites could be used as an index of abundance. However, DNA from a large subsample of pellets counted would have to be analysed in order to determine that they are all from the target species.

If the study area and number of sites is moderately large, and able to be accessed twice then occupancy modelling from sensor cameras would be optimal. Methods similar to those used in this study would be suitable; with pellets for DNA analysis collected at the time of camera setup/collection, although there is always the possibility that *P. burbidgei* and *P. concinna* are sympatric at sites. The estimates of ψ and p produced by this study can be used to optimise the study design according to Donovan and Hines (2007a) and Linkie *et al.* (2007) and if the sample size is sufficiently large the R-N (Royle and Nichols 2003) model type is recommended. Careful planning should be made with respect to how the cameras are placed and how site variables are measured. To maximise the information generated this should be used in conjunction with GIS mapping.

A good starting place to look for *P. concinna* would be at the sites in Prince Regent Nature Reserve where it has been previously recorded, especially the mainland sites where it was recorded in the last 20 years (Figure 1.3). However, I will reiterate that any survey should use molecular genetics to confirm species as all three rock-wallaby species are sympatric in this reserve. Work to locate *P. concinna* should also extend to the Northern Territory populations which most likely have similarly disappeared. Further research should include a re-assessment of all museum specimens to clarify correct labeling, potentially with the aid of molecular genetics, in order to obtain the best knowledge of the former distribution of *P. concinna* and help locate remaining populations.

Less urgently, further research should be done to clarify the intraspecific genetic relationships of *P. burbidgei.* As reported by Potter *et al.* (2012c) and as shown in this study, the north-eastern (Mitchell Plateau) *P. burbidgei* have very different mitochondrial DNA sequences compared with the south-western *P. burbidgei* of the Prince Regent Nature Reserve and of this study area. In fact, in the *cytochrome b* fragment, there were more base pair differences between *P. burbidgei* from the two areas than between any of the three species. The south-western animals possibly represent a new subspecies or even species of *P. burbidgei*, however more work is needed as only mitochondrial DNA has so far been sequenced for this 'new' variant. It would be particularly useful to sample individuals where the two haplotypes meet, most likely in the northern Prince Regent Reserve, to determine if reproductive isolation has occurred.

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Appendix A: Tables of Site Names and Localities

Table A.1. Dambi Sites

Site	Date	GPS Location	Habitat Grade (1-5)
A	4-Jul-13	16°1'30.9"S 124°45'55"E	4.5
B	4-Jul-13	15°50'42.7"S 125°0'15.1"E	3.5
B	4-Jul-13	15°50'45.0"S 125°0'14.6"E	3.5
C	4-Jul-13	15°34'4.7"S 125°7'0"E	2
D	4-Jul-13	15°33'6.9"S 125°10'19.5"E	4

Table A.2 Derby Sites

Site	Date	GPS Location	Habitat Grade (0-5)
A	30-May-13	16°11'31.2"S 123°38'54"E	3.5
A	30-May-13	16°11'31.0"S 123°38'52.3"E	3.5
B	30-May-13	16°12'57.7"S 123°45'41.9"E	3
C	30-May-13	16°17'2.7"S 124°2'59.5"E	3
D	31-May-13	16°27'34"S 124°36'52.5"E	3
D	31-May-13	16°27'33.4"S 124°36'52.6"E	3
E	31-May-13	16°19'4.3"S 124°40'47.4"E	4
F	31-May-13	15°20'30.5"S 124°32'28.3"E	0.5
G	31-May-13	15°39'37.3"S 124°54'39.4"E	4

Table A.3 Kalumburu Sites

Site	Date	Camera Set?	GPS Location	Habitat Grade (0-5)
A	10-Jun-13	Yes	14°16'41.6"S 126°37'33.5"E	4
B	11-Jun-13	Yes	14°12'33.3"S 126°37'39.1"E	3
C	12-Jun-13	Yes	14°16'26.9"S 126°37'11.6"E	2.5
C	12-Jun-13	Yes	14°16'24.3"S 126°37'9"E	2.5
D	12-Jun-13	No	14°12'36.6"S 126°37'29.7"E	5
E	13-Jun-13	No	14°9'9.4"S 126°39'36.3"E	1.5
F	12-Jun-13	Yes	14°11'32.4"S 126°39'7"E	2
G	13-Jun-13	Yes	14°16'57.2"S 126°39'58.1"E	3.5
G	13-Jun-13	Yes	14°16'57.0"S 126°39'57.5"E	3.5
H	14-Jun-13	No	14°18'18.9"S 126°41'21.3"E	3.5
I	15-Jun-13	Yes	14°13'26.6"S 126°38'19.3"E	3
J	16-Jun-13	No	14°12'13.37"S 126°39'3.27"E	0.5
K	17-Jun-13	Yes	14°32'57.7"S 126°33'54.8"E	1.5
K	17-Jun-13	Yes	14°33'0.6"S 126°33'56"E	1.5

L	17-Jun-13	Yes	14°26'15.0"S 126°40'26.5"E	4
M	18-Jun-13	Yes	14°31'4.4"S 126°35'57.2"E	3
M	18-Jun-13	Yes	14°31'3.4"S 126°35'58.0"E	3
N	19-Jun-13	Yes	14°33'21.88"S 126°34'22.52"E	3
O	20-Jun-13	Yes	14°14'40.1"S 126°37'53.6"E	2.5
O	20-Jun-13	Yes	14°14'39.26"S 126°37'51.93"E	2.5
P	20-Jun-13	No	14°13'51.55"S 126°38'9.76"E	4
Q	20-Jun-13	Yes	14°17'13.0"S 126°41'21.4"E	4.5
R	21-Jun-13	No	14°28'59.63"S 126°37'31.38"E	3
S	23-Jun-13	Yes	14°16'48.1"S 126°39'48.1"E	1.5

Appendix B: Examples of Camera Setup

Photos: Jessica Koleck/Stephanie Todd

Appendix C: Examples of Sites with Different Habitat Variables

Figure C.1. Site Derby F. Habitat variables: low (height=1), almost flat (steepness=1), with some rock structure (structure=2), generally poor vegetation (veg=1). Overall habitat grade was 0.5/5. Photo: Jessica Koleck

Figure C.2. Site Derby A. Habitat variables: high (height=3), almost vertical (steepness= 3), some structure and fissures in cliff (structure=2). Overall habitat grade was 3.5/5.

Figure C.3. Site Kalumburu Q. Habitat variables: moderately high (height=2), almost vertical (steepness= 3), highly structured rock with many caves and crevices (structure= 3), high diversity and quality of vegetation (veg=3). Overall habitat grade was 4.5/5. Photo: Jessica Koleck

Appendix D: Table of Faecal Pellet Sub-sampling

Table D.1. Number of samples of faecal pellets collected from each site that met the criteria for DNA extraction, number of samples randomly selected for DNA extraction and sequencing and the number of samples that were successfully sequenced. Kalu=Kalumburu

Area	Site	Camera	Met Criteria	Selected	Sequenced
Dambi	A	YES	36	10	10
Dambi	B	YES	39	10	10
Dambi	C	YES	14	5	5
Dambi	D	YES	23	10	10
Derby	A	YES	2	2	2
Derby	B	YES	3	3	2
Derby	C	YES	7	5	5
Derby	D	YES	13	5	4
Derby	E	YES	2	2	1
Derby	F	YES	13	5	5
Derby	G	YES	26	10	10
Kalu	A	YES	11	5	5
Kalu	B	YES	3	3	2
Kalu	C	YES	27	10	9
Kalu	D	NO	4	0	0
Kalu	E	NO	1	0	0
Kalu	F	YES	11	5	3
Kalu	G	YES	12	5	4
Kalu	H	NO	2	0	0
Kalu	I	YES	2	2	2

Kalu	J	NO	0	0	0
Kalu	K	YES	29	10	7
Kalu	L	YES	12	5	5
Kalu	M	YES	32	10	10
Kalu	N	YES	19	5	5
Kalu	O	YES	missing		
Kalu	P	NO	0	0	0
Kalu	Q	YES	28	10	7
Kalu	R	NO	9	1	1
Kalu	S	YES	3	3	3
TOTAL			383	141	126

Appendix E: Table of Faecal Pellets Extracted and Corresponding Species Identifications

Area	Site	Sample	Species (*Petrogale)*
Dambi	A	2	*burbidgei*
Dambi	A	3	*burbidgei*
Dambi	A	5	*burbidgei*
Dambi	A	16	*burbidgei*
Dambi	A	17	*burbidgei*
Dambi	A	18	*burbidgei*
Dambi	A	20	*burbidgei*
Dambi	A	23	*burbidgei*
Dambi	A	34	*burbidgei*
Dambi	A	39	*burbidgei*
Dambi	B	3	*burbidgei*
Dambi	B	4	*burbidgei*
Dambi	B	6	*burbidgei*
Dambi	B	7	*burbidgei*
Dambi	B	19	*burbidgei*
Dambi	B	22	*burbidgei*
Dambi	B	32	*burbidgei*
Dambi	B	33	*burbidgei*
Dambi	B	34	*burbidgei*
Dambi	B	40	*burbidgei*
Dambi	C	2	*burbidgei*
Dambi	C	5	*burbidgei*
Dambi	C	8	*burbidgei*
Dambi	C	9	*burbidgei*
Dambi	C	10	*burbidgei*
Dambi	D	2	*burbidgei*
Dambi	D	5	*burbidgei*
Dambi	D	6	*burbidgei*
Dambi	D	8	*burbidgei*
Dambi	D	13	*burbidgei*
Dambi	D	17	*burbidgei*
Dambi	D	26	*burbidgei*
Dambi	D	29	*burbidgei*
Dambi	D	30	*burbidgei*
Dambi	D	31	*burbidgei*
Derby	A	5	*brachyotis*
Derby	A	7	*brachyotis*
Derby	B	1	*brachyotis*

Area	Site	Sample	Species
Derby	B	10	*brachyotis*
Derby	C	5	*brachyotis*
Derby	C	6	*brachyotis*
Derby	C	8	*brachyotis*
Derby	C	11	*brachyotis*
Derby	D	3	*burbidgei*
Derby	D	9	*burbidgei*
Derby	D	10	*burbidgei*
Derby	D	18	*burbidgei*
Derby	E	6	*brachyotis*
Derby	F	1	*concinna*
Derby	F	2	*concinna*
Derby	F	6	*concinna*
Derby	F	15	*concinna*
Derby	F	16	*concinna*
Derby	G	1	*burbidgei*
Derby	G	4	*burbidgei*
Derby	G	7	*burbidgei*
Derby	G	12	*burbidgei*
Derby	G	13	*burbidgei*
Derby	G	15	*burbidgei*
Derby	G	17	*burbidgei*
Derby	G	19	*burbidgei*
Derby	G	24	*burbidgei*
Derby	G	30	*burbidgei*
Kalu	A	2	*brachyotis*
Kalu	A	3	*brachyotis*
Kalu	A	6	*brachyotis*
Kalu	A	11	*brachyotis*
Kalu	A	14	*brachyotis*
Kalu	C	3	*brachyotis*
Kalu	C	5	*brachyotis*
Kalu	C	6	*brachyotis*
Kalu	C	11	*brachyotis*
Kalu	C	14	*brachyotis*
Kalu	C	20	*brachyotis*
Kalu	C	22	*brachyotis*
Kalu	C	30	*brachyotis*
Kalu	C	32	*brachyotis*
Kalu	C	35	*brachyotis*
Kalu	C	42	*brachyotis*
Kalu	F	1	*brachyotis*
Kalu	F	4	*brachyotis*
Kalu	F	9	*brachyotis*
Kalu	F	16	*brachyotis*

Area	Site	Sample	Species
Kalu	G	1	*brachyotis*
Kalu	G	9	*brachyotis*
Kalu	G	10	*brachyotis*
Kalu	G	17	*brachyotis*
Kalu	I	1	*brachyotis*
Kalu	I	2	*brachyotis*
Kalu	K	3	*brachyotis*
Kalu	K	4	*brachyotis*
Kalu	K	5	*brachyotis*
Kalu	K	6	*brachyotis*
Kalu	K	26	*brachyotis*
Kalu	K	29	*brachyotis*
Kalu	K	35	*brachyotis*
Kalu	K	42	*brachyotis*
Kalu	L	12	*brachyotis*
Kalu	L	17	*brachyotis*
Kalu	L	18	*brachyotis*
Kalu	L	22	*brachyotis*
Kalu	L	26	*brachyotis*
Kalu	M	6	*brachyotis*
Kalu	M	8	*brachyotis*
Kalu	M	10	*brachyotis*
Kalu	M	11	*brachyotis*
Kalu	M	14	*brachyotis*
Kalu	M	17	*brachyotis*
Kalu	M	21	*brachyotis*
Kalu	M	24	*brachyotis*
Kalu	M	25	*brachyotis*
Kalu	M	28	*brachyotis*
Kalu	M	37	*brachyotis*
Kalu	M	45	*brachyotis*
Kalu	N	3	*brachyotis*
Kalu	N	7	*brachyotis*
Kalu	N	8	*brachyotis*
Kalu	N	10	*brachyotis*
Kalu	N	22	*brachyotis*
Kalu	Q	2	*brachyotis*
Kalu	Q	13	*brachyotis*
Kalu	Q	14	*brachyotis*
Kalu	Q	16	*brachyotis*
Kalu	Q	19	*brachyotis*
Kalu	Q	22	*brachyotis*
Kalu	Q	24	*brachyotis*
Kalu	Q	26	*brachyotis*
Kalu	R	1	*brachyotis*

Kalu	S	1	*brachyotis*
Kalu	S	2	*brachyotis*
Kalu	S	3	*brachyotis*

Appendix G: Sensor Camera Images of Monjon (*Petrogale burbidgei*)

Figure G.1. Site: Dambi B; Date: 29/07/13; Time: 15:42

Figure G.2. Site: Dambi B; Date: 30/07/13; Time: 08:17

Figure G.3. Site: Dambi B; Date: 25/07/13; Time: 22:19

Figure G.4. Site: Dambi B; Date: 30/07/13 Time: 00:37

Figure G.5. Site Dambi A; Date: 11/07/13; Time: 17:42

Figure G.6. Site Dambi A; Date: 11/07/13; Time: 17:49

Figure G.7. Site: Dambi A; Date: 26/07/13; Time: 04:19

Figure G.8. Site: Dambi D; Date: 19/0713; Time: 12:32

Figure G.9. Site: Dambi D; Date: 19/0713; Time: 12:32

Figure G.10. Site: Dambi D; Date: 18/07/13; Time: 12:33

Figure G.11. Site: Dambi D; Date: 28/07/13; Time: 17:48

Figure G.12. Site: Derby D; Date: 07/07/13; Time: 17:09

Appendix H: Sensor Camera Images of Nabarlek (*Petrogale concinna*)

Figure H.1. Site: Derby F; Date: 01/07/13; Time: 03:01

Figure H.2. Site: Derby F; Date: 25/07/13; Time: 04:15

Appendix I: Examples of Other Species Detected by the Sensor Cameras

Figure I.1. Wild Dog (*Canis lupus*)
Site: Kalumburu I; Date: 18/06/13; Time: 23:23

Figure I.2. Wild Dog (*Canis lupus*)
Site: Kalumburu M; Date: 17/07/13; Time: 10:32

Figure I.3. Feral Cat (*Felis catus*)
Site: Dambi D; Date: 20/07/13; Time: 20:14

Figure I.4. Feral Cat (*Felis catus*) (same animal)
Site: Dambi D; Date: 20/07/13; Time: 20:15

Figure I.5. Golden-backed Tree Rat (*Mesembriomys macurus*)
Site: Derby B; Date: 22/06/13; Time: 21:47

Figure I.6. Golden-backed Tree Rat (*Mesembriomys macurus*)
Site: Dambi B; Date: 20/7/13; Time: 01:27

Figure I.7. Delicate Mouse (*Pseudomys delicatulus*)
Site: Dambi D; Date:15/07/13 Time: 04:19

Figure I.8. Kimberley Rock Rat (*Zyzomys woodwardi*)
Site: Dambi D; Date: 16/07/13; Time: 03:38

Figure 0.9. Common Rock Rat (*Zyzomys argurus*) or Central Pebble-mound Mouse (*Pseudomys johnsoni*)

Site: Derby A; Date: 27/07/13; Time: 01:38

Figure 0.10. Rock Rat (*Zyzomys* sp.)
Site: Kalumburu A; Date: 14/06/13; Time: 04:40

Figure I.11. Short-beaked Echidna (*Tachyglossus aculeatus*)
Site: Kalumburu M; Date: 18/07/13; Time: 01:45

Figure 0.12. Northern Quoll (*Dasyurus hallucatus*)
Site: Derby F; Date: 19/07/13; Time: 06:48

Figure 0.13. Euro (*Macropus robustus*)
Site: Kalumburu M; Date: 26/07/13; Time: 07:05

Figure 0.14. Scaly-tailed Possum (*Wyulda squamicaudata*) Adult & Juvenile
Site: Dambi B; Date: 20/07/13; Time: 03:29

Figure I.15. Scaly-tailed Possum (*Wyulda squamicaudata*)
Site: Derby F; Date: 02/06/13; Time: 03:02

Figure 0.16. Rock Ringtail Possum (*Petropseudes dahli*)
Site: Kalumburu I; Date: 15/06/13; Time: 20:14

Figure 0.17. Rock Ringtail Possum (*Petropseudes dahli*) Adult & Juvenile
Site: Kalumburu G; Date: 19/06/13; Time: 14:48

Figure 0.18. Short-eared Rock-wallaby (*Petrogale brachyotis*)
Site: Derby C; Date: 12/07/13; Time: 06:51

Figure I.19. Short-eared Rock-wallaby (*Petrogale brachyotis*)
Site: Derby A; Date: 31/05/13; Time: 07:33

Figure 0.20. Pied Butcherbird (*Cracticus nigrogularis*)
Site: Derby A; Date: 12/06/13; Time: 07:15

Figure I.21. White Quilled Rock Pigeon (*Petrophassa albipennis*)
Site: Dambi B; Date: 25/7/13; Time: 07:35

Figure 0.22. Sandstone Shrike-thrush (*Colluricincla woodwardi*)
Site: Derby E; Date: 31/05/13; Time: 16:36

Figure I.23. Great Bowerbird (*Ptilonorhyncus nuchalis*)
Site: Kalumburu I; Date: 28/06/13; Time: 06:39

Figure 0.24. Variegated Fairy-wren (*Malurus lamberti,* race *rogersi*) Female
Site: Dambi D; Date: 05/07/13; Time: 11:22

Figure 0.25. Variegated Fairy-wren (*Malurus lamberti,* race *rogersi*) Male
Site: Dambi D; Date: 05/07/13; Time: 11:22

Figure I.26. Singing Honeyeater (*Lichenostomus virescens*)
Site: Kalumburu I; Date: 17/06/13; Time: 15:20

Figure 0.27. Torresian crow (*Corvus orru*)
Site: Kalumburu C; Date: 12/06/13; Time: 10:14

Figure 0.28. Southern Boobok Owl (*Ninox novaeseelandiae*)
Site: Kalumburu Q; Date: 20/06/13; Time: 17:46

Figure 0.29. Willy Wagtail (*Rhipidura leucophrys*)
Site: Kalumburu G; Date: 19/06/13; Time: 14:48

Figure I.30. Bar-shouldered Dove (*Geopelia humeralis*)
Site: Derby B; Date: 11/06/13; Time: 16:44

Figure 0.31. Magnificent Tree Frog (*Littoria splendida*)
Site: Derby F; Date: 03/06/13 Time: 17:57

Figure 0.32. Magnificent Tree Frog (*Littoria splendida*)
Site: Derby F; Date: 21/06/13 Time: 18:16

Figure 0.33. ***Gehyra xenopus*** **or** ***Oedura gracilis***
Site: Kalumburu A; Date: 16/06/13; Time: 04:38

Figure 0.34. Kimberley Rock-monitor (*Varanus glauerti*)
Site: Kalumburu S; Date: 25/06/13; Time: 12:07

Figure 0.35. Children's Python (*Antaresia childreni*)
Site: Kalumburu B; Date: 17/06/13; Time: 01:14

Figure I.36. Black-palmed Rock Monitor (*Varanus glebopalma*)
Site: Derby A; Date: 14/06/13; Time: 09:12

Figure I.37. Fire

Site: Kalumburu M; Date: 30/07/13; Time: 14:23