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Department of **Biodiversity,**
Conservation and Attractions

An assessment of ngwayir (western ringtail possum) in the Perup Sanctuary, to inform management and potential future translocations: Final report.

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June 2023

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This document is available in alternative formats on request.

Cover image: Adult female and subadult ngwayir (western ringtail possum, *Pseudocheirus occidentalis*) in the Perup Sanctuary, Western Australia.

Contents

Acknowledgments

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Summary

The ngwayir (western ringtail possum, *Pseudocheirus occidentalis*) is currently listed as *Critically Endangered.* This report broadly informs the conservation and management of ngwayir with a particular focus on the translocated population in the Perup Sanctuary and wild population within the southern forest region, Western Australia. This principally involved a regionally contextualised demographic and genetic assessment of the ngwayir within the Perup Sanctuary.

A survey to determine the current spatial distribution and abundance of ngwayir (western ringtail possum, *Pseudocheirus occidentalis*) within the Perup Sanctuary was conducted in November 2022. Pedestrian-based spotlight surveys along 24 transects totalling 39.6 km resulted in a total of 126 ngwayir individuals and 217 koomal (common brushtail possum, *Trichosurus vulpecula*) being detected. Distance sampling model estimates of the population size were 156 ngwayir individuals (95% Confidence Interval 127 – 192) and 350 koomal individuals (95% CI 272 – 452) across 423 ha. Twenty-eight individual ngwayir were hand-captured to take tissue samples for genetic analysis and record demographic and biometric information.

Predation and competition are likely to be key factors in the distribution and abundance of ngwayir and their habitat use in the sanctuary. The lack of terrestrial predators (feral cats, foxes and chuditch) is likely to be the main reason why ngwayir densities within the sanctuary are higher than the immediate surrounds. Competition from the more dominant and abundant koomal may explain why ngwayir densities were not greater in the more mesic/productive parts of the Sanctuary and why ngwayir densities in the Sanctuary overall are less than contemporary peak densities observed elsewhere in the Upper Warren. It is also possible that the ngwayir population is at or close to carrying capacity given the relatively modest minimum growth rates required to reach the estimated 156 ngwayir (95%CI: 126-192) in 2022, however adequate ongoing monitoring is necessary to confirm whether this is the case.

To understand the genetic diversity and relatedness of the ngwayir population within the Perup Sanctuary in 2022, new and existing genome-wide SNP data was collected from the Perup Sanctuary founder cohorts (Busselton Hospital, Upper Warren, and Upper Donnelly), and samples from across the Southern Forest. The Perup Sanctuary population had observed heterozygosity (0.06), expected heterozygosity (0.07) and inbreeding coefficient (0.08) values that were intermediate between other subpopulations. Effective population size was relatively low (13.4, 95% CI 13.3-13.5). Genetic distance and ordination analyses showed that the Perup Sanctuary animals are admixed and represent all founder subpopulations. Further admixture analysis showed that genomic contribution from the three founder populations is 47% from Busselton Hospital, 44% from Upper Warren and 9% from Upper Donnelly. A genomic relatedness matrix network suggested that the maximum number of successful breeders in the founders was 10: five from Upper Warren, four from Busselton Hospital and one from Upper Donnelly. Considering there were genetic data for 34/38 founder animals, this suggests at least around 30% of the translocated ngwayir successfully reproduced. Overall, genetic analysis has shown the Perup Sanctuary to be relatively genetically diverse. However, we recommend

ongoing genetic and inbreeding monitoring. Supplementation with unrelated Upper Donnelly animals and geographically distant Upper Warren animals could be done to increase the effective population size, after appropriate population viability modelling.

Key recommendations for the management of the ngwayir population in Perup Sanctuary relate to regulating predation and competition to optimise the size of the ngwayir population, genetic management to optimise the quality and value of the ngwayir population, maintaining an adequate monitoring and surveillance program to have timely and sufficient information on spatiotemporal changes in the ngwayir population and their habitat across the southern forest region and within the Perup Sanctuary, improving the understanding of the risks to these populations (e.g. the current gumleaf skeletonizer outbreak, fire, predators, climate change and other outbreaks and tree decline), and seeking to improve the effectiveness of management and conservation activities (e.g., introduced predator control, fire management, dieback hygiene) to deliver better conservation outcomes for this and other threatened species.

1 Introduction

This report is intended to broadly inform the conservation and management of ngwayir (western ringtail possum, *Pseudocheirus occidentalis*) with a particular focus on the wild population within the Upper Warren region and to assess the merits and feasibility of improving the value of the ngwayir population in the Perup Sanctuary. This includes having a better understanding of,

- the status of the wild ngwayir population within the Upper Warren, which has undergone >95% decline in the last 20 years (e.g., what are the conservation values of the population, what are the risks to the population, how great is the need for an insurance population, etc),
- what is the status and characteristics of the ngwayir population within the Perup Sanctuary (e.g., what is the size of the population, is it at or close to carrying capacity, what is the genetic diversity, representativeness, and relatedness to other ngwayir populations)?
- whether genetic augmentation / supplementation is needed to improve the value of the ngwayir population within the Perup Sanctuary, and if so, how is this best done? For example, provide an indication of appropriate potential source sites and help determine safe harvest numbers for a possible translocation for the purposes of genetic augmentation from the wild to the Perup Sanctuary.

The specific purpose of the project being reported here was to conduct a demographic and genetic assessment of the ngwayir within the Perup Sanctuary.

Project questions were;

- What is the current size and distribution of ngwayir population within the Perup sanctuary?
- What has been the survivorship and relative genetic contribution of the ngwayir individuals and cohorts translocated into the sanctuary?
- What is the health, condition, and genetic diversity of ngwayir within the Perup Sanctuary?
- To assess the potential for interspecific competition, how does the ngwayir population compare to the koomal population with respect to size, distribution and habitat use?
- For context, what available data and information exists for the ngwayir and koomal populations within the Perup Sanctuary?

The aims of the genetic investigation component of this study have two main components,

1) Determine the genetic characteristics of ngwayir within the Perup Sanctuary. Specifically, this includes;

- Population characterization: quantify general genetic metrics, including observed (H_O) and expected (H_E) heterozygosity, inbreeding coefficient (F_{IS}), mean number of alleles per marker (N_a), estimate of effective population size.
- Founder contribution assessment: compare current genetic characteristics to the genetics of the founders to determine the relative contribution by individuals and cohorts (Busselton versus Upper Warren and Upper Donnelly founders), i.e., to what extent do the different founder cohorts contribute to the current genetics in the Perup Sanctuary? And how many individuals have contributed to the current gene pool (e.g., what % of Busselton founders contributed to the current gene pool?)?
- Ngwayir population assessment: relate the genetic characteristics of the Perup Sanctuary population to the source populations (Busselton, Upper Warren, and Upper Donnelly) and the species as a whole. i.e., how closely related (F_{ST}) and how representative is the Perup Sanctuary population of the genetic diversity of the Busselton, Upper Warren and Upper Donnelly populations and the species as a whole?

2) To contextualise the genetic characteristics of the Perup Sanctuary, describe the genetic characteristics of the broader ngwayir population in the Warren (Southern Forests) Region. Specifically, this includes;

- Population characterization: quantify general genetic metrics, including observed (H_O) and expected (H_E) heterozygosity, inbreeding coefficient (F_{IS}), mean number of alleles per marker (N_a), estimate of effective population size
- Determine how the individuals sampled in this study relate to the genetics and genetic structure of the species as characterized in White et al. (2021).

This work is part of a collaborative project (2021 – 2023) involving the Department of Biodiversity, Conservation and Attractions, under contract with South West NRM (formerly South West Catchments Council, SWCC) with funds from the Commonwealth's National Landcare Program (NLP2).

2 Background

2.1 The ngwayir

The ngwayir (pronounced n-wa-ear; or western ringtail possum, *Pseudocheirus occidentalis*) is one of 21 mammals identified as a priority species in the Australian Government's Threatened Species Action Plan 2022-2032 (DCCEEW 2022). Endemic to southwestern Australia, the ngwayir has substantially declined in numbers and range since European settlement (1826) and is currently listed as 'Critically Endangered' under the Western Australian *Biodiversity Conservation Act of 2016* and the Australian *Environment Protection and Biodiversity Act of 1999.*

The current threatened status is due in large part to the rapid and substantial decline of the species in the Upper Warren region (an area roughly between Lake Muir and the towns of Manjimup, Bridgetown and Boyup Brook, in southwestern Australia; Wayne et al. 2012, 2015, 2017; Woinarski et al. 2014). Of the eight genetic clusters identified for the species, the Upper Warren cluster has the greatest genetic diversity (heterozygosity and mean number of alleles per marker; White et al. 2021). The key threats to this folivorous, arboreal, and nocturnal marsupial include habitat loss and fragmentation as a direct result of human development, introduced predators, climate change, timber harvesting and fire (Department of Parks and Wildlife 2017).

2.2 The Upper Warren

The Upper Warren region is one of Australia's most important areas for the conservation of threatened native mammals, supporting some of the largest and genetically most diverse remnant populations of species, several of which previously had ranges across much of southern Australia (e.g., the Critically Endangered woylie (*Bettongia penicillata*), the Endangered numbat (*Myrmecobius fasciatus*), and Vulnerable chuditch (*Dasyurus geoffroii*)).

2.3 The ngwayir population in the Upper Warren

- The ngwayir is one of at least seven species of native mammal to have undergone rapid and catastrophic declines (80-100%) since the mid-1990s in the Upper Warren region of southwestern Australia (Wayne et al. 2017).
- The best estimate for the size of the ngwayir population is 7,103 (6,052 – 8,335 95%CI) in 2022, within an area of 38,349 ha containing the current 'hotspot' with the highest ngwayir densities and constituting the vast majority of the extant population within the Upper Warren region (Wayne et al. 2022b).
- The Upper Warren region supports the largest remaining ngwayir population in the jarrah forest and a substantial portion of the extant population and its genetic diversity (White et al. 2021).
- The ngwayir population in the Upper Warren remains very important to the conservation and recovery of the species given its size and genetics. Despite having undergone substantial declines (>99% in some areas) since 1998

(Wayne et al. 2005, 2012 and 2017), the Upper Warren population remains large relative to other extant populations on the west coast and south coast of Western Australia, which combined, total more than 20,000 individuals (Teale and Potts 2020).

- Introduced predators, particularly the cat (*Felis catus*) are considered the most likely common factor in the recent declines of mammals, including ngwayir, in the Upper Warren (Wayne et al 2017). Ngwayir abundance has also been positively associated with fox control (Wayne et al. 2006).
- Fire is also an important factor affecting ngwayir abundance in the Upper Warren (Wayne 2006, Wayne et al. 2006, 2022b). Fire intensity has been repeatedly demonstrated to be a negative factor in the abundance of ngwayir, the effects of which can be significant for at least 20 years (Wayne et al. 2006, 2022b).
- Forest fragmentation and timber harvesting are also negatively associated with ngwayir abundance (Wayne et al. 2000, 2006).

2.4 The ngwayir population within the Perup Sanctuary

- Ngwayir were frequently detected in the 1990s and 2000s during ad hoc and informal surveys in and around the Perup Sanctuary area prior to its construction in 2010. In the early 2000s ngwayir were abundant around the Perup Nature's Guesthouse accommodation (adjacent to the Perup Sanctuary site) but began to decline in the mid-late 2000s to undetectable levels (Julia Wayne pers. obs.).
- Perup Sanctuary was originally established in late 2010 to support an insurance population of the critically endangered woylie (Wayne et al. 2013; Yeatman and Wayne 2015; Harrison et al. in review).
- It is thought that ngwayir were either absent or at very low (undetectable) numbers when the Perup Sanctuary was established, given that surveys immediately prior to and after the establishment of the Perup Sanctuary did not detect ngwayir.
- Twenty (20) ngwayir individuals (7 male,13 female, plus 10 pouch young) from the Busselton hospital development site (<5 ha) were translocated 130 km to the Perup Sanctuary in August-September 2012 (Figure 1; Appendix 1a). A subset of five males and six females, were collared with mortality-sensitive radio transmitters (equipped with a VHF core and brass band antenna, made by Sirtrack Ltd.) to monitor their survival and movements within the sanctuary (Figure 2). Of the 11 individuals collared, eight of the western ringtail possums from the Busselton cohort died (August 2012 – January 2013). The three remaining radio collars lost signal in April 2013, at the end of the monitoring period, and after failed attempts to recover the collars. However, there were a few confirmed records of radio-collared and uncollared but tagged ngwayir over the subsequent nine months (up to January 2014).
  -One of the three remaining collared animals were sighted on 3rd December

  2013 during spotlighting (capture was attempted but unsuccessful).
  -Through remote sensor cameras, a collared animal was detected on 29th June 2013 and another on 24th January 2014 (approximately 900m apart) and an uncollared but tagged mother and young (sanctuary-born) were detected on 19 July 2013.
- Eighteen (18) ngwayir individuals from across the Upper Warren and Upper Donnelly (within 45 km of the Perup Sanctuary) were translocated to the Perup Sanctuary between 21st March 2016 and 26th April 2017 (Figure 1, Appendix 1b). These were sourced from sites where animals were most readily seen (i.e., considered local hotspots), based on local knowledge at the time. Four males and three females were radio-collared to monitor their survival and movements within the sanctuary (Figure 2). Of the seven radio-collared individuals from the Upper Warren-Upper Donnelly cohorts, one died, one removed its collar (left in a tree hollow) and a third collar was unable to be retrieved from a tree hollow where it had gone into mortality mode. The remaining four collars were removed from the animals (alive) between 370 and 551 days after their initial release.
- The Busselton and the Upper Warren-Upper Donnelly cohorts were released at different points within the Perup Sanctuary but within close proximity of each other and all within the vicinity of the creek line north of where it is intersected by Alf Road. Radio- tracking was conducted both during the day and at night to estimate the day and night refuges.
- Survivorship overview: Of the 11 ngwayir collared from the Busselton cohort, 8 (73%) died on average 67 days (SD=50.8, range 0-120 days, n=8) post-translocation. Three died within the first two weeks and five died 91 – 120 days (23rd November 2012 - 7th January 2013) after release. The one known fatality of a radio-collared animal from the Upper Warren – Upper Donnelly (out of 7 radio-collared animals) was confirmed dead 153 days after release (September 2016) (Appendix 1). In summary, the maximum number of animals that may have survived the first few months of the translocation was 12/20 (60%) from Busselton, 10/11 (91%) from Upper Donnelly and 7/7 from Upper Warren.
- Habitat use by radio-collared ngwayir was significantly different to what was expected based on percentage area of each vegetation complex within the Perup Sanctuary for both the Busselton cohort (Chi square $X^2 = 283.02$, 5 degrees of freedom, $p<0.0001$), and the Upper Warren-Upper Donnelly cohort (Chi square $X^2 = 73.66$, 5 degrees of freedom, $p<0.0001$). In particular, Busselton ngwayir in 2012 were found in Yerraminnup Flat (YEf) on 46% of observations (n=204), which is substantially more than expected (11% of the Perup Sanctuary). Upper Warren-Upper Donnelly ngwayir in 2016-2017 were found in Bevan 2 (BE2) vegetation complex 48% (n=169) of the time, which is substantially more than expected (25% of the Perup Sanctuary).

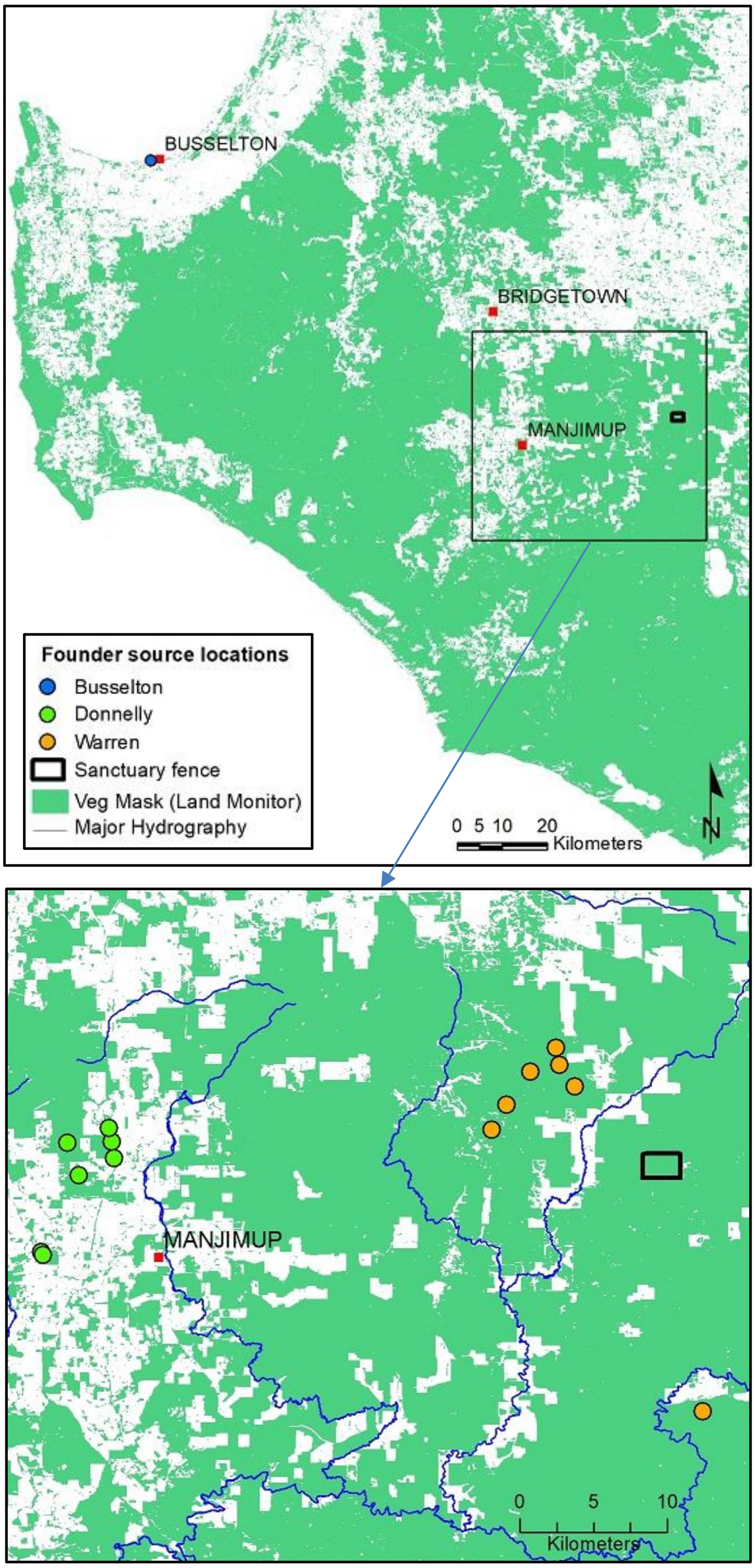

Figure 1. *Map of the location of founders sourced from Busselton (2012) and across the Upper Warren (orange dots) and Upper Donnelly (green dots)(2016-2017), in relation to Perup Sanctuary (black outlined rectangle).*

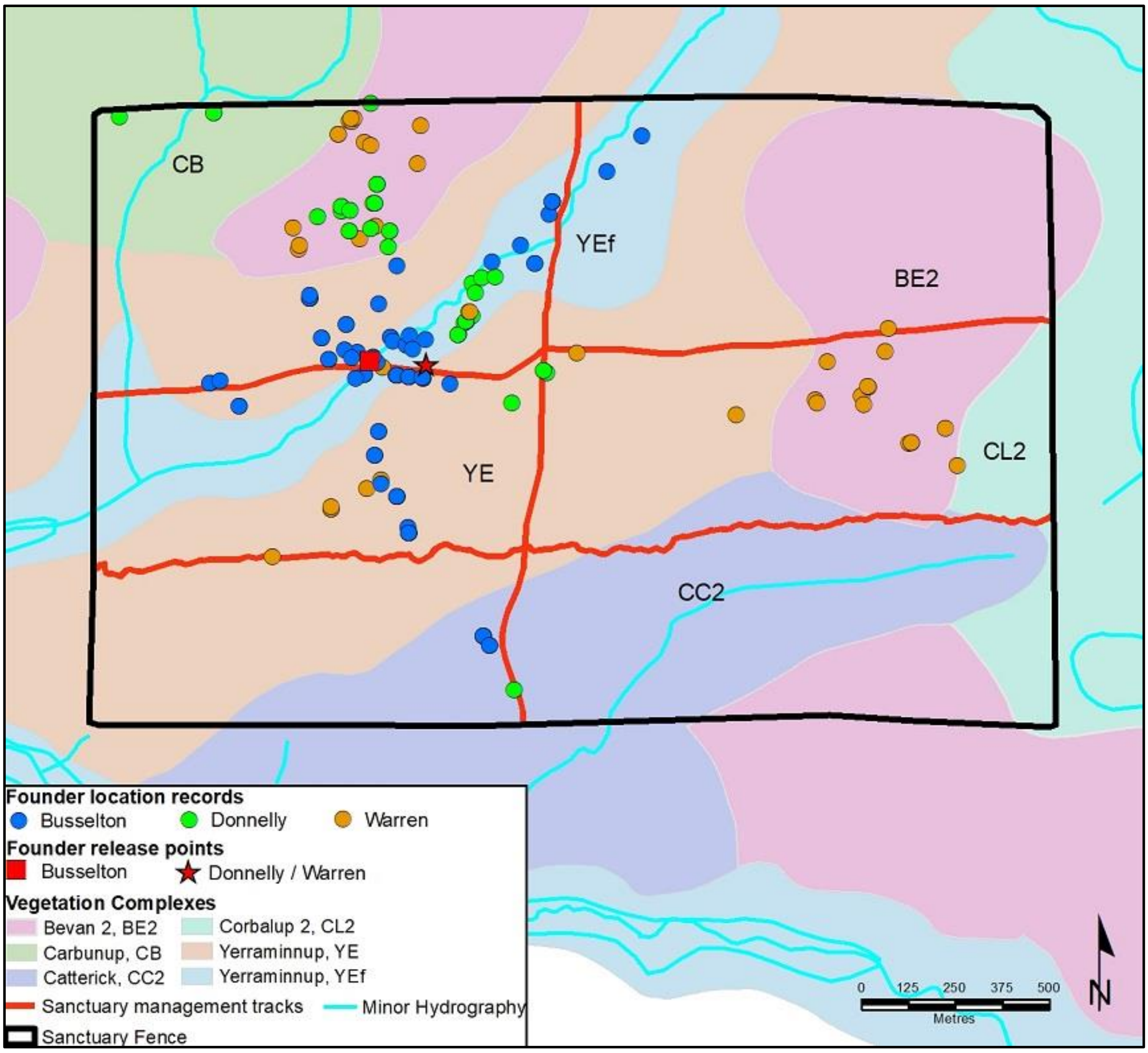

***Figure 2.** Map of the location records in the Perup Sanctuary of radio-collared founder individuals from Busselton, Upper Warren, and Upper Donnelly. Release points are also represented in red.*

2.5 Overview of past possum survey and monitoring in Perup Sanctuary

2.5.1 Science surveys

Vehicle based spotlight surveys were conducted (2010 – 2013 & 2015) using similar methodology as the Kingston spotlight program (BCS Science Project Plan 2012-038; Wayne et al. 2005): 10.9 km transect (Figure 3), three repeat surveys per Spring or Autumn season (four repeat surveys in Autumn 2015), involving two observers located on a purpose-built seated observation platform mounted to the roof rack of a four-wheel drive vehicle, using handheld spotlights (100 watt white light, Lightforce), travelling approximately 5 km per hour. No ngwayir were detected prior to the translocation of animals from Busselton in 2012 (Table 1). Seven detections of at least five individuals were recorded three months after their release, but no ngwayir were detected a further 3-4 months later, in autumn 2013 (Table 1). Two ngwayir were also detected in Autumn 2015 (assumed to be of Busselton pedigree), prior to the translocation in 2016-2017 of ngwayir sourced from the Upper Warren-Upper Donnelly.

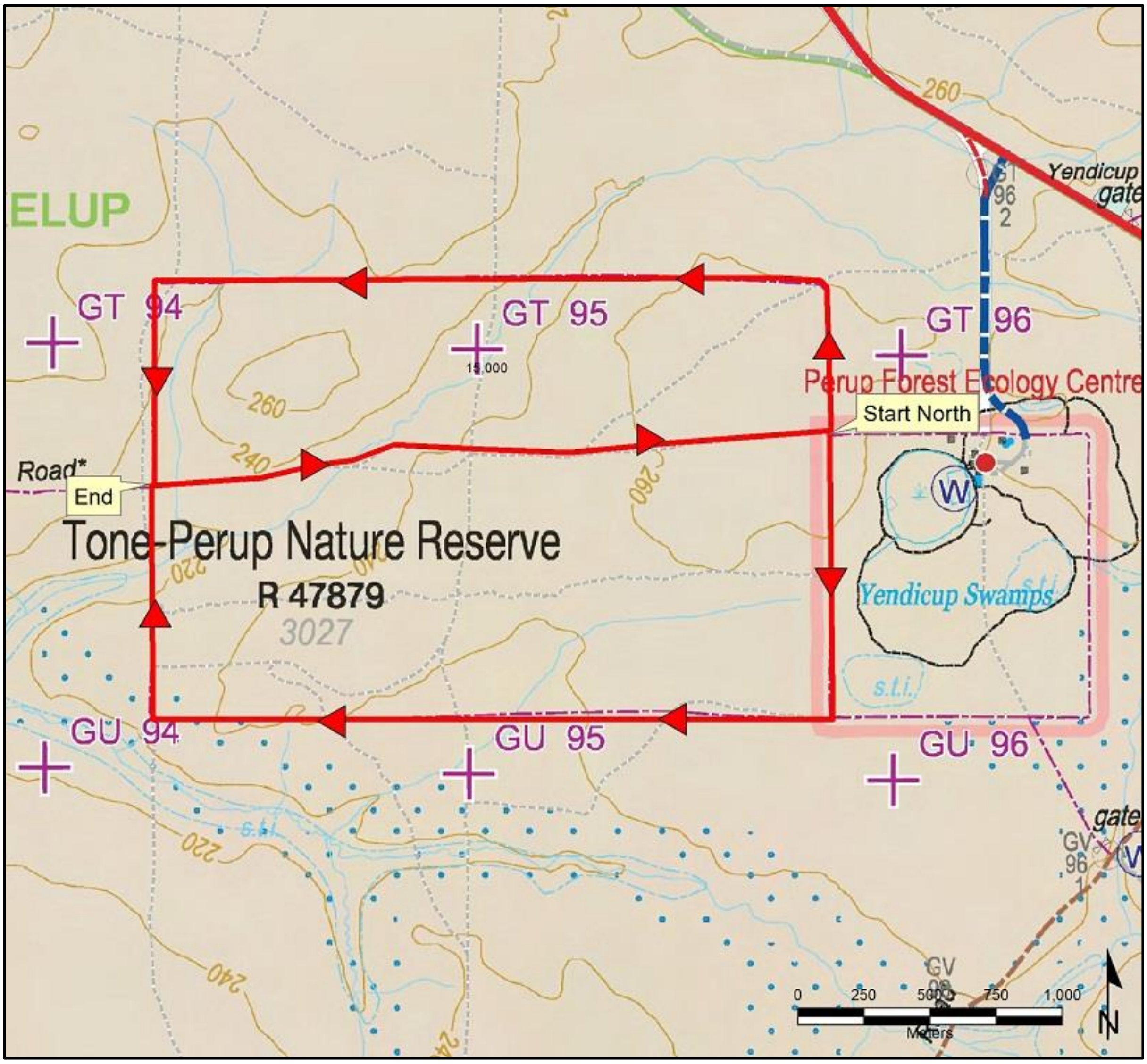

Figure 3. *Location of the vehicle-based spotlight survey transect in the Perup Sanctuary (2010 – 2013 and 2015).*

Table 1. *Summary of ngwayir and koomal detections during the vehicle-based spotlight surveys in the Perup Sanctuary (2010-2015).*

Date	Ngwayir	Koomal
1/10/2010	0	14
2/10/2010	0	8
3/10/2010	0	13
28/03/2011	0	6
18/04/2011	0	8
20/04/2011	0	13
21/11/2011	0	5
23/11/2011	0	11
29/11/2011	0	7
7/03/2012	0	5
28/03/2012	0	5
10/04/2012	0	6
3/12/2012	1	9
5/12/2012	1	2
7/12/2012	5	8
27/03/2013	0	7
3/04/2013	0	11
4/04/2013	0	10
24/3/2015	0	26
25/3/2015	0	12
26/3/2015	0	16
28/4/2015	2	13
Total	**9**	**215**

2.5.2 Barrett surveys (Honours project)

Pedestrian-based spotlight surveys by two observers over four nights in Autumn 2015 focused on creek line areas within the sanctuary to optimise the likelihood of encountering possums. Surveys began approximately 30-45 minutes after sunset and lasted approximately four hours (limited by the battery life of spotlights). Observers used the same handheld spotlights used during the vehicle-based surveys (Lightforce with 100-watt white light, connected to a 12-volt motorcycle battery carried in a backpack) and walked approximately 50 m apart in parallel lines to maximise the chance of detection (Barrett, 2016). Only two ngwayir were detected during these surveys (Table 2).

Table 2. *Count of ngwayir and koomal recorded in Perup Sanctuary from pedestrian -based spotlight surveys (April 2015). Source: Barrett (2016).*

Date	Ngwayir	Koomal
31/03/2015	2	8
1/04/2015	0	5
2/04/2015	0	6
3/04/2015	0	4
Total	**2**	**23**

Density estimates of koomal in the Perup Sanctuary in March-April 2015 varied between 0.37 ha^{-1} (0.23 – 0.61 95% CI; i.e., 156 (97 – 258 individuals)) based on distance sampling modelling using vehicle-based spotlight data and 1.15 ha^{-1} (0.76 – 1.74 95% CI; i.e. 486 (321 – 736 individuals)) using Spatial Explicit Capture Recapture modelling using cage trap data (Barrett 2016).

2.5.3 District surveys

Spotlighting:

Ngwayir detected during nocturnal activities (e.g., radiotelemetry and non-systematic spotlighting) conducted by Donnelly District also confirmed that some individuals were present in the Perup Sanctuary at least one year after the Busselton animals were released in August – September 2012. There were more records of ngwayir in the year after the release of ngwayir sourced across the Upper Warren and Upper Donnelly areas (March- 2016 – April 2017) (Table 3).

Table 3. *Summary of ngwayir opportunistically detected during nocturnal activities conducted by the Donnelly District in the Perup Sanctuary. Note: survey effort and method are not consistent between evenings. Grey shaded rows indicate observations prior to 18 ngwayir individuals from across the Upper Warren and Upper Donnelly were translocated to the Perup Sanctuary between March 2016 and April 2017.*

Date	No. tagged Adult	No. untagged Adult	No. untagged Subadult
3/12/2013	3	1	2
4/12/2013	0	1	1
30/04/2014	0	0	0
1/05/2014	0	0	0
21/11/2016	0	1	0
24/11/2016	0	6	0
3/04/2017	0	3	1
9/05/2017	0	2	0
5/09/2017	0	1	0
7/09/2017	0	2	0
30/10/2017	1	4	1
31/10/2017	0	3	2
15/11/2018	0	2	0

Remote Sensor Camera trapping:

Routine remote sensor camera trapping has been conducted within the Perup Sanctuary for the main purpose of surveillance for potential introduced predator incursion. A total of 14 cameras in single or paired arrangements have been used at 8locations around the internal perimeter of the Perup Sanctuary (Figure 4), generally focussing along the internal fence line. At each corner of the fence, paired cameras are placed with one directed along each of the boundaries. At each end of the central north-south track, paired cameras are placed with one directed down the internal track and the other directed along the boundary track. There are cameras located at each end of the central east-west track directed toward the access gates. This data crudely indicates that ngwayir from Busselton persisted at low numbers, but numbers have tended to increase since the release of ngwayir from across the Upper Warren and Upper Donnelly in 2016-2017 (Table 4). Caution is needed in interpreting this data any further given that:
1) the survey effort varied to some extent over time with some reduction in recent years (not quantified here),
2) the frequency of camera checks decreased over time (2013-June 2015 was twice weekly, July 2015 -December 2017 was weekly and January 2018 to current was fortnightly), and,
3) the data only quantifies the number of cameras that detected at least one ngwayir within a given monitoring period.

A 300m x 300m grid of 54 remote sensor cameras were deployed (June – September 2013) in the Perup Sanctuary for the purposes of monitoring woylies. There were three incidental detections of ngwayir during this time: a collared animal on 29th June 2013, an uncollared but tagged mother and young (sanctuary-born) on 19th July 2013, and an uncollared individual (not sure if tagged) on 21st August 2013.

Cage trapping koomal:

Koomal have been recorded during cage trapping designed for monitoring woylies since 2011 (Harrison et al. *in press*). This data is however insufficient to derive reasonable estimates of the abundance or density of koomal within the Perup Sanctuary due to low capture and recapture rates. Although koomal are normally readily caught in live cage traps, reduced trap availability (i.e., trap saturation) by woylies in the Perup Sanctuary has likely significantly limited the capture of koomal. The greatest number of individuals caught during these trapping sessions was 54 (plus 9 recaptures; April 2011).

A total of 147 koomal have been removed from the Perup Sanctuary and translocated elsewhere. This includes 43 individuals in September-October 2010, 38 in 2016, 20 in 2017, 41 in 2018 and 5 in March 2022.

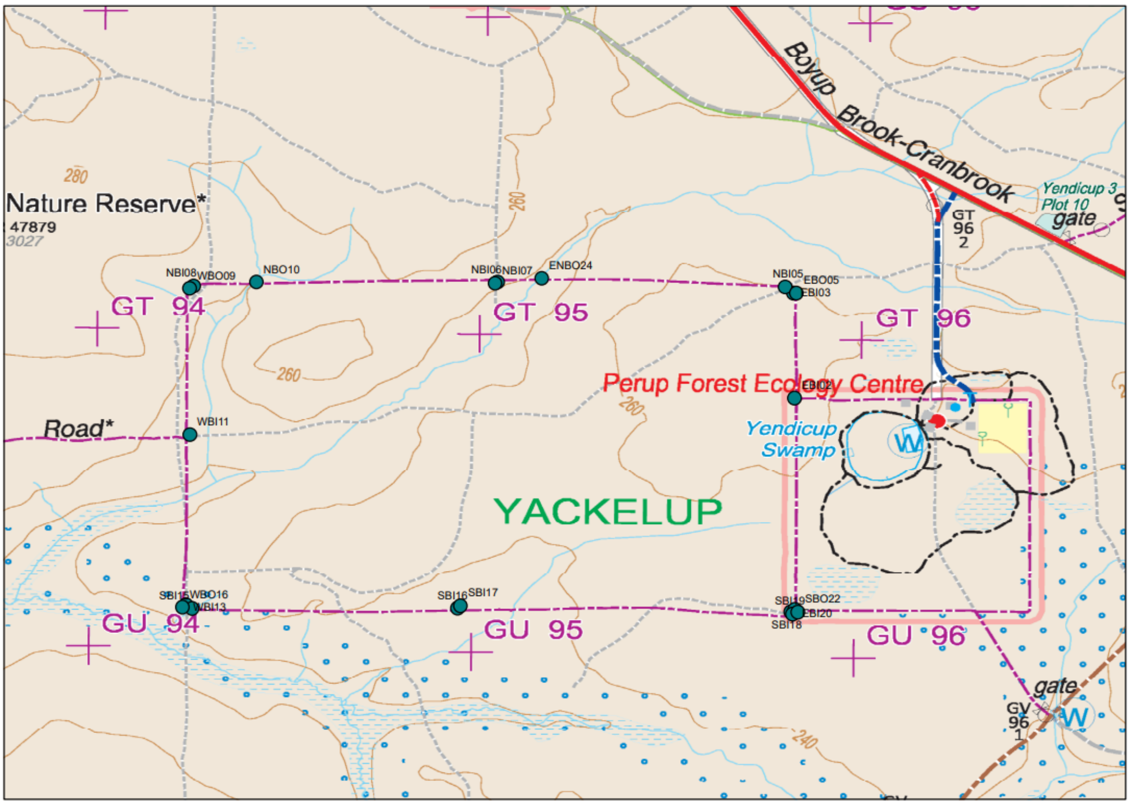

Figure 4. *Map showing the location of regular remote sensor camera monitoring points. Note this map also shows some camera locations external to the fence (data not provided here).*

Table 4. *Summary of ngwayir records during predator incursion monitoring conducted by the Donnelly District using remote sensor camera traps in the Perup Sanctuary.*

Note: detection rate is the sum of the number of monitoring locations (14) that detected at least one ngwayir within a given camera check period and summed over a calendar year.
** = 1 individual was detected with radio-collar and ear tags (i.e., Busselton founder).*

Year	Detection rate
2013	6
2014	2*
2015	1
2016	17
2017	7
2018	20
2019	14
2020	20
2021	25
2022	42

3 Methods

3.1 Study area

The Perup Sanctuary is located 40 km east of Manjimup in the Tone-Perup Nature Reserve, in the Upper Warren region, southwestern Australia. Adjacent to the Perup Nature's Guesthouse accommodation and education facility, the Perup Sanctuary was established in 2010 and has an introduced predator exclosure fence encompassing 423 ha (Wayne et al. 2013). Part of the Southern Jarrah Forest IBRA subregion (JAF02), the forests and woodlands of the area are dominated by jarrah (*Eucalyptus marginata*), marri (*Corymbia calophylla*) and wandoo (*Eucalyptus wandoo*), with some yate (*Eucalyptus decipiens*) and flooded gum (*Eucalyptus rudis*) (Figure 5). Six main vegetation complexes (Mattiske and Havel 1998) have been identified in the Perup Sanctuary: one upland (Bevan 2, BE2), four valley types (Carbunup, CB; Caterick, CC2; and Yerraminnup, YE and YEf) and one type of 'Depressions and Swamps on Uplands' (Corbalup 2, CL2). Elevation range is between 215 and 270 m above sea level. Riparian vegetation within the sanctuary includes thickets of *Melaleuca viminea*. Thickets of heartleaf (*Gastrolobium bilobum*) are also prominent in some areas (Department of Environment and Conservation 2012). The area experiences a Mediterranean-type climate (with warm dry summers and cool wet winters) with a long-term annual average rainfall of around 700 mm.

Foxes (*Vulpes vulpes*), cats (*Felis catus*), emus (*Dromaius novaehollandiae*), and chuditch (*Dasyurus geoffroii*) were removed or confirmed absent from the Perup Sanctuary at the time of establishment. While most were herded out, several (>5) yongka (western grey kangaroo, *Macropus fuliginosus*) and one kwara (western brush wallaby, *Notamacropus irma*) are known to remain within the Perup Sanctuary in 2023. Other non-volant native mammals recorded in the Perup Sanctuary include woylie (*Bettongia penicillata*), ngwayir, koomal, quenda (*Isoodon fusciventor*), tammar wallaby (*Notamacropus eugenii*), numbat (*Myrmecobius fasciatus*), wambenger (*Phascogale tapoatafa wambenger*), dunnart (*Sminthopsis spp.*), mundarda (western pygmy possum, *Cercartetus concinnus*), and mardo (*Antechinus flavipes*). Some removal of yongka, kwara and koomal individuals remains ongoing (Wayne et al. 2013).

Fire management within and around the Perup Sanctuary includes conducting planned fuel reduction burns to reduce the risks of large and intense wildfires (Figure 6). Where ngwayir are present, this is guided by species-specific fire management information (DPAW 2008). The general approach for fire management within the Perup Sanctuary is to subdivide the area into four quadrats demarcated by the main management tracks and to burn three of the cells on successive rotation with a minimum 2–3-year interval between burns and to maintain these within an approximate fire interval of 6 - 12 years. The plan is to exclude fire from the northwest quadrat for the time being, thereby providing a range of fire ages within the Perup Sanctuary while also reducing the risks of wildfire impacts on the infrastructure and wildlife. Fire management outside the Sanctuary is done in accordance with the Department's broader Bushfire Risk Management Framework

(DBCA 2019), and as specified in the Regional Fuel Management plan (DBCA 2020). The aim is to reduce the risk of bushfire to people, communities, infrastructure, the economy, and the natural environment. The tolerable fuel age[1] for dry eucalypt forest in and around the Perup Sanctuary is 6 years (DBCA 2020).

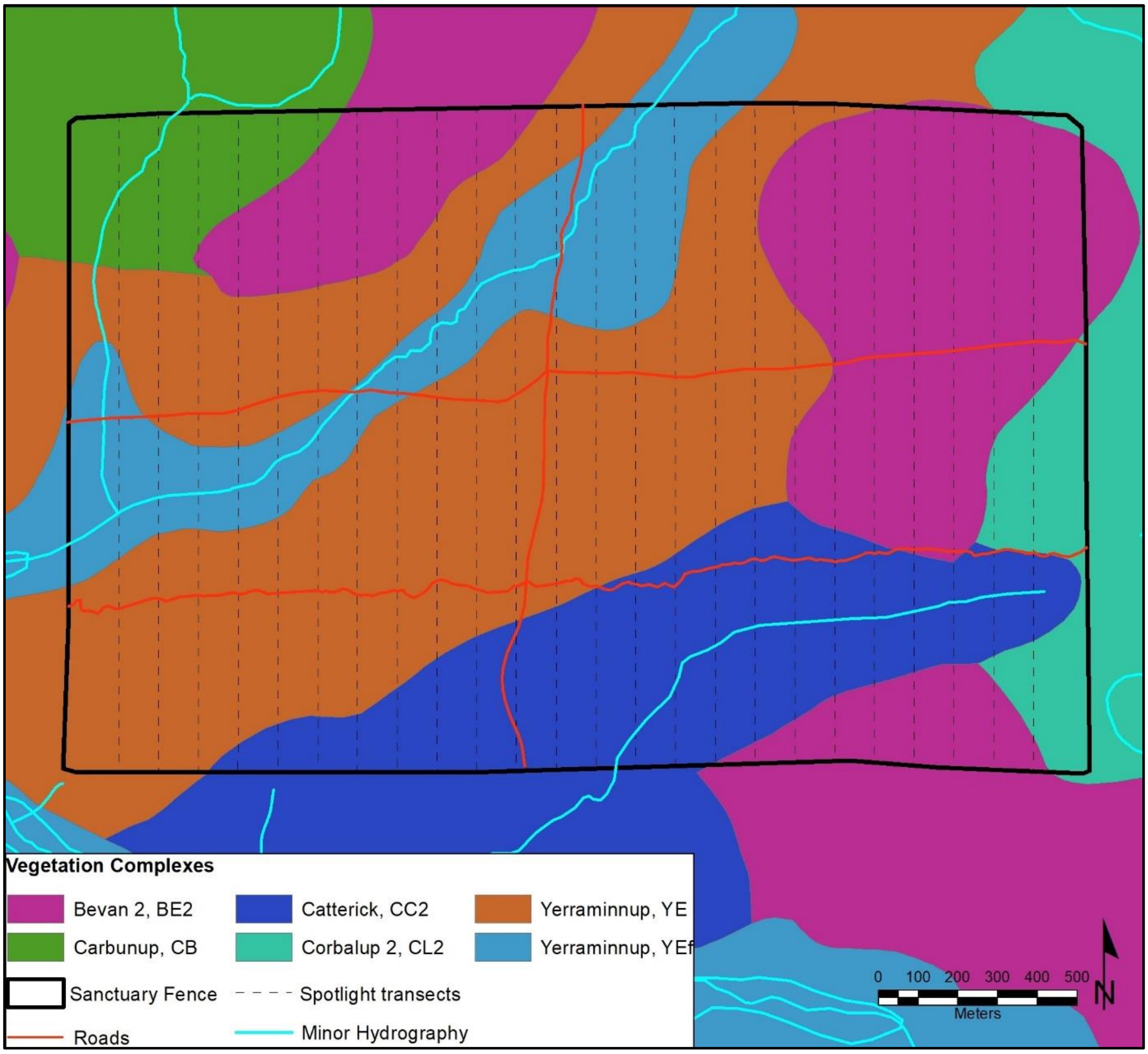

Figure 5. *The 2022 pedestrian spotlight transects and main vegetation associations in the Perup Sanctuary.*

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[1] the maximum age at which fuel is judged to be in a managed state. It is defined as the age at which the fuel will burn with an intensity that is double the upper limit at which machine and tanker attack on the head fire is possible under 95th percentile Fire Danger Index (FDI) weather conditions.

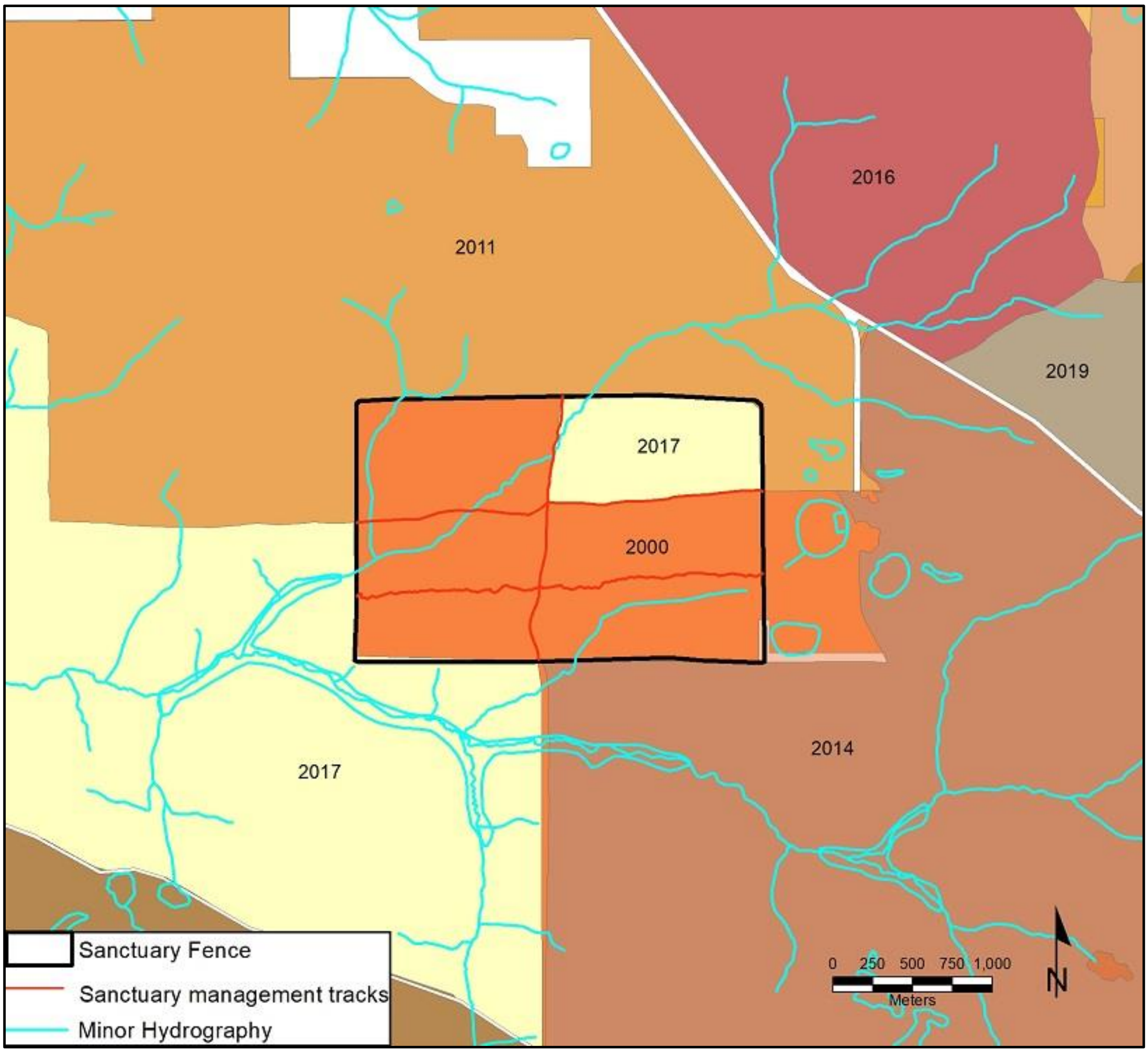

Figure 6. *Fire history (year last burnt) in and around the Perup Sanctuary.*

3.2 Spotlight survey method

Line transect distance sampling surveys were undertaken on 7th – 9th November 2022 using methods described in Teale and Potts (2020) and Wayne et al. (2022a & b). Twenty-four transects (average 1660 m each) running north-south were spaced 100 m apart within the Perup Sanctuary, aligned with the established grid markers (metal fence droppers) spaced 100 m apart (Figures 5 & 7). Surveys began approximately 1 hour after sunrise and finished before sunrise.

Each transect was surveyed by a single observer, following the transect using GPS track files and walking quietly at approximately 1 km per hour. Three observers co-ordinated the concurrent survey of adjacent transects to reduce the chances of the same possum individuals having enough time to travel and be recorded on separate transects. Animals were searched for using a high-powered head torch (Led Lenser XEO 19R or H19R Core models). For each animal observation the following information was recorded; animal location (using a GPS), species (principally medium-sized and larger mammals and some larger birds), time, number of individual animals, animal position (e.g., ground, tree), tree species and size class (based on diameter at breast height over bark (DBH: 1.3m above ground), and other information about the animals (e.g., adult / independent animal, female with joey on back, female with joey at heel). Data validation included checking that the same possum individual was not recorded more than once from adjacent transects (i.e., the location of possums was not close to other records made from adjacent transects).

3.3 Hand capture

Spotlighters assessed whether the ngwayir individuals they detected were feasible for hand capture (e.g., within 5 m of the ground and in an isolated shrub or tree with limited connectivity for escape). If the animal was within easy reach and it was safe to do so, the spotlighter would immediately capture the individuals, having a handling bag at the ready. However, spotlighters generally contacted a dedicated capture team of six to eight people via UHF and VHF radio to relay the location of candidate ngwayir. To avoid the disturbance of animals along the transects, the capture team followed behind the spotlighters, maintaining a suitable distance (usually >100m), and remained only in areas that had already been surveyed by the spotlighters. The capture team used pruning loppers and ropes to manipulate the vegetation to aid in the capture of ngwayir using nets and/or by hand. Captured animals were placed into handling bags and immediately processed on site. Basic biometrics (weight, head length, sex, breeding condition, gross signs of health and body condition) were recorded and small biopsies of ear tissue for genetic analysis were collected. Captured animals were released at point of capture immediately upon completion of processing.

3.4 Demographic analysis

Distance sampling analysis (Buckland *et al.* 2001) was undertaken using the ‘Distance’ package (v. 1.0.4, Miller *et al.* 2019) in R (v. 4.1.2, R Core Team, 2022). The perpendicular distance of the possum’s location from the transect were used to estimate the Probability Detection Function (i.e., the probability of detecting a possum, given it is *x* m from the transect line). Model selection using an information theoretic approach was used to identify the detection function that best fit the data (i.e., the model with the lowest Akaike Information Criterion (AIC) was selected; Burnham and Anderson, 2002). These included the half-normal and hazard-rate keys, both with and without the respective adjustments for each function (cosine adjustment for half-normal and polynomial adjustment for hazard-rate (Buckland et al. 2001)). To evaluate the fit of the most parsimonious model, we visually inspected quantile-quantile plots (where a good fit is indicated by the cumulative distribution function of the fitted model against the empirical distribution model falls on, or close to, the 1-1 line of the plot (Buckland et al. 2004)) and performed Cramer-von Mises goodness of fit tests (de Tores & Elscot 2010). We tested for any potential observer differences by building an additional model with observer (factor) as a covariate and comparing this model to the base model.

To investigate the effect of environmental covariates, each transect was partitioned into 100m segments, and possum counts, and respective covariate data were attributed to each segment. The covariates examined included fire age (whether the habitat was last burned in 2000 or 2017), vegetation complex (one of 6 Mattiske and Havel (1998) complexes) and the presence of competing possum species (koomal for the ngwayir models or ngwayir for the koomal models) based on detections from the line transect sampling. Density surface models from the package dsm (Miller et al. 2022) were used to model possum counts as a function of space. For each species, Poisson, negative binomial, and Tweedie distributions were compared by visually inspecting plots of standardised residuals against fitted residuals. Each covariate was then systematically added, and the percentage of deviance explained was examined to select the best model.

To compare the height and tree diameter of detection events between species, linear mixed effects models from the package ‘lme4’ (Bates et al. 2015) included observer and transect ID as random effects to control for the non-independence of observations. Model assumptions were assessed by comparing standardised residuals to fitted values (Ferrari & Cribari-Neto 2004).

3.5 Genetics

Samples

To complete the genetic component of this project, new genotype sequencing data was required from the Perup Sanctuary population (n=28 individuals, collected in November 2022) and the Busselton Hospital founder population (n=20 individuals, collected in 2012 at the time of translocation). In addition, opportunistically collected samples from the Upper Warren and Upper Donnelly regions over the last two years were also sequenced to increase the sample size of reference locations. Tissue samples were collected by taking 2 mm ear biopsy and immediately stored in 70% ethanol. Genetic data from newly acquired samples were combined with genetic data from the Upper Warren and Upper Donnelly founder animals, as well as further reference locations, generated from a previous study (White et al. 2021).

Sequencing and SNP calling

A total of 67 samples were available for genotyping by sequencing. Genomic DNA was extracted from these samples using a standard ‘salting out’ method (Sunnucks & Hale 1996) with the addition of 3 µl of 10 mg/ml RNase to the TNES buffer to remove RNA contamination. Extracted DNA was normalised to a concentration of 10 ng/ul and 20 µl of each sample was sent for SNP genotyping using DArTseq^{TM} High Density sequencing (DArT Pty Ltd, Canberra, Australia). DArTseq^{TM} is a genome complexity reduction technique that relies on restriction enzymes to fragment genomes for sequencing and SNP calling (Kilian et al. 2012; Sansaloni et al. 2011). Restriction enzymes are chosen dependent on the fraction of the genome represented, number of polymorphic loci and average read depth of fragments. The enzymes deemed most appropriate for our species were *PstI* and *NlaIII*. To call

SNPs, sequences from the genomic fragments generated by the DArTseq^{TM} western ringtail possum assay as part of this study were combined with relevant sequences generated by White et al. (2021) and processed using analytical pipelines proprietary to DArT Pty Ltd (but for details see Georges et al. 2018). In total, sequences from 190 samples were processed which generated 24,805 SNP markers.

Data filtering

Genotype data along with locus and individual metadata were loaded into the dartR v2.7.2 package (Gruber et al. 2018) and converted into a genlight data object, run in R version 4.3.0 (R Core Team 2020). Filtering of data is required to ensure the dataset is of high quality and robust to potential biases. While different analyses require specific filtering parameters, a preliminary set of filtering steps was, i. remove all loci that did not provide 100% reproducibility, ii. remove monomorphic loci, iii. remove loci that have more than 10% missing data, iv. keep one locus per sequence tag, v. remove individuals with greater than 20% missing data, vi. re-check that all loci are polymorphic and have no more than 10% missing data, and vii. prune loci that are in linkage disequilibrium in R using SNPRelate v1.24 (Zheng et al. 2012). The number of markers may vary between analyses dependent on any further filtering such as minor allele frequencies (admixture analysis) and population size (diversity analysis).

Population genetic analyses

Descriptive genetic diversity statistics were estimated for sample locations that had at least two individuals. Observed, expected and unbiased expected (which controls for the impact of small sample sizes using the multiplier $n/(n-1)$) heterozygosities, as well as inbreeding coefficients, were estimated for all locations using dartR. The mean number of private alleles per sample per population were estimated after exporting the filtered genlight object into GenAlEx v 6.51 (Peakall and Smouse 2012; Peakall and Smouse 2006). Allelic richness, or the mean number of alleles per locus corrected for sample size, was estimated for all subpopulations with n >= 6 using Hierfstat v0.5-11 (Goudet 2005). We estimated the genetic effective population size (Ne) using the linkage disequilibrium method (Hill 1981, Waples 2006, Waples 2010), excluding singleton alleles (those that occur in one copy in one heterozygote) to prevent an upward bias in Ne estimation, as implemented in NeESTIMATOR v2.1 (Do et al 2014). The minimum minor allele frequency for the dataset used for Ne estimation was 0.003.

To get an overview of the genetic distance between the Perup Sanctuary and its founder populations, as well as reference locations within the Southern Forest, a genetic distance matrix at the population level was created based on the Euclidean distance method using dartR. A principal coordinates analysis (PCoA) ordination was done on the resulting distance matrix using the R package adegenet v2.1.10 (Jombart 2008; Jombart and Ahmed 2011). This also allowed confirmation of how well the Upper Warren and Upper Donnelly founder populations represent their parental locations.

To assess in more detail the relationship of the Perup Sanctuary animals to their founder populations, individual-based PCoAs were run based on allele frequencies, also run in adegenet v2.1.10 (Jombart 2008; Jombart and Ahmed 2011). To determine how well the Busselton Hospital founder population represented the Busselton population, a final PCoA was done at the population level using all Busselton animals sequenced by White et al. (2021), also run in adegenet v2.1.10 (Jombart 2008; Jombart and Ahmed 2011).

To estimate the proportion of genomic contribution from each of the founding populations to the Perup Sanctuary animals, an admixture analysis was run in Structure v2.3.4 (Pritchard et al. 2000), after removal of loci with minor allele frequency less than 0.01, as rare alleles can lead to biases in structure analysis (Linck and Battey 2019). ‘Structure’ uses a Bayesian clustering method to assign individuals to one of k populations and to estimate the degree of inter-population admixture. As our analysis was focused on the Perup Sanctuary, which is an admixed population, and distinct founding populations, we tested performance of the admixture ancestry model with both correlated and independent allele frequency models. Population pre-assignment (Busselton Hospital, Perup Sanctuary, Upper Donnelly founder or Upper Warren founder) was used as a location prior. After preliminary assessment of convergence times for the Monte Carlo Markov chain, a burn-in period of 10,000 steps was chosen, followed by 20,000 steps of the chain (30,000 steps in total). Five replicate runs with k pre-set to values of 2 and 3 were executed. ‘Structure’ figures were generated using Distruct v1.1 (Rosenberg 2004) in Clumpak (Kopelman et al. 2015).

Relatedness analysis

To estimate the number and origin of founding individuals that have contributed to the current population, an additive genomic relationship matrix between all individuals was generated by calculating the probability of identity by descent (IBD) across loci. This was done in R using the package rrBLUP v4.6.2 (Endelman 2011, Endelman and Jannink 2012). From the relationship matrix a network diagram was created, using the Fruchterman-Reingold layout, where nodes in the network are individuals and links between nodes indicate relatedness.

4 Results

4.1 Demographic survey results

Three observers walked 24 separate line transects totalling 39.6 km (Tables 5 & 6). There was a total of 126 ngwayir individuals detected in 94 events (Table 7, Figure 7). In 28 of these events two ngwayir were detected and on four occasions three individuals were together. Of the 217 koomal individuals detected in 174 events (Table 7, Figure 8), there were 39 occasions when a pair of koomal were detected and two occasions when three individuals were detected together.

4.1.1 Population estimates (Distance sampling modelling)

Distance sampling modelling used a truncation distance (*w*) of 50 m. That is, observations greater than 50 m were discarded from the analysis, representing 0% and 2.3% of the total ngwayir and koomal observations, respectively. While these values were under the recommended rough rule-of-thumb that the top 5% of distance observations be truncated, they meet as close as practically possible the recommendation for the estimated detection probability at the truncation distance to be 0.15 (i.e., $\hat{g}(w) \approx 0.15$, Buckland et a. 2001, p. 151). This differs slightly from the 55 m truncation distance used by Teale and Potts (2020) on ngwayir detection data from the Upper Warren and the 45 m truncation distance used for the ngwayir survey of the Upper Warren hotspot by Wayne et al. (2022a).

No observer differences were found for the detection of both possum species, based on a visual comparison of the detection frequency histograms for the three spotlight observers (Figure 9) and the observer models were less parsimonious (had a higher AIC value) than models without observer included as a covariate. The histograms of the detection distances for ngwayir and koomal (all observers combined) are provided in Figure 10.

A hazard-rate detection function with a polynomial adjustment had the best fit for the ngwayir data, and a half-normal detection function with a cosine adjustment was the best for the koomal data (Figure 11).

The distance sampling models (Buckland *et al.* 2001) estimated the density at 0.37 ngwayir ha^{-1} (95% CI: 0.30 – 0.45) and the population size in the Perup Sanctuary to be 156 ngwayir individuals (126.6 – 192.4, 95% Confidence Interval). The density of koomal inside Perup Sanctuary was estimated to be 0.83 possums ha^{-1} (95% CI: 0.64 – 1.10) and 350 koomal individuals (95% CI 271.7 – 452.5).

A simplistic estimated minimum average population growth rate of 1.23 per annum would be required to account for the current ngwayir population size assuming the maximum possible number of surviving founders (12 Busselton animals in 2012 and 17 Upper Warren-Upper Donnelly animals in 2016-2017), and there being no resident ngwayir extant in the Perup Sanctuary at the time of the introduction of the first cohort of founders in 2012. Given that this growth rate is biologically plausible it is possible that the ngwayir population is already at or close to carrying capacity.

Table 5. *Summary of spotlight surveys conducted in the Perup Sanctuary 2022.*

Number of observers	3
Number of transects surveyed	24
Transect spacing (m)	100
Total length surveyed (km)	39.6
Distance Sampling Truncation distance (m)	45
Total area surveyed (ha)	356
Survey region (ha)	423
% of survey region actually surveyed	84%

Table 6. *Summary of which observers surveyed what transects, the total distance walked by each observer and the number of detection events (and number of individuals detected) for the two possum species.*

Observer	Transects surveyed	Distance surveyed	Ngwayir detection events	Koomal detection events
A. Barrett	4, 7,10,13,16,19, 22, 25	13.3 km	29 (40)	54 (66)
A. Wayne	3, 6, 9, 12, 15, 18, 21, 24	13.3 km	26 (38)	71 (93)
M. Maxwell	2, 5, 8, 11, 14, 17, 20, 23	13.3 km	39 (52)	49 (58)
Total		**39.6 km**	**94 (126)**	**174 (217)**

Table 7. *Summary of species detected during spotlight surveys on transect in the Perup Sanctuary, November 2022.*

Species	Number of detection events	Total number of individuals
Ngwayir	94	126
Koomal	174	217
Woylie	30	34
Western grey kangaroo	2	2
Wambenger	3	3
Quenda	1	1
Tammar wallaby	2	2

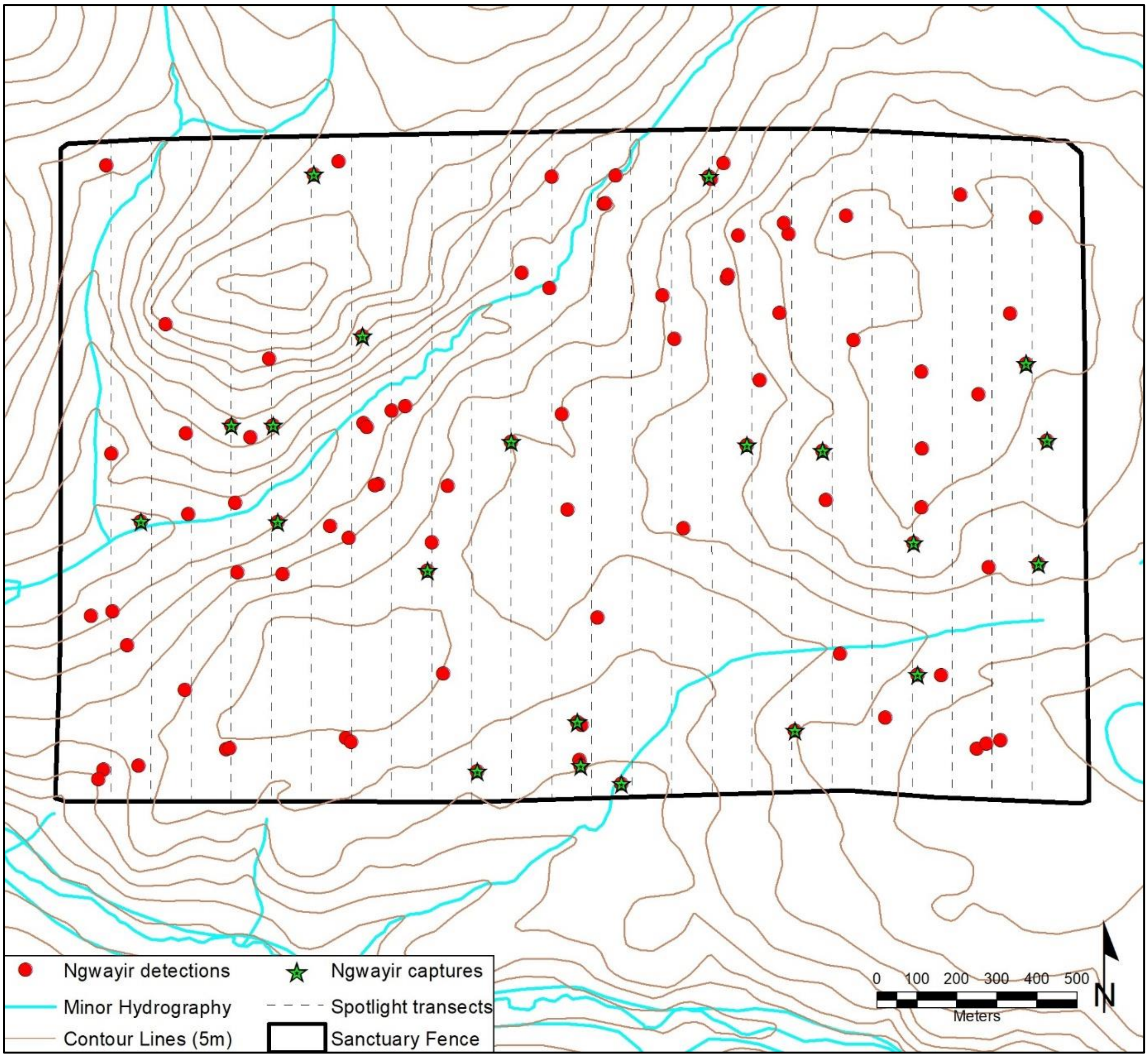

Figure 7. *Independent detections of ngwayir during distance sampling spotlight surveys along transects conducted in Perup Sanctuary (November 2022).*

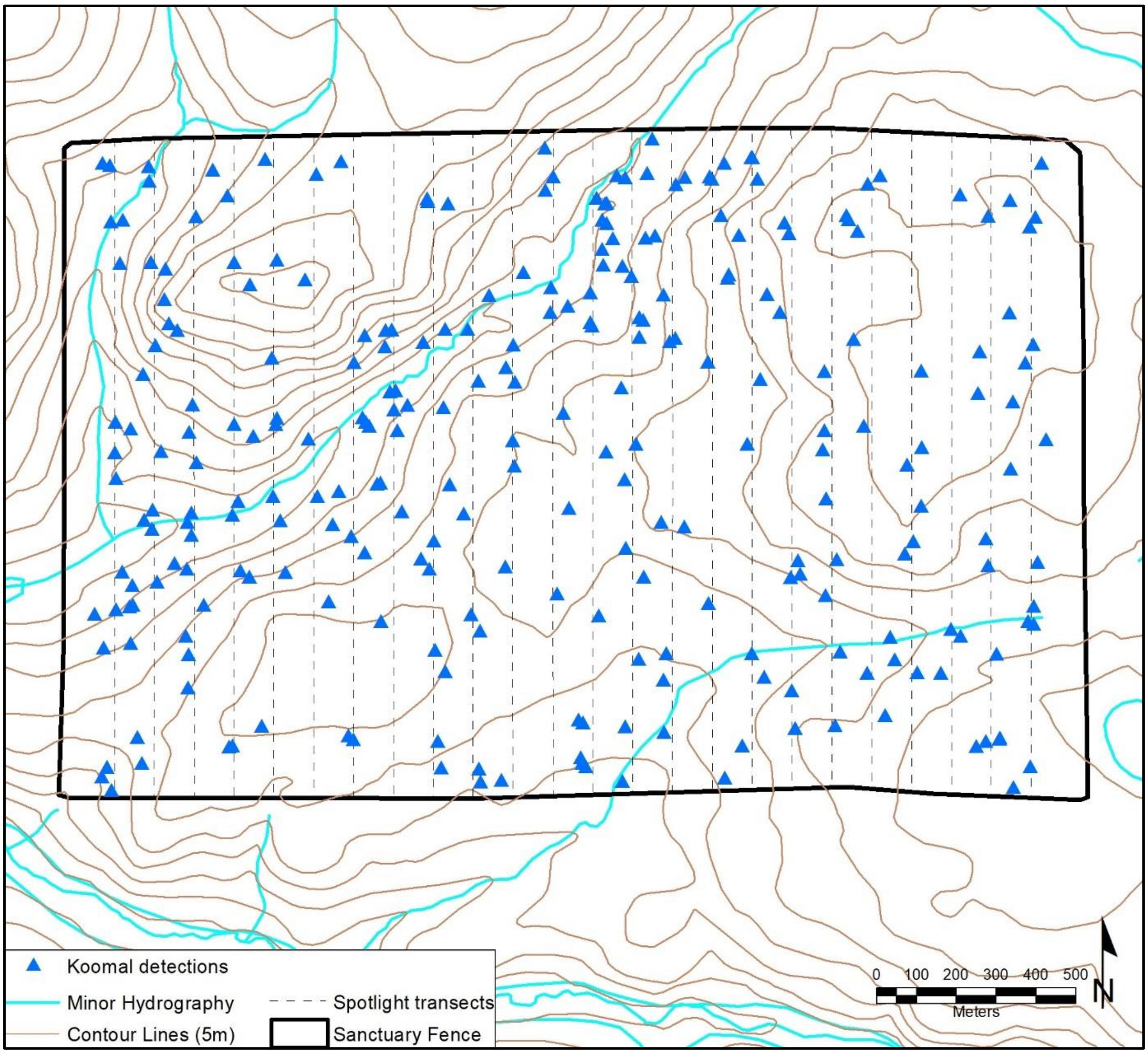

Figure 8. *Independent detections of koomal during distance sampling spotlight surveys along transects conducted in Perup Sanctuary (November 2022).*

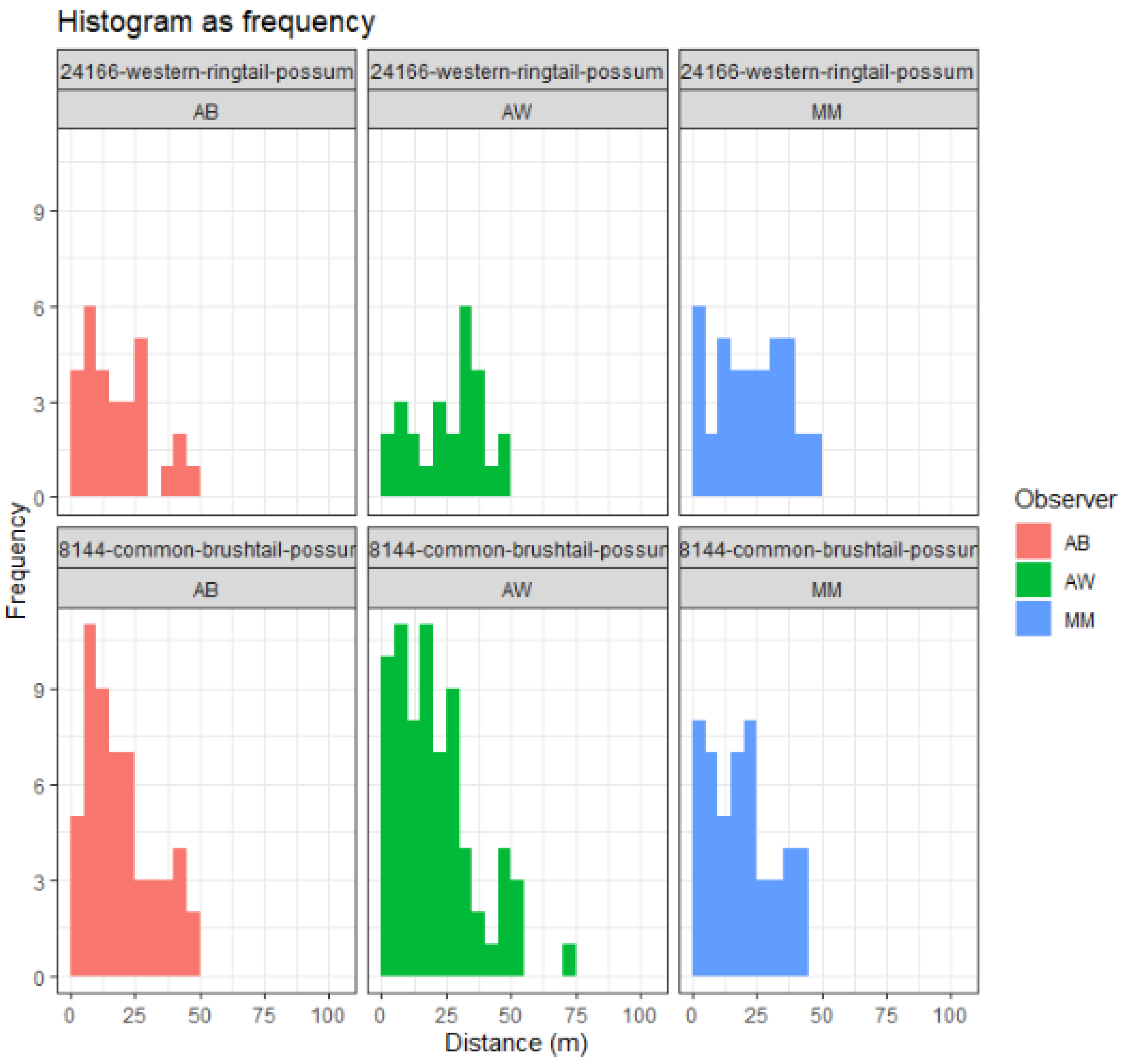

Figure 9. Frequency histograms of detection distances for both possum species and by each spotlight observer, Perup Sanctuary (November 2022).

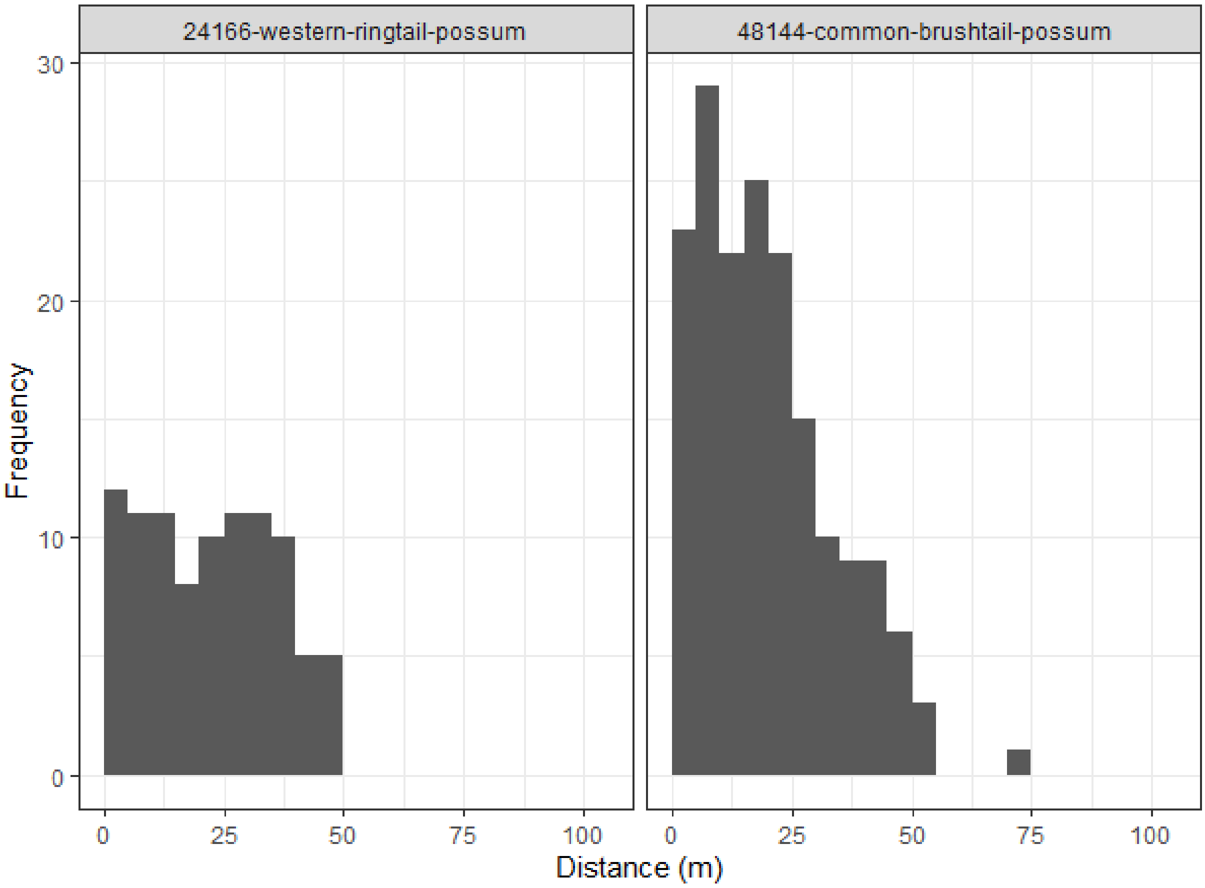

Figure 10. *Histograms of detection distances of ngwayir and koomal in the Perup Sanctuary (November 2022) from the three observers combined.*

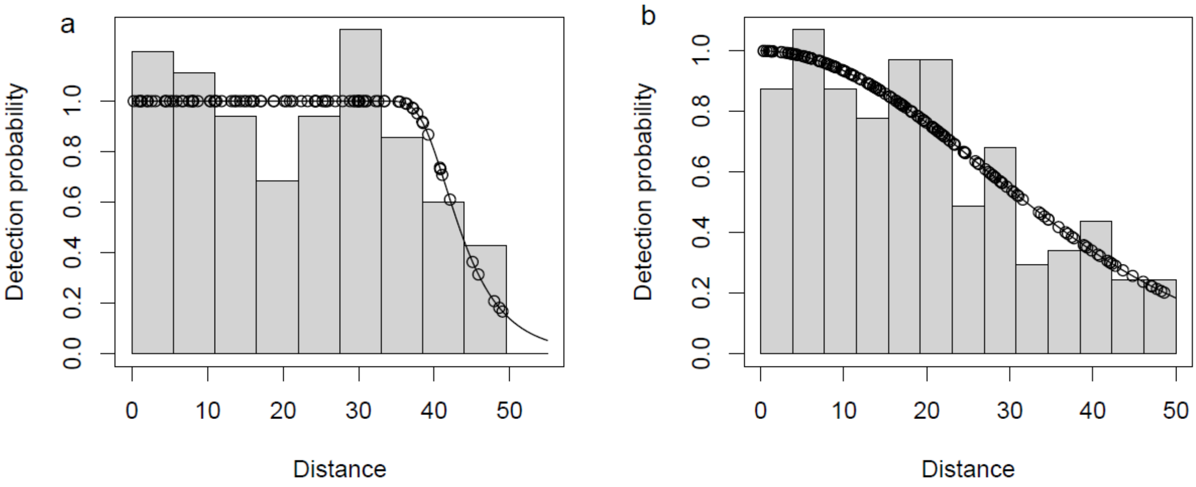

Figure 11. *Fitted detection curve in relation to distance from transect (m), with truncation distance of 50 m, for (a) ngwayir and (b) koomal.*

4.1.2 Habitat associations (Density surface modelling)

When examining the habitat covariates, the Tweedie density surface models performed best for both species. The model including all three covariates explained the most variation in both species and accounted for 6.42% and 15.5% of deviation explained in ngwayir and koomal distribution, respectively. Burn age and vegetation complex did not affect the abundance (counts) of ngwayir (Table 8). The presence of koomal had a weak positive effect on the abundance of ngwayir. Koomal abundance was highest in the Yerraminnup flats vegetation class, followed by Yerraminnup and Catterick, which had weak positive effects. Burn age and ngwayir presence did not affect koomal abundance (Table 8). Modelled densities of ngwayir did not vary greatly throughout the Perup Sanctuary, whereas there was greater variation in koomal densities (Figure 12c & d).

Table 8. *Effect of burn age and habitat complex on abundance of possums inside Perup Sanctuary.*

	Ngwayir			**Koomal**		
Parameter	**Slope**	**S.E.**	**p-value**	**Slope**	**S.E**	**p-value**
Intercept	-10.516	0.311	<0.001	-10.022	0.221	<0.001
LastBurn_2017	0.099	0.417	0.813	0.013	0.268	0.960
VegClass_CB	-1.308	1.287	0.310	0.427	0.477	0.371
VegClass_CC2	-0.357	0.503	0.478	0.720	0.340	0.035
VegClass_CL2	-0.049	0.853	0.954	0.368	0.587	0.532
VegClass_YE	0.208	0.406	0.611	0.480	0.282	0.090
VegClass_YEf	0.672	0.470	0.153	1.568	0.298	<0.001
Koomal_present	0.449	0.249	0.072	-	-	-
Ngwayir_present	-	-	-	-0.171	0.179	0.341

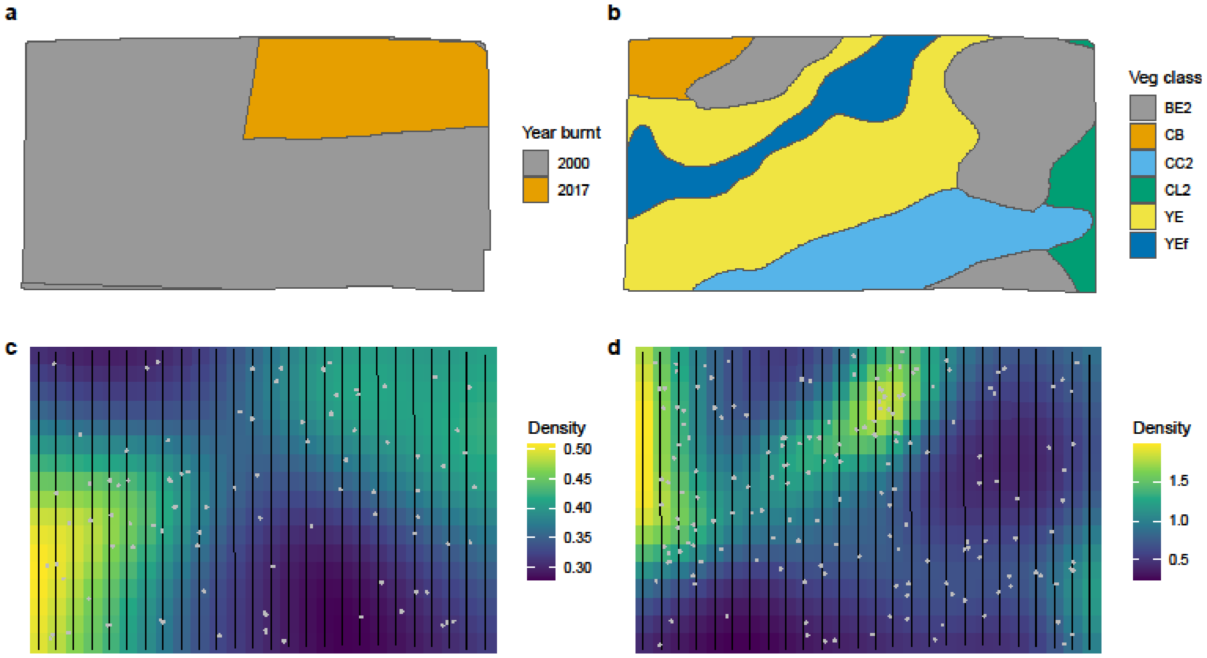

Figure 12. *Map of Perup Sanctuary (a) fire age; (b) vegetation complexes; (c) ngwayir density; and (d) koomal density. In (c) and (d), the lines represent transects (spaced 100m apart) and the dots represent individual possums detected. Lighter colours indicate higher densities of individual possums.*

4.1.3 Possum habitat use

Most ngwayir and koomal were observed in arboreal locations.

Koomal were detected at greater heights than ngwayir (Figure 13; Table 9), with median detection heights at 10 m (mean=11, SD=5.8) and 6 m (mean =6.8, SD=4.7) for koomal and ngwayir, respectively. Put another way, 67% of ngwayir were 10 m or less above ground and 65% of koomal were greater than 10 m above ground (Figure 13).

Koomal also were observed in significantly larger diameter trees than ngwayir (Table 10, Figure 14). About two thirds (68%) of ngwayir were detected in trees with less than 40 cm diameter at breast height over bark (DBHob, 1.3m above ground). The location of koomal differed, with 73% being in trees larger than 40 cm DBHob (Table 11a). There was no major difference in the species of tree used by both possum species (Table 11b).

Table 9. *Effect of possum species on the height of spotlight detection events in the Perup Sanctuary*

Parameter	**Slope**	**S.E.**	**p-value**
Intercept	6.728	1.242	0.018
Species_Koomal	4.503	0.676	<0.001

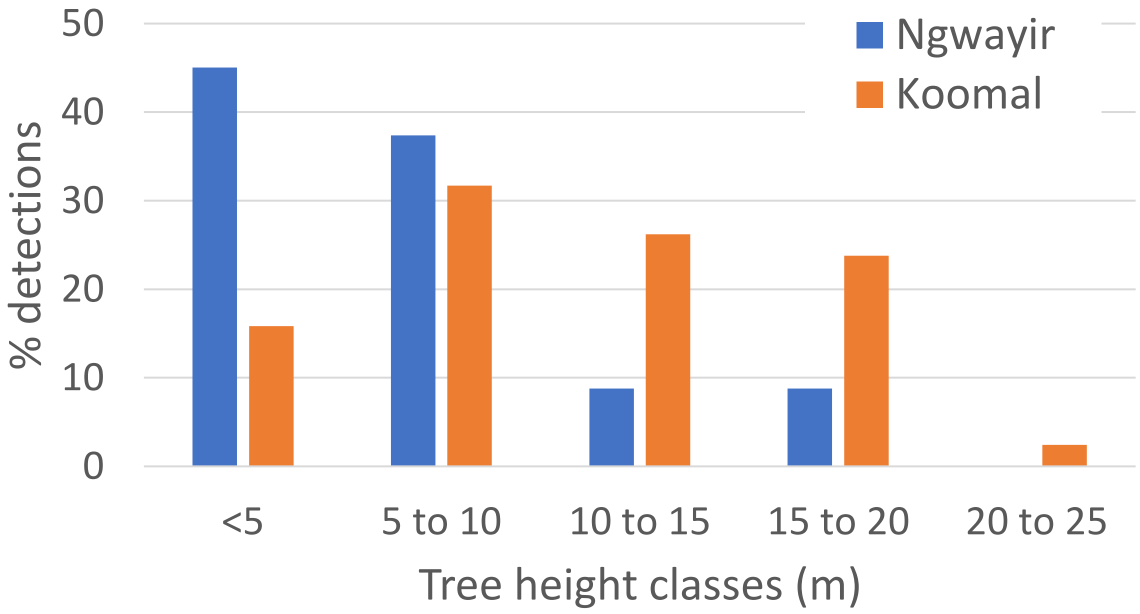

Figure 13. *Height of ngwayir (n=92) and koomal (n=174) detected during spotlight surveys of Perup Sanctuary, 2022.*

Table 10. *Effect of possum species on the diameter of trees in which the possums were detected during spotlight surveys in the Perup Sanctuary, November 2022.*

Parameter	**Slope**	**S.E.**	**p-value**
Intercept	25.96	1.47	<0.001
Species_Koomal	13.56	1.83	<0.001

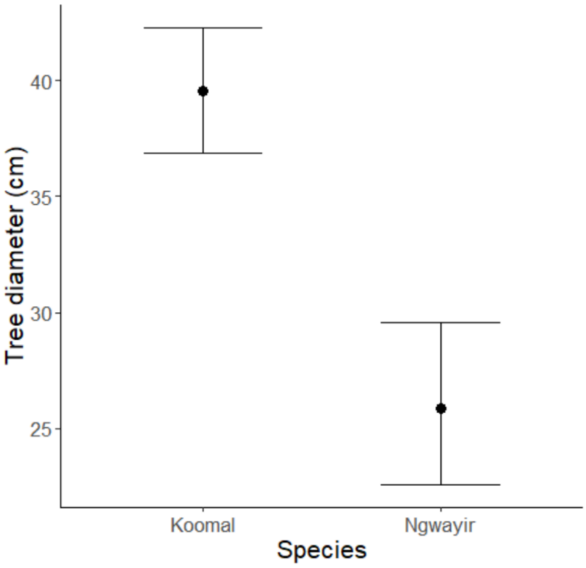

Figure 14. *Mean and 95% confidence intervals for the tree diameters in which ngwayir (n=94) and koomal (n=174) were detected during spotlight surveys of Perup Sanctuary, 2022.*

Table 11. *Summary of the location of possums detected during the 2022 spotlight survey of the ngwayir in the Perup Sanctuary.*

a) Substrate structure

		Terrestrial	**Arboreal**				
Species	**n**	**Ground / log**	**Shrub**	**Tree sapling (<15 cm d.)**	**Tree pole (15-40 cm d.)**	**Large tree (>40 cm d.)**	**Dead**
Ngwayir	94	3.2%	7.4%	41.5%	19.1%	27.7%	1.1%
Koomal	171	4.7%	1.8%	6.4%	12.3%	73.1%	1.8%

b) Tree species. Note ‘Other’ includes *Eucalyptus rudis*, *E. decipiens*, *Banksia*, *Hakea*, *Melaleuca spp.*, *Xanthorrhoea preiseii*, and *Bossiaea linophylla*.

Species	**n**	**Marri**	**Jarrah**	**Wandoo**	**Melaleuca**	***Other**
Ngwayir	90	38.9%	40.0%	5.6%	5.6%	10.0%
Koomal	166	31.3%	43.4%	17.5%	2.4%	5.4%

4.2 Ngwayir captures

Twenty-eight ngwayir were captured and tissue samples taken for DNA analysis (Figure 7, Table 12). Four of these were individuals considered to be dependent young of an adult mother that was also captured. All captured animals appeared in general good health with no obvious significant health issues. Adult weights ranged from 980g to 1330g, with body condition scores ranging from 3 (moderate condition) to 5 (very good condition). All captured adult females (6) had young at heal and two were also carrying a second small pouch young. All captured animals had very low ectoparasite loads.

Table 12. *Summary demographic and biometric information of the 28 ngwayir hand-captured in the Perup Sanctuary (November 2022).*

Left ear tag	Right ear tag	Sex	Age	Breeding	Wt. (g)	BC	Comments/Relationship
DL8312	DL8313	F	A	LacYP	1170	4	Mother of DL8326/27
DL8326	DL8327	F	J	U	530	4	Young of DL8312/13
DN8752	DL8314	M	A		1140	3	In same tree as DL8312/13. Previous capture 11/4/18 on Alf Grid.
DL8115	DL8116	M	A			4	
DL8117	DL8118	M	A			4	
DL8328	DL8329	M	A		1280	5	
DL8330	DL8331	M	A		1090	4	
DL8119	DL8120	F	A	B - 1 x 60mm		5	Mother of DL8121/22
DL8121	DL8122	F	J	U		3	Young of DL8119/20
DL8349	DL8350	M	A		1120	3	
DL8123	DL8124	F	A	B - 1 x 60mm		5	Sub-adult in tree with it - not captured.
DL8332	DL8333	M	A		1080	3	
DL8334	-	M	S		780	3	only tagged 1 ear.
DL8125	DL8173	F	A	LacYP		3	Mother of DL8126/DL8127
DL8126	DL8127	M	J		450	4	Young of DL8125/DL8173
DL8336	DL8337	F	A	LacYP	1240	4	Mother of DL8338/39
DL8338	DL8339	M	J		520	3	Young of DL8336/37
DL8128	DL8129	M	A		1190	3	
DL8149	DL8150	M	A		1020	4	
DL8130	DL8131	M	S		1270	4	small testes
-	-	F	J	U	370	4	Young of DL8132/8133 - too small to tag
DL8132	DL8133	F	A	LacYP	1070	3	Mother of animal above (22DN71R)
DL8134	DL8135	M	A		1030	3	
DL8136	DL8137	M	A		1190	3	
DL8138	DL8139	M	A		1090	3	
DL8140	DL8141	M	A		980	4	
-	-	F	J		520	4	Was in tree with mother - not captured.
DL8142	DL8143	M	A		1330	5	

Age: A=adult, S=subadult (independent but sexually immature), J=juvenile (dependent)
Breeding: LacYP = Lactating with young at heal present, U = undeveloped pouch (subadult), B= both young at heal and suckling pouch young present (crown-rump length measurement provided in millimeters)
BC= Body Condition (1=very poor, 2=poor, 3=moderate, 4=good, 5=very good)

4.3 Ngwayir genetic analysis

The Perup Sanctuary has an observed heterozygosity (Ho) of 0.06 and an unbiased expected heterozygosity (uHe) of 0.07. These values are between the Upper Donnelly founders (0.05, 0.06) and Upper Warren founders (0.07, 0.08), and greater than estimates for Busselton Hospital (0.04, 0.05), although standard deviations are relatively large due to small sample sizes (Table 13). Allelic richness analysis shows Perup Sanctuary to have 1.13 alleles per locus (rarefied to a sample size of n=6), less than the Upper Warren founders (1.14) but greater than the Upper Donnelly (1.11) and Busselton Hospital (1.08) founders. Combining all three founder subpopulations gives Ho of 0.05 and uHe of 0.06, and allelic richness of 1.11. Perup Sanctuary heterozygosity values lie within the range of values from other Southern Forest locations (Figure 15). The unbiased inbreeding coefficient (uFIS) in the Perup Sanctuary is 0.10, which is less than the Upper Donnelly founders (0.16), the same as the Upper Warren founders (0.10) and greater than the Busselton Hospital founders (0.06) (Table 1). Perup Sanctuary has a relatively low uFIS compared to other Southern Forest subpopulations, Manjimup has the highest (0.27) (Table 13, Figure 15).

Private allele analysis shows that Perup Sanctuary has the greatest number per sample (17.0), which is significantly greater than the mean across all 14 subpopulations (5.7. ± s.d 4.0, Figure 16). This number is likely inflated by the small sample sizes of regional subpopulations reducing the likelihood of sharing rare alleles. Further, greater numbers of private alleles will likely be reported in the founder populations when Perup Sanctuary is removed from the analysis, in particular Busselton Hospital. Nonetheless, if genetic variation occurs in Perup Sanctuary that is not present in other subpopulations this is surprising considering both founder populations are included in this analysis. However, not all founders for the Perup Sanctuary were sequenced – four are missing – and some of the reported unique genetic variation may have come from these individuals. In particular, one individual from Talling was not sequenced and Talling is geographically distant from other source locations. A further, less likely, possibility is that there has been some integration of local animals from the Yackelup region with the Perup Sanctuary animals.

Table 13. *Summary diversity statistics based on up to 4573 SNP loci. nInd: no. individuals; nLoc: no. loci analysed; polyLoc: no. of polymorphic loci; Na: mean number of alleles per locus; AR: allelic richness (Na controlled for population size) for subpopulations with n >= 6; Ho: observed heterozygosity; He: expected heterozygosity; uHe: unbiased heterozygosity; SD: standard deviation; uFIS: inbreeding coefficient calculated as 1 – Ho/uHe; Ne: effective population size; CI: confidence intervals; UW: Upper Warren; UD: Upper Donnelly; B: Busselton*

Site	**Region**	**nInd**	**nLoc**	**polyLoc**	**Na**	**AR**	**Ho**	**HoSD**	**He**	**HeSD**	**uHe**	**uHeSD**	**FIS**	**uFIS**	**Ne (95% CIs)**
Perup S	UW	28	4573	1910	1.53	1.13	0.06	0.11	0.07	0.12	0.07	0.12	0.08	0.10	13.4 (13.3, 13.5)
Bsltn Hosp	UW	20	4573	1046	1.50	1.08	0.04	0.12	0.05	0.11	0.05	0.12	0.03	0.06	51.6 (48.2, 55.5)
Upper Don. F	UD	8	4573	1120	1.26	1.11	0.05	0.11	0.06	0.12	0.06	0.12	0.10	0.16	4.0* (3.4, 4.4)
Upper War. F	UD	6	4573	1316	1.23	1.14	0.07	0.13	0.07	0.12	0.08	0.13	0.01	0.10	1879.4* (151.3, ∞)
Camelar	B	6	4573	1323	1.29	1.14	0.07	0.13	0.07	0.12	0.07	0.13	0.02	0.11	n/a
Corbal	UD	4	4573	945	1.21	n/a	0.06	0.14	0.06	0.13	0.07	0.15	-0.02	0.11	n/a
Kingston 99	UW	5	4573	924	1.24	n/a	0.05	0.13	0.06	0.13	0.07	0.14	0.09	0.18	n/a
Kingston NP	UW	2	4570	608	1.12	n/a	0.07	0.19	0.05	0.14	0.07	0.18	-0.24	0.08	n/a
Manjimup	UW	21	4573	1904	1.51	1.12	0.05	0.09	0.06	0.11	0.06	0.11	0.25	0.27	33.8 (32.9, 34.7)
Orient Rd	UW	58	4573	2925	1.77	1.14	0.07	0.09	0.07	0.10	0.07	0.10	0.10	0.11	54.7 (54.3, 55.0)
Palgarup	UW	4	4573	826	1.20	n/a	0.05	0.13	0.05	0.12	0.06	0.14	0.06	0.18	n/a
Pemberton	UD	3	4573	730	1.13	n/a	0.06	0.16	0.06	0.13	0.07	0.16	-0.07	0.11	n/a
Yackelup	UW	2	4541	676	1.11	n/a	0.07	0.19	0.06	0.14	0.08	0.19	-0.25	0.07	n/a
Yendicup	UW	4	4566	978	1.12	n/a	0.06	0.14	0.06	0.13	0.07	0.15	0.03	0.16	n/a
Mean			4570	1231	1.50	1.12	0.06	0.13	0.06	0.12	0.07	0.14	0.01	0.13	

* Ne estimates were based on low sample size (n<10). Genetic diversity estimates for sites with n<5 should be treated with caution.

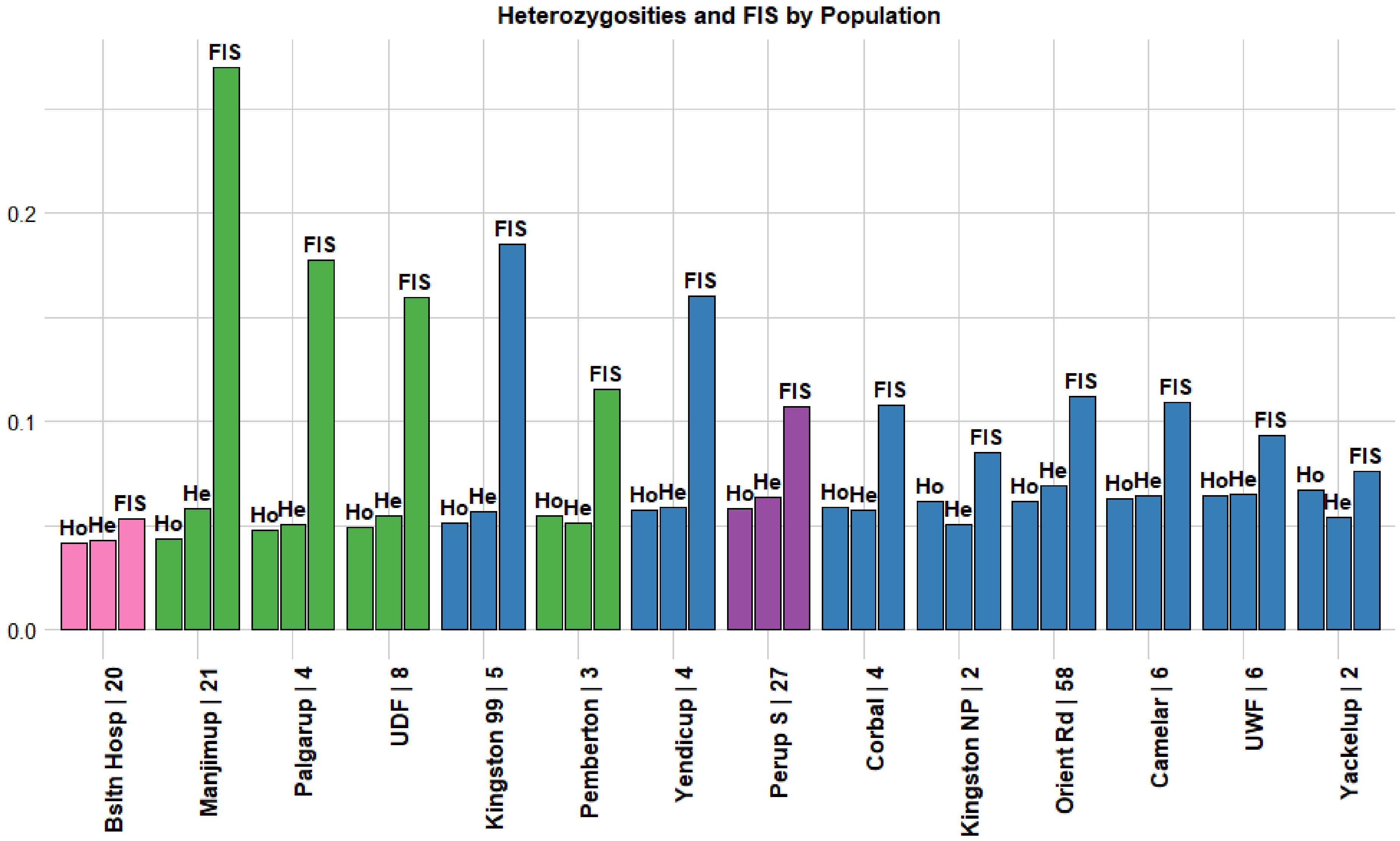

Figure 15. *Summary of population diversity estimates. UDF: Upper Donnelly founders; UWF: Upper Warren founders*

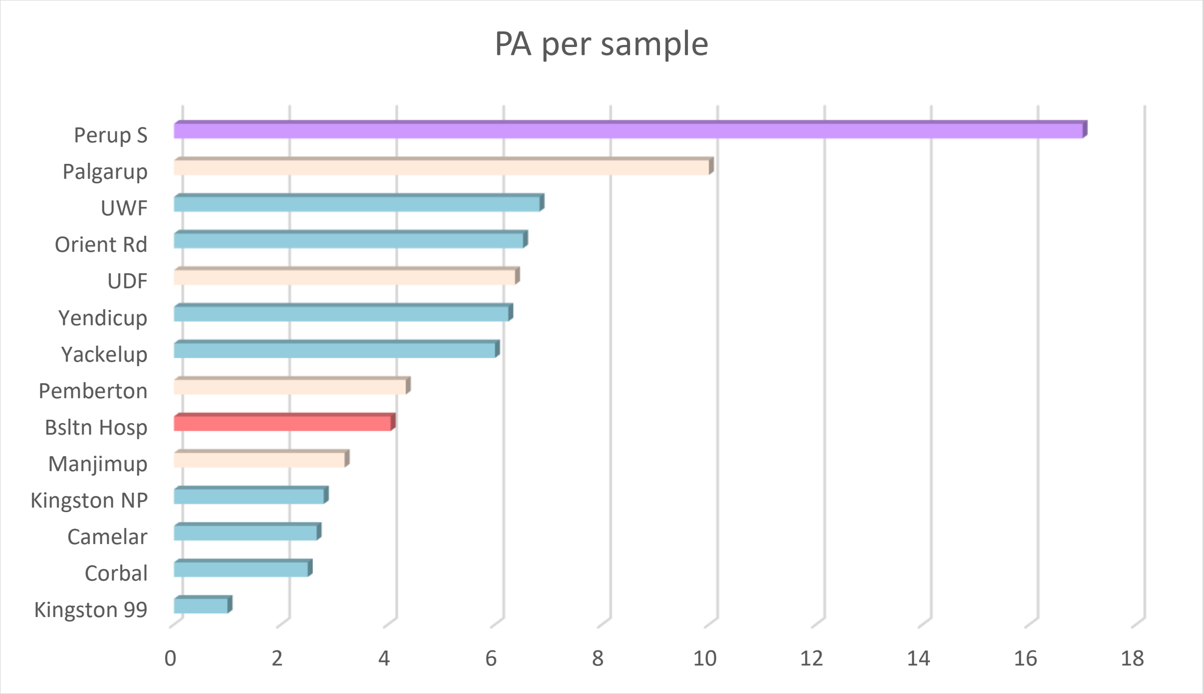

Figure 16. *Private allele analysis. UDF: Upper Donnelly founders; UWF: Upper Warren founders*

A review of Euclidean genetic distances between locations with n>=5 revealed that Perup Sanctuary had p-distances ranging from 8.18 (v Busselton Hospital) to 12.59 (v Kingston 99)). The p-distance between Perup Sanctuary and the Upper Warren founders was slightly higher than with Busselton Hospital and was higher still between the Perup Sanctuary and Upper Donnelly founders. The latter (p-distance between Perup Sanctuary and Upper Donnelly founders) was higher than the p-distance between Perup Sanctuary and both Manjimup and Orient Road. The highest p-distances are associated with Busselton Hospital (except with the Perup Sanctuary) as expected, with values ranging from 12.2 to 14.1 (Table14). The smallest p-distance was between Camelar and Orient Road which are less than 10km apart.

Table 14. *Population genetic distance matrix on populations with n >= 5 using Euclidean method. UDF: Upper Donnelly founders; UWF: Upper Warren founders*

	Bsltn Hosp	Camelar	Kingston 99	Manjimup	Orient Rd	Perup S	UDF	UWF
Bsltn Hosp	0.00							
Camelar	13.20	0.00						
Kingston 99	14.14	11.90	0.00					
Manjimup	12.21	9.05	11.74	0.00				
Orient Rd	12.87	7.21	11.72	7.36	0.00			
Perup S	8.18	10.85	12.59	9.09	9.64	0.00		
UDF	12.83	9.79	11.77	7.76	8.81	10.44	0.00	
UWF	13.05	9.00	10.80	8.78	7.80	8.98	9.67	0.00

Ordination analyses at the population level of all locations shows Perup Sanctuary to lie midway between the Busselton Hospital founders and all Southern Forest locations including the Upper Donnelly and Upper Warren founders (Figure 17). When this analysis is run on just Perup Sanctuary and the founder populations at the individual level only, relationships of the Perup Sanctuary animals to the founder populations can be viewed in more detail (Figures 18a and 18b). When the first two coordinate axes are plotted, it is apparent that the Perup Sanctuary animals are spread between the Busselton Hospital animals and the founders from Upper Donnelly and Upper Warren, with some overlap of distributions (Figure 18a). However, when the first three coordinate axes are plotted together it is clear the Upper Donnelly founder animals cluster independently with no overlap, suggesting no pure Upper Donnelly provenance animals remain in the Perup Sanctuary population (Figure 18b). The PcoA analyses were also able to show that the Southern Forest founders (Upper Donnelly and Upper Warren, Figure 17) and Busselton Hospital founders (Figure 19) represent their source locations well.

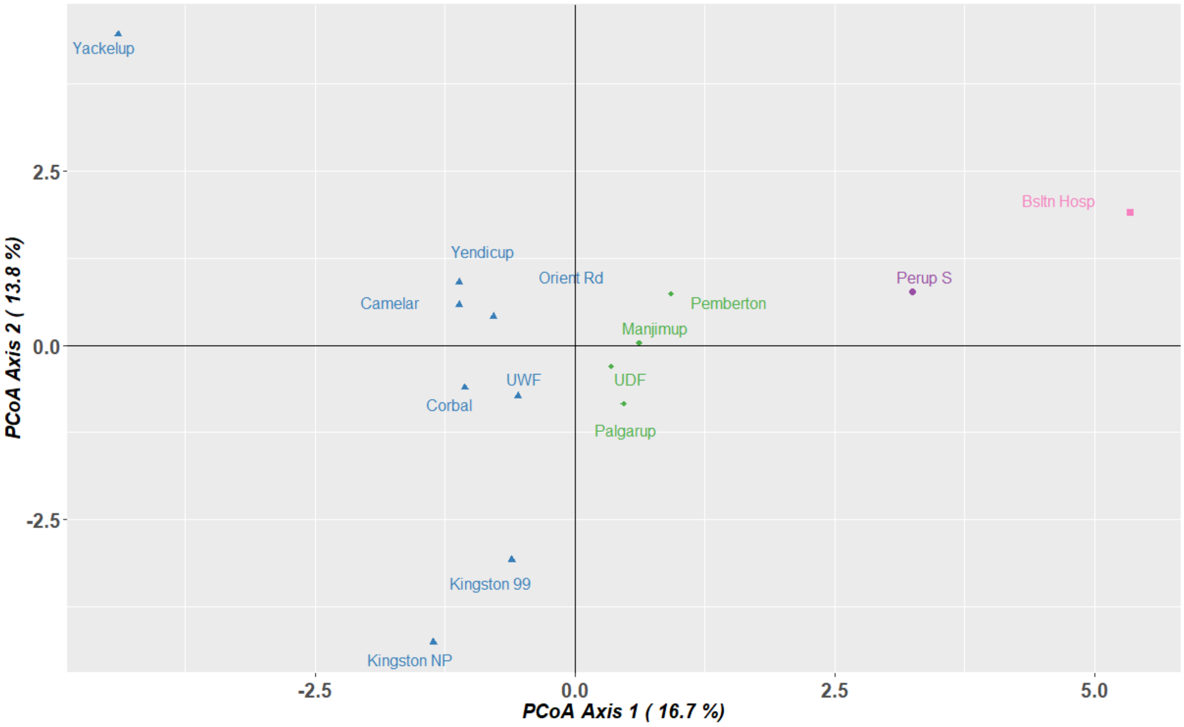

Figure 17. *Principal coordinate analysis (PcoA) of Southern Forest locations, Upper Donnelly founders, Upper Warren founders and the Busselton Hospital founders at the population level. Blue triangles are Upper Warren locations; green diamonds are Upper Donnelly locations.*

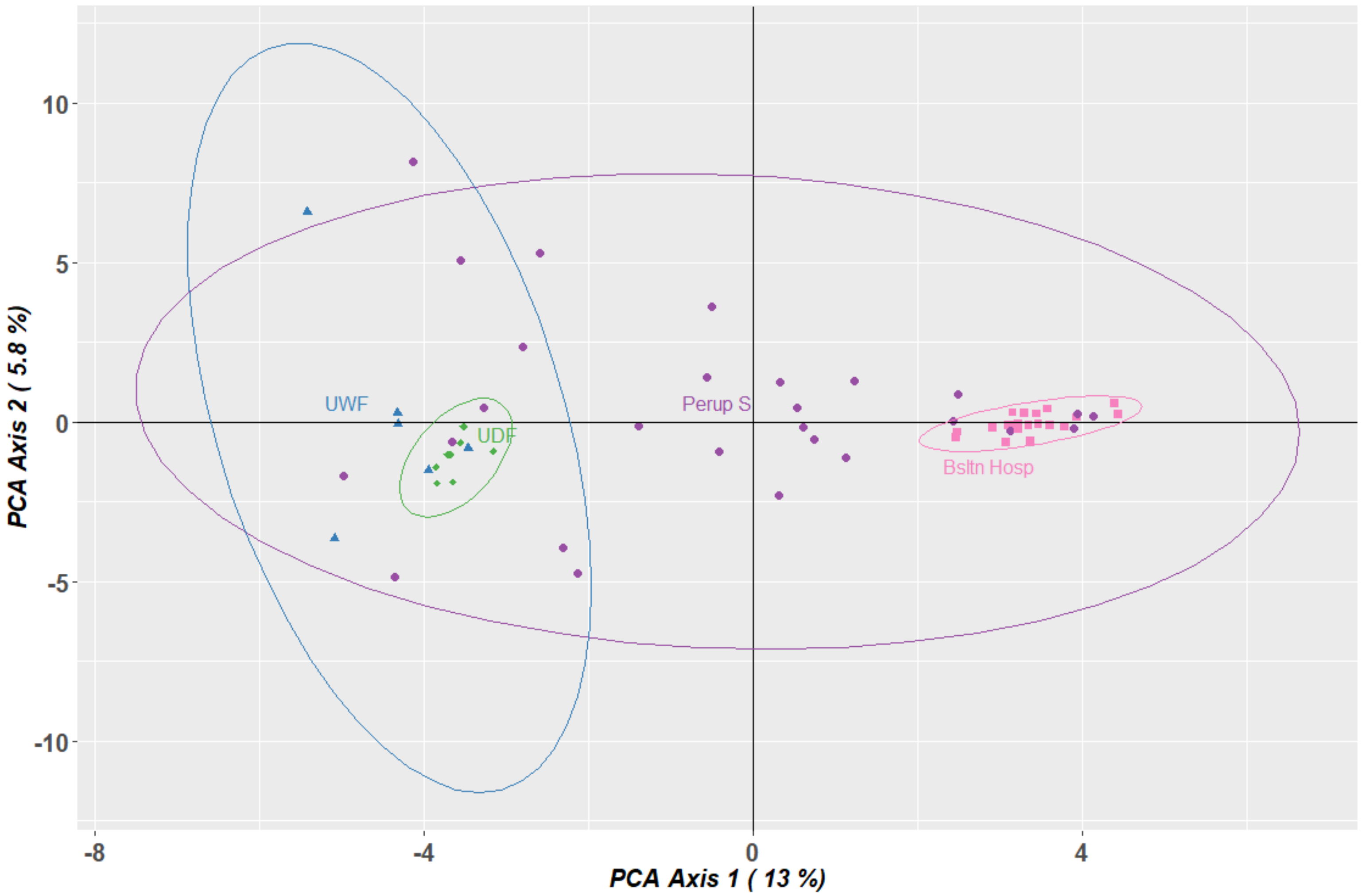

Figure 18a. *PcoA of Perup Sanctuary and founder populations at the individual level showing 95% confidence ellipsoids. X and Y axes are the first and second principal coordinates, respectively, with percentage of variation explained in brackets. Blue triangles: Upper Warren founders; green diamonds: Upper Donnelly founders; purple circles: Perup Sanctuary; pink squares: Busselton Hospital.*

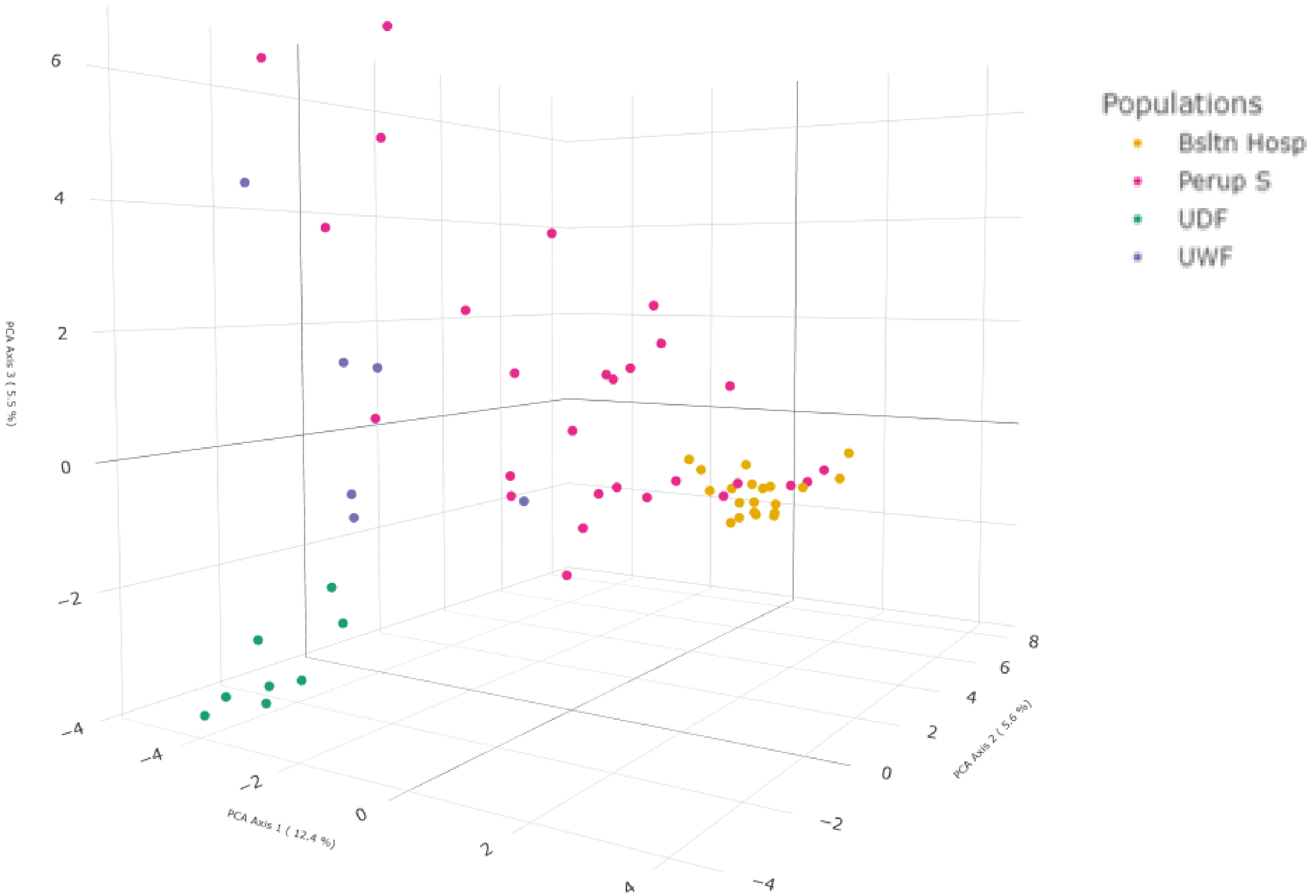

Figure 18b. *PcoA of Perup Sanctuary and founder populations at the individual level X, Y and Z axes are the first, third and second principal coordinates, respectively, with percentage of variation explained in brackets.*

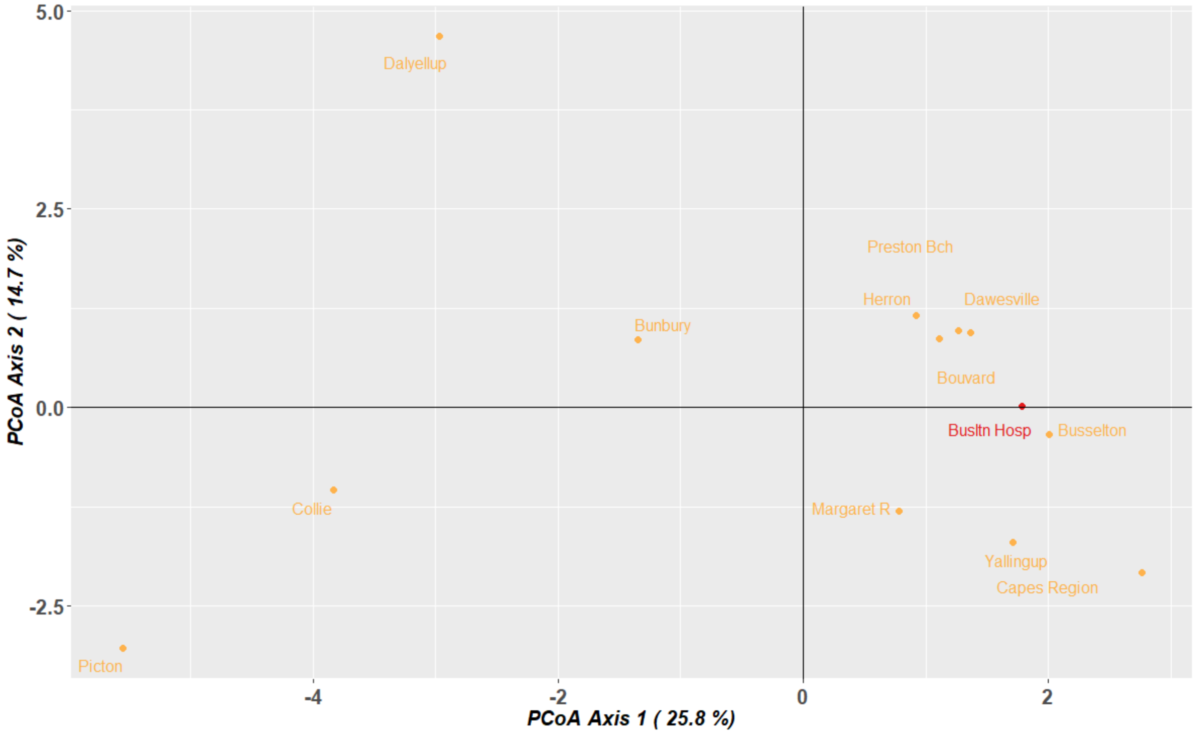

Figure 19. *Principal coordinate analysis (PcoA) of West Coast locations including the Busselton Hospital founders, at the population level. X and Y axes are the first and second principal coordinates, respectively, with percentage of variation explained in brackets.*

Structure analysis revealed the contributions from each of the founder populations to Perup Sanctuary. When the number of genetically defined clusters, k, was set to 2 the two genetic clusters were defined by the Busselton Hospital population and the Southern Forest populations (Figure 20). When k was set to 3, two genetic clusters were defined clearly by the Busselton Hospital population and the Upper Donnelly founders, and the third genetic cluster was defined by Upper Warren which shows some admixture with the Upper Donnelly animals (Figure 21). Interestingly, the Q value (or percentage contribution) in the Perup Sanctuary from the Busselton Hospital population was almost identical for both k=2 and k=3 at 46.2% and 46.7%, respectively.

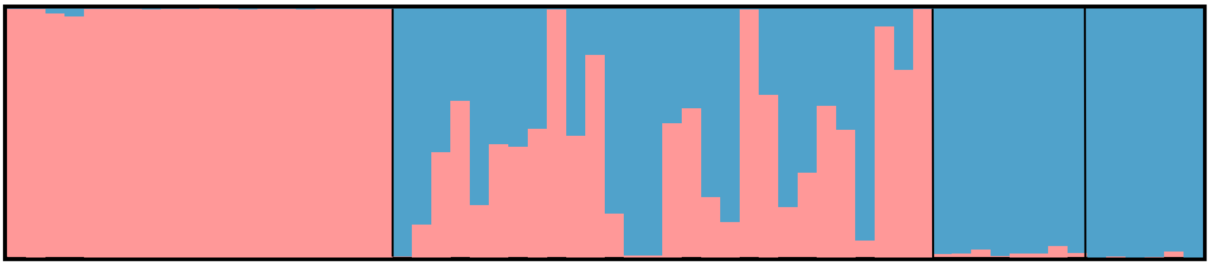

Figure 20. *Structure analysis for Busselton Hospital, Perup Sanctuary, Upper Donnelly founders (UDF) and Upper Warren founders (UWF), k = 2. Each vertical line represents an individual grouped by pre-assigned population, with Q scores (measure of admixture) on y axis. Population-level Q scores tabulated below.*

	% Genetic cluster 1 (pink)	% Genetic cluster 2 (blue)
Busselton Hospital	99.7	0.3
Perup Sanctuary	46.2	53.8
UDF	2.8	97.2
UWF	0.9	99.1

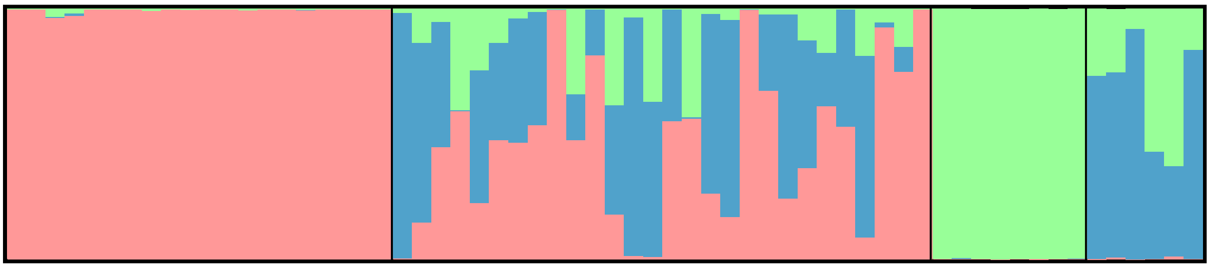

Figure 21. *Structure analysis for Busselton Hospital, Perup Sanctuary, Upper Donnelly founders (UDF) and Upper Warren founders (UWF), k = 3. Each vertical line represents an individual grouped by pre-assigned population, with Q scores (measure of admixture) on y axis. Population-level Q scores tabulated below.*

	% Genetic cluster 1 (pink)	% Genetic cluster 2 (green)	% Genetic cluster 3 (blue)
Busselton Hospital	99.4	0.6	0.1
Perup Sanctuary	46.7	9.2	44.1
UDF	0.0	99.9	0.1
UWF	0.1	28.2	71.7

The genomic relatedness matrix network using the most relaxed relatedness threshold (r=0.004), suggests the maximum number of breeders from the founder animals is ten in total: five from the Upper Warren, one from the Upper Donnelly and four from Busselton Hospital (Figure 22). The relatedness threshold does not alter how individuals are clustered, but rather determines whether a link is drawn between individuals.

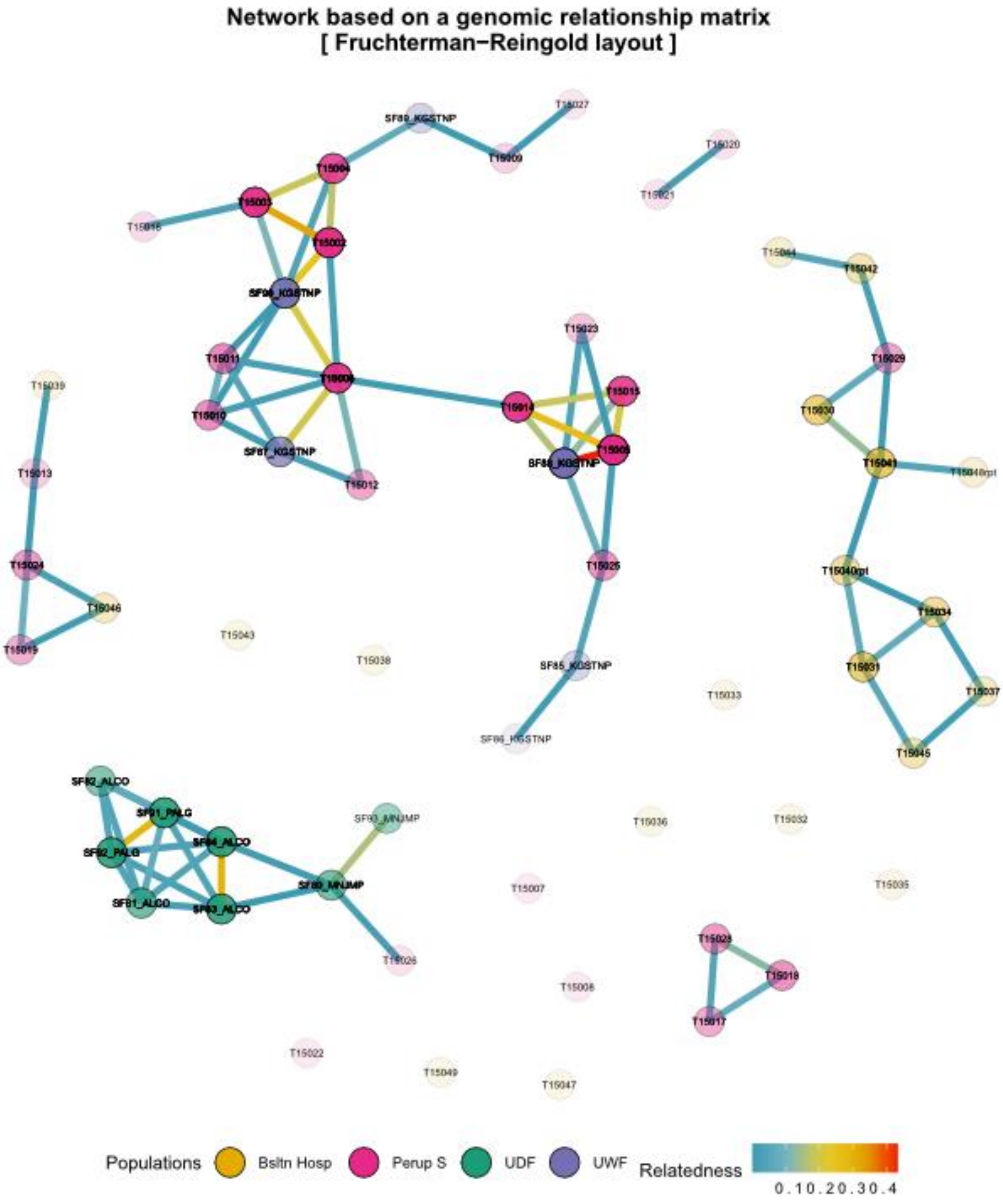

Figure 22. *Genomic relationship among the Perup Sanctuary and founder animals (Busselton Hospital (Bsltn Hosp), Upper Donnelly (UDF) and Upper Warren (UWF)) as a network diagram. Each circle represents an individual, colours indicate subpopulation and shade of colour represents how many relatives an individual has (the darker the shade the more relatives). Codes indicate individual ID. The colour of the link between individuals indicates the degree of relatedness.*

5 Discussion

This report constitutes the most comprehensive study of ngwayir in the Perup Sanctuary and the most intensive survey of ngwayir in the southern forests to date. It demonstrates that the translocated ngwayir population in the Perup Sanctuary has been a success with the successful establishment of founders from three different sources, subsequent recruitment and growth in the population and comparably good levels of genetic diversity. Robust population estimates for ngwayir and koomal are based on a systematic survey of 85% of the sanctuary (423 ha) using pedestrian-based spotlight methods and distance sampling modelling. It provides an insight into the spatial distribution of both possum species throughout the sanctuary, their associations with habitat types and fire history and identifies species differences in habitat use. Temporal changes in these populations can also be inferred using other available evidence. Biometric data indicates that ngwayir individuals are in good condition and the adult females are breeding. Genotyping a sample of the ngwayir population using powerful modern methods provides a good understanding of the genetic characteristics of the Perup Sanctuary and how it relates to the source populations of founders – Busselton, Upper Warren, and Upper Donnelly. Evidence from the genetics and radio-telemetry monitoring of the founders following their release provides an indication of the survivorship of the founders and the genetic information demonstrates that all three cohorts are represented to varying degrees in the current population. All these insights are directly relevant to informing the ongoing management of the Perup Sanctuary and the ngwayir population within. This body of work also constitutes a good baseline for ongoing monitoring, which is essential for the efficient and effective delivery of important conservation outcomes.

5.1 Ngwayir population

Density

The density of ngwayir within the Perup Sanctuary (0.37 ngwayir ha^{-1} (95% CI: 0.30 – 0.45)) is substantially greater than the estimates from similar methodology for wild ngwayir in comparable habitat surrounding the Perup Sanctuary (0.004 – 0.2 ngwayir/ha in 2019 and 0 – 0.1 ngwayir/ha in 2022 (Wayne et al 2022b)). The lack of terrestrial predators (feral cat, fox and chuditch) within the Perup Sanctuary is the main difference with the habitat outside the Perup Sanctuary, suggesting that predation may be a major limiting factor in wild ngwayir populations. However, the densities within the Perup Sanctuary are also well below the peak densities found in the Upper Warren region (<1.3 ngwayir ha^{-1} in 2022), just 10 km to the north of the Perup Sanctuary, suggesting that the carrying capacity within the Sanctuary may be potentially higher than current densities. It is also possible that the ngwayir population is at or close to carrying capacity given the relatively modest minimum growth rates required to reach the estimated 156 ngwayir (95%CI: 126-192) in 2022, however adequate ongoing monitoring is necessary to confirm whether this is the case. To what extent competition from koomal may also be limiting the ngwayir population in the Perup Sanctuary remains unknown. Nonetheless, the Perup Sanctuary provides an opportunity to investigate further the role of predation and competition in limiting ngwayir populations in this habitat.

Health and condition

All 28 ngwayir individuals captured during the 2022 survey were in good health with no obvious significant health issues and low ectoparasite loads. Evidence of breeding included numerous observations of young at heal with adults and all captured adult females (6) had young at heal and two were also carrying a second small pouch young.

5.2 Koomal population

The koomal population inside Perup Sanctuary in 2022 is estimated to be 0.83 possums ha^{-1} (95% CI: 0.64 – 1.10) and 350 koomal individuals (95% CI 271.7 – 452.5). This is more than double the population estimate in 2015 by Barrett (2016; (0.37 koomal ha^{-1}, 95% CI: 0.23-0.61). It is also higher than the wild koomal average density estimate of 0.5 koomal ha^{-1} (95% C 0.45 – 0.56 ha^{-1}) derived from similar methodology on across 38,349ha of DBCA-managed lands surrounding the Perup Sanctuary, but less than maximum modelled densities in some areas of up to 1.3 koomal ha^{-1} (unpublished data associated with Wayne et al 2022b).

5.3 Habitat use

Understanding the factors that affect the distribution and abundance of ngwayir and that drive population change are fundamental to inform effective management and conservation efforts for this species. Research 20 years ago found that the relative abundance of the ngwayir across the Upper Warren was patchy and related to fire, timber harvesting, fox control and forest fragmentation (Wayne et al. 2006). However, the ngwayir population in the region is expected to have changed considerably since, with declines continuing or being sustained in some areas.

The ngwayir population in the Perup Sanctuary in 2022 appears to be relatively evenly distributed throughout (Figures 7 and 12). Ngwayir densities did not vary significantly with fire age (5 versus 22 years) or vegetation complex and there was a possible weak positive association of ngwayir density with koomal density. By comparison koomal densities were spatially more variable and positively associated with the three valley vegetation complexes (particularly Yerraminnup flats but also Yerraminnup and Catterick). These results vary somewhat from our *a priori* expectations that ngwayir densities would be at lower densities in the younger fire age and positively associated with valley vegetation complexes.

The lack of a fire regime effect on the ngwayir density within the Perup Sanctuary is particularly helpful for informing sustainable fire management that is conducive with ngwayir conservation. Given the results from this study it suggests that fire can be applied in a manner that does not have significant adverse effects on the ngwayir population in the medium term (5 years post burn). The low fire intensity and the absence of terrestrial predators (i.e., removing the potential for adverse interactive effects between fire and predators) are likely to be particularly important factors here. However, well-designed scientific studies are needed to better understand ngwayir responses to fire and the relative importance of the factors involved and their interactions (e.g., fire intensity, temporal attributes (frequency, duration, timing), spatial attributes (extent, patchiness, grain size), other environmental factors (e.g., weather) and context (e.g., landscape factors, other threats and drivers of population change, disturbance history).

The results demonstrate that ngwayir can and do occupy vegetation across the landscape including valleys, midslopes, and uplands alike. It is also plausible that competition from koomal may have limited the densities of ngwayir in at least the more mesic / productive areas (i.e., valley vegetation complexes), which are otherwise expected to be their preferred habitat (e.g. Jones et al. 1994). Supporting evidence for this includes ngwayir founders from Busselton having showed a clear and strong preference for Yerraminnup flat vegetation complex (Yef) relative to its proportional representation within the Sanctuary during their initial months after their introduction in 2012. This was at a time when no other ngwayir were known within the Perup Sanctuary and when koomal numbers are thought to have been less than half their densities in 2022. The disproportionate use by the Upper Warren-Upper Donnelly cohort of the Bevan 2 (upland) vegetation complex in 2016 – 2017 most likely reflects the limited availability of the preferred habitats through competition with both the existing resident ngwayir population and the increased density of koomal at the time.

Similarly, differences between ngwayir and koomal in their nocturnal use of arboreal habitat (height above ground and DBH of occupied trees), suggests that there may be some resource partitioning, however the degree to which this may be a result of habitat preferences versus competition is not clear. Nonetheless, the differences in habitat use observed in the Perup Sanctuary are very similar to the observations made elsewhere in the Upper Warren region including the so called ‘hotspot’, where most ngwayir used trees <40 cm DBH, and most koomal used trees greater than 40 cm DBH (Wayne et al. 2022b), and Greater Kingston area (Wayne et al. 2005, unpublished data). Other examples of habitat use differences between these species include the characteristics of den trees used as diurnal shelter in the Upper Warren (Wayne 2005); and nocturnal heights above ground in tuart (*Eucalyptus gomphocephala*) and peppermint (*Agonis flexuosa*) woodland in Tuart Forest National Park, near Busselton (Grimm & de Tores 2009).

5.4 Ngwayir Survivorship

The survivorship of Busselton founders translocated in 2012 was less than that of the subsequent founders from the Upper Warren-Upper Donnelly, translocated in 2016-2017. Differences in the habitat and environment and/or timing of the releases are the most likely reasons for these differences.

Differences between the Busselton and Upper Warren-Upper Donnelly source sites relative to the receiving site (Perup Sanctuary) were substantial. The Busselton site was an urban coastal remnant peppermint tree woodland with a maritime-moderated climate. The diet of ngwayir would have been predominantly peppermint trees (*Agonis flexuosa*) and dreys were probably the main form of diurnal shelter (Jones and Hillcox, 1995; Jones et al., 2004). The Upper Warren-Upper Donnelly source sites would have had comparatively very similar habitat and climate to that of Perup Sanctuary; intact jarrah/marri dominated native forest, with an inland climate (i.e., higher daily and seasonal variation in ambient temperature), in which the diet is predominantly jarrah, marri and Melaleuca, and diurnal shelter sites used by ngwayir are predominantly tree hollows (Wayne et al. 2000, Wayne 2005). Evidence for the potential additional challenges that these environmental differences may have had on Busselton founders include observations shortly after translocation of ngwayir resting during the day either on the ground at the base of trees (in the first few days after release) or on the open limbs of branches about two to four meters off the ground. This was never observed of the Upper Warren-Upper Donnelly founders (observed using tree hollows six or more metres above ground).

The founder cohorts were also released at different times (years and seasons): Busselton in Spring 2012 and Upper Warren-Upper Donnelly years later in Autumn (15 in March – May 2016 and 3 in February – April 2017). However, the extent to which this may have disadvantaged the Busselton cohort may have been negligible for the first month or two. On the contrary, Upper Warren-Upper Donnelly animals may have been disadvantaged by the drier seasonal conditions of autumn compared with spring and the site having already been occupied by Busselton founders and their offspring (i.e., both factors limiting the availability and quality of important resources such as food, shelter, water, and space). Well-designed scientific studies are needed to better understand the factors determining the success and survivorship of ngwayir during translocations/relocations, including habitat differences between source and recipient sites, time of year, intra and interspecific competition and predation.

While a number of ngwayir translocations have been conducted over the last 40 years, few have reported the outcomes. Many are thought to have not been successful. At Locke Nature Reserve, a site with high density resident populations of ngwayir, a trial translocation failed with four of the five radio-collared ngwayir being predated within six weeks of release in 1991 (cited in Clarke 2010). Releases in Yalgorup National Park 1995-2001 resulted in a persistent population at the White Hills site, but undetectable levels at other Yalgorup sites (Jacques 2012 pers com). Translocations to Leschenault Peninsula Conservation Park (1991-1995) were initially successful but this was followed by major declines for unknown reasons but thought to be linked to predation and mesopredator release (de Tores 2009). Translocations to Lane Poole and Keats Block were deemed to have failed because of predation, principally by chuditch and fox. Clarke (2010) aimed to determine which factors limit translocation success at three locations and found that mortality rates were high, and most deaths were attributed to predation (foxes, cats, pythons, and raptors) but involved complex interactions of health, predation, habitat quality and inter-specific competition. The study concluded that the complete exclusion of exotic predators (cats and foxes) from translocation sites may be necessary in the future coupled with selecting sites with larger carrying capacities, and reduced numbers of brushtail possums (Clarke 2010). While the translocated ngwayir population in the predator-free Karakamia Sanctuary (275 ha) was frequently detected for 17 years, no animals have been detected for more than 5 years: 55 individuals were released between 1995 and 2012, the last record was in 2017 and repeated surveys since have not found any ngwayir; Nieuwhof, 2022; Amanda Bourne *pers. Comm.*).

5.5 Ngwayir genetics

Genetic diversity analysis has shown that the Perup Sanctuary lies within the range of diversities seen within the subpopulations, in terms of both expected heterozygosity and observed heterozygosity. In fact, these values are higher in Perup Sanctuary than in both the Upper Donnelly and the Busselton Hospital founders, but not the Upper Warren founders. This is also reflected in allelic diversity. Interestingly, the inbreeding coefficients based on the unbiased estimate of expected heterozygosity were positive for every subpopulation. Values were a lot closer to 0 when He was not corrected for sample size. Further, Ho and He were not significantly different from each other in any of the subpopulations. This suggests that the sample size correction used in the unbiased estimate of He may be over correcting, leading to overestimates of He and consequently inbreeding coefficients. Nonetheless, when considering FIS estimates based on uncorrected He (Table 1), Manjimup remains an outlier at 0.25, and the Upper Donnelly founders and Orient Road subpopulation are also relatively high (0.10). The high value in Manjimup is consistent with previous findings (White et al. 2021) and suggests there is inter-relative breeding in Manjimup ngwayir. The Perup Sanctuary FIS is below these at 0.08, but higher than most other Upper Warren subpopulations, and the Busselton Hospital founders. However, considering the Perup Sanctuary is an admixed population and may still retain structure, this FIS estimate may be an overestimate due to the Wahlund effect (Hedrick 2011). Repeating this measurement after several generations when founder individuals, and their genomes, are more mixed will give a more reliable result. Effective population size estimates were possible for five populations, ranging from as high as 1879.4 (95% CI 151.3 - ∞) in the Upper Warren founders to as low as 13.4 (95% CI 13.3 – 13.5) in the Perup Sanctuary. The Perup Sanctuary Ne (13.4) is 39% of the total number of founders (Nf=38), and 9% (13.4/156) of the estimated number of ngwayir in the Perup Sanctuary in 2022. The relatively low Ne of 13.4 is likely a result of the small Nf for Perup Sanctuary with a subsequent lack of immigration. However, this result should also be treated with caution as population substructure can lead to downward biases in estimates of Ne. The Ne for Busselton Hospital is 64% (51/80) of the census population estimate for the site at the time (Williams and Barton, 2012) and the Ne for the Upper Warren region is 26% (1894/7103) of the estimate of the census population within the Upper Warren hotspot in 2022 (38,349 ha; Wayne et al. 2022b).

The relatively large number of private alleles in Perup Sanctuary indicates that genetic variation exists in the sanctuary that has not been sampled in any of the other populations. This is surprising, considering that all founder populations were present in the analysis. It may be that multiple factors are giving rise to this result.

For example, as the Southern Forest subpopulations are being analysed separately the number of private alleles that each harbour will be kept low as these subpopulations are genetically similar and likely share much genetic variation. In addition, it was not possible to sequence four Perup Sanctuary founders and at least one of them (sourced from Talling, 23km from the nearest cofounder) was likely a source of unique genetic variation. Of interest, private allele analysis did not show Pemberton to be an outlier as in previous work (White et al 2021). This could be due to larger sample sizes being used for Upper Warren and Upper Donnelly subpopulations in the current analysis, in particular both Manjimup (21 v 8) and Orient Road (59 v 20) sample sizes are substantially larger in the current analysis.

The Perup Sanctuary is currently an admixed population, and its animals show varying amounts of genetic similarity to each of its founder populations. Admixture analysis (Figures 20 and 21) shows 46% to 47% of current genomic material is contributed from the Busselton Hospital founders, and 53% to 54% is contributed from the Southern Forest founders. Of the Southern Forest founders, around 44% of genomic material originates from the Upper Warren and 9% from the Upper Donnelly. Overall, this suggests a relatively equal contribution from Busselton Hospital and the Southern Forest founder animals, but that the Upper Warren animals have been more successful breeders than the Upper Donnelly animals.

Interestingly, although the sample size of Yackelup is small (n=2), it appears that Perup Sanctuary is distinct from Yackelup, the Upper Warren subpopulation that surrounds the Perup Sanctuary. This supports the original observation that there were no local residents extant at the site prior to the translocations and provides evidence that there may have been little or no genetic exchange ‘over the fence’ since translocation, particularly immigration into the Sanctuary.

The relatedness analysis suggests that the Perup Sanctuary population has derived from at most ten of the founder individuals, mostly from Upper Warren and Busselton Hospital and one from Upper Donnelly. This is consistent with our estimate of Ne for Perup Sanctuary, and with our admixture analysis. Considering there were 34 founder animals, this suggests around 30% of the translocated ngwayir successfully reproduced. These results also demonstrate that there is no obvious barrier to inter-breeding between ngwayir from different genetically defined clusters of Busselton and the Southern Forest subpopulations.

5.6 Gumleaf skeletonizer outbreak

An outbreak of gumleaf skeletonizer (GLS; *Uraba lugens*) across approximately 100,000 ha of the Upper Warren region in Spring 2022 – Summer 2022-23 (Figures 23 & 24; Wills 2023), is likely to have a major impact on the ngwayir population. The effects of tree decline on fauna have been demonstrated elsewhere (Flemming et al. 2021) and are expected to be significant for arboreal folivores such as the ngwayir and koomal, that are dependent on the tree canopy for their staple diet. Depending on the severity, extent, and duration of the outbreak it could take many years or decades for the ngwayir population to recover. There is also a risk of local extinction.

The GLS outbreak effects on the forests within the Perup Sanctuary was relatively minor in 2022-2023, however the effects were severe in forests 5 km to the west and south of the Sanctuary (personal observations). The location, extent, and severity of GLS outbreaks are largely driven by climatic conditions: outbreaks occur in normally wetter areas that were subject to an unusually dry autumn (Wills and Farr, 2017; Wills 2023). Historically these outbreaks have been more severe and frequent west of Manjimup. However, climate change trends may mean the Upper Warren region, including the Perup Sanctuary, is at increasing risk of GLS outbreaks in the future. Most of the current hotspot for ngwayir in the Upper Warren region (north of the Perup Sanctuary; Wayne et al. 2022a&b) was less severely impacted by the current GLS outbreak, but a future outbreak in this area could be catastrophic for the population and of major significance to the conservation of the species. Similarly, there is a risk to the viability of the ngwayir population within the Perup Sanctuary, were a severe GLS outbreak to occur. A greater understanding of the risks to the ngwayir population in the Upper Warren and Perup Sanctuary is urgently needed and essential for the effective conservation and recovery of these populations.

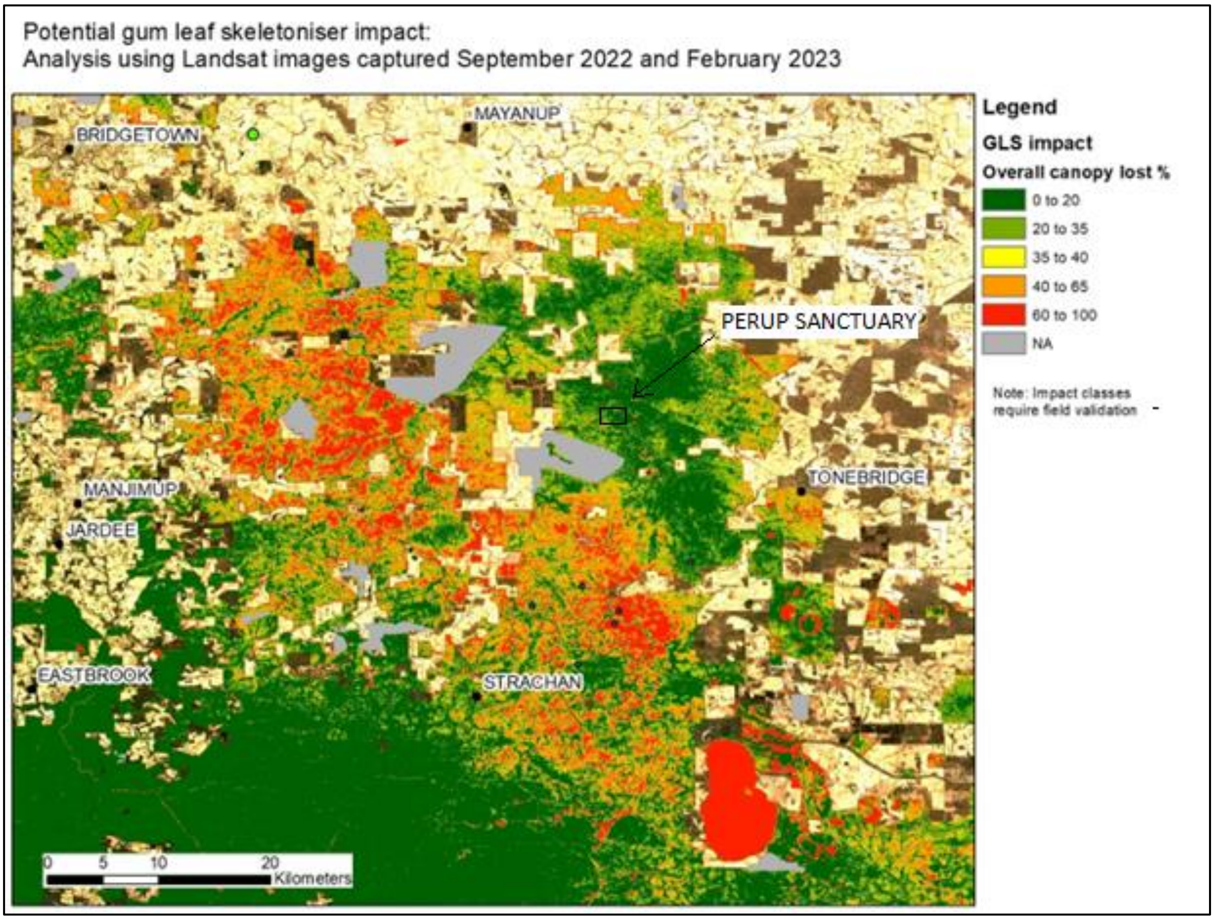

Figure 23. *An unfiltered map of potential gum leaf skeletonizer impact on canopies in the Upper Warren, east of Manjimup. Some canopy loss is attributable to normal summer and autumn leaf loss, also to drought effects on the shrub layer in the northeast woodlands. Unmasked water bodies and silviculture tend to show as red. Masked areas in grey are areas that were prescribed burnt in the year prior to the Landsat images recorded in September 2022 and February 2023, used to assess the loss of leaf area. Image courtesy Ricky Van Dongen, DBCA. Source: Wills (2023)*

Figure 24. *Defoliated canopies of jarrah along Mordalup Rd in Tone-Perup Nature Reserve, February 2023. Photo courtesy Adrian Wayne.*

5.7 Recommendations:

Key recommendations for the management of the ngwayir population in Perup Sanctuary relate to regulating predation and competition to optimise the size and security of the population, genetic management to optimise the quality and value of population, maintaining an adequate monitoring and surveillance program to have timely and sufficient information on spatiotemporal changes in the ngwayir population and their habitat across the southern forest region and within the Perup Sanctuary, improving the understanding of the risks to these populations, and continue to improve the effectiveness of management and conservation activities to deliver better conservation outcomes for this and other threatened species.

5.7.1 Introduced predator management

Mitigating the effects of introduced predators on the wild and havened ngwayir populations is imperative to their ongoing persistence. Lethal control of foxes and feral cat is a major component of integrated introduced predator management. But it also involves co-ordinating these control activities with other management activities and disturbance factors such as prescribed burning and wildfire, respectively. Co-ordinated management across the landscape involving all land tenures is also expected to be more effective. Management also includes reducing food resources for introduced predators and limiting recruitment through breeding and emigration. Improving habitat quality for ngwayir can also improve the resilience of their populations by reducing their risks of predation, increasing their avoidance of predators, and increasing their breeding and recruitment potential sufficiently to withstand the predation losses that do occur.

Maintaining the exclusion of introduced predators within the Perup Sanctuary is essential to maximise the populations of ngwayir and woylies. This includes maintaining the integrity of the terrestrial predator exclosure fence (including the ‘hot’ wire [electric] system) and access gates, limiting human access, monitoring for predator incursions, and having an adequate preprepared rapid response plan and resources to remove an introduced predator in the event there is an incursion.

5.7.2 Management of the Perup Sanctuary and ngwayir population

A management plan for the Perup Sanctuary is needed to set clear directions on the objectives and priorities for the facility and the species it supports. This is particularly so for the insurance population of the *Critically Endangered* woylie and the translocated ngwayir population. This includes determining whether the latter should be managed as an insurance population and for what purpose. The management of these populations should be strongly guided by the conservation and recovery strategies for these species. The management plan should also address the other recommendations made here in this report, relating to predators, fire, forest diseases and other risks to the integrity of the sanctuary and the viability of the priority species and habitat within, and monitoring of the priority species, the habitat and factors potentially driving change.

With terrestrial predators excluded, it is expected that the ngwayir population is regulated by ‘bottom-up’ (e.g., food resources) rather than ‘top-down’ (e.g., predation) factors. Such populations maybe at risk of overshooting the food resource supply needed to support the population, causing the population to decline as well as habitat change or degradation. Similarly, when at carrying capacity the ngwayir population will be more strongly regulated by the factors that affect the quantity and quality of their food resources, such as seasonal and climate changes and the abundance of other consumers competing for the same food resource (e.g., gumleaf skeletonizer). The risks and consequences of these phenomena need to be carefully considered with respect to the management of the sanctuary and the ngwayir population.

If the priority is to optimise the conservation value of the ngwayir population within the Perup Sanctuary, then the reduction or removal of koomal from the Perup Sanctuary may be the most feasible and cost-effective way to increase the size (and value) of the ngwayir population. It also presents a unique opportunity to investigate the extent to which ngwayir are limited by competition with koomal. It is also an opportunity to assist in the recovery of koomal distribution and abundance having been lost from more than half of their former range, by providing a ready source of founders for reintroduction or supplementation elsewhere. Any genetic supplementation of the ngwayir population in the Perup Sanctuary should immediately follow the removal of the koomal to maximise their prospects of survival when interspecific competition for resources (principally food and shelter) is least and before the resident ngwayir population increases in response to the increased accessibility to these resources (i.e., before intraspecific competition increases).

Maintenance of the Sanctuary fence has been effective in reducing or preventing the emigration of ngwayir and to a lesser extent koomal. The main barriers for possum movement in and out of the Sanctuary are the internal and external electric ‘hot’ wires and the outward facing floppy top. Nonetheless, ngwayir and koomal have been observed climbing up and back down the inside of the fence, seemingly impeded to go beyond the floppy top. The pressure to emigrate is expected to be greater when the population is at or above carrying capacity. Allowing some ‘leakage’ of animals may be a simple and ‘passive’ way to manage the ngwayir population within the Sanctuary. It may also facilitate an increase in the ngwayir population in the surrounding forest (i.e., source-sink dynamics). Regardless, careful consideration is needed regarding the implications of Sanctuary ‘escapees’ into the surrounding population, particularly regarding the introduction of locally novel genetics into the wild indigenous population, including genotypes from Busselton and Upper Donnelly. Consideration should also be given to the merits of using the ngwayir population within the Sanctuary as a source of ngwayir for deliberate translocations elsewhere.

5.7.3 Genetic management of ngwayir in the Perup Sanctuary and elsewhere

Genetic diversity estimates (observed heterozygosity and allelic diversity) are relatively good in the Perup Sanctuary. Values of these parameters were intermediate between Upper Warren (most diverse) and Busselton (least diverse) populations, however the inbreeding coefficient (FIS) is moderate, and effective population size (Ne) is relatively low. Whilst admixture between differentiated populations may bias these latter estimates, continued genetic and life history monitoring for signs of inbreeding is recommended for the Perup Sanctuary, especially considering this population appears genetically isolated from the broader UW population. Although there are no obvious signs of inbreeding depression, ongoing monitoring is also recommended for the Manjimup subpopulation due to the extreme FIS values detected at this site. Similarly, the Busselton subpopulation appears to be experiencing genetic erosion, likely because of ongoing habitat loss and fragmentation in this region. Further conservation attention may need to be paid to this population in the context of climate change.

To increase Ne and genetic diversity in Perup Sanctuary, and lower the inbreeding coefficient, supplementation is recommended. This is best achieved using animals unrelated to the founders and from as genetically different from the Perup Sanctuary population as possible, depending on the objectives for the Perup Sanctuary population. For example, if the objective is to have a population that is genetically representative of the three original sources of founders, then the priority would be in the following order: unrelated animals from Upper Donnelly, Busselton, and Upper Warren locations geographically distant to the Upper Warren founders (e.g., Talling). However, Upper Donnelly and Busselton populations appear to exhibit lower genetic diversity than the Upper Warren populations. If prioritising genetic diversity over representation, then sourcing from additional Upper Warren populations would provide the quickest improvement in heterozygosity and effective population size. A genetically informed population viability analysis would assist in evaluating these scenarios and determining the numbers of animals needed from each source population to maximise genetic diversity and/or representativeness, as well as predict any impact of such a harvest on the source populations.

Between 20% and 60% of Busselton founders (2012), 9% and 91% of Upper Donnelly founders (2016-2017) and 57% and 100% of Upper Warren founders (2016-2017) subsequently contributed to the current gene pool within the Perup Sanctuary having survived (the first estimate for each cohort being based on the maximum number of breeders identified by the relatedness analysis and the second estimate being based on the survivorship result from radiotelemetry). Several factors may affect founder survivorship and reproductive success. The Busselton founders came from different habitat and were introduced into an area with relatively low competition (low abundance of koomal and no conspecifics) and no terrestrial predators. While the Upper Donnelly and Upper Warren founders came from similar habitat with relatively higher competition and at a different time of year. The differential survivorship and reproductive successes observed in the Perup Sanctuary may help inform future ngwayir translocations, considerations of acceptable survival rates for translocation and relocation activities, modelling of adequate founder sizes needed to establish desirable levels of genetic representativeness and diversity, and identifying important factors related to the success and survivorship of translocated/relocated ngwayir.

Continued genetic monitoring of the Perup Sanctuary ngwayir population will further elucidate the population trajectory and may act as a trigger for further population intervention. It is also recommended that further genetic samples and analyses focus on the current knowledge gaps in the ngwayir distribution and includes sites that are underrepresented (e.g., small sample sizes) and/or are currently considered genetic outliers identified in this study (Yackelup, Picton, Collie, and Dalyelup) and in White et al. (2021; Lower Blackwood River, Pemberton, and Two People’s Bay). Genetic samples from outside the Perup Sanctuary would also confirm whether there has been any emigration and/or immigration between the Perup Sanctuary and the surrounding wild population.

Efforts should also be made to prevent substantial and irreversible loss of the high levels of genetic diversity observed in the Upper Warren, which are at risk due to recent declines and the existence of current and future risks (e.g., introduced predators, gumleaf skeletonizer outbreaks, etc).

5.7.4 Monitoring and surveillance

Adequate monitoring and surveillance of the spatiotemporal changes in the ngwayir population and their habitat across the southern forest region and within the Perup Sanctuary is fundamental to effective and efficient natural resource management and threatened species conservation. Knowing population trends can help identify circumstances, such as population declines, that may require intervention. It can also inform whether the ngwayir population within the Perup Sanctuary is at carrying capacity, which can have implications for the success of any potential genetic augmentation efforts. It is similarly important to know whether there are changes in the condition of the habitat and the drivers behind these changes. For example, whether the ngwayir population is having an adverse effect on parts of the ecosystem (e.g., preferred food sources) and whether insect outbreaks may affect the resources available to the ngwayir population. Good monitoring and surveillance data used in conjunction with good covariate data can be a powerful scientific way of identifying what are the major drivers responsible for the spatio-temporal differences in the ngwayir population (e.g., predators, climate change, fire, insect outbreaks) and their responses to management activities such as introduced predator control, prescribed burning, and harvesting koomal within the Perup Sanctuary. Other important aspects of the ngwayir population worth monitoring include health and condition, breeding, and genetics.

5.7.5 Improved understanding of the risks to ngwayir populations and the drivers of population change

The main threats to the conservation of the ngwayir are broadly recognised as including habitat loss and fragmentation as a direct result of human development, introduced predators, climate change, timber harvesting, fire, competition, tree decline and disease (Parks and Wildlife 2017). However, detail is needed on the nature of these threats, to better inform on-ground management and conservation. This includes the need for a better understanding of how these threats vary over space and time and how they interact with each other. Associative evidence derived from monitoring and surveillance data, as discussed above (section 5.7.4), can be a practical and useful way to improve our knowledge base. However, scientific evidence that demonstrates causation and the mechanisms and processes driving these changes/differences can verify these associations and can be used with more confidence to inform management to deliver better outcomes with more confidence. Targeted research using well-design experimental and manipulative approaches to investigate the roles of the most important drivers of population, are therefore recommended.

The current GLS outbreak is of immediate concern for the potential viability of the ngwayir populations across the Upper Warren and Perup Sanctuary. A clearer understanding of the risks and consequences of the current and future outbreaks is necessary for the management of these areas and the conservation of these populations. A better understanding of the role of fire in GLS outbreaks would also be potentially instructive in terms of providing direction on how best to moderate potential future GLS outbreaks, which may then help mitigate the impacts on affected species such as the ngwayir. Having spatio-temporal predictive modelling of the short- and longer- term risk of GLS outbreaks and adequate monitoring of GLS and the effects on ngwayir are also needed, particularly if the risks and consequences of these outbreaks is significant as expected.

5.7.6 Improved management and conservation of ngwayir populations

Continue to improve the effectiveness of management and conservation activities to deliver better conservation outcomes for this and other threatened species is also recommended. Planned burning for multiple objectives (e.g., wildfire mitigation, asset protection and promoting conservation values) is especially relevant here. Managing forest health and hygiene and reducing the impacts of forest diseases such as jarrah dieback (*Phytophthora cinnamomi*), jarrah leaf miner (*Perthida glyphopa*), GLS and myrtle rust (*Puccina psidii s.l.*) is also locally important. With the cessation of native timber harvesting beginning in 2024, consideration should also be given to enhancing the habitat values of the large areas of forest that have been subject to timber harvesting in the past.

The recovery plan for the ngwayir (Parks and Wildlife 2017), provides relevant detail here, particularly in relation to Recovery Objectives,

1. “Habitat critical for survival for western ringtail possums is identified and protected…
2. “Threatening processes that are constraining the recovery of western ringtail possums are mitigated…”
3. “An evidence-based approach is applied to the management and recovery of western ringtail possums….”

A strategic assessment is also recommended with respect to whether insurance populations are needed for the ngwayir. This would include a risk assessment of the loss of abundance, distribution and genetic diversity at the species and subpopulation levels. On first principles the conservation and recovery of these subpopulations should preferentially be done in situ. However, in extreme cases, it may well be that for some subpopulations their viability cannot be satisfactorily secured *in situ* and an insurance population or translocation may be justified. While the results of this study demonstrate that admixture from different subpopulations from different habitats can be successful, it remains to be determined whether this is the best option for the conservation of the species. Where an insurance population is justified for one or more subpopulations, an evidence-based approach is needed to determine whether or not these be managed separately and located in similar environments (habitat, climate, etc.) to those that the source populations have behavioural and genetic adaptations for. These considerations should include a long-term view that addresses the threats to these populations and changing environmental conditions, including climate change and habitat change.

6 Photo Evidence

Figure 25. *Spotlighters that conducted the pedestrian-based spotlight surveys in the Perup Sanctuary, November 2022.*

Figure 26. *Catching team, November 2022*

Figure 27. *Captured ngwayir being processed.*

Figure 28. *Ngwayir being released.*

Figure 29. *Pair of ngwayir being released.*

7 References

Barrett A. (2016). Population estimates of the threatened western ringtail possum (*Pseudocheirus occidentalis*) and common brushtail possum (*Trichosurus vulpecula*) in conservation and harvested forests. Honours thesis. (Murdoch University, Western Australia.

Bates D, Mächler M, Bolker B, Walker S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67:1-8.

Buckland, S.T., Anderson, D. R., Burnham, K. P., Laake, J. L., Borchers, D. L. and L. Thomas (2001). Introduction to Distance Sampling. Oxford University Press. UK.

Burnham KP, Anderson DR (2002). ‘Model selection and multimodel inference: a practical information-theoretic approach.’ (Springer: New York, USA)

Buckland ST, Anderson DR, Burnham KP, Laake JL, Borchers DL, Thomas L (2004). Advanced Distance Sampling. Estimating Abundance of Biological Populations., Oxford University Press: Oxford, UK.

Clarke JR (2010). Translocation outcomes for the western ringtail possum (*Pseudocheirus occidentalis*) in the presence of the common brushtail possum (*Trichosurus vulpecula*): Health, survivorship, and habitat use. PhD thesis, Murdoch University.

DBCA (2019). Bushfire Risk Management Framework: Managing fuel hazards on public lands to control bushfire risk. Version 2.8. Department of Biodiversity, Conservation and Attractions, Perth, Western Australia.

DBCA (2020). Draft Regional Fuel Management Plan: Warren Region. Version 1. Department of Biodiversity, Conservation and Attractions, Perth, Western Australia.

DCCEEW (2022). Threatened Species Strategy Action Plan 2022–2032, Department of Climate Change, Energy, the Environment and Water, Canberra, September. CC BY 4.0.

DPAW (2008). Fire information note S8 Western Ringtail Possum. Fire Policy and Planning, Department of Parks and Wildlife. (Perth, Western Australia).

Department of Parks and Wildlife (2017). Western Ringtail Possum (*Pseudocheirus occidentalis*) Recovery Plan. Wildlife Management Program No. 58. Department of Parks and Wildlife. (Perth, Western Australia).

de Tores, P. J. (2009). A summary of research by Department of Environment and Conservation (DEC), Murdoch University and Curtin University on the western ringtail possum (*Pseudocheirus occidentalis*) on the southern Swan Coastal Plain and recommendations relevant to current research proposals *Australia.* Dept. of Environment, Water, Heritage and the Arts.

de Tores PJ, Elscot S. (2010). Estimating the population size of a threatened arboreal marsupial: use of distance sampling to dispense with *ad hoc* survey techniques. *Wildlife Research* **37**:512-523.

Do, C., Waples, R.S., Peel, D., Macbeth, G.M., Tillett, B.J., and Ovenden, J.R. (2014). NEESTIMATOR v2: Re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. *Molecular Ecology Research* 14, 209–214, doi:10.1111/1755-0998.12157.

Endelman, J.B. (2011). Ridge regression and other kernels for genomic selection with R package rrBLUP. *Plant Genome* 4:250-255. doi:10.3835/plantgenome2011.08.0024

Endelman, J.B., and Jannink, J.-L. (2012). Shrinkage estimation of the realized relationship matrix. *G3: Genes, Genomes, Genetics* 2:1405-1413. doi:10.1534/g3.112.004259

Ferrari S, Cribari-Neto F. (2004). Beta regression for modelling rates and proportions. *Journal of Applied Statistics* 31:799-815.

Fleming, P.A., Wentzel, J.J., Dundas, S.J., Kreplins, T.L., Craig, M.D., and Hardy, G.E.S.J., (2021). Global meta-analysis of tree decline impacts on fauna. *Biological Reviews*, *96*(5), pp.1744-1768.

Georges, A., Gruber, B., Pauly, G. B., White, D., Adams, M., Young, M. J., Kilian, A., Zhang, X., Shaffer, H. B., Unmack, P. J. (2018). Genomewide SNP markers breathe new life into phylogeography and species delimitation for the problematic short-necked turtles (Chelidae: *Emydura*) of eastern Australia. *Molecular Ecology* 27:5195-5213 doi:10.1111/mec.14925

Goudet J. (2005). Hierfstat, a package for R to compute and test variance components and F-statistics. *Molecular Ecology Notes* 5:184-186

Grimm HL, de Tores PJ. (2009). Some aspects of the biology of the common brushtail possum (*Trichosurus vulpecula*) and the threatened western ringtail possum (*Pseudocheirus occidentalis*) in a pine plantation scheduled for harvesting and in adjacent tuart and peppermint woodland near Busselton, Western Australia.

Gruber B., Unmack P.J., Berry O.F., Georges A. (2018). DARTR: An R package to facilitate analysis of SNP data generated from reduced representation genome sequencing. *Molecular Ecology Resources* 18:691-699 doi:10.1111/1755-0998.12745

Harrison, N.D., Thorn, S.M., Maxwell, M.A., Ward, C.G., Wayne, J.C., Wayne, A.F., (in review). Insuring woylies (*Bettongia penicillata ogilbyi*) against extinction: Establishment of Perup Sanctuary. *Wildlife Research.*

Hedrick P. W. (2011). Genetics of Populations 4th Edition. (Jones and Bartlett Publishers: Sudbury, MA)

Jombart, T. (2008). adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* 24: 1403-1405. doi:10.1093/bioinformatics/btn129

Jombart T, Ahmed I (2011). adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. *Bioinformatics* 27: 3070-3071 doi:10.1093/bioinformatics/btr521

Jones B and Hillcox S (1995). A survey of the possums *Trichosurus vulpecula* and *Pseudocheirus occidentalis* and their habitats in forest at Ludlow, Western Australia. *Western Australian Naturalist* **20**, 139-150.

Jones BA, How RA, and Kitchener DJ (1994). A field study of *Pseudocheirus occidentalis* (Marsupialia: Petauridae). II. Population studies. *Wildlife Research* **21**, 189-201.

Jones BA, Meathrel CE, and Calver MC (2004). Hypotheses arising from a population recovery of the western ringtail possum *Pseudocheirus occidentalis* in fire regrowth patches in a stand of *agonis flexuosa* trees in south-western Australia. In 'Conservation of Australia's forest fauna'. (Ed. D Lunney) pp. 656-662. (Royal Zoological Society of New South Wales: Sydney)

Kopelman N.M., Mayzel J., Jakobsson M., Rosenberg N.A., and Mayrose I. (2015). Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources* 15:1179-1191 doi:10.1111/1755-0998.12387

Linck E. and Battey C.J. (2019). Minor allele frequency thresholds strongly affect population structure inference with genomic data sets. *Molecular Ecology Resources* 19:639-647 doi:10.1111/1755-0998.12995.

Mattiske, EM and Havel JJ (1998). Vegetation Mapping in the South West of Western Australia and Regional Forest Agreement vegetation complexes. Map sheets for Pemberton, Collie, Pinjarra, Busselton- Margaret River, Mt Barker, and Perth, Western Australia. Scale 1:250,000. Department of Conservation and Land Management, Perth.

Miller DL, Rexstad E, Burt L, Bravington MV, Hedley S, Ferguson M, Kelly N. (2022). dsm: Density Surface Modelling of Distance Sampling Data. R package version 2.3.3.

Miller, D. L., Rexstad, E., Thomas, L., Marshall, L., Laake, J. L. (2019). Distance Sampling in R. Journal of Statistical Software, 89(1), 1-28. doi: 10.18637/jss.v089.i01 (URL: https://doi.org/10.18637/jss.v089.i01).

Nieuwhof N (2022). Karakamia Sanctuary Western Ringtail Possum & Rakali 2022 Survey Report. Australian Wildlife Conservancy, Perth.

Peakall, R.; Smouse, P. (2012). GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research—An update. *Bioinformatics* 28: 2537–2239. doi:10.1093/bioinformatics/bts460.

Peakall, R.; Smouse, P.E. (2006). GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6: 288–295. doi:10.1111/j.1471-8286.2005.01155.x.

Pritchard J.K., Stephens M., and Donnelly P. (2000). Inference of population structure using multilocus genotype data. *Genetics* 155:945-959.

R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rosenberg N.A. (2004) DISTRUCT: a program for the graphical display of population structure. *Molecular Ecology Notes* 4:137-138 doi:10.1046/j.1471-8286.2003.00566.x

Sunnucks P. and Hales D.F. (1996) Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus *Sitobion* (Hemiptera: Aphididae). *Molecular Biology and Evolution* 13 (3), 510-524. doi:10.1093/oxfordjournals.molbev.a025612

Teale, R. and Potts, J. (2020). Western Ringtail Possum, Pseudocheirus occidentalis Regional Surveys. Biota Environmental Services. (Leederville, Western Australia.)

Waples, R.S. (2006). A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked gene loci. *Conservation Genetics* 7, 167–184. doi:10.1007/s10592-005-9100-y.

Waples, R.S.; Do, C. (2010). Linkage disequilibrium estimates of contemporary Ne using highly variable genetic markers: A largely untapped resource for applied conservation and evolution. *Evolutionary Applications* 3, 244–262. doi:10.1111/j.1752-4571.2009.00104.x.

Wayne AF (2005). The ecology of the koomal (*Trichosurus vulpecula hypoleucus*) and ngwayir (*Pseudocheirus occidentalis*) in the jarrah forests of south-western Australia. PhD Thesis, The Australian National University, Canberra.

Wayne, A. F. (2006). Fire management guidelines for the ngwayir (*Pseudocheirus occidentalis*). A Report for Fire Management Services. Department of Conservation and Land Management, Western Australia.

Wayne, A. F., Cowling, A., Rooney, J. F., Ward, C. G., Wheeler, I. B., Lindenmayer, D. B., and Donnelly, C. F. (2005). Factors affecting the detection of possums by spotlighting in Western Australia. *Wildlife Research* 32, 689-700.

Wayne, A. F., Cowling, A., Lindenmayer, D. B., Ward, C. G., Vellios, C. V., Donnelly, C. F., and Calver, M. C. (2006). The abundance of a threatened arboreal marsupial in relation to anthropogenic disturbances at local and landscape scales in Mediterranean-type forests in south-western Australia. *Biological Conservation* 127, 463-476.

Wayne, A.F., Maxwell, M.A., Ward, C.G., Vellios, C.V., Wilson, I.J., Dawson, K.E., (2013). Woylie Conservation and Research Project: Progress Report 2010-2013. Department of Parks and Wildlife, Perth, Western Australia.

Wayne, A. F., Maxwell, M. A., Ward, C., Vellios, C., Ward, B., Liddelow, G. L., Wilson, I., Wayne, J. C., and Williams, M. R. (2013). The importance of getting the numbers right: quantifying the rapid and substantial decline of an abundant marsupial, *Bettongia penicillata*. *Wildlife Research* 40, 169-183.

Wayne, A. F., Maxwell, M. A., Ward, C. G., Vellios, C. V., Wilson, I., Wayne, J. C., and Williams, M. R. (2015). Sudden and rapid decline of the abundant marsupial, Bettongia penicillata in Australia. *Oryx* 49(1), 175-185.

Wayne, A. F., Maxwell, M. A., Ward, C. G., Wayne, J. C., Vellios, C. V., and Wilson, I. (2017). Recoveries and cascading declines of native mammals associated with control of an introduced predator. *Journal of Mammalogy* 98(2), 498-501. doi: DOI:10.1093/jmammal/gyw237.

Wayne, A. F., Maxwell, M. A., Barrett, A., and Potts, J. (2022a). A survey of ngwayir (western ringtail possum) in the Upper Warren, Western Australia, to inform a potential translocation to the Perup Sanctuary. Report. Department of Biodiversity, Conservation and Attractions. (Perth, Western Australia.)

Wayne, A. F., Maxwell, M. A., Potts, J., Barrett, A., and Geary, W. L. (2022b). Ngwayir associations with management and environmental factors in the Upper Warren. Report. Department of Biodiversity, Conservation and Attractions. (Perth, Western Australia.)

Wayne, A. F., C. Ward, J. Rooney, and I. Wheeler. (2000). The immediate impacts of timber harvesting and associated activities on the ngwayir (*Pseudocheirus occidentalis*) in the jarrah forest of Kingston State Forest Block. Unpublished Progress Report. Western Australian Government Department of Conservation and Land Management, Manjimup.

Wayne, A. F., Ward, C., Vellios, C., Maxwell, M., Wilson, I., Wayne, J., Ward, B., Liddelow, G., Renwick, J., and Orell, P. (2012). Ngwayir (Pseudocheirus occidentalis) declines in the Upper Warren, the issue in brief. Department of Environment and Conservation. (Manjimup, Western Australia.)

White, D., Comer, S., and Wayne, A. F. (2021). Understanding genomic variation in the western ringtail possum and its application to effective conservation management - Final Report. NESP Threatened Species Recovery Hub Project 4.1.8 report. (Brisbane, Australia.)

Williams, K. and Barton, B. (2012). Western Ringtail Possum Pseudocheirus occidentalis translocation proposal for various locations. Internal Report. Western Australian Department of Environment and Conservation.

Wills, A.J. and Farr, J.D., (2017). Gumleaf skeletonizer *Uraba lugens* (Lepidoptera: Nolidae) larval outbreaks occur in high rainfall Western Australian jarrah (Eucalyptus marginata) forest after drought. *Austral Entomology*, *56*(4), pp.424-432.

Wills, A. (2023). Notes on ecological and fire management implications of Gumleaf Skeletonizer outbreak 2022/23. Report Department of Biodiversity, Conservation and Attractions, Brain Street, Manjimup, Western Australia.

Woinarski, J. C. Z., Burbidge, A. A., and Harrison, P. (2014). 'The action plan for Australian mammals 2012.' (CSIRO Publishing: Melbourne.)

Zheng X.W., Levine D., Shen J., Gogarten S.M., Laurie C., and Weir B.S. (2012). A high-performance computing toolset for relatedness and principal component analysis of SNP data. *Bioinformatics* 28:3326-3328 doi:10.1093/bioinformatics/bts606

Yeatman, G. J. and Wayne, A. F. (2015). Seasonal home range and habitat use of a critically endangered marsupial (*Bettongia penicillata ogilbyi*) inside and outside a predator proof sanctuary. *Australian Mammalogy* 37, 157-163. doi: http://dx.doi.org/10.1071/AM14022.

8 Appendices

Appendix Table 1a. Ngwayir founders translocated from Busselton into the Perup Sanctuary

Animal ID	Left ear	Right Ear	Sex	Age	Mass (g)	Repro	Release date	Release Location	Collared	Last date KTBA	Fate
		532	F	A	1240	PY	22/08/2012	TB09	No		Unknown
		543	F	A	1240	PY	22/08/2012	TB09	No	Poss 25/8/12	Unknown
		534	F	A	1150	PY	22/08/2012	TB09	No		Unknown
		533	F	A	1060	LAC	23/08/2012	TB09	No		Unknown
	536		M	A	990		23/08/2012	TB09	No		Unknown
	542		M	A	790		23/08/2012	TB09	No	24/08/2012	Unknown
		573	F	A	1240	PY	23/08/2012	TB09	No		Unknown
		535	F	A	1010	PY	23/08/2012	TB09	No		Unknown
		WL2803	F	A	940		6/09/2012	TB09	No		Unknown
WRP3A		540	F	A	970		24/08/2012	TB09	Yes	31/08/2012	Dead 3/9/12
WRP4		544	F	A	1010	PY	24/08/2012	TB09	Yes	23/11/2012	Predated (wedgetail?)
WRP5		541	F	A	1020		24/08/2012	TB09	Yes	23/11/2012	Predated (wedgetail?)
WRP1	538		M	A	1000		24/08/2012	TB09	Yes	17/04/2013	Unknown
WRP6	539		M	A	1240		29/08/2012	TB09	Yes	3/12/2012	Predated (bird of prey)
WRP7		WL2801	F	A	1240	PY	29/08/2012	TB09	Yes	24/12/2012	Found remains 4/1/13
WRP2	WL2802		M	A	1030		29/08/2012	TB09	Yes	10/04/2013	Unknown
WRP3B	WL2809		M	A	1170		6/09/2012	TB09	Yes	6/09/2012	Dead 7/09/12
WRP10		WL2806	F	A	1050	YAH	6/09/2012	TB09	Yes	21/09/2012	Dead 24/9/12
WRP9		WL2805	F	A	1025	YAH	6/09/2012	TB09	Yes	4/01/2013	Dead 7/1/13
WRP8	WL2804		M	S	845		6/09/2012	TB09	Yes	17/04/2013	Unknown

Repro = reproduction status: PY = pouch young, YAH = young at heal, LAC = Lactating but no PY present.

KTBA = Known to be alive

Appendix Table 1b. Ngwayir founders translocated from Warren region into the Perup Sanctuary

Cohort	Animal ID	Left ear	Right Ear	Sex	Age	Mass (g)	Repro	Release date	Release Location	Collared	Last date KTBA	Fate
UD	MRP1	DL0991	DL0992	F	A	920	YAH	21/03/2016	TB08B	No	21/03/2016	Unknown
UD	MRP2	DL0989	DL0990	F	J	360		21/03/2016	TB08B	No	21/03/2016	Unknown
UD	MRP3	DL0341	DL0342	F	A	960		5/04/2016	TB08B	No	5/04/2016	Unknown
UD	MRP5	DL0343	DL0344	M	S	510		5/04/2016	TB08B	No	5/04/2016	Unknown
UD	MRP6	DL0345	DL0346	M	A	720		6/04/2016	TB08	No	6/04/2016	Unknown
UD	MRP8	DL0347	DL0348	F	A	1010		6/04/2016	TB08	No	6/04/2016	Unknown
UD	MRP17	DL2302	DL2303	F	A	990		Apr-2017	TB08	No	Apr-2017	Unknown
UD	MRP18	DL2304	DL2305	F	S	860		Apr-2017	TB08	No	Apr-2017	Unknown
UD	MRP13	DL0864	DL0865	M	A	890		27/04/2016	TB08	Yes	23/09/2016	Found dead 27/9/16
UD	MRP14	DL0866	DL0867	F	A	1070		27/04/2016	TB08	Yes	30/10/2017	Collar removed
UD	MRP15	DL0868	DL0869	F	A	970		9/05/2016	TB08	Yes	21/07/2017	Collar in mort mode in tree hollow
UW	MRP4	DL0676	DL0677	F	A	1210		11/04/2016	TB08B	No	11/04/2016	Unknown
UW	MRP9	DL0856	DL0857	M	A	1150		17/04/2016	TB08B	No	17/04/2016	Unknown
UW	MRP12	DL0862	DL0863	F	A	1105		21/04/2016	TB08	No	21/04/2016	Unknown
UW	MRP7	DL0854	DL0855	M	A	1100		17/04/2016	TB08B	Yes	17/04/2016	Unknown
UW	MRP11	DL0860	DL0861	M	A	1090		21/04/2016	TB08	Yes	21/04/2016	Collar retrieved from hollow 30/6/17 - no signs of death - collar came off?
UW	MRP10	DL0858	DL0859	M	A	1100		21/04/2016	TB08	Yes	26/04/2017	Collar removed
UW	MRP16	DL2201	DL2202	F	A	900		28/02/2017	TB08	Yes	12/02/2018	Collar removed

Cohort: UD = Upper Donnelly (west of Southwestern Highway), UW – Upper Warren (east of Southwestern Highway)

Repro = reproduction status: PY = pouch young, YAH = young at heal, LAC = Lactating but no PY present.

KTBA = Known to be alive