The Woylie Conservation Research Project: investigating the cause(s) of woylie declines in the Upper Warren region

Progress Report

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Photo: Sabrina Trocini



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Summary

At a species level the woylie (*Bettongia penicillata*) declined by around 90% between 1999 and 2010. Since 2006, the woylie conservation research project (WCRP) has investigated these declines with a focus on the populations within the Upper Warren region, east of Manjimup, Western Australia. The Upper Warren region supports two (Perup and Kingston) of the four remaining extant indigenous woylie populations and constituted up to 80% - 90% of all estimated woylies in 2001. There has been an overall 95% decline in woylie numbers across the Upper Warren region from an estimated peak of 213,000 in 1999. The characteristics of the decline have been similar across sites throughout the Upper Warren and a clear spatio-temporal pattern in the spread of the areas affected by the decline (1999 – 2008) is clearly evident.

Around 0.5 predators km⁻² was estimated necessary to account for the peak rates of woylie decline (assuming all woylie deaths involved predation and each predator killed one woylie per night). This is >5 times greater than introduced predator density estimates elsewhere in the jarrah forest but are comparable or less than other non-urban areas including those adjacent to or dominated by farmland.

The history of fox-baiting is summarised and some operational issues have been quantified. While the mean interval between aerial fox-baiting events was close to the target 90 days, the range was 40 - 189 days and 25% of baiting events were greater than a month from the 90 day interval. Since 1996, three planned aerial baiting events were not conducted. Fox bait longevity and uptake trials indicated that 81% of the 100 baits monitored were removed within 4 days, and 98% by 15 days. More than 37% of baits were first consumed partially and less than 2% of the baits (1/61) were taken by a fox, when it consumed only half of a bait. One bait was also consumed by a cat. Non-target native species, particularly koomal, were responsible for the removal of all other baits monitored by remote sensor (infra red and motion) cameras.

Predator activity indices (AIs) derived from sandpad monitoring has shown significant variation in fox AI across the region and an increasing trend over time since monitoring began in 2006. There has been no major change in cat AI over time and no significant difference across the region. There was no significant evidence that cat AI and fox AI was related (i.e. no evidence consistent with mesopredator release although the available evidence is limited). Estimates of cat and fox density have not been determined but remain a priority.

Cats were identified as the primary predator/scavenger of 62% of the mortalities (n=17) that occurred while woylie declines were underway. Foxes (24%), raptors (12%) and chuditch (3%) were attributed to the other mortalities during woylie declines. The frequency of fox associations with woylie mortalities has progressively increased over time. A comparative monitoring

program in and outside of the Perup Sanctuary is underway and early results have shown that predation is a major factor limiting the recovery of affected wild woylie populations.

Routine health assessments and extensive sampling for pathology, clinical and specific disease investigations have been collected since 2006. Key associations identified with declining woylie populations include skin and fur condition prevalence and severity, some haematological attributes (such as lymphocytosis), *Trypanosoma* prevalence and parasitemia and *Toxoplasma* prevalence. A fatal case of aspirated pneumonia caused by oesophageal myopathy is also of particular interest given that an ongoing review of the pathology evidence indicates that there may be some commonality with other woylie bodies that have been recovered from declining populations. The significance of these associations as possible agents of decline remains to be determined. Other disease-related activities are summarized, including an external review of the woylie disease research, the appointment of a manager of disease investigation for 12 months beginning in 2010, disease risk analysis and action plan, *Theileria*, genetics, population viability analyses, viruses, and the discovery of novel organisms including *Trypanosoma*, several novel strains of *Toxoplasma*, a papilloma virus, two *Bartonella* bacteria species, a new tick and flea species and new records for host-ectoparasite associations.

While predation, particularly by cats, is a key factor in the decline of the woylie, it is unlikely to be the only agent of decline. The weight of evidence indicates that probably some other factor is also principally involved. The leading hypothesis remains that animals were made more vulnerable to predation as a result of a cryptic disease that has characteristically spread through affected populations. The extent and nature of how disease may be involved in the declines remains to be verified.

Other conservation priority species have also undergone substantial declines in the Upper Warren region, from which they have not recovered, including wambenger (*Phascogale tapoatafa*) and dunnarts (*Sminthopsis* spp.) in the mid 1990s. Ngwayir (western ringtail possum, *Pseudocheirus occidentalis*) have declined since 1998 to levels that are now undetectable in most areas. Quenda (southern brown bandicoot, *Isoodon obesulus*) declines have also been associated with the woylie declines. The community-level declines are of particular concern given that the Upper Warren has long been recognised as one of the most important fauna conservation areas in southwestern Australia.

Recommendations are provided regarding clarifying the significance of the declines of multiple conservation-listed species across the Upper Warren region, the importance of verifying the causes of the woylie decline, understanding the factors limiting recovery, resolving issues associated with effective control and monitoring of introduced predators and the key priorities for disease investigation.

The extensive and close collaborations with other organisations and the involvement of volunteers (0.8 FTE since 2006) have been critical to the success of this program. Twenty four student projects have been involved with the WCRP. More than 158 media articles have been published or broadcast across all major media since 2006. Data from media monitors indicates that quarterly audiences have at times been greater than 2 million. More than 100 presentations have been made on aspects of WCRP since 2006 at national and international conferences, symposia and other forums by DEC staff and collaborators. Three workshops have been convened and more than 31 reports produced. Twelve scientific papers have been published in international journals, and many more will be forthcoming.

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1 Introduction

The woylie conservation research project (WCRP) began in July 2006 in response to extensive, rapid and substantial declines of woylies throughout southwestern Australia and South Australia. The project was incorporated as a fifth component of the pre-existing Western Shield research program (also called the mesopredator release research program), to facilitate collaboration with related investigations and to be subject to ongoing independent and external review (Western Shield Research Advisory Panel). An interim progress report for the WCRP was published in January 2008. While some elements of woylie conservation research continue, this report constitutes a brief summary of progress to June 2011.

2 The hypotheses and study sites

The principal aims of the woylie conservation research project (WCRP) are;

a) Determine the causal factors responsible for the recent woylie declines in southwestern Australia;

b) Identify the management required to ameliorate these declines; and,

c) Develop mammal monitoring protocols that will better inform factors associated with future changes in population abundances.

Based on the 'declining population paradigm' and related scientific approaches (e.g. Caughley 1994; Caughley and Gunn 1996; Peery *et al.* 2004) the diagnosis framework used to investigate the recent woylie declines is;

- 1. Confirm that the population has declined.
- 2. Determine the spatial, temporal and demographic characteristics of the observed decline.
- 3. Understand the species' ecology.
- 4. Identify all potential causes.
- 5. Use circumstantial evidence to help shortlist the potential causes.
- 6. Seek direct evidence test putative causes.
- 7. Given the evidence, determine the most appropriate conservation and management responses within an active adaptive management framework.

The WCRP uses a hypothetico-deductive approach (as recommended by Caughley, 1994) involving parallel lines of enquiry addressing the main possible agents of decline, most of which can be broadly classified into four major groups;

- 1. Resources particularly food depletion
- 2. Predation including native and introduced species, and effectiveness of current control measures.
- 3. Disease using clinical, pathology and epidemiological approaches and targeted research into likely agents including viruses, parasites, and bacterial diseases.

4. Direct human interference – e.g. possible negative consequences of trapping (over-harvesting for translocations, disrupted breeding success, reduced condition, injuries, increased stress and susceptibility to other mortality factors).

The research has a specific focus on the Upper Warren region (Figure 2.1 and 2.2) to concentrate limited resources in the one area while declines were current, to improve the chances of success. Information from other woylie populations has been incorporated through collaborations, particularly the mesopredator research programs in Dryandra and Tutanning (Marlow *et al.*) and monitoring at Karakamia Wildlife Sanctuary (AWC) and Batalling (DEC, Wellington District) (Figure 2.1).

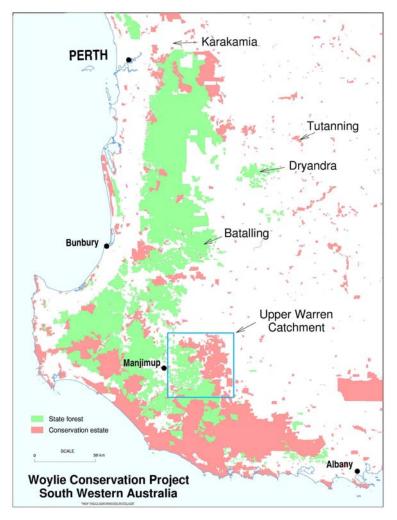


Figure 2.1. The location of important woylie populations involved in the Woylie Conservation Research Project.

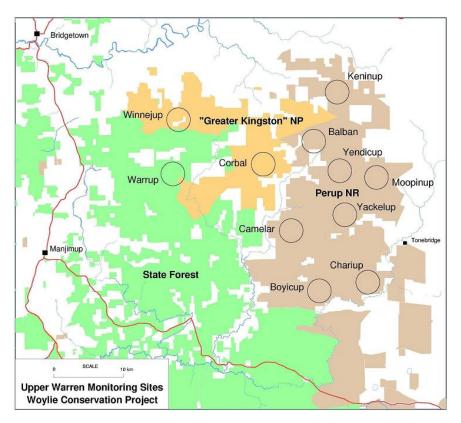


Figure 2.2. Key monitoring locations within the Upper Warren region involved in the Woylie Conservation Research Project.

3. Personnel and Funding

The Woylie Conservation Research Project has been led by the Forest Ecology Research Team (FERT): Adrian Wayne as the Chief Investigator, Technical Officers Colin Ward, Chris Vellios, and Marika Maxwell and casual employee Jamie Flett. Occasional assistance from other staff has included Bruce Ward, Richard Robinson, Graeme Liddelow, Julie Fielder, John Rooney and Matthew Williams. Science Division expenditure to this project is summarised in Table 3.1. Excluded from Table 3.1 are the contributions of Dave Algar, Neil Hamilton, Mike Onus, Steffie Hilman, Katrin Koch and Jim Rolfe, who assisted with attempts to develop an effective cat trapping methodology in Keninup during the woylie declines and verified the predator-free status of the Perup Sanctuary before woylies were introduced (Hamilton and Rolfe 2011). **Table 3.1. Summary of Science Division expenditure on woylie monitoring and research as part of the Woylie Conservation Research Project. Figures are summarised as \$1,000 units.** * The Saving Our Species Biodiversity Conservation Initiative provided \$300,000 in 2006/07, the balance of which was directed to Donnelly District.

DEC, Science	05/06	06/07	07/08	08/09	09/10	10/11	TOTAL
FTE	1.95	1.95	4.1	3.2	2.4	2	
Salaries/Wages	\$74	\$191*	\$237	\$211	\$159	\$139	\$1,011
22% Salary Overheads	\$16	\$42	\$52	\$47	\$35	\$31	\$223
Operating Costs	\$94	\$288*	\$53	\$40	\$22	\$25	\$522
TOTAL	\$184	\$521	\$342	\$298	\$216	\$195	\$1,756

The Donnelly District and Warren Region have provided around \$320,000 in kind contribution over the 6 years from 2005, principally through ongoing monitoring programs throughout the Upper Warren region. Other districts, principally Wellington, have also been involved in providing assistance in the field, monitoring data and woylie samples.

Around \$800,000 from external collaborators (in kind and material costs) and external funding sources over the 6 years from 2005 have also contributed to the Woylie Conservation Research Program. Sources of external funds have included Wildlife Disease ARC Project funds (MU/DEC), Wildlife Conservation Action (Perth Zoo), The Australian Academy of Sciences, South Coast NRM, and Environment Division of the United Nations Association of Australia (WA) Incorporated. Principal collaborators have been Murdoch University, Perth Zoo, and Australian Wildlife Conservancy. Other important collaborators and contributors include South Australian Department of Environment and Heritage, Australian Wildlife Health Network, Wildlife Disease Association (Australasia), University of Adelaide, Data Analysis Australia and various professionals across Australia, New Zealand and United States of America.

The WCRP was also principally involved in all stages of the development and establishment of the Perup Sanctuary, which has cost approximately \$1.5m including \$500,000 from State NRM and \$61,000 from the Perth Zoo. An affiliated woylie conservation project led by Warren Catchments Council (WCC) in collaboration with DEC began in November 2010 with federal CFOC funding (\$408,000 over three years). The focus of this project is aiding the survival of woylies principally by increasing/improving native habitat suitable for woylies through increased introduced vertebrate pest control.

Twenty four student projects have collaborated with the WCRP, including 13 completed and 11 current projects (13 PhD, 1 Masters, 8 Honours, 2 undergraduate independent study projects; Appendix 1).

Volunteers have made a critical contribution to the project, particularly assisting with field work. An equivalent of 0.8 FTE has been directly contributed by 171 volunteers over five years (Table 3.2). An additional 61 volunteers (approximately 400 hours)

assisted 87 DEC staff with the 'fauna muster' ('herding' out of large macropods and emus from within the enclosure) as part of the establishment of the Perup Sanctuary.

Total	171	1000	7293
2010	36	171	1134
2009	44	244	1650
2008	15	116	796
2007	47	257	1925
2006	53	212	1788
Year	# individuals	# days	Total hours

Table 3.2. Summary of the volunteer contribution to the Woylie Conservation
Research Project not including collaborators and affiliated student projects.

4. Methodology

There have been several phases to the WCRP; initial situation assessment (2005/06), major investigative phase (2006-08), smaller-scale investigations, reduced ongoing monitoring and development of funding proposals (2008-present).

2005/06	Woylie situation assessment; convened a recent mammal decline workshop; verification that the woylie declines were real
2006/07	Commencement of the WCRP (BCI funded) involving major data collection and collation - standardised monitoring protocols established; population comparison study conducted; database development and corporate data management; meta-analysis
2007/08	Interim analysis and reporting; development of proposals for 'Phase 2', predator control experiment and Keninup intensive study; seeking funding (DEC); ongoing monitoring
2008/09	Keninup intensive study; ongoing monitoring; funding proposal development (DEC-internal, Australian Biosecurity CRC, CFOC, WCA, Wildlife Disease ARC); establishment of Woylie Recovery Team
2009/10	Ongoing monitoring; funding proposal development (DEC, State NRM, Genetics ARC, Translocation ARC, CFOC, WCA, Biodiversity CRC); commencement of Perup Sanctuary development
2010/11	Ongoing monitoring; Perup Sanctuary establishment; commencement of CFOC-funded woylie project

The main results of the major investigative phase were reported elsewhere (Wayne 2008) and detail on the research approach and methodology is provided in the DEC Science Project Proposal (SPP 2007/02).

Upper Warren Monitoring

Standardised protocols for monitoring woylies and associated wildlife were developed through collaboration of the Science Division and Regional Services staff at Manjimup. The operations handbook summarising these protocols has been provided for use by other groups including the mesopredator project collaborators (particularly Marlow *et al.* and de Tores *et al.*), WCRP collaborators including AWC, Murdoch University, University of Western Australia and DEH (South Australia), and various other DEC programs (e.g. Lorna Glen) and external users.

Cage trap monitoring

Eleven key cage trap transects (50 traps spaced 200 m apart; Figure 2.2) formed the basis for monitoring woylie and other native medium-sized mammal populations throughout the Upper Warren region. These transects were all surveyed biannually for two years (2005-2007), after which there has been a progressive reduction in the frequency to annual monitoring in most cases, except Keninup and Warrup (where woylie populations were most abundant and dynamic) that have remained biannually monitored (Table 4.1). These surveys have been conducted variously by DEC Science, Donnelly District, Fauna Management Course and Bushranger programs. Each survey consists of four consecutive nights. Further details on the methodology are provided in Wayne (2008).

Block	Keninup	Warrup	Balban	Camelar	Boyicup	Moopinup	Yendicup	Yackelup	Chariup	Winnejup	Corbal
First Survey	1999	1994	2000	2000	1974	1999	1977	2000	1998	1994	2005
# Surveys pre '05	6	34	6	5	68	7	59	10	8	29	0
Spr-05	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Aut-06	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Spr-06	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Aut-07	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Spr-07	Y	Y	Y	Y			Y	Y			
Aut-08	Y	Y	Y		Y	Y				Y	
Spr-08	Y	Y	Y	Y							
Aut-09	Y	Y			Y	Y	Y	Y	Y		
Spr-09	Y	Y	Y	Y							
Aut-10	Y	Y			Y	Y	Y	Y	Υ	Y	
Spr-10	Y	Y	Y	Y							Y
Aut-11	Y	Y			Y	Y	Y	Y	Y		
# Surveys since '05	12	12	9	8	8	8	8	8	7	6	5

Table 4.1. Summary of the survey history of the key Upper Warren cage trapmonitoring transects.

Predator activity

Predator activity has been monitored using five principal sandpad arrays (25 sandpads (1 m x ~4 m) across forest tracks, spaced 500 m apart), plus a more recent addition (Moopinup). The sandpad arrays were systematically surveyed pre and post Western Shield aerial baiting for four consecutive baiting events between spring 2006 and winter 2007, with alternate sandpads having lures (FAP and fish oil; Maxwell *et al.* 2008). Since 2008 surveys using entirely passive sand pads (i.e. no lures) have been conducted opportunistically as resources have been available, with efforts to conduct at least annual surveys in autumn (a key time for monitoring, along with Spring) on as many arrays as possible. Each survey session went for 4-9 nights (Table 4.2).

Table 4.2 Summary of monitoring predator activity using sand pads in the Upper Warren. The values indicate the number of nights surveyed per session (including days for which the data has subsequently been removed from analysis due to poor weather conditions). Highlighted sessions indicate key periods for sampling in relation to foxes, spring (green) and autumn (orange). Surveys were conducted either immediately prior to aerial fox-baiting (pre) or 10-28 days after aerial fox-baiting (post).

Baiting	Block	Keninup	Balban	Warrup	Boyicup	Winnejup	Moopinup
Pre	Aug-06	9	9	9	9	9	
Post	Oct-06	4	4	4	4	4	
Pre	Dec-06	4	4	4	4	4	
Post	Jan-07	4	4	4	4	4	
Pre	Feb-07	6	6	4	4	4	
Post	Apr-07	4	4	4	4	4	
Pre	Jun-07	4	4	4	4	4	
Post	Jul-07	4	4	4	4	4	
Post	Feb-08	6	6	6	6	6	
Pre	Aug-08	4	4				
Pre	Mar-09	6	6	6			
Pre	Jun-09	6					
Pre	Mar-10	6	6	6	6	6	6
Pre	Mar-11	6	6	6	6	6	6

Keninup Intensive study

Keninup was the best opportunity to collect detailed data and evidence, immediately prior to and during an entire decline cycle of a large, wild woylie population. It was the last area known to be affected by the recent declines in Western Australia and its imminent decline could be anticipated based on the spatial and temporal pattern of the decline in Perup (see results and Figure 5.7). The significance of this opportunity is highlighted by the prospects of identifying the causes of decline being immeasurably greater if investigated while the declines are occurring rather than retrospectively (Caughley 1994, Caughley and Gunn 1996, Peery *et al.* 2004).

A proposed predator control experiment in Keninup was recognised as the most powerful means of determining the key factor(s) involved in the woylie decline. By removing (or at least substantially reducing) introduced predators from a portion of the population prior to or immediately after woylie declines began in the area, the significance of predators as an agent of decline could be quantified by comparing with the woylie population responses in the remainder of Keninup where predator numbers remained unchanged. It would also substantially increase the likelihood of detecting other agents of decline, given that prior experience had shown that woylie carcasses were completely consumed within hours of death thereby destroying the critical evidence necessary for examination (pathology, forensics, etc) before the bodies could be recovered. The use of a fenced enclosure (at least 400 ha) to provide a partial or complete barrier to predator incursion, was deemed the most practical and effective means of conducting the experiment, however the resources for the project were not secured in time.

An alternative observational study was conducted instead. The primary aim of the 'Keninup intensive study' was to closely monitor the population during decline to provide a more detailed account of the process of decline and potential factors associated with mortalities. It also aimed to increase the likelihood of detecting and recovering compromised woylie individuals in the presence of predators by means of more frequent surveillance (9 trapping sessions over 11 months, August 2008 to June 2009) than had been previously possible. Other key points of difference from previous endeavours included the greater emphasis on collecting longitudinal ante-mortem evidence from a subset of individuals (radio-collared and uncollared cohorts) and detailed clinical assessments and sampling conducted in the field, with the assistance of wildlife vets from Perth Zoo.

Survivorship and mortality monitoring of 14 radio-collared woylies along the Keninup transect (at least 3 times per week, August 2008 to June 2009) was done using similar methods used in the population comparison study (Ward *et al.* 2008).

Fox-baiting history in Upper Warren

The standard *Western Shield* aerial fox-baiting regime (i.e. four baiting events per year) has been applied across most of the DEC-managed land in the Upper Warren (Figure 4.1). Notably it did not include northern parts of Keninup.

Ground baiting by the Donnelly District has also been routinely associated with the aerial baiting. This supplementary baiting has principally targeted the forest perimeter adjacent to private property to increase the efficacy of aerial baiting and to intercept fox movements from adjacent agricultural land into the forest. Since October 2010, additional ground baiting within the core of Perup (Yendicup, Yackelup, Balban and part of Moopinup), has also been conducted on a monthly basis (with the exception of one month). This was originally associated with the reintroduction of dalgyte (*Macrotis lagotis*) into Perup but has subsequently been extended to provide a 5-10 km buffer around the Perup Sanctuary.

There have been a total of 57 aerial baiting events for the Manjimup baiting cell from November 1996 to April 2011. Three Western Shield aerial baiting sessions were missed

completely during this period (Summer 1997, Summer 1998, Winter 2008). District ground baiting was however conducted on each of these occasions.

The period between successive aerial baiting sessions ranged from 40 to 189 days (Mean=92.5, SE=5.1). 34% of baiting events were between 80-100 days and 25% were greater than a month from the 90 day interval (Figure 4.2). The average intervals were particularly high in 1997, 1998 and 2008 with an increasing trend over time between 1999 and 2008 and 2009-2011 (Figure 4.3).

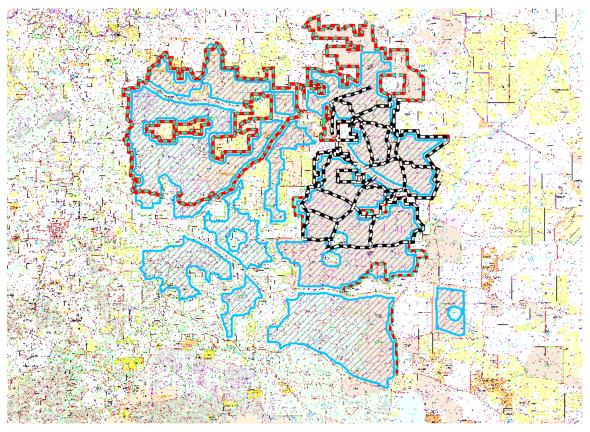


Figure 4.1. Map of the aerial Western Shield fox-baiting (blue hatching) and supplementary Donnelly District ground baiting for the Upper Warren region. Red/green dashed lines indicate ground baiting transects associated with aerial baiting. Black/white dashed lines indicate additional, generally monthly, baiting within the core of Perup (conducted since October 2010). (Map provided by Ian Wilson).

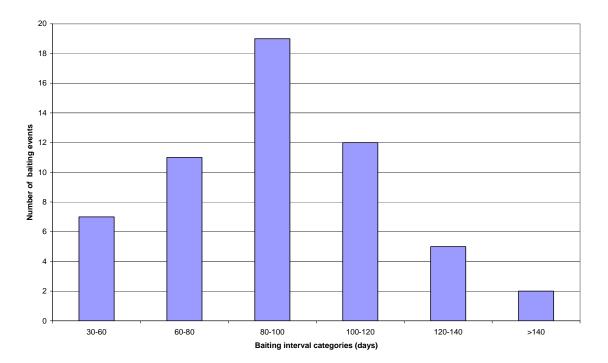


Figure 4.2. The frequency distribution of aerial fox-baiting intervals for the Manjimup flight cell, November 1996 to April 2011.

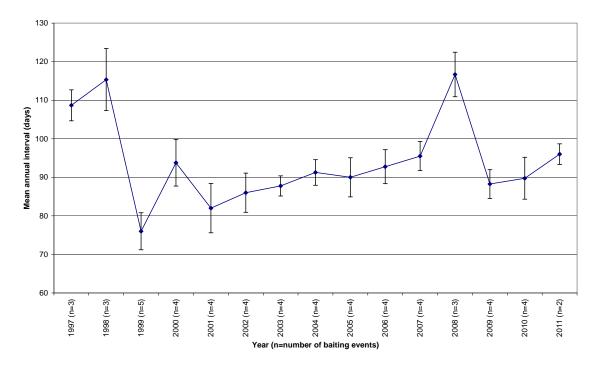


Figure 4.3. Average interval per year for aerial fox-baiting for the Manjimup flight cell, November 1996 to April 2011.

Regular ground baiting within the Kingston and Perup areas began in conjunction with the *Western Shield* aerial baiting in November 1996. Since then only two sessions have been missed at Kingston and four sessions at Perup. Aerial baiting was, however, conducted on each of these occasions. Ground baiting has occurred consistently four times per year since 2001. The period between successive ground baiting sessions ranged from 25 to 217 days (Mean=94.5, SE=5.7) for Kingston and 52 to 298 days (Mean=97.7, SE=6.2) for Perup.

The interval between aerial and ground baiting ranged from -62 to 58 days (Mean=3.8, SE=4.2) for Kingston and -59 to 42 days (Mean=10.3, SE=4.1) for Perup. Ground baiting for Perup and Kingston was achieved within a month of the aerial baiting 92% and 91% of the time, respectively, and within 10 days of aerial baiting 41% and 53% of the time respectively (Figure 4.4).

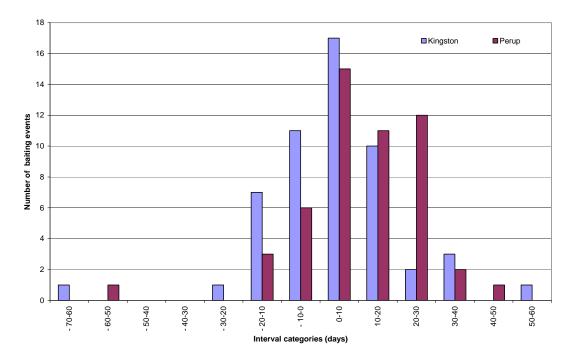


Figure 4.4. Interval between aerial and ground fox-baiting for the Manjimup flight cell, November 1996 to April 2011.

5. Results

Woylie population change in the Upper Warren

Verification that the declines observed in trap capture rates were corresponding to real population changes was established in Wayne (2006). Evidence included high correlations between capture rates and more sophisticated population modelling based on

trapping data (number of captured individuals per session, minimum number known to be alive (MNA or KTBA) and mark-recapture population modelling). Similarly there were correlations with other independent (non-trapping related) methods including sandpads, digging and nest densities and spotlighting data (Wayne 2006, in prep). Trap success rates were established as an accurate but relatively conservative measure of population change compared with other methods and was used here given that it was the only measure common to all key monitoring datasets within the Upper Warren.

Individual monitoring transects in the Upper Warren showed increases in woylies between the 1970s and 2007 (Figures 5.1 and 5.2). Pre-decline woylie capture rates ranged 40% – 80%. The first detectable declines on individual transects ranged between 1999 (Warrup, Greater Kingston) and 2007 (Keninup, northern Perup) (Figure 5.3 – note the start of the decline in Winnejup was based on reliable spotlight data collected throughout the year since trapping data was limited). Warrup began the first apparent recovery in 2005 but, having recovered to a 40% capture rate, subsequently commenced a second decline in 2009 to 18% capture rate in 2010 (Figure 5.1) and continue to decline (i.e. 4.5% capture rate in April 2011). Following a similar pattern to Warrup, after four years at low post-decline numbers, Boyicup and Camelar displayed the first signs of a possible recovery beginning in 2009, however the modest increases have not continued at either Camelar (i.e. 1%, 5.5% and 4% in 2008 – 2010 respectively) or Boyicup (i.e. 3%, 5%, 10.5% and 9% in 2008 – 2011, respectively).

At a regional level, the core Upper Warren woylie population (110,000 ha) remained relatively stable between 1998 and 2003, with mean and median trap rates typically between 50% and 60% (Figure 5.4), which correlates to densities of up to 2 woylies per hectare (Wayne *et al.* in prep, Table 5.1). Since then the median population decline has been 95% to around 10,000 individuals in 2010 from the 1999 peak of approximately 213,000 woylies (Figure 5.5). Following a sigmoid pattern of decline, the greatest declines occurred between 2003 and 2005 (i.e. 79% decline and ~147,000 net woylies in 2 years). Regional-level rates of decline were up to 73% per annum (i.e. net loss of ~107,000 woylies 2004-2005).

The capture rates of woylies on the 10 key Upper Warren transects surveyed in 2010 were similar to those observed more extensively from an additional 21 comparable transects across the region conducted in October – December 2010 used to source 54 woylies for the Perup Sanctuary, Perth Zoo insurance population and Native Animal Rescue facility. The overall mean and median woylie captures across the 31 sites surveyed in 2010 were 4.1% and 2.5%, respectively.

The characteristics of the decline were similar across sites throughout the Upper Warren. Prior to the decline all sites had a capture rate >40% (i.e. >1.3 woylies per ha; mean = 61% or 2.0 woylies/ha). The decline typically took four years (range 3-5 years), once underway the annual rates of decline were 25%-95% per annum, the progressive pattern in the annual rates of decline over time were similar across sites (Figure 5.6), the outcome was an average 96% reduction (range 87%-100%), and the post-decline numbers remained low (<5% capture rate) for at least four years. The rate of spread of first impact of the declines in Perup was on average around 4 km per year, beginning in Camelar (2002) in the south and finishing 22 km to the north in Keninup (2007)(Figure 5.7).

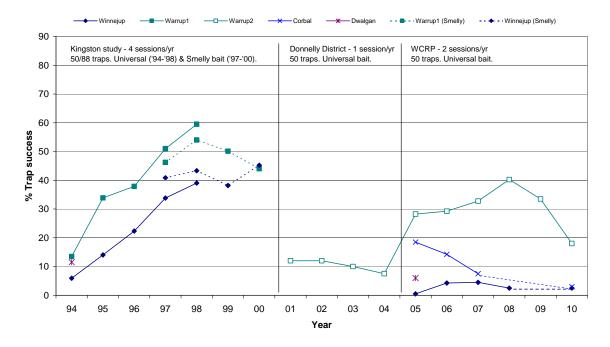


Figure 5.1. Annual trap capture rates of woylies along monitoring transects in Greater Kingston (western Upper Warren) 1994-2010

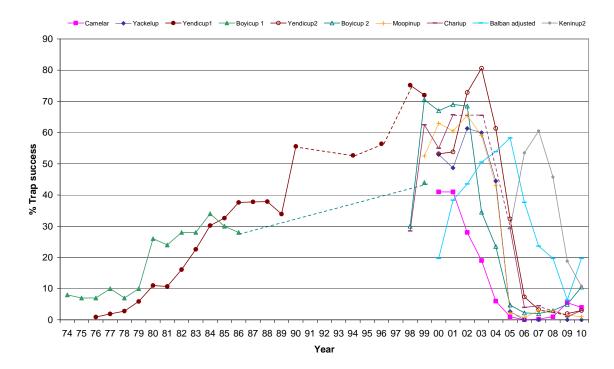


Figure 5.2. Annual trap capture rates of woylies along monitoring transects in the Perup (eastern Upper Warren) 1994-2010.

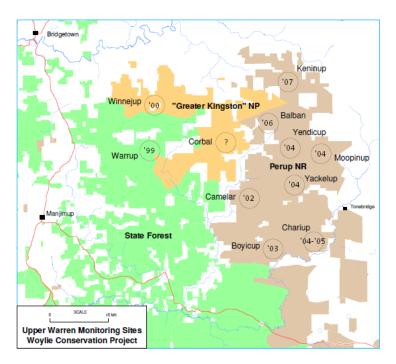


Figure 5.3. Key woylie monitoring sites (circles) in the Upper Warren region, southwestern Australia, and the year in which recent substantial declines began (>10% decline in capture rates within a year). The region includes Perup Nature Reserve (brown), 'Greater Kingston' Proposed National Park (orange), and State Forest (green). White areas are freehold land (generally agricultural or eucalypt plantations). Note the start of the decline in Winnejup was based on reliable spotlight data, since no trapping was done here May 2000 – October 2005.

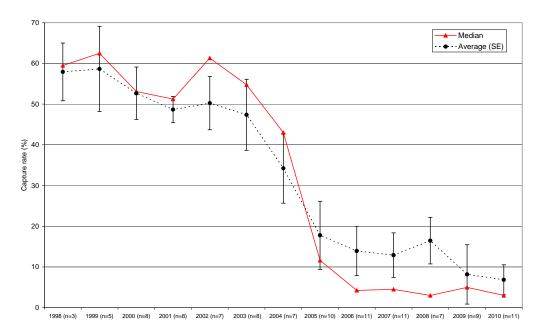


Figure 5.4 Annual trap capture rates of woylies in the Upper Warren 1998-2010 (n= 3-11 monitoring transects per annum)

Table 5.1. Density (ha⁻¹) estimates of woylies in the Upper Warren; based on overall median capture rates and a correlation between capture rates along a monitoring transects and density estimates (based on spatially explicit capture-recapture modelling in Density) from associated PCS grids ($n = 12, a = 0, b = 0.031, R^2 = 0.92, p<0.0001$).

	'9 9	'00	'01	'02	'03	'04	'05	'06	'07	'08	'09	'10
Density	1.94	1.65	1.59	1.90	1.70	1.33	0.36	0.13	0.14	0.09	0.16	0.09
Lower 95%	1.56	1.33	1.28	1.53	1.37	1.07	0.29	0.11	0.11	0.07	0.12	0.07
Upper 95%	2.32	1.97	1.90	2.27	2.03	1.59	0.43	0.16	0.17	0.11	0.19	0.11

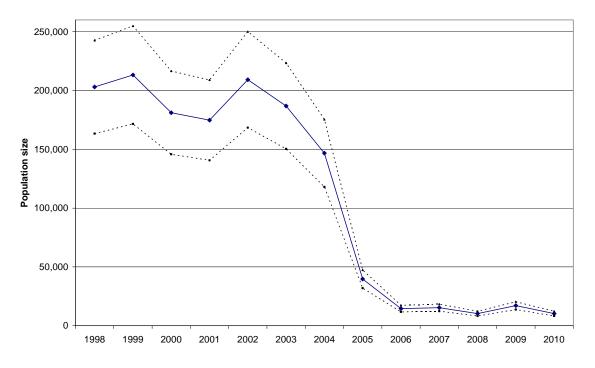


Figure 5.5 Woylie population estimate for the Upper Warren (core 110,000 ha only), based on a conversion of median capture rate to density (R^2 = 0.92). The lower and upper 95% confidence intervals for the regression co-efficient for the relationship between capture rate and density are presented as dashed lines.

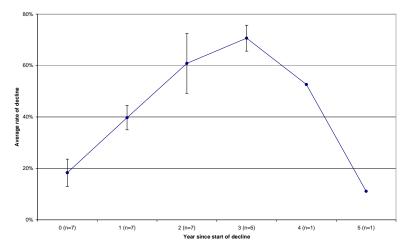


Figure 5.6. Average rate of decline in woylie trap capture rates in Perup - adjusted to year since start of decline

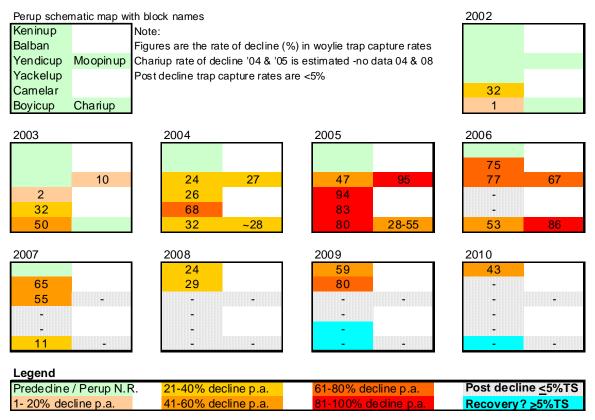


Figure 5.7. Schematic spatial model of the annual rates of woylie decline in Perup, Upper Warren. Note: annual rates of decline are calculated on changes in the average capture rate across multiple trapping sessions within a given year and do not indicate the first detection of declines in any given area. For example, at Keninup, the first detection of a decline in capture rates was in November 2007 but the first decline in the annual average capture rate was recorded in 2008.

Predator activity in the Upper Warren

The average detected fox activity index (AI) generally increased across the Upper Warren region since monitoring began in August 2006 (Figure 5.8). There has been no major sustained change in detected cat activity index (AI) over the same period across the region (Figure 5.8). Nonetheless there has been considerable spatial and temporal variation in detected fox AI and cat AI (Figures 5.9 and 5.10). Fox AI differed significantly between areas (ANOVA, p=0.015) and was greatest in northern Perup (Keninup and Balban) and least in Winnejup. Cat AI did not differ significantly between sites (ANOVA, p=0.82) but on average tended to be least in Keninup and greatest in Winnejup. Variation in cat AI tended to be greater between surveys within the same area than observed for foxes. There was a marginally significant inverse relationship between the average fox AI and average cat AI at the five main sandpad monitoring sites (regression, n=5, p=0.062, adjusted R² = 0.65). However, a more reliable general linear model (GLM) between fox AI and cat AI, using each individual session and site as a covariate, found no significant relationship (p=0.100) and no significant site differences (p=0.968) (n=60, R² = 0.079).

There was a significant negative relationship (p=0.004) between woylie capture rates and fox AI and significant site differences in woylie capture rates (p<0.000, GLM, n=38, $R^2 = 0.62$). It is important to note that this is based on data between August 2006 and March 2011, and therefore after the majority of the decline in the Upper Warren had already occurred (i.e. a 93% decline 1999-2006 and a subsequent 2% decline from the 1999 peak between 2006 and 2010).

There was no significant relationship between woylie capture rates and cat AI (p=0.169, GLM with site as a covariate, n = 38, $R^2 = 0.53$).

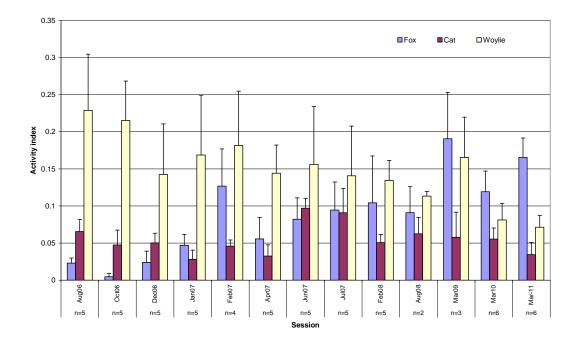


Figure 5.8. Average predator and woylie activity indices (AI) across the Upper Warren region using sand pad arrays (n=2-6 monitoring areas, each with 25 sand pads) since August 2006 (not including June 2009 when only Keninup was surveyed).

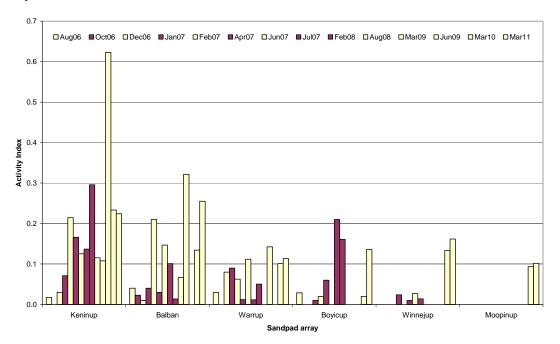


Figure 5.9. Fox activity index (AI) derived from sand pad arrays at the 6 Upper Warren monitoring sites since August 2006. Light bars indicate pre fox-baiting surveys and dark bars indicate post fox-baiting surveys. Note: Moopinup was only surveyed in March 2010 and 2011 and only 1-3 sites were sampled in August 2008, March 2009 and June 2009.

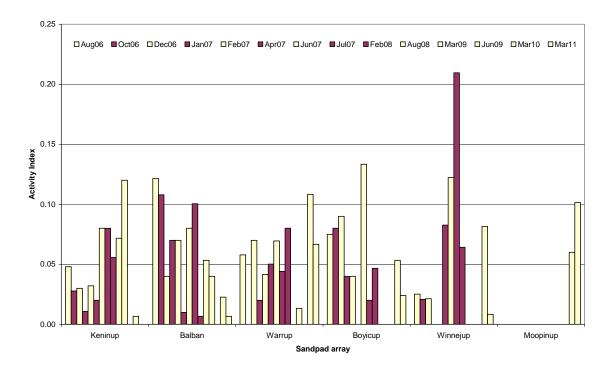


Figure 5.10. Cat activity index (AI) derived from sand pad arrays at the 6 Upper Warren monitoring sites since August 2006. Light bars indicate pre fox-baiting surveys and dark bars indicate post fox-baiting surveys. Note: Moopinup was only surveyed in March 2010 and 2011 and only 1-3 sites were sampled in August 2008, March 2009 and June 2009.

Longevity and uptake of fox baits

An observational study of the longevity and uptake of fox baits was conducted as part of routine ground baiting activities in March 2009. This involved tracking the fate of a subset of 100 sausage baits in Balban (northern Perup). This revealed that 81% of the baits were removed within 4 days of being laid, and 98% were removed within 15 days (Figure 5.11). In other words, on any given day an average of 30% of baits available were removed. More than 37% of baits were first consumed partially. Concealed sensor (infra red / motion) cameras were used to identify visitors and consumers at 61 of the 100 baits. Most baits were consumed or removed by non-target native fauna, predominantly koomal (Figure 5.12). Less than 2% of the baits (1/61) were confirmed taken by the target species, the fox. In this case it consumed only half of a bait. Another bait was visited by a fox but not consumed. One bait was consumed by a cat, only after numerous visits to an adjacent bait on preceding nights.

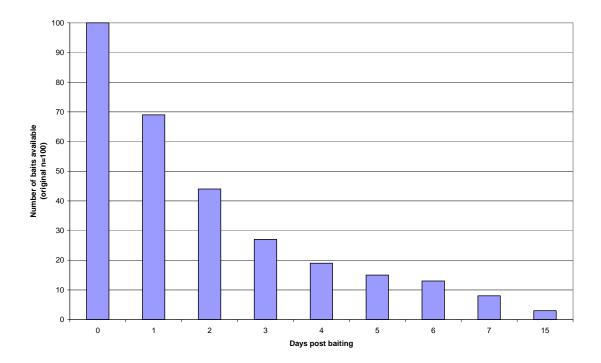


Figure 5.11. Longevity of 1080 fox baits laid in Balban as part of routine ground baiting operations.

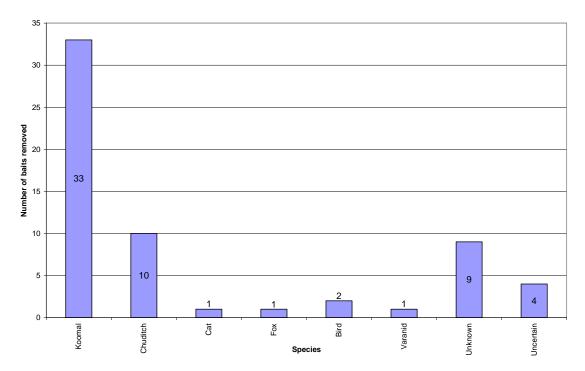


Figure 5.12. Species responsible for 1080 fox bait removal out of 100 laid in Balban as part of routine ground baiting operations in March 2009. Note Unknown = bait removed without sighting of species responsible, Uncertain = unclear as to which species sighted at bait was responsible for its removal.

Preliminary estimates of predation densities and rates required to result in observed declines

A high density of predators and/or a very high predation rate would be required to entirely account for the peak magnitude and rate of the declines observed in the Upper Warren (2004-2005). It is estimated that the predator density would need to be around 0.5 per 100 ha, assuming that it kills at least one woylie per night throughout the year (i.e. sufficient for nutritional needs but not considering surplus killing), and based on a median 2004 woylie density of 1.3 woylies ha⁻¹ within the area of active declines and a corresponding median density of 0.08 woylies ha⁻¹ in 2005, a 3.3 annual birth rate per female, gender parity in breeding adults and no consideration of population changes in other prey species). This estimation model does not include the mortality of breeding adults by causes other than predation.

Keninup Intensive Study

Declines in woylie capture rate began along the Keninup monitoring transect by November 2007 (Figure 5.13). Having reduced by up to 88%, capture rates appear to have stabilised around 9% since May 2009. Mark-recapture modelling (Robust model, program MARK) indicate that the estimated population size (N^) and apparent survivorship (φ) may have already begun to decline by March 2007, when capture rates peaked at 64%. Nonetheless, there was a strong and significant relationship between capture rates and MARK-derived population estimates (log(N^); R² = 0.65, p<0.0001). The Keninup intensive study was conducted during the second year of the decline, between August 2008 and June 2009 and provides more temporal detail of a decline than achieved elsewhere. The apparent minor seasonal pattern to the decline in capture rates (low points in late autumn-early winter (May-June of 2008 and 2009) followed by unsustained partial recoveries in spring), generally correspond to survivorship (φ) but were not as pronounced in the mark-recapture population abundance estimates (N^).

Possible spatio-temporal patterns to the decline along the Keninup transect were investigated across the successive surveys conducted since March 2006. Some patchiness was apparent with some areas retaining woylies while elsewhere they remained undetectable (Figure 5.14).

Extensive health checks and sampling of woylies were conducted providing an important resource for ongoing and future investigations as opportunities and priorities present (Table 5.2 and Figure 5.17). This included 2-9 serial samples for each of 14 radio-collared woylies regularly monitored to determine their survivorship over time and factors that may be associated with their fate. Other regularly trapped but uncollared woylies were also repeatedly sampled over time to provide an important resource for longitudinal and case studies, including 47 individuals with 5-16 sampling events, 99 individuals with 2-4 sampling events and another 105 individuals that have been sampled only once, between March 2006 and June 2009. The key results from specific disease-related investigations using some of these data and samples are presented below.

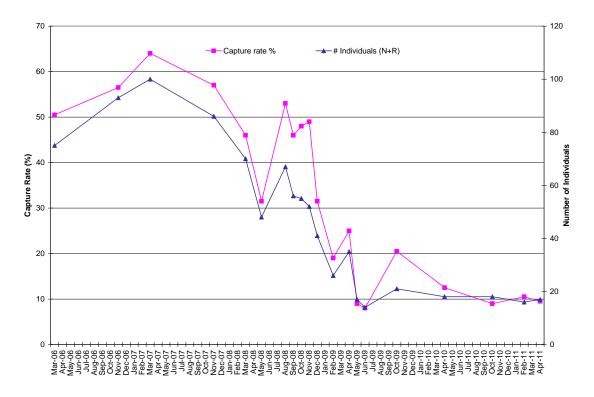
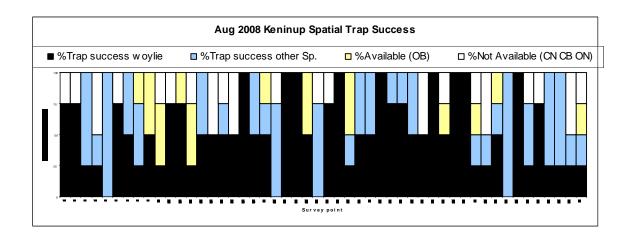
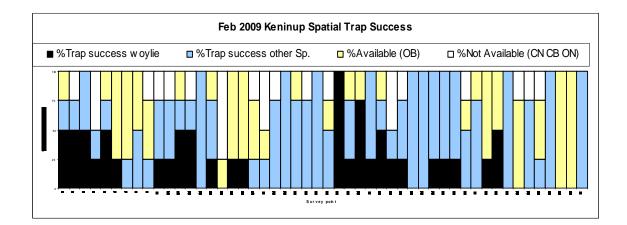


Figure 5.13 Woylie capture rate and number of individuals trapped along the Keninup monitoring transect.





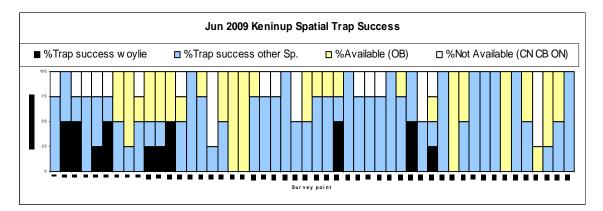
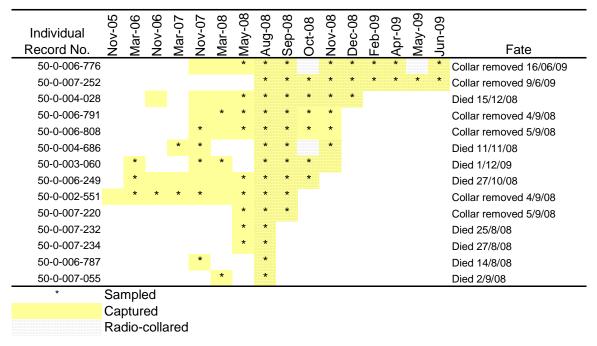


Figure 5.14. An example of the spatio-temporal pattern to the decline of woylies along the Keninup transect from three of the 20 surveys conducted since Mar 2006

Table 5.2. Individual woylie history of trapping, health checks and disease samplingfor the 14 radio-collared woylies in the Keninup Intensive Study (August 2008-June2009).



Survivorship and mortality

The population comparison study (2006-07) resulted in 21 mortalities out of 58 collared woylies across the five sites (Keninup, Balban, Warrup, Winnejup and Boyicup). The subsequent Keninup Intensive Study (2008-09) involved 8 mortalities out of 14 collared woylies. In 27/29 cases the carcasses were largely consumed, resulting in most of the remains being destroyed except for some skin and bone (i.e. organs and most muscle tissue absent) to the extent that necropsy was not possible. Of the two remaining cases one body was too autolysed for pathology (predation/scavenging possible but not confirmed) and the other was sent for necropsy (only tail missing, predation/scavenging confirmed). Reliably distinguishing predation from scavenging was fundamentally not possible. The independent corroboration between field evidence, laboratory assessment, classification based on common carcass characteristics, DNA analysis and forensic odontology was used to identify the primary predators/scavengers associated with each carcass with generally high confidence.

Cats were identified as the primary predator/scavenger of 62% of the mortalities (n=17) that occurred while woylie declines were underway (Figure 5.15). Foxes (24%), raptors (12%) and chuditch (3%) were attributed to the other mortalities during woylie declines. The frequency of fox associations with woylie mortalities progressively increased over the stages of decline in woylie populations (Figure 5.15). Overall cats were attributed to 50% of all mortalities, foxes 26%, raptors 17% and chuditch 3%.

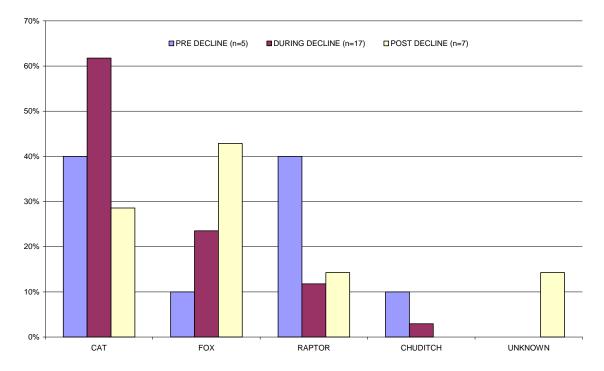


Figure 5.15. Proportion of woylie mortalities (n=29) in the Upper Warren attributed to specific predators/scavengers in relation to the decline status of the population at the time of mortality.

Woylie ecology and food resources

The dietary ecology of the woylie and its possible role in current population declines has been investigated as part of a collaborative PhD project (Kerry Zosky, Murdoch University). Specific aims were to examine temporal and spatial variation in the diet of the Woylie, examine changes in woylie diet in relation to population decline, and investigate food resource availability.

The study involved two components, an assessment of diet using faecal material collected during woylie population monitoring and seasonal fungi surveys to assess food resource availability. A comparative paper on dietary analysis based on different faecal preservation methods has been published (Zosky *et al.* 2010). Fifty-six species of hypogeal fungi have been identified (three novel). Results indicate fungi constituted the dominant dietary component throughout southwestern Australian populations but also included plant, invertebrates and seeds. There was limited spatial variation in diet at regional and subregional scales but there were strong seasonal changes with fungi being greatest in winter. There were no indications that diet or food resources were associated with the recent woylie declines in the Upper Warren (Zosky PhD thesis submitted 2011 and papers within in preparation).

Disease

Field health checks, sampling and clinical cases

Routine general health checks of woylies have resulted in 2,285 assessments in the Upper Warren and 301 from Karakamia, between 2006 and 2010 (Table 5.3). Extensive collections of blood, ectoparasites, faeces, DNA and other samples have accompanied many of these checks. Some comparable data and samples from other woylie populations have also been collected in collaboration with field staff working in those areas. Much of this material has been made directly available to collaborating research, particularly student projects. Many of the samples also remain in storage as an important resource available for retrospective analysis if and when new and compelling evidence of the agents of decline are identified that require further testing.

	Keninup	Perup / Kingston	Karakamia	SA	Dryandra /Tutanning	Batalling	TOTAL
Blood - EDTA	272	369	48	146	17	35	877
Blood - Smears	417	291	3	165	18	33	927
Blood - Sera	599	454	145	144	20	38	1400
Ectoparasites	626	568	303	105	18	34	1654
Scats - Endoparasites	539	480	52	135	12	21	1239
Scats - Diet	648	523	240	128	20	27	1586
Scats - Salmonella	19	122	27	0	0	0	168
DNA	245	525	150	156	11	32	1119
Other*	19	6	0	0	0	0	25
Routine health checks	1049	1236	301	17	22	20	2645

Table 5.3 Summary of health and disease sampling completed as part of the WoylieConservation Research Project (2006-2010). * Other sample types include biopsies,skin scrapes and urine.

Clinical cases

There have been a total of 18 woylie clinical cases (six from Upper Warren) 2006-2011. Principally all cases have been debilitated individuals, with no suggestion of a consistent, underlying disease process. Of the 17 woylies sent to the Perth Zoo for assessment, five animals were ultimately returned to their site of origin, 11 were euthanased and sent to Murdoch University for necropsy and one was kept at the Perth Zoo as part of the captive insurance population. In addition, in 2010 one woylie was euthanased at a Busselton veterinary clinic and sent immediately to Murdoch University for necropsy. The animal presented with pneumonia symptoms and pathology revealed oesophageal myopathy. The implications of which require further examination. Necropsies were conducted on all euthanased woylies. Twenty woylies from the Upper Warren had skin scrapes and/or skin biopsies collected to investigate a variety of skin conditions observed. Results showed non-specific chronic changes, most likely associated with tick and other ectoparasite burdens. Self-trauma and fighting may have also contributed to some of these skin conditions. There was no evidence of an underlying primary pathogen or disease causing the skin changes (Eden *et al.* 2010).

		#		
Origin	Year	cases	Clinical summaries	Fate
Upper Warren	2006	1	Trauma injury	Returned to field
Upper Warren	2007	1	Poor condition/ neurological	Returned to field
Upper Warren	2008	1	External lesions	Returned to field
Upper Warren	2009	2	1 Poor condition, 1 Trauma injury	2 Euthanased / Necropsy
Upper Warren	2010	1	Trauma injury	Perth Zoo insurance population
Karakamia	2006	3	3 External lesions / abscesses	1 Returned to field, 2 Euthanased/Necropsy
Karakamia	2007	2	1 Neurological, 1 External lesion	Euthanased/Necropsy
Karakamia	2008	3	1 Sternal abnormality, 2 Poor condition / lesions	1 Returned to field, 2 Euthanased/Necropsy
Karakamia	2009	1	Poor condition / trauma injury	Euthanased/Necropsy
Private/Other	2008	1	Poor condition / abscesses	Euthanased/Necropsy
Private/Other	2010	2	1 External lesion, 1 Pneumonia*	2 Euthanased/Necropsy

Table 5.4. Summary of woylie clinical cases administered by Perth Zoo veterinary
staff 2006- July 2011

Pathology

Commentary by Phil Nichols (May 2011)

Establishing cause of death, or refining provisional diagnoses

The necropsy (post-mortem examination) programme has several roles. The examinations aim to confirm or refute the suggested cause of death, or to uncover unsuspected causes of death or disease. For example, in animals found dead on the road, and presumed to be roadkill, gross and microscopic examinations post-mortem aim to determine if predisposing factors were present.

Establishing a tissue reference bank for disease investigation

A key aim of the necropsy programme is to build a tissue bank of frozen and formalinfixed tissues for reference. Such a reference collection can be used to investigate disease hypotheses, such as the presence or absence of a suspected disease agent, genetic feature, or toxin. The tissues are able to be used for molecular diagnostic techniques in addition to the light microscopic examinations performed at the time of necropsy. A sufficiently large reference database is required to be able to determine associations in a statistical manner, since the mere presence of a disease agent is rarely enough to establish causality. Roadkill animals provide a useful source of control tissues when investigating proposed disease hypotheses.

Challenges

Animals that are not recovered promptly after death can be predated and/or undergo significant post-mortem decay (autolysis). This makes establishing the cause of death difficult, such as distinguishing between predation and scavenging. Tissues from such animals are nevertheless useful for tissue reference purposes. There are time and cost restraints on necropsy examinations, but the fee for the post-mortem investigations has been waived. Staff movements have hindered continuity of the necropsy programme, but this is being addressed by a retrospective evaluation of all necropsies to date by a single pathologist, to ensure a consistent approach and to look for missed features or themes. A backlog of histology examinations is currently being processed.

Outcomes

In addition to the information on causes of death obtained to date (Table 5.5), the reference collection is a significant outcome of the programme. The detection of muscle degeneration in one animal that suffered aspiration pneumonia is of potential significance, since the degeneration of tongue and oesophagus musculature could have been a predisposing factor to the inhalation pneumonia that proved fatal in this individual. Molecular diagnostics are in progress to look for an associated agent.

	Purpose of necropsy			
Origin	Specific cases*	Study cohort (radio- collared)	Opportunistic (e.g. road kill)	Total
Upper Warren	5	4	7	16
Batalling	2	-	3	5
Karakamia	10	-	2	12
Dryandra / Tutanning	-	15	-	15
Private colonies	4	-	-	4

Table 5.5. Summary of woylie necropsies conducted by Murdoch UniversityPathology Unit 2005-2011.

*Specific cases represent individuals of interest with clinical symptoms found either dead or moribund.

Pathology cases summary

A total of 52 necropsies have been conducted from 2005 to current. In most cases, diagnosis of cause of death was either straight forward (mostly road vehicle trauma, trap-

related trauma, predation trauma) or was not possible due to the advanced state of autolysis of the carcass.

Empirical evidence suggests that the greatest chance of detecting significant pathology associated with a disease-driven population decline is shortly before, during, and shortly after the period of decline. The "window of opportunity" is dependent on the characteristics of the disease in question, and cannot be accurately determined at this time, for woylie declines. The investigation continues to be hampered by a lack of bodies, in good state of tissue preservation, from key geographic areas, and at key times of decline.

In a handful of cases, unusual or unexplained histological findings have been noted and are considered worthy of further investigation. Ongoing investigation of these cases may lead to greater understanding of disease processes contributing to the decline, or at a minimum will further our understanding of health and disease and interpretation of pathology findings in woylies.

All pathology material is currently being reviewed in light of the 2010 oesophageal myopathy case and early indications that there may be other examples of the same condition in other cases collected since 2006.

Key associations with declines

Skin and fur conditions

Preliminary analyses of the health check data revealed that skin and fur conditions had a strong association with populations currently declining (Wayne *et al.* 2008). Preliminary results from Pacioni (2010) highlighted that prevalence of health problems recorded at physical examinations (the majority of which were various types of skin lesions) increased significantly immediately before the detection of the decline and were moderately (but significantly) correlated with the intensity of the decline. In other words, the quicker the population was declining, the higher the prevalence of health problems. Eden *et al.* (2010) clinically examined some of the skin conditions (reported above) and also showed an increase in the prevalence of skin conditions between October-November 2006 and November 2008 in Keninup (immediately prior to and initial stages of decline) and Warrup (post-decline recovery and immediately prior to the beginning of a second decline). These findings merit a more comprehensive and rigorous epidemiological analysis to resolve the exact nature of these associations and further investigate the pathogenesis of skin lesions.

Trypanosoma

A novel, host-specific trypanosome has been identified in woylies as part of the WCRP (Smith *et al.* 2008, Averis *et al.* 2009). A comparison of the prevalence (by PCR) and parasitemia levels (by light microscopy) revealed a positive association with the declining woylie populations within the Upper Warren compared with the stable population at Karakamia (Smith and Averis 2008; Table 5.5). A subsequent detailed analysis of 503 samples from Keninup between March 2006 and June 2009 was made possible as a result of the Keninup Intensive Study, Wildlife Conservation Action

funding and considerable investment by collaborators at Murdoch University. It has provided further evidence of an association in the prevalence of *Trypanosoma* with the progression of the decline of woylies in that area, whereby trypanosome prevalence was 0-10% prior to the commencement of the decline after which the prevalence reached up to 62% and followed a similar pattern to changes in woylie capture rates (Wayne *et al.* in prep; Figure 5.16). Albeit a smaller and temporally more limited dataset, a similar association was also evident at Balban – the only other site where samples have been collected during a decline.

A new project began in 2010 on the transmission dynamics of trypanosomes in declining, stable and enclosed populations of woylies (Craig Thompson, Murdoch University PhD project). Another project is looking at the genetic characterization of trypanosomes (Adriana Botero, Murdoch University PhD project).

 Table 5.5. Association of *Trypanosoma* with recent woylie declines: a comparison between Upper Warren and Karakamia populations.

	Upper Warren	Karakamia
Population state	Declining	Stable
n	124	123
Prevalence - PCR	49%	13%
Parasitemia - Microscopy	High	Not detected

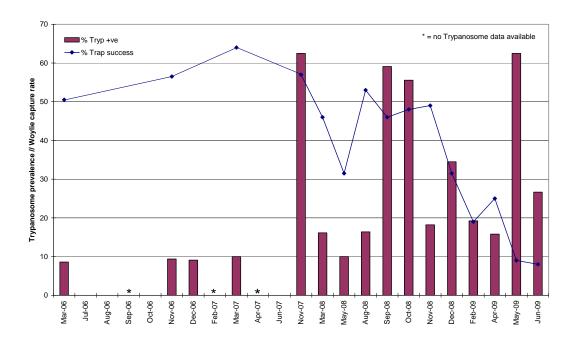


Figure 5.16. Prevalence of trypanosome infections in woylies at Keninup in relation to capture rates.

Toxoplasma

Initial serological work (MAT – modified agglutination tests) from samples collected in 2006 identified seropositive woylies from the Upper Warren but not at other stable and declined populations elsewhere in Western Australia (Table 5.6. Parameswaran *et al.* 2008, Parameswaran 2008 (PhD thesis)), however, the extent to which this may be a function of sample size is not resolved.

Subsequent PCR tests for *Toxoplasma* infection in the bodies of woylies and other native wildlife sourced across numerous sites including Upper Warren and other sites in southwestern Australia, as well as Karakamia and the eastern states, has revealed that *Toxoplasma* occurs frequently in native wildlife usually not associated with any clinical disease. Numerous novel genotypes (strains) of *Toxoplasma* not previously recorded in any other hosts or geographical areas have been found (Parameswaran *et al.* 2010; Pan *et al.* submitted (part of a current Murdoch University PhD project)). These findings have raised questions about the origin of *Toxoplasma* in Australia, its transmission and most importantly in the context of the woylie decline, the nature of the virulence phenotypes of the 'novel' Australian strains of *Toxoplasma* and the circumstances that give rise to clinical toxoplasmosis.

A new project commenced in 2011 on the effect of *Toxoplasma* on woylie behaviour (Amanda Worth, Murdoch University PhD project).

Location	Seropositive	Total tested
Upper Warren - March 06	9	153
Upper Warren - Jul-Dec 06	0	143
Karakamia – Jul 06	0	81
Dryandra - Nov 06	0	12
Tutanning - Nov 06	0	8
Batalling - Nov 06	0	17
St Peters Is., S.A.	1	73
Wedge Is., S.A.	0	14

 Table 5.6. Toxoplasma seroprevalence (MAT) in woylies across Western Australian populations (from Parameswaran 2010).

Haematology

Several haematological attributes (e.g. lymphocytosis) and health factors (e.g. skin and fur conditions) of woylies were statistically significantly associated with the contemporary rates of decline (Pacioni 2010). Funds from 'Woylie Rescue' extended the haematological data analysis to South Australian woylie populations and other WA populations (Pacioni *et al.* submitted). The populations affected by the decline presented clear haematological signs of immune system stimulations, which were not detected in non-declining woylie populations. The biological significance of these associations, whether they are coincidental or related to population declines and individual mortality need to be investigated further.

Other disease work

External review of disease components of WCRP

An external review panel (Dr Rupert Woods, Dr David Obendorf, Dr Lee Skerratt and Pam Whiteley) undertook a review of the woylie disease investigation components following the interim report (Wayne 2008) and woylie symposium in February 2008 (Woods *et al.* 2008). Key points from this review included that there was insufficient information at the time to determine whether or not disease was a cause of the declines, that disease must be considered as a likely cause, that the highest priority was for an epidemiological framework to be applied to the disease investigation and that this would best be achieved by the appointment of a dedicated epidemiologist and disease investigation manager to co-ordinate the disease investigation components of the research program. In response, considerable effort was made to secure a means to make such appointments. This was met in part by the appointment of a manager of woylie disease investigation in 2010-11.

Manager of woylie disease investigation

A part-time manager of woylie disease investigation (Dr Andrea Reiss, with assistance from Dr Carlo Pacioni) was hosted for 12 months (2010-11) by the Perth Zoo with funds provided by the 'Woylie Rescue' sponsorship program. The role of the manager was to facilitate collaborative endeavours to determine the role of disease in the recent woylie declines. This included assisting in organising and synthesising existing disease-related data and information generated to date, develop a woylie disease investigation plan, develop and assist collaborations regarding woylie disease investigations (including clinical assistance in the field during ongoing monitoring), and identify potential sources of funding and apply for funds where appropriate. Substantial and significant progress on all of these objectives was achieved however a cessation of funds for this position has meant that this work has no longer continued.

Disease risk analysis

A Disease Risk Analysis, seeking to identify likely pathogens which may have been contributing to the woylie decline was developed by Carlo Pacioni (2007) as part of his PhD thesis. This desk top exercise gathered information from publications, grey literature, anecdotal reports and personal experiences and developed a risk analysis matrix. This information helped to guide priorities for field and laboratory investigations into woylie disease.

The disease risk analysis was reviewed and updated in 2010-11 (Reiss and Pacioni) to establish disease priorities for further investigation and is currently being prepared as a submission for publication.

Woylie disease investigation action plan

A detailed investigation action plan was developed by Andrea Reiss in her role as Manager, Disease Investigation, with input from all members of the woylie disease investigation team. This action plan outlined all major areas of investigation, provided preliminary detail on how each area of investigation could best proceed, and attempted to prioritise the programs. A short list of high priority action programs was agreed upon.

Papilloma virus

A novel papilloma virus was detected in several woylies as part of the Keninup intensive study and subsequent monitoring. The complete genomic characterization of the papillomavirus (BpPV1) is a first for an Australian marsupial (Bennett *et al.* 2010). While the consequences of this epitheliotropic virus in woylies remain unresolved, it is considered incidental to the causes of the recent woylie decline.

Theileria

An intra-erythrocytic protozoan parasite belonging to the family *Theileriidae* (piroplasms) was identified in several woylie populations, including Perup, Kingston, Karakamia in WA and St Peter Island and Venus Bay Conservation Park in South Australia. An initial investigation into the piroplasm was completed in 2009 (Jia Rong's Honours project). Molecular identification is underway to confirm that this parasite is the same species identified in woylies from the Avon Valley (i.e. *Theileria penicillata*, Clark and Spencer 2007). The investigation undertaken revealed unreported morphological findings of the erythrocytic cycle of the piroplasm in the woylie. Additionally, it suggested that the parasite is responsible for haematological changes in infected individuals as well as the overall populations. These findings are potentially the first report of clinical consequences of piroplasm infections in Australian marsupials and, although direct evidence of association between the parasite and woylie declining populations was not found, this study demonstrated that the presence of high parasite prevalence and/or parasitemia could reduce woylie survival (Rong *et al.* submitted, Basile *et al.* in prep).

Woylie Genetics

Genetic profiles of extant indigenous and translocated woylie populations were examined to assess whether woylie populations were suffering from reduced genetic "health", as a consequence of past bottlenecks. To do this, suitable microsatellite primers for genetic investigation were first identified (Pacioni and Spencer 2010).

Genetics were found not to be a contributing factor to the present woylie decline with relatively high heterozygosity ($H_E \sim 80\%$) and allelic richness ($N_{AR} = 9-12$) in the Dryandra and Upper Warren populations (Pacioni *et al.* 2011). However, substantially reduced genetic diversity was found at Tutanning Nature Reserve (Pacioni *et al.* 2011), and on the South Australian islands (Pacioni 2010).

Other insights into woylie population structure and dynamics include there being four genetically distinct indigenous populations (i.e. Dryandra, Tutanning, Kingston and Perup) and that current gene flow between Kingston and Perup is in the order of 2-3% migration rate (Pacioni *et al.* 2011). The evidence of current gene flow within and between populations (i.e. up to 60 km) signifies that direct transmission of an aetiological agent would be possible throughout the whole Upper Warren region within the time frame experienced in the decline (Pacioni 2010).

Using ancient DNA techniques on archaeological collections it has been shown that woylies began declining around the time of European settlement, having previously been relatively stable, and have declined by around 90% and lost up to 50% of their genetic diversity since. It is also evident that historically there was considerable gene flow across more than 1000 km of southwestern Australia (Hunt 2010, Pacioni *et al.* in prep).

Molecular data also confirmed female philopatry with an apparent dispersal range of less than 1 km (i.e. females are settling within or adjacent to their mother's home range). Average male dispersal ranges were apparently 1-3 km for males (Pacioni 2010). Also, it was demonstrated that the decline has caused changes in the genetic spatial structure of woylie populations in the Upper Warren region (Pacioni 2010).

Viruses

Based on the results of the disease risk assessment and haematological analysis, the serological response to Macropod Herpesvirus (MaHV 1 and 2), Encephalomyocarditis virus (EMCV) and Orbivirus (Wallal and Warrego serogroups) was investigated. There was no serological evidence of any of these viruses affecting woylie populations but sample size limitations cannot confirm the absence of these diseases with a high level of confidence (i.e. >90%; Pacioni 2010). Additionally, the absence of detection of seropositive individuals does not necessarily imply absence of the pathogen(s) in the population given that, for example, infected animals may die before developing a detectable seroresponse. In collaboration with Dr Cheryl Johansen (UWA), an initial screening of Alphaviruses and Flaviviruses has been conducted. While no positive individuals were detected, final analysis of the data is still pending (C. Pacioni *pers. comm.*).

Population viability analysis

A population viability analysis (PVA) demonstrated that the main potential threat to woylie populations is the interaction of various variables (in particular predation and reduced fitness) that acquire a considerable strength together, whilst not being greatly significant by themselves (Pacioni 2010). It also quantified the minimum mortality rates necessary for the decline to occur (an average juvenile and subadult mortality rate of 28% and 22% for adults per 91 day time period) (Pacioni 2010). The minimum viable population size (MVP) estimated through PVA was consistent with the empirical evaluation based on molecular data (i.e. 1,000-2,000 individuals) (Pacioni 2010).

Other disease investigations

Other disease investigations associated with woylies include;

- Enteric parasites, with a particular focus on protozoa including *Blastocystis*, *Cryptosporidium* and *Giardia* (Unaiza Parkar Murdoch University PhD project; Thompson *et al.* 2010)
- Ectoparasites, including the discovery of a novel species of tick and flea and other new host-parasite associations (Halina Burmej and Yazid Abdad Murdoch University PhD projects)

- Bacteriology, including the discovery of two novel species of *Bartonella* (Kaewmongkol *et al.* 2011; Gunn Kaewmongkol, Murdoch University PhD project) and *Rickettsia* infections in ectoparasites removed from woylies and other wildlife (Yazid Abdad, Murdoch University PhD project)
- A review of all pathology evidence to identify other cases of oesophageal myopathy and to verify the aetiological agent in this and other possible cases (including Phil Nicholls, Andrew Thompson, and Andrea Reiss)
- The suspected detection of *Leishmania* in a moribund woylie from near Margaret River has prompted a review of all pathology evidence to determine whether there are similar cases from elsewhere and to verify the aetiological agent in this and other possible cases (including Phil Nicholls, Andrew Thompson, and Andrea Reiss)
- Woylie monitoring and associated disease sampling has been done in collaboration with an ARC-funded project (2006-2009) and ongoing research investigating the nature, diversity and potential impact of infectious agents in Western Australian threatened mammals (Thompson, Lymbery, Smith, Morris, Wayne, Burmej, *et al.*).
- A comparative health and disease investigation in the woylie captive versus free-range enclosure (Perup Sanctuary) versus wild, began in 2011 (Kim Skogvold, Perth Zoo / Murdoch University Masters Project). It includes an investigation of haematology, biochemistry, antioxidants and potentially corticosteroids as well as gross clinical assessments and any possible emerging issues.

Northern Perup - Case study synthesis

Balban and Keninup are the only sites where corresponding survivorship (radiotelemetry), predator activity (sandpads), woylie abundance (trapping) and individual woylie condition (health checks, haematology and disease sampling) has been closely monitored throughout most of the woylie decline cycle. A comparative analysis of the diet of woylies (2006-2007) and the abundance of hypogeal fungi (staple diet; 2007-2009) coincided with the decline in Balban (2006-2009) but precede or coincide, respectively, with the early stages of the declines in Keninup (2008-ongoing). As such these sites represent the best opportunity to examine the characteristics, associations and possible factors related to the woylie declines at the site level.

Nearly three quarters of the 29 woylie mortalities observed as part of the survivorship and mortality components of the project occurred in Balban (n=7, 2006-2007) and Keninup (n=14, 2006-2008). Cats were primarily associated with up to 78% and 50% of mortalities in Balban and Keninup, respectively. Foxes were primarily associated with 0% and up to 37% of mortalities in Balban and Keninup, respectively. The remainder were predominantly associated with raptors and occasionally chuditch.

While cats have been associated with most woylie deaths during woylie declines the positive association between cat AI and woylie capture rates was significant at Balban (regression: n=7, p=0.04, adjusted $R^2=0.51$) but not at Keninup (regression: n=9, p=0.3,

adjusted $R^2 = 0.03$). The inverse association between fox AI and woylie capture rates was not significant at either Balban (regression: n=7, p=0.24, adjusted $R^2 = 0.12$) or Keninup (regression: n=9, p=0.14, adjusted $R^2 = 0.18$) (Figures 5.17, 5.18 and 5.19). The interpretation of these results need to consider the small number of data points, the linear interpolation used to get corresponding measures given that the woylie and predator indices were not simultaneous and the possible nature of the relationship between indices and actual abundance.

Relative to other sites in the Upper Warren, fox AI was significantly the greatest in Keninup and Balban and cat AI tended (i.e. insignificant) to be the highest in Balban and the lowest in Keninup.

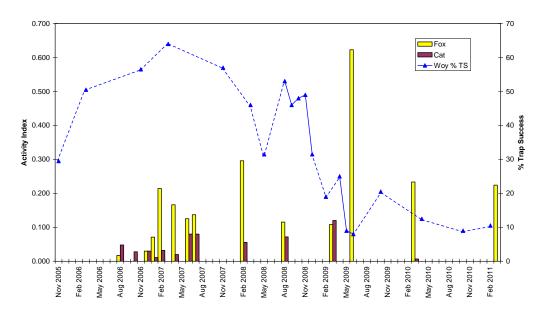


Figure 5.17 Predator activity index (AI) and woylie capture rates at Keninup

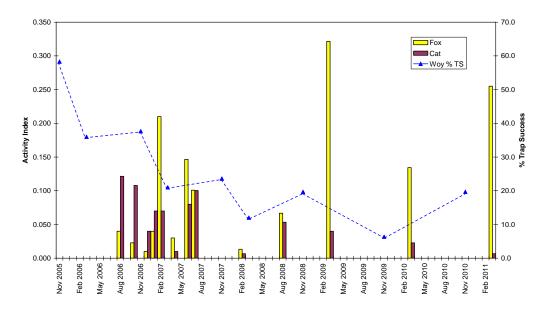


Figure 5.18 Predator activity index (AI) and woylie capture rates at Balban

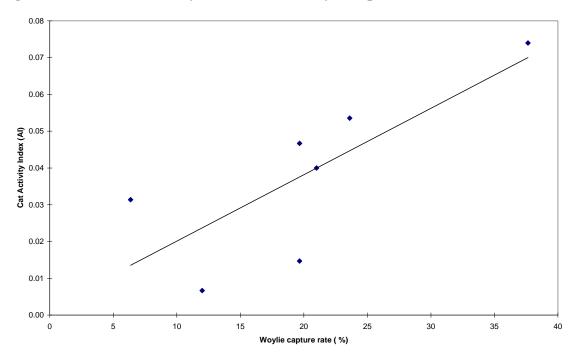


Figure 5.19 Association between cat activity index (AI) and woylie capture rates (%) at Balban (regression: n=7, p=0.04, adjusted $R^2=0.51$)

Other species declines

Several other native species have declined in the Upper Warren prior to, or during the recent woylie declines. The wambenger (*Phascogale tapoatafa*) and dunnarts (*Sminthopsis spp.*) declined in the mid 1990s (Wayne et al. 2001). Ngwayir (western ringtail possum, *Pseudocheirus occidentalis*) (discussed below) and quenda declines (Henstridge et al. 2008) appear to be of similar magnitude and timing as the woylie declines. Other critical weight range native species, such as the koomal and chuditch, do not appear to have declined or have increased during the same period.

Ngwayir (Pseudocheirus occidentalis)

The best ngwayir monitoring dataset available from the region comes from routine spotlight surveys along three transects in Warrup, Winnejup and Kingston forest blocks (1996-2011, however 1996 data is not included here given the different methodology used). Conducted as part of the 'Kingston Study' (Wayne et al. 2001, 2005), the data indicates that ngwayir began declining in Warrup and southern Kingston forest blocks in 1998 and simultaneously on the other two transects in 1999. Ngwayir have remained undetectable within the greater Kingston area since 2002, except for one sighting in 2006 (Figure 5.20). Annual or biannual spotlight surveys (2000 - 2010) in Yendicup and Yackelup forest blocks indicate that ngwayir began declining in these areas in 2002 and 2003 respectively (Bushranger program, B. Ward and G. Liddelow, unpublished data). No ngwayir have been detected on either of these transects since 2007 (Figure 5.21). Spotlight surveys conducted by the Donnelly District along several transects (Boyicup, Chariup, Keninup, Moopinup) across the Upper Warren (2004-2009) indicate that ngwayir have been declining since surveys began. No ngwayir have been detected on these transect since 2006 except for Keninup, which detected seven individuals in 2007 and one individual in 2009 (J. Wayne and I. Wilson, unpublished data). The results for the surveys across different transects conducted by the Donnelly District have been combined given the limited nature of the data (Figure 5.22). The DEC Fauna Management Course have conducted annual spotlight surveys (late October/Early November 2001-2011) along four transects per year. There has been some variation in the location of these transects over time. The transects have covered parts of central Perup (Balban, Yendicup, Moopinup, Yackelup and Camelar forest blocks). The length (generally around 10 km each) of the transects and number/experience of observers per transect have also varied between years. Nonetheless the data clearly indicates a decline in ngwavir detection rates, with no ngwavir having been detected since 2004 (Figure 5.23).

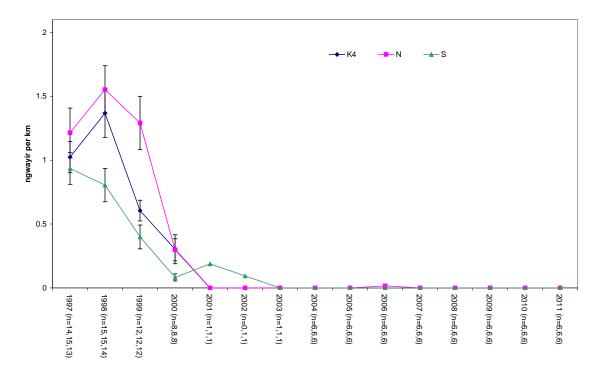


Figure 5.20. Annual average spotlight detection rate (individuals per km, +/-SE) of ngwayir (*P. occidentalis*) along three transects in the greater Kingston area – 'K4' = Kingston 4 forest coupe, Northern (N) = Winnejup & northern Kingston, Southern (S) = Warrup and southern Kingston (Source: Kingston study, Wayne et al. 2005 and unpublished). Note: n = total number of surveys conducted within a given year on each of the three transects: K4, N, S respectively.

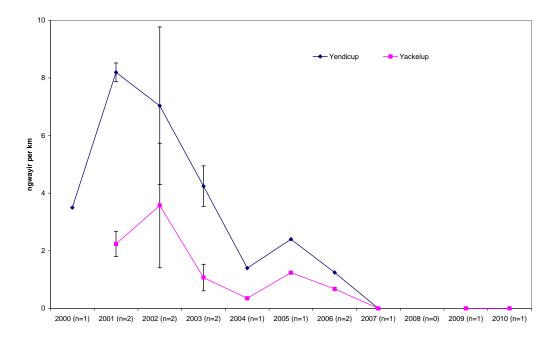


Figure 5.21. Annual average spotlight detection rate (individuals per km, +/- SE) of ngwayir (*P. occidentalis*) along two transects in central Perup (Source: Bushranger surveys, B. Ward and G. Liddelow). Note: n = number of repeat surveys along the same transect in any given year.

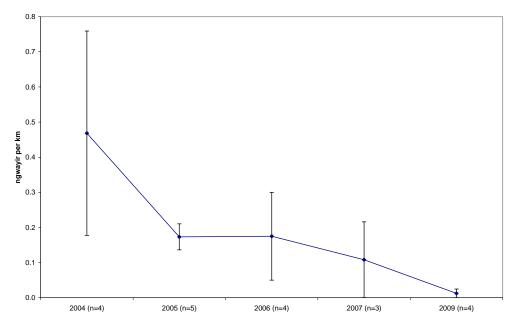


Figure 5.22. Average (+/- SE) detection rate of ngwayir (*P. occidentalis*) from Donnelly District spotlight records within the Upper Warren region (transects from Boyicup, Chariup, Keninup and Moopinup forest blocks combined) (Source: Donnelly District monitoring, I Wilson and J. Wayne). Note: n = number of transects surveyed in any given year. Each transect was generally surveyed twice (range 1-3) within a year.

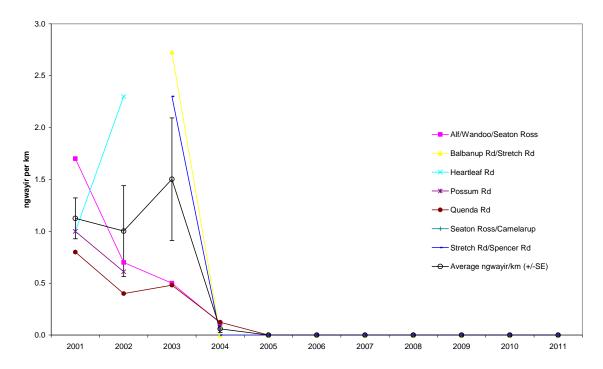


Figure 5.23. Detection rate of ngwayir (*P. occidentalis*) from annual spotlight surveys (n=4 transects per year) conducted by the Fauna Management Course in the Perup (Source: Species and Communities Unit, J. Renwick and P. Orell).

Media

Media coverage relating to the woylie declines and the Woylie Conservation Research Project has been an important part of the project in increasing public awareness and interest in biodiversity conservation and the activities of DEC and key collaborators on this issue. Data from media monitors indicates that quarterly audiences have been greater than 2 million. An average 29 articles have been published per annum since 2006 (Table 5.7). ABC regional radio (Bunbury and Albany) have been particularly active in reporting the woylie story as well as other state and national ABC and commercial radio stations. Television broadcasts have included repeated news articles on all Western Australian commercial and ABC stations, 7:30 Report (ABC), Today Tonight (Channel 9), and Totally Wild (Channel 10). Newsprint articles have been dominated by local regional newspapers, The West Australian, Sunday Times (WA), Australian and Sunday Telegraph (UK). Magazine articles have included, The Bulletin, Australian Geographic, RMW Outback, Living Planet (WWF), NewsPaws (PZ), Wildlife Matters (AWC), Murdoch Campus (MU) and Environment and Conservation News (DEC). Other print articles have included society newsletters including Australasian Wildlife Disease Association, Society for Conservation Biology, Save the Tasmanian Devil Program and WA Naturalists Society.

	2006	2007	2008	2009	2010	2011	TOTAL
Gov. Media Release	s 3			2	1	1	7
Television	1	2	3	5	1	1	13
Radio	3	16	2	13	2	1	37
Newspaper	12	6	11	28	12	5	74
Other Print	3		5	10	2	3	23
DEC Web	2		2				4
ΤΟΤΑ	L 24	24	23	58	18	11	158

 Table 5.7. Summary of media articles relating to the woylie declines and the Woylie

 Conservation Research Project 2006 to June 2011

Publications and presentations

To date there have been 12 peer-reviewed papers published in scientific journals, five non-peer reviewed publications (principally in *Landscope*), more than 31 reports, 14 student theses, more than 45 conference proceedings, four prizes and 22 research proposals and funding applications since 2006 (Appendix 2). More than 100 oral presentations and 7 posters have also been delivered on aspects of the woylie conservation research project at national and international conferences and symposia and other forums by DEC staff and collaborators. Three workshops have been convened; Recent mammal declines in southwest forests, Perup (February 2006), Wildlife forensics workshop and course: Applying science and rigour to fauna mortality events, Kensington (October 2006), and Woylie symposium and workshop, Murdoch University (February 2008). A guest lecture to the Wildlife Biology (Bio317) Course at Murdoch University has been delivered most years since 2007.

6 Discussion

Woylie declines

The Woylie Conservation Research Project (WCRP) has clearly demonstrated that there has been a rapid and substantial decline in woylies in the Upper Warren resulting in a 95% decline by the end of 2010, from an overall population peak in 1999 of around 213,000 woylies. The characteristics of the decline provide essential evidence of the possible causes of the decline. Key points include;

Mechanics of decline

- Mortality is a principal driver in the decline (primarily based on evidence from radio-telemetry).
- The rates of decline are greater than what can be expected if there was complete recruitment failure (i.e. by default population loss must be involved). The proportion of adult females with pouch young remain high and are not associated with the declines.
- Emigration is not associated with the declines (based on evidence from trapping and radio-telemetry).

Patterns and attributes of decline Australia

- Substantial detectable declines began 1999 in Greater Kingston (Upper Warren), 2001 in Dryandra, 2002 in Perup (Upper Warren), 1999-2003 in Boyagin West, 2003 in Batalling, 2005 in Venus Bay Peninsula, South Australia, and about 2010 in Tutanning.
- All extant indigenous populations have declined since 1999 (Kingston, Perup, Dryandra, Tutanning).
- All populations that have declined are on mainland Australia with foxes and cats present.
- Most declined populations were large, including 7/10 populations with >200 individuals in 2001.
- Substantial populations that have not declined are physically isolated by fences (Karakamia) or ocean (St Peter's and Wedge Islands) and effectively free of introduced predators.

Regional-level patterns across the Upper Warren

- A spatial pattern to the timing of when sites first underwent substantial declines (>10% per annum) is clearly evident in Perup; starting in Camelar (2002), spreading at an average of 3.7 km per year, ending in Keninup (2008)
- Substantial and rapid declines occurred net loss of around 200,000 individuals in the 8 years from 2002 (<73% decline and <107,000 net loss of individuals within any given year) within the core population extent of 110,000 ha
- The greatest declines occurred between 2003 and 2005 (i.e. 79% decline or approximately 147,000 net woylies in 2 years)

Site-level within the Upper Warren

- All sites had greater than 40% capture rates prior to decline (i.e. >1.3 woylies per ha)
- The duration of the decline at particular sites was typically 4 years (range 3-5 years)
- Successive annual rates of decline across years was similar at all sites
- Rates of decline were rapid (25%-95% per annum)
- Declines have been substantial average 96% declines per site (range 87-100%)
- Post-decline capture rates have been typically <5% and sustained for at least 4 years

Associations with the woylie declines in Upper Warren

Woylie mortalities

- 97% of the bodies of radio-collared woylies that have been recovered (n=29) have had strong evidence of predation/scavenging
- Cat predation/scavenging were associated with 62% of radio-collared woylie mortalities during declines. Foxes were associated with a quarter of mortalities.

Population/site declines

• Skin and fur condition prevalence and severity

- Some haematological attributes such as lymphocytosis
- Trypanosome prevalence and parasitemia
- Toxoplasma prevalence (weak association based on incomplete information)

Not associated with woylie declines in Upper Warren

- Habitat loss/modification (including logging)
- Fire or timber harvesting
- Direct human interference (live-trapping)
- Food resources

Factors limiting recovery in Upper Warren

• Cat predation and increasing levels of fox predation in post-decline woylie populations are likely to be major factors limiting recovery.

Other species declines in the Upper Warren

The decline of quenda (*Isoodon obesulus*) has previously been associated with the woylie decline (Henstridge et al. 2008) but requires review and further investigation. The decline of ngwayir (western ringtail possum, *Pseudocheirus occidentalis*) has also been previously reported (Wayne et al. 2001, 2005) and appears to coincide with the decline of the woylie. Although the level of detail is not as great or complete for ngwayir as it is for woylie, available records indicate the first ngwayir declines began in 1998 in Warrup, where woylie declines were first detected in 1999. Ngwayir are now undetectable by spotlighting across most of the Upper Warren, where once it was common or abundant. The only records of ngwayir from routine spotlight transects since 2006 have been from Keninup (7 in 2007 and 1 in 2009), where woylie populations were the last affected within the region (woylie declines began in Keninup in 2007).

The decline of ngwayir to generally undetectable levels is of high conservation concern given that the Upper Warren supports the largest extant inland population of the species. Other species have also substantially declined and not recovered, such as the wambenger and dunnart which declined in 1994-1995 (Wayne et al. 2001), while others have apparently remained stable or have increased (e.g. koomal and chuditch).

There is serious concern that community level fauna declines have been occurring. This is of particular significance given that the Upper Warren has long been recognised as one of the most important fauna conservation areas in southwestern Australia (DEC 2011). It is a rare national example of an area that supports a high diversity and abundance of conservation-priority medium-sized mammals within an ecosystem that supports an almost intact indigenous marsupial fauna assemblage (dalgyte were recently reintroduced, the boodie is locally extinct). This includes a range of species that have contracted in range to a limited number of sites (e.g. Upper Warren and Dryandra) and/or that have historically declined (e.g. numbat, woylie, ngwayir, tammar, chuditch, wambenger and quenda) (Wayne and Moore 2011).

The apparent associated declines of quenda, ngwayir and woylie needs clarification. This is important for the conservation of the individual species concerned, ecosystem and habitat management across the Upper Warren region and the successful diagnosis of the

causes of these declines. This includes resolving the nature of these declines and associations (e.g. spatial and temporal patterns, decline characteristics, related versus coincidence, cause versus effect, etc) and determining whether there are significant changes in other co-occurring species (e.g. numbat, koomal, chuditch, phascogale, dunnart, etc). For example, this would help verify whether decreased woylie densities has resulted in koomal increases in response to increased available food resources, which in turn has resulted in decreased ngwayir as a result of increased competition with koomal for hollow resources. Similarly it would help clarify whether it is likely that a common factor(s) is responsible for the multi-species declines, such as predation, and/or help explain why some critical weight range species have declined while others have increased.

Of the species that have recently declined, the woylie is by far the more readily studied: it is more abundant, more readily caught, and has more historical data available across space and time. Therefore the woylie serves as a practical model that may help to inform the causes of declines in other species. Conversely, incorporating evidence from other species may assist in the identification of the factors driving woylie declines.

Predators - the case for their role in the woylie declines

Around 0.5 predators per km² has been estimated necessary to reflect peak rates of decline observed in the Upper Warren if no other mortality factors were involved. These densities are high considering the area is routinely fox-baited, but they are possible given the abundance of prey and particularly if fox control has not been effective or mesopredator release of cats has occurred. The density of predators in the Upper Warren are unknown. Problematic as it is, inference from activity indices (AIs) derived from sandpad surveys since 2006 (i.e. after the 2005 peak in woylie declines) are not likely to suggest densities as great as this. Density estimates in the northern jarrah forest were <0.05 foxes per km² and <0.04 cats per km² at unbaited sites and <0.04 foxes per km² and <0.03 cats per km² at fox-baited sites (de Tores 2009).

Although highly variable, notoriously difficult and error prone, most non-urban density estimates in areas without predator control are 1-4 foxes per km² (Carter 2010 and references within) and typically around 1 cat per km² (0.03-7 feral cats per km²) where cats rely on hunting with very little or no food subsidy such as rubbish tips and human settlement (Denny and Dickman 2010 and references within). Around 0.5 foxes per km² were recorded from the interface of farmland and forest around the Beverly and Bannister districts in Western Australia (N. Marlow *pers. comm.*).

The spatial behaviour and ecology of the introduced predators is also not known for anywhere in the southern jarrah forest, but observations elsewhere may be similar with what might be expected in the Upper Warren. Although fox home ranges can be in the order of 1000's ha in arid environments (Burrows et al. 2003) and around 1000 ha in the WA farmland/forest interface (N. Marlow *pers. comm.*), the average size in predominantly natural temperate coastal habitats in NSW (including forests) has been 135 ha to around 370 ha (range 60 ha and 520 ha; Phillips and Catling 1991, Meek and Saunders 2000). Fox homeranges are typically exclusive. Individuals have been typically observed moving around 10 km or greater per evening. Dispersal distances of young are typically 10-30 km in rural settings but may be less than 4 km in urban settings and greater than 300 km in some circumstances (Carter 2010 and references within). Male cat home ranges are on average 3.5 times larger than those of females and there is overlap in the home ranges of both males and females (Liberg and Sandell 1994). Home ranges recorded across a range of habitats have varied between 41 ha to >20,000 ha for males (typically less than 700 ha) and 29 ha to <700 ha for females (Denny and Dickman 2010 and references within).

There is a high level of confidence in the identity of the predators/scavengers associated with woylie bodies based on the integration of multiple independently derived lines of evidence (DNA, forensic odontology, laboratory review and classification of carcass characteristics, field evidence, necropsy, and results from Rogers 2009).

While most woylie deaths have been primarily associated with cats (especially while declines have been underway), there were no significant differences in cat AIs between sites and there were no clear associations between cat AI and the different stages of woylie decline, either between sites or within sites over time. The exception to this is the significant positive association between cat AI and woylie capture rate at Balban. However, there are limitations to this analysis including the low number of observations (n=7) and the same associations were not significant at either Keninup (n=9) or using all data combined across the Upper Warren region (n=38; site included as a covariate).

The sensitivity of the methodology used to derive predator AIs and the nature of their relationship to actual predator abundance or density within the Upper Warren is unknown, but a high correlation has been found elsewhere (e.g. WA ranglelands, D. Algar et al. *pers. comm.*). Nonetheless, the predator AIs are measures of encounter rate that can be inferred as predator encounter probabilities, which can be considered an index of predation risk.

Fox AIs were significantly different between sites and greatest in northern Perup (Balban and Keninup) where contemporary declines were occurring. Parts of Keninup, the last area in the Upper Warren to undergo woylie declines, were also not subject to aerial foxbaiting. Nonetheless, foxes were primarily associated with zero and 37% of the woylie deaths during population declines in Balban and Keninup, respectively. Furthermore foxes account for no more than about a quarter of all observed woylie mortalities across the Upper Warren (2006-2009). The significant negative association between woylie capture rates and fox AI, based on all Upper Warren data combined (August 2006 -March 2011), relates to the very final stages of the woylie decline, by which time woylies had already declined by 93%. Therefore, with all available evidence considered, there is no direct evidence that foxes were the primary causal agent to the woylie declines in the Upper Warren. Foxes were nonetheless a substantial factor associated with woylie mortalities during the declines. There is also clear evidence that foxes are becoming an increasingly more important factor in limiting the recovery of woylies in the Upper Warren. This is particularly important in light of fox control being one of the most readily available management tools, which if effectively applied, is potentially one of the key strategies for promoting the recovery of the woylie and other species.

There is stronger evidence that cats are more closely associated with the woylie declines (e.g. mortalities and AI association with woylie declines at Balban) and therefore remain as one of the primary putative agents of decline. Cat predation is also a likely limiter to

woylie recovery. The effective control of cats within key woylie populations is therefore necessarily a key priority for the conservation and recovery of the woylie.

The limited available scientific evidence from the Upper Warren is not consistent with a release of cats in lieu of a reduction of foxes by baiting. It nonetheless remains possible - particularly in light of the evidence from the northern jarrah forest (P. de Tores *pers comm.*). Therefore it is recommended that there is an integrated approach to the control of foxes and cats and the careful monitoring of predators, woylies and other wildlife to verify i) the effectiveness at reducing both target predators, ii) the population responses by woylies, and iii) that there are no perverse outcomes, particularly for other conservation priority species.

Early results from the Perup Sanctuary indicate that in the absence of introduced predators the survivorship of woylie individuals and population growth is substantially greater than concurrently observed in the wild elsewhere in Perup, in the presence of introduced predators.

Other possible agents of decline

Not all of the evidence is consistent with predation being the only or primary factor causing the woylie declines and the weight of evidence indicates that other factors may be involved. For example, the striking spatial and temporal pattern of the decline across the Upper Warren populations is difficult to explain by predation alone. The spread through Perup, from a point source beginning in Camelar (2002) and ending in Keninup (underway), is not easily explained by predation when cats and foxes have long been established throughout the region. It is also uncertain whether the seemingly high estimated density of predators required to produce the rate and magnitude of the observed woylie declines is greater than what might be expected for the area.

Similarly, the fine spatial scale of the progressive spread of the declines through Perup is not consistent with our current understanding of the territoriality and scales of movement by foxes and cats. For example, the decline of woylies in Balban (by 91%, 2005-2007) to very low densities was not detected 4 km north in Keninup (where woylies were more abundant and increasing) for at least another two years. This spatial scale is comparable to the nightly movements, homeranges and dispersal distances observed for foxes and cats in various habitats across Australia, albeit highly variable (previously discussed). It is difficult to account for why predators would continue to prey upon the last remaining woylies in one area when nearby they remain in abundance without other factors being involved that can counter the effect of reduced predator efficiency (i.e. increased search effort) corresponding with reduced prey density. Similarly, the apparent lack of prey switching to other critical weight range species, such as koomal and chuditch, as woylies become increasingly rare is also difficult to explain by predation alone (although there may be some evidence of concurrent declines in some CWR species such as ngwayir and quenda).

The declines of woylies across a range of spatially isolated sites (e.g. Kingston, Perup, Dryandra, West Boyagin, Batalling) all began within a few years (1999 - 2003). It is difficult to account for this by predators alone given the diversity of habitats and predator control histories across these sites. Examples of this diversity include, duration of baiting,

baiting frequency, degree of forest fragmentation by agriculture, size and shape and degree of isolation of contiguous habitat, climatic differences, vegetation structure and floristics, site productivity, density and diversity of other prey species.

Also the predation associations with woylie mortalities, predator AIs and woylie population status do not consistently or clearly correspond within and between sites over time. Therefore the most parsimonious explanation is that predation (principally by cats), in conjunction with at least one other major factor, is the cause of the decline of woylies.

The spatial pattern and magnitude of the declines and the associated differences in health indicators (skin and fur conditions, haematology), are consistent with disease being an important or critical factor in the declines. Associations with various parasites (e.g. *Trypanosoma* spp. and *Toxoplasma* strains) are apparent but remain to be more rigorously investigated. Furthermore, not all likely disease agents have yet been adequately examined.

New woylie conservation research

A new 3-year project, "Using well managed habitat to rescue woylies from the brink of extinction', led by Warren Catchments Council in collaboration with DEC and funded by a Caring for Our Country Federal (CFoC) grant began in November 2010. It integrates with and enhances existing DEC activities in the Upper Warren directed toward woylie conservation. The project has three major components;

1) Introduced vertebrate pest (IVP) control and monitoring – principally wild dogs, rabbits and pigs

2) Comparative monitoring of woylies in the Perup Sanctuary and free-ranging populations.

- Monitoring of the sanctuary woylies (individual survivorship and population growth) to verify the success of establishing a secure woylie colony within the sanctuary and to provide a timely alert to any possible problems such as predator incursions
- Health monitoring a continuation of existing protocols plus hair and faeces samples for cortisol stress analysis and EDTA for antioxidant studies
- In the absence of predators it is an opportunity to examine whether other factors may be limiting woylie recovery
- Baseline biological surveys to be able to quantify the impact of anticipated high densities of medium-sized mammals within the sanctuary in the next decade

3) Increasing community awareness and engagement in threatened species conservation. This includes encouraging volunteer participation in conservation and natural resource management, greater opportunities for indigenous people to be involved and to promote indigenous perspectives on woylies and wildlife.

A PhD project focusing on a comparative study of wildlife ecology in and outside the Perup Sanctuary began in 2011 (Georgina Yeatman, University of Western Australia). It includes a comparison of woylie home ranges derived from radio-telemetry and environmental factors (such as habitat attributes, fire and logging history) associated with the abundance of woylies in the Upper Warren based on available trapping data prior to, during and after the decline. The project will also be conducting the baseline biological surveys component of the CFoC project (described above) for small terrestrial vertebrates, within the framework of a comparative investigation of responses to habitat types.

Predator control and management

Key points regarding the management of predators include;

Fox control and management

- Operational issues with timing and intervals of aerial baiting need to be resolved.
- Fox activity is significantly different across the Upper Warren region but overall it has increased substantially over time, particularly since 2007.

Effectiveness of fox-baiting

- There is a very high uptake of baits by non-target native species, which substantially reduces the number of baits available to foxes. An increased density of baits may be an important part of the strategy to overcoming this.
- The high incidence of partial bait consumption increases the potential for sublethal doses to foxes, which may result in increased tolerance to 1080 and/or increased bait shyness. The reduced toxicity of baits from 4.5 mg to 3.0 mg further increases these risks.
- The monitoring of predator activity or abundance is important to verify the sustained effectiveness of control activities and to alert managers of possible emerging issues.

7 Recommendations

Multiple species / community level declines in the Upper Warren

The recent substantial declines of multiple conservation-listed species throughout the Upper Warren (e.g. woylie, ngwayir, wambenger, quenda) remains an urgent and important conservation priority given the significance of the area and these populations to the viability and conservation of these species. A synthesis and review of all of the available fauna data for the region is required to more completely assess the extent and nature of the declines and the strength of the possible associations between species. Additional data may be required to assess the status of some priority species such as the numbat, ngwayir and phascogale. These results should inform management on how best to respond to the situation and be integrated into the investigations of the causes of the woylie declines and the factors limiting their recovery.

Causes of decline

Determining the causes of the declines of woylie populations remains critical to successfully and efficiently managing a robust and sustained recovery of the species. Key priorities in the diagnosis of the woylie declines include;

- a. Improving the characterisation of the declines of woylies and co-occurring species (e.g. case definition, spatio-temporal analysis and epidemiological analysis).
- b. Rigorously determining the strength and nature of the known associations. This includes determining whether the associations are i) coincidence or related, ii) if related whether it is a cause or effect of the decline, and iii) if it is a cause, then its significance.
- c. Close monitoring of key woylie populations, other declining species and potential agents of decline, such as predators and disease. Monitoring declining species should include detecting potential clinical cases, moribund individuals and recently deceased animals that can be thoroughly investigated (i.e. the strongest line of evidence to identify agents potentially responsible for population declines).

Factors limiting woylie recovery

Effective woylie conservation now also depends on understanding the factors that limit the recovery of post decline populations; given that most large woylie populations have now declined. This explicitly recognises that the factors that limit recovery may be different from those that reduced populations – i.e. the distinction between the declining population paradigm from the small population paradigm (Caughley 1994). For instance, predation is likely to be an even greater factor in limiting recovery than it was in the recent declines. Verification that this is the case can be achieved by adequate comparative monitoring of woylies and predators under different predator control regimes within an adaptive management framework. The basis of this approach is already in place within the Upper Warren region with monitoring occurring within the Perup Sanctuary (predator free), core Perup area around the sanctuary (monthly ground baiting), and within the remaining DEC-managed land (quarterly baiting by ground and aerial delivery) (note some other smaller areas are either only aerially baited or only ground baited or not baited at all). Integrating, developing and maintaining this program across key woylie populations, is therefore a key ongoing priority.

Predators

Key priorities regarding introduced predators include;

- Integrated and effective cat and fox control is needed in areas with high conservation values such as the Upper Warren and other key woylie populations.
- Other bait related issues to be resolved include; reduced availability of baits for introduced predators due to high interference by non-target species, high incidence of partial bait consumption and the associated risk of sublethal bait encounters (also a function of bait toxicity) that may lead to bait shyness and/or resistance to the 1080 toxin.

- Analyse the risk to wildlife conservation of distributing potential pathogens or other disease agents within the baits used for predator control (e.g. *Toxoplasma*, novel bacteria, toxins, etc).
- Operational issues associated with the timing of bait delivery need to be resolved and assurance measures in place to maintain a higher standard of delivery.
- Determine the density, spatial behaviour and ecology of introduced predators within the Upper Warren. This can also be used to calibrate and assess the predator monitoring methods based on sand pads and activity indices and improve the monitoring methods for broader operational use where appropriate. This would complement and build on the achievements of the other mesopredator projects (Marlow et al., de Tores et al. Algar et al., Morris et al.) and may include the use of DNA capture-recapture methods and radio-telemetry. This is also an opportunity to assess the effectiveness of the different predator control regimes within the region.
- Effective monitoring of predators in conjunction with the monitoring of woylies and other wildlife. This includes having a capacity to identify and respond appropriately to changes in predator abundance in a timely manner. Where possible the methodology used should be standardised to enable comparability between areas and make possible a more powerful meta-analysis of predator ecology and their responses to control measures.

Disease

The highest priorities for determining the role of disease in the declines and recovery of woylies includes;

- The timely detection, recovery and examination of compromised, moribund and recently deceased woylies remains singularly the most powerful means to assess and identify whether predators, disease or other factors are key agents of decline and/or limiters to population recovery. Adequate surveillance and monitoring of key woylie populations that improves the probability of detection of such cases is critical in this regard.
- Epidemiological expertise to assist in the completion of a woylie decline case definition, spatio-temporal analysis of the declines and an epidemiological analysis of all available evidence with a focus on the key associations detected to date (skin conditions, haematological attributes, the blood borne parasites *Trypanosoma* and *Theileria* and the systemic parasite *Toxoplasma*).
- Completion of the review of the pathology evidence in light of the fact that the recent case of oesophageal myopathy may be common to other woylie mortalities and given that Leishmania may be the aetiological agent
- Continue health and disease monitoring of woylie populations in the Perup Sanctuary and Upper Warren and develop similar monitoring in other key populations. This includes the development of a reference library of material for future analysis.

• Maintain ongoing support to collaborating expertise investigating disease aspects that may be directly related to woylie conservation

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9 Appendices

Appendix 1 – Student projects collaborating with WCRP

Completed student projects

- Basille, S. (2011) The epidemiology of piroplasm infection in the woylie (*Bettongia* penicillata ogilbyi). Undergraduate Project (Independent Study Contract). Murdoch University
- 2. Eikelboom, T. (2010). A field comparison of survey methods for estimating the population density of woylies (*Bettongia penicillata*) at Karakamia Wildlife Sanctuary. Honours Thesis, University of Western Australia.
- 3. Hide, A. (2006). Survival and dispersal of the threatened woylie *Bettongia penicillata* after translocation. Honours Thesis, University of Western Australia.
- 4. Hunt, H. (2010). A temporal assessment investigating the effects of population declines on genetic diversity, in the critically endangered woylie (*Bettongia penicillata ogilbyi*). Honours Thesis, Murdoch University.
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- 8. McCalmont, J. (2010). Evaluation of conservation measures for a specific endangered species, *Bettongia penicillata*. 3rd year BSc (Hons) project. Institute of Biological, Environmental and Rural Science, University of Wales, Aberystwyth.
- 9. O'Brien, R. Christopher (2008). Forensic animal necrophagy in the south-west of Western Australia: Species, feeding patterns, and taphonomic effects. PhD thesis, University of Western Australia.
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- 11. Parameswaran, N. (2008). *Toxoplasma gondii* in Australian marsupials. PhD thesis, Murdoch University.
- 12. Rogers, P. (2009). Predator profiling as a tool for the conservation of the woylie (*Bettongia penicillata*). Honours thesis, University of Western Australia.
- 13. Yeatman, G. (2010) Population demographics of a fenced population of woylie. Honours Thesis, University of Western Australia.

Current student projects

- 1. Abdad, Y. (PhD, MU) Rickettsial infections in wildlife and humans in Western Australia
- 2. Botero, A. (PhD, MU) Genetic characterisation of trypanosomes
- Burmej, H. (PhD, MU) Ectoparasites of threatened mammals in Western Australia: Biodiversity and impact

- 4. Kaewmongkol, G. (PhD, MU) Bartonella infections in wildlife and domestic animals in Western Australia
- 5. Pan, S. (PhD, MU) *Toxoplasma gondii* infection and atypical genotypes in Western Australian wildlife species.
- 6. Parkar, U. (PhD, MU) Blastocystis in humans and other mammals
- Skogvold, K. (MVSc) A comparative health and disease investigation in the woylie captive vs free-range enclosure vs wild
- 8. Thompson, C. (PhD, MU) Trypanosome effects on woylies and their vectors
- 9. Worth, A. (PhD, MU) Toxoplasma effects on woylie behaviour
- 10. Yeatman, G. (PhD, UWA) Woylie and wildlife ecology in the Upper Warren
- 11. Zosky, K. (PhD, MU) Food resources and woylie declines in south-western Australia

Appendix 2 List of woylie conservation research project (WCRP) publications

Note: some lists are incomplete

Peer-reviewed publications

- Averis, S., R. C. A. Thompson, A. J. Lymbery, A. F. Wayne, K. D. Morris, and A. Smith. 2009. The diversity, distribution and host-parasite associations of trypanosomes in Western Australian Wildlife. *Parasitology* **136** 1269-1279.
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- Thompson, RCA, A. Smith, A. J. Lymbery, S. Averis, K. D. Morris A. F. Wayne 2010. *Giardia* in Western Australian wildlife. *Veterinary Parasitology* **170**: 207-211.
- Zosky, K., K. Bryant, M. Calver, and A. F. Wayne. 2010. Do preservation methods affect the identification of dietary components from faecal samples? A case study using a mycophagous marsupial. *Australian Mammalogy* **32**:173-176.

Papers submitted

- Pacioni, C., Wayne, A., Maxwell, M., Marlow, N., Thomas, N., Spencer, P. (in review). Integrated genetic and demographic comparisons across space and time reveal important insights into a mammalian species (Bettongia penicillata) undergoing rapid decline. *Molecular Ecology.*
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- Woylie Conservation Research Project Progress summary to Woylie Recovery Team 23 January 2009
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- Thompson et al. (2006) ARC Linkage Project: The nature, diversity and potential impact of infectious agents in Western Australian threatened mammals. [Successful]

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2010

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- Lymbery et al. (2011). ARC Linkage (LEIF) project: Breaking the vicious cycle: translocation stress, parasitism and disease in threatened species
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