In situ conservation funding
Annual report to Woylie Conservation Action

Project title and location

Woylie Conservation Research Project, Manjimup, Western Australia

Background

The principal aim of Woylie Conservation Research Project (WCRP) is to determine the causal factors responsible for the recent woylie declines. Using a decline diagnosis framework broadly based on the 'declining-population paradigm' (Caughley, 1994), the WCRP focussed primarily at the declines in the Upper Warren and consists of three major components;

1. Upper Warren Fauna Monitoring that built on, enhanced and co-ordinated previously independent existing activities,
2. Meta-analysis of existing datasets that were aggregated into a single database, and
3. A Population Comparison Study (PCS) designed to discriminate factors and attributes associated with contemporary declines. The PCS has five main lines of enquiry; Woylie components -
    a) woylie density and demographics,
    b) woylie survival and mortality,

    Key putative agents of decline -
    c) predators,
    d) resources, and
    e) disease.

So far the weight of evidence indicates that the declines have been principally mortality driven. While scavenging/predation (especially by cats) has been associated with the death of most radio-collared animals there is evidence indicating that individuals may be made more vulnerable to predation as a result of other factors, principally some form of disease(s). The key associations with the declines identified through the population comparison study include the prevalence and degree of skin and coat conditions, the prevalence and parasitemia levels of Trypanosoma and the prevalence of Toxoplasma.

Project Objectives for 2008/09

The Woylie Conservation Action Funds ($10,500) were specifically directed toward assisting in the investigations into determining the role of disease in the woylie declines. Specifically, WCA funds were directed toward developing a better understanding of the nature of skin and coat conditions, the relationship of these and trypanosome infections with the declines, a review of the woylie pathology cases to date, development of the disease risk analysis, and finally support for the development of normal haematological values for the assessment of woylie health as part of

Carlo Pacioni's PhD and other related ongoing work. Much of this work was done by engaging the time and expertise of Perth Zoo staff, namely Paul Eden and Andrea Reiss, as well as the material costs associated with running PCR tests for trypanosomes by collaborators at Murdoch University.

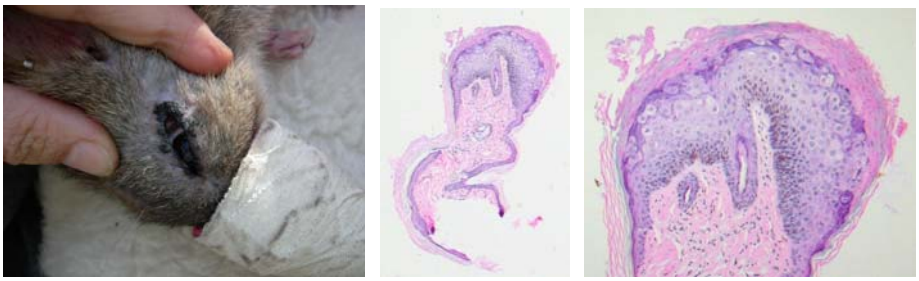
Report on conservation outcomes of the project objectives for 2008/09

A summary of the key outcomes from this project include;

1. **Investigation of skin diseases observed in woylies from the Upper Warren Region** – Lead by Dr Paul Eden (PZ) this work involved Perth Zoo vets, vet-nurses and keepers assisting in woylie monitoring in the field and undertaking clinical examinations, some under anaesthesia, biopsies and other sampling and the subsequent pathology investigations of the collected material. Analysis of the prevalence of skin conditions in relation to the declines was also conducted. The outcomes of these activities were reported (Eden et al. Mar 2010 – attached) and serve to develop our understanding of the nature and association of skin conditions associated with declines.

Examples of clinical field examinations and skin condition investigations by Perth Zoo and DEC staff and volunteers including woylies with typical periocular fur loss and crusting observed during woylie declines, a Chiggers mite found on skin scrape from a woylie with skin disease and the release of woylies at the point of capture after examination and processing.

2. **Review of woylie Pathology cases** – Led by Dr Andrea Reiss (PZ) a review was undertaken of all 39 post mortem reports from examinations conducted on woylies by Murdoch University veterinary pathology department (MUP) from 2005 to 2009 (Reiss Mar 2010 – attached). This synthesis was considered critical given that pathological evidence is key to understanding the role of disease in woylie declines. It provided a significant increase in our understanding of health and disease and interpretation of pathology findings in woylies and included recommendations for future work including following-up on unusual findings of interest that were indentified during this work.

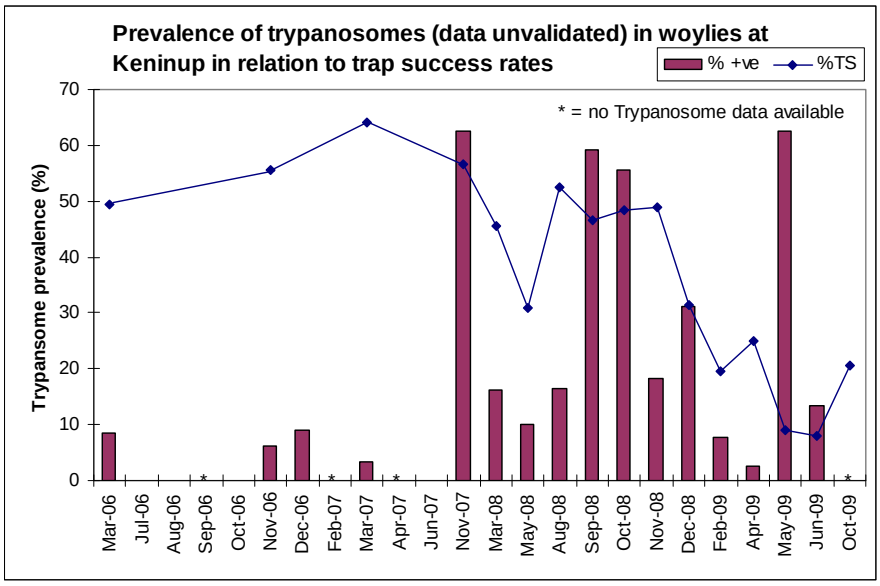


A novel papilloma virus detected by DEC field staff, clinically examined by PZ staff, pathology conducted by Murdoch University staff and subsequently genotyped and confirmed (see Bennett et al. 2010).

Examples of a gross examination as part of the post mortem and pathology of deceased woylies investigated as part of the Woylie Conservation Research Project

3. **Disease Risk Analysis** – Led by Dr Andrea Reiss (PZ) this work involved the co-ordination and development of a Disease Risk Analysis with existing collaborators. This is considered fundamentally important in informing priorities for disease investigation.
4. **Haematology** – Dr Andrea Reiss and Dr Paul Eden (PZ) provided assistance to Carlo Pacioni as part of his PhD project to review all current haematology results to date, and to create normal reference ranges for free living woylies in swWA.

5. **Trypanosoma association with woylie declines in Keninup** – led by Dr Andrew Smith (Murdoch University) this study investigated of the prevalence of Trypanosome infection (by PCR analysis of blood) over time and during the decline of woylies at Keninup – a site subject to an intensive study. The preliminary results from the 540 samples analysed provide the most compelling evidence to date of an association of a potential disease agent during a population decline. Validation of the preliminary results is in progress, the results of which are likely to be extremely important for future research. Publication of this work in an international scientific journal is planned within the next 12 months.



Attachments

i) Copy of financial statement

ii) Documents relating to the achievement of this project
- Eden et al. Mar 2010 – skin condition report
- Reiss Mar 2010 – Pathology review

iii) A bibliography of material published from the Woylie Conservation Research Project to date. Copies happily provided on request.

Signed

Department of Environment and Conservation
Our environment, our future

Dr Adrian Wayne
Research Scientist and Chief Investigator of the Woylie Conservation Research Project
Department of Environment and Conservation
Locked Bag 2 Brain St, Manjimup, 6258
10 August 2010

Specific Purpose Trust Account Reports as of Tuesday, 20 July 2010

Job: YPE Project Manager: WAYNE,AF

Woylie Disease Investigation – Woylie Conservation Action Funds

Tenure: 0000

Expenditure Type: Other Services & Contracts **FlexField: 535-06-24-FD1-1784-0000-YPE**

Date:	Journal Name:	Description of Service:	Payment To:	Debits:	Credits:
24/08/2009	ETJ118081	AUGUST 2009 NAB VISA STATEMENT		$135.44	$0.00
18/09/2009	ETJ118092	SEPTEMBER 2009 NAB VISA STATEMENT		$36.91	$0.00
4/02/2010	Purchase Invoic	WOO69822 REIMB SALARY & ONCOSTS WOYLIE DISEASE INV	PERTH ZOO	$5,000.00	$0.00

FlexField Summary: Sum of Debits: $5,172.35 Sum of Credits: $0.00 Balance: -$5,172.35

Expenditure Type: Materials **FlexField: 535-06-24-FD1-1938-0000-YPE**

Date:	Journal Name:	Description of Service:	Payment To:	Debits:	Credits:
26/11/2009	Purchase Invoic	SUS69226-DETECTION - TRYPANOSOMES FROM WOYLIE SAMP	MURDOCH	$5,000.00	$0.00

FlexField Summary: Sum of Debits: $5,000.00 Sum of Credits: $0.00 Balance: -$5,000.00

Tenure Summary: Sum of Debits: $10,172.35 Sum of Credits: $0.00 Balance: -$10,172.35

Woylie Disease Investigation

Opening Balance:	$10,223.47
Debits:	$10,172.35
Credits:	$0.00
Balance:	$51.12

Investigation of skin diseases observed in Woylies (*Bettongia penicillata ogilvyi*) from the Upper Warren Region, WA.

Paul Eden, Andrea Reiss, Phil Nicolls, Adrian Wayne

Introduction

Numbers of woylies (*Bettongia penicillata ogilvyi*) in wild populations around the Upper Warren region have been undergoing dramatic declines (average 85-90%) over the last 10 years, with Keninup undergoing a rapid decline in 2007. Observations from field researchers have reported a high level of a skin condition prior to and during declines (Wayne et al. 2008), but this has not been investigated to date. Skin conditions have also been noted in animals trapped as part of population monitoring efforts, but also have not been investigated. This project will aim to investigate the nature of observed skin conditions in woylies and interpret potential significance in relation to population declines.

Methodology

Retrospective data analysis

Woylies from the Upper Warren region were trapped and monitored as part of a population monitoring program run by the Western Australian Department of Environment and Conservation (DEC). Woylies were examined by field workers, which included assessment of the health of the skin and pelage. Lesions were noted on a standardized health assessment form and data entered into a Microsoft® Access 2003 database. Data from two transects (Keninup and Warrup) was used in a cross-sectional study to investigate for differences in prevalence of skin disease between the populations of woylies from these transects and to assess for changes in prevalence over time. These two transects were chosen as representative of a high density population that underwent decline (Keninup) and a moderate density population that is considered stable (Warrup). Subjects were selected on the basis of transect (Keninup vs Warrup) and trapping period (Oct/Nov 06 vs Nov 08 – pre-decline and post-decline for Keninup based on population monitoring data). Data was transformed to indicate presence/absence of skin disease. Prevalence of skin disease was then determined for a given transect in a given year and comparisons made using Chi-squared tests in Microsoft® Excel 2003. Statistical significance was determined for p-values$\leq$0.05. Overall prevalences for the total database, all animals sampled in a given year and all animals sampled for a given transect were not established due to these being open populations sampled over a period of 2 years.

A subset of the above raw data was developed focusing on woylies that presented with evidence of skin disease. This data was analysed after transformation to assess for a pattern of distribution of skin lesions over the woylie. The body of the woylie was divided into a number of descriptive areas and a score of 1 given to a lesion being recorded in a particular area for a particular woylie. The results were then totalled for each body area and proportions calculated for numbers of animals with lesions in that area.

Field Health Assessment

During one monitoring session in February 2009, woylies from the Keninup area with evidence of skin disease were identified for further investigation. This involved general anaesthesia using

Isoflurane in oxygen, delivered via face mask. Once anaesthetized, each woylie underwent veterinary examination, including mapping of skin lesions on a standard diagram (Fig 1) to assess distribution. Photographs of typical lesions were taken where possible, and presence of ectoparasites was noted, identifying the type of parasite observed. Fungal culture samples were collected using a new, clean toothbrush, to brush the skin and coat, focusing on areas of lesions, around the inguinum and the axillae. Skin scrapes were collected from the margins of lesions using liquid paraffin and a fresh scalpel blade, and samples were examined under a microscope using 4x and 10x objectives. Skin biopsies of 6mm diameter were collected from lesions as well as one biopsy of normal skin from each individual. One biopsy was sectioned in half, with half being placed in sterile gauze soaked with sterile saline, to submit for bacterial culture, and the other half placed in an Eppendorf tube for freezing at -80ºC. Remaining biopsies were placed in 10% buffered neutral formalin for histopathology, which was performed by Phil Nicholls (Murdoch University). Biopsy site collection was standardized across woylies where possible.

Results

Retrospective data analysis

The prevalence of skin disease for given transects in a given year are presented in Table 1. Subjectively there appears to be a difference in prevalence between Keninup and Warrup as well as a change in prevalence over time for Keninup. Chi-squared tests comparing transects at a given time revealed statistically significant differences in prevalence between Keninup and Warrup for both October/November 2006 and November 2008 ($p<0.001$ and $p=0.029$ respectively). Chi-squared tests comparing changes in prevalence over time for a given transect also revealed statistically significant differences for Keninup between 2006 and 2008 ($p=0.001$), however this was not the case for Warrup ($p=0.2$).

	Keninup 2006	**Keninup 2008**	**Warrup 2006**	**Warrup 2008**
Skin disease absent (# cases)	63	22	14	10
Skin disease present (# cases)	21	25	23	31
TOTAL (# cases)	84	47	37	41
Prevalence % (skin disease absent)	75	47	38	24
Prevalence % (skin disease present)	**25**	**53**	**62**	**76**

Table 1. Prevalence of skin disease for a given transect in a given sampling period

Data analysis to assess for distribution of skin lesions in woylies is presented in Table 2. A number of animals presented with skin lesions in more than one location. This data suggests the observed skin lesions are noted particularly around the head, eyes and tail.

[redacted]	Head	Eye	Ear	Mouth	Nose	Throat	Forelimb	Chest	Abdomen	Back	Hindlimb	Rump	Tail	number of affected woylies
Keninup 2006	0	1	2	0	0	1	0	0	0	2	0	2	13	18
Keninup 2008	6	10	3	0	0	5	0	1	0	2	0	0	14	24
Warrup 2006	4	1	7	0	0	3	0	1	1	2	2	7	7	23
Warrup 2008	16	19	7	0	0	0	1	1	0	0	0	3	10	31
TOTAL	26	31	19	0	0	9	1	3	1	6	2	12	44	96
Proportion	**27**	**32**	**20**	**0**	**0**	**9**	**1**	**3**	**1**	**6**	**2**	**13**	**46**	[redacted]

Table 2. Distribution of skin lesions in affected woylies from Keninup and Warrup

Field Health Assessment

A total of nine woylies were examined for the field health assessment component of this study, with eight of these animals undergoing anaesthesia for sample collection and one animal (WC0878) examined under manual restraint (Table 3). Eight woylies showed evidence of skin lesions; one woylie was sampled as a reference for a 'healthy' woylie (WC 0879). Table 4 shows results of ectoparasite observations from examined woylies.

Sample Number	Woylie ID	Date	Skin exam + map	Photos	Skin scrape	Fungal culture	Skin Biopsy culture	Skin Biopsy Eppendorf	Skin biopsy Histo
WC 0729	DO8083/DN0853	3/02/2009	Y	-	pos	Y	Tail base	Tail base	R hip
WC 0730	DO8271/DO8272	4/02/2009	Y	-	neg	Y	Tail base	Tail base	L hip, Tail base
WC 0873	DO7974/DO7975	4/02/2009	Y	x8	pos	Y	Tail base	Tail base	L hip, R periocular, tail base
WC 0875	DO6669/DO6668	4/02/2009	Y	-	neg	Y	Tail base	Tail base	R hip, L periocular, tail base
WC 0876	DO1493/DO1494	4/02/2009	Y	-	neg	Y	Tail base	Tail base	R hip, tail base
WC 0735	DO3757/DO3756	5/02/2009	Y	-	neg	Y	Tail base	Tail base	L hip, tail base
WC 0736	DN0856/DN0857	5/02/2009	Y	y	neg	Y	N	N	R hip, tail base
WC 0878	DN0801/DN0802	5/02/2009	Y		neg	N	N	N	N
WC 0879	DO7985/DO7986	5/02/2009	Y	-	-	Y	Tail base	Tail base	R hip, tail base

Table 3. Summary of sampling of field health assessments of woylies, February 2009.

Woylie ID	Ectoparasite presence			
	Ticks	Fleas	Lice	Mites
DO8083/DN0853	X	X		
DO8271/DO8272				X
DO7974/DO7975	X	X	X	X
DO6669/DO6668	X		X	X
DO1493/DO1494				
DO3757/DO3756	X		X	
DN0856/DN0857	X		X	
DN0801/DN0802				
DO7985/DO7986	X		X	X

Table 4. Ectoparasite observations from field health assessments of woylies, February 2009

Lesion distribution typically involved the periocular skin and dorsal tail base (Table 5). Periocular lesions involved hair loss with minimal changes to the underlying skin; one woylie showed evidence of thickening of the skin at the medial canthus of both eyes. Lesions of the dorsal tail base typically showed evidence of fur loss, with thickening of the skin and varying degrees of scale formation. The extent of the lesions was variable – most were contained within the proximal quarter of the tail; one lesion extended approximately half way down the dorsal surface of the tail. Figures 2 and 3 show some typical lesions observed during field examinations.

Sample Number	Woylie ID	Date	Skin lesion distribution						
			R periocular	L periocular	R hip	L hip	Dorsal Tail base	Ventral neck	L stifle
WC 0729	DO8083/DN0853	3/02/2009	1	1	1	1	0	1	0
WC 0730	DO8271/DO8272	4/02/2009	1	1	0	0	1	0	0
WC 0873	DO7974/DO7975	4/02/2009	1	1	0	0	1	0	1
WC 0875	DO6669/DO6668	4/02/2009	1	1	0	0	1	0	0
WC 0876	DO1493/DO1494	4/02/2009	1	1	1	0	1	0	0
WC 0735	DO3757/DO3756	5/02/2009	1	1	0	0	1	0	0
WC 0736	DN0856/DN0857	5/02/2009	1	1	0	0	1	0	0
WC 0878	DN0801/DN0802	5/02/2009	0	0	0	0	1	0	0
WC 0879	DO7985/DO7986	5/02/2009	0	0	0	0	0	0	0
TOTAL			7	7	2	1	7	1	1

Table 5. Distribution of skin lesions (1 = present; 0 = absent); only affected areas are presented in the above table.

Two out of eight animals returned positive results for mite infections on skin scrapes. One woylie (WC 0729) was found to have a fur mite; another woylie (WC 0873) was found to have Chiggers mite infestation (Figure 4). No evidence of demodectic or sarcoptid mites were found in these woylies.

Bacterial culture and identification was undertaken for three of the affected woylies. Samples were selected from woylies with typical skin lesions. Culture results identified a range of bacteria, including alpha-haemolytic Streptococcus spp, Coryneform bacilli, Baccillus cereus, and Pasteurella spp.

Fungal culture was undertaken for the same three animals as above. Culture results identified evidence of *Microsporum gypseum* infection in one woylie; the other two woylies isolated mixed environmental fungi only.

Histopathology examination was undertaken for all animals that were biopsied. These revealed varying degrees of hyperkeratosis and acanthosis (thickening of the epithelium), some with mixed inflammatory infiltrates. Period Acid-Schifft staining did not reveal any evidence of yeasts or fungal hyphae. There was a lack of neutrophilic inflammation, suggesting bacterial infection is unlikely as a significant factor in these skin lesions. Neoplastic changes were not evident, however one woylie showed a focal area of excess collagen stroma – possibly recent scar tissue formation. There was no evidence of mites seen in any samples examined. Skin glands and hair follicles were all normal on histopathology, suggesting hair loss is secondary to rubbing or self trauma.

Discussion

Skin disease has been a factor in poor health and population declines of a range of wild terrestrial mammals. Well described examples include Sarcoptic mange in common wombats (*Vombatus ursinus*) in Australia (Skerratt et al 1998) and Parapoxvirus infection of red squirrels (*Sciurus vulgaris*) in the UK (Tompkins et al 2002). In both of these scenarios, the aetiological agent was identified in response to observation of skin lesions through pathology of affected cases fitting a particular case description, and then assessed to determine involvement in population declines. This study has taken a somewhat different approach, looking first at evidence of skin disease being a factor (causative or result) in woylie declines, then assessing particular individuals to determine the nature of the skin disease.

Statistical analysis of survey data indicates a significant difference in the prevalence of skin disease between woylies from Keninup and Warrup, as well as indicating a significant increase in the prevalence of skin disease for Keninup woylies from Oct/Nov 2006 to November 2008. Prior anecdotal reports have suggested a high prevalence of skin disease in woylies in the lead up to a decline; however these results do not support this. Skin disease appears more prevalent in the Warrup populations during both survey periods. Given this is considered a moderately stable population, it suggests that there can be a high prevalence of skin disease without evidence of population decline. An increase in the prevalence of skin disease at Keninup from 2006 to 2008 also suggests skin disease is not likely to be associated with causal factors for population declines, and is perhaps a symptom/result of these causal factors.

Distribution of skin lesions appears to typically affect the head region (including around the eyes and ears) as well as the tail base/rump region (Figures 2 and 3). This is supported with the results from field health assessment data. Although not typical for a particular aetiology, this information assists in developing a case definition for woylies seen with skin disease.

Ectoparasite infections were very common in woylies examined in the field, with 7 of 9 woylies infected with one or more types of ectoparasite. The most common parasites found were ticks and lice, with mites also being regularly found. Skin scrapes from one woylie were positive for a Chiggers-type mite infection (Figure 4). There is a large range of different species of Chigger's mites that have been identified from Australian marsupial hosts (Domrow and Lester, 1985), which can cause skin irritation in some species. Identification of the species of mite was not able to be performed due to deterioration of the sample. The significance of ectoparasite infections as a primary cause of disease in Australian marsupials is variable. Many marsupials may be infected with ectoparasites without overt clinical signs, however some ectoparasites, such as *Sarcoptes* spp of mites, can cause severe debilitation (Ladds 2009; Skerratt et al 1998). The authors were unable to find any reports in the literature of particular ectoparasites causing skin disease in woylies, however identification of ectoparasites from wild woylies of the Upper Warren region is being undertaken as part of a PhD project by Halina Burmeij.

Histopathology and culture results revealed a consistent pattern of thickening and hyperkeratosis of the skin, with mild to moderate inflammatory changes. There appears to be little to no involvement of the glands or hair follicles in the skin, suggesting hair loss is likely to be secondary to irritation and self trauma. A lack of neutrophilic inflammation, and culture of a range of bacterial isolates from different individuals, suggests primary bacterial infection is not a likely cause of skin disease in these woylies. *Microsporum gypseum* was isolated from one affected animal – this organism is a common saprophyte, and although capable of causing disease in its own right (Carter 1986), is likely to be a secondary invader in this particular case. These findings are non-specific and do not identify a particular aetiology for this condition, however would be consistent with hypersensitivity, self trauma or ectoparasite infections, the latter of which is supported by examination findings and skin scrape results. There does not appear to be any subjective correlation between the severity of skin changes on histopathology and the distribution of lesions, or the severity of ectoparasite infection, however the latter was not objectively assessed for these cases.

The term 'skin disease' encompasses a large array of aetiological agents and presenting signs, and it is beyond the scope of this investigation to examine all potential agents. The sample size included in this study, particularly in relation to the field health assessments, is also somewhat limited and does not accurately reflect the varying degrees of skin lesions anecdotally reported in wild woylies. However the results from this study help to define a case definition for this particular presentation of skin disease in these animals, and presents some of the potential aetiologies that may cause these lesions. Other agents that have been identified as causing skin lesions in woylies include a poxvirus, however the presentation of lesions associated with this infection are clinically quite different to the presentations described in this study, and have only been described in two cases to date.

Conclusion

Although skin disease has been anecdotally reported in high levels in the lead up to a population decline for woylies, data from this study does not support this, instead suggesting skin disease may occur at a high prevalence without signs of a population decline, and that prevalence of skin disease may change as a result of population decline. The nature and distribution of the skin lesions investigated in this study is non-specific, and may be the result of a range of causative agents, including ectoparasites. Affected woylies that underwent field examination tended to be infected with at least one type of ectoparasite, and it is likely that ectoparasites are involved with the presence of these skin lesions in wild woylies.

Acknowledgements

This work was funded by Wildlife Conservation Action as part of the Woylie Conservation Research Project with considerable support and associated data from the Department of Environment and Conservation, Perth Zoo and Murdoch University.

References

Carter, G.R. 1986. *Essentials of Veterinary Bacteriology and Mycology*. Lea and Febiger, Philadelphia. pp228-231.

Domrow, R. and Lester, L.N. 1985. Chiggers of Australia. *Australian Journal of Zoology Supplementary series No. 114*. Melbourne:CSIRO Australia.

Ladds P (2009), *Pathology of Australian Native Wildlife*, CSIRO Publishing, Collingwood.

Skerratt LF, RW Martin, KA Handasyde. 1998. Sarcoptic mange in wombats. *Australian Veterinary Journal* 76(6): 408-410.

Tompkins DM, AW Sainsbury, P Nettleton, D Boxuton, J Gurnell (2002), Parapoxvirus causes a deleterious disease in Red Squirrels Associated with UK Population Declines, *Proceedings: Biological Sciences* 269(1490): 529-533, Royal Society of London.

Wayne, A. F., Swinburn, M., Ward, C., Wayne, J., Whittred, B., Maxwell, M. and Vellios, C. (2008). Chapter 5.2: Field health and disease sampling. In 'Progress report: woylie conservation research project'. (Ed. A. F. Wayne) pp. 207-216.

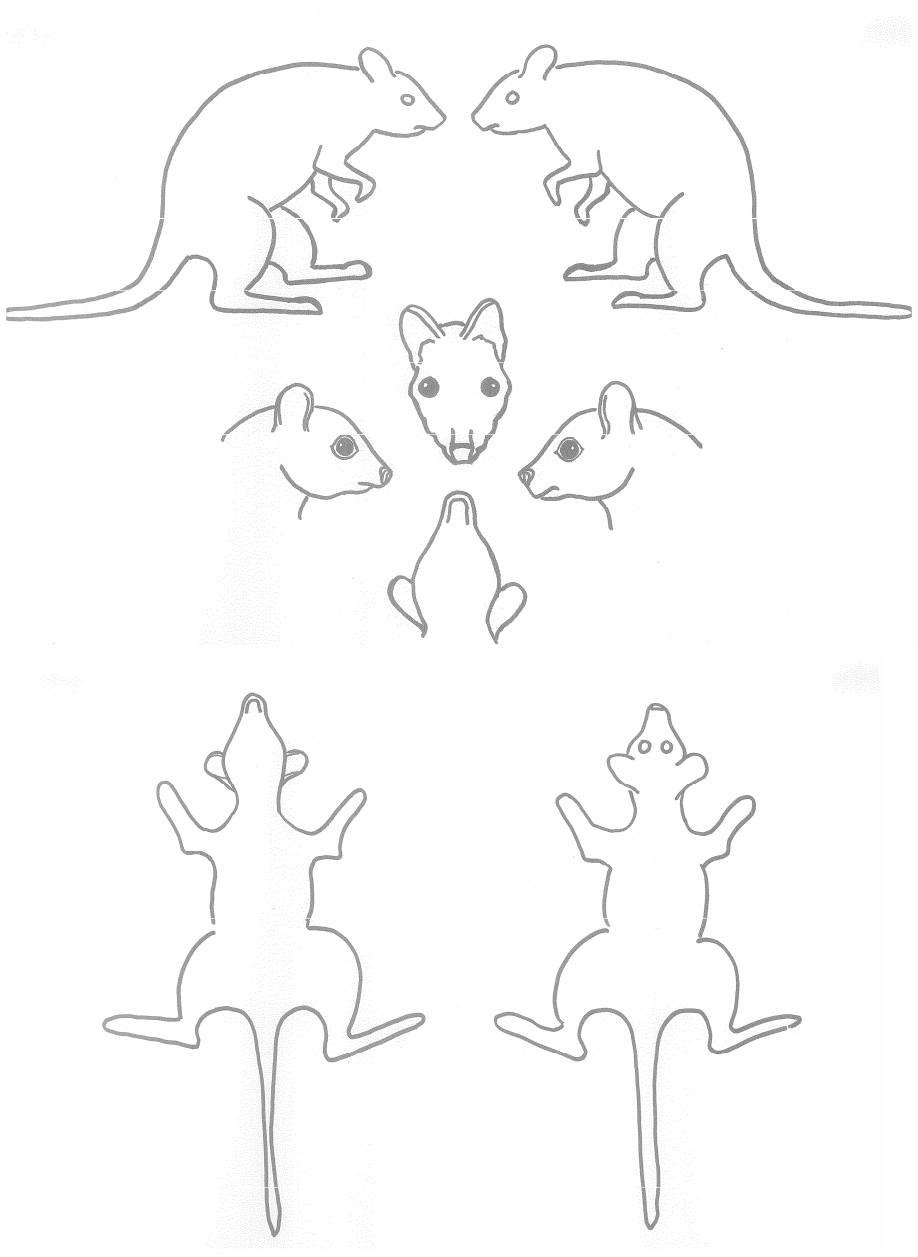


Figure 1. Standard diagram for mapping distribution of skin lesions in Woylies.

Figure 2. Tail lesion during field examination of a woylie, showing fur loss and hyperkeratosis (thickening of the skin).

Figure 3. This woylie presented with periocular fur loss and crusting, typical of anecdotal reports of skin disease observed in woylies in the lead up to a decline. Also note the crusting and ectoparasite infection of the pinna.

Figure 4. A Chiggers mite found on skin scrape from a woylie with skin disease.

Woylie Pathology Review and Summary - March 2010

Andrea Reiss, Regional Veterinary Projects Officer, Perth Zoo

Background

From September 2009 to March 2010 a review was undertaken of all post mortem reports from examinations conducted on woylies by Murdoch University veterinary pathology department (MUP) from 2005 to 2009 as part of the Woylie Disease Reference Council.

The objectives of the review were:

- To summarise all post mortem cases and results and associated information from this period into a readily accessible format (Excel spreadsheet).
- To review all cases and reports and identify areas of outstanding or incomplete information (reports pending, etc) and create a plan (with the help of MUP) to finalise these reports.
- To compile data on the spreadsheet on useful parameters such as evidence of predation, motor vehicle trauma, level of autolysis etc.
- To review all cases with the aim of identifying patterns or syndromes worthy of greater focus and to create a list of recommendations for future action and investigation.
- To create a list of cases where tissues have been archived for possible future investigation (histopathology or other). This list of cases and tissues will be prioritised as low, medium or high priority for future investigation as time and resources allow, or if new information indicates more detailed investigation is required.
- To review individual cases with the aim of identifying interesting or clinically relevant findings worthy of greater focus or independent review. This list will be prioritised and will suggest possible avenues of future investigation.
- To review (with the assistance of MUP) existing pathology protocols and practices with the aim of ensuring best outcomes from future endeavours.

A summary spreadsheet containing relevant information on all post mortem cases is attached (Attachment 1: Woylie Post Mortem cases at Murdoch Uni 2005-2009; necropsy 2005-2009). Information relating to useful parameters (evidence of predation, evidence of motor vehicle trauma, evidence of trap-related trauma, level of autolysis, type of tissues collected, tissues examined etc) has been included in new columns. The spreadsheet also identifies case numbers of archived glass slides and fixed tissues. Many areas of outstanding or incomplete information have been addressed during the review process. Areas which still require completion have been identified and highlighted on a separate page of the spreadsheet.

Analysis of post mortem cases

Post mortem cases were reviewed and analysed by year, location, evidence for trauma-related findings, autolysis, gender and age and original presentation.

Table 1: Breakdown of cases by year

YEAR	2005	2006	2007	2008	2009	Total 2005-09
NUMBER OF POST MORTEMS	2	18	13	3	3	39

Live cases: 39 bodies were submitted for post mortem, including 16 (41%) animals initially submitted as live clinical cases (to Perth Zoo or elsewhere) and subsequently submitted for post mortem following euthanasia or death.
Comment: The ability to examine animals whilst alive, and collect both clinical data and samples ante mortem adds greatly to the value of post mortem investigations.

Histopathology examination: 36 of 39 bodies submitted for post mortem (92%) had some tissues collected for histopathology. In 5 of 36 cases, tissues were not examined at the time but were archived for possible future investigation. During the review, many case reports were tidied up and missing sections of reports were finalised. Only 2 of 36 cases remain where histopathology data is still to be pursued.
Comment: Murdoch University's policy of retaining, for perpetuity, glass slides and fixed pathology tissues from woylie cases is an extremely valuable insurance tool. Ongoing collection of a full range of tissues from all carcases, along with perpetual archives of tissues is vital to this investigation. It allows for tracking and re-examination of samples when new evidence comes to light.

Post mortem autolysis: A large percentage (24/39 or 62%) of bodies had evidence of post mortem autolysis: mild autolysis 6/39 (15%); moderate autolysis 7/39 (18%), advanced autolysis 11/39 (28%). 6/39 (15%) consisted only of pieces of autolysed bodies. Only 15 (38%) had no or minimal evidence of post mortem autolysis of tissues.
Comment: moderate to advanced autolysis of carcases severely limits the diagnostic usefulness of a case. In almost all cases, it is not useful to submit bodies with evidence of moderate or advanced autolysis.

Age and gender: 38 adults and 1 pouch young were presented for post mortem. There were 16 males, 19 females, two carcasses which could not be sexed and two on which data is incomplete (Tutanning cases).
Comment: There is an even distribution of sexes in the surveyed cases, which is desirable. Greater representation of immature animals, although practically difficult to achieve, could prove rewarding as immature animals are often more susceptible to infectious disease processes.

Trauma: 10/39 (26%) of bodies had direct evidence of predation (as classified by puncture wounds to skin or deeper tissues) and another 8 (21%) showed less conclusive evidence suggestive of to predation. 4 (10%) had direct evidence of being hit by a motor vehicle. There were another 7 (18%) cases where it was not possible to definitively rule out motor vehicle trauma. 6 (15%) had evidence of death associated with trapping (and a history which supported either trap injury or death in handling bag) with a further 2 cases where it was not possible to rule out trap-associated death.
Comment: Trauma related death does not preclude the presence of subtle pre-existing disease. Investigation of trauma cases, in particular predation cases and trap associated deaths, is still warranted and if focussed on areas where declines have occurred or are occurring, may provide much needed data on health and disease of animals from these areas.

Skin disease: 17/39 (43%) had evidence of some type of skin disease, which varied from case to case.
Comment: In almost all cases, skin disease is a non-specific finding. A more detailed investigation of skin disease in both living and post mortem cases is ongoing and is outside the scope of this report.

Causes of death: 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 carcase.
Comment: As mentioned previously, a traumatic cause of death does not preclude significant other pathology findings

Location: 25 of 39 post mortem cases originate from areas where significant population declines have occurred and 14 (36%) cases were from captive colonies or areas where populations have been stable (Table 2). Of the 25 cases from "decline areas", only 5 or 6 cases were collected during, or shortly after, the time of key decline. 9 cases (23%) originate from the Upper Warren area, which has been the focus of investigation for woylie declines since 2005. Only 4 or 5 of these cases were collected during population decline years.
Comment: 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. It is apparent that 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.

Table 2: Post mortem cases by location, year and key decline years

Location	**Key decline years**	**Numbers of post mortems 2005-9**	**Numbers of post mortems per year**	**Numbers of post mortems in key decline years**
Karakamia and Paruna	No decline	12	2005 (1), 2006 (5), 2007 (3), 2008 (2), 2009 (1)	N/A
Captive animals	No decline	2	2006 (1), 2008 (1)	N/A
Dryandra	2000-2002	8	2006 (3), 2007 (5)	0
Tutanning	2009	7	2006 (3), 2007 (4)	0
Batalling	2002-07	1	2006 (1)	1
Upper Warren animals				
Balban	2004-07	1	2005 (1)	1
Chariup	2004-06	1	2006 (1)	1
Corbal	Not known	1	2006 (1)	?
Warrup	Prior to 2004	1	2007 (1)	0
Keninup	2008-09	5	2006 (3), 2009 (2)	2
TOTALS		39		**5 or 6**

.

Parastism: Many cases had both internal (gastro-intestinal) and external parasites, which were routinely collected during necropsy and submitted for specialist identification. In most cases these parasites were not considered to be contributing to a clinical or subclinical disease condition as most animals were in good body condition and showed no evidence of clinical disease associated with parasitism.

General comments on pathology findings:
In most cases, pathological findings were "routine". No necropsies found definitive evidence of a disease condition likely to be contributing to the population declines. All necropsy cases, including those returning normal findings, have served the important purpose of developing a level of expertise, and specific experience and understanding of both gross and histological anatomy and commonly-seen pathological changes in woylies. This expertise and understanding is necessary for interpretation of all pathological findings and its importance in the process of disease investigation in a little-studied wildlife species cannot be overemphasised.

In a handful of cases, unusual or unexplained histological findings have been noted and are considered worthy of further investigation (Attachment 1: Woylie Post Mortem cases at Murdoch Uni 2005-2009; noteworthy cases 2005-2009). 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.

Recommendations

- Develop plans for ongoing and increased efforts to collect appropriate woylie carcases from recognised decline areas for full post mortem investigation. A sample size of 15-20 good quality cases from identified "high-risk" populations would be a reasonable target under ideal conditions. To date the study has only achieved 5 or 6 of such cases over 4 ½ years.
- At the same time, continue to be discerning about the state of carcases which are presented for post mortem. Necropsies are costly in time and dollar resources and moderate to advanced autolysis of carcases will severely limit the diagnostic usefulness of a case. In almost all cases, it is not useful to submit bodies with evidence of moderate or advanced autolysis.
- Review communication channels; ensure good documentation and understanding of responsibilities by all parties. Ensure DEC, PZ and MU staff can easily access necessary protocols and information. (DEC or PZ vets attach documents to each case submitted to MU?).
- Create and refine a list of "noteworthy" findings, both clinically, at necropsy and at tissue review which trigger a more detailed investigation process.
- Collate cases of interest for review by MU pathologists (either by one individual reviewing all cases for consistency, or a consistent group working together to discuss each case).
- Discuss possibility of review of significant pathology findings by third party pathologists, preferably those with expertise in particular area of wildlife pathology.

- Consider conducting on-line wildlife pathology rounds session (hosted by Australian Registry of Wildlife Health) dedicated to review and discussion of woylie pathology.
- Discuss possibility of review of skin parasites and associated pathology by parasitologists with expertise in marsupial pathology.
- Prepare (if not already in existence) a work-flow document for submission, handling, storage, examination and reporting on necropsy specimens submitted to MU, for the benefit of those in the project working outside MU.
- Collation and continued co-ordination of all samples from woylies stored at MU.
- Ensure a well-labelled designated area in wet tissue storage facility of pathology department at MU for easy storage and retrieval of specimens.
- Continue to promote discussion and collaboration between pathologists including those based at DAF-WA, other universities, research facilities and in the private sector.

Woylie Conservation Research Project Bibliography

PUBLICATIONS

Averis, S., Thompson, R. C. A., Lymbery, A. J., Wayne, A. F., Morris, K. D. and Smith, A. (2009). The diversity, distribution and host-parasite associations of trypanosomes in Westerna Australian Wildlife. *Parasitology*.

Bennett, M. D., A. Reiss, H. Stevens, E. Heylen, M. Van Ranst, A. F. Wayne, M. Slaven, J. N. Mills, K. S. Warren, A. J. O'Hara, and P. K. Nicholls. (2010). Genomic characterization of a novel marsupial papillomavirus: Bettongia penicillata papillomavirus type 1. *Journal of Virology* **84**:5448-5453.

Clark, P., and P. B. S. Spencer. (2007). Description of three new species of *Theileria* Bettencourt, Franca & Borges, 1907 from Macropodidae in Western Australia. *Transactions of the Royal Society of South Australia* **131**:100-106.

Groom, C. (2010). Justification for continued conservation efforts following the delisting of a threatened species: A case study of the woylie, *Bettongia penicillata ogilbyi* (Marsupialia: Potoroidae). *Wildlife Research.* **37**: 183-193.

Mitchell, S., and A. F. Wayne. (2008). Down but not out: solving the mystery of the woylie population crash. *Landscope* **25**:10-15.

Pacioni, C. and Spencer, P. (2010). Capturing genetic information using non-target species markers in a species that has undergone a population crash. *Australian Mammalogy* **32**: 33-38.

Parameswaran, N., O'Handley, R. M., Grigg, M. E., Wayne, A. F. and Thompson, R. C. A. (2009). Vertical transmission of *Toxoplasma gondii* in Australian Marsupials. *Parasitology* **136**:939-44.

Parameswaran, N., A. Thompson, N. Sundar, S. Pan, and M. Grigg. (2010). Nonarchetypal Type II-like and atypical strains of *Toxoplasma gondii* infecting marsupials of Australia. *International Journal for Parasitology*. **40**: 635-640.

Richards, J., Gardner, T. and Copley, M. (2009). Bringing back the animals. *Landscope* **24(3)**, 54 - 61.

Smith, A., P. Clark, S. Averis, A. J. Lymbery, A. F. Wayne, K. D. Morris, and R. C. A. Thompson. (2008). Trypanosomes in a declining species of threatened Australian marsupial, the brush-tailed bettong *Bettongia penicillata* (Marsupalia: Potoroidae). *Parasitology* **135**:1-7.

Smith, A., Lymbery, A. and Thompson, A. (2009). Meet the parasites. *Landscope* **24(4)**, 24 - 29.

Thompson, RCA, Kutz, SJ, Smith, A. (2009). Parasite zoonoses and wildlife: Emerging issues. *International Journal of Environmental Research and Public Health* **6**: 678-693.

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., Bryant, K., Calver, M., and Wayne, A. F. (in press). Do preservation methods affect the identification of dietary components from faecal samples? A case study using a mycophagous marsupial. *Australian Mammalogy*. **XX**: XXX

PAPERS SUBMITTED

Glen, A., A. F. Wayne, M. Maxwell, and J. Cruz. (in review 2010). Comparative diets of chuditch (*Dasyurus geoffroii*) in the northern and southern jarrah forests, Western Australia. *Australian Journal of Zoology*.

Pacioni, C., Wayne, A. F. and Spencer. P. (Under review). Effects of habitat fragmentation on population structure and long distance gene flow. Journal of Zoology **XXX**:XXX.

THESES

Madon, E. (2006). Mating systems and reproductive anatomy in marsupials: explaining the evolution of the anterior vaginal expansion of *Bettongia penicillata*. Page 66. School of Animal Biology. Honours thesis. University of Western Australia, Perth.

Cherriman, (2007). Simon C. Territory size and diet throughout the year of the Wedge-tailed Eagle *Aquila audax* in the Perth region, Western Australia. Honours thesis. Curtin University. [not directly involved in WCRP – Upper Warren but is with Karakamia and Dryandra]]

Eikelboom, T. H. 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.

Hide, A. (2006). Survival and dispersal of the threatened woylie *Bettongia penicillata* after translocation. Honours thesis, University of Western Australia.

Hunt, H. (2010). A temporal assessment investigating the effects of population declines on genetic diversity, in the critically endangered woylie (*Bettongia penicillata ogilbyi*). School of Biological Sciences and Biotechnology. Honours thesis. Murdoch University, Murdoch, Western Australia.

O'Brien, R. Christopher, B.A., M.F.S. (2008) Forensic Animal Necrophagy in the South-west of Western Australia: Species, feeding patterns, and taphonomic effects. Doctor of Philosophy thesis, University of Western Australia.

Rogers, P. (2009). Predator profiling as a tool for the conservation of the woylie (*Bettongia penicillata*). Honours thesis, University of Western Australia.

Rong Ho, J. (2009) Investigation of the potential pathogenicity of the erythrocyte piroplasm *Theileria penicillata* (Clark & Spencer, 2007). Honours thesis, Murdoch University.

Parameswaran, N. (2008). *Toxoplasma gondii* in Australian marsupials. PhD Thesis, Murdoch University.

REPORTS

2006

Wayne, A.F. (2006). Science Project Plan, Department of Environment and Conservation, Woylie conservation research project: An investigation into the declines of woylies (Bettongia penicillata) in south-western Australia.

Bougher, N. L.(2006). Identity and taxonomy of truffle fungi from an initial survey at Karakamia Wildlife Sanctuary. Department of Environment and Conservation.

Wayne, A. F. (2006). Interim assessment of the evidence for a recent decline in woylie abundance in south-western Australia. Pages 1-28. CALM, Perth, Western Australia.

Wayne, A. F., I. Wilson, J. Northin, B. Barton, J. Gillard, K. Morris, P. Orell, and J. Richardson. (2006). Situation report and project proposal: identifying the cause(s) for the recent declines of woylies in south-western Australia. A report to the Department of Conservation and Land Management Corporate Executive. Pages 1-28. CALM, Perth, Western Australia.

Wayne, A. F. 2006. Woylie Conservation Research Project Progress Notes: Mesopredator External Review – 7 & 8 Nov 2006. DEC Mesopredator External Review. Department of Environment and Conservation.

2007

Robinson, R., J. Fielder, M. Maxwell, N. L. Bougher, W. Sicard, and A. Wayne. 2007. Woylie conservation research project: Preliminary survey of hypogeous fungi in the Upper Warren region. Department of Environment and Conservation.

2008

Eden, Paul and Wayne, Adrian (2008). Metabolic bone disease of woylies.

Pacioni, C., Spencer, P.B.S. and Wayne, A.F. (July 2008). "Conservation Conundrum: The population dynamics associated with recent decline of woylies (*Bettongia penicillata)* in Australia. Final report to the Australian Academy of Sciences.'

Pacioni, C., Spencer, P.B.S. and Wayne, A.F. (Dec 2008). "Conservation Conundrum: The population dynamics associated with recent decline of woylies (*Bettongia penicillata)* in Australia. Interim report to the South Coast Natural Resource Management Inc.'

DEC. 2008. Diagnosis of recent woylie (*Bettongia penicillata ogilbyi*) declines in southwestern Australia. Progress report of the Woylie Conservation Research Project (Final Draft). Perth, Western Australia, Western Australian Government Department of Environment and Conservation: 1-314.

DEC WCRP, I. Wilson Ed. 2008. Woylie Conservation Project Operations Handbook. Perth, Western Australia, Western Australian Government Department of Environment and Conservation: 1-158.

Freeguard, C. (2008). '*Bettongia penicillata ogilbyi* – nomination of a Western Australian species for listing as threatened, change of status or delisting. 'Dept. Conservation and Land Management, Perth, Western Australia.

Mawson, P., Atkins, K. and Goldberg, J. (2008). '*Bettongia penicillata obilbyi* –woylie: Species Information Sheet.' Department of the Environment and Water Resources.

2009

DEWHA (2009). Department of the Environment, Water, Heritage and the Arts (2009). Bettongia penicillata ogilbyi in Species Profile and Threats Database, Department of the Environment, Water, Heritage and the Arts, Canberra. Available from: http://www.environment.gov.au/sprat.

Orell, P. (2009). 'Current Status of the woylie: based on an update of the species information sheet submitted to the Department of Environment and Water Resources in 2008.' Deptartment of Environment and Conservation, Perth, Western Australia.

Threatened Species Scientific Committee (TSSC) (2009). Commonwealth Listing Advice on Bettongia penicillata ogilbyi (Woylie). [Online]. Department of the Environment, Water, Heritage and the Arts. Available from: http://www.environment.gov.au/biodiversity/threatened/species/pubs/66844-listing-advice.pdf.

Pacioni, C., Spencer, P. B. S. and Wayne, A. F. (2009). 'Conservation Conundrum: The population dynamics associated with recent decline of woylies (*Bettongia penicillata*) in Australia. Final report to the South Coast Natural Resource Managment Inc.'

Wayne, A. F., Friend, T., Burbidge, A., Morris, K. and van Weenen, J. (2008). Bettongia penicillata. In: IUCN 2009. IUCN Red List of Threatened Species. Version 2009.2. <www.iucnredlist.org>.

Wayne, A. F. and Maxwell, M. (2009). 'Examples of woylie skin and fur conditions associated with declines in the Upper Warren populations.' Deptartment of Environment and Conservation.

CONFERENCE PROCEEDINGS

2006

Wayne, A. F. 2006. The Post-Recovery Collapse of the Woylie: Diagnosing their recent declines in south-western Australia. Australian Mammal Society Scientific Meeting and Macropod Symposium. Australian Mammal Society, Melbourne, Victoria.

2007

Abdad, Y., P. Adams, L. Pallant, A. Li, A. F. Wayne, and S. Fenwick. 2007. Potential bacterial pathogens in Woylies from SW WA (ABSTRACT). Page 50. Wildlife Disease Association (Australasian Section), Dryandra Woodland.

Burmej, H, Smith, A, Lymbery, A, Wayne, A, Morris, K, Fenwick, S, Thompson, RCA. The biodiversity and host-specificity of fleas in the woylie (Bettongia penicillata). ASP & ARC/NHMRC Research Network for Parasitology Annual Conference, Canberra, ACT, 8-11 July, 2007.

Burmej, H., A. Smith, A. J. Lymbery, A. F. Wayne, K. D. Morris, S. Fenwick, and A. Thompson. 2007. The biodiversity, ecology and importance of ectoparasites in the woylie (Bettongia penicillata) and sympatric species (ABSTRACT). Page 51. Wildlife Disease Association (Australasian Section), Dryandra Woodland.

Clark, P., A. Thompson, A. F. Wayne, and S. Averis. 2007. Trypanosomes in relation to the diagnosis of woylie declines: prevalence and molecular characterisation (ABSTRACT). Page 47. Wildlife Disease Association (Australasian Section), Dryandra Woodland.

Eden, P., K. Payne, R. Vaughan, S. Vitali, and A. F. Wayne. 2007. Clinical aspects of disease investigations in population declines of woylies (Betongia penicillata ogilbyi) (ABSTRACT). Page 44. Wildlife Disease Association (Australasian Section), Dryandra Woodland.

Knowles, G., P. Eden, and A. F. Wayne. 2007. Results of woylies (Betongia penicillata ogilbyi) submitted for necropsy to Murdoch University, September 2005 to May 2007 (ABSTRACT). Page 45. Wildlife Disease Association (Australasian Section), Dryandra Woodland.

Pacioni, C., P. B. S. Spencer, and A. F. Wayne. 2007. Woylie (Betongia penicillata ogilbyi) conservation genetics project (ABSTRACT). Page 46. Wildlife Disease Association (Australasian Section), Dryandra Woodland.

Parameswaran, N, O'Handley, R, Thompson, RCA. Development of an ELISA for the detection of Toxoplasma gondii antibodies in macropod marsupials. ASP & ARC/NHMRC Research Network for Parasitology Annual Conference, Canberra, ACT, 8-11 July, 2007.

Parameswaran, N., R. O'Handley, A. F. Wayne, A. Smith, P. Eden, A. J. Lymbery, K. D. Morris, and A. Thompson. 2007. Toxoplasma in woylies (ABSTRACT). Page 49. Wildlife Disease Association (Australasian Section), Dryandra Woodland.

Smith, A., A. J. Lymbery, A. Elliot, U. Parkar, A. F. Wayne, and A. Thompson. 2007. Prevalence and diversity of woylie endoparasites: from individuals to populations and sympatric species (ABSTRACT). Page 48. Wildlife Disease Association (Australasian Section), Dryandra Woodland.

Wayne, A. F., A. Thompson, G. Knowles, P. Eden, M. Swinburn, and K. Morris. 2007. Diagnosing recent woylie declines in south-western Australia: Situation assessment, research approach and disease investigations (Paper). Wildlife Disease Symposium, Perth Zoo, Western Australia.

Wayne, A. F., C. Ward, M. Maxwell, C. V. Vellios, I. Wilson, J. Wayne, M. Swinburn, B. Ward, K. Morris, A. Thompson, G. Knowles, P. Eden, A. Dugand, J. Williams, and T. Gardiner. 2007. Diagnosing the recent woylie (Bettongia penicillata) collapse in south-western Australia. Ecological Society of Australia Annual Conference. Ecological Society of Australia, Perth, Western Australia.

Wayne, A. F., C. Ward, M. Maxwell, C. V. Vellios, I. Wilson, J. Wayne, M. Swinburn, B. Ward, K. Morris, A. Thompson, G. Knowles, P. Eden, A. Dugand, J. Williams, and T. Gardiner. 2007. Diagnosing the

recent woylie declines in south-western Australia (ABSTRACT). Page 43. Wildlife Disease Association (Australasian Section), Dryandra Woodland, Western Australia.

2008

Averis, S. (2008). Characterisation of Trypanosomes from Australian Marsupials. (ABSTRACT). In 'Proceedings of the European Multicolloquium of Parasitology'. Paris, France.

Burmej, H. I. (2008). Describing ectoparasite biodiversity in threatened Western Australian mammals: new methods and challenges. (Abstract). In 'Proceedings of the Australasian Wildlife Management Society, 21st Annual Conference'. Fremantle, Western Australia.

Pacioni, C., Spencer, P. and Wayne, A. F. (2008). Understanding woylie decline: a molecular perspective. (Abstract). In 'Proceedings of the Australasian Wildlife Management Society, 21st Annual Conference'. Fremantle, Western Australia.

Pacioni, C., Spencer, P. B. S. and Wayne, A. F. (2008). Unravelling the contribution of genetics in the decline of the woylie (ABSTRACT). In 'Proceedings of the Wildlife Disease Association (Australasian section)'. Kioloa, New South Wales.

Parameswaran N., Grigg, M., O'Handley, R., Thompson, RCA.. Molecular characterisation of *Toxoplasma gondii* DNA in wild Australian marsupials. ASP & ARC/NHMRC Research Network for Parasitology Annual Conference, Glenelg South Australia, 6-9 July, 2008.

Parameswaran, N., Pan, S., Lymbery, A., Smith, A., Wayne, A. F., Morris, K., Grigg, A. H. and Thompson, R. C. A. (2008). Toxoplasma in Australian wildlife – food for thought? (Abstract). In 'Proceedings of the Australasian Wildlife Management Society, 21st Annual Conference '. Fremantle, Western Australia.

Parkar, U., Traub, R., Vitali, S., Wayne, A. F., Morris, K. and Thompson, R. C. A. (2008). Characterisation of *Blastocystis* isolates from zoo animals and native wildlife. (Abstract). In 'Proceedings of the Australasian Wildlife Management Society, 21st Annual Conference '. Fremantle, Western Australia.

Reiss, A., Eden, P., Wayne, A. F., Nicholls, P. and Thompson, R. C. A. (2008). Veterinary investigation of population declines of the woylie (*Bettongia penicillata*) in south-west Western Australia (Abstract). In 'Proceedings of the Australasian Wildlife Management Society, 21st Annual Conference '. Fremantle, Western Australia.

Rose, K., McCallum, H., Wayne, A. F. and Speare, R. (2008). Wildlife Health and Biosecurity in Australia, Federal Parliamentary Forum on Biosecurity. Canberra.

Siah, W. S., Woods, R., Rose, K., Skerratt, L., Wayne, A. F., Mckenzie, J. and Field, H. (2008). Protecting Wildlife Health in Australia: how will we do it better in a 'One Health' setting? In 'Proceedings of the Wildlife Disease Association Conference'. Kioloa, New South Wales, Australia.

Smith, A., Clark, P., Averis, S., Lymbery, A., Wayne, A. F., Morris, K. and Thompson, A. (2008). A novel *Trypanosoma* sp. and its role in the decline of a threatened species of Australian marsupial, the brush-tailed bettong (*Bettongia penicillata*) (ABSTRACT). In 'Proceedings of the European Multicolloquium of Parasitology'. Paris, France.

Smith, A., Clark, P., Averis, S., Lymbery, A., Wayne, A. F., Morris, K. and Thompson, R. C. A. (2008). The role of trypanosomes in the decline of a threatened species of Australian marsupial, the brush-tailed betong (*Bettongia penicillata*) (Abstract). In 'Proceedings of the Australasian Wildlife Management Society, 21st Annual Conference '. Fremantle, Western Australia.

Thompson, R.C.A. Transmission and life cycle patterns of *Toxoplasma*. Joint International Tropical Medicine Meeting, Bangkok, Thailand, 13-14 October, 2008.

Wayne, A. F., Ward, C., Maxwell, M., Vellios, C., Wilson, I., Wayne, J. C., Thompson, R. C. A., Reiss, A., Eden, P. and Richards, J. (2008). Diagnosing the recent woylie (*Bettongia penicillata*)

collapse in south-western Australia. (Abstract). In 'Proceedings of the Australasian Wildlife Management Society, 21st Annual Conference'. Fremantle, Western Australia.

2009

Armstrong, T., Best, W., Charman, S., Don, R., Laverty, C., Luna G., Scaffidi, A., Sims, C., Thompson, R.C.A., White, K. (2009). A novel and highly potent class of compounds for the treatment of trypanosomiasis. Keystone Symposia on Molecular and Cellular Biology, Drug Discovery for Protozoan Parasites, Breckenridge, Colorado, USA. 22-26 March, 2009.

Smith, A., Lymbery, A., Averis, S., Wayne, A., Morris, K., Thompson, R.C.A. (2009) Low host specificity in trypanosomes associated with native Australian wildlife In 'Proceedings of the World Association for the Advancement of Veterinary Parasitology'. Calgary Alberta, Canada. 9 – 13 August, 2009.

Pan, S., Thompson, A., Lymbery, A., Smith, A., Morris, K., Wayne, A. F. and Grigg, M. E. (2009). Atypical *Toxoplasma gondii* genotypes in Western Australian wildlife species. In 'Proceedings of the World Association for the Advancement of Veterinary Parasitology'. Calgary Alberta, Canada.

Parkar, U., Traub, R., Wayne, A., Morris, K., Thompson, R.C.A. (2009). The prevalence and molecular characterization of *Blastocystis* isolates from wild Australian native fauna. ASP/NHMRC Research Network for Parasitology Annual Conference, University of Sydney, New South Wales, 12-15 July, 2009.

Smith, A., Lymbery, A., Averis, S., Wayne, A., Morris, K., and Thompson, R.C.A. (2009) Low host specificity in trypanosomes associated with native Australian wildlife. In "Proceedings of the World Association for the advancement of Veterinary Parasitology'. Calgary Alberta, Canada. 9 – 13 August, 2009.

Thompson, R.C.A. (2009). The molecular ecology of *Toxoplasma* in wildlife. The Royal Golden Jubilee PhD Congress X, Jomtien Palm Beach Resort, Pattaya, Chonburi, Thailand. 3-5 April, 2009.

Reiss, A., Vitali, S., Eden, P. and Wayne A (2009) Perth Zoo Vet Involvement In Conservation Programs – Recent Developments. In Proceedings of the Wildlife Disease Association- Australasian Section Annual Conference, Catlins, New Zealand

Wayne, A. F. (2009). Where have all the woylies gone? Is predation, food or disease the primary agent of population collapse? In 'Proceedings of the Dwellingup Seminar Series, Science Divsion, Department of Environment and Conservation'. Dwellingup, Western Australia.

Wayne, A. F., Thompson, A., Reiss, A., Eden, P., Pacioni, C., Smith, A., Nicholls, P., Page, M., Van Weenen, J., Morris, K., Marlow, N. and Orell, P. (2009). Investigation of woylie (brush-tailed bettongs) population declines – a critical and challenging problem. In 'Proceedings of the Australian College of Veterinary Scientists, Scientific Meeting'. Surfers Paradise, Queensland.

Wayne, A. F., Thompson, A., Reiss, A., Page, M., Van Weenen, J., Maxwell, M., Ward, C. and Vellios, C. (2009). A wildlife pandemic? Are introduced predators and disease the causes of the collapse of woylie (brush-tailed bettong) populations? In 'Proceedings of the Australian Mammal Society, Scientific Meeting'. Perth, Western Australia.

2010

Pan, S., Thompson, R.C.A., Lymbery, A.J., Smith, A., Morris, K., Wayne, A.F. and Grigg, M.E. Non-archetypal *Toxoplasma gondii* genotypes in Western Australian wildlife species. XIIth International Congress of Parasitology (ICOPA) Melbourne, Aug 2010

Parkar, U., R. J. Traub, N. Hijjawi, A. Wayne, K. Morris, and R. C. A. Thompson. 2010. The development of a molecular-based multiplex method to detect *Blastocystis, Cryptosporidium* sp. and *Giardia duodenalis*. XIIth International Congress of Parasitology (ICOPA) Melbourne, Aug 2010

POSTERS

Burmej, H., A. Smith, A. J. Lymbery, A. F. Wayne, K. D. Morris and A. Thompson. 2007. The Biodiversity and Host-specificity of Fleas in the Woylie (*Bettongia penicillata*) (POSTER) Australian Society of Parasitology, Canberra, Australian Capital Territory

Burmej, H. I., Wayne, A. F., Abdad, Y., Lymbery, A., Morris, K. and Thompson, R. C. A. (2009). Ectoparasites and the woylie (*Bettongia penicillata ogilbyi*): biodiversity and ectoparasite burden. (Poster). In 'Proceedings of the Australian Mammal Society'. Perth, Western Australia.

Pacioni, C. (2006). A conservation conundrum: the population and epidemiological dynamics associated with recent decline of woylies (Bettongia penicillata) in Australia (Poster). Department of Environment and Conservation, Murdoch University, Perth, Western Australia.

Pacioni, C., Spencer, P., Wayne, A. (2007). Assessing the gentic costs of recurring population crashes in the woylie (*Bettongia penicillata*). (Poster). Post graduate poster day. Murdoch University, Perth, Western Australia

Pacioni, C., Spencer, P., Wayne, A. (2008). Modelling real-time migration rates during a population decline (Poster). Post graduate poster day. Murdoch University, Perth, Western Australia

Wayne, A. F., Ward, C., Maxwell, M., Vellios, C., Wilson, I. and Wayne, J. (2008). Where have all the woylies gone? (Poster) Department of Conservation and Land Managment, Woylie Conservation Symposium and Workshop.

Zosky, K., Wayne, A., Bryant, K. and Calver, M. (2009). Variation in the diet of the woylie, Bettongia penicillata. (Poster). In 'Proceedings of the Australian Mammal Society'. Perth, Western Australia.

PRIZES

Pacioni, C., Best post graduate introductory student poster, first year (2006). (Sponsored by Oz Biotech). Post-graduate poster day, Murdoch University

Caccianaga, Rian. EJG Pitman Prize, Australian Statistical Conference (2008). Analysis of upper warren woylie data.

Pacioni, C. (2008). Best student presentation. Wildlife Disease Association (Australasian section). Kioloa, New South Wales.

Australian Wildlife Conservancy, Biodiversity Conservation Award, Western Australian Environment Awards (2009).

COMMITTEE/WORKSHOP REPORTS

2006

The Way Forward: A meeting to respond to the Workshop on recent mammal declines - 17 February 2006

2008

Western Shield Research Advisory Panel Dwellingup meeting 12-13 February, 2008

Woylie Conservation Workshop: Where to from here? Murdoch University 14 February 2008

Woods, R., Obendorf, D., Skerratt, L.F., Whiteley, P. Woylie Conservation Research Project - Woylie (*Bettongia penicillata*) disease review – Report to Department of Environment and Conservation, 12 March 2008.

Science in Public Report – Australian Biosecurity Forum, Parliament House Canberra 16 September 2008

Western Shield Research Advisory Panel Perup meeting 18-20 November, 2008

2009

Woylie Conservation Research Project – Progress summary to Woylie Recovery Team 23 January 2009

Woylie Conservation Research Project – Update report to the woylie recovery team 1 December 2009

RESEARCH PROPOSALS

2008

Wayne, A.F. (Jun 2008). Response to Woylie Declines, Phase 2 Proposal (July 2008-June 2011)

Wayne, A.F. (Aug 2008). Woylie Conservation Research Project, Interim to Phase 2 (Aug-Dec 2008) [Keninup Intensive Study]

Wayne, A.F. (Aug 2008). Australian Biosecurity CRC Partnership with DEC: Proposal [AB-CRC Proposal 1]

Wayne, A.F. (Sept 2008). Wildlife Conservation Action Funding Application

Wayne, A.F. (Dec 2008). SPP Predator Control Experiment: Keninup, Upper Warren (& Pilot Study)

Wayne, A.F. (Dec 2008). Woylie Conservation Research Project, Phase 2 Proposal (2009-2011) Prospects for Sponsorship, Bringing the woylie back from the brink

2009

Wayne, A.F. (Jan 2009). Caring for our country funding proposal. Bouncing back from the brink: the recovery of the woylie and other threatened fauna by vitiating the critical threats [EOI for CFOC, Jan 09]

Wayne, A.F. (Mar 2009). Australian Biosecurity CRC/Woylie Consortium Partnership: Determining the role of disease in woylie declines [AB-CRC Proposal II]

Chapman et al. (April 2009). Caring for our country. Improving native habitat resilience in south-west Western Australia – Australia's only international biodiversity hotspot

Wayne, A.F. (2009). Prospective student projects. Help bring the woylie back from the brink.

Prowse et al.(Mar 2009). Cooperative research centres program 11th Selection Round (2009)

Orell, Wayne, A.F. & Marlow (May 2009). Securing critically important woylie populations from extinction

Thompson, R.C.A. et al. (2009), Murdoch University, Australian Research Council, Linkage Projects 2010, Round 1

Herbert, C. et al. (2009). The University of Sydney, Australian Research Council, Linkage Projects 2010, Round 2

OTHER ARTICLES

Parkar, U., R. J. Traub, S. Vitali, A. Elliot, B. Leveke, I. Robertson, T. Geurden, J. Steele, B. Drake, and A. Thompson. 2010. Molecular characterisation of Blastocystis isolates from zoo animals and their animal keepers. *Veterinary Parasitology* **169**:8-17. [not directly on woylies]

Richards, J. D. 2007. Karakamia: a stronghold for the woylie. Page 27. Wildlife Matters: Newsletter of Australian Wildlife Conservancy.

Richards, J. D. 2008. Paruna Wildlife Sanctuary. Pages 12-13. Wildlife Matters: Newsletter of Australian Wildlife Conservancy.

Wayne, A. F. (2009). 'Woylie declines: what are the causes? Science Division Information Sheet 7/2009.' Department of Environment and Conservation, Perth, Western Australia.

Wayne, A. F. (2010). Woylie Conservation Research (Fact Sheet). South Coast Natural Resource Management. Albany, Western Australia