SUPPLEMENTARY MATERIAL for WCRP Progress Report 2010 – 2013

Selected reports associated with the WCRP (2010-2013)

October 2013

Department of Parks and Wildlife

WARREN CATCHMENTS COUNCIL

State Natural Resource Management

CARING FOR OUR COUNTRY

Selected reports associated with the Woylie Conservation Research Project (2010-2013)

Reports contained in this supplement

Basille, S. (2010). The epidemiology of piroplasm infection in the woylie *(Bettongia penicillata ogilbyi).* Undergraduate Project (Independent Study Contract). Murdoch University, Western Australia.

Bennett, K. (2012). 'An evaluation of two methods for assessing population densities of introduced predators in southwest Western Australia. Undergraduate independent study report.' Biology SIT Study Abroad Program, Cairns, Australia.

Edwards, S., (Oct 2012). Balban predator control program. Wildthings Animal Control Solutions.

Edwards, S., (March 2013). Boyicup predator control program. Wildthings Animal Control Solutions.

Hamilton, N. and Rolfe, J. (2011). *Assessment of introduced predator presence within the Perup Sanctuary, Western Australia. Unpublished report.* Department of Environment and Conservation, Woodvale, Western Australia.

Harradine, E., White, L., Madsen, A., McMahon, S., Borkowski, K., Pinto, E. (2012). Perup – Nature's Guesthouse: Strategic destination management plan (2012-2016). Tourism Management course unit (TOU303) group project, Murdoch University.

Jaimes, S. (2010). Where's the Woylie? Possible factors affecting post-decline Woylie (*Bettongia penicillata ogilbyi*) abundance in the Upper Warren Region of South West Australia. Undergraduate independent study report. Pacific Lutheran University, Biology SIT Study Abroad Program, Cairns, Australia.

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.

Yeatman, G.J., Wayne, A.F., Mills, H., (2013). Terrestrial vertebrate assemblage and species level patterns between major habitat types inside and outside a fenced exclosure in south-western Australia, p. 22. University of Western Australia, Perth.

The epidemiology of Piroplasm Infection in the Woylie

(*Bettongia penicillata ogilbyi*).

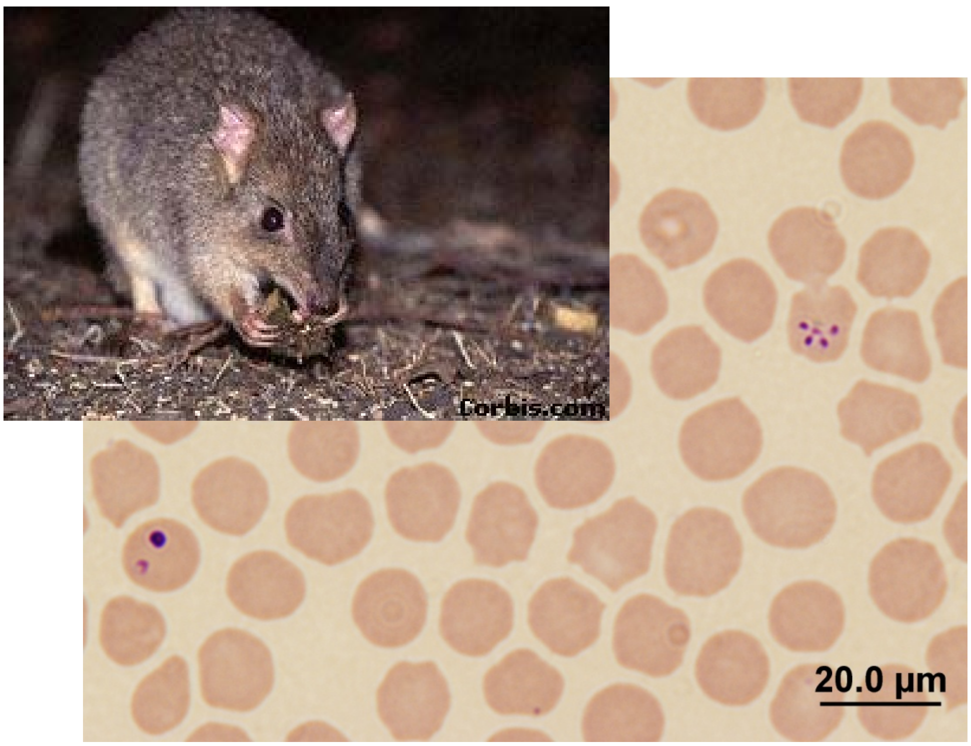

Photo: Stefania Basile

Stefania Basile (30648234)

Independent Study Contract
Murdoch University
2010

Supervisors: Irwin Peter & Pacioni Carlo

Abstract

The woylie (*Bettongia penicillata ogilbyi*) is an endangered endemic species of the south-west of Western Australia that has experienced a 70-80% decline in the last five years. Among the potential agents for this event, infectious diseases are strongly suspected.

The aim of this study was to define the epidemiology of the haemoparasite, *Theileria penicillata*, in four localities: Karakamia, Keninup, Balban and Warrup. Light microscopy examination (LME) of 274 woylie blood smears was used in the study to establish *T. penicillata* parasitaemias (via count) and to detect any erythrocyte and leukocyte morphological changes. The protozoa prevalence and average parasitaemias (AP) were considered in relation to gender, location, and body condition; while AP was assessed in relation to haemoglobin (Hb) concentrations, haematocrit (HCT) and red blood cell (RBC) counts to evaluate possible clinical outcomes of the infectious agent.

The study highlighted previously unreported morphological findings of the erythrocytic cycle of *T. penicillata* in the woylie. Parasite infection did not account for any morphological alterations of the RBC and leukocytes. Piroplasm prevalence did not significantly vary between males and females, but was strongly associated with the locality of sampling. Higher and similar AP was detected in Balban and Keninup, while Warrup presented the lowest AP. AP was also strongly associated with Hb only rather than Hb, HCT and RBC count altogether. The reason why this occurred is uncertain and requires further investigation.

The establishment of fenced predator-free areas could assist in the study of the woylie biology as well as future conservation and management.

Keywords: piroplasm, woylie, decline, endangered species, wildlife disease, haematological profile, body condition

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1. Wildlife Extinctions and Their Underlining Causes.

Extinctions have been a constituent component of the evolutionary history on Earth (Pimm *et al.*, 1995). Current rates though have worryingly escalated 100 to 1000 times in the last centuries, accelerating incredibly the process of biodiversity loss (Pimm *et al.*, 1995; Smith *et al.*, 2009). Establishing the causes of decline in a wildlife population is a fundamental step for efficacious conservation and management plans (Caughley, 1994). Nevertheless it is the most demanding task. A population decline can be triggered by multiple variables, whose interactions are possibly complex and not completely understood. Fragmentary knowledge of the demographic dynamic of the endangered species further complicates the challenge (Caughley, 1994).

The main driving forces implicated in the loss of biodiversity have been identified in the following categories: anthropocentric exploitation of the environment with habitat loss, fragmentation, pollution and over-harvesting of natural resources; introduction of non-native species; and loss of genetic variation (Pimm *et al.*, 1995; Smith *et al.*, 2009). The threat to wildlife by infectious agents may appear as a minor problem (Cleaveland *et al.*, 2001). However, if combined to any of the previously mentioned factors, “there is substantial evidence that they can cause temporary or permanent decline in local species abundance” (Cleaveland *et al.*, 2001).

Emerging infectious diseases have been at the centre of the public concern whenever wildlife has been implicated as reservoir of pathogens easily transmissible to humans and domestic animals (Daszak *et al.*, 2000; Cleaveland *et al.*, 2001). The opposite process though has gained consideration especially when managing endangered species (Daszak *et al.*, 2000). The consequences of spill-over of diseases from domestic to wild populations and of introduction of new pathogens in naive populations cannot be underestimated anymore (Daszak *et al.*, 2000).

2. **Disease Impact on a Wildlife Population.**

Several cases can be referred where infectious agents have concomitantly contributed to the decline and/or extinction of local wildlife populations (Smith *et al.*, 2006, 2009).

The Tasmanian tiger, *Thylacinus cynocephalus*, is an Australian example (Bulte *et al.*, 2003; Smith *et al.*, 2006, 2009). The thylacine was already rare or extinct on the Australian mainland before the arrival of the first settlers and survived only in Tasmania. The over-harvesting policy implemented by governmental bounties have been often blamed as major cause of its extinction, but a distemper-like disease could have acted in concert with the introduction of exotic species, such as the domestic dog, and human invasion of its habitat (Bulte *et al.*, 2003; Smith *et al.*, 2006, 2009).

The Tasmanian devil facial tumour (TDFT) is another illustration of how an infectious agent combined with habitat loss, over-exploitation and genetic impoverishment can drive a population to decline (Lachish *et al.*, 2010). The Tasmanian devil, *Sarcophilus harrisii*, has experienced a 60% decrease in its population size since 1996 and is presently at threat of extinction (McCallum, 2008). The risk of population extinction is inherent in its low numbers and is conceptually formalised by Caughley's (1994) small population paradigm. The genetic diversity of an isolated population, as in the case of the Tasmanian devil, decreases with time, as a result of genetic drift (Caughley, 1994; Frankham, 2005). 'Rare alleles that contribute little to heterozygosity are more easily lost during population size reductions' (Lachish *et al.*, 2010). However also, reproduction between related animals (inbreeding) causes an increase in homozygosity and can be responsible for the fixation of deleterious traits over time (Caughley, 1994; Frankham, 2005). The loss of genetic diversity via genetic drift and inbreeding impacts negatively on the population fitness (inbreeding depression) and can ultimately influence the population's ability to adapt to environmental changes (Caughley, 1994; Frankham, 2005). This self-perpetuating harmful cycle to potential extinction is summarised in Figure 1.

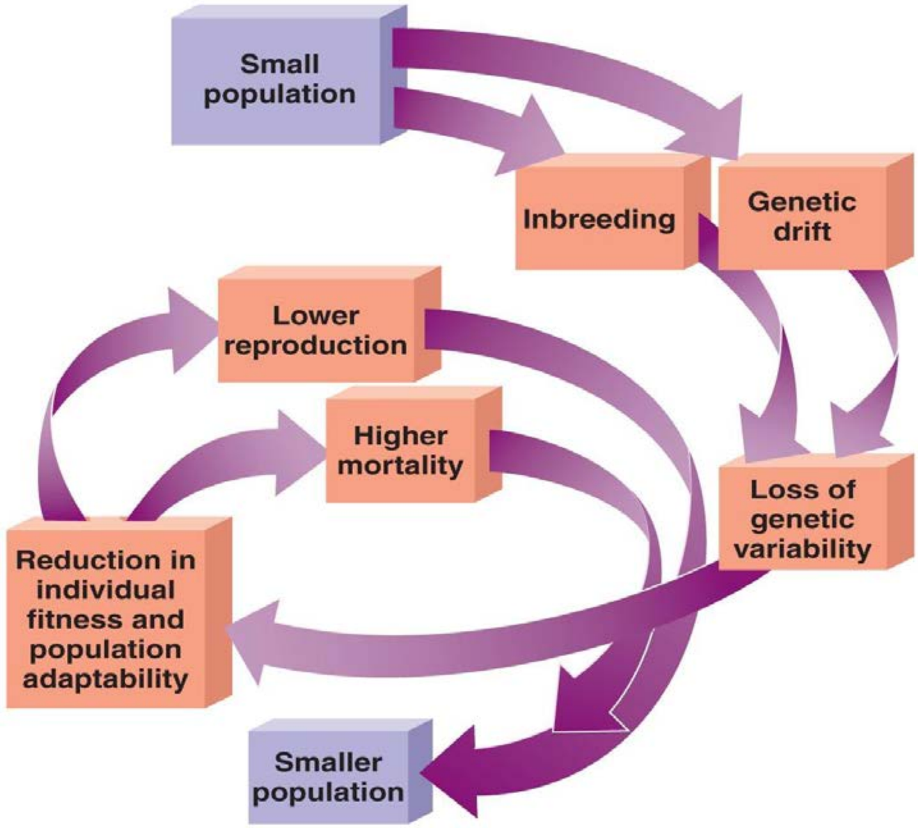

Figure 1 The small population paradigm
(http://apbiosemonefinalreview.pbworks.com/Ecology%20%28Ch%2054-56%29).

3. The Recent Decline of the Woylie.

The brush-tailed bettong (*Bettongia penicillata ogilbyi)* could become another Australian example of native species decline linked to infectious disease (DEC, 2008). Since 2001, the extant populations present in the southwest of Western Australia have experienced a rapid and intense decrease, estimated around 70-80% (DEC, 2008). Multi-factorial causes have been contributing to the woylie decline, such as habitat degradation and patchiness as well as introduction of feral cats and foxes (Maxwell *et al.*, 2008). Other elements that need to be considered are: alterations of fire regimes (Wayne *et al.*, 2008); variation in diet and food resource availability (Rodda *et al.*, 2008); rainfall and climatic fluctuations (Orell, 2008); and human intervention ('trapping intensity, live harvest for translocation; trapping consequences: deaths, predations and pouch young intervention; and illegal killing/harvesting, and road kills') (Wayne and Wilson, 2008).

The suspicion that infectious agents could be the cause of the recent decrease was raised based on the characteristic of the decline (Wayne in prep.). Figure 2 summarises the "untested hypothesis of the causes of woylie declines" by the Department of Environment and Conservation (DEC) (2008). Protozoal haemoparasites, like *Toxoplasma gondii* and *Trypanosome* spp., are under current investigation, as well as the potential impact of endoparasites and/or ectoparasites. Tick or flea burden could directly influence the health of the single animal or act as vectors for the transmission of other infectious agents (DEC, 2008). Additional "diseases considered being significant for future investigations include: Chlamydiales; Macropod Herpesvirus and Orbivirus; encephalomyocarditis virus; *Neospora caninum"* (Pacioni *et al.*, 2008).

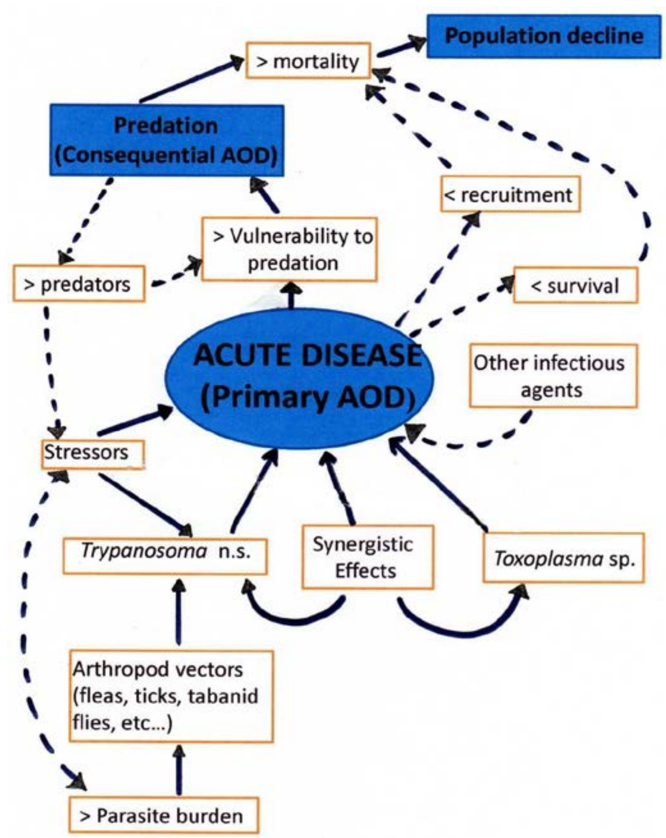

Figure 2 The untested hypothesis of the causes of woylie declines in the Upper Warren region (WA) based on preliminary inferences (adapted from DEC, 2008). Legend: AOD = Agent of Decline; → = 1st order factor/higher confidence based on available evidence; - -> = 2nd order factor/ likely relationship but less evidence available; stressors = predation; competition; climate factors/ extreme weather; nutrition; high density woylie population; ectoparasites; disease reservoirs in sympatric species; concurrent infections.

This study focuses on the possible threat that a piroplasm infection, described recently by Clark and Spencer (2007), could directly or indirectly pose on the declining woylie population and it represents a component of the ongoing investigation on the potential role of infectious agents in the woylie decrease.

4. The Woylie (*Bettongia penicillata ogilbyi)*: Past and Present Distribution.

"Woylie" (stick carrier) is the Noongar word used to describe the ability of the brush-tailed bettongs (*Bettongia penicillata*; Gray 1837) to carry leaves and sticks with their prehensile tail (Whitehurst, 1997). It is a small marsupial endemic to Australia and belonging to the Potoroidea family (Start *et al.*, 1995; Groves, 2005). Its body length spans between 300 and 380mm, while its weight ranges between 1.1 and 1.6 Kg (Christensen, 2004).

Before the arrival of the first European settlers, the woylie inhabited most of the Australian

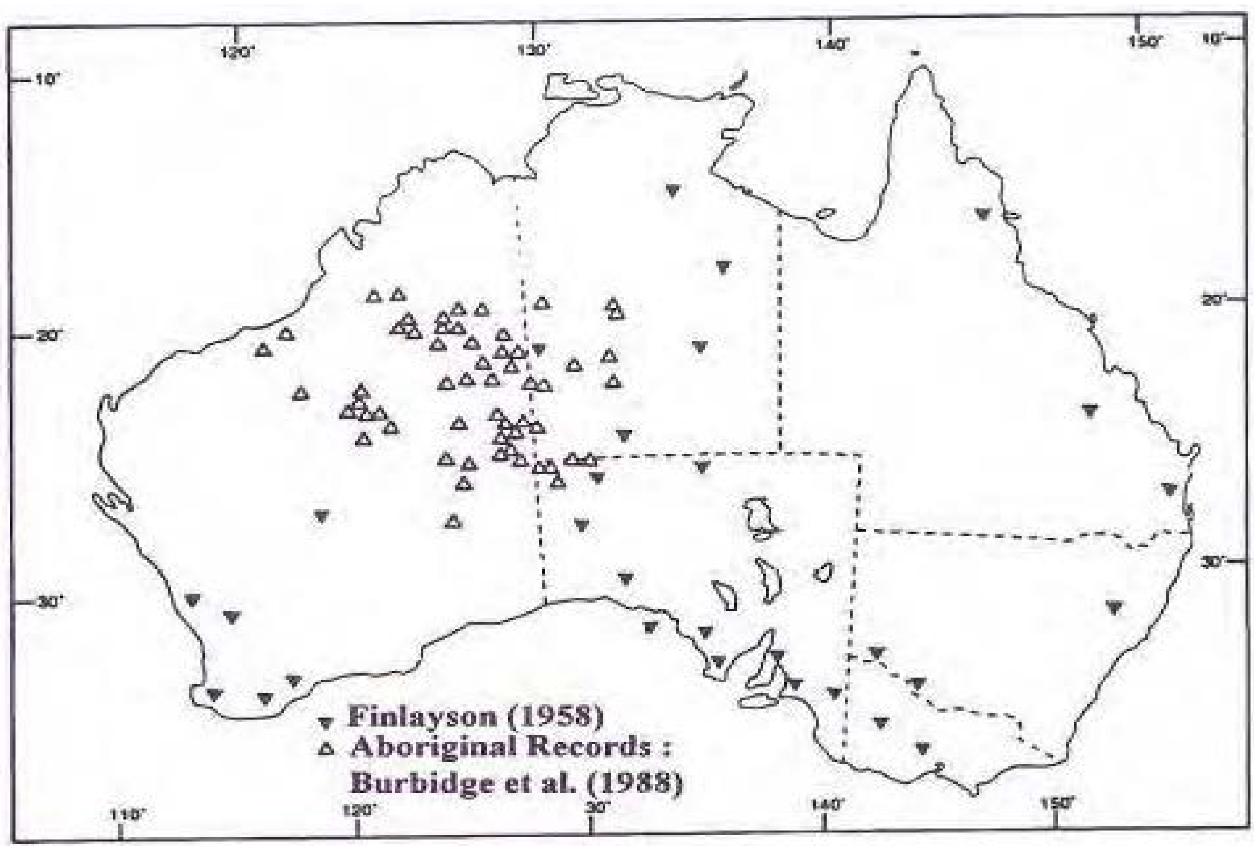

Figure 3 Historic distribution of woylies (*Bettongia penicillata*) (adapted from Start *et al.*, 1995).

continent south of the tropics, including the central desert areas, as determined from the Aboriginal oral history (Burbidge *et al.*, 1988).

By the 1960's though, the number of brush-tailed bettongs had rapidly declined, surviving only in the Perup/Lake Muir area, Tutanning Nature Reserve and Dryandra woodland, in WA (Wayne, 2008; Groom, 2010). Huge efforts have been directed towards woylie reintroductions in Western Australia, NSW and SA and fox control, in the 1970's (Wayne, 2008). The creation, in 1996, of the 'Western Shield' conservation program has contributed dramatically to the recovery of the brush-tailed bettong, which then became the first Australian mammal to be downgraded in its protection status on Commonwealth and State conservation lists (Start *et al.*, 1998). Since 2001, however, a new decline (up to 80%) has occurred in the remnant woylie populations, forcing the need to relist the species as 'critically endangered' in the IUCN Redlist (Wayne, 2008; Wayne *et al.*, 2009). A concomitant decrease has also been recorded in the populations translocated in SA mainland, but the South Australian island populations appear to have remained relatively stable (Wayne, 2008).

The causes of the decline are yet to be determined and are at the heart of the Woylie Conservation and Research Project (WCRP), established by the Department of Environment and Conservation (http://www.dec.wa.gov.au/content/view/3230/97/ accessed 25/08/2010).

5. Brief Overview of the Woylie's Ecological Functions.

"Research, especially in the last two decades, has led to a better understanding of the important ecological roles played by woylies" (Groom, 2010). The small marsupial is mycophagous. The main components of its diet are the fruiting bodies of hypogeous fungi, which it actively seeks by excavation. While digging, the brush-tailed bettong can displace on average 4.8 tonnes of soil annually, therefore improving incredibly soil and nutrient turnover, water penetration and ectomycorrhizal fungi dispersal (Lamont *et al.*, 1985; Garkaklis *et al.*, 1998, 2000, 2003). Whereas the seed-hoarding behaviour, the handling and storage of food items in hollows then covered by leaf litter or soil, aids seed dispersal, facilitates recruitment and regeneration of vegetation and ultimately influences fire regimes by constant reshaping of the understorey (Lamont *et al.*, 1985; Murphy *et al.*, 2005).

In the context of the ecological functions associated to the woylie, concerns may arise for "the far-reaching impacts on the ecosystem" that their decline could have (Groom, 2010).

6. Potential Causes of the Woylie's Decline.

Historically, multiple causes have contributed to the decline of the woylie. The habitat alteration that reshaped the Australian landscape since the arrival of the European settlers strongly restructured the environment available to the species (Burbidge and McKenzie, 1989; Start *et al.,* 1995). Land clearing, livestock grazing and altered fire regimes have directly affected vegetation composition, leading to changes in the abundance, availability and/or suitability of resources such as water, food, shelter, reproductive mates and territory (Groom, 2010). The introduction of the European fox (*Vulpes vulpes*) and feral cat (*Felis catus*) has increased the rate of predation and hastened the marsupial decline, already hunted by native predators like Carpet Python (*Morelia spilota*) and large birds of prey (Burbidge and McKenzie, 1989; Start *et al.,* 1995; Kinnear *et al.*, 2002; Groom, 2010). Other biotic factors, such as food competition with the rabbit (*Oryctolagus cuniculus*) and other introduced grazing animals, must have escalated the process (Start *et al.*, 1995). Abbott (2006) hypothesises that disease could have contributed to the decline of many Australian marsupials towards the end of the nineteenth century, but there is only anecdotal evidence, for this theory.

7. Piroplasm Taxonomic Classification.

The collective name 'piroplasm' describes the pear-shaped phenotype commonly observed in the erythrocyte of the mammalian host (Mehlhorn and Schein, 1984; Homer *et al.*, 2000; Bowman, 2003; Lee, 2004; Uilenberg, 2006; Lee *et al.*, 2009). Piroplasms are vector-borne intracellular protozoa (Allsopp *et al.*, 1994). They belong to the Phylum *Apicomplexa,* Order *Piroplasmida,* which contains two main families, the *Babesiidae* and *Theileriidae* (Homer *et al.,* 2000; Morrison, 2009). The main morphological distinctive features of the phylum are the presence of an apical complex, specialised secretory organelles (rhoptries and micromene) and a three stage reproduction life cycle, which requires both a vertebrate host and a non-vertebrate vector (Homer *et al.*, 2000; Shaw, 2003).

8. Piroplasm Morphology.

8.1. Size Matters.

The traditional classification of babesial and theilerial species has relied primarily on morphological, behavioural and biological characteristics (Lee, 2004). Phenotypic features, such as size, shape, and absence of pigment formation, have been easily identifiable from microscopic examination. Light microscopy of blood smears of infected animals has been the major diagnostic tool prior to the discovery of molecular biology techniques (Homer *et al.*, 2000; Kunz, 2002; Lee, 2004).

Based on subjective investigation, babesial species have then been classified into ‘small’ and ‘large’ groups in relation to the pyriform body size (Homer *et al.*, 2000; Lee, 2004; Morrison, 2009). The large *Babesia* spp. are characterised by intra-erythrocytic bodies whose length varies from 2.5 to 5 micron and is twice or five times the size of the so-called small *Babesia* spp., which instead measure between 1 and 2.5 micron. *Babesia bigemina, B. major, B. caballi* and *B. canis* are classic examples of ‘large’ Babesia; while *B. bovis, B. ovis, B. gibsoni, B. felis* and *B. divergens* are representatives of the ‘small’ Babesia (Barnett, 1977; Mahoney, 1977; Mehlhorn and Schein, 1984; Caccio’, 2002).

Theileria species have intraerythrocytic bodies of 1-2 micron (Mehlhorn and Schein, 1984; Homer *et al.*, 2000; Lee, 2004). *Theileria* spp. cannot be reliably differentiated from ‘small’ *Babesia* spp. by LME (Irwin, 2009).

On morphological basis alone, the same protozoa could have presented with different morphological appearances in different hosts; or different phenotypes within the same host; or belonged to a different species but be phenotypically very similar in the same host (Homer *et al.*, 2000; Kunz, 2002; Lee, 2004).

8.2. Shape.

As mentioned previously, the common name ‘piroplasm’ describes the prevalence of pear-shaped bodies in parasitised red blood cell (RBC). The haemoparasites are also rather pleomorphic. Their appearance in peripheral blood or bone marrow varies, as represented in Figure 4 (Backhouse and Bollinger, 1957; Clark *et al.*, 2004).

The *Theileria* spp. may be found as comma, bayonet, round, ovoid, rod-like, amoeboid or irregularly shaped in erythrocytes and lymphocytes (Barnett, 1977; Clark *et al.*, 2004). *Babesia* spp. are equal or relatively larger and round, pyriform or irregular and only within the RBC (Barnett, 1977; Clark *et al.*, 2004). This variety of shapes could help distinguish the two genera, but individual piroplasms are impossible to differentiate (Barnett, 1977). The elongated bacillary or bayonet forms are typical of *Theileria* as are the tetrad or Maltese cross (Barnett, 1977).

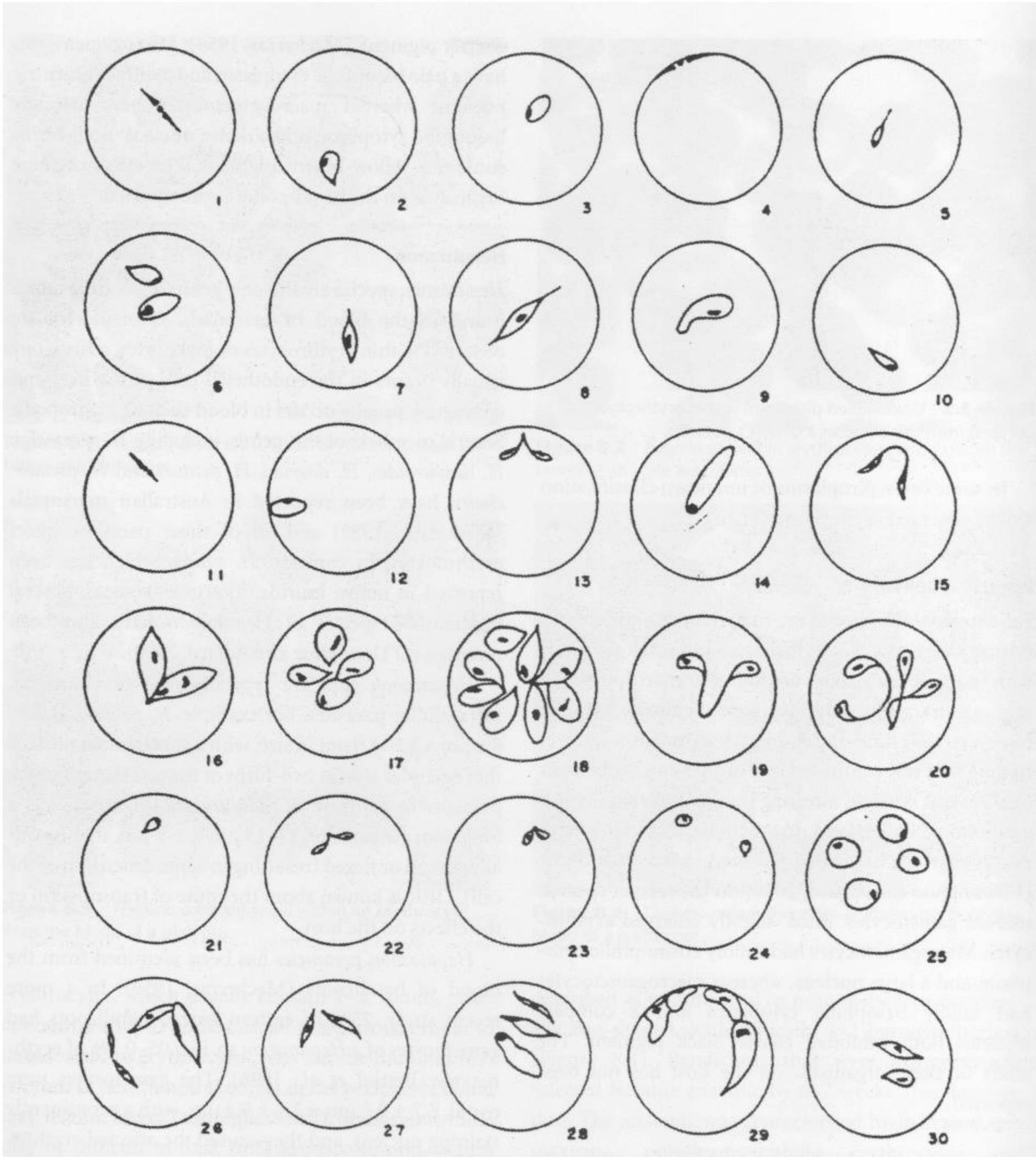

Figure 4 Line drawings of piroplasms of echidna, *Babesia tachyglossi* and *Theileria tachyglossi*, which show the range of parasites forms that may be encountered. Figures 1-30. Forms of the parasites seen in RBCs and bone marrow of echidna. **Figures 1-5**. Commonly occurring forms. Particularly frequent are marginal forms as in Fig. 4. Often thicker than that shown and reminiscent of the 'appliquee' form of malarial plasmodia. **Figure 6-10**. Larger pleomorphic forms as observed mainly in echidna 2. **Figures 11-15**. Blood of echidna 3. Small, slender, wispy, 'duplex' forms as in Fig. 13 and Fig. 15 were present. **Figures 16-20**. Large forms seen only in bone marrow smears of echidna 3. Bigemminate Babesia-like organisms as in Fig. 16 were common. Cells containing from four to eight parasites (Figs. 17 and 18) and curved attenuated structures (Figs. 19 and 20) were also features of this animal. **Figures 21-24**. Minute forms in blood of echidna 6, which were numerous. In the bone marrow occasional red cells contain larger ovoid forms as in Fig. 25. **Figures 26-30**. Echidna 10. A heavy infection exhibiting many minute forms as well as those depicted (adapted from Backhouse and Bollinger, 1957, and Clark *et al.*, 2004).

8.3. Absence of Pigment Formation.

The other common feature of *Babesia* and *Theileria* is the absence of pigment production (Mehlhorn and Schein, 1984; Uilenberg, 2006). The two genera digest completely the haemoglobin of the targeted erythrocyte, without leaving any residue, although some *Theileria* species (*T. veliferea*, *T. separata*, *T. buffeli*) partially digest haemoglobin, forming crystallised compounds. This feature distinguishes the Piroplasmida order from genera like *Plasmodium* and *Haemoproteus* (Uilenberg, 2006).

8.4. Piroplasm Life Cycle: Biological Similarities and Differences.

The life cycle of Apicomplexans is quite complex. It consists of three reproductive stages (gamogony, sporogony and merogony), and relies on intermediate and definitive hosts (Barnett, 1977; Mahoney, 1977; Homer *et al.*, 2000). Gamogony is a form of sexual reproduction. It occurs in the gut of the intermediate vector, normally a tick of the genus Ixodid, and aims to the formation of gametes, which then fuse inside the non-vertebrate host. Sporogony is an asexual duplication, which occurs in the vector salivary glands. Merogony is another asexual replication that takes place in the vertebrate host (Mehlhorn and Schein, 1984; Homer *et al.*, 2000).

The classical biological differences between *Theileria* and *Babesia* life cycles are: the absence in *Babesia* species of a pre-erythrocytic schizogonic replication in the mammalian host, which is instead distinctive of the theilerial genus; and the spread of *Babesia* from one infected tick generation to the following one via transovarial transmission. This vertical transmission is absent in *Theileria*. The protozoan sporogonic multiplication occurs exclusively at the vector's salivary gland (Barnett, 1977; Mahoney, 1977; Mehlhorn and Schein, 1984; Homer *et al.*, 2000; Uilenberg, 2006).

In *Babesia* the division into the definitive host's RBCs occurs by budding and often results in two pyriform daughter cells (merozoites), while in *Theileria* up to four or more merozoites are formed within the parasitized erythrocyte, giving rise to the typical Maltese cross disposition (Uilenberg, 2006).

The life cycle of *Theileria* species is described in more detail in the following paragraph, while a genus general example is visually represented in Figure 5.

8.5. *Theileria* species Life Cycle.

Sporozoites are transmitted to the definitive host's blood stream by inoculation of infected saliva from a feeding tick (Barnett, 1977; Mehlhorn and Schein, 1984; Homer *et al.*, 2000; Uilenberg, 2006; Lee, 2004). The non-motile sporozoites come into contact with a circulating

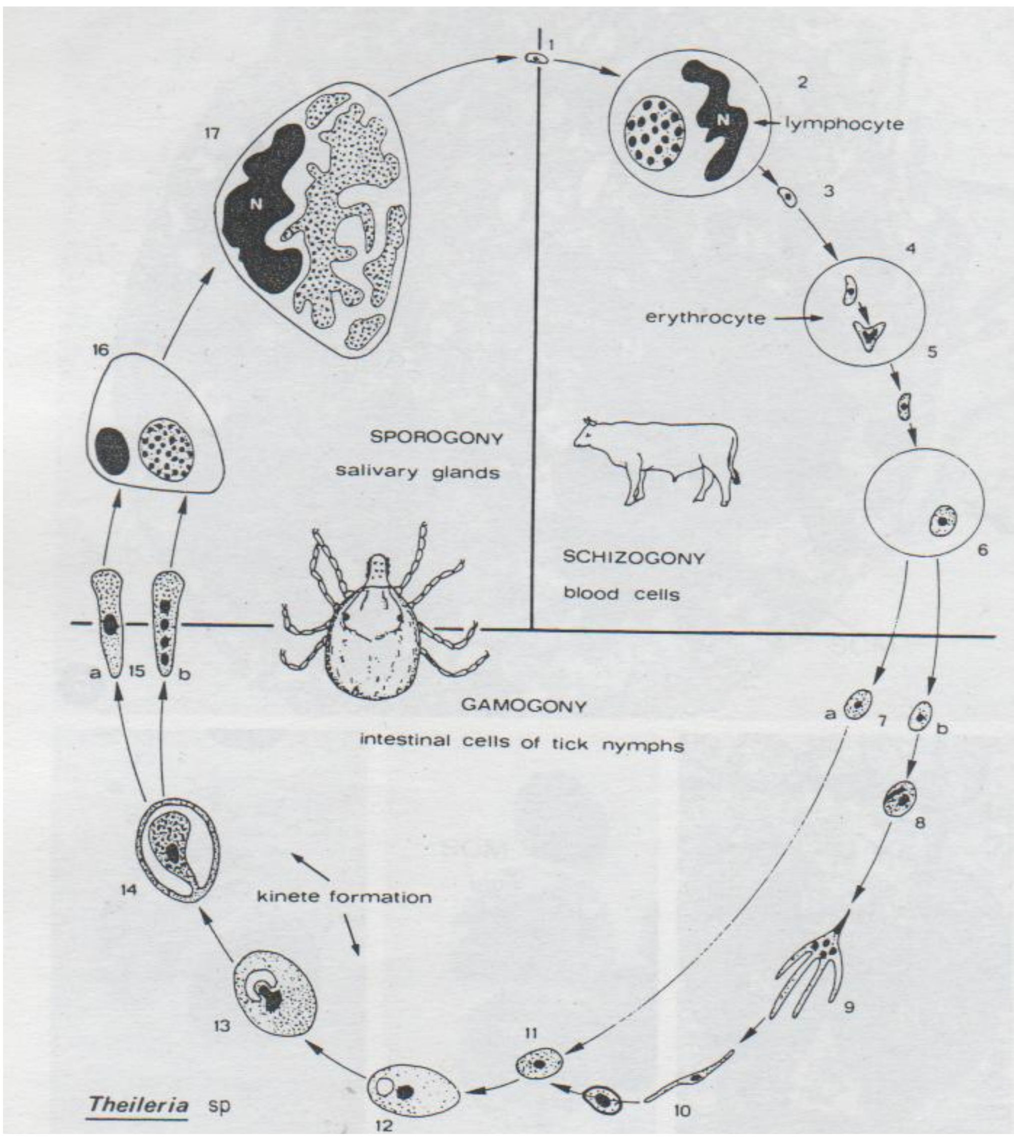

Figure 5 Diagram of the life cycle of *Theileria* species (Mehlhorn and Schein, 1984).

leukocyte, macrophage or lymphocyte, by pure chance. Once attached, they penetrate the host cell by a gradual circumferential 'zippering' and develop into multinucleated syncytial schizonts (Shaw, 2003). A percentage of schizonts divide (merogony) and form merozoites that are then released in the blood stream and invade additional RBCs (Barnett, 1977; Mehlhorn and Schein, 1984; Homer *et al.*, 2000; Uilenberg, 2006; Lee, 2004). Within the host erythrocytes, the merozoites described in previous sections, undergo a further binary fission, forming the typical non-pigmented pear-shaped bodies (Homer *et al.*, 2000; Uilenberg, 2006).

A new tick becomes infected after ingesting a blood meal containing RBC with merozoites. The infected erythrocytes are digested and lysed in the tick gut, releasing the piroplasms (Mehlhorn and Schein, 1984; Homer *et al.*, 2000; Uilenberg, 2006). At this stage the protozoa can undertake gametogony, producing macrogametes and microgametes, which then fuse forming a motile zygote (ookinete). Ookinetes migrate and reach the tick's salivary gland to undergo asexual reproduction, without invading any other organs (Mehlhorn and Schein, 1984; Homer *et al.*, 2000). Tick feeding initiates rapid sporogony in the salivary glands, and infective sporozoites are injected during the later stages of the blood meal (transstadial transmission), restarting the life cycle (Shaw, 2003).

The tick loses its theilerial infection after having transmitted it. The infection does not endure to the next stage or even to the next generation (Mehlhorn and Schein, 1984; Uilenberg, 2006). "When the larva becomes infected the nymph is infective, when the nymph is infected the adult tick is infective. Newly hatched larvae are never infected or infective" (Uilenberg, 2006).

8.6. *Babesia* species Life Cycle.

As mentioned previously, the babesial sporozoites are transmitted to a new vertebrate host by infected tick saliva, as observed with *Theileria* species. Almost immediately they invade the host's RBC to produce the pear-shaped merozoites, without any intra-lymphocytic replication. Once a new vector attaches to the infected definitive host and ingests the circulating merozoites, the gametogonic reproduction occurs, exactly as seen in *Theileria* species. In

Babesia, though, the ookinetes enter various organs, including salivary glands and ovaries, to undertake sporogony. In this way the infection is passed by vertical transmission to the next tick generation. When female ticks become infected, sporogony takes place in the salivary glands of larval, nymphal and- or adult ticks of the following generation (Mehlhorn and Schein, 1984; Homer *et al.*, 2000; Uilenberg, 2006). Certain species of *Babesia* can carry on over numerous tick generations, even without new infections (Uilenberg, 2006).

9. ***Babesia* and *Theileria* species in Australian Native Fauna.**

Comprehensive reviews on protozoan parasites in Australian marsupials are sparse and this becomes particularly evident when compared to studies on piroplasms infecting domesticated animals such as companion animals and livestock (O'Donoghue, 1997; Lee, 2004). The observation of *Babesia* and *Theileria* species in native Australian wildlife has been mainly associated to incidental findings or individual case reports (O'Donoghue, 1997; Lee, 2004). Very little is known about the actual incidence of naturally occurring protozoan infections and their impacts on the native fauna (O'Donoghue, 1997).

The first identification of a piroplasm in an Australian animal dates back to 1915 (Priestly, 1915). Priestly (1915) consistently found an intra-erythrocytic parasite in blood films from the short-beaked echidna (*Tachyglossus aculeatus*). He would later name it *Theileria tachyglossi* (Priestly, 1915). Since then, knowledge of the range of infected marsupials has widened, as has the identification of new piroplasms. From the first isolations in the monotremes, the short-beaked echidna and the platypus (*Ornithorhynchus anatinus*), up to ten marsupials have been reported to present *Babesia* and/or *Theileria*-like organisms in blood or tissue samples (Priestly, 1915; Backhouse and Bollinger, 1957; Mackerras, 1958; Mackerras, 1959). The most recent investigations have been conducted by Clark and Spencer (2007) and Lee (2004, 2009). Clark and Spencer (2007) identified and named three new *Theileria* species in three distinct macropods. They are: *Theileria brachyuri* in the quokka (*Setonix brachyurus); Theileria penicillata* in the brush-tailed bettong (*Bettongia penicillata ogilbyi*); and *Theileria fuliginosa* in the Western grey kangaroo (*Macropus fuliginosus*). *Theileria* *gilberti* n.sp. is the name recommended for the protozoa described from the Gilbert's potoroo (*Potorous gilbertii*) (Lee, 2004; Lee *et al.*, 2009). Table 1 lists the piroplasm infections reported in Australian native animals to date (2010).

Table 1 Overview of the reported cases of piroplasm infection in Australian native animals (adapted from: O'Donoghue and Adlard, 2000; Clark *et al.*, 2004; Lee, 2004).

Host species	Piroplasm species	References
Platypus (*Ornithorhynchus anatinus)*	*Theileria sp.* *Theileria ornithorhynchi*	Mackerras, 1958 Mackerras, 1959; McMillan and Bancroft, 1974
Short-beaked echidna (*Tachyglossus aculeatus)*	*Babebsia sp.* *Theileria tachyglossi* *Babesia tachyglossi*	Backhouse and Bollinger, 1957; Mackerras, 1959 Priestly, 1915; Seddon, 1952; Mackerras, 1959; Seddon and Albiston, 1966 Backhouse and Bollinger, 1957; Ristic and Lewis, 1977
Brown antechinus (*Antechinus stuartii)*	*Babesia sp.*	Arundel *et al.*, 1977
Northern brown bandicoot (*Isoodon macrourus)*	*Theileria sp.*	Seddon and Albiston, 1966
Southern brown bandicoot (*Isoodon obesulus)*	*Babesia thylacis* *Theileria peramelis*	Mackerras, 1959 Mackerras, 1959; Munday, 1978, 1988
Long-nosed bandicoot (*Perameles nasuta*)	*Theileria sp.* *Theileria peramelis*	Mackerras, 1958; Munday, 1978, 1988 Mackerras, 1959
Long-nosed potoroo (*Potorous tridactylus*)	*Theileria sp.* *Theileria peramelis*	Mackerras *et al.*, 1953; Mackerras, 1958; Munday, 1978; Speare *et al.*, 1989 Mackerras, 1959
Proserpine rock-wallaby (*Petrogale Persephone*)	*Babesia sp.*	O'Donoghue, 1997
Quokka (*Setonix brachyurus)*	*Theileria brachyuri*	Clark and Spencer, 2007
Brush-tailed bettong (*Bettongia penicillata ogilbyi*)	*Theileria penicillata*	Clark and Spencer, 2007
Western grey kangaroo (*Macropus fuliginosus*)	*Theileria fuliginosa*	Clark and Spencer, 2007
Gilbert's potoroo (*Potorous gilbertii*)	*Theileria gilberti* n.sp.	Lee *et al.*, 2009

10.Piroplasmosis Diagnosis.

10.1. Microscopy.

Microscopic examination of stained blood smears is the routine protocol for the diagnosis of piroplasmosis (Barnett, 1977; Mahoney, 1977; Homer *et al.*, 2000; Clark *et al.*, 2004; Lee, 2004). Depending upon the magnitude of the parasitaemia, the merozoites should be detectable within the host's red blood cells (Clark *et al.*, 2004; Lee, 2004). Their morphology varies from the classic 'ring with a stone' appearance or the tetrads (Maltese cross) in *Theileria*, to single or paired pear-shaped merozoites in the *Babesia* genus (Homer *et al.*, 2000). As described previously, the assumption that absence of lymphocytic schizogony implies babesial piroplasmosis only is rather misleading (Uilenberg, 2006). The extra-erythrocytic multiplication that *Theileria* species undertake before invading the RBCs is difficult to find, even in some unquestionable species of *Theileria* (Uilenberg, 2006). Phagocytosis of an infected RBC by a macrophage could be misinterpreted as intralymphocytic schizogony (Callow, 1984).

The analysis of blood films is a subjective process, which relies on the experience and knowledge of the observer and a reasonable amount of time spent to examining the smear (Homer *et al.*, 2000). Regardless, as noted earlier, an absolute discrimination between genera and species is unreliable if exclusively based on morphological resemblance, even for the most experienced parasitologist (Barnett, 1977; Mahoney, 1977). Low parasitaemias (i.e., less than 0.1% of erythrocytes infected), are difficult to detect by routine microscopy, hence the need to combine light microscopy inspection with complementary tests, such as serology or, as illustrated in more detail in the following paragraph, molecular technologies (Callow, 1984; Gaunt, 2000; Homer *et al.*, 2000; Lee, 2004).

10.2. Polymerase Chain Reaction (PCR) Diagnosis.

In the last two decades, molecular techniques, such as polymerase chain reaction (PCR), have been more often used to complement microscopic screening of blood films in the detection and identification of haemoprotozoa (Kunz, 2002; Lee, 2004).

PCR is an extremely sensitive method capable to assess parasitaemias of *Babesia* sp. of approximately 0.000003% (Jefferies *et al.*, 2003). The fault associated to such high sensitivity is the likelihood of false positives and negatives (Yang and Rothman, 2004). The “background contamination from exogenous sources of DNA is a common cause of false-positives [...] the “carryover” of products from earlier PCR reactions can be harboured and transmitted through previous PCR reagents, tubes, pipettes, and laboratory surfaces.” (Yang and Rothman, 2004). “Very minor amounts of carry-over contamination may serve as substrates for amplification and lead to false-positive results.” (Yang and Rothman, 2004). False-negative findings are instead associated with the concentration and purification of the DNA sample prior to the beginning the amplification process (Yang and Rothman, 2004). “Three of the most commonly encountered problems are in fact: inadequate removal of PCR inhibitors in the sample, such as haemoglobin, blood culture media, urine, and sputum; ineffective release of microbial DNA content from the cells; or poor DNA recovery after extraction and purification steps.” (Yang and Rothman, 2004).

The selectivity of PCR results from the use of primers that are complementary to the DNA region targeted for amplification under specific thermal cycling conditions (Yang and Rothman, 2004). A schematic representation of the three major steps in each PCR cycle is illustrated in the following figure. As PCR progresses, the DNA template is exponentially amplified (Yang and Rothman, 2004).

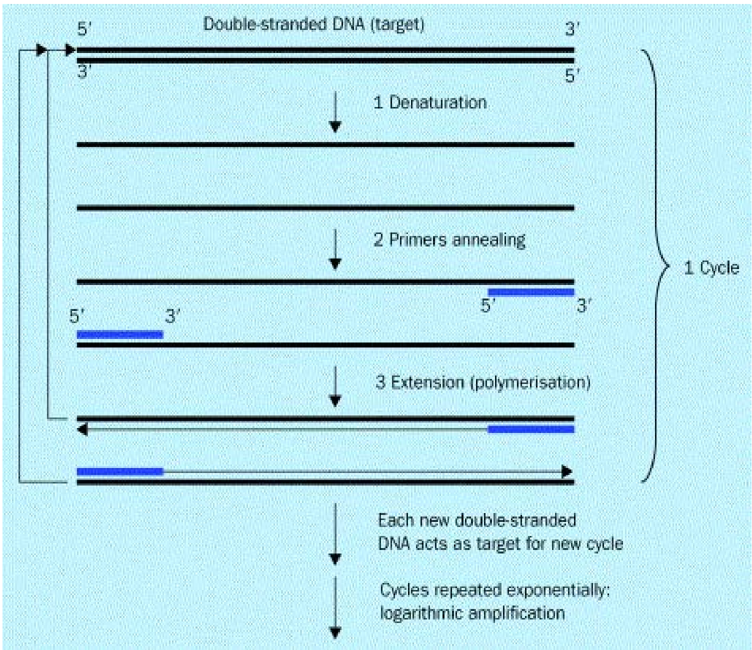

Figure 6 Schematic of PCR. The PCR reaction takes place in a thermocycler. Each PCR cycle consists of three major steps: (1) denaturation of template DNA into single-stranded DNA; (2) primers annealing to their complementary target sequences; and (3) extension of primers via DNA polymerisation to generate new copy of the target DNA. At the end of each cycle the newly synthesised DNA act as new targets for the next cycle. Subsequently, by repeating the cycle multiple times, logarithmic amplification of the target DNA occurs (adapted from Yang and Rothman, 2004).

10.3. Molecular Tools for Phylogenetic Analysis.

The use of PCR to amplify and sequence highly conserved genes is a more objective technique in species characterisation than morphology and life history (Barta, 2001; Kunz, 2002; Criado-Fornelio *et al.*, 2003). In the case of protists, some of the highly conserved genes are the sequences associated to the subunit 18 of the ribosomial RNA (18SrRNA) genes (Barta, 2001; Caccio', 2002). These are most commonly used to infer evolutionary correlation on a molecular basis (Barta, 2001; Caccio', 2002; Schnittger *et al.*, 2003).

The application of molecular technology to redefine traditional taxonomy has not been without frictions or confusion (Kunz, 2002) and the Order *Piroplasmida* has not been left out of this debate. In recent years, a new family, the *Nicollidae*, has been proposed to include *Babesia equi, B. rodhaini* and *Cytauxzoon felis,* which have intermediate properties of the two genera (Allsopp *et al.*, 1994). Other studies have suggested up to three or four new taxa of piroplasms based on the sequencing of 18SrRNA from various babesial and theilerial isolates (Criado-Fornelio *et al.*, 2003).

It has been suggested that the piroplasms are quite ancestral haemoparasites (Criado-Fornelio *et al.*, 2003). They must have developed in their mammalian or arthropod host in Africa, during the Pleocene, around 60-55 million years ago (Criado-Fornelio *et al.*, 2003).

The current biogeographical knowledge of piroplasms is still incomplete, lacking the sequencing of species from South America and Australia. More comprehensive approaches and techniques will improve the final evolutionary hypothesis (Criado-Fornelio *et al.*, 2003).

11. *Theileria penicillata* and the Woylie.

Clark and Spencer (2007) have described *Theileria penicillata* as a new species "during an assessment of blood samples as part of an investigation of animal health" in the woylie. A predictable finding has been the impossibility to distinguish *T. penicillata* from other piroplasms merely on a morphological basis (Clark and Spencer, 2007). The pleomorphic aspect and the reduced size, 0.4-1.2 µm x 0.8-1.5 µm, were not indicative of the species when compared to the piroplasms in other Macropodoidea (Clark and Spencer, 2007). By relying on molecular analysis though, these authors have ascertained the monophyletic origin of the organism within the *Theileriidae* family and suggested the new species name (Clark and Spencer, 2007).

12. Objectives and Aims of the Study.

The specific aims of this study were:

a. To describe morphological variations of (i) the piroplasms and (ii) the RBC and WBC by LME in selected blood films.

b. To investigate piroplasm prevalence by gender and location.

c. To estimate AP and determine its correlation with woylie gender, location and body condition.

d. To estimate AP variation for Keninup, pre- and post-decline.

e. To relate AP to Hb, HCT and RBC count.

f. To correlate haemolysis of the whole blood samples to piroplasm infection or alternative causes.

13. Material and Methods.

13.1. Blood Samples.

A total of 274 blood smears (112 female and 162 male adult woylies) were selected for this study from the woylies trapped in Karakamia Sanctuary and in the Upper Warren region between 2006 and 2009 as part of WCRP (Pacioni, 2010). Only adults were included in the study, excluding any juveniles or sub-adults.

Blood samples were collected from the lateral caudal vein and preserved in EDTA in commercial tubes (as described by Pacioni, 2010). Between 2 to 3 blood films per animal were prepared and air dried in the field, while the whole blood samples were submitted to Murdoch University Clinical Pathology Laboratory (MUCPL) within 36 hours from collection and processed (Pacioni, 2010). Morphology of cells, including examination for red blood cell parasites, was assessed (Pacioni, 2010). Blood smears were stained with Wright's/Giemsa stain soon after collection (Pacioni, 2010) however a few unstained ones were submitted to Murdoch University Clinical Pathology Laboratory to be processed with Wright's/Giemsa stain in September 2010 for this study. The number of blood smears screened classified by gender and location is summarised in Table 2.

Table 2 Number of blood samples screened classified by gender and location.

LOCATION	FEMALE	MALE	TOTAL
Balban	7	22	29
Karakamia	13	13	26
Keninup	59	77	136
Warrup	33	50	83
GRAND TOTAL	112	162	274

Our study referred to the haematological profiles processed by MUCPL between 2006 and 2009 (as described by Pacioni 2010) when analysing Hb, HCT and RBC count. The samples that were haemolysed before reaching the laboratory were identified and a correlation to piroplasm infection or alternative causes was considered in our study.

13.2. Woylies' Population Locations.

Four locations were chosen: Balban, Karakamia, Keninup and Warrup. Their selection was based on: the animals' density per single location (close to or below carrying capacity), which warranted a representative sample size; and the variation of these same densities by location over time and at different stages of the decline.

The specific locations are visualised in the following figures (Figure 7 and 8).

Karakamia Sanctuary is located 50 Km North-East of Perth. The woylie population here resident extends on a 275 ha property that is completely predator-proof (Pacioni, 2010). Originally the land was used for livestock grazing and Jarrah timber production, but the majority of the sanctuary has kept or restored its original Jarrah forest (*Eucalyptus marginata*) and mixed woodlands of Marri (*Corymbia calophylla*) and Wandoo (*E. wandoo*) (Pacioni, 2010).

Keninup and Balban are situated in the eastern side of the Upper Warren region, 300K Km in the south-west of Western Australia, while Warrup belongs to the western side of the area. The vegetation is characterised by Karri forest (*E. diversicolor*), interspersed by Jarrah and Marri woodland (Pacioni, 2010).

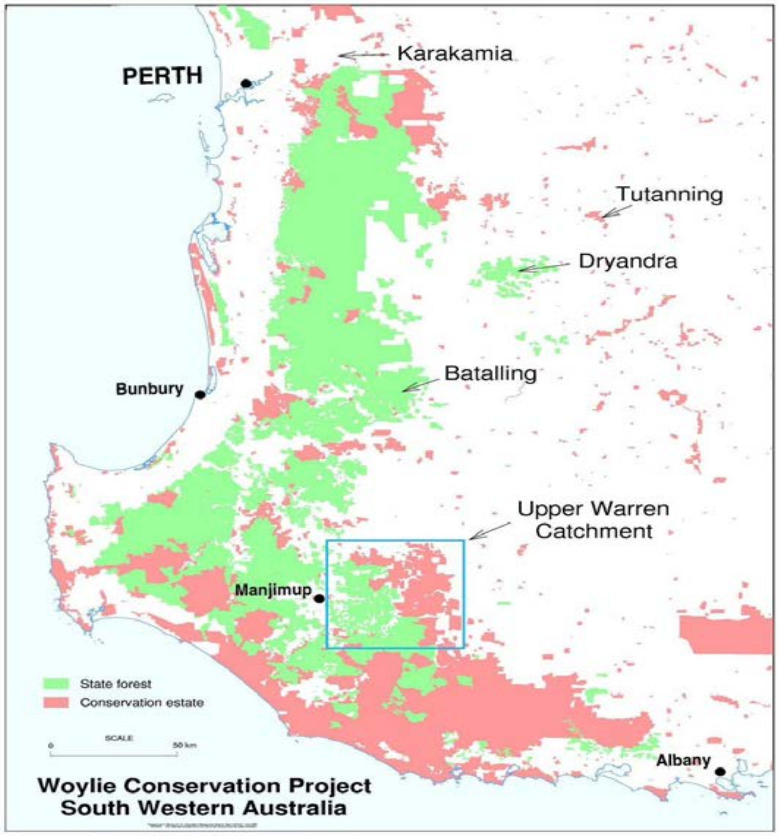

Figure 7 Karakamia and the Upper Warren region: locations of the woylie populations selected by this study (adapted from Wayne, 2008).

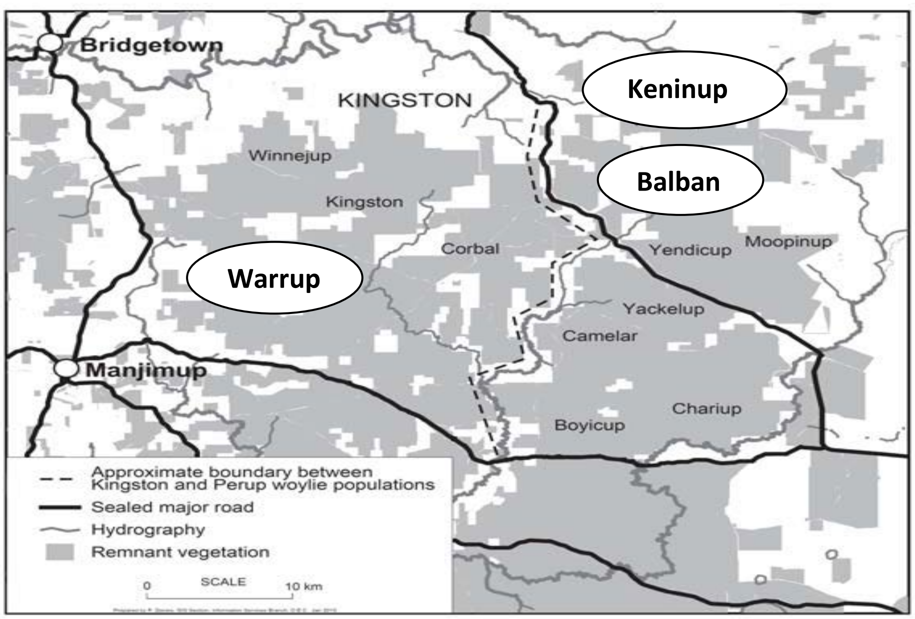

Figure 8 Keninup, Balban and Warrup locations in the Upper Warren region (adapted from Pacioni, 2010).

13.3. Light Microscopy Examination of the Blood Films.

Every blood smear was examined under light microscopy in accordance to the following protocol:

1. The sample identification number was noted and recorded

2. First, 5-10 fields at 200x magnification were examined in the blood film, to estimate any qualitative or quantitative abnormality of RBC and/or white blood cells (WBC), such as signs of regenerative anaemia or increase/decrease of WBC

3. At 400x magnification 5-10 fields were examined to establish presence/absence of piroplasms within the RBC, noting down any other haemoparasites encountered, such as *Trypanosoma* spp., or other interesting morphological findings

4. With the oil-immersion (100x) objective lens, 50 high power (HP – x1,000) fields for the piroplasm-negative slides and 100 HP fields for the piroplasm-positive samples were examined, and each piroplasm was counted (see below)

5. Digital photographs were taken of the protozoa and host cells to create an image library.

13.4. Parasite Counts (Estimation of Parasitaemia).

"An estimate of the degree of infection is established by enumerating the parasites in blood smear in relation to RBC" (Callow, 1984). At HP magnification all the piroplasms present in 100 fields were counted. All the RBC present in 10 fields were then counted, always at HP. The percentage of RBC infected by piroplasm equalled the total number of parasites (from 100 fields) divided by the total number of RBC (average number of RBC counted in 10 fields multiplied by 10) (Callow, 1984).

13.5. Data Analysis.

The statistical analysis was performed using SPSS 17.0 (SPSS Inc., 2008).

To evaluate potential relationships among the three categorical variables, piroplasm prevalence, woylie gender and population locations, a loglinear analysis has been conducted.

The normality of the AP was inspected and the Kolmogorov-Smirnof test and both z-skewness and z-kurtosis were conducted to confirm a non normal distribution. The AP was transformed via square root transformation to obtain a normal distribution. The fit of regression model for the transformed parasitaemiae, gender and location variables were then assessed using multiple regression and ANOVA. A one-way ANOVA analysis was used to detect the differences in levels of AP in relation to the different locations, while a Spearman's test was performed to detect potential influence of parasitaemia on the animals' body and coat conditions in each single location.

An independent t-test has been performed to evaluate a longitudinal study of piroplasm parasitaemia in the remnant woylie population present in Keninup.

A loglinear analysis was performed to correlate haemolytic blood samples to the presence or absence of the haemoparasite and in relation to the different locations.

Finally, multiple linear regressions were performed to establish correlations between the square root of the AP and haemoglobin concentration, haematocrit levels and red blood cell counts.

14. Results.

14.1. Morphological Findings at the Light Microscopy.

While estimating average parasitaemia (AP), a variety of morphological features were noted, such as pleomorphism of the *Theileria* sp., as well as unreported findings regarding *Theileria penicillata* morphology. Some digital photographs of *T. penicillata* intra-erythrocytic infection in the brush-tailed bettong are reproduced below (Figures 9 to 13). Other findings are included, such as detection of *Trypanosoma* haemoparasites, shown in Figure 14 and 15.

No significant RBC and WBC morphological abnormalities were encountered in the light microscopy examination (for example, there were no signs of regenerative anaemia or leukocytosis).

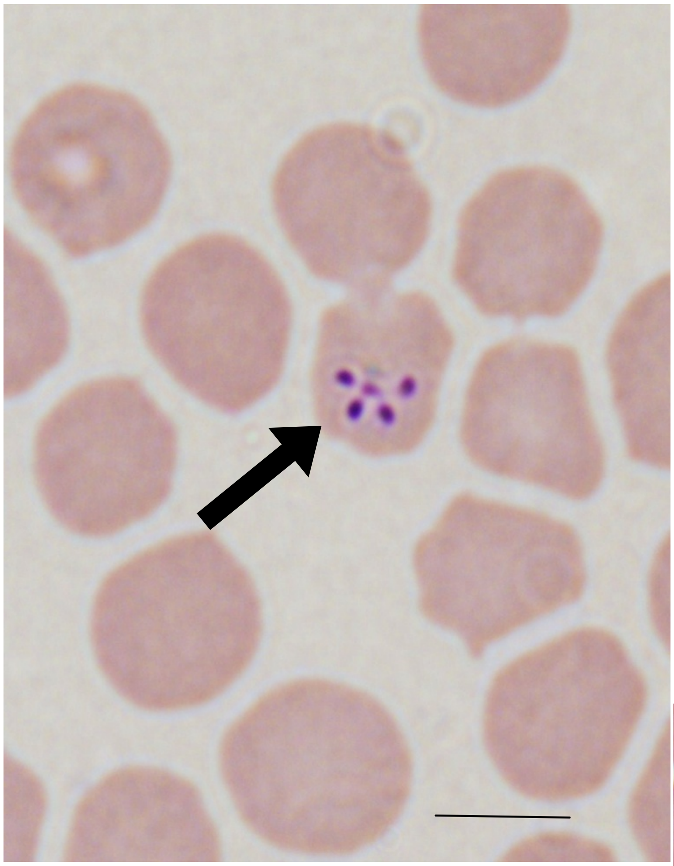

Figure 9 ***Theileria penicillata*** **(arrow) dividing within woylie's RBC: Maltese cross feature. Bar = 10 µm (photo by Stefania Basile).**

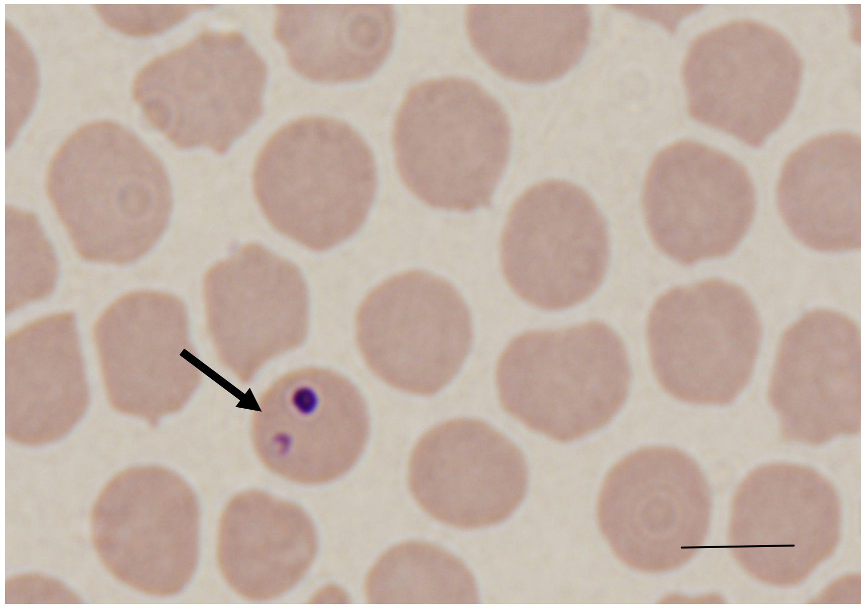

Figure 10 ***T. penicillata*** **(arrow) with the typical 'stone and a ring' shape in proximity of a Howell Jolly body. Bar = 10 µm (photo by Stefania Basile).**

Figure 11 ***T. penicillata*** **(arrow) with the ovoidal shape. Bar = 10 µm (photo by Stefania Basile).**

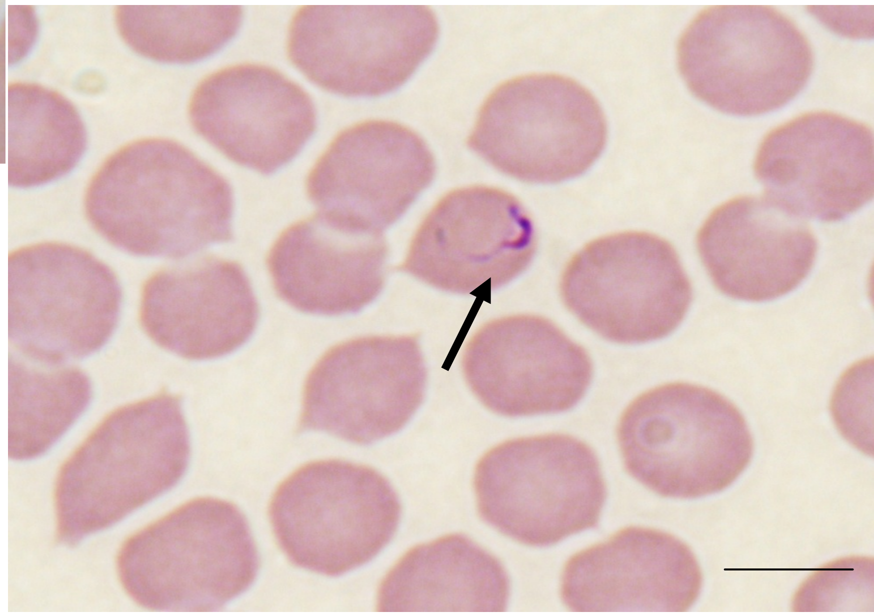

Figure 12 ***T. penicillata*** **(arrow) with the 'bayonet' shape. Bar = 10 µm (photo by Stefania Basile).**

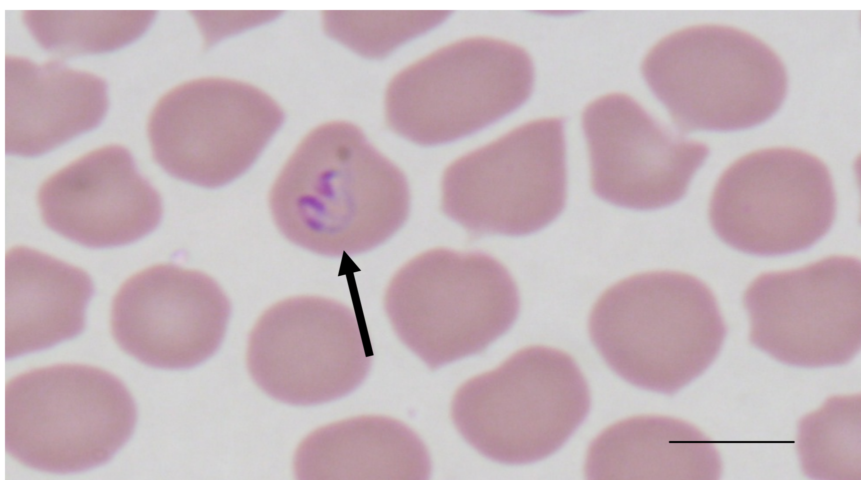

Figure 13 Dividing forms of ***T. penicillata*** **(arrow) within a RBC. Bar = 10 µm (photo by Stefania Basile).**

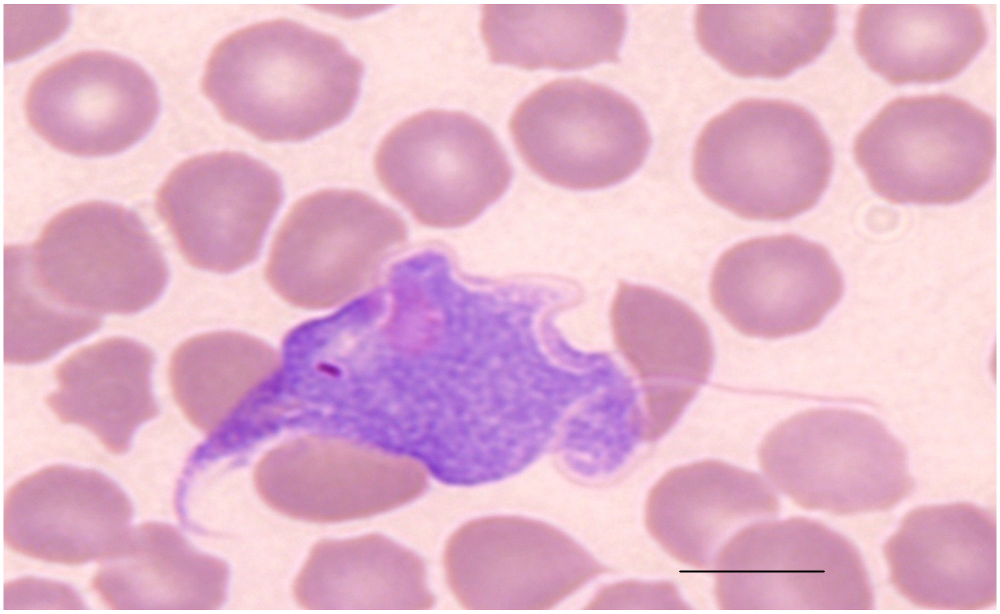

Figure 14 ***Trypanosoma*** **sp. encountered at LME (Bar = 10µ) (photo by Stefania Basile).**

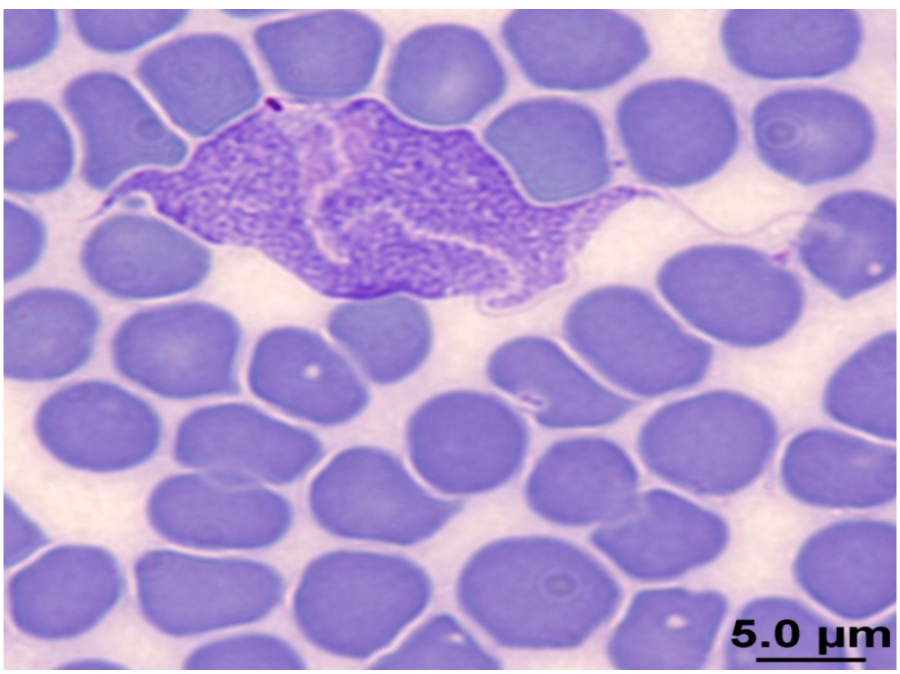

Figure 15 ***Trypanosoma*** **sp. with its undulating membrane across the middle (photo by Stefania Basile).**

14.2. Piroplasm Prevalence by Gender and Location.

The piroplasm prevalence by gender and location is summarised in Table 3. Higher prevalence was recorded for Keninup, while Balban, Karakamia and Warrup had similar overall lower prevalences. The two-way loglinear analysis generated a final model that retained only two effects. The K-way Effects likelihood ratio of the model had Chi-square = 102.405, df = 7 and $p < 0.0005$. This indicated that the variables piroplasm prevalence x location was highly significant, when compared to the prevalence x gender interaction, which was not significant ($p > 0.05$).

Table 3 Piroplasm prevalence (%) by gender and location

LOCATION	FEMALE PREVALENCE (%)	MALE PREVALENCE (%)	TOTAL PREVALENCE (%)
Balban	2.5	8.3	10.8
Karakamia	6.4	6.4	12.8
Keninup	20.6	27	47.6
Warrup	2.9	5.4	8.3

14.3. Piroplasm Parasitaemia Correlation to Gender, Location and Body Condition.

The multiple regression model revealed that population location accounted for 32% of the variability of the transformed AP, while gender had no influence at all. The ANOVA indicated that AP is strongly correlated to location ($p < 0.05$) rather than gender. In the one-way ANOVA, the homogeneity of variances of transformed AP by location has been met (Leven’s test was not significant, $p > 0.05$), while the transformed AP among locations were significantly different (ANOVA test, $p < 0.0005$). Hochberg’s post-hoc test highlighted a significant difference ($p < 0.0005$) among the transformed AP of the various locations, with the highest and similar levels in Balban and Keninup, followed by Karakamia, while the transformed AP of the woylies at Warrup were significantly lower. This is summarized in Figure 16.

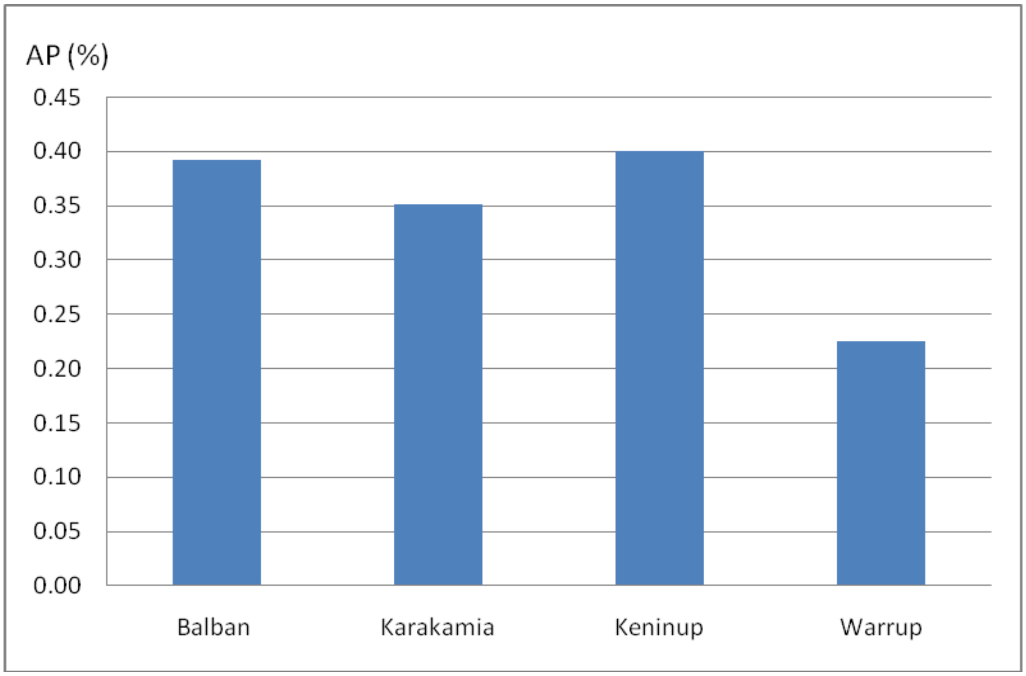

Figure 16 AP by location.

The Spearman's test was not significant ($p < 0.05$) for every location, underlining that the transformed AP were not affecting woylies' body condition and coat status.

14.4. Piroplasm Parasitaemia Variation in Keninup over Time.

There was no significant ($p > 0.05$) difference in transformed AP in woylie cohorts before and after the decline at Keninup, yet there was a tendency for the transformed AP to be higher before the decline (M= 6.5; SE= 0.45) compared with later (M= 5.9; SE= 0.27).

14.5. Piroplasm Parasitaemia Correlation with Haemoglobin (Hb), Haematocrit (HCT) and RBC Counts.

The two-way loglinear analysis generated a final model that retained only two effects. The K-way Effects likelihood ratio of the model had Chi-square = 117, df = 10 and $p < 0.0005$. This indicated that the two-way interaction (haemolysis x location) was highly significant when compared to the haemolysis x piroplasm infection interaction, which was not significant ($p > 0.05$). These results suggest that the parasite infection was not responsible of the haemolysis of some of the blood samples collected, but rather the blood collection procedures in some locations could have not been optimal.

The multiple regression assessed that Hb accounted for 15.2% of the variability of the transformed AP, while HCT and RBC accounted only for 4.3% and 6.8% respectively. The ANOVA indicated that the transformed AP is strongly correlated to Hb ($p < 0.005$) rather than HCT and RBC ($p > 0.05$).

15. Discussion.

15.1. Light Microscopy.

The LME findings on *Theileria penicillata* in the woylie confirmed the previously reported information about the morphology of this parasite (Clark and Spencer, 2007). *T. penicillata* is a small pleomorphic piroplasm (0.4-1.2 µm x 0.8-1.5 µm) (Clark and Spencer, 2007). The variation in shape described by the same authors (Clark and Spencer, 2007) was also observed in this study (Figure 11, 12 and 13). An interesting discovery was the detection of the characteristic 'Maltese cross' during the erythrocytic cycle of the piroplasms in the RBC. Observation of this morphological form could be predicted on the basis of what is known about the normal reproductive behaviour of the *Theileriidae* family, but was confirmed during this project by frequent observation of the form during LME (Figure 9). Another finding was the presence of multiple 'ring-with-a-stone' protozoa within the same erythrocyte. These presented with differing sizes (Figure 13) and it is hypothesised that these are simply morphological variations of *T. penicillata* which naturally occur within the species and most likely reflects normal maturation of the parasite and different trophozoite stages. Alternatively, the different morphological forms could represent different *Theileria* spp., or instead be *Babesia* spp. The impossibility to distinguish *T. penicillata* from other piroplasms merely on a morphological basis has been previously discussed (Clark and Spencer, 2007; Irwin, 2009) and molecular analysis is necessary to ascertain the monophyletic origin of the parasite(s) (Clark and Spencer, 2007).

Occasional findings of *Trypanosoma* parasites was also reported (Figure 14 and 15), suggesting multiple haemoparasite infections in individual woylies. Further investigation is needed to confirm the identity of these parasites, but the morphology was consistent with previously reported trypanosomes observed in the blood of woylies in the Upper Warren region (where these samples were obtained during allied studies under the WRCP) (Smith *et al.*, 2009).

15.2. Statistical Analysis.

15.2.1. Piroplasm Prevalence by Gender.

In our study, there was no significant difference in piroplasm prevalence between male and female woylies, contradicting the findings of Wilson *et al.* (2002) in other species. These authors described the tendency of male mammals to be more prone to higher parasite burdens due to their behaviour; males are more likely than females to cover larger home ranges for territorial, reproductive and feeding purposes (Wilson *et al.*, 2002). This behaviour was confirmed in the case of male woylies by Pacioni (2010); where females had the tendency to remain within or close to their mother's home range (less than 1km), males were more mobile, dispersing up to 3km (Pacioni, 2010). Longer distances (more than 6km) were also covered by male woylies but more rarely (Pacioni, 2010). In spite of behavioural differences between the two woylie genders, variation in home ranges did not seem to affect the prevalence of piroplasms in males and females in our study, highlighting that other factors may contribute to this such as the distribution and/or density of the (presumed) intermediate host, the Ixodid tick.

Establishing piroplasm prevalence across age groups, including sub-adults and juveniles for examples, should be investigated to broaden our knowledge of the parasite epidemiology. The challenge, though, will be to tailor capture techniques for these categories. Females with pouch young should be also included in the demographic study but the ethical issue of pouch young rejection by the mother and consequent pouch young survival should not be underestimated.

15.2.2. Is Piroplasm conducive to the Woylie Decline?

As Pacioni described (2010) there is a spatial pattern in the decline of the woylie. The eastern regions of the Upper Warren (Balban and Keninup) were the first to report a decrease; then the decline progressed north at a rate of 5-10 Km per year (Pacioni, 2010). This spatial model is supported by the fact that Karakamia is the only location in WA to be still stable over time.

The pattern of decline may mimic the spreading of an infectious agent, from the southern to the northern regions (Pacioni *et al.*, 2010). However our findings do not support this hypothesis. If piroplasm infection was the causative agent of the decline, still to be experienced in the northern location (Karakamia), AP in Karakamia might be predicted to be significantly lower than those in Balban and Keninup. This was not the case; northern (Karakamia) and southern (Balban and Keninup) locations presented with almost equal AP. Furthermore, no cytological evidence of red cell insult (for example, regenerative changes within erythrocytes suggesting anaemia) was detected in any of the samples examined. Although it might be argued that only healthy individuals were sampled, it seems unlikely that at least some indication of red cell insult was not found if the parasites were causing illness. Possible conclusions that we could derive from this are: (i) piroplasm infection is not the primary decline drive; (ii) piroplasm could interact synergistically with other elements (climate, nutrition, predation, fire) and be conducive to decline in the long term.

The *Theileriidae* family is an ancient group of parasites (Criado-Fornelio *et al.,* 2003). It must be presumed that the host-parasite relationship between *T. penicillata* and *Bettongia penicillata olgilbyi* has co-evolved over many millennia, potentially establishing equilibrium. This could be supported by the AP in woylies trapped in Keninup pre- and post-decline, which did not vary significantly. Investigating similar parameters in Balban, Warrup and Karakamia would be interesting, but inadequate sample sizes were available. Similarly estimating prevalence and AP of piroplasm in the woylies translocated in South Australia could represent an important term of comparison.

As mentioned previously, concomitant intrinsic or extrinsic causes are more likely to perturbate the host-parasite balance and affect the woylie population fitness (Spalding and Forrester, 1993; Wobeser, 2007).

Subtle effects of an infectious agent on survival and/or fecundity could have important consequences on the woylie population (Spalding and Forrester, 1993; Wobeser, 2007). For example the cow-pox virus in rodents is asymptomatic and does not influence the new born survivorship. The infection however delays the reproduction onset by one month, reducing by 25% the reproductive output in such short-lived animals (Wobeser, 2007). A mild dysfunction due to sub-clinical or sub-lethal disease could indirectly influence survival by increasing the vulnerability to predation or other factors (Spalding and Forrester, 1993; Wobeser, 2007).

In regards to predation, Karakamia represents a peculiar setting when compared to the other locations. It is a vermin-proof area, where the woylies reached their carrying capacity (400-600 subjects), and remained stable during the decline (Pacioni, 2010). Analogously, this time due to geographic isolation, there is also an absence of introduced predators in the in the South Australian islands (Pacioni, 2010). The translocated island population, similarly to the protected Karakamia woylies, remained constant over time (Pacioni, 2010). This has some implications for future management. As Finlayson *et al.* (2008) suggested secure and self-sustaining populations of threatened species, like the woylie, through the establishment of fenced areas could assist in the study of the woylie biology as well as future conservation and management.

When considering the general wellbeing of the animals, body and coat condition were not affected during and after the decline (Pacioni, 2010) and were not significantly associated to AP. The next reliable index of wildlife general health status was haematology (Clark, 2004). In our study Hb, HCT and RBC count were considered.

Critical to this discussion about potential pathogenesis of *Theileria penicillata*, it was necessary first to exclude piroplasm-induced haemolysis of the collected blood samples (since the pathology of piroplasms is usually associated with haemolysis). The interesting finding was that haemolysis was more significantly caused by inappropriate blood sample handling rather than the haemoparasite itself; and that the higher percentage of haemolysed samples was associated with the Karakamia field site. This highlighted the necessity to review and improve the infield procedures of blood collection in the immediate future.

Once haemolysis by piroplasms was ruled out, possible correlations between AP and the above described haematological parameters were investigated. Surprisingly enough, our study demonstrated a stronger association between AP and Hb only rather than Hb, HCT and RBC count altogether. These three parameters are strictly correlated (Stockham and Scott, 2008). Hb is a more direct measure of blood's oxygen carrying capacity than HCT and RBC count, but notwithstanding they should finally reflect each other (Stockham and Scott, 2008). Hb and HCT can be both determined by conductivity methods, which vary in accordance to the analyser used (Stockham and Scott, 2008). Why this finding occurred is yet to be understood completely.

16. Acknowledgements.

This independent study contract could not have been possible without the support and guidance of many people.

I would like to thank my two supervisors, Peter Irwin and Carlo Pacioni from Murdoch University, for the patient, passion and availability, they have demonstrated along the project, and in spite of all the concomitant commitments. Their constant feedback and encouragement has increased my confidence in critical thinking, writing skills and understanding of statistical analysis. Thank you for sharing your knowledge.

I also thank Adrian Wayne from the Western Australia Department of Environment and Conservation (DEC) and Andrew Thompson from Murdoch University, for allowing me to access the Woylie Conservation and Research Project (WCRP) field samples; and to Murdoch University Clinical Pathology Laboratory for the haematology work.

My acknowledgment is addressed to Aileen Elliot from Murdoch University, for the patient she has shown while improving my light microscopy technique.

A sincere thank-you goes to Andrew Smith and Marika Maxwell from DEC, in helping me sorting out the missing data.

A special recognition goes as well to all the people involved in the collection of the field samples and data, without which this study could not have happened: all the WCRP volunteers; Australian Wildlife Conservancy; and DEC.

And last but not the least I express my love and gratitude to my parents and my partner, Jeremy Sinclair, who have always trusted my decisions, supported my efforts and transmitted the necessary sense of humour to deal with life.

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An evaluation of two methods for assessing population densities of introduced predators in Southwest Western Australia

Kevin Bennett
Project advisor: Adrian Wayne, Ph.D.
Department of Environment and Conservation
Manjimup, WA

Academic advisor: Tony Cummings
Home institution: Colby College
Major: Biology

Submitted in partial fulfillment of the requirements for Australia: Rainforest, Reef, and Cultural Ecology, SIT Study Abroad, Spring 2012

Abstract

A study of introduced predator activity in the jarrah forest of the Upper Warren region of Western Australia found that density estimates cannot be accurately drawn from activity indices on sandpads. Estimates of 0.08-0.14 foxes per km^2 and 0.02-0.20 cats per km^2 were calculated. These estimates are well below those from other sources, due to inherent limitations of sandpad surveys. These are discussed. Motion-sensor cameras, however, may be able to accurately estimate population densities, as their ability to detect animals overall and distinguish between individuals is superior to that of a sandpad. A comparison is made of the cost and time investment necessary between studies involving sandpads and cameras. The relative effectiveness and costs of alternate methods for estimating density in relation to the study area and species are also considered.

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1. Introduction

Non-marsupial mammalian predators first arrived in Australia in 1788 (Abbott 2002). At the establishment of the British in Sydney, there are several early reports of cats (*Felis catus*) kept as pets (Abbott 2002). Cats were subsequently introduced to Perth and Albany in the early 1830s, and had established feral populations in the surrounding forests by the mid-1840s (Abbott 2002). Foxes (*Vulpes vulpes*) arrived first in Victoria, successfully introduced for hunting in 1871—around the same time as their natural prey, the European rabbit (Rolls 1969). By 1893, they were already considered a pest in that area, with a bounty being placed on fox heads in three Victorian shires (Rolls 1969). Foxes were first recorded in Western Australia in the early 20th century (again, following the rabbits), and had made it to the Southwest coast by the 1930s, about 100 years after their feline counterparts (Long 1988).

With the arrival of Europeans, native fauna numbers quickly began to decline. Burbidge and McKenzie (1989) compared Western Australian island populations of small mammals on islands with and without disturbance (including fire, housing, mining, introduction of cats, introductions of foxes, etc.), finding that on islands without human disturbance, no mammal within the “critical weight range” (Burbidge and McKenzie 1989) declined or became extinct, whereas on islands containing cats and/or foxes islands and no rock piles for shelter, at least one native mammal declined or went extinct. Kinnear *et al.* (1988) determined that fox predation was the most significant factor hindering the population growth of threatened black-flanked rock wallabies in the wheatbelt region of Western Australia (~200 km east of Perth). Many recent successes in recovery of native WA fauna has been on the heels of large-scale fox-control efforts (Friend 1990, Start *et al*. 1998, Morris *et al*. 2003). Likewise, feral cats have been implicated in species decline through circumstantial and historical evidence (Finlayson 1961, Spencer 1991, Calver and Dell 1998), but it is often difficult to isolate effects of feral cats from those of foxes. Risbey *et al.* (2000), however, displayed the effects of rapid cat population growth (by fox removal) on a small isolated area. They found that trapping success for small mammals dropped by 80% over two years. The Department of Environment, Water, Heritage, and the Arts *Threat Abatement Plan for predation from feral cats* (2008) lists 34 bird, 37 mammal, 7 reptile, and 3 amphibian species as being at risk from cat predation. In a cat- and fox-free enclosure in South Australia, Moseby *et al.* (2009) found that small mammal and reptile populations increased by as much as 15 times over outside populations.

Among the species commonly considered as having suffered at the "hands" of introduced predators is the critically endangered woylie, or brush-tailed bettong (*Bettongia penicillata ogilbyi*). Woylie pre-European settlement distribution is thought to have covered much of the Australian continent, generally in semi-arid scrub or forest habitats (de Tores and Start 2008). Today, its natural range is restricted to three small areas of schlerophyll woodland in Southwest Western Australia: Tutanning Nature Reserve, Dryandra Woodland, and the Perup Nature Reserve and surrounding jarrah (*Eucalyptus marginata*) forests (de Tores and Start 2008).

The population of the woylie has undergone three major changes: an initial major decline from the late-19th century through mid-20th century, a recovery from the mid-1970s through the early 2000s, and a second decline lasting the majority of the first decade of the millennium. The initial major decline may be attributable to fox predation (among other human-related factors), and the 30-year resurgence has been linked to fox-control efforts (Start *et al.* 1998). However, the causes of the most recent woylie decline are not clear. One of the most evident deficiencies of information is the lack of known population densities of foxes and cats in woylie habitat, particularly in its historical stronghold, Perup Nature Reserve. Wayne *et al.* (2011) determined that the minimum population density to solely account for the woylie decline in this region would be 0.5 predators per km^2 assuming one woylie killed per predator per night and no woylie deaths from means other than predation. To understand the role of predators in the most recent woylie decline, it is necessary to determine how close current predator population densities are to that minimum necessary density estimate.

There are several possible methods that can be used for approximating fox and cat populations: DNA capture-mark-recapture, index-manipulation-index, intensive shooting, trapping, and poisoning, spotlighting, sandpad surveying, and sandpad surveying with the addition of motion sensor cameras.

DNA capture-mark-recapture (CMR)—luring animals to a bait station with a sticky pad or barbed wire to catch some hair—is a potentially promising method because it yields an estimate of absolute abundance. However, models associated with CMR techniques assume geographic closure (no random movements into or out of the study area). Boulanger *et al.* (2002) evaluated two methods for carrying out DNA CMR studies on grizzly bears in British Columbia, finding that large grids with widely-spaced bait stations more closely conformed to the geographic closure estimates of their models, but were more likely to miss individual animals within their study area. Likewise, smaller grids accounted for more of the individuals present, but more poorly predicted the movement of bears in and out of the grids. Ideally, grids for animals with potentially low populations densities and large home ranges, like cats (Jones and Coman 1982, Edwards *et al.* 2001) and foxes (Coman *et al.* 1991, Phillips and Catling 1991), would be extensive and intensive. In other words, the grids area should be large and the spacing of the bait points should be tight. However, depending on habitat, this may be difficult given budget and manpower constraints. It has also been noted that detection probability should be calculated for each species to give more accurate results (Forsyth *et al.* 2005).

Index-manipulation-index (IMI) methods have been used previously in Australia to give population estimates of predators. Short and Turner (2005) used spotlighting and poisoning with cyanide as index and manipulation, respectively to estimate feral cat populations at Shark Bay. Their estimate was reasonable and was supported by observational evidence. However, their study site was almost entirely enclosed by water and fences, greatly increasing the confidence in their estimate. Additionally, spotlighting is not a feasible method to index a cat population in jarrah forests, as are cats secretive and almost never observed (Wayne *et al.* 2001). Marks *et al.* (2009) used IMI methods for assessing fox abundance in Victoria with sandpads (more feasible for jarrah forests) along with spotlighting as abundance indices and poisoning with cyanide as manipulation. However, as has been noted previously (Allen *et al.* 1996, Edwards *et al.* 2000), after the removal of some individuals by poisoning, the activity of the remaining population increased and skewed the sandpad results.

Short and Turner (2005) also undertook a successful density estimation effort through intensive trapping, shooting, and poisoning in a 12 km^2 fenced reserve. They successfully removed all cats over a two-year period and obtained a former population density. Intensive removal efforts will ensure that the estimated population density is correct (or at least very accurate), as all or almost all individuals will be accounted for. However, it is very labor-intensive and difficult in the large-scale, especially in very large unfenced areas like the jarrah forest. Marlow *et al.* (2000), however, successfully removed almost all foxes from area of 200-km^2 to obtain a density estimate along mid-coastal Western Australia.

Spotlighting has often been used as an index of population abundance (Risbey *et al.* 2000, Short *et al.* 1997, Marlow *et al.* 2000, Short and Turner 2005). However, it will not yield an absolute measure of abundance without being paired and "calibrated" with another method that does provide an absolute measure of abundance (Bayliss *et al.* 1986, Risbey *et al.* 2000, Short and Turner 2005). Additionally, multiple studies have shown that spotlighting is a less effective population index for predators than sandpad surveys (Mahon *et al.* 1998, Edwards *et al.* 2000).

Track counts using sandpads have often been used as a method of assessing relative predator activities (Marks *et al.* 2009, Mahon *et al*. 1998, Edwards *et al.* 2000, Thompson *et al.* 2000, Allen *et al.* 1996). To date, there have been no attempts to derive estimates of population density from sandpad survey data. This study attempts to derive a population density estimate for foxes and cats in the jarrah forest of Southwest Western Australia from sandpad survey data. It also compares the effectiveness and efficiency of using sandpads and the effectiveness and efficiency of using motion-sensor cameras to assess the activity and movement of predators to contribute to that density estimate.

2. Methods

2.1. Study Area

The study took place in the jarrah forest ecoregion of Southwest Western Australia, mostly open *Eucalypt* woodland dominated by jarrah, marri (*Corymbia calophylla*), and wandoo (*E. wandoo*). The region has a warm Mediterranean climate, with an inland annual rainfall of 600-700 mm. The sandpad survey sites were spread over a an area of about 875 km^2 including land in the Tone Perup Nature Reserve, the "Greater Kingston" National Park (unofficial name), and Kingston state forest just east and northeast of Manjimup, WA. Together, these areas of forest managed by the Western Australian government's Department of Environment and Conservation (DEC) make up the Upper Warren region.

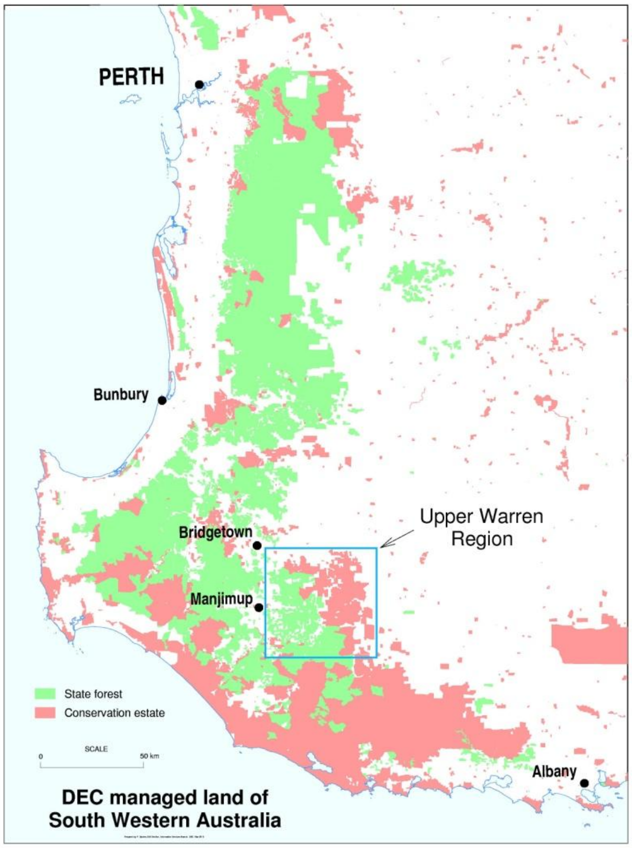

Figure 1. Conservation land and state forest in Southwest WA. Sandpads were all located within the Upper Warren.

There were six sandpad arrays, each named after the forest block where it was located: Keninup, Balban, Boyicup, Moopinup, Winnejup, and Warrup. All arrays had generally similar habitats and climates due to their proximity, but there were small differences in rainfall, vegetation, proximity to private pasture land, and area covered by the array that are not discussed in this report.

2.2. Sandpads

Sandpads were laid out, for the most part, in accordance with the suggestions of Allen and Engeman (1995). Each array contained 25 sandpads—a strip of sand one meter-wide dug across the width of the road. Pads were spaced 500 meters apart, and each array followed seldom-driven park roads. All tracks were checked and recorded in the morning and sand was smoothed with a rake and broom. The process was repeated every morning for six mornings from 27 March through 1 April, 2012. Tracks were divided into seven categories: cat, fox, chuditch (Western quoll), woylie, other macropod, possum, and other. Each set of tracks

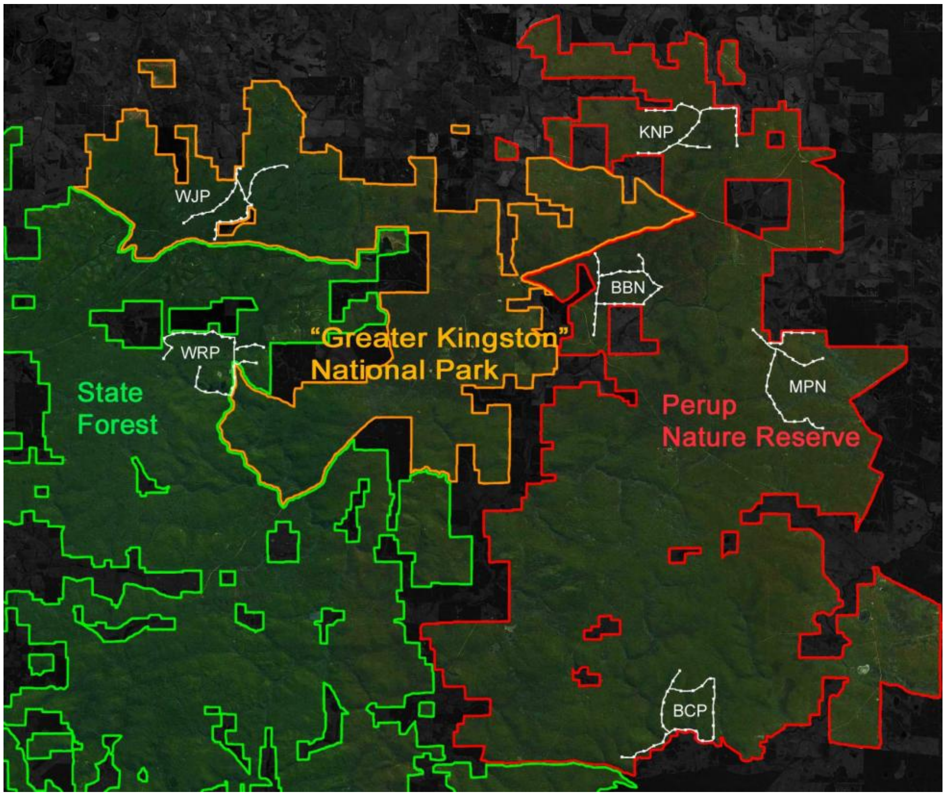

Figure 2. Sandpad arrays and their arrangement in the Upper Warren. WJP = Winnejup, KNP = Keninup, WRP = Warrup, BBN = Balban, MPN = Moopinup, BCP = Boyicup.

recorded was given a certainty rating from one to three, one being certain, two being probable, and three being possible. Tracks recorded under "other" were also identified and given a certainty rating. For cats and foxes, direction and activity on the pad was also recorded. On every pad, a clarity marker (a handprint) was placed in the morning over the road wheel rut after the pad had been smoothed over. If the marker was no longer visible the following morning, whether because of vehicle tracks, wind, rain, etc., that pad was removed from consideration for that day.

2.3. Analysis

Calculating an index for activity was based on Allen and Engeman (1995), called the Allen's Activity Index. Calculating an index for each study day requires dividing the total number of pads with recorded activity of a given species by the total number of available pads for that day. The overall activity index (AI) for a single session is the average of the indices from each day.

The convention (for foxes and cats) for attempting to determine how many individuals have been active in a sandpad array is to assume that every break in activity of more than one kilometer is a break between two individuals (Allen *et al* 1996). The possible flaw in this method is that enough activity will eventually result in a sole individual being identified, rather than many. Therefore, in order to determine if this is an effective way of assessing individual activity, AIs were compared to number of estimated individuals for each day at each site from the March 2012 survey and from previous surveys (2008-2011), with the assumption that a strong relationship implied a good estimate of number of individuals.

2.4. Cameras

For three of the nights of during the study, one Reconyx HC600 Hyperfire High Output Covert IR motion sensor camera was placed, facing along the sandpad at each of 25 total pads across five of the six sites. Batteries for the cameras were changed whenever necessary. Because no past data exists for cameras and because there were only 25 total cameras to cover 150 sandpads, data from cameras were not used to contribute to a density estimate, but rather just as a comparison between camera and sandpad in terms of effectiveness and efficiency in order to inform a recommendation regarding the more extensive use of cameras in the future.

3. Results

3.1. Sandpads

The average fox AI was 0.127 for the March 2012 session and 0.131 for all sessions combined (Feb ’08, Aug ’08, Mar ’09, Mar ’10, Mar ’11, Sep ’11, Mar ’12). Average cat AI was

0.046 for the March 2012 session and 0.047 for all sessions combined. For all sessions, average fox AI was 0.182 at Keninup, 0.162 at Balban, 0.100 at Boyicup, 0.103 at Moopinup, 0.136 at Winnejup, and 0.081 at Warrup. Average cat AI was 0.050 at Keninup, 0.034 at Balban, 0.043 at Boyicup, 0.085 at Moopinup, 0.036 at Winnejup, and 0.051 at Warrup.

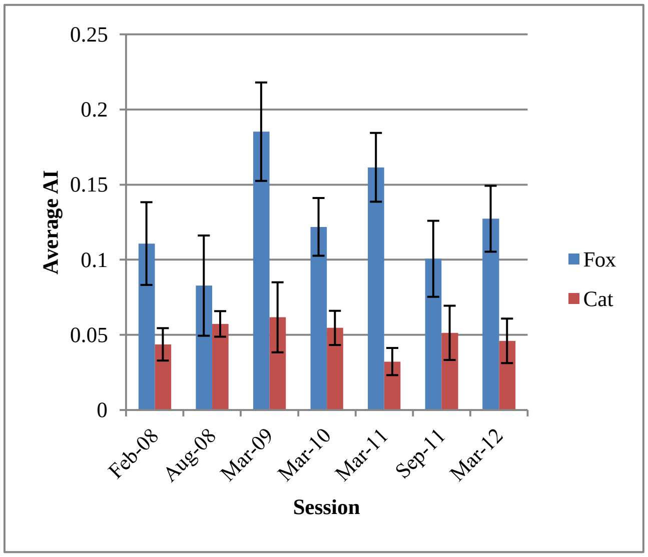

Figure 3.a. Average fox and cat AI by session

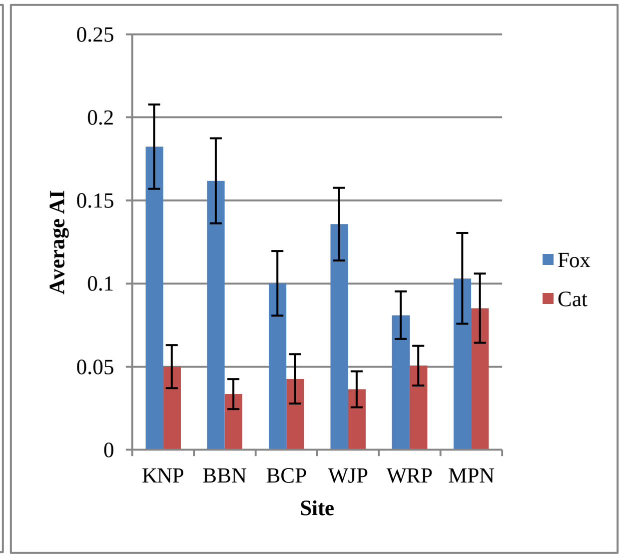

Figure 3.b. Average fox and cat AI by site

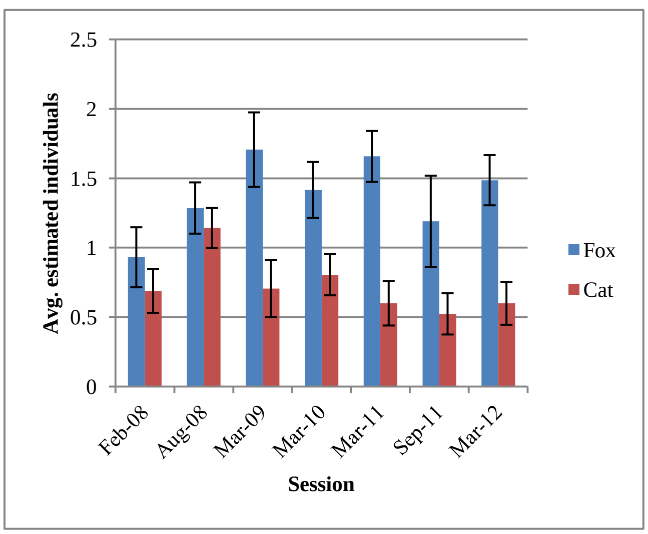

Figure 4.a. Average estimated fox and cat individuals by session

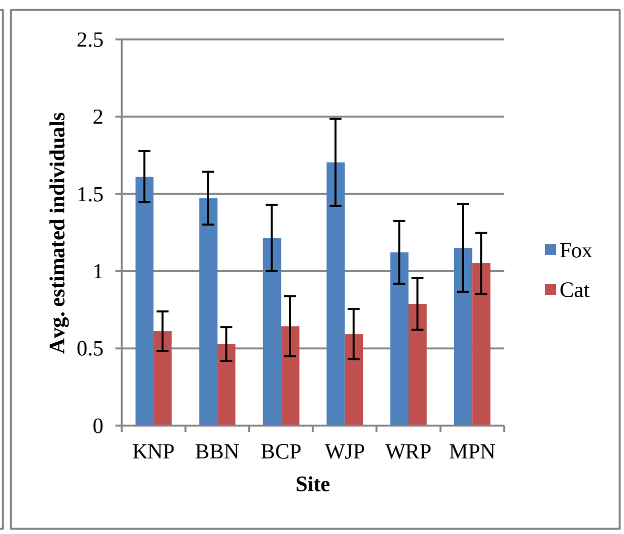

Figure 4.b. Average estimated fox and cat individuals by site

Fits of the linear regressions of AI to estimated individuals were variable between sites. For foxes, the comparison was worst at Keninup (R^2=0.387) and Balban (R^2=0.584) and best at Moopinup (R^2=0.868) and Winnejup (R^2=0.726). Cats, the comparison was worst at Keninup (R^2=0.612) and Moopinup (R^2=0.626) and best at Boyicup (R^2=0.878) and Winnejup (R^2=0.866). It was determined that sites appropriate to attempt to derive a density estimate were Winnejup and Moopinup for foxes and Balban, Boyicup, and Warrup for cats.

Dice's (1938) method was used to estimate density using average home range size. However, no home range studies for predators have been published for the jarrah forest, so a range was used based on previous studies in Australia (foxes: Meek and Saunders 2000, Coman *et al.* 1991, Phillips and Catling 1991; cats: Bengsen *et al.* 2012, Smucker *et al.* 2000, Edwards *et al.* 2001, Langham and Porter 1991) of 1.2-4.0 km^2 for foxes and 1.7-5.0 km^2 for cats. From this range, a fox density of 0.081-0.119 km^{-2} for Winnejup and 0.097-0.140 km^{-2} for Moopinup and a cat density of 0.073-0.107 km^{-2} for Balban, 0.136-0.204 km^{-2} for Boyicup, and 0.019-0.028 km^{-2} for Warrup was calculated. All regressions were calculated with Microsoft Excel 2007.

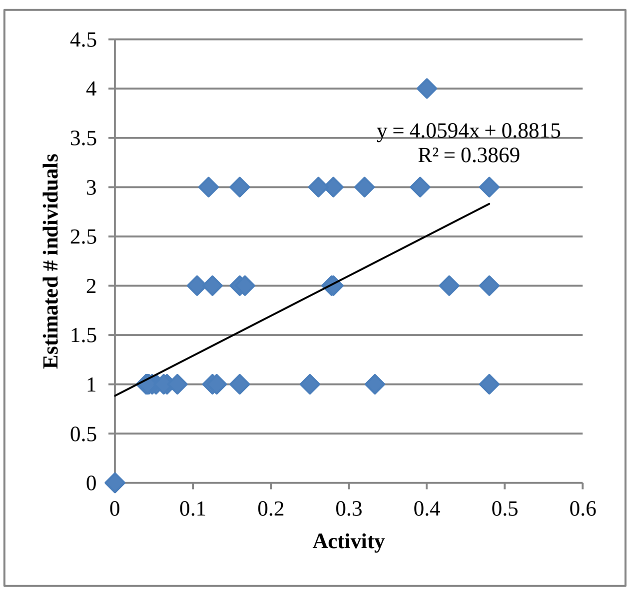

Figure 5.a. Estimated fox individuals versus activity at Keninup across all sessions

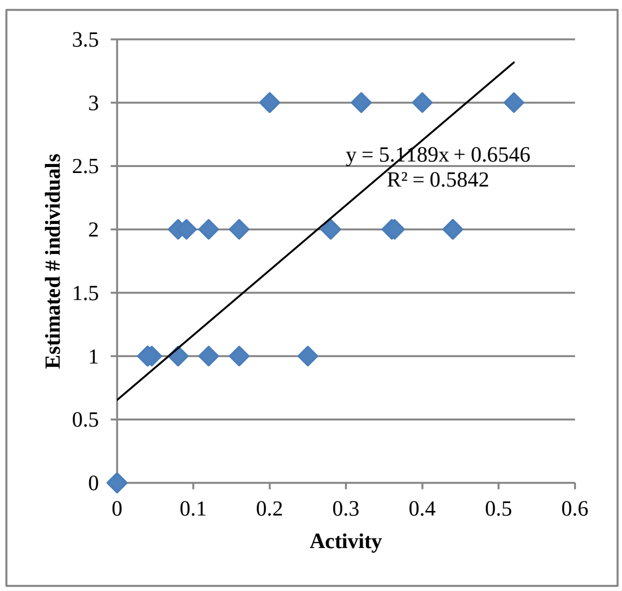

Figure 5.b. Estimated fox individuals versus activity at Balban across all sessions

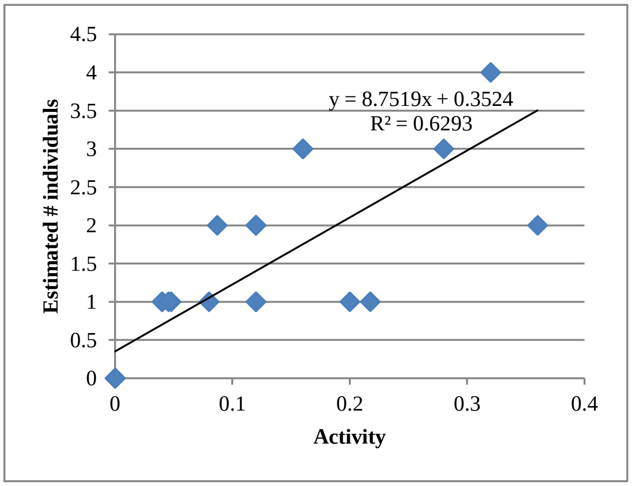

Figure 5.c. Estimated fox individuals versus activity at Boyicup across all sessions

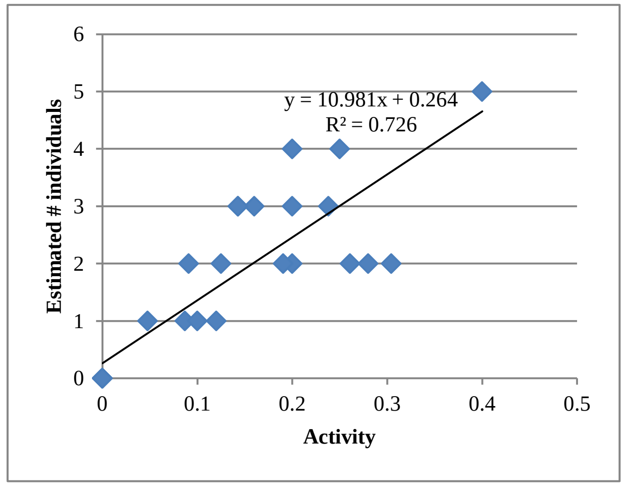

Figure 5.d. Estimated fox individuals versus activity at Winnejup across all sessions

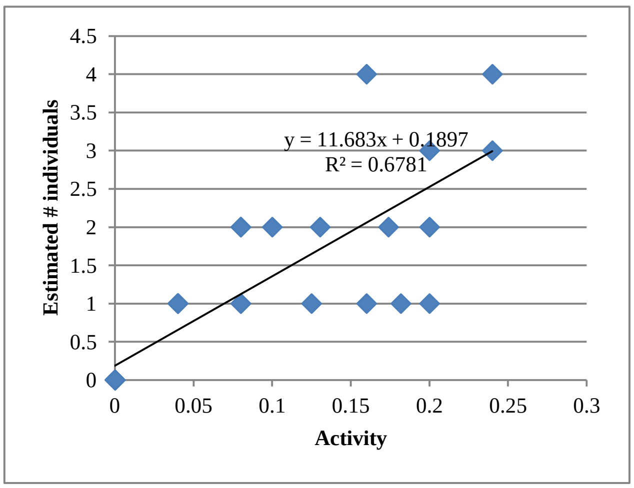

Figure 5.e. Estimated fox individuals versus activity at Warrup across all sessions

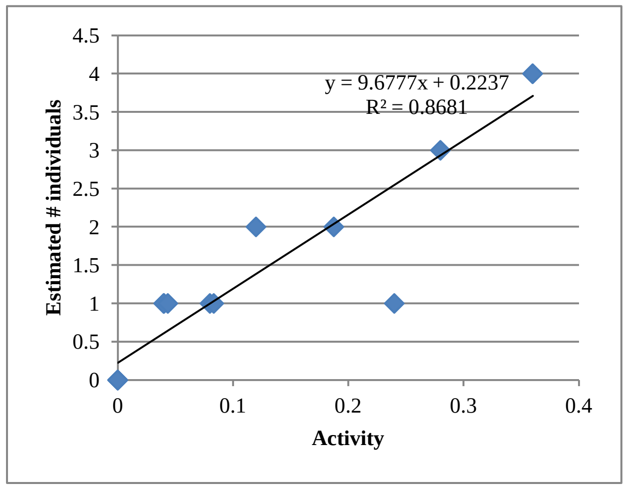

Figure 5.f. Estimated fox individuals versus activity at Moopinup across all sessions

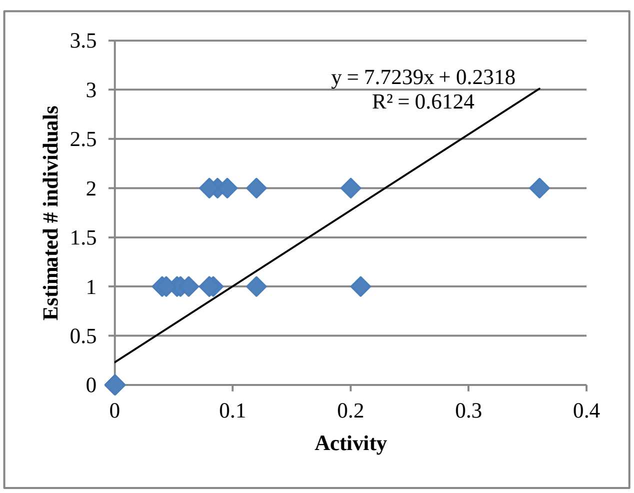

Figure 6.a. Estimated cat individuals versus activity at Keninup across all sessions

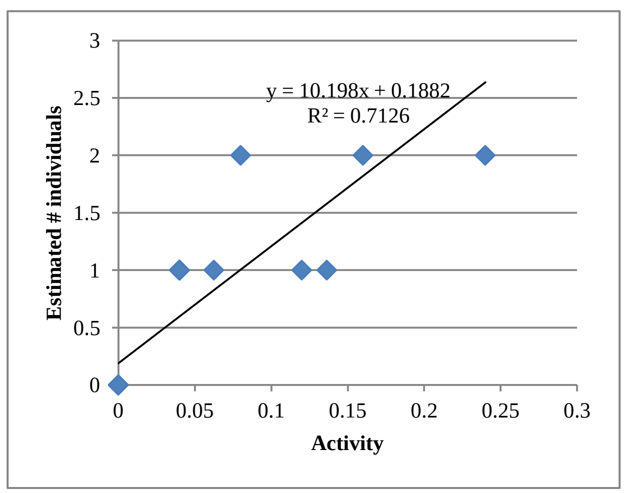

Figure 6.b. Estimated cat individuals versus activity at Balban across all sessions

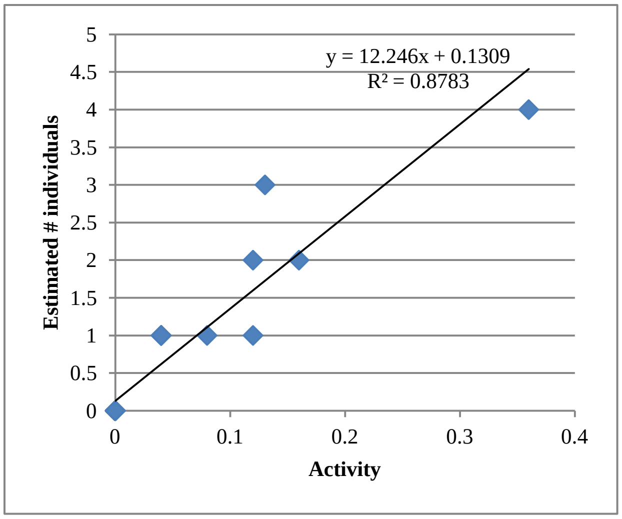

Figure 6.c. Estimated cat individuals versus activity at Boyicup across all sessions

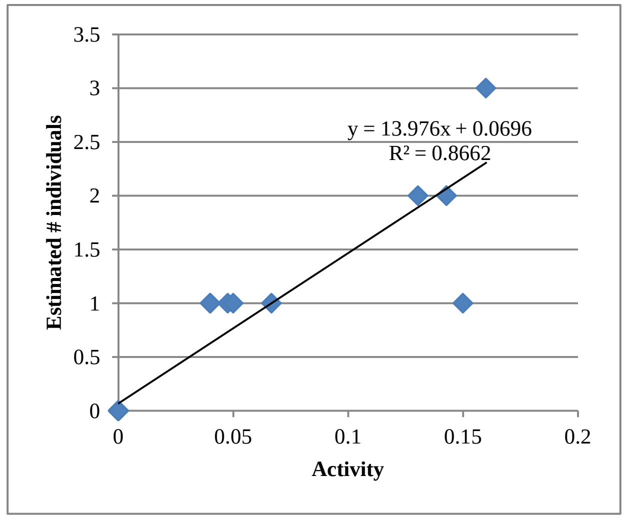

Figure 6.d. Estimated cat individuals versus activity at Winnejup across all sessions

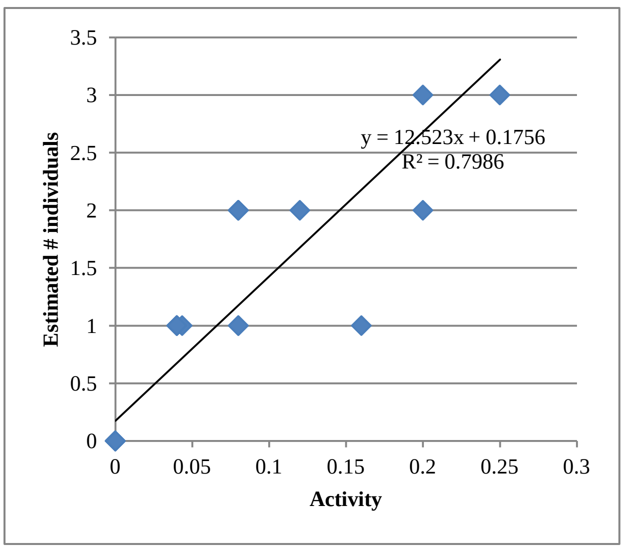

Figure 6.e. Estimated cat individuals versus activity at Warrup across all sessions

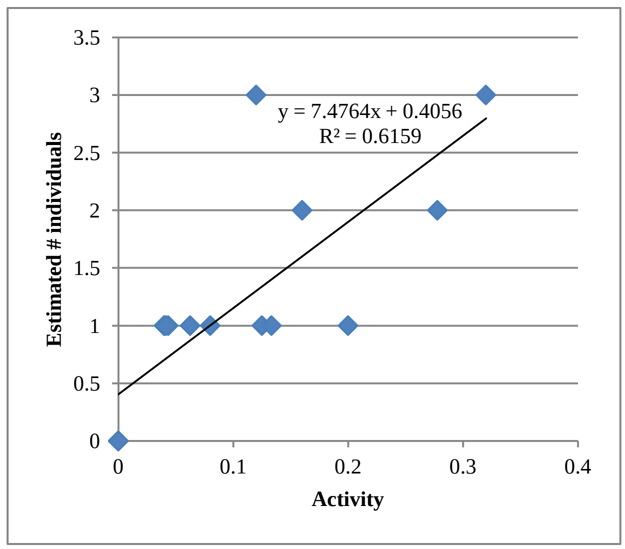

Figure 6.f. Estimated cat individuals versus activity at Moopinup across all sessions

3.2. Cameras

Overall, cameras detected more individual animals than sandpads. Sandpads (that were paired with a camera) detected 10 foxes (76.9% of total), none of which were not also detected by cameras. Cameras detected 13 foxes (100% of total), 3 of which were not also detected by sandpads. Sandpads detected 4 cats (100% of total), one of which was not also detected by a camera. Cameras detected 3 cats (75% of total), none of which were not also detected by sandpads. However, it is likely (though not certain) that the one night in which a cat was detected on the sandpad but not the camera, there had been an error in camera setup.

Figure 7. Example of a fox that was detected by a camera, but not by the sandpad. In this case, the fox was not detected because it did not cross over the pad.

There were also two instances in which two foxes were detected by the camera. In one of these instances, no foxes were detected by the sandpad, and in the other, one fox was detected. However, without any method to identify separate individuals, both instances were recorded as two crossings of one fox.

4. Discussion

4.1. Sandpads

The derived density estimates for both foxes and cats were significantly lower than previous estimates for predator density necessary to account for the recent woylie decline (Wayne *et al.* 2011). This could imply that there are other factors, such as disease, negatively impacting woylie survival. This could also imply that the method used to derive these low estimates requires too great a leap from recorded data to estimate.

Given the discussed limitations of sandpads, it is very likely that numbers of individual have been underestimated due to large amounts of activity leading to many consecutive sandpad detections and fewer individuals estimated. It is also possible that the method for deriving density from number of individuals overestimates catchment area due to the non-grid nature of the sandpad arrays. It is also likely that a six-day survey is not enough time to produce accurate estimates of predator activity. Predator activity varied greatly from day to day.

4.2. Cameras

4.2.1. Effectiveness

The addition of cameras proved an effective way of detecting predators. Once technical issues are resolved, cameras have a better detection ability than sandpads, as they are not limited by wind and rain, vehicles, or space. Identifications even of clear tracks are certain, rather than relying on inferences. Cameras are also effective at differing between individual cats by markings, and, if cameras were placed at every point along an array, individual foxes by direction and timing. They have the ability to detect if two animals are together or interacting. In this study, a fox was captured on camera holding a predated possum in its mouth. Other animal species were captured together on camera as well (possums, woylie and Western grey kangaroo, emus).

The greatest limitations in deriving density estimates from sandpad data alone stem from the fact that sandpads only provide activity levels, rather than individuals. With a grid or array of cameras, individuals, activity, and behavior could all be positively identified.

4.2.2. Efficiency

Cameras for this study required a one-time cost of about $17,000. In the future, that cost would apply to enough cameras to cover each sandpad at a single site. Running costs (not including salaries) for seven days at a single site (including setup) would be about $1,000, assuming cameras were checked every day by one vehicle and about $300, assuming the cameras were only accessed when being set up and taken down, as well as about $1,000 for batteries per session.

Sandpads require a one-time construction cost of about $5,000 and running costs (not including salaries) for a session of about $1,000 per site for vehicles and about $2,500 for sand.

Cameras require a much larger initial cost, and, if paired with sandpads, do not reduce running costs. However, cameras not paired with sandpads require significantly less running costs and will be less expensive and time-consuming in a long-term study.

5. Conclusions

This study adds to the body of work involved in ultimately determining the impact of feral animals on native Australian ecosystems. The findings above imply that density estimates based on sandpad data alone may not be sound enough to inform management decisions. However, the addition of remote sensor cameras may be a more effective and more efficient means of deriving these estimates.

The efficiency and cost comparison of cameras to other, previously mentioned methods of estimating density will be relatively similar to the comparison to sandpads. Sandpads are the most effective, practical method in the jarrah forest for calculating a relative activity index, but they should be used in an IMI study in order to determine density. Trapping, shooting, and poisoning is more difficult in the jarrah forest (especially for cats) and is very labor-intensive, but it produces a good absolute abundance estimate.DNA CMR is expensive and labor-intensive, but is one of only a few methods that can be effective for cats. Further studies should test the utilization of cameras in producing an independent density estimate, perhaps paired with another method of density-estimation. Based on the results of this study, cameras should be effective to this end.

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Balban Predator control program

October 2012

Thank you for the opportunity; it was a pleasure to be involved in such a challenging exercise. A quick thanks to all DEC staff, Warren Catchment Council and the Local Farming Community for all the support given. It really was a terrific environment to work in.

Issues that could have influenced the results:

- More time to plan the logistics
.
- The first two weeks were heavily weather affected.
- The vixens were denned up with cubs.
- Movement of DEC staff through the core area (collecting sand pad data for the duration of the trapping program).
    - When foxes are marking trap sites with faeces, as they were marking sand pads, it is generally a sign that the animal is suspicious of the site and can be difficult to trap.
- No control of track access/ option of shutting tracks to vehicle traffic as needed.
- Unnecessary access, student groups etc. compromising trap locations.

Techniques and strategies that could be modified to improve the integrity of the program if it is repeated:

- Flexibility of trapping period, to minimise the impact of extended rain events.
- Shut down sand pad data collection throughout the trapping period to lessen the human impact on fox behaviour.
- Allow contractors to control track access as needed
.
- Maintain stricter protocols with the baiting program.
    - Ensure any uneaten baits are removed from the site to reduce the risk of foxes receiving sub lethal bait.
    - All foxes were extremely cautious and wouldn't come to food lure and were hard to trap.
- Remove the need to put traps in areas with refuge.
    - Refuge creates an opportunity to entangle the trap, chain, swivels and fox in vegetation.
    - This can cause injury to the animal. See image 1.
    - It would be more prudent to ensure traps are checked within a reasonable time frame to manage the animals exposure to extreme weather.

 Image 1

- Change lure medium (maybe use liquid or tethered baits)

  - Maintain a viable trap site and combat non-target animals removing lures and baits.

  - At one point during the trapping 70% of lures were being taken by non-target animals each night. See image 2.

 Image 2

- Access data on cat and fox movement either by DEC's cameras or contractor to install own camera monitoring sites before the trapping commences.

- Use of night vision to target hard to trap animals.

- No rubber jawed traps to be used.

  - Highly modified offset laminate traps suited to the off-target conditions are the preferred choice. See image 3 for example offset and laminated trap.

 image 3

- See image 4 for example of trap jaws closed with hair of non-target animal

 Image 4.

- Images 5 showing off target capture having pulled out of trap without creating a catch circle.
  - This indicates the animal was not held in the trap for any length of time.
  - It is very likely that another style of trap would have resulted in the native animal being captured, injured and destroyed.


Image 5

- Extend the program over five weeks with a week off in the middle.

- Take advantage of new technologies, M-44 ejectors.

  - For example - limit the trapping effort to 6000 ha and deploy M-44 ejectors with cyanide throughout the remainder of the 30,000 ha.

- DEC not to use inexperienced trappers unfamiliar with trapping amongst the specific off target animals.

    - Poor trap selection, trap location, set design, trap set up etc. can result in large numbers of off-target animals being captured and injured.

    - This not only compromises the native animals throughout the program but may jeopardise future control programs or scientific effort.

    - See image 5 for possum released after capture in foothold trap.

    - The release was due to a well set up trap capturing the animal in a manner that the possum could be released unharmed.


image 6

Effort and Results

Spotlighting

39 hours spotlight surveying for foxes

5 fox sightings

2 on Hazled's Property

3 on Lloyd's

2 individual animals?

2 shots taken, 1 on Hazled's property 1 on Lloyds

1 fox shot, 4th October, Lloyds

Foothold Trapping

669 trap nights with foot hold traps.

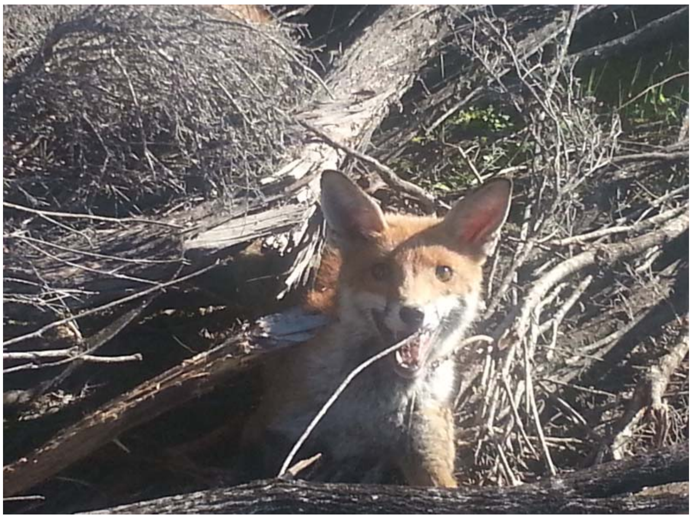
Foothold traps were set from the 17th of September until the 16th of October in 33 locations.

217 hours searching for fox and cat sign, setting traps, maintaining trap sites etc.

Four foxes captured and dispatched,

- One fox captured using food lure .
- One fox captured with curiosity/food lure.
- One fox trapped with passive/blind set.
- One fox caught with social scent.

1st October, trap 18



9th October, trap 17

11th October, trap 11

14th October, trap 23

Off target animals

2 tammar wallabies captured, dispatched

3 possums captured, released

1 echidna captured, released

Cage Traps

50 cage trap nights

2 chudich, released

1 Dickenson's property

1 Askew's property

3 possums, released

1 Dickenson’s property

2 Simcock’s property

Native sightings

1 numbat

3 woylies

1 brush tail phascogale

GPS locations of sightings

Numbat

-34 6.632 116 33.438

Woylie 1

34 6.035 116 30.263

Woylie 2

34 6.617 116 31.959

Woylie 3

34 11.8 116 35.375

1 brush tail phascogale

34 6.933 116 31.87

Wild Things
ANIMAL CONTROL SOLUTIONS

Boyicup predator control program

Feb-Mar 2013

Thank you for the opportunity; it was a pleasure to be involved in such a challenging exercise. Thanks to all DEC staff, Warren Catchment Council and the Local Farming Community for all the support given. It is a terrific environment to work in, and the cooperation between parties should be commended.

Effort and Results

The program was a joint initiative between DEC and the Warren Catchment Council and the works were conducted on both DEC and private property. The control techniques were chosen at each site based on a risk assessment. The hazards being injury or death to people, domestic animals and native animals and the risk being likelihood of each consequence occurring.

All spotlight shooting was carried out in open paddocks on private property. Private property allows better control of both authorised and unauthorised access.

Foxes and cats could be spotted and shot whilst in open ground, in a forested environment both cats and foxes will utilise cover and observe the spotlight and vehicle without being detected.

Foothold trapping was conducted on both DEC estate and private property. All though it should be noted, that six foothold traps were set on one farm for one night only. On the last night of the program Murray Muir invited Gavern to inspect the cage trap he had set and the fox tracks surrounding the cage. On inspection the tracks were deemed to be feral cat. It was mentioned that the trap had been set off and rolled over on one occasion. Foothold traps were deployed immediately, but neither feral cat nor fox, visited the area that night. Unfortunately, in this instance the traps were originally loaned to Rod Muir who was shown how to set them, but the traps were then subsequently passed onto Murray Muir. Hence, there was not the opportunity to ensure that the trap was set effectively. There also would have been a much better opportunity to catch the cat if Wildthings had been made aware of the fresh cat activity sooner. As a future recommendation, the Landholder Information night would also have been a good opportunity to demonstrate the effective setting of cage traps for landholders.

Only cage traps were used by land holders around dwellings and where feral cats were likely to reside. Cage traps take considerably less skill to set than leghold traps and reduce the potential risk of injuring possums and domestic animals.

Three cage traps were set at the Tone Perup Bridge for three nights to attempt to capture a cat scavenging off the bins. On the fourth night the parking area was occupied by campers so the trapping was aborted.

Wildthings effort would be most accurately divided between DEC and Landholders as;

DEC - 150 hours spent trapping for 403 trap nights

Landholders – 41 hours spotlight searching and 6 hours trapping for 6 trap nights

Spotlighting

- 41 hours searching
- No foxes sighted
- One cat sighted
- One cat shot (Photo not taken in the field)

Foothold Trapping

- 409 foot hold trap nights were set between the 19th Feb to 10th Mar in 36 locations.
- 34 offset laminated Bridge traps #2 sized and two rubber jawed 1.5 sized traps used
- A total of 156 hours was spent searching for fox and cat sign, setting traps, maintaining trap sites etc.

Four foxes captured and dispatched,

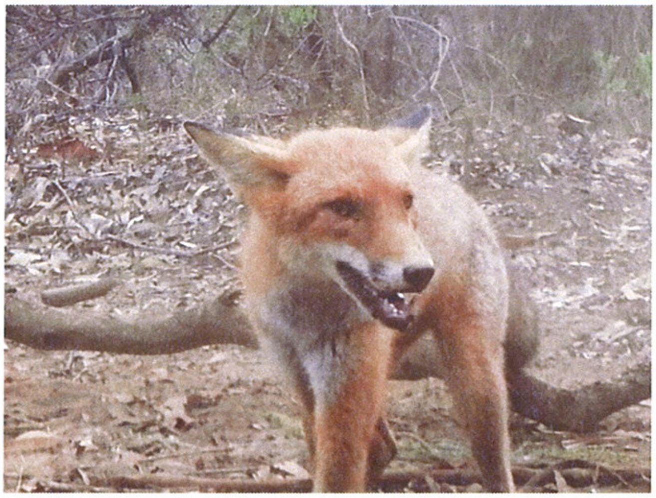
- One on a remade set that had previously captured a tammar.
- One on a remade set that had previously captured both tammar and fox.
- One on a food lure.
- One with a passive/blind trap set at the same site as the fox was caught on the food lure.

The estimation at the end of the program is two foxes remaining; one appeared on the river every 5- 7 days and one on Glendale Rd near De Landgrafft Rd of which tracks were sighted only twice during the program.

27th February, trap 7

28th February, trap 19

3rd March, trap 19



7th March, trap 7

Cage Traps

Three cages were set at Tone Perup Bridge for three consecutive nights.

Two traps were loaned to land holder Rod Muir and subsequently reloaned to Jan and Murray Muir.

Feral cats

One feral cat was shot throughout the program.

Cats were hard to monitor without camera technology due to the soil medium (mostly gravel). Cats generally don't leave much of an imprint on the soil but on a gravel track or road it is almost impossible to find sign consistently.

DEC allowed WildThings to access camera data throughout the program. This led Wildthings to set four traps to target photographed cats in locations that would not have been set without the data. Unfortunately, unlike the cats in the previous Balban program, the Boyicup cats showed no regular patterns in road usage and infrequently returned to these sites if at all. If a pattern had been detected from the cameras then this information would have been used to target passive placement of traps on roads, as was conducted at Balban. The camera data did not influence the placement of traps for foxes, again as a regular pattern was not detected from the cameras.

Feral cats visited traps (within 30 cm) on three known occasions.

- On the first occasion a cat stepped on the back of the pan which meant that the pressure needed to fire the trap was increased and subsequently the trap didn't trigger. See photos below of this trap location.


Cat tracks at trap site


The site as first found


The site set with trap

- On the second instance, the set was arranged for the animal to walk up to the lure, investigate and walk onto the trap (this arrangement is one of the methods used to deter non target captures, since the lure is some distance from the trap), the cat investigated the lure and for reasons unknown, then backed out of the set, which was unusual.
- The third instance was at site seven which was a remade set that had captured a Tammar and fox. As the set had been remade, there was a less defined focal point, the cat walked over the trap circle and onto the trap but didn't step on the pan.

Whilst no cats have been captured live on either program, Balban or Boyicup, there have been enough visits and near captures to prove that cats can be lured to a trap in the southern forests.

Non targets

Over the four hundred trap nights five non-target animals were captured. Three were released unharmed, one sheep and two brushtail possums. A woylie was euthanized and a tammar was found deceased in the trap due to predation by fox, this animal however would have required euthanizing as a result of trap injuries. These were unfortunate deaths and were not taken lightly, however when setting foothold traps in the presence of these animals there will always be an inherent risk of capturing and injuring an animal. These risks need to be acknowledged. On two occasions, where native animals were captured, one brushtail possum and one chuditch, the offset jaws allowed the animals to pull free.

Possum fur in a trap where the possum was captured and pulled free

Trapped sheep, released unharmed

Issues that could have influenced the results:

- The animals didn't appear to have a steady rhythm or use any particular roads on a nightly basis
    - Since one of the techniques used to reduce non-target capture is to only trap on regular sign this made choosing trap sites difficult. This also reduced the total number of trap nights and lessened the exposure of predators to traps.

- Roads that were cleared by hand were a potential tyre staking hazard to a quad bike
    - If a machine was used to clear the road this would have had a twofold effect of making those tracks more accessible and therefore a potential trap site and would have brought those roads up to a point where they could be read for tracks.

- Moon phase
    - The program was undertaken without considering the moon phase and its effect on animal movements and behaviour.
    - The full moon is generally the hardest time to trap and shoot cats and foxes, ideally the program should be scheduled to take advantage of the new moon period.

- All animals were shy of lures
    - It is hard to qualify why this is, it could be the excess of live food, a bad experience with sub lethal baits or another negative experience. I have seen this behaviour in areas with high food resources that is heavily baited.
    - This led to trap sites being set with more than one trap and exposing natives to more risk, without damage on those occasions.
    - This also meant that some traps sites were set passively, without lures, which captured one possum (released without harm).

- Non targets
    - Since the area is inhabited by species that are protected and in decline, a considerable effort was needed to reduce the impact of the control program on those species.

- This lessened the number of traps deployed, and reduced the exposure of predators to traps
- The setup of the traps reduces the likelihood of catching non target animals, but this setup will make the area the animal needs to step on smaller. On three occasions (one cat and two foxes) stepped on the back of the pan but didn't produce enough down force to fire the trap.
- Experience from the previous program dictated that the lure selection to exclude solid food and some visual lures.
- The native animals were less attracted to the lures used than previous food lures.

Potential improvements to the next project:

- Land holders
  - While most landholders were supportive and cooperative, some were less so. This should be qualified earlier.
  - A higher priority should be given to the landholder information night; this is the best opportunity to build rapport and enthusiasm for the project.
  - The list of land holders should be completed and be ready for the contractor before the program starts.
  - All external groups need to be informed of control activities. In this case, the feral pig eradication group was checking water holes with dogs within the control area on at least one occasion. Whilst the dogs were on the back of a ute, the ute was parked within meters of a trap site.

- Move trial to a location where tracks are easily identified.
  - It was difficult to read tracks in a large part of the study site at Boyicup due to the gravely and rocky substrate.

  - Maintain stricter protocols with the baiting program.
    - All foxes were extremely cautious and wouldn't come to food lure and were hard to trap. The removal of any uneaten, partially consumed baits from the area, whilst impractical on such a large scale, may reduce the risk of foxes receiving sub lethal 1080 dosages and hence the potential for wariness to meat lures.

  - Take advantage of new technologies, M-44 ejectors when available.
    - For example - limit the trapping effort to 6000 ha and deploy M-44 ejectors with cyanide throughout the remainder of the 30,000 ha.

  - More time to organise logistics.
    - The traps and supplies for this job are extremely specialised and need to be manufactured and imported specifically for these conditions, the successful applicant would need at least four weeks to allow for manufacture and transport issues.

Finally, how could we best maximise the opportunity to successfully capture a cat in the southern forests;

- Choose sites with sandy tracks.
- Select sites with areas of dense vegetation, whereby allowing for more effective targeting of predators compared to open forest.
- Utilise cameras to scope large areas to select the control site.
- Place cameras at sites and process the data well in advance of control. This would allow for targeting cats with regular visitation patterns.
- Allow for greater scoping of the chosen site for signs prior to commencement of the control.
- Select sites with the lowest non-target densities.
- Select control site with greatest number of resident cats.
- Upgrade tracks with a machine rather than by hand to improve readability of the ground for tracks (as well as for ease of travel with a quad bike).
- Trial new trap types to reduce the risk of trap injury to non-targets allowing for greater trap saturation of the area.
- Greater landholder trapping effort. Encourage coordinated trapping with landholders and provide training.
- Target landholders rubbish tips with cage traps.
- Provide education to landholders about the impacts on fauna from cats on their properties (i.e. shed cats).

GPS locations of native fauna sightings (lat/long).

Numbat-
34 6.632 116 33.438

Woylie
34 6.035 116 30.263

Woylie
34 6.617 116 31.959

Woylie
34 11.8 116 35.375

Phascogale
34 6.933 116 31.87

Assessment of introduced predator presence within the Perup Sanctuary, Western Australia

N. Hamilton and J. Rolfe

Department of Environment and Conservation, Science Division, P.O. Box 51 Wanneroo, Western Australia 6946

Plate 1. Entry gates to the 400 ha introduced predator-proof enclosure.

Recommended citation: Hamilton, N. and Rolfe, J. (2011). Assessment of introduced predator presence within the Perup Sanctuary. Unpublished Report, Western Australian Department of Environment and Conservation.

Abstract

The Upper Warren Region is home of one of the last remaining Woylie populations in the south west of Western Australia. To ensure the successful breeding and recovery of Woylies, a 400 ha ‘introduced predator-proof’ enclosure has recently been constructed. The aim of this work was to quantify and eradicate feral cats and foxes in this enclosure, prior to the introduction of Woylies. This program was conducted following the removal of a number of native species from within the area.

After an extensive trapping exercise and track surveys, which included monitoring sand plots and deployment of remote cameras for seven days, no sign of either foxes or feral cats was found. Seven Brushtail Possums and one Australian Raven were captured in leg-hold traps and released unharmed outside the enclosure. The sand plots, remote cameras and opportunistic sightings indicated the presence of a number of native animals still within the enclosure and included: Chuditch, Brushtail Possums, Tammar Wallabies, one Woylie and Monitor lizards.

Introduction

The Woylie (*Bettongia penicillata*) has declined by more than 80% since 2001 (Orell 2009). The largest and most important populations have generally been the most affected, each experiencing greater than 93% losses within two to five years with few or no signs of a subsequent recovery. There is now less than an estimated 1300 individuals remaining within the last four indigenous populations (Perup, Greater Kingston, Dryandra and Tutanning) (op. cit.). In an attempt to establish and secure ‘insurance’ populations one of several decisions was to re-introduce a minimum of 40 Woylies into a 400 ha introduced ‘predator-proof’ enclosure at Perup (the Perup Sanctuary) (Wayne 2010).

Before any Woylie translocations into the enclosure could be undertaken, it was critical that any introduced predators were removed. The timing of the translocation was dependent on the confirmation of the Perup Sanctuary’s ‘introduced predator-free’ status, but was planned to be undertaken between September and December of 2010 (Wayne 2010). This report summarises the program of monitoring for presence and removal of these predators from what can be considered a ‘fenced island’.

Material and Methods

Study site

Perup Nature Reserve (34^{0} 10.515’ S, 116^{0} 34.279’ E) is located 50 km to the north-east of the town of Manjimup in the south west of Western Australia. It is part of the Upper Warren region, lying between the Perup and Tone Rivers. The region experiences a moderate Mediterranean-type climate with warm dry summers and cool wet winters. It supports dry sclerophyll forest and woodland dominated by jarrah (*Euclyptus marginate*), marri (*Corymbia calophylla*) and wandoo (*E. wandoo*) at canopy level. Common understorey species include *Leucopogon*, *Hakea, Acacia*, *Bossiaea*, *Macrozamia, Gastrolobium*, *Xanthorrhoea, Hibbertia*, *Pteridium* and *Billardiera* spp (Strelein 1988). Average annual rainfall varies along a north-south gradient of increasing rainfall but is about 700 mm, mostly falling between May and August (Burrows and Christensen 2002).

Trapping exercise

The trapping technique utilizes padded leg-hold traps, Victor ‘Soft Catch’® traps No. 3 (Woodstream Corp., Lititz, Pa.; U.S.A.), a Felid Attracting Phonic (FAP) that produces a sound of a cat call, and a blended mixture of cat faeces and urine (Pongo) (for details see Algar *et al*. 2010). Sixteen leg-hold traps were set around the inside fence area at 500 m intervals, eight of these with FAPs (alternate traps) and all with pongo. In addition, seven leg hold traps were set on the internal tracks; all with a FAP and pongo (see Figure 1). Four Sheffield cage traps baited with apple and carrot as lure were set to try to remove remaining Brushtail Possums (*Trichosurus vulpecula*) from the enclosure. The trap locations were recorded on a GPS and are provided in Figure 1. Traps were checked twice daily due to weather conditions during the monitoring process (see below).

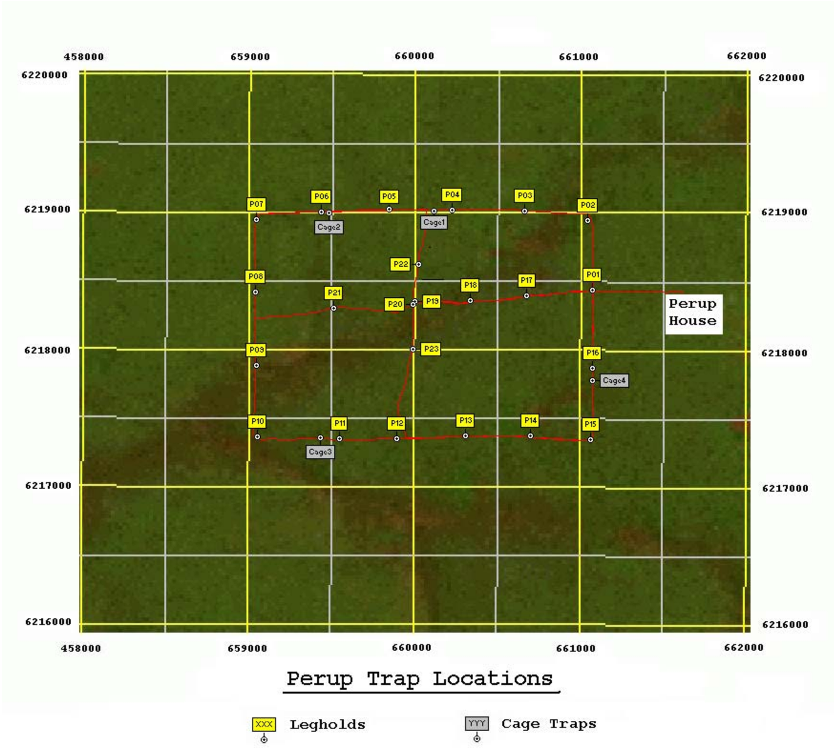


Figure 1. Leg-hold and cage trap locations at the Perup Woylie Enclosure

Monitoring

The enclosure was examined twice daily (06:00 h and 16:00 h) for feral cat and fox activity by walking the internal road network (8 km). This monitoring technique was used to avoid spreading dieback by vehicle as well as it increased the possibility of observing any introduced and native animal activity. Furthermore, 20 sand plots with Pongo as an attractant were established along the road transects between the trapping transects (see Figure 2) and monitored daily over a six day period. These plots were used to identify feral cat and fox activity as well as that of native animals. Sand was also placed around the two artificial watering points within the enclosure to monitor animal activity. Five remote sensor cameras (Reconyx) were set up over seven days at each corner of the enclosure and one at a water point (WP2 on Figure 2). The photographs were downloaded and analysed every few days.

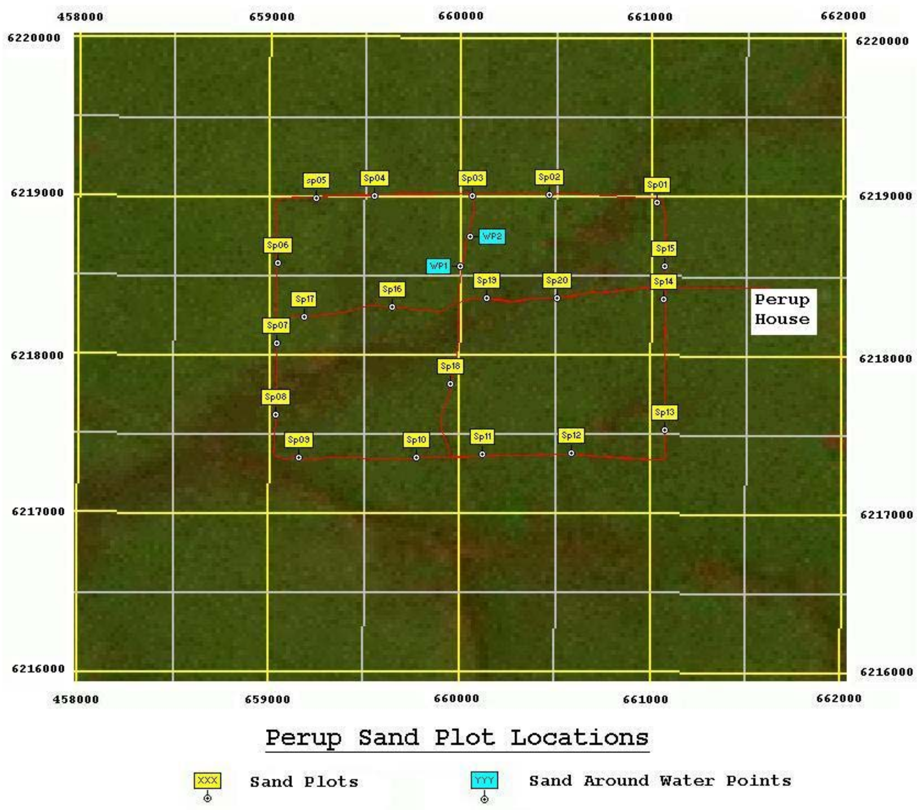


Figure 2. Sand plots and water point locations within the Perup Woylie Enclosure

Results

During the course of this exercise, no sign of feral cat or fox activity was observed within the enclosure. Over ten nights of trapping, seven Brushtail Possums and one Australian Raven (*Corvus coronoides*) were caught in leg-hold traps without any injuries and released outside the enclosure. No animals were captured in the cage traps.

During the monitoring of the road network, one Brush Wallaby (*Macropus irma*), two Tammar Wallabies (*M. eugenii*) and one Woylie were sighted. The sand plot monitoring indicated the presence of a number of native species: - Brush Wallaby, Chuditch (*Dasyurus geoffroii*), Tamar Wallaby, Brushtail Possum, Southern Brown Bandicoot (*Isoodon obesulus*), Heath Monitor (*Varanus rosenbergi*.) and various bird species. The remote cameras captured the presence of one Chuditch, one Brushtail Possum and one Australian Raven.

Discussion

After an extensive trapping exercise and track surveys, which included monitoring sand plots and deployment of remote cameras no evidence of feral cat and fox activity was detected suggesting these introduced predators were not present within the enclosure. Any feral cats and foxes present in the area may have moved out prior to the fence closure, during the *battue* conducted several weeks prior to this program. During the final stages of fence construction, following the *battue*, no evidence of fox or feral cat activity was observed in the area by operation staff (B. Whittred pers. com.).

To ensure that the fenced area remains 'introduced predator-free', a dedicated monitoring effort will need to be undertaken. Whether the fence construction provides an effective, long-term barrier to fox and feral cat movement can only be determined if routine surveys for footprints are conducted within the fenced area and in particular along the fence-line. The use of remote cameras along the fence-lines should also be considered.

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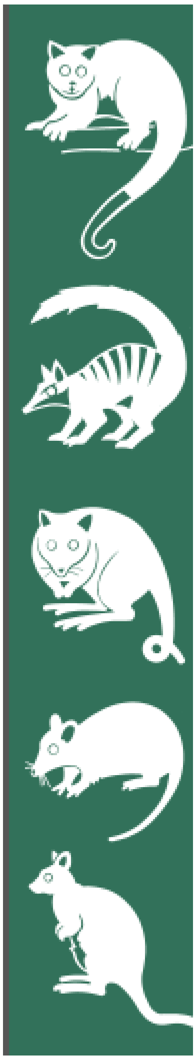
STRATEGIC DESTINATION MANAGEMENT PLAN

2012 - 2016



Perup - Nature's Guesthouse

TOU303
Tourism Management
Supervisor: Dave Cooper

Department of
Environment and Conservation

PERUP - NATURE'S GUESTHOUSE

ECOTOURISM DESTINATION

Strategic Destination Management Plan 2012-2016

Prepared by:

Elysia Harradine
Liz White
Anette Madsen
Sean McMahon
Kasia Borkowski
Elise Pinto

Developed for:

Department of Environment and Conservation

Warren Catchments

…Where nature welcomes <u>you</u> to its backyard.

OUR VISION

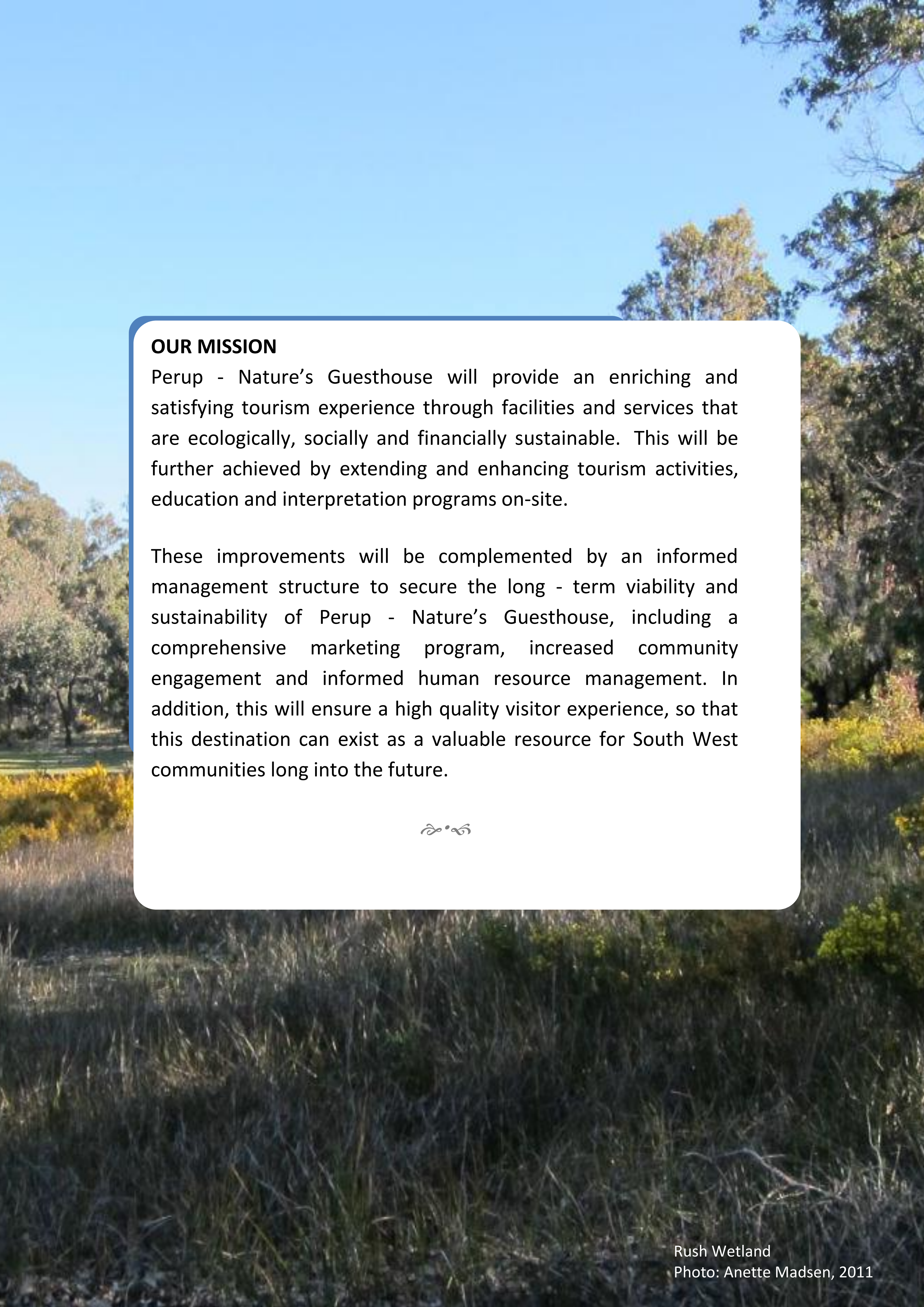
To provide a unique and sustainable forest experience to visitors of the Perup region in the South West by offering an invaluable eco-education and eco-tourism experience.

The Jarrah Forest
Photo: Anette Madsen, 2011

OUR MISSION

Perup - Nature's Guesthouse will provide an enriching and satisfying tourism experience through facilities and services that are ecologically, socially and financially sustainable. This will be further achieved by extending and enhancing tourism activities, education and interpretation programs on-site.

These improvements will be complemented by an informed management structure to secure the long - term viability and sustainability of Perup - Nature's Guesthouse, including a comprehensive marketing program, increased community engagement and informed human resource management. In addition, this will ensure a high quality visitor experience, so that this destination can exist as a valuable resource for South West communities long into the future.



Rush Wetland
Photo: Anette Madsen, 2011

DISCLAIMER

Implementation of recommendations made by the project team and overall destination management is at the discretion of the DEC and other stakeholders of Perup - Nature's Guesthouse. Destination planning is limited to the progress of each planning stage and its success rate. Implementation of the strategic destination management plan is to be carried out over a five year period that incorporates continual review for each stage of the development process.

Table of Contents

Brush-tailed Possum
Photo: Elysia Harradine, 2011

Acknowledgements

We would like to acknowledge the following people who have made it possible to produce this report, in particular:

Bev Gardiner

Kathy Dawson

Dave Cooper

Peter Clay

and everyone not acknowledged by name that helped in one form or another;

Thank you.

The Perup Region Strategic Planning Group

Executive Summary

While Perup - Nature's Guesthouse (PNGH) provides a number of basic facilities and services to visitors, the destination has yet to develop an 'identity'. As a result, PNGH has not yet reached its full potential. The following management plan provides information and strategic recommendations for perusal by PNGH stakeholders. Over the next five years, it is anticipated that the strategies identified in the strategic management plan will encourage an increased environmental awareness in visitors, whilst developing the business into a sustainable eco-tourism destination.

Destination Goals

1. Increase the financial viability of Perup - Nature's Guesthouse so that it is sustainable, offsetting running costs and assisting in future sustainable development and conservation efforts in the Perup region.
2. Improve and expand the eco-tourism product along with the identity and services of Perup - Nature's Guesthouse through a variety of media.
3. To become an established facility for eco-educational experiences for all interest groups and tourists who visit the Perup - Nature's Guest House.
4. Expanding the tourism product by providing activities that will encourage increased environmental awareness and enrich the nature-based and ecotourism tourism experience.
5. Improve infrastructure, services, technology and access to allow for increased visitation to PNGH whilst remaining environmentally, economically and socio-culturally sustainable.
6. Develop and maintain strong relationships with local and regional businesses, tourism operators and the non-business community, such as schools, interest groups and residents. Engage the local community and develop partnerships with local and regional businesses to expand knowledge of the destination and encourage funding and sponsorship.
7. Ensure sufficient human resources for site planning and management, to ensure a positive experience for visitors.

Key Strategic Issues

Several key strategic priorities, as identified from the goal set, have been selected to enhance the identity and management of Perup - Nature's Guesthouse over a 5 year period. It is anticipated that the running costs of the facility will be offset as a result of the recommendations being implemented in this plan.

Priority areas include:

- Financial Viability
- Marketing
- Education and Interpretation
- Attractions and Experiences
- Facilities, Services and Visitor Management
- Community Engagement
- Resource Management

1. Introduction

1.1 Destination Background

Perup - Nature's Guesthouse is situated within the South West region of Western Australia, 267km south of Perth (See Figure 1) and is managed by the Department of Environment and Conservation (DEC). The region encompasses a number of nature reserves and State Forest, including the Tone-Perup Nature Reserve and Perup Sanctuary; all of which have significant environmental and historic value (Wayne and Moore, 2011). The Guesthouse has the potential to serve as an access point for visitors to experience and learn about the culture and nature of Western Australia's South West.

1.2 Historical Context

The initial function of PNGH was to provide a base for conservation research of the flora and fauna in the surrounding Jarrah forest . In the 1980s, nature-based tourism activities were introduced at PNGH. As a result, basic housing was installed to accommodate visitors who participated in conservation activities such as spotlighting, radio tracking, and trapping of fauna species . In addition, two commercial tour operators (CTOs) began visiting PNGH, and schools and universities started to use the site for field- based education activities . The site was redeveloped in 1993 and 2003 to update PNGH's facilities to those seen today.

1.3 Current Tourism and Use

PNGH provides the opportunity for guests to experience the Jarrah forest through recreational walking trails, night spotlighting walks and trapping tours . Visitors at the site also have the opportunity to spot a range of wildlife around the facilities. The site has a range of accommodation types, and a classroom which is available for group activities. For a detailed list of the facilities available at PNGH, please refer to Appendix 1.

However, PNGH has low visitation rates, and is no longer used by CTOs. This may be a consequence of

- The isolation of the destination,
- Limited available tourist activities,
- Limited facilities,
- Little/ no marketing, or
- Competition from nearby tourist regions.

Photo: Lee Griffith, 2011

1.4 Future Direction

Perup - Nature's Guesthouse provides a valuable education service to both the local and wider community, and allows visitors to enjoy and experience the surrounding forest environment. It is also a resource for tourism activity, and promotion of this facility could enhance tourism throughout the South West and the Great Southern region. Managed tourism growth may also be required in order for the Guesthouse to facilitate continued conservation research. Ensuring the viability and sustainability of Nature's Guesthouse into the future will secure a valuable resource for the community and the state. This plan suggests strategic actions in order to remediate current problems that may come between PNGH and long-term viability.

Photo: Elise Pinto, 2011

1. Introduction

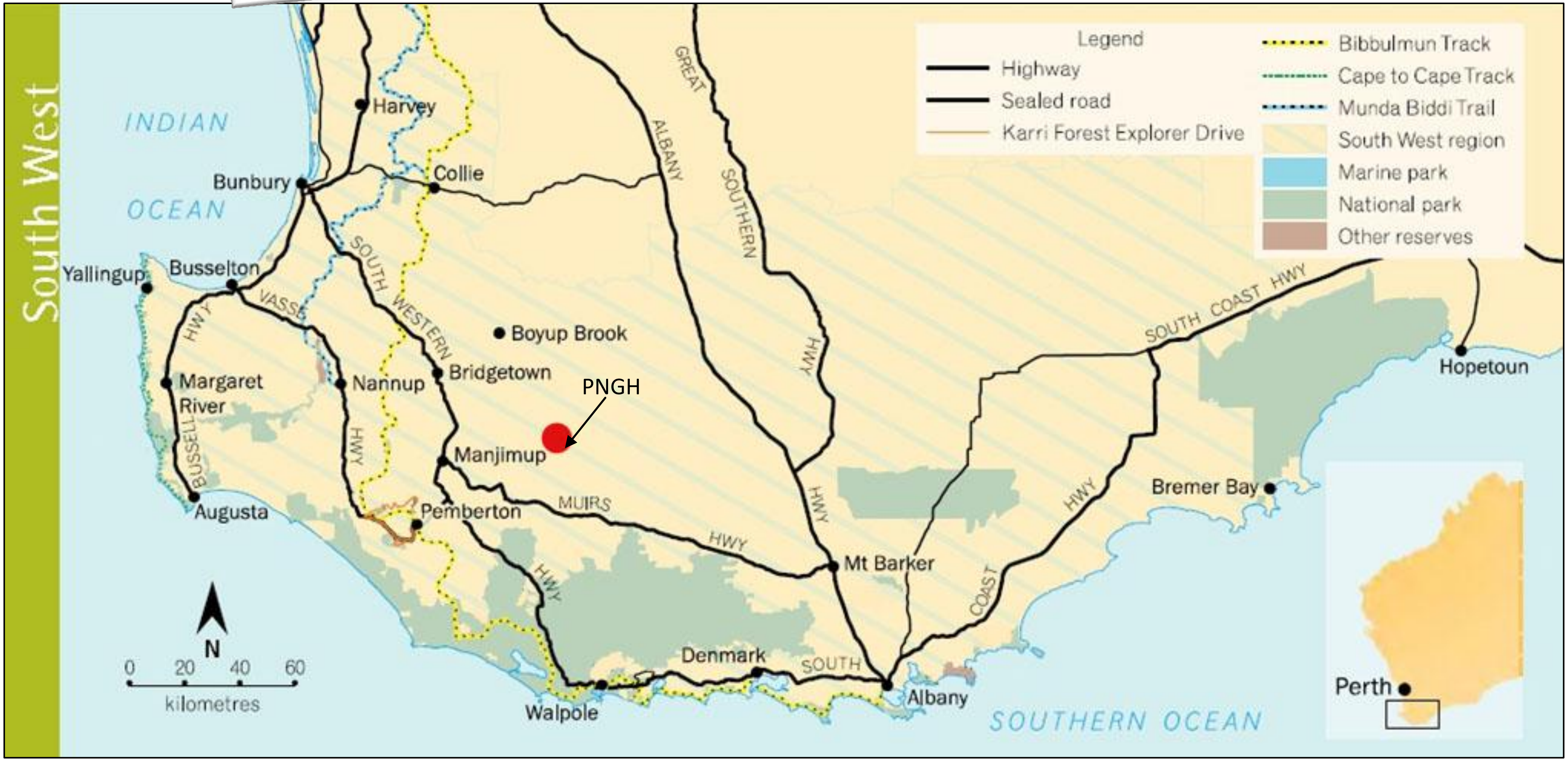


Figure 1: Location of Perup - Nature's Guesthouse (Adapted from DEC, 2010)


Lake outside Wilderness Lodge
Photo: Elise Pinto, 2011

2. Sustainable Tourism

(Literature Review)

2.1 Sustainable Tourism

Tourism was once thought to 'do no damage' to the environment, especially given attitudes implying natural destinations as 'free' resources, available to and used by everyone (Howie, 2003). Through recent years this attitude has changed, and currently acknowledges that tourism activities and development generate both positive and negative impacts on the natural, socio-cultural and economic aspects of a destination (Weaver, 2006). As a result, sustainable destination management and tourism planning approaches have been developed. Not only do these approaches incorporate sustainable development principles, but they also provide a means for implementing effective management strategies for a specific attraction or destination (Weaver, 2006).

The idea of sustainable development was first recognised by the United Nations World Commission on Environment and Development in 1987, with the publication of the Brundtland Report. The report outlined the damages occurring to the world's natural resources as a result of human use, and suggested that these precious environments should be preserved for the benefit of future generations (WCE, 1987). This new focus and direction was aimed at minimising negative human impacts whilst preserving the value of the natural resource (Hunter, 1997). Since its establishment, sustainability concepts have been used in a number of different industries and sectors, including tourism (Gössling, 2009). The introduction of sustainable development initiatives in tourism management and destination planning has not only added value to the tourism industry, but ensured its existence over the long term (Howie, 2003).

Photo: Anette Madsen, 2011

2.2 Tourism Models and Frameworks for Destination Management

To become recognised as an eco-tourism destination, management and planning must identify specific areas in which sustainable development theories should be applied. This report harnesses ideas from several theoretical frameworks including Butler's Destination Lifecycle Model (1980), Getz's Framework (2007), The Marketing Mix (2003) and Miossec's Model for tourism development (2003). These frameworks provide a guideline for managers of PNGH to secure its financial viability and long-term future as an eco-tourism destination.

Butler's Destination Lifecycle model (See Figure 2) (Butler, 1980, p.7) provides a basis for the scope of research in this strategic plan. According to Butler's Model, PNGH is currently undergoing a transitional stage, and is sitting between the Exploration-Involvement phases. This indicates that PNGH is currently in a relatively early phase of potential growth. Thus, before visitation can increase, research must clarify appropriate ways to involve, develop and consolidate positive tourism growth. Specifically, target markets and the physical features required for tourism development must be examined.

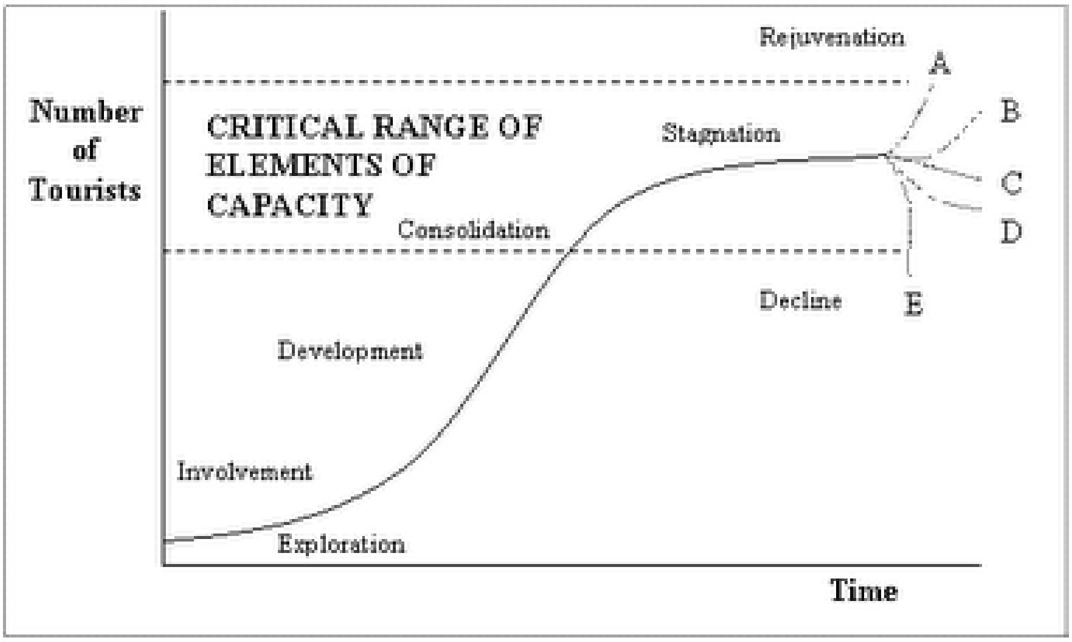


Figure 2: Butlers Destination Lifecycle Model (Source: Butler, 1980)

2. Sustainable Tourism
(Literature Review)

The Antecedents and Decision Making Process Framework (See Figure 3) (Getz, 2007, p.236) encapsulates the motivations and behaviours behind tourists visiting an event. In relation to the strategic planning and management of PNGH, the term 'event' will be replaced with the word 'destination'. The motives of visitors can be identified by applying Butler's Lifecycle. As target markets consist of school groups and nature-based visitors, initiatives to increase visitation at PNGH should focus on an early 'Involvement' phase.

The Antecedents Framework therefore outlines ways in which these visitors will feel encouraged to visit PNGH. Furthermore, identification of potential markets has also been aided by this framework, as removing barriers and constraints will open the visibility of PNGH to other visitors.

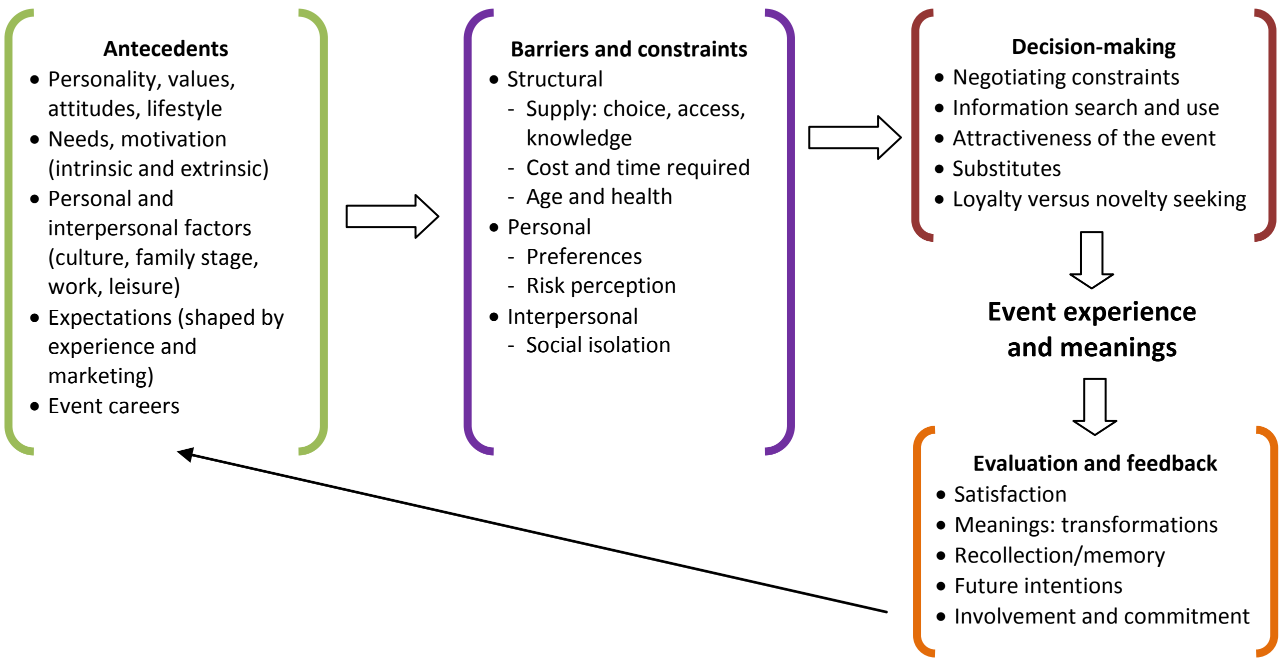


Figure 3: The Antecedents and Decision Making Process Framework (Adapted from Getz, 2007)

The four P's or the 'Marketing Mix' (See Table 1) (Howie, 2003, pp 149-150) consist of the Product, Price, Promotion and Place, which allow for an analysis of current marketing existence. In relation to PNGH, the product is the destination itself; the price is value for money (which can be subjective due to dependence on levels of monetary support). Promotion is the actual marketing process and therefore is vital to the destination management plan, as it cannot be sampled before hand and a range of materials have to be made available. The Place is where the decision to purchase the product is made. By addressing these points, it is possible to create viable marketing strategies for the destination. Authenticity is also important, as it contributes to the creation of an induced image.

Table 1: The 4 P's (Product, Price, Place and Promotion) of the 'Marketing Mix' in relation to PNGH.

'P'	RELATIONSHIP TO PERUP - NATURE'S GUESTHOUSE
Product	The PNGH unique natural environment. Surrounded by jarrah forests and home to endemic species this is a rare opportunity to experience a native area of the South West
Price	Through various incentives (see other strategies) will it be possible to create a product which is unique and competitive.
Place	Distribution is non-existent as it is nature and educational experiences which is offered.
Promotion	Brochures, internet, information sessions and word of mouth

Miossec's Model (Howie, 2003, p.58) considers business and community involvement, and examines a destination's characteristics (See Figure 4). If managed correctly, business and community involvement could aid positive and sustainable growth in PNGH. This model also indicates the role of transport in tourism, the decision maker's attitudes toward tourism, identification of competition in services and attractions close to the destination, and the involvement of all residents and stakeholders. All these aspects need to be addressed in the management of PNGH.

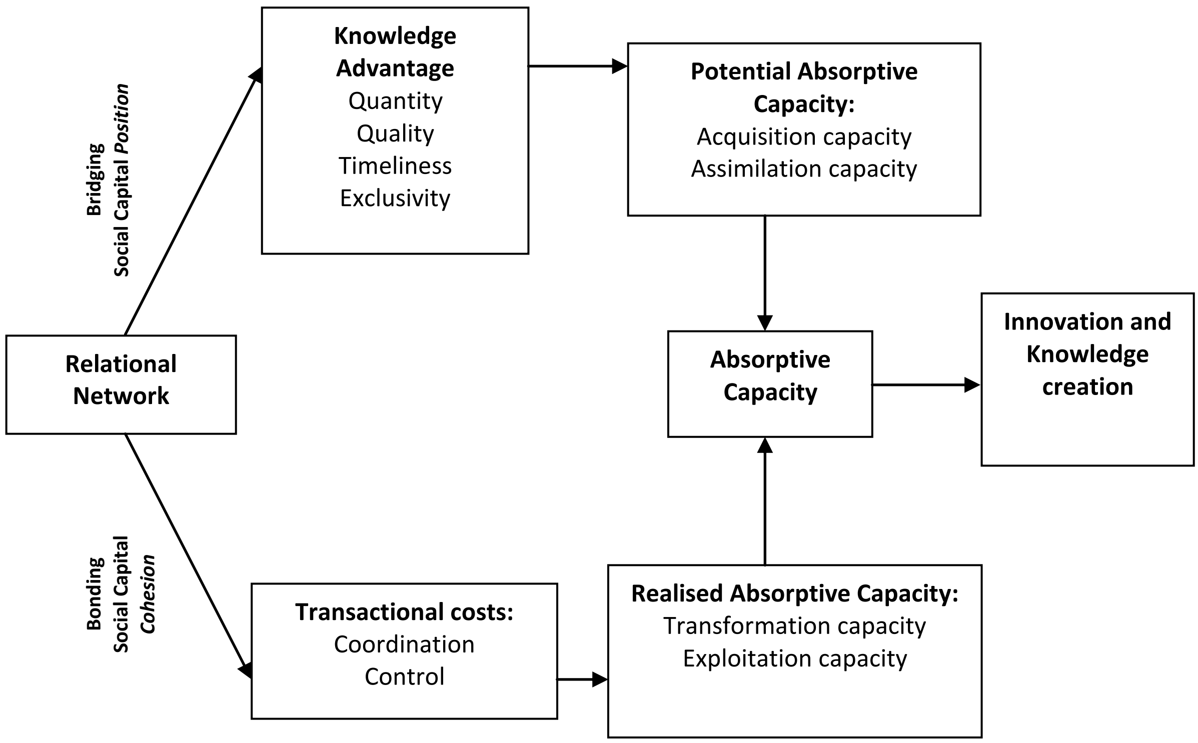


Figure 4: Miosecc's Tourism Development Model (Adapted from Howie, 2003)

Nature's Guesthouse backyard
Photo: Anette Madsen, 2011

3. SWOT Analysis of Perup

Strengths, Weaknesses, Opportunities and Threats

Table 2: SWOT Analysis

For SWOT analyses that address the specific components of PNGH, please refer to Appendix 2.

STRENGTHS	WEAKNESSES
- Unique opportunities to see rare and endangered native flora and fauna - Located within a dieback-free jarrah forest - 5 nature trails - Research site for forest management and nature conservation - Pre-existing self contained accommodation - Renewable energy and recycling systems with no dependence on main power grid - Potable water via rainwater collection - Community involvement – volunteering programs - DEC staff supply knowledgeable and accurate information (authenticity of experiences withstands) - Lack of distractions for visitors so they can learn and appreciate the natural surroundings - Established environmental and sustainability values	- Limited access - No public transport - Weak product identity and marketing - Limited advertising strategies - Current energy and water systems are insufficient for increased accommodation use and services - Limited opportunities to see fauna - Limited available activities - Limited services for accommodation: i.e. internet connection, mobile coverage, provision of linen - No nearby medical facilities - Limited disabled access - Leniency in payment rates for local organisations
OPPORTUNITIES	**THREATS**
- Guided walking tours: Day and Night - Further development of accommodation for increased visitation - Provision of unique nature-based and recreational activities and experiences (including cultural and historical) - Corporate use and educational facilitation - Focus on niche tourism markets - Increased and improved marketing - Increased business opportunities with external tour operators and shire partnerships - Research and education partnerships - Aboriginal interpretation and education - Improved renewable energy systems - Provision of interpretative signage - Eco-tourism certification - Upgrading current road access - Incorporation of local attractions and products/services with PNGH	- Natural disasters: fire, flooding, etc - Spread of dieback - Competition with other attractions - Carrying capacity is exceeded - Possible lack of knowledge from personnel taking tours (volunteers) leading to inauthentic experiences - Conflict between stakeholders, tour operators, business owners and local community - Uncontrolled visitor access to restricted zones - Physical impacts to environment, e.g. soil erosion and compaction

4. Strategic Plan

(Recommendatioms)

4.1 Budget Strategy

Goal: Increase the financial viability of Perup - Nature's Guesthouse so that it is sustainable, offsets operational costs and assists in future sustainable development and conservation efforts in the Perup region.

Current Situation:

The current financial disposition of Perup - Nature's Guesthouse may not be conducive to long- term viability (Figure 5; Figure 6). This is due to a number for reasons. Low visitor numbers and high outgoing expenditures over past years have led to a decline in available funds and income, and therefore a lack of financial sustainability. Over the years 2008 - 2010, revenue has amounted to less than half of PNGH's expenses (Gardiner and Norrish, n.d). Seasonal visitation may also challenge financial stability, as some months during 2008 - 2010 received no visitation, especially during January, December and July (Gardiner and Norrish, n.d).

A narrow visitor market consisting mainly of school and special interest groups account for the majority of PNGH's visitation. However, there is often a leniency towards payment rates for local school groups, which has decreased the chances of financial viability at PNGH quite dramatically. Some visitation exists as opportunistic bookings from tourists, but most of these are made *ad hoc*, and are not predictable. However, there is increasing repeat visitation from local special interest groups who use PNGH for nature-based activities and as a meeting place.

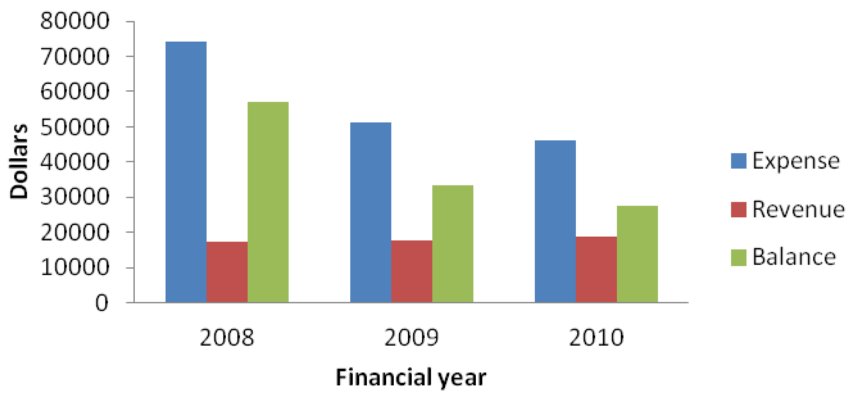


Figure 5: Book-keeping data from PNGH between the financial years of 2008 and 2010 (Source: Gardiner and Norrish, n.d)

4. Strategic Plan
(Recommendatioms)

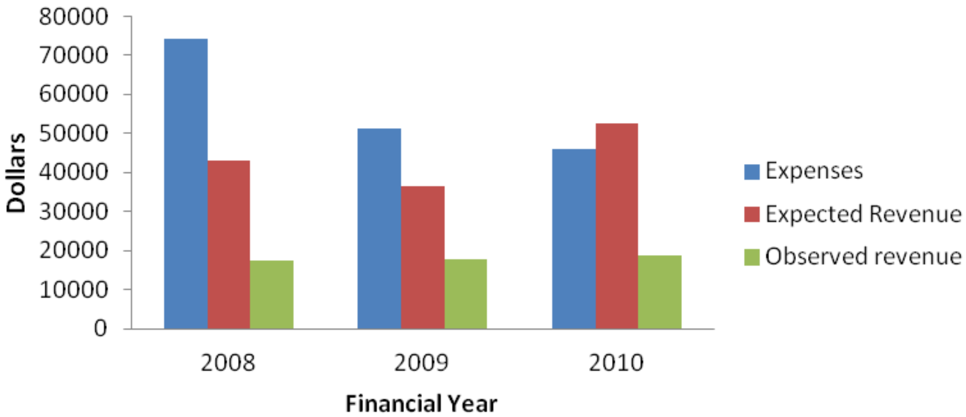


Figure 6: Expenses, expected revenue and observed revenue using book-keeping and visitor data from Perup - Nature's Guesthouse over the years 2008- 2010. Expected revenue was calculated by multiplying each recorded visitor with the accommodation rate associated with their visitor type, as defined in Appendix 6 (price listings for accommodation).

Future Direction:

In order to secure PNGH as a long-term eco-education resource for the public, financial viability and sustainability must firstly be achieved (Weaver, 2006). This opinion is widely supported throughout sustainable tourism and ecotourism- based literature, in which it is recognised that economic and financial viability is vital to overall sustainability, and can provide direct financial benefits to conservation and resource management (Newsome *et al*, 2002). For this reason and to ensure its contribution to environmental education and ecotourism in the South West indefinitely, the financial viability of PNGH is of the upmost importance. The strategies detailed below aim to offset annual expenses and enable PNGH to become financially self-sufficient by 2014.

Table 3: Key objectives and planning criteria for budget strategy.

OBJECTIVES	SUCCESS CRITERIA (TARGET)	SUGGESTED MONITORING PROTOCOL
Secure funding.	Funding is secured to facilitate development projects over the next 5 years.	Annual monitoring of funding allocations and identification of future funding needs.
Increase revenue.	Equal to or higher than annual expenditure. Revenue is equal to or higher than expenses by 2014.	Annual monitoring of expenditure and revenue.
Further research.	Benefit - Cost Analysis (BCA) of recommendations is undertaken and used to inform decision-making. The implementation of a monitoring system to measure annual visitation trends and activity use.	Not Applicable. Regular updating of visitation records, with an annual evaluation period.

4. Strategic Plan

(Recommendatioms)

Table 4: Grants available to Perup - Nature's Guesthouse.

ORGANISATION	GRANT	LIMIT	PROJECTS COVERED
Dept. Regional Development and Lands, Community Development Division.	Royalties for Regions.	No limit imposed.	Infrastructure development, sustainability initiatives, business expansion that creates jobs and increases tourism in a regional area.
	RFR: Regional Grants Scheme.	No limit imposed.	Projects to expand social and economic opportunities (including employment), and that plan for a sustainable economic and social future.
Tourism WA.	TQUAL	$100 000	Collaborative community tourism industry development projects.
Lotterywest Commission.	Heritage and Conservation: conserving natural heritage.	$15 000 per annum. For unincorporated groups, otherwise unlimited.	Activities aiding conservation of natural habitats through planting, protecting, and increasing awareness.
	Heritage and Conservation: Interpreting cultural heritage.	$15 000 per annum. For unincorporated groups, otherwise unlimited.	Signage for heritage trails, information and education mediums, public education programs.
	Organisational Development.	$15 000 per annum. For unincorporated groups, otherwise unlimited.	BCAs, economic plans, marketing research, strategic plans, feasibility studies, training and development, establishing best practice.
	Trails.	$15 000 per annum. For unincorporated groups, otherwise unlimited.	Trail planning, construction, marketing and upgrades, for various types of trail.
Local Government.	Environmental Community Grants Program.	No limit imposed.	Includes biodiversity conservation, sustainable catchment management, fauna rehabilitation, and interpretation and sustainable recreation in natural areas.

4. Strategic Plan
(Recommendatioms)

Table 5: Sponsorship programs available to Perup - Nature's Guesthouse.

ORGANISATION	LIMIT	FEATURES OF SUCCESSFUL PROJECTS
Rio Tinto WA Community Investment.	No limit imposed. Sponsorships over $5000 require a project proposal.	• Benefit communities that neighbour Rio Tinto operations in WA. • Will enhance Rio Tinto's reputation amongst stakeholders and communities.
Water Corporation.	None specified.	• Events and activities that focus on sport, youth, education, the environment, and cultural activities.
McDonald's.	None specified.	• Benefit the wider community • Promote balanced and active lifestyles for children. • 'Grassroots' community focus.
Alcoa.	No limit imposed. Sponsorship includes financial or in- kind support, or volunteering.	• Clear strategy. • Community benefit. • Sustainability and environmental focus.
Peet Limited - WA office.	No limit imposed.	• Environmental sustainability. • Family and community-based activities.
Satterley Property Group.	4 sponsorship types: Small Equipment Grants up to $500. Matching grants up to $2000 Small Event Sponsorship up to $5000. Large Event Sponsorship up to $20 000.	• Environmental sustainability focus. • Community based. • Education opportunities. • Youth development programs. • Sport, recreation and health lifestyle. • Self-sustaining in the long-term.
Chevron.	Not specified.	• Protects the environment and reflect other Chevron values.

Photo: Elise Pinto, 2011

4. Strategic Plan
(Recommendatioms)

Table 6: Strategies to achieve financial viability.

STRATEGIES	ACTIONS	PRIORITY 1: 1-6 months; 2: 6 months – 3 years 3: 3 years – 5 years
Secure Funding		
Identify needs for funding.	Identify areas which may benefit from improvement or maintenance and would require extra funding to undertake (this will influence which grants are applied for).	1
Apply for grants.	Refer to Table 4 for grants available to PNGH. Grants are available to support: Sustainable development. Tourism development. Indigenous tourism activities. Community-based conservation. Environmental education/interpretation. Research for economic viability. Trails and other recreational resources.	1-3
Explore sponsorship opportunities from private sources.	There are a large number of corporations that are willing to sponsor projects or organisations that benefit the community and their reputation (Refer to Table 5).	1
Provide opportunity for other contributions.	Provide a donation box on- site for donations to improve services or contribute towards conservation programs.	1
	Initiate an ‘adopt a woylie/ numbat/ wallaby’ program: Identify an appropriate species to become adoption candidates (e.g. from Perth Zoo). Compile information and photos. Create information pamphlets and web pages. Create a website ‘adoption’ link on PNGH websites. Install information in guest rooms.	2
Increase revenue from visitation		
Apply a consistent minimum rate per night for schools and other groups.	Meet with local organisations that return regularly e.g. local schools and special interest groups, to discuss a minimum rate, or to negotiate a special price. Update information brochures with new rates.	1-2
Introduce activities that will generate revenue.	See strategic recommendations for tourism activities and education/ interpretation (pp. 32 - 39).	1-3

4. Strategic Plan
(Recommendatioms)

STRATEGIES (CONT'D	ACTIONS (CONT'D)	PRIORITY (CONT'D) 1: 1-6 months; 2: 6 months – 3 years 3: 3 years – 5 years
Even out seasonality effects.	Introduce events and activities over periods of low visitation. Marketing initiatives (See strategic recommendations for marketing Table 8).	1-3
Increase visitation.	Identify the carrying capacity of PNGH for visitation (e.g. maximum level of monthly visitation). Promotion through marketing initiatives.	1 1
Research and Analysis		
Benefit- Cost Analysis (BCA).	Organise for a BCA to consider recommended strategies that may involve a level of financial investment. Use the outputs from BCA to inform decision making and prioritisation of recommendations.	1-2
Monitoring Systems.	Continue a monitoring system to evaluate annual visitation trends, consistent with records from 2008-2010.	1

Photo: Otters Adrift, 2011

4.2 Marketing

Goal: Improve and expand the eco-tourism product along with the identity and services of Perup - Nature's Guesthouse through a variety of media.

Current Situation:

Butler's Destination Lifecycle Model (See Figure 2) identifies the evolution of a destination in regards to visitor numbers. PNGH is currently in the exploration and development phase, where marketing initiatives are minimal and further action is required (refer to p. 19 for an explanation of the model). It is therefore not surprising that current marketing initiatives for PNGH are restricted to a handful of websites. There is also no signage on surrounding roads and crossings, or in nearby towns, and although there is a degree of local school involvement and community awareness, it is partial and requires rejuvenation. Advertising is also lacking at surrounding visitor centres such as Bridgetown, Manjimup, Boyup Brook and Walpole, where information in regards to PNGH is almost non-existent.

Future Direction:

Over the next five years, it is expected that the visibility of PNGH will expand and provide for a niche tourism market where self-guided tourists and a range of educational and other special interest groups can enjoy an eco and nature-based tourism experience.

Several initiatives may be implemented to improve overall marketing and visibility of PNGH. These include the breakdown of barriers as indicated by the Antecedents Framework (See Figure 3). This would require extensive internet advertising, the construction of more prevalent road signage, as well as brochure distribution to local visitor centres and attractions. Marketing for school groups and tertiary students would also need to become more focused given the percentage of students which have previously visited PNGH in the last two-three years (See Figure 7). While the main focus group will remain school and special interest groups, with increased marketing efforts it is expected that numbers of self-guided tourists will substantially increase.

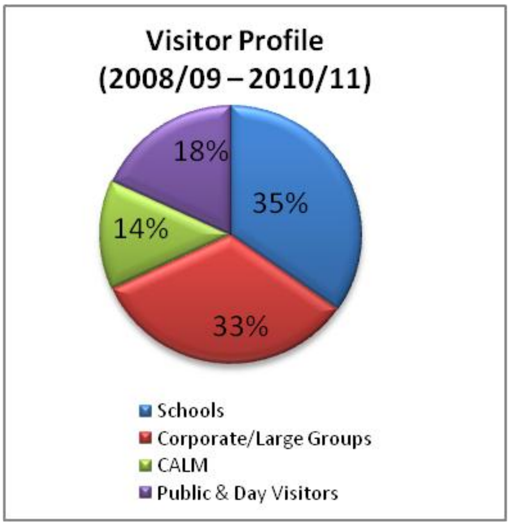


Figure 7: Comparison of visitor types to PNGH in the last 3 years. (Adapted from Gardiner and Norrish, 2010)

4. Strategic Plan
(Recommendatioms)

Table 7: Key objectives and planning criteria for marketing.

OBJECTIVES	SUCCESS CRITERIA (TARGET)	SUGGESTED MONITORING PROTOCOL
Increased local awareness.	All visitor centres and destinations containing information on PNGH, which is utilised by tourists.	Visitor surveys, check with visitor centres and local destinations annually.
Public access to information.	The majority of visitors (70%) hearing about PNGH from online sources and half (50%) using online booking systems.	Visitor surveys.
Increased road-side visibility.	Visitors finding their way to PNGH easily. Some clientele 'dropping' past after observing signs.	Visitor surveys.

Table 8: Strategies for successful marketing.

STRATEGIES	ACTIONS	PRIORITY 1: 1-6 months; 2: 6 months – 3 years 3: 3 years – 5 years
Create a website for PNGH.	Create and maintain a specific website for PNGH where potential visitors can read all about attractions and unique environment. Offer internet bookings for convenience to all visitors.	1,2
Produce a brochure.	Secure involvement with tourist centres in Manjimup, Nannup, Busselton, Collie, Bunbury and Albany.	1
	Partnerships with surrounding local business and wineries e.g. Ferngrove and Alkoomi Wineries to promote PNGH.	2
Internet Advertisements and Destination Guide of the South West.	Advertise in conjunction with other tourist websites and schemes e.g. the Destination Guide of the South West.	2
Signage on nearby roads.	Increase signage on surrounding roads in order to create awareness and prevent confusion when trying to visit PNGH.	2
Increase school visitation.	Distribution of brochures and creation of tab on website specifically for schools, educational groups and educational organisations.	1
	Providing information sessions for teachers, coordinators and students so that they obtain a 'feel' for PNGH before visiting.	2

4. Strategic Plan
(Recommendatioms)

4.3 Education and Interpretation

Goal: To become an established eco-educational facility, and provide interpretative experiences for all interest groups and tourists who visit Perup - Nature's Guesthouse.

Current Situation:

Perup - Nature's Guesthouse provides all the basic facilities to cater for small and large groups, including a range of accommodation, facilities and teaching areas (See Appendix 1). Currently, the main visitation groups are schools, community groups and self-guided eco-tourists (Gardiner and Norrish, n.d). Although school groups have been the centre of focus for PNGH, there is currently a lack of comprehensive educational programs and activities on offer. Interpretation is also limited to the information provided to visitors before the walking trails commence.

Future Direction:

Over the past few years, a number of tertiary students and corporate groups have visited PNGH. These markets may therefore be considered a potential growth area. PNGH should thus attempt to maintain relationships with school groups, and pursue tertiary students, as PNGH is an ideal facility for practical coursework or post-graduate research. Programs which are established for older school students could also be adapted to provide for corporate groups as well, in the form of staff training and education. Similarly, PNGH provides suitable facilities for corporate groups wishing to go on an overnight excursion for team-building exercises, training days, or conferences.

The strategies recommended aim to establish and maintain informative, interesting and challenging eco-education programs in which sustainability concepts are promoted and a sense of environmental responsibility is fostered (DEC, 2011). For the self-guided tourists, erection of interpretation panels around the area and especially around the walk trails may enable the tourists to engage and enhance the knowledge of the unique flora and fauna of PNGH. Well made sited panels can also be highly effective, as illustrated by Scottish Natural Heritage (See Appendix 3). Additionally, these strategies are linked to strategies for Marketing, Activities and Community Engagement.

Following the implementation of marketing strategies and further educational programs, it should become substantially easier to attract regular school visits from years 1-12. These programs are also intended to facilitate long-term relationships with a number of educational institutions, encouraging annual revisitation. For an example of a successful eco-education program in another DEC - operated educational facility, please refer to Appendix 4. These examples could be used and adapted to Perup - Nature's Guesthouse.

Photo: Save Warrup, 2010

4. Strategic Plan

(Recommendatioms)

Table 9: Key objectives and planning criteria for education and interpretation.

OBJECTIVES	SUCCESS CRITERIA (TARGET)	SUGGESTED MONITORING PROTOCOL
Increase eco-education programmes.	Implementation with DEC's Eco-education programs by 2013.	Continuously. Surveys to be filled out by teachers and students after visit.
Increase eco-education visitation.	Increase in eco-education visitation by 15% by 2016.	See above protocol.
Implement interpretative panels for self-education.	Interpretative panels on all walks by 2014.	Continuously. Have voluntary questionaries visitors can fill out at the end of visit.

Table 10: Strategies to achieve education and interpretation.

STRATEGIES	ACTIONS	PRIORITY 1: 1-6 months 2: 6 months-3 years 3: 3-5 years
Create classroom activities appropriate for school years.	Activities focusing on the flora and fauna will be produced in conjunction with Department of Education in order to gain basic knowledge of PNGH.	1
Create teaching packs for classroom activities prior to visit.	In conjunction with above strategy, Department of Education will also generate a teaching pack which will enhance teachers' knowledge of PNGH prior to visit.	1
Develop informative and educational on-site day/overnight educational programs for early childhood.	In order to get most out of PNGH it is imperative that appropriate educational programs are in place. Thus it will become an interactive learning experience which will create a bond with the natural environment.	2
Develop programs for primary schools (early to mid childhood).	(Cont. from previous strategy) Department of Education along with Department of Environment and Conservation have developed eco-educational programs for other areas in W.A (See Appendix 4). Trough cooperation with this established collaboration of departments it is possible to establish appropriate eco-educational programs for all school ages at PNGH.	2
Develop interactive programs for secondary school groups.	See above strategy.	2
Develop educational programs to enhance the knowledge of the indigenous culture in the area.	Through hiring and working with local indigenous persons it is possible to create educational programs unique to PNGH.	2
Develop evaluation protocols	Produce feedback sheets to be filled out by students, teachers, corporate groups and self-guided tourists which are to be on a voluntary basis for the continuous improvement of PNGH.	2

4.4 Activities and Experiences

Goal: To expand the tourism product by providing activities that will encourage increased environmental awareness and enrich the nature-based tourism experience.

Current Situation:

Surrounding nature reserves such as Tone-Perup Nature Reserve, Great Kingston National Park, and Unicup Nature Reserve (See Appendix 5) indicate the potential for providing a range of recreational activities. Currently, these reserves mainly cater for bushwalking. New recreational activities are being considered at this time for the future, such as horse-riding and bike trails.

CTO use of PNGH has declined in the past, from 12 overnight visits in 1999 to none in 2010. Most likely, this is a direct result of competition with other more popular tourist destinations, for example Pemberton or Walpole. Ideologically this is not a concern for DEC, as the guesthouse's primary objective is to provide eco- education for local communities, and to facilitate environmental research. There may be a need in the future to encourage tourism use of PNGH, to provide a supplementary income which may also take the pressure off local schools as the main provider of revenue at Perup.

Currently, Perup - Nature's Guesthouse offers a limited range of tourist activities. These include:

- 5 themed walking trails (See Appendix 6)
- Self- guided spotlighting tours
- Guided spotlighting tours, and
- Trapping tours (available to schools and special interest groups)

A price list for these activities can be found in Appendix 7.

Photo: The Hot Rock, 2010

4. Strategic Plan
(Recommendatioms)

Future Direction:

To encourage visitors to Perup for one night or more, it may be beneficial to increase the opportunities for tourists to partake in nature-based activities. Although the establishment of activities will incur initial financial and time costs, a two-phase plan has been proposed in an attempt to reduce these cost and energy outputs. The phases are as follows:

1) The implementation of new activities, events and partnerships that will attract visitors (and increase revenue) while limiting cost and energy outputs. The estimated timeframe for Phase 1 is 1-2 years. These activities include:

- Interpretative, themed trails
- Geo-caching
- National Park Passport Stamps
- Orienteering
- Art workshops
- Things to do in Perup – Pamphlet
- Increasing the use of PNGH by CTOs

2) Activities associated with a higher implementation cost would be introduced at approximately 3-5 years, once additional funding has been secured. These activities include:

- Push Bike Trails
- Indigenous Tours and Cooking Classes
- Guided Tours
- Bird watching
- Blindfold Course
- Woylie Rehabilitation Centre

Photo: Sabrina Trocini, 2011

4. Strategic Plan
(Recommendatioms)

Table 11: Description of Proposed Tourism Activities.

ACTIVITY	DESCRIPTION
Interpretative Trails.	The trails currently in use would be adapted to educate visitors on different aspects of the natural environment. The trails would take on themes and provide written interpretation (signage). Other activities such as rubbing plates (image impressions) and touching stations (samples of native vegetation and other materials), would provide a hand on experience for inspection by visitors at their own leisure.
Geo-caching.	This is a low-cost, low-impact activity, in which players use a Global Positioning System (GPS) to locate hidden treasures (caches). These cases are often hidden in areas that are culturally, historically or naturally significant. Once found, a player will fill out the logbook, and may swap the treasure items, usually inexpensive trinkets.
National Park Passport Stamps.	Stamps would placed at points within the park or across a number of parks in the Perup region, in which visitors then attempt to visit as many places as they can to collect a series of stamps in their 'passports'.
Orienteering.	Orienteering requires navigation through areas to particular checkpoints, with the aid of only a map and a compass. Although this activity can be done recreationally, it is also a competitive sport. In the future, PNGH may provide a base for orienteering competitions.
Art Workshops.	Workshops would provide a medium (photography, art or craft) that encourages visitors to appreciate the natural aspects of the Perup Sanctuary. Artist-in-residence programs could also be provided, in which an artist would attend and teach their own workshop for a selected period, usually 1-2 weeks.
Things to do in Perup – Pamphlet.	This pamphlet would outline the experiences and activities available to tourists visiting the Perup Region. Partnerships between other businesses would ensure that PNGH would also be promoted.
Push-bike Trails.	Push-bikes would be hired to visitors as an alternate way of experiencing the natural area. Specific trails would need to be provided, in conjunction with horse riding trails.
Indigenous Tours and Cooking Classes.	Indigenous tours would involve the knowledge and require participation of local indigenous people to provide information on the local environment as well as the Aboriginal culture. The cooking classes would demonstrate the use of traditional recipes, ingredients and methods.
Bird Watching Hide.	A camouflaged bird watching hide would provide avid bird watchers with a place where they can observe the bird life at PNGH. The provision of information sheets and binoculars would also encourage interested parties to participate in this activity.
Blindfold course.	In this activity, visitors are encouraged to disconnect their sense of sight, by wearing a blindfold, to enhance other senses. At specific points along a rope (indicated by a symbol carved into a wooden post), the visitor is required to stop and smell, listen, touch objects, or navigate obstacles.
Woylie rehabilitation and Interpretation Centre.	Woylie joeys dropped from the pouch during trapping exercises are often not viable for release. Subsequently, they may go to wildlife parks, or contribute to breeding programs. An on-site rehabilitation centre for Woylies at PNGH would contribute to the environmental education of guests visiting PNGH.

4. Strategic Plan

(Recommendatioms)

Table 12: Key objectives and planning criteria for activities and experiences.

OBJECTIVES	SUCCESS CRITERIA (TARGET)	SUGGESTED MONITORING PROTOCOL
2-phase process.	In each phase, introduce a further 2 activities.	In each phase, at least 2 new activities are introduced.
	Expenses do not exceed revenue by more than 10%.	Book-keeping of activities and costs, annual evaluation.
Increase use by CTOs.	2 CTOs organize regular bookings by the end of second year, consistent with Perup's carrying capacity.	Annual evaluation.
Offering activities to enrich the nature-based experience of all visitors.	All new activities are used or booked regularly (once/ twice per fortnight).	Visitor feedback surveys, annual evaluation of bookings and income from activities.

Table 13: Strategies to achieve activities and experiences.

STRATEGIES	ACTION	PRIORITY 1: 1-6 months 2: 6 months-3 years 3: 3-5 years
Phase 1: Expanding available activities		
Interpretative trails.	Design and create interpretive signs for each trail. Ideas for trail themes are: • Nocturnal mammals. • Termites and fungi (microorganisms): drivers of the bush. • Frogs- identifying calls. • The flora or the Jarrah Forest.	2
	Place signs along the walks currently available, using natural props such as trees of interest and Woylie diggings.	2
Geo-caching.	Place caches in areas near PNGH, and enter GPS details onto the Geo-caching website (www.geocaching.com) and associated Perup websites.	1
	Purchase 2 GPS devices to loan visitors without a GPS who want to try geo-caching while at PNGH.	1
	Return to cache positions at semi-regular intervals to maintain caches.	2
National Park Passport Stamps.	Place stamps in natural areas surrounding PNGH and provide a map (passport) that tourists purchase to locate and collect stamps.	1

4. Strategic Plan
(Recommendatioms)

STRATEGIES (CONT'D)	ACTION (CONT'D)	PRIORITY (CONT'D) 1: 1-6 months 2: 6 months-3 years 3: 3-5 years
Orienteering.	Meet with local orienteering organisations to plan an annual event at Perup. Things to consider: • Date (could be used to even out seasonality effects). • Entrance fees. Work in conjunction with interested organisations to choose an orienteering route through nearby forest areas.	1 1
Art Workshops.	Meet with schools, local community art groups or organisations (e.g. TAFE) to suggest PNGH as a weekend workshop site. This can also be used to even out seasonality effects.	1
Increase use of PNGH by Commercial Tour Operators		
Increased CTO use.	Identify optimal level of CTO use- e.g. 2 nights per fortnight. Meet with nearby CTOs to propose organised tours through Perup. Suggestion: tours associated with backpacker accommodation in Margaret River and Busselton, Go West Tours, Donnelly River Cruises, Pemberton Discovery Tours, and Birding South West.	1,2
Phase 2: Expanding available activities		
Increased activities in forest areas.	The opportunity for bike riding is recommended, especially if horse-riding becomes permitted, as both activities have common impacts and can be dealt with collectively.	1
Bush tucker tour and cooking class.	The kitchen located within the large living area could provide a great environment for a cooking class. The opportunity is available to combine this with indigenous tours, and finish with a demonstration of indigenous cooking.	3

4. Strategic Plan
(Recommendatioms)

STRATEGIES (CONT'D)	ACTION (CONT'D)	PRIORITY (CONT'D) 1: 1-6 months 2: 6 months-3 years 3: 3-5 years
Guided Tours.	Tours may be cultural (indigenous, bush tucker) or nature-based, and run at intervals compatible with PNGH (twice a week in heavy seasons/ once a fortnight in low seasons). Alternatively, tours may be provided by CTOs.	3
Nature-based workshops.	Workshops could occur 4 weekends per year, and could cover bird-watching, flora of south-western forests, etc. A guest speaker and tour guide may be involved.	1,2
Blindfolded tours/ obstacle course.	Choose an area not far from PNGH buildings, and construct a rope fence along a trail.	3
On-site Woylie rehabilitation centre.	Construct rehabilitation centre and provide interpretation covering breeding programs, possibly in conjunction with Perth Zoo and other conservation organisations. Design of the centre could also restrict human proximity to animals, and charge an entry fee (suggestion is $20 per adult).	3

Photo: Rob Simson, 2005

4. Strategic Plan
(Recommendatioms)

4.5 Facilities, Services and Visitor Management

Goal: Improve infrastructure, services, technology and access to allow for increased visitation to PNGH which is environmentally, economically and socio-culturally sustainable.

Figure 8: Current Solar Array (Photo: Elise Pinto, 2011)

Current Situation

Perup - Nature's Guesthouse provides all rudimentary services that visitors require for short stays – accommodation, cooking facilities, power supply and ablution facilities. Running independent off the main power grid, PNGH is self-energy efficient, using a 4.2kWh and 1.6 kWH solar panel array (See Figure 8) in association with a 1kWh wind turbine and 12.5kVA diesel generator (Murdoch RISE, n.d). Hot water is supplied via solar heating. While the system can accommodate current visitation numbers, any future increases will cause stress on the system, requiring another upgrade or alternative source of power due to its age.

Perup - Nature's Guesthouse uses rainwater as a potable water source (See Figure 9) with supplementary water supplied from wetlands on-site for bathroom use, which is used in many other tourism destinations (Kelly and Williams, 2007). Visitors are encouraged to use water wisely with signs located water points, indicating that rainwater is used and limited in supply. Similarly, existing waste management strategies require visitors to take their rubbish with them when they leave, as there are no waste removal services to the facility. DEC encourages visitors to minimise waste, and separate it from recyclable material.

Figure 9: Current Rainwater Tank (Photo: Elise Pinto, 2011)

Visitor capacity is an important concept at PNGH, due to the ecologically- significant surroundings and limits to energy and water supply. At present, a visitor carrying capacity, or maximum level of visitation, has not been identified for the destination.

Future Direction

With an updated renewable energy system, Perup - Nature's Guesthouse will have a sufficient energy supply to meet the demand of increased visitation. Interpretation and education of these services may also help visitors to understand and appreciate sustainable technology, which they may adapt to their own homes. A carrying capacity for visitation whether it is monthly or annually, will also ensure that the environmental values of DEC are sustained, and that energy requirements do not exceed the levels provided on- site.

4. Strategic Plan
(Recommendatioms)

Table 14: Key objectives and planning criteria for facilities, services and visitor management.

OBJECTIVES	SUCCESS CRITERIA (TARGET)	SUGGESTED MONITORING PROTOCOL
Develop and improve infrastructure for visitor satisfaction and safety.	Infrastructure accommodates visitor needs and wants.	Not required.
Sustainable energy and water systems.	Diesel generator is not needed for additional power supply. Half dependency on on-site lake for supplementary water supply.	Automated or manual recording of supply and demand for energy and inflow and outflow of water.
Sustainable waste management practice.	Not applicable.	Visitor feedback.
Visitor Impact Management.	Visitors have the ability to contact staff if they require it whilst out on the trails. Carrying capacity of natural environment is not exceeded.	Visitor feedback of service. Environmental Impact Assessment.

Table 15: Strategies for facilities, services and visitor management.

STRATEGIES	ACTIONS	PRIORITY 1: 1-6months; 2: 6months– 3years; 3: 3years-5 years
Develop and improve infrastructure for visitor satisfaction and safety		
Contract third party to operate a café selling locally produced/sourced food and handicrafts.	Open project up to tenders and shift all operational and logistical responsibility to third party contractor. Liaise with local community to source products and handicrafts.	1
Construct bird hide to attract bird watchers.	Conduct feasibility study in conjunction with activity objectives (p. 37) to determine potential financial returns from construction of a bird hide.	2
Upgrade trails for better safety and clarity.	Identify signage that lacks clarity and rectify. Define trail and make it safe and accessible for different age groups. Investigate possibility of disabled access to part of the trails.	1
Seal access roads from Bridgetown and surrounding areas.	Liaise with Mainroads/Department of Transport to consider sealing access Gommes-Kingston Rd and adjoining roads from Yornup and Bridgetown (See Appendix 8).	1
Place more signage across the surrounding region.	In conjunction with marketing objectives (p. 31), strategically place more branded signage at key visibility points across the region to advertise and direct visitors to the site.	1

4. Strategic Plan
(Recommendatioms)

STRATEGIES (CONT'D)	ACTIONS (CONT'D)	PRIORITY (CONT'D) 1: 1-6months; 2: 6months– 3years; 3: 3years-5 years
Install assistance locators on trails (if in need, visitors push button that alerts site caretaker of location).	Consider feasibility of this approach in contributing towards overall objectives and goals.	2-3
Sustainable water and energy systems		
Assess and monitor demand, and supply of energy needs.	Conduct an analysis of energy supply and demand every quarter for performance review. Monitor trends in visitation.	1
	Technology appraisal for any additional upgrades and replacements.	3
Install water efficient toilets.	Bathroom services appraisal for either installing composting toilets or toilets where the wash basin is combined with the flushing mechanism of the toilet (Kelly and Williams, 2007).	3
Timed showers and monitored temperature.	Use a portable timer (5mins) for education for tourists or install coin slots for 5 minute showers.	2
	Ensure temperature of solar hot water system is set to its most efficient setting (usually 60 degrees and above).	1
Introduce waste-water recycling system (grey-water).	Investigate viability of grey-water system.	3
Sustainable waste management practice		
Increase accessibility and ease of waste disposal following increased visitation.	Viability assessment of waste collection and disposal by an external waste disposal operator.	1
Visitor Impact Management		
Identify carrying capacity.	Organise for staff or a third party to identify the maximum level of visitation agreed on by DEC, based on environmental tolerance for change and renewable energy provisions.	1-2

Photos: Elise Pinto, 2011

Forest
Photo: Elise Pinto, 2011

4. Strategic Plan
(Recommendatioms)

4.6 Community Engagement

Goal: Develop and maintain strong relationships with local and regional businesses, tourism operators and the non-business community, such as schools, interest groups and residents. Engage the local community and develop partnerships with local and regional businesses to expand knowledge of the destination and encourage funding and sponsorship.

Current Situation:

Currently little community engagement exists at PNGH outside cooperation with local schools, and there is a significant lack of cooperation with and awareness amongst local businesses regarding PNGH.

Future Direction:

The Perup region offers great potential for a community engagement strategy to be implemented, increasing the marketing leverage of PNGH, as well as allowing other businesses to benefit from PNGH's expected success. Developing and maintaining engaging relationships with the surrounding community requires significant work to build up momentum, and then ongoing effort in order to sustain relationships (Fitch, 2009). Greater awareness and understanding of what activities and services offered by PNGH, as well as increased uptake of these activities and services by the local community will heighten awareness and enhance community engagement.

Table 16: Key objectives and planning criteria for community engagement.

Objectives	Success criteria (Target)	Suggested Monitoring Protocol
Develop relationships with schools.	Provide schools with a tactile learning experience year-round.	Quantitative analysis of school involvement supplemented by qualitative feedback.
Develop supplementary onsite services.	Work with local producers to ensure economic "trickle-down" effects to local businesses.	Measureable accounting practices will allow community producers to receive negotiated cuts of profit from total revenue.
Secure promotion by local visitor centres.	Visitor centre employees present PNGH in positive light.	Guest surveys ascertaining where/how they heard about the site; monitor allocated space and location of promotional materials in visitor centres.
Develop partnerships with local organisations.	Allow for guests to enjoy the services/products that both organisations offer.	Quarterly review of contractual obligations.

Photo: Elise Pinto, 2011

4. Strategic Plan
(Recommendatioms)

Table 17: Strategies to achieve community engagement.

STRATEGIES	ACTIONS	PRIORITY 1: 1-6months; 2: 6months– 3years; 3: 3years-5 years
Develop relationships with schools		
On-site day/overnight programs.	Work with schools to develop a cost-effective program that complements their curriculum while not compromising the economic viability of PNGH.	2
Holiday camps.	Build on previous strategy to offer camps over school holiday periods.	2
Animal adoption program for classes, students and parents.	Promote program to schools and parents, tie into overnight programs and accommodation offers for further leverage (in conjunction with animal adoption as covered in the budget strategy section, pg 28).	2
School visits with an animal, or eco talks.	Develop an eco-education program consisting of monthly presentations by DEC staff in classrooms – based on flora/fauna of the Perup region.	2
Develop supplementary onsite services		
Contract third party to operate a café selling locally produced/sourced food and handicrafts.	Open project to tenders to operate as separate business entity. If suitable contractor is found, they are to source goods from producers in the community and develop relevant contractual relations, as well as determining what financial returns local producers will receive.	1
Secure promotion by local visitor centres		
Provide visitor centre employees with a first-hand experience.	Bring visitor centre employees to the site to experience what PNGH has to offer.	1
Maintain communications with visitor centres.	Monthly updates, as well as face to face interaction.	2
Develop partnerships with local organisations		
Enter into promotional agreement with wineries and restaurants.	Display site promotional material at wineries, and cross-promote wineries at site as a day-attraction.	1
Include Perup on regional tour itineraries.	Approach regional and tour operators regarding including Perup on day trips and multi-day itineraries.	1
Manjimup Chamber of Commerce and Industry.	Develop relationships with key members of MCCI, such as wineries, restaurants and other tourist attractions, to strengthen PNGH's regional network and profile amongst fellow organisations.	1

4.7 Human Resources

Goal: Ensure sufficient human resources for site planning and management to ensure a positive experience for visitors.

Current Situation:

One full time staff member is currently employed on site, and works 30 hours per fortnight maintaining the site and, if requested, conducting activities such as guided spot light tours. The people who carry out trapping activities are outsourced, trained professionals.

Future Direction:

Although hiring and training staff will initially incur a financial setback, it will add to long term financial benefits and result in a reliable working staff base. Human resources in the fields of hospitality and the running of nature-based activities will attract more visitors, increase the income of PNGH and contribute to an overall enjoyable experience, which will essentially motivate visitors to visit again (Howie, 2003, p. 147). It would thus be beneficial to hire an additional caretaker so that two staff members would be present onsite. These caretakers would have to be competent in supervising and hosting activities and education programs. The proposed café will have to be outsourced by an external company who will sell prepared food along with local arts and crafts. This will provide a beneficial relationship for both in terms of promotional purposes and the benefit of having hospitality services available when required without hiring permanent staff.

While initiatives may be employed to ensure the process of attracting, hiring and retaining of staff is effective, the change between employees must also be managed. Waddell *et al* (2007, pp. 24-27) argues that current and potential employees need to be given the opportunity to acquire and strengthen existing knowledge, experience and capabilities. This should be provided in the form of training programs which will build confidence in employee self-efficacy levels and in turn result in productive job performance (Wood *et al*, 2010, p. 125).

Table 18: Key objectives and planning criteria for human resources.

Objectives	Success criteria (Target)	Suggested Monitoring Protocol
Retain and attract staff	Offer attractive wage premium Provide payment for: -Food -Accommodation -Travel costs -Health insurance	Annually assess staff turnover and successes in retention
Hospitality Service	Outsourced externally	Not Applicable
Activities/Tour guides/Trapping	Hiring appropriate staff Involvement of existing staff	Quarterly training programs or meetings to keep on track with current requirements and feedback from staff

4. Strategic Plan
(Recommendatioms)

Table 19: Strategies to achieve human resource management.

STRATEGIES	ACTIONS	PRIORITY 1: 1-6 months 2: 6 months- 3 years 3: 3-5 years
Decision making process for hiring of new staff.	Involve: Current employees, Volunteers, Friends of Perup, and The Perup Wildlife Trust, To establish a process and criteria for the hiring of new staff.	1-3
Hiring new staff.	Attracting appropriate staff via newspaper, internet and local radio for: • Running activities, and • Running a café.	 1 3
Training programs.	Providing appropriate training for knowledge base, hospitality services and site activities for current and new staff. Community/stakeholder/volunteer involvement to offer deeper understanding of the area.	1 3
Compensation/ staff retention/ remuneration.	Employ initiatives to retain staff by providing: Accommodation Fuel Food Extra remuneration (wage premium)	1-3


Photo: Elysia Harradine, 2010

5. Timeframe for Strategy Implementation

		2012				2013				2014				2015				2016			
Topic	**Action**	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Budget Strategy	Identify areas in need of funding	■						■				■				■					
	Research and apply for grants and/or sponsorship	■	■	■					■	■			■	■			■	■			■
	Identify other contributors for funding and/or have a donation box		■	■	■																
	Initiate the adoption program			■	■	■	■	■	■	■	■	■	■	■	■	■	■				
	Organise BCA report for recommendations				■	■															
	Annual evaluation of revenue + expenses			■				■				■				■				■	
Marketing	Create a website for PNGH	■	■	■	■	■	■														
	Produce a brochure	■																			
	Internet advertising and destination guide			■	■	■	■	■	■	■	■	■	■	■	■						
	Signage on nearby roads	■	■																		
	Distribute information packs to schools	■	■			■	■	■	■	■	■	■	■	■	■	■	■				
	Have information session for teachers	■	■			■	■	■	■	■	■	■	■	■	■	■	■				
Education and Interpretation	Create classroom activities for appropприate year group	■	■																		
	Prepare teaching packs	■	■																		
	Educational programs on-site for all age groups			■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	Interactive programs for secondary schools			■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	Cultural Indigenous education program			■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	Develop after-visit factsheet			■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Expanding Tourism Activities	Interpretive trails			■	■																
	Set up geo-caching		■																		
	National Park Passport Stamp		■	■	■																
	Orienteerig	■																			
	Art workshops	■																			
	Creating 'Things to do in Perup' activity list	■																			
	Meetings with CTO operators				■	■															
	Increased activities in nearby forest					■	■														
	Bush tucker tour and cooking class (unless CTO- operated)										■										
	Guided tours (unless CTO- operated)										■										
	Nature-based workshop					■															
	Blindfolded tours/obstacle course																■				
	On-site woylie rehabilitation centre																	■	■		
	Annual evaluation of activity use			■				■				■				■				■	
Facilities and Services	Construction of a bird hide							■													
	Upgrade trails and signage	■	■																		
	Installation of assistance locators (x3) for visitor safety			■																	
	Place more signage for direction	■	■									■	■	■	■						
	Analysis of energy demand and supply		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	Bathroom services appraisal for installation of water efficient toilet													■	■	■	■				
	Installation of shower timers or coin operation for showers													■							
	Grey-water system appraisal													■							
	Viability assessment of waste collection and disposal			■	■																
	Identify carrying capacity	■	■	■	■	■	■	■	■	■	■										
Community Engagement	On-site day/overnight programs and holiday camps			■	■	■	■	■	■	■	■	■	■	■	■	■	■				
	Animal adoption program for classes			■	■	■	■	■	■	■	■	■	■	■	■	■	■				
	School visits with an animal			■	■	■	■	■	■	■	■	■	■	■	■	■	■				
	Bring visitor centre staff to site for tour and activities	■	■																		
	Maintain communication with visitor centres			■	■	■	■	■	■	■	■	■	■	■	■	■	■	■			
	Enter into promotional agreements with surrounding attractions	■	■																		
	Become involved with Manjimup Chamber of Commerce and Industry	■	■																		
Human Resources	Hire new staff	■	■	■	■																
	Training programs	■			■	■			■	■			■	■			■	■			■

Figure 10: Timeframe for implementation of strategies


Tree Bark remains
Photo: Anette Madsen, 2011

6. Conclusion

Perup - Nature's Guesthouse provides a tranquil and unique experience for visitors in a truly extraordinary environment. However, due to lack of focused management, limited tourism product, and lack of marketing, the Perup - Nature's Guesthouse is currently running at an annual loss. This plan recognises that PNGH has the potential to successfully generate revenue, yet remain an ecologically and socially sustainable establishment. To achieve this, it has been acknowledged that strategic changes to the following areas need to be addressed:

- Financial Viability
- Marketing
- Education and Interpretation
- Tourism Activities
- Facilities and Services
- Community Engagement
- Human Resources

While various approaches outline possible future development and management opportunities, the suggested recommendations also attempt to uphold sustainability concepts and values. It is therefore possible for PNGH to establish itself in the wider community as an eco-educational and conservation institution through the implementation of various tourism and community activities as well as eco-educational programs. The implementation of the management plan will be successful if all recommendations are attended to as several strategies are interlinked to other key areas and reliable to the overall achievements. Thus, to achieve a well-balanced and sustainable facility it is recommended that this 5-year management plan be applied.

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42	*Elise Pinto, 2011*	*– Classroom/Cottage/Bunkhouse*
43	*Elise Pinto, 2011*	*– Perup - Nature's Guesthouse backyard*
44	*Elise Pinto, 2011*	*– Sedge Wetland*
47	*Elysia Harradine, 2010*	*– Brushtail Possum*
48	*Danny Id, 2010*	*– Ringtail Possum*
49	*Anette Madsen, 2011*	*– Tree bark remains*
50	*ZooAquarium, 2010*	*– Numbat*
51	*Anonymous, 2011*	*– Grasstree*
58	*Perth Zoo, 2011*	*– Chuditch*
59	*Project Numbat, 2011*	*– Numbat Habitat*
59	*Elise Pinto, 2011*	*– Sedge Wetland*
60	*Anette Madsen*	*– Possum in stove*
61	*Elysia Harradine, 2011*	*– Roads*
63	*Elise Pinto, 2011*	*– Picnic space*
64	*Anette Madsen, 2011*	*– Marshland*
66	*Matt Prophet, 2010*	*– Little Corella*
69	*Anette Madsen, 2011*	*– Lake Unicup*
69	*Derani Sullivan, 2010*	*– Lake Muir*
70	*Nannup Visitor Centre*	*– Red capped Wren*
70	*Elise Pinto, 2011*	*– Trail information Sign*
72	*Bundacrreek Country Lodge, 2011*	*– Black cockatoo*
71	David Molach, 2008	*– Bobtail Lizard*

Glossary

List of Definitions

Ecotourism – "Ecotourism is ecologically sustainable tourism that fosters environmental and cultural understanding, appreciation and conservation" – as defined by the Ecotourism Association of Australia (2006)

Nature-based tourism – Tourism that features 'nature' – as defined by Tourism Western Australia (2006)

Self-efficacy – refers to a person's belief that they can perform adequately in a situation (Wood *et al*, 2010, p. 105)

Sustainable development – "*Sustainable development is development that meets the needs of the present without compromising the ability of future generations to meet their own needs"* – as defined by the Brundtland Report (WCE, 1987)

Triple bottom line- A sustainable development concept that requires a holistic approach to planning that acknowledges the environmental, social and economic aspects and impacts in an area. (Dwyer, 2005)

SWOT analysis: An analysis which identifies the strengths, weaknesses, opportunities and threats of a destination or activity.

Acronyms

CTO – Commercial Tour Operators

DEC – Department of Environment and Conservation

PNGH – Perup - Nature's Guesthouse

RISE – Research Institute of Sustainable Energy

TQUAL – Tourism Quality Projects

Appendices

Appendix 1: Site Inventory

Accommodation:

1. **Homestead and Bunkhouse:** The homestead consists of a large kitchen and living area with 3 bedrooms and 2 bathrooms, with disabled access. The building overlooks a natural wetland, surrounded by natural forest. Neighbouring the homestead is the bunkhouse, which consists of 7 bedrooms and 2 bathrooms, and can sleep up to 28 people. Hot water for both buildings run on solar energy, and buildings are constructed from local materials, including rammed earth, plantation pine, and jarrah.

2. **Cottages:** These are booked by families or small groups, and contain a kitchen, living area, 2 bedrooms and 1 bathroom. 2 Cottages are available at PNGH.

3. **Workers Quarters:** Workers Quarters consists of a self-contained 3 bedroom cottage, supplied for researchers or to accommodate visitor overflow in large group situations.

Table 20: Visitor numbers and accommodation types at the guesthouse

Accommodation Type	Number Accommodation Types	Number People Catered	Number of Beds	Features
Cottage	1	7 pax	1 double bed 1 double bunk 1 bunk bed (3 person capacity)	Kitchenette Bathroom Toilet Solar Hot Water System
Bunkhouse/ Dormatory	1	28 pax	9 bunk beds 4 trundle beds 5 single beds	Male/Female Bathrooms Solar Hot Water System
Wilderness Lodge	1	9 pax	2 double bed 3 single bed 1 double bunk	Ensuite Disabled access bathroom Fireplace Solar Hot Water System

Energy and water:

PNGH operates off the power grid, sustained by renewable energy sources such as solar and wind. Natural gas is used for cooking and as a backup source for hot water. For very large groups a generator is often used to supplement energy requirements.

Other Facilities:

1. Classroom: Also a functional laboratory, the classroom is situated beside the homestead, and facilitates large group activities and functions.
2. Barbeque and Picnic Area: This area extends from the homestead building, consisting of picnic-style tables and a large barbeque.

On-site Recreation:

1. Walking Trails: 4 trails of varying length take visitors through different habitats of the jarrah forest. All ages and fitness levels are accommodated.
2. Spotlighting: Self-guided night spotlight walk along one of the 4 trails, torch provided.

Appendices

Appendix 2: SWOT Analyses

Table 20: SWOT Analysis of Destination Marketing and Business Enterprise

STRENGTHS	WEAKNESSES
- Unique 'Wilderness' experience offered - See WA's State Fauna Emblem (iconic species) - Ecologically sustainable and aesthetically designed accommodation and Infrastructure - Offers relaxation and escape from city and suburbia - Mention of attraction in list of activities to do in Boyup Brook Visitor Centre Hand-out	- Remote location - Accessibility - Current product identity and promotion is weak - Limited advertising strategies for target market/s - No clear marketing niche - Value as a corporate showcase not recognized - Risk of destination being Sold Off - Primarily a night experience
OPPORTUNITIES	THREATS
- Stronger online presence - Use of destination as a 'detour/passing through' destination/attraction - Develop partnerships with outside region operators - Promotion Days/Weekends - Focus on niche tourism markets - Develop new activities and experiences - Aboriginal involvement/interpretation - Increase public awareness of DEC roles - Encourage local community involvement with operations and management - Fundraising Campaigns	- Ability to cater for opportunities and deliver the experience marketed - Changes in priority and loss of support from destination managers and other stakeholders - Leasing of facilities to other than DEC - Greater Warren-Blackwood Region (outside tourism attraction and destinations with more appeal)

Photo: Elise Pinto, 2011

Table 21: SWOT Analysis for Attractions, Tours and Activities

STRENGTHS	WEAKNESSES
- Ecologically sustainable and aesthetically designed accommodation and infrastructure - Offers relaxation and escape from city and suburbia - 5 walking trails - 52,000 ha of forest - See one or more of the 27 species of native mammals found in this reserve	- Mostly a night experience - Limited activities to do during day - Limited time/opportunities to see most fauna
OPPORTUNITIES	**THREATS**
- Utilisation of Perth Tour Operators - Information/education systems for visitors : provide interpretative panels for flora and interesting facts of wildlife and other areas - Incorporate indigenous interpretation of nature and their environment, culture and history - Recreational activities – i.e. canoeing on lake	- Weather conditions for night experiences not always ideal for visitors or wildlife to be visible - Wildlife parks with all "sought after" Aussie animals in one place (e.g. Whiteman Park) - Greater Warren-Blackwood region (outside tourist destinations within) - Visitor numbers exceed carrying capacity - Degradation of natural area through development, and thus tourist experience - Disruption on wildlife from visitors - Impacts on environment from visitors - Uncontrolled visitor access to restricted zones/areas

Photo: Anette Madsen, 2011

Table 22: SWOT Analysis of Economic Viability

STRENGTHS	WEAKNESSES
- Independent of grid power and therefore costs of electricity are negligible	- Expensive to service the facility and provide staff due to distance from Manjimup and other town sites - Ratio of government funding versus donations received
OPPORTUNITIES	**THREATS**
- Possibility of offsetting running costs of centre with improved destination management and implementation of recommendations provided - Donation Box - Creating a business that is economically viable to run - Ability to pay both full-time/casual staff	- Loss of profit and inability to recover expenses - Unlikelihood of returning profit (DEC, 2007)

Photo: Elysia Harradine, 2011

Table 23: SWOT Analysis of Facilities and Services

STRENGTHS	WEAKNESSES
- Renewable energy supplies - No-dependence on electrical power grid - Telstra services for landline - Sanitary disposal services - Rainwater collection and use - Supplement water source – on-site lake - On-site accommodation facilities - Per Christensen Classroom - Walking trails - Guided night walks with caretaker - Solar hot water systems - Recycling systems - Take rubbish with you (also possible weakness)	- No internet connection for visitors - No technological interpretation and information sources for visitors – environmental/historical - No indigenous interpretation - Mobile coverage - Nearby medical facilities - Lack of disabled access - Provision of linen and other basic needs i.e. towels, soap and clothes washing services - Increased demand of energy not supported by current energy system supplies - Limited choice of activities - Limited opportunities to see fauna - No security (locks) on bunkhouse rooms for separate groups staying - Separate group bookings not yet possible – few individual groups able to stay
OPPORTUNITIES	**THREATS**
- Provision of internet, computer, interpretation - Communicate with Telstra for mobile coverage - On-site medical facilitation or services to next available medical facility - Upgrade renewable energy systems to be more energy efficient and supply increased demand - Develop on-site database to monitor tourism and research data - Increased range of activities and tourism facilities - Upgrade road access and infrastructure	- Lack of visitation for viability on installing/supplying such services for visitors - Possible lack of knowledge and correct information from guides and staff/volunteers - Seasonal changes and effects - Visitor numbers exceeding carrying capacity - Limits of acceptable change reached and exceeded - Health and safety issues

Photo: Elise Pinto, 2011

Table 24: SWOT Analysis of Corporate and Educational Value

STRENGTHS	WEAKNESSES
- Per Christensen Classroom with internet access - On-site Accommodation - Wilderness, values of conserving the environment - Renewable Energy and Sustainable Development practices - Lack of distractions – perfect for training purposes - Almost any land use or conservation issue has relevance to area (DEC, 2007) - Conservation efforts of flora and fauna - Research site for conservation and forest management	- Lack of identity and appeal for value - Lack of interest from school groups to visit (DEC, 2007) - Distance from schools/universities - Possible costs for experience - No current internet services for visitors in accommodation buildings - No computers provided for visitor use - Communication services limited - Low occupancy by school groups and visitors in general (DEC, 2007)
OPPORTUNITIES	**THREATS**
- Become THE centre for ecological research and studies in the southwest of WA - Research Partnerships with universities - Post-graduate use for research and data collection - Primary and Secondary School Programs - Partnership with the Department of Education - Provision of internet and computer services - Catering services for corporate use and other profit organizations (DEC, 2007) - Provision of computers depending on demand - Upgrades to facility depending on promotion and use - Educational focus and commercial operators work together (DEC, 2007) - Feedback programs - Ability to incorporate educational activities with collection of valuable monitoring data for the area (DEC, 2007)	- Outside region educational experiences and resource allocation to schools - Continued lack of interest to visit centre by both educational and corporate entities - Ability to cater for experience and provide/deliver opportunities and the experience - Ability to meet all sustainability criteria in the triple bottom line (environment, socio-cultural, and economic contexts)

Table 25: SWOT Analysis of Industry and Community Involvement

STRENGTHS	WEAKNESSES
- Friends of Perup Non-profit Organisation - Volunteer base - DEC involvement and management - Unique experience/destination - Educational and research values - Established environmental and sustainability values	- Lack of identity - Funding for projects - Isolation from surrounding region
OPPORTUNITIES	**THREATS**
- Encourage more community involvement - Partnerships with surrounding property owners - Protection Program for flora and fauna with monitoring - Restoration and maintenance groups	- Risk of been sold off (DEC, 2007) - Reluctance of working together

Photo: Elise Pinto, 2011

Table 26: SWOT Analysis of Human Resources (Staffing and Volunteers)

STRENGTHS	WEAKNESSES
- Government support (though possibly underprovided) - Existing personnel experience – ability to train new potential development staff - DEC staff – knowledgeable and accurate information suppliers (Authenticity of experience withstands) - Guides for night tour and trapping experiences	- Possible bureaucratic control by government - On-site/nearby accommodation for staff - Distance from 'nearby' towns for volunteers and staffing
OPPORTUNITIES	**THREATS**
- Work experience programs - Volunteer campaigning - Canteen/café staff - Training of current staff and volunteers - Gift Shop Staff - Site monitoring staff - Maintenance staff - Recruitment of staff and volunteers online - Online training programs - Cleaning Staff	- Possible over dependence on volunteers - Possibly too much responsibility laid on caretaker - Health and Safety issues - Change in management may cause tension amongst staff and volunteers - Lack of ecotourism training

Photo: Elysia Harradine, 2011

Scottish Natural Heritage examples of good interpretation panels.

Appendices

Appendix 4: DEC Educational Programs

Department of Environment and Conservation Eco-education programs 2011. An example of successful eco-education programs in DEC operated facilities.

Appendices

Appendix 5: Surrounding Region Attractions

Surrounding State forest and Nature Reserves

1. **Greater Kingston National Park (unofficial name):** Includes Heartlea picnic area, visitor information and lookout.

2. **Tone-Perup Nature Reserve:** PNGH and surrounding bushland.

3. **Unicup Nature Reserve:** Includes Lake Unicup, opportunities for bird-watching, walk trail, boardwalk, picnic area and toilets.

4. **Lake Muir National Park (unofficial name):** Includes Lake Muir, toilets, information facilities, lookout and picnic area

Figure 12: Lake Unicup
(Photo: Anette Madsen, 2011)

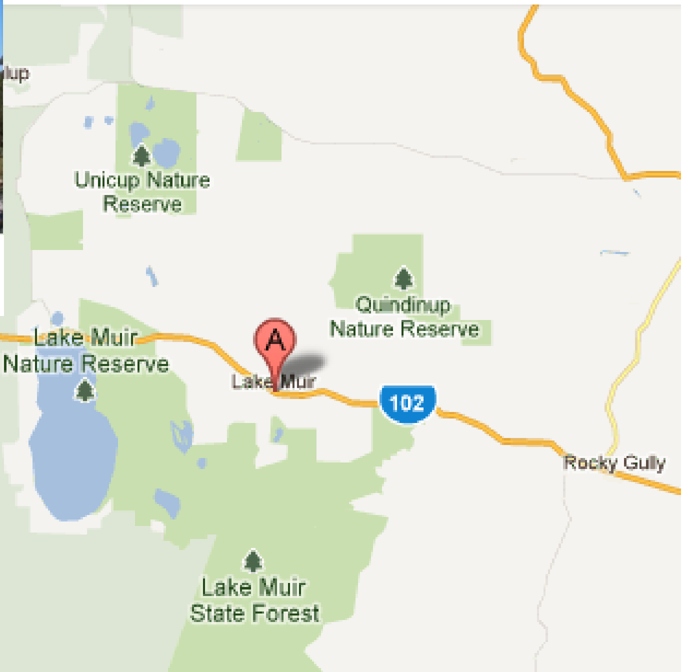

Figure 11: Lake Muir and Lake Unicup
(Note: Scale is approximate)

(Source: Google Maps, 2011)

Figure 13: Lake Muir
(Photo: Derani Sullivan, 2010)

Appendices

Appendix 6: Current themed walk-trails

Perup Ecology Centre, Guest House and Trails

Figure 14: Aerial view map of Perup - Nature's Guesthouse current walk trails (Source: Google - Imagery, 2011)

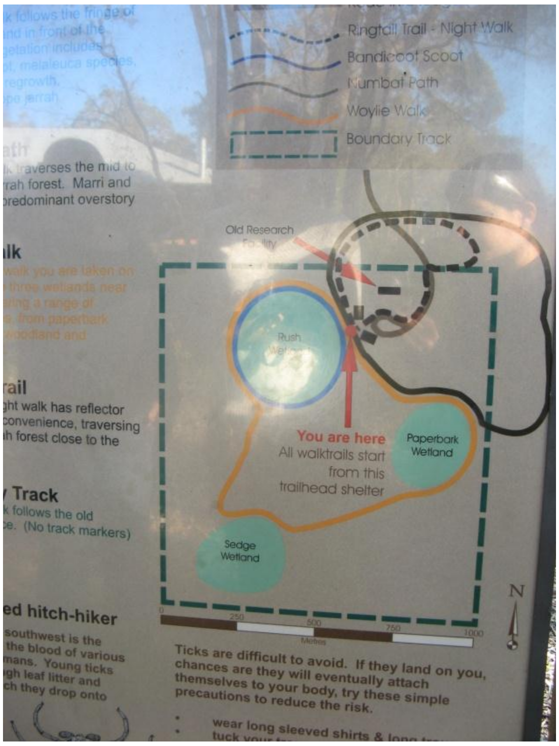

Figure 15: Walk-trail information Sign at start of all trails (Photo: Elise Pinto, 2011)

Woylie Walk
On this walk you are taken on a journey to three wetlands near the facility, covering a range of vegetation types from paperbark swamps, open woodland and re-growth forest.

Boundary Walk
This 4km walk follows the old boundary fence.

Ringtail night walk
This 1.2 km night walk has reflector signs, traversing mid-slope Jarrah forest close to the facilities.

Bandicoot Scoot
This 1.2 km walk follows the fringe of the sedge wetlands in front of the facility.

Numbat Path
This 2.1 km walk traverses the mid to upper slope jarrah forest.

(Source: Google - Imagery, 2011)

Appendices

Appendix 7: Pricing structure for facilities

Pricing Structure as of the 30th January 2009

ACCOMODATION

Cottage: (can sleep up to 7 people)		$80.00 per night
Scientist Cottage: (can sleep up to 7 people)		$80.00 per night
Lodge and Bunkhouse:	DEC Staff and Volunteers	$20.00 per night
	General Public	$20.00 per night
	Community groups, non-profit organisations	$20.00 per night
	Tour Operators	$20.00 per night
	University/Tafe Groups (over 18yrs old)	$20.00 per night
	School Children (under 18yrs old)	$15.00 per night
	School Adults (teachers, parents etc)	$15.00 per night
	Children (under 18 years old)	$15.00 per night

However if a group only contains minimal numbers of people, eg: 2 or 3, the minimum amounts are to be applied.

Lodge and Bunkhouse:	Minimum of $150.00 per night for both or	$75.00 for one or the other
Caravans: (2 max at any one time)	$10.00 per person per night	
Classroom: (payable for day use only, not if staying night)	$40.00 for up to 4 hours	$60 for the day

ACTIVITIES

Activity Prices are to be used as a **GUIDE ONLY** at this stage. Confirmation will still follow the same principle of Bev organising once established via shared calendar and price confirmed.

Self guided Spotlighting including Presentation	$25.00
Guided spotlighting including presentation (not promoted)	$50.00
Trapping with ranger	$250.00
Ranger Talk	$150.00
Package - including talk, trapping and spotlighting	$400.00

(Source: DEC, 2010)

Appendices

Appendix 8: Map of unsealed roads

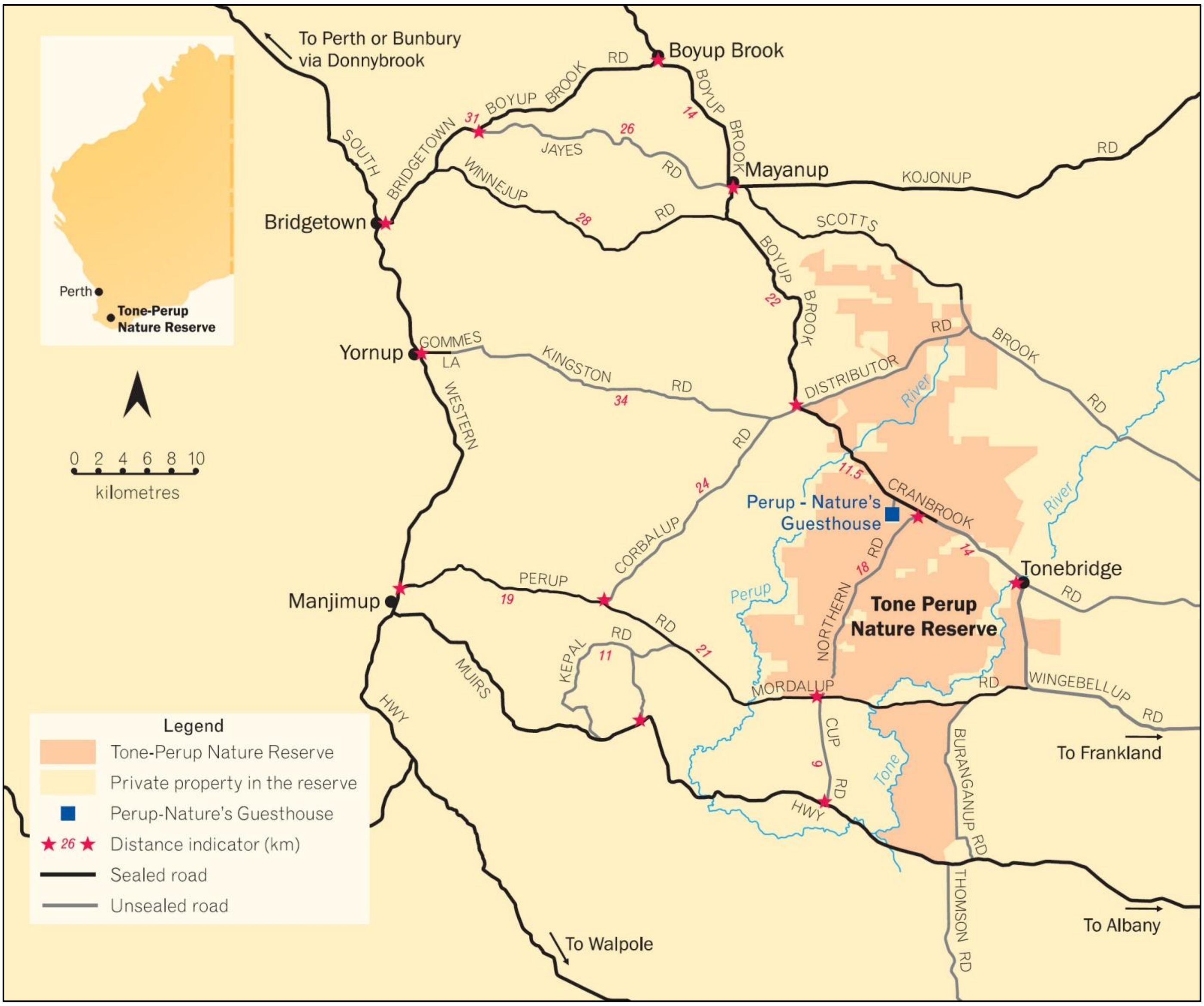

Figure 13: Map showing sealed and unsealed roads which access Perup - Nature's Guesthouse. (Source: DEC, 2011)

This map indicates all access roads to Perup – Nature's Guesthouse and the town sites of which they pass through. For increased access to the destination, it is recommended for that roads starting from Yornup and Manjimup are sealed.

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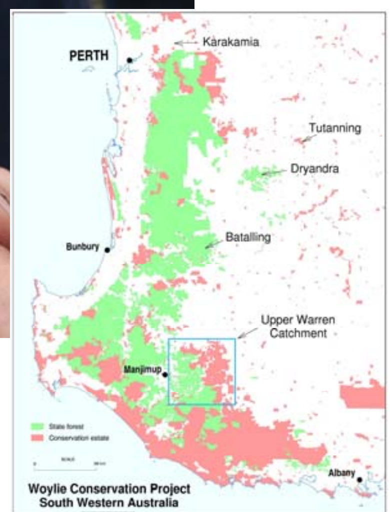
Where's the Woylie?

Possible factors affecting post-decline Woylie (*Bettongia penicillata ogilbyi*) abundance in the Upper Warren Region of South West Australia



By: Sydney Jaimes

Pacific Lutheran University, Biology
Submitted in fulfillment of requirements for Cairns, Australia: Rainforest, Reef and Cultural Ecology
SIT Study Abroad, December 2010
Academic Director: Tony Cummings
Advisor: Adrian Wayne
Department of Environment and Conservation, Science Division
Warren Region
Manjimup, Western Australia

Cover Photo by: Sabrina Trocini and Department of Environment and Conservation

Keywords: Woylie, abundance, population, population decline, factors

Abstract:

The woylie (*Bettongia penicillata ogilbyi*), also known as the Brush-tailed Bettong, has been declared as "*fauna that is rare or likely to become extinct"* not once, but twice. The woylie used to cover over 70% of the Australia, but over the past century the population has declined to populate only 1% of Australia. It currently exists predominantly in the Upper Warren Region of the south west region of Western Australia. Studies involving the factors causing woylie population declines began in the 1970's. These studies examined woylie habitats and established which vegetation types, groups and landscapes were preferred by woylies. While these studies were being conducted, a fox baiting program in the 1980's was established and the scientific and conservation community saw a miraculous increase in woylie abundance in the 1990's. But in 2001, woylie populations declined once again and declined farther than they did in the 1970's. The main factors thought to be the cause of the second population decline are disease caused by two parasites *Toxoplasma* and *Trypanosoma sp. nov.* and increased mortality of adult woylies. In response to these drastic population declines, the Warren District Western Australia Department of Environment and Conservation (D.E.C.) developed the Woylie Conservation Research Project which involves a wide range of disciplines to determine the main factor or factors affecting woylie abundance. Recently, D.E.C. developed a Woylie Translocation Project which involved construction of the Perup Sanctuary. The goal of the project is to translocate 40 individuals into the sanctuary.

For my Independent Study Project (ISP), I worked with D.E.C. in Manjimup, Western Australia for three weeks on the Woylie Translocation Project. I collected primary data which included trapping data and habitat surveys for 50 trapping sites in 13 transects equaling 650 data points. I asked the question: What factors may be affecting post-decline Woylie *(Bettongia penicillata ogilbyi)* abundance in the Upper Warren Region of South West Australia? I investigated this question by looking at several possible factors including habitat surveys used to observe any woylie habitat preferences, the presence of other mammals, distance from paddocks and private property, vegetation groups, landscapes, and category of forest. Several trends were noticed, but none of the study factors were claimed as a major factor affecting the present decline in woylie abundance. My study verified previous studies involving habitat preferences, vegetation groups and landscapes suited for woylies. Also in my study, possible competition between brush-tail possums (*Trichosurus vulpecula*) was observed, but no solid conclusions were made about brush-tail possums being a major factor for population decline. Many woylies were trapped in trap sites that were in close proximity to paddocks and private property. This trend is interesting since paddocks are becoming universally understood as a habitat that provides resources for a variety of species. Woylie abundance was high in both National Park and Nature Reserves which is worthy to note, since National Parks are claimed to have the highest abundance of woylies.

It has been a difficult process, in the past and presently, to find one solid factor affecting woylie abundance. It is something that may take many more years to solve. Currently, the best efforts are being made by scientists and conservations to find an answer. It is important to not disregard any of the study factors but to continuously investigate multiple factors as a change in habitat preferences or the presence of other small mammals may affect woylie population declines.

Acknowledgements:

First, I would like to thank my supervisor, Adrian Wayne, for being so willing to help me and assist me with my Independent Study Project. His insight was extremely useful in the completion of my study. Second, I would like to thank Cassidy Newland, a conservation officer and friend, for gathering and organizing copious amounts of data in a couple of days which would have taken me months to collect on my own. Also, I would like to thank Ian Wilson (Willow), Brain, Chris (Mad Macedonian), Wayne (Foxy), Zoey (Zo) and Collin (Col) for being so patient with me and helping me collect data for my habitat surveys. Also thank you Marika and Robyn for helping me settle into the Kingston Home and for their willingness to help me with anything I needed help with. I also have much gratitude to the numerous volunteers who took time out of their own schedules to assist in collecting trapping data for the Woylie Translocation Project. Also thank you to the various partners and sponsors for supporting and funding this Woylie Translocation Project. I want to thank Simon Evans, a post-graduate student from Oxford, England for giving me valuable guidance with the statistical analysis of my data.

I would also like to extend my appreciation to Tony Cummings for supporting through the entire ISP process and helping me get to my final destinations. Also, thank you Maria Rubinsky for traveling so far with me and being a part of my unforgettable journey to Western Australia.

I'll be forever grateful to the entire Science Division Crew at the Warren District Department of Environment and Conservation (D.E.C)- you have all helped make my experience in Western Australia that much more meaningful and memorable.

Introduction:

1.1 Woylie Ecology and Habitat Preference

The woylie (*Bettongia penicillata ogilbyi*) is a nocturnal rat-like kangaroo. It is commonly called a brush-tail bettong and woylie refers to its name from the Nyoongar, indigenous south west Australians. Other common and indigenous names include Brush tailed Bettong, Rat Kangaroo, Brush trailed Rat Kangaroo, Woylyer, and Karpitchi (De Torres 2008). There are two currently recognized subspecies *Bettongia pencillata ogilbyi* which populates south west Australia and has been reintroduced into parts of South Australia and New South Wales and *Bettongia pencillata pencillata* which is an extinct woylie originally endemic to south-eastern Australia (Department of Environment and Conservation 2002). The woylie has a brown dorsal side and a yellow-grey ventral side with a black crest on its tail which is the meaning of *penicillata* ('black crest') (Troughton 1941). When the woylie moves, it bounds with its head held low, back arched and the tail is held straight behind it. The tail is used for digging and collected nesting materials. The woylie diet consists of tubers, bulbs, and grain/seeds and sometimes resin exudate from *Hakea* species (De Torres 2008). Woylies are typically known to be mycophagous, feeding primarily on underground fruiting bodies of ectomysorrhizal fungi (Christensen 1980, Lamont et al. 1985, Malajczuk et al. 1987, Claridge & May 1994). A main part of their diet includes a common native truffle of south west Australia called *Hysterangium* (Mitchell *et al* 2008). The digging involved in finding these truffles, increases water infiltration and traps seed and organic matter which aids in dispersing fungal spores and seeds (De Torres 2008). These various contributions to significant environmental services hails the woylie as a keystone species to the Jarrah woodlands of south west Australia (Garkaklis 2001).

Different sources note the *bettongia penicillata ogilbyi* lives in various habitats. In a population of *B. penicillata ogilbyi* in Western Australia, scrub density and bare ground were identified as important characteristics of the preferred habitat for this species, with animals absent from open areas and areas with extremely dense ground cover (Christensen 1980). Woylies are known to inhabit areas where *Xanthorrhoea* (grass trees) (De Torres 2008), *Gastrolobium* (Christensen 1980; Mitchell 2008), various types of *Leucopogon* (Christensen 2008), *Hakea* (Straham 1984), *Pteridium esculentum* (bracken fern) and *Bossi ornata* (A. Wayne pers. comm.). Woylies use these types of vegetation for nesting, nutrients, and protection. *Pteridium esculentum* (bracken fern), *Bossi ornata, and Gastrolobium* are thought to be classic indicators of a good woylie habitat (A. Wayne pers. comm.).

1.2 Woylie Population Declines

Evidence from Aboriginal people and early settlers' records suggest the woylie was abundant throughout most of Australia as they once occupied south-eastern Queensland, eastern and southern New South Wales, western Victoria, most of South Australia, much of the Northern Territory outside the Wet Tropics and the south-west of Western Australia. In the 1880's woylies were quoted to be 'swarming'. In the early 1900's the number of woylies in South Australia were so high, people sold them by the dozen for coursing (hunting) using greyhounds (Wood-Jones 1925). Within the last century woylies have had numerous peaks and lows in their abundance (Figure 1).

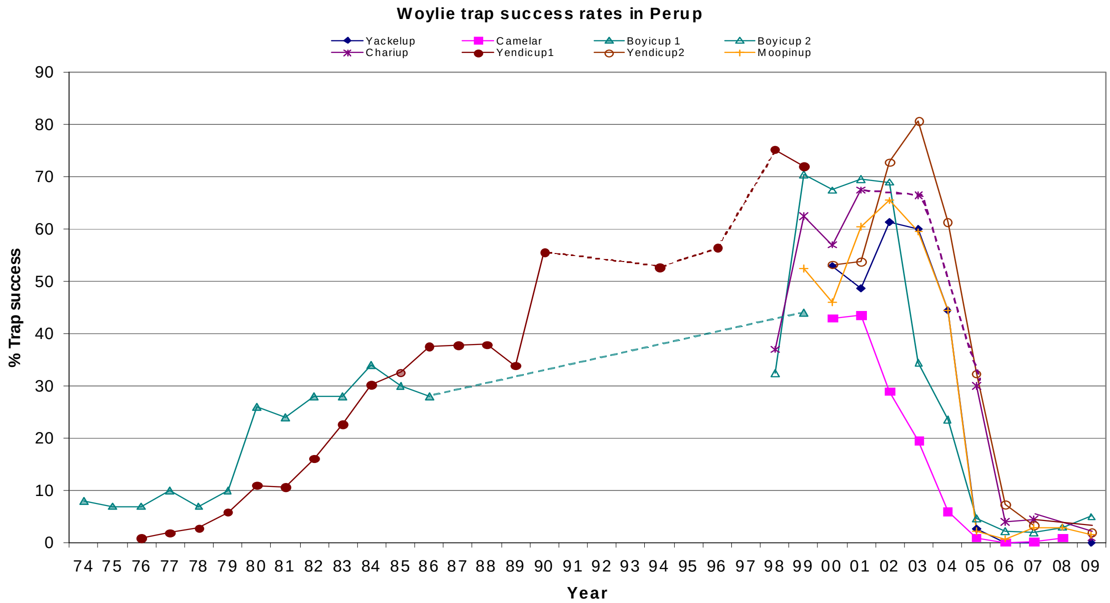


Figure 1: Trap success of woylies from the first population decline in the 1970's and second population decline, in the early 2000's.

In the 1970's a new interest in the woylie populations was sparked by a Conservation and Land Management (C.A.L.M.) scientist, Per Christensen. Christensen determined exotic diseases, land clearing and the spread of the introduced fox led to accelerated declines and by the 1960's, the woylie became restricted to three areas in southwestern Australia ---Dryandra, Tutanning and Upper Warren (Christensen 1980). In 1973, the woylie was listed as a threatened species in Western Australia (Wayne J. 2010). It was suspected that foxes were a factor in the decline of woylie populations. In 1977, D.E.C. and C.A.L.M. started a fox baiting program using 1080. After the fox baiting program started, a dramatic increase in the numbers of woylies and other medium sized marsupials was observed in 1996 (Wayne J. 2010). The recovery of the woylie was hailed as a resounding success. Populations of this native marsupial had recovered to the extent that it was removed from both State and Commonwealth threatened species categories-the first species in Australia to be de-listed. The woylie became the ‘pinup’ animal for other conservation efforts throughout Australia (Mitchell *et al* 2008).

But in 2001 as the population was thought to continue increasing, woylie numbers were observed to have once again to plummeted. Statistical analysis’ predicted that populations declined by 93 per cent at Dryandra, 95 per cent in Upper Warren River Catchment east of Manjimup and 97 per cent at Batalling (Mitchell *et al* 2008). Overall, the woylie population has declined by more than 80 per cent since 2001 (Wayne J. 2010) as the number of woylies have decreased any where from 25 to 95 per cent each year. Currently there are estimated to be less than 1,300 woylies remaining within the last four local populations- Perup, Kingston, Dryandra and Tutanning (Wayne J. 2010). As a result, in 2008 the Western Australia Wildlife Conservation Act re-listed the woylie as “*fauna that is rare or likely to become extinct*” as was written in the Wildlife Conservation Act of 1950 (Department of Environment and Conservation, “Minimum Standards” 2008). The Australian Federal conservation status has the woylie listed as *Endangered* and the World Conservation Union lists the woylie as *Critically Endangered*.

In response to these most recent population declines, the Department of Environment and Conservation established a recovery team and a project called the Woylie Conservation Research Project. The project speculates the most recent decline is due to several factors. One is the increased rate of adult mortality and whether reduced recruitment into the adult breeding population involved can not be established (Department of Environment and Conservation, Woylie Conservation Project Report 2008). Other possible causes of the recent population decline include a disease carried by to two parasites called *Toxoplasma* and *Trypanosoma sp. nov.* as well as the increased predation of feral cats (Department of Environment and Conservation, Woylie Conservation Project Report 2008). Although it is not certain, there may be a synergistic effect between the two parasites and various stressors are thought to trigger the disease (Department of Environment and Conservation, Woylie Conservation Project Report 2008). Climate and extreme weather conditions are also thought to be an agent of decline. Habitat loss, habitat modification, fire, direct human interference from trapping and lack of food resources have been dubbed as unlikely factors effecting recent population declines (Department of Environment and Conservation, Woylie Conservation Project Report 2008).

1.3 Woylie Translocation Project

In response to drastic woylie declines, government and national park groups have organized re-introduction or translocation projects. In 1983 the woylie was reintroduced and monitored in the Perup River Fauna Priority area and the same was done in 2001 in the Gammon Ranges National Park of South Australia. A successful woylie translocation project was conducted in the Karakamia Wildlife Sanctuary in 2008 (Richards et al 2009). The current Woylie Translocation Project run by the Department of Environment and Conservation aims to

> "translocate woylies (*Bettongia pencillata ogilbyi*) from wild populations within the Upper Warren Region (Greater Kingston and Perup areas) to the Perup Sanctuary, Perth Zoo and Malaga Native Animal Rescue in an attempt to establish and secure 'insurance' populations of the species and to further investigate the factors involved in the species decline" (Wayne J. 2010).

The goal of the project is to re-introduce at least 40 woylies into the Perup Sanctuary, 25 to the Perth Zoo and 8 to the Malaga Native Animal Rescue. The Perup Sanctuary was recently constructed for the purpose of securing an 'insurance population' of woylies. The translocation project is important to "securing critically important woylie populations from extinction" (Ordell *et al*., 2009). The sanctuary is a 400 hectare (ha) predator-free enclosure in a forest block of Yackelup which was home to a large woylie population prior to the population declines in 2005 (Wayne J. 2010). The individuals translocated to the Perup Sanctuary will be tagged with radio collars and monitored to determine whether the woylies have an increased survival rate. I was involved in the first three weeks of the translocation project. At the end of three weeks 27 woylies were successfully translocated to Perup Sanctuary, 7 to Malaga and 3 to the Perth Zoo (e-mail from Adrian Wayne 1/12/2010).

1.4 My ISP

My Independent Study Project (ISP) involved me volunteering for three weeks with the Warren District Department of Environment and Conservation during the Woylie Translocation Project. My work lead me to the Shire of Manjimup in Western Australia and into the Bush, or forest, of the Upper Warren Region. I was a nomadic member of the trapping teams. I helped find, measure, GPS record and clear various transects. I also helped teams set, collect and clean traps . During the translocation project, I helped trap and process woylies and other species such as brush-tail possums, chuditchs, bobtails and quendas. In the field, I collected data on habitat surveys and tree basal areas. At the end of each trapping day, I recorded all the data collected at each transect and infiltrated it into my study. Throughout the course of three weeks I reviewed literature about woylies and the various factors affecting their population declines. I used both field data and various woylie resources to investigate the question of: *What factors may be affecting post-decline Woylie (Bettongia penicillata ogilbyi) abundance in the Upper Warren Region of South West Australia?* The possible factors studied included habitat surveys used to observe any habitat preference, the presence of other small mammals, distance from paddocks and private property, vegetation group, landscape, and category of forest.

Methods:

2.1 Transects, Sites and Trapping

Woylie trapping for the translocation project officially begun on November 8th, 2010 and will continue for 8 weeks. Multiple transects were established throughout the Upper Warren Region (in the Greater Kingston and Perup areas) which were set at least 1-2 km away from the already existing Upper Warren woylie population monitoring sites. This was done to maximize genetic diversity and capture woylies in areas suspected to have high population densities. Each trapping transect was at least 10 km in length with 50 trap sites, 2 m apart. Fifty Sheffield wire cage traps and a peanut butter-based bait was used at all sites. During the three weeks I volunteered, multiple trapping transects were run concurrently. Each site was trapped for up to four consecutive nights. At 5:30 A.M, four to five teams (made up of a scriber and an animal handler) drove out to a designated transect, collected and recorded all trapped animals before 10 A.M. All animals captured, including woylies and non-target species (i.e. Common Brush-tail Possums and Southern Quolls), were processed and recorded with standard trap monitoring protocols. These protocols include recording species, weight, sex, breeding condition, age, standard body measurements and individual identifiers (i.e. ear tags). Woylies that were not female with dependent young, were processed, recorded and sent to the Perup Ecology Center for further processing. This collected data will be used to explore how woylie populations may be effected by the presence of brush-tail possum and chuditch populations.

2.2 Habitat Surveys

Many studies involving woylie population declines have involved habitat site-vegetation studies or habitat surveys. Per Christensen did a habitat site-vegetation study in the 1980's involving woylies and tammars. He hypothesized woylie populations are "centered around certain site types which may be defined by distinctive plant associations" (Christensen 1980). Per Christensen did habitat surveys to determine if suitable habitats was an important factor in woylie abundance and distribution. Christensen's methods included a principal component analysis, (P.C.A.), to reveal any associations between the study species and site-vegetation types. A P.C.A. was also done in this study to analyze any associations present between woylies and the basal areas of Jarrah (*Eucaylptus marginata)*, Marri (*Eucalyptus camaldellensis)*, Wandoo (*Eucaylptus wandoo)* and other species identified in the 13 transects.

Over the course of three weeks, I did habitat surveys for all 13 trapping transects at all 50 sites equaling 650 data points at the end of the study. The forests of the Upper Warren Region are mostly Jarrah and Marri Forests with low under story scrub, and open Wandoo woodland. Tree basal area measurements using a Factor 2 prism were made for Jarrah (*Eucaylptus marginata)*, Marri (*Eucalyptus camaldellensis)*, and Wandoo (*Eucaylptus wandoo)* trees as well as other species identified such as Flooded Gum (*Eucalyptus grandis)* and *Bull banksia* trees. The amount of prism contacts were recorded and these numbers were multiplied by two to give the value of m^2/ha. I also recorded which vegetation was present in both the middle and lower canopy of the forest. One part of Christensen's study involved him taking note of the density and sparseness of vegetation present. Density and sparseness in my study was also noted, but inconsistently and only in when very obvious density and sparseness was observed. Obvious can be denoted as 'dominant flora covering 40-60% of the ground.' The soil color and type (brown/red/grey/yellow and sand/gravel) was also noted, but not used in the final analysis.

2.3 Other Possible Factors

At every trap site, the different types of vegetation were recorded. After all the vegetation was recorded, eight dominant types of vegetation was chosen out of the habitat surveys completed at each transect. These eight types of vegetation were chosen due to the *Bossiaea ornata, Eucalyptus wandoo, Hakea, Gastrolobium bilobum, Leucopogon capitalatus, Leucopogon juniperinus, Pteridium esculentum* and *Xanthorrhea preissii.* Each of the eight floral types were given one mark as a means to denote that the vegetation was present. The other data including landscape, vegetation group, category of forest and the distance from paddock was collected by a colleague using a variation of computer programs and DEC corporate data bases. All trap sites were matched up using an ARCH Pad GPS system and ARCH view GSI which are E.S.R.I. programs. All 650 GPS points were matched up with DEC Corporate data bases.

During analysis, the data was separated into sites that did and did not trap woylies. They are classified as captured and non-capture sites. This was done to determine any trends or patterns in the possible factors affecting woylie abundance.

Results:

3.1 Habitat Surveys

Per Christensen's P.C.A. run on his habitat site-vegetation studies revealed woylies favored sites with a variety of vegetation including *Leucopogon capitellatus, Eucalyptus marginata,* a variety of *Hakea* and other varieties of *Leucopogon* present in well drained areas and deep soils. The P.C.A. run for this study (Table 1) reveal woylies had a negative correlation with transects with high basal areas of Marri, Wandoo and Other identified species and a positive correlation with those areas consisting of a high Jarrah basal area.

	PC1	PC2	PC3	PC4
Jarrah	0.5304	-0.33589	0.57979	0.51932
Marri	0.48211	0.34981	-0.65494	0.46505
Wandoo	-0.61634	-0.42104	-0.23705	0.62182
Other	-0.32614	0.76651	0.42274	0.3569

Table 1: P.C.A. results for Tree basal area of Jarrah, Marri, Wandoo and other species identified.

In Figures 2 and 3 it reveals the amount of each vegetation recorded at sites where woylies were and were not captured. The results of this particular study revealed woylies were captured in sites with high levels of *Leucopogon capitalatus*, *Hakea* and *Bossinea ornata.* Where woylies were not caught, high levels of a varying type of *Leucopogon*, *Leucopogon juniperinus* was observed. The ground cover density of various types of vegetation was recorded as dense in different transects (Table 2).

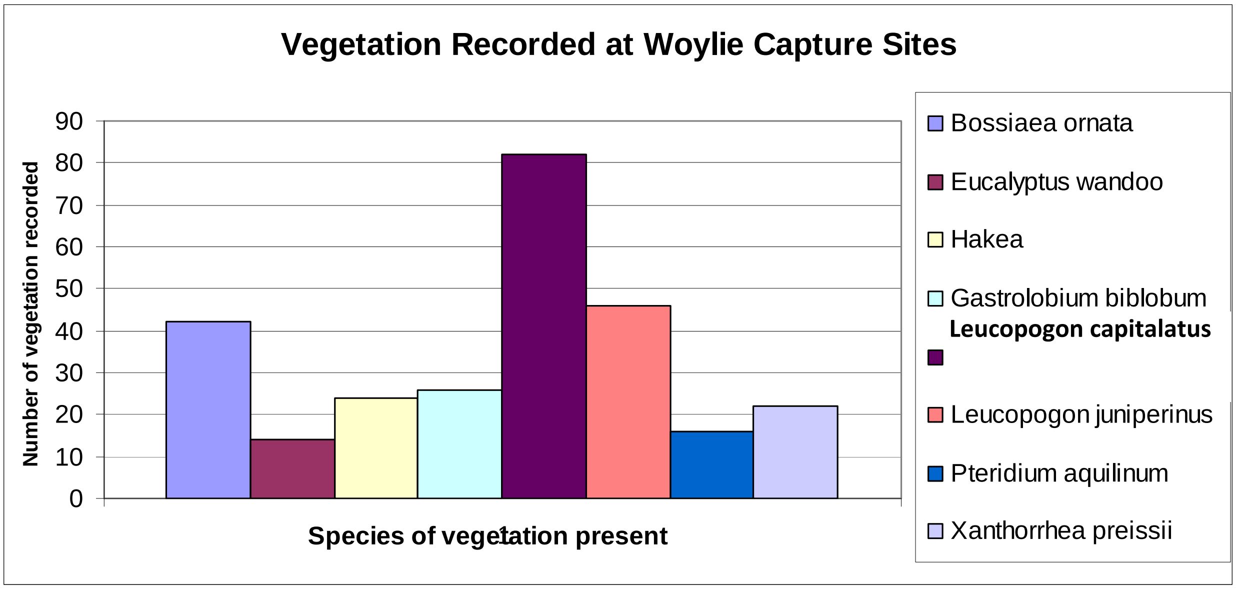


Figure 2: Graph of the 8 dominant vegetation species present at woylie capture trap sites.

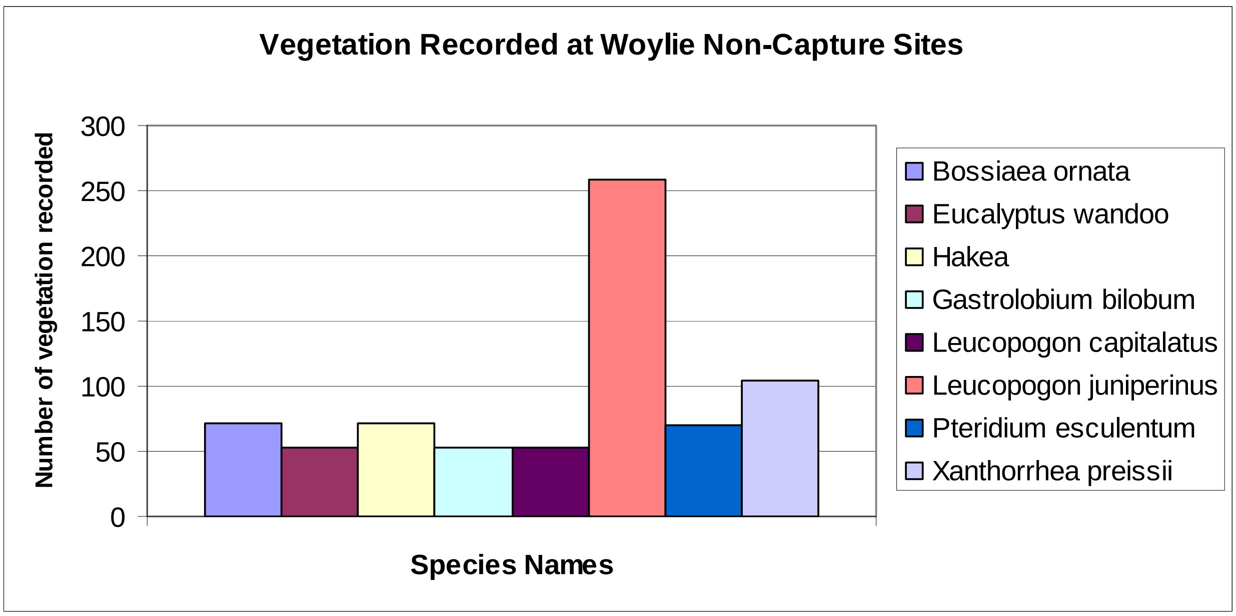


Figure 3: Graph of the 8 dominant vegetation species present at woylie non-capture trap sites

Vegetation Type	**Coverage**	**Transect Name**
Leucopogon capitalatus	Dense	Dwalgan, Corbal and Boyicup 2 and Yackelup 3

Gastrolobium bilobum (heartleaf)	Dense	Kenninup 2
Xanthorrhea	Dense	Kingston 9
Pteridium esculentum (bracken fern)	Dense	Coonan, Coonan 2, Corbal
Bossiaea ornata	Dense	Dwalgan, Boyicup 2, Yerramin

Table 2: Dense vegetation at various transects. Notice that Dwalgan and Boyicup 2 have high densities of *Leucopogon capitalatus* and *Bossiaea ornata.*

3.2 Presence of Other Small Mammals

Figure 4 displays how many woylies, brush-tail possums and chuditchs were caught in three weeks at all 13 transects. Generally transects where high numbers of woylies were trapped, trapped fewer brush-tail possums and fewer or equal numbers of chuditchs. The amount of brush-tail possums trapped were more than double the amount of woylies trapped. Overall, the brush-tail possum had the greatest abundance in all 13 transects.

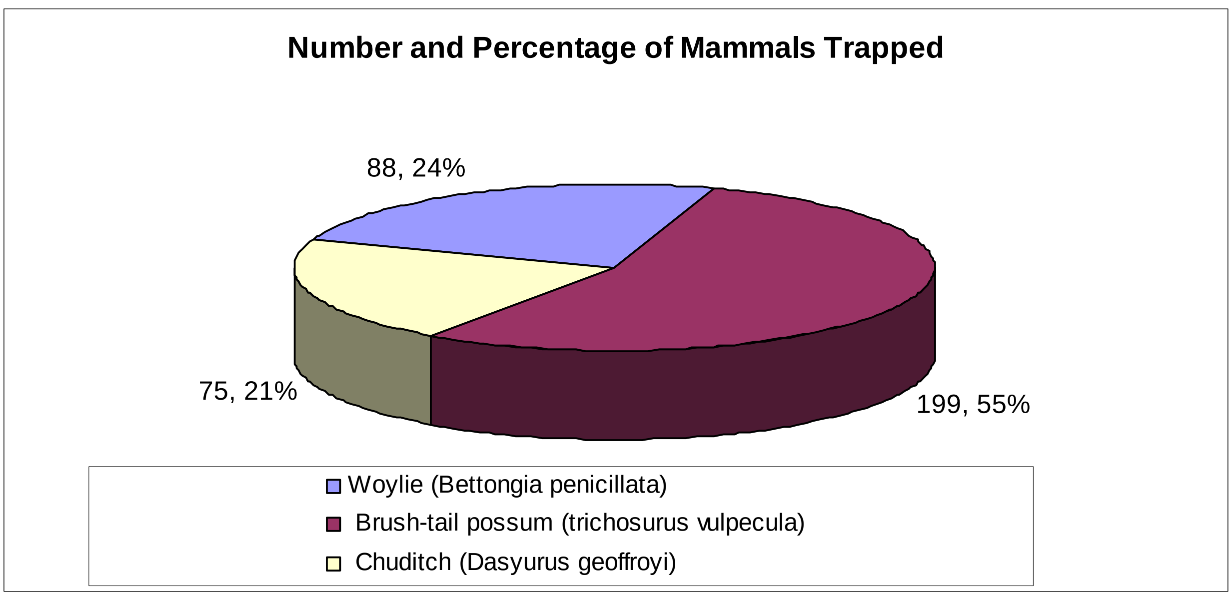


Figure 4: Pie chart showing how many woylies, brush-tail possums and chuditchs were caught in all 13 transects.

Transect Name	Woylie (Bettongia penicillata oygli)	Brush-tail Possum	Chuditch
Balban 2 (BAL2)	4	6	17
Boyicup 2 (BOY2)	19	12	1
Coonan (COO)	0	5	4
Coonan 2 (COO2)	0	12	1
Corbal (CORBAL)	7	34	6
Dwalgan (DWAL)	28	12	4
Kenninup 2 (KEN2)	5	16	12
Kenninup 3 (KEN3)	8	23	1
Kingston (KING)	3	25	14
Meribup (MER)	3	9	1
Yackelup 2 (YAK2)	1	13	2
Yackelup 3 (YAK3)	1	14	5
Yerramin (YER)	9	18	7
TOTAL	88	199	75

Table 3: Total number of mammals caught at each transect. Dwalgan and Boyicup 2 are in bold to emphasize the high number of woylies captured at that site.

3.3 Distance from paddocks

A great majority of the transects (~43%) were within 100-500 m to a paddock or some form of private property (Figure). 41% of the woylies captured were within this same distance from paddock.

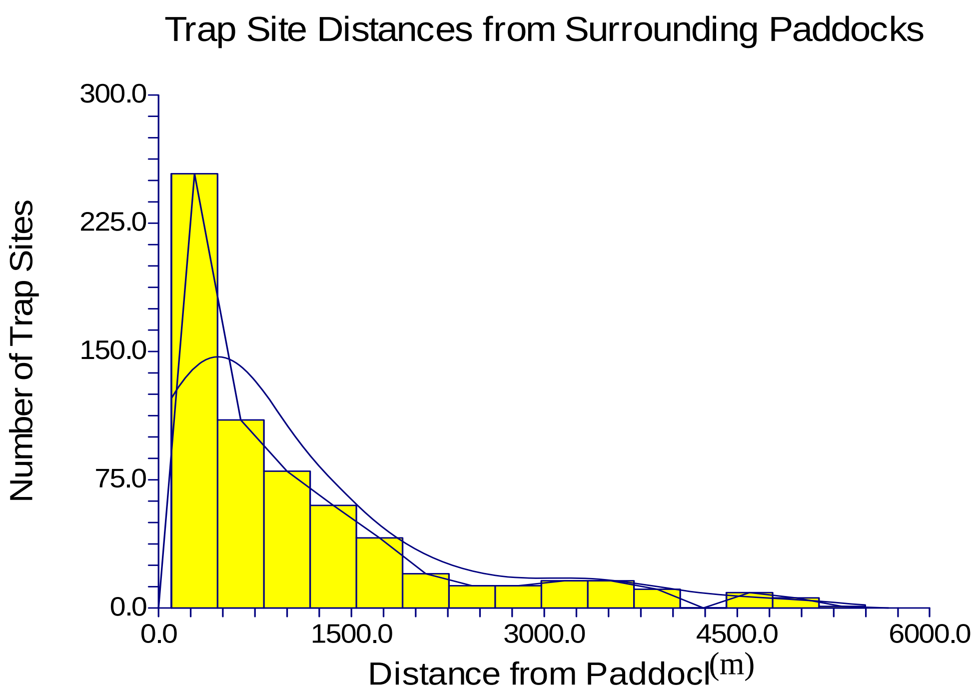


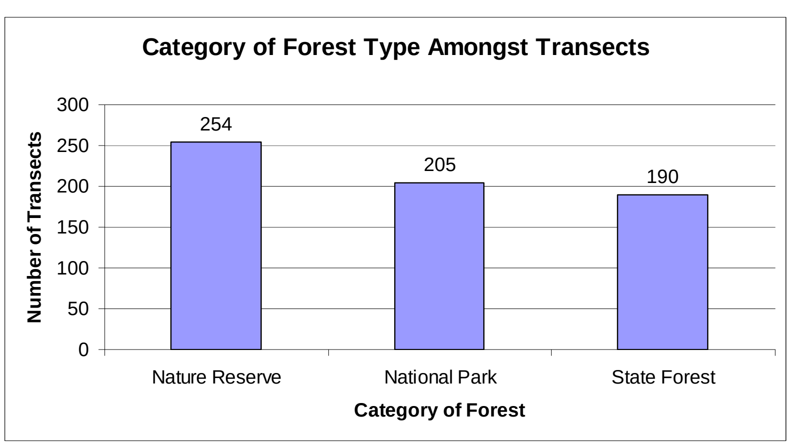
Figure 5: Chart displaying the distances each trap site was from paddock or private property.

3.4 Vegetation Groups

The vegetation groups of each trap site fell underneath the category of depressions/swamps, Jarrah uplands, Jarrah Valleys, Wandoo uplands and Wandoo Valleys. As was previously stated, the data was separated into sites that did and did not trap woylies. They are classified as captured and non-capture sites. The per cent of each vegetation type was calculated for capture and non-capture sites. They are the following: Captured sites: Depressions/swamps: 29%, Jarrah uplands: 43%, Wandoo uplands: 8.3 %, Wandoo valleys: 15.7% Non-captured sites: Depressions/swamps: 22.1%, Jarrah Uplands: 34.9%, Jarrah Valley: 11%, Wandoo uplands: 12%, Wandoo valleys: 18.8%

3.5 Landscape

Figure 6:



Simple bar

graph showing the how many transects were in each category of forest.

The landscapes included depression, uplands and valleys. The percentage of these land forms present in woylie capture and non-capture sites was calculated. The results are the following: Captured: Depression: 29%, Uplands: 48.6%, Valleys: 19.4% and Non-captured sites: Depressions: 29%, Uplands: 48.6%, Valleys: 19.4%.

3.6 Category of Forest

There were three different categories of forest including National Park, Nature Reserve and State Park (Figure) Again, the percentage of these categories were calculated for woylie capture and non-capture sites. The results are the following: Captured: National Park: 53%, Nature Reserve: 38.8 %, State Forest: 5.5% and Non-Captured: National Park: 33%, Nature Reserve: 36%, State: 31%.

Discussion:

4.1 Habitat Surveys and Habitat Preference

The results of my habitat surveys and tree basal areas revealed similar results to Per Christensen's habitat site-vegetation studies. Both Christensen's and my study revealed that woylies are found in habitats with high levels of Jarrah, *Gastrolobium bilobum* and *Leucopogon capitalatus,Bossi ornata* and *Hakea*. The results of my study are evidence that woylie habitat preference is the same as it was 30 years ago.

4.2 Presence of Other Mammals

During the translocation project trapped other mammals including brush-tail possums (*Trichosurus vulpecula*) and chuditch also known as Western quolls (*Dasyurus geoffroyi*). The abundance woylies, brush-tail possums and chuditch are all low to moderate in the Upper Warren Region (Wayne J. 2010). The fox baiting program established in the 1980's saw in increase in all Upper Warren small mammal populations, especially the woylie and the chuditch (Start *et al.* 1996). The chuditch, like the woylie, used to populate about 70% of Australia but is now reduced to the South West corner of Australia. The chuditch is a natural predator to the woylie but its main diet consists of invertebrates and plants. According to the data collected in this study, chuditch numbers were either equal or less than woylie numbers. Therefore, chuditch do not seem to be a major factor in the post decline woylie populations.

Usually the densities of small mammal populations have tended to fluctuate together, but recently a new trend as arisen between woylie and brush-tail possum abundance. It has been observed that when woylie populations are low, brush-tail possum populations tend to increase (A. Wayne per. comm.). This trend was evident in the trapping data collected during the translocation project. There is no published literature discussing this recent trend, but it may be due to the brush-tail possums' ability to adapt to a variety of habitats ranging from forests, woodlands, ground hollows, hollow logs, ground with no trees and cities (How 1995) therefore adding an element of competition to woylies in preferred habitats.

4.3 Distance from Paddocks

A recent study in the Upper Warren Region involving Western Ring-tail possums (*Pseudocherius occidentalis*) found an increased abundance associated with cleared land that was agricultural or an exotic tree plantation. A variety of animal populations across the world have had increased abundance due to increases in forest edges (Wayne 2006). This is thought to be explained by the selective nature of agricultural development (Finlayson 2008; A. Wayne per. comm.). Cleared land for agriculture is generally picked due to its close proximity to water sources and good soil quality. The high percentages of woylie captures were close to paddocks and some woylies upon release were observed to retreat into blue eucalypt farms. Studies of the wildlife values of blue eucalypt plantations concluded that blue gums provide a habitat or resources for a range of species (Lindenmayer 2004) including woylies which helps explain the high percentage of woylie capture sites near paddocks.

4.4 Vegetation Groups and Landscape

The majority of woylies caught were in the Jarrah uplands and depression/swamp vegetation groups and in upland landscapes. Christensen's study found woylies prefer well drained areas and deep soils which consists of uplands and depressions. Woylies are generalists in that they prefer drier open woodlands which include upland Jarrah forests. Therefore, this data is further evidence that woylie habitat preference remains the same.

4.5 Category of Forest

The Conservation Commission of Western Australia is the vesting body for State forest, national parks, conservation parks and nature reserves and the Department of Environment and Conservation (D.E.C.) (Australia's Forest 2009). In 2001, the Forest Management Plan 2004-2013 (F.M.P.) was established by the State Government of Western Australia. With the F.M.P. the State Government implemented the *Protecting our old-growth forests policy* which brought an end to logging in all old-growth forests managed by DEC. These areas include the National Park areas of Dwalgan and Perup which were transects included in the translocation project. These areas were chosen due to the high abundance of fauna, such as woylies, while Nature Reserve and State Forest have lower fauna abundance. This explanation may give the impression logging is a major effect of low woylie abundance, but many studies have proved logging is not a major factor in woylie population declines (A. Wayne per. comm.).

Conclusion:

In conclusion, the study did not verify any of the possible factors to be a main factor affecting woylie abundance. The study did ensure that woylie habitat preference has not changed and remains to be arid upland habitats consisting of Jarrah. Also, the collected data verified woylies continue to prefer ground vegetation consisting of *Bossiaea ornata, Hakea, Gastrolobium bilobum, Leucopogon capitalatus,* and *Eucalyptus marginata* (Jarrah). Woylie abundance may be affected by competition with brush-tail possums for preferred habitats. Competition may be amplified in areas where woylie habitat preference is enclosed or bordered by a variety of habitats suitable for possums.

The transects with the highest numbers of trapped woylies were Dwalgan and Boyicup 2. Both of these transects contained habitats preferred by woylies. There was not an immense amount of trap sites in Dwalgan and Boyicup 2 that were close to paddocks but it important to note that about 50% of the trap sites in Dwalgan and Boyicup 2 were 100-500m away from paddocks. Another interesting observation is all Dwalgan trap sites were in National Park while Boyicup 2 trap sites were predominantly in Nature Reserve. Perhaps woylie abundance in Boyicup 2 are recovering in Nature Reserves where abundance is thought to be low.

Although no factors led to any definite conclusions, none of the studied factors should be completely disregarded. The collected data may have been more useful if a more in-depth statistics analysis was conducted. In the end, looking at multiple factors is important especially when developing a successful conservation plan for endangered species. Many studies that investigate multiple factors affecting rare animal abundance, speculate many factors tend to have a synergistic effect on the abundance of the threatened species. The Department of Environment and Conservation woylie translocation project plans to do habitat surveys for the remaining trapping transects and sites so as to investigate further whether woylies have changed their habitat preferences. Currently, the main factor is most likely due to disease and increased mortality of adult woylies.

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INSTITUTE OF BIOLOGICAL, ENVIRONMENTAL AND RURAL SCIENCE

University of Wales
Aberystwyth

Evaluation of conservation measures for a specific endangered species

Bettongia penicillata

Jon Paul McCalmont

11/23/2010

An exercise in mathematical population modeling and its relationship to the conservation of an endangered species. Carried out as part of a 3rd year BSc (Hons) in Countryside Management

Contents

Bettongia penicillata ogilbyi (Brush Tailed Bettong, Woylie)

Class – Mammalia

Order – Diprotodontia

Suborder – Macropodiformes

Family – Potoroidae

Genus – *Bettongia*

Species – *penicillata*

Description and Ecology

Woylie is the Nyoongar aboriginal name for *Bettongia penicillata*; also called the brush tailed Bettong; this is a small, ground dwelling, nocturnal marsupial growing to approximately 40cm long and weighing up to 1600g (Claridge et. al. 2007). It belongs to a group of animals sometimes known as "rat-kangaroos" and fulfils an important role in the ecology of the regions in which it lives. Once widespread across Australia, it distribution is currently much reduced, primarily to the eucalypt woodlands of southwestern Australia. This is an area of seasonal wet and dry, comparable to a southern European Mediterranean climate; soils in these regions are generally lateritic and can become very impermeable to water and nutrient poor during the dry season with attendant problems of water runoff and poor penetration in wet periods. The Woylies habit of digging for the sporocarps of hypogeous fungi, which comprise the major part of its diet, provides a more broken topsoil which helps both with topsoil health/biodiversity (Martin 2003) and infiltration of water into the subsoil (Garkaklis et.al. 1998) though there is also some evidence that some of their subsoil tunnels may create localised water repellent patches (Garkaklis et.al. 1999). The foraging activities of this animal also help with re-establishment of vegetation after the regular bush fire events common to this region (Lamont et.al. 1985). Large numbers of ectomycorrhizal fungi spores were found in faecal material; when pellets of Woylie faeces were applied to important local tree species, *Eucalyptus calophylla* and *Gastrolobium bilobum* they showed marked increases in growth rate, control studies demonstrated that stratification through the gut of the Woylie appeared to be the usual pre-treatment for this process. Seed caching by Woylies was also shown to be helpful in tree distribution, demonstrated in sandlewood species by Murphy et. al.(2005). Mitochondrial DNA (mtDNA) analysis (Pacioni et.al. 2010) has shown that there was, historically at least, long distance interbreeding, upwards of 150km between populations, demonstrating the vagile nature of this species; however, habitat fragmentation has made this process increasingly unlikely now as populations have become more isolated.

Current conservation status, threats

This small marsupial species has led a chequered life since European habitation of Australia, very nearly joining the estimated 50% of all the world's mammal extinctions that have occurred in Australia in the last 200 years (Short and Smith 1994). Once widespread it was decimated by habitat destruction as native forests were cleared to produce farmland, compounded by introduced predators such as cats and red foxes; by 1975 only three natural populations remained; the Dryandra woodlands and the Tutanning and Perup (Upper Warren) nature reserves and it was considered extinct from central Australia. However, through conservation efforts such as Western Shield (DEC 2010a) to exclude non-native predators (fencing and poison baiting) and re-introduction programmes the Woylie recovered to a great extent and became the first species to be removed from the Commonwealth Endangered Species Act 1992. Unfortunately though, since 2001, monitoring of trapping rates for Woylies combined with population modelling, began to indicate numbers undergoing a huge decline; up to 95% in its areas of highest abundance and around 80% across its entire range (Figure 1).

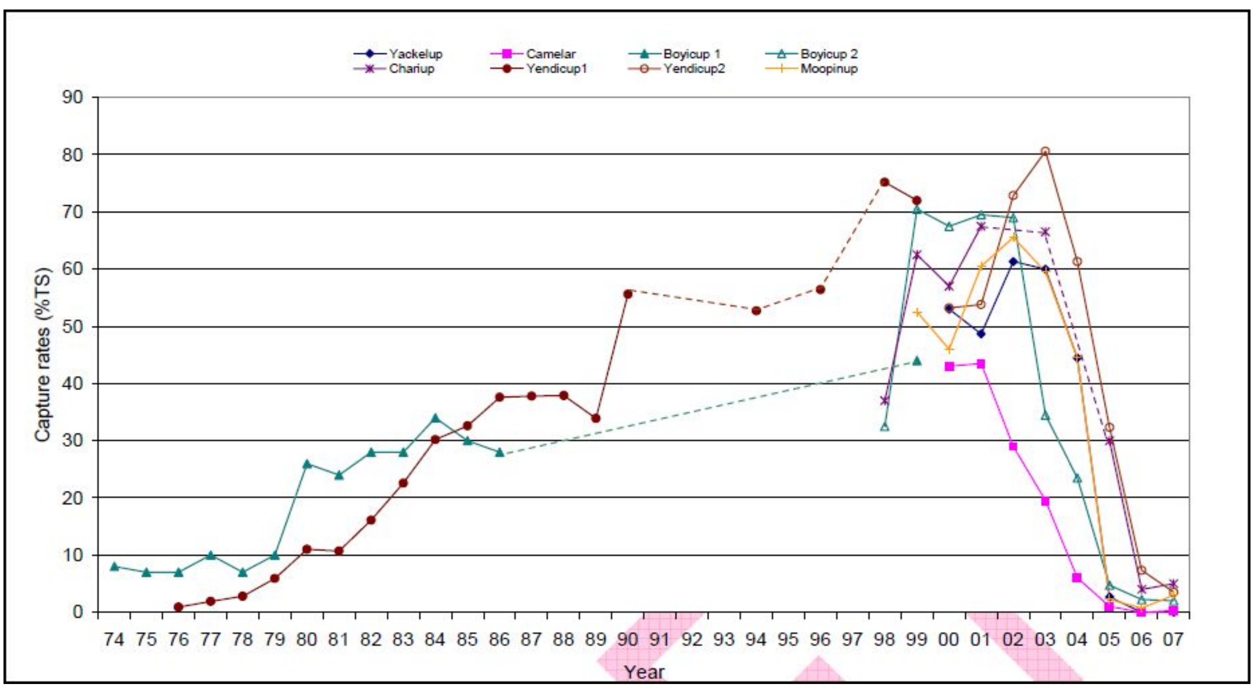

Figure 1 ***Capture rates of Woylies in Perup nature reserve (Upper Warren)*** **(WCPR 2010)**

In 2008, the Woylie was relisted as endangered under the State Wildlife Conservation Act 1950 (DEC 2010b) and it is listed on the IUCN red data list as critically endangered (IUCN 2010). Areas of its highest density, and seeming successful reintroduction, have shown the most acute declines and five areas of the Upper Warren region (Keninup, Balban, Warrup, Boyicup and Winnejup) were chosen for the main investigative effort. One area of high density Woylie population, Karakamia approximately 200km north of the Upper Warren (Karakamia 2010), has remained free of any signs of decline. This 275 ha sanctuary has a predator-proof fence around its entire perimeter and provides an excellent comparison population operating at its carrying capacity.

Conservation effort and policy

The major part of the current conservation effort is given to studying and understanding the mechanisms of the acute population declines of the last decade. The Woylie Conservation Progress Report (WCRP 2010) is the result of a huge collaborative effort both within the Australian Department of the Environment and with outside academic institutions such as Murdoch University in Perth; figure 2, below, shows a draft summary of the findings of these investigations. As can be seen, the primary hypothesis suggests that disease, spread through the faecal material of introduced predators, may be the ultimate agent of decline. It would appear that possible synergistic effects of infection by *toxoplasma* sp. and *trypanosoma* sp. nov may be leading to a reduced fitness in the Woylies resulting in them being far more susceptible to predation, particularly by cats. However, it remains unclear whether predators such as cats and foxes are taking advantage of moribund dying animals or whether these animals might have recovered given the chance.

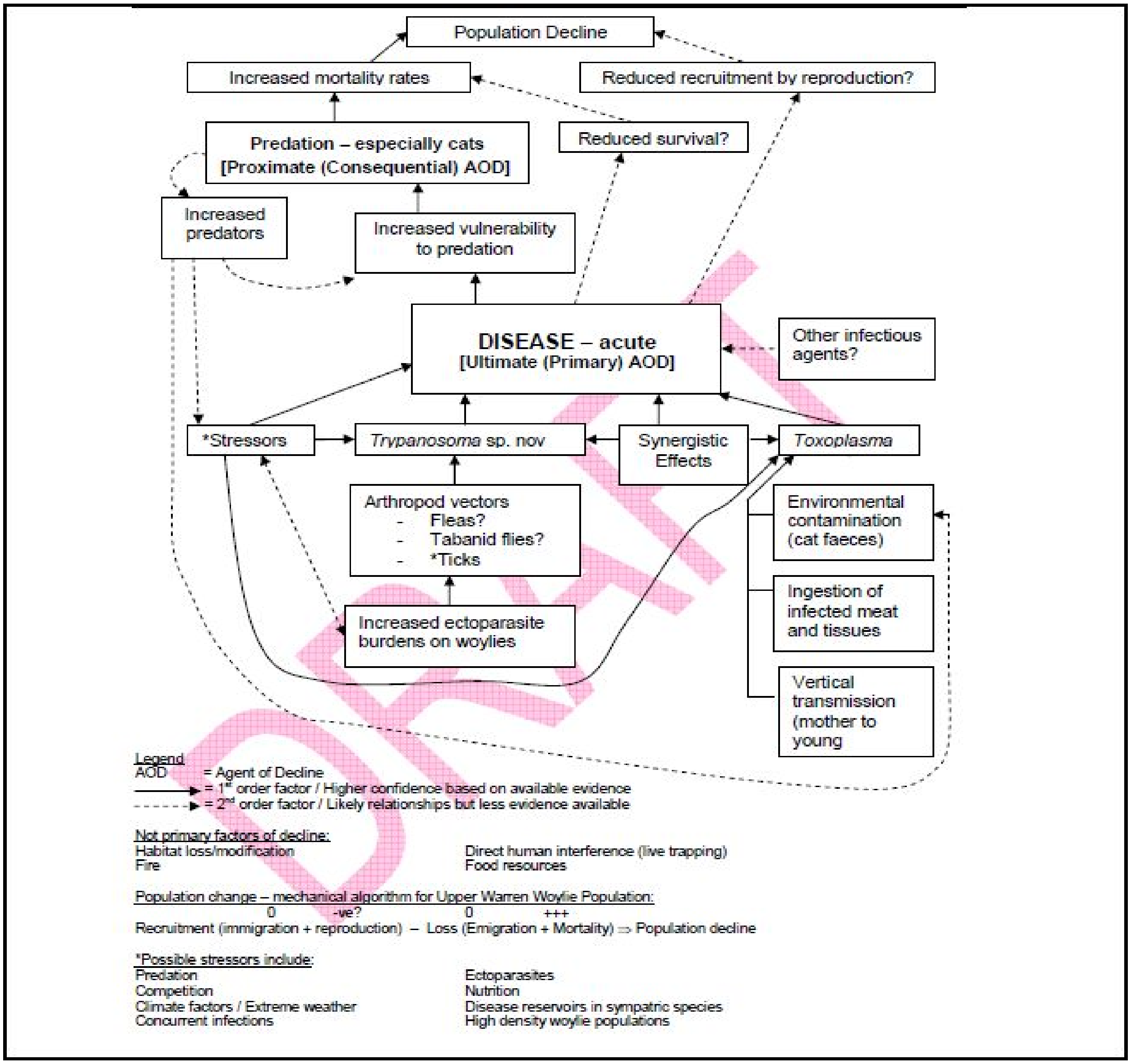

Figure 2 ***Schematic showing the leading hypothesis of causes of Woylie declines in the Upper Warren region; based on preliminary and untested inferences.*** **(WCPR 2010)**

Given that the main contributing factors seem to be directly linked with introduced predator species, both as predators and as disease vectors, the main practical conservation effort must lie with control, or preferably total exclusion of these species. Beyond developing increasingly sophisticated monitoring programmes to identify specific areas of Woylie ecology to target, the continuation of the Western Shield predator control programme forms the main focus of conservation effort. In their official conservation advice (Env. 2010), the Australian government also recommend reintroduction programmes and management of fire regimes to consider the importance of understorey cover for these and other ground dwelling species along with efforts to raise public awareness.

Population model

The population model will be concerned with the five areas of the Upper Warren region (Keninup, Balban, Warrup, Boyicup and Winnejup) and will assume an estimated starting population.
Data for inclusion in this model has been taken from the draft edition of the progress report from the Woylie Conservation Research Project; produced by the Science Division of the Department of Environment and Conservation (WCRP 2010). An online link to this document can be found in the bibliography below and page numbers within the progress report for specific data results can be found in the text. Research for this study was undertaken primarily at the five areas of the Upper Warren region of southwestern Australia, with a comparative population (Karakamia) located further North; 50km east of Perth. Decline rates vary across the Upper Warren regions with Balban still undergoing acute losses and Boyicup/Winnejup numbers at such low levels that they seem to have fallen below a density dependent effect on the decline vector. The comparative population at Karakamia shows no sign of declines and Woylie populations seem be at carrying capacity for this closed area (p.142).

a. Initial population and age demographics (WCRP 4.2, Demographics)

Even though the Upper Warren actually consists of semi-discrete populations undergoing differing survivorship/declines rates, it was considered, for the purposes of this model, as one area with an initial population suggested, based on an estimate of a post decline population for the region. It has been suggested that, pre-decline, there were approximately 20,000 individuals present in the Upper Warren (p.96); decline rates since 2001 give a median of 95% (p.2) therefore an initial population for the model was set at 1000 individuals across the region.

Age demographics are very difficult to ascertain from the Upper Warren trapping study, very few sub-adults were trapped over the year, e.g. 4.2% of trapped Woylies at Keninup, 10 individuals, Warrup 2 individuals and the remaining three areas none at all (p. 126), possibly influenced by the very short time taken from emergence to maturity (30-60 days). Therefore, given these low sample rates it was decided to distribute the initial population across an estimated life span of up to six years (p. 131) by considering the initial population of 1000 individuals as the area under a triangle and distribute them across six intervals.

b. Adult survival (WCRP 4.3, Survival and Mortality)

Adult survival rates of *B. penicillata* were investigated in the Upper Warren over the year between July 2006 and June 2007, carried out by a radio collar survey of 58 individuals. These collars were mortality sensitive and monitored every weekday, mainly from a fixed wing aircraft. 21 of these individuals were found to be dead by the end of the study giving an average adult survival rate across the region of 63.79% However, when the Kaplan-Meir survival function was applied to the figures, considering population density, it was found that areas still suffering the acute population declines had a higher mortality rate than areas which had already undergone significant declines. This indicated that vectors of the very high population declines were density dependent and ceased operating after those populations reached ~5% of their initial size. For the purposes of this model it is assumed that initial adult mortality would be taken at 66.67%; the Balban rate (which was still undergoing acute decline) and reduced to 22.22%; the Keninup rate (post decline) once numbers reach 5% of initial population (p. 142).

c. Numbers of females (WCPR, 4.2, Demographics)

Sex ratios within trapped samples proved to be area specific, the Upper Warren region showed the M/F ratio averaging 2.1:1 whereas the high density comparison site at Karakamia showed 0.7:1 (P. 126). It was considered that while this did provide some indication of gender demographics, sample sizes were very low at 2 of the Upper Warren sites and this bias may be indicating gender specific behaviour with respect to density and trapping success. Therefore, for the purposes of this model it was considered that M : F will be 1:1.

d. Numbers of pouch young (WCPR, 4.2, Demographics)

Reproduction rates remained high at all Upper Warren sites, an average of 89% of trapped females was found to be carrying pouch young and this was aseasonal across the year (p.129).

e. Survival of pouch young to maturity (WCPR, 4.2, Demographics)

Observations in this study were said to be in broad agreement with earlier studies (Christensen 1980) which showed a relatively high survival rate for pouch young up to the age of emergence (100-110 days), put at 82-91% but an extremely low rate of survival through to maturity once emerged from the pouch, 11-15% (p. 131). The model will consider averages of these values.

f. Numbers of offspring per year (WCPR, 4.2, Demographics)

While it is not uncommon for captive bred animals to produce twin offspring a single joey per breeding cycle is more usual in the wild though more than one cycle per year is the norm. Reproduction rates in the Upper Warren do not appear to be affected by seasonal variations as do the Karakamia populations whose reproduction rates drop significantly during the much hotter summer months. From birth to sexual maturity is assumed to be an average of 150 days; time spent in the pouch is between 100-110 days and from emergence to sexual maturity is between 30-60 days (p.131), giving the opportunity for at least 2 breeding cycles in each year. Assuming one offspring per cycle and aseasonal reproduction; the model incorporates 2 generations of offspring per adult per year with the first of these generations also having opportunity to breed in the same year. Again assuming 1:1 sex ratio in the offspring this gives 0.5*1st generation female with 89% of these likely to be breeding, therefore combined offspring going forward to next year = survival to maturity*2.45.

Model Parameters

Estimates of demographic survival rates

Age Distribution *Initial population of 1000 individuals*

1 yr = 305.57	4 yrs = 138.90
2 yrs = 250	5 yrs = 83.35
3 yrs = 194.45	6 yrs = 27.76

Adult Survival

Initial = 33.33%

Post population decline to 5%, survival = 77.78%

Numbers of females

Surviving adults / 2

Pouch young

Females*89% (assuming one joey per breeding cycle)

Survival to maturity

(Pouch young*86%)*13%

Combined reproduction

(Survival to maturity)*2.45

Results

Population Model 1

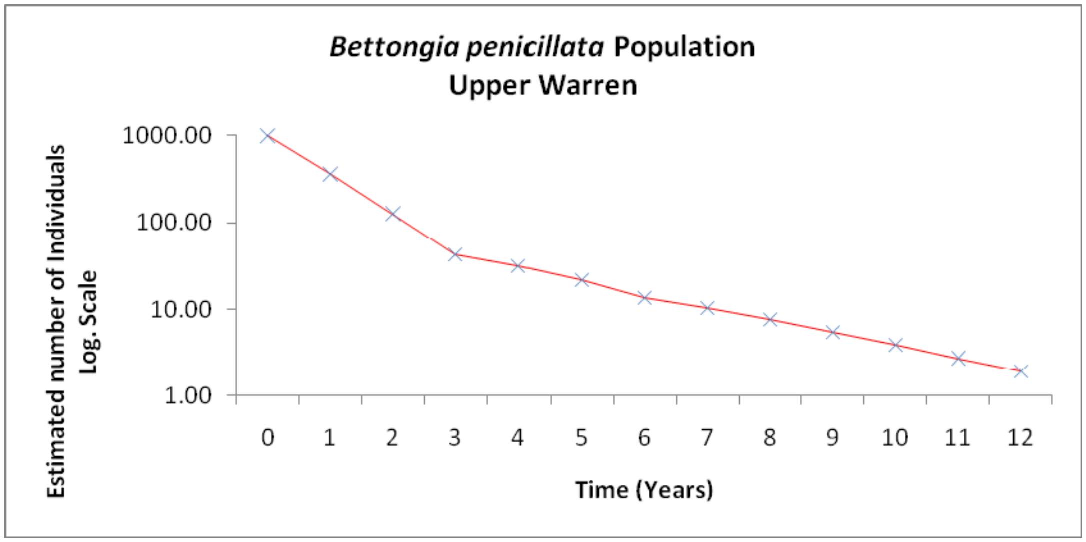

Figure 3 ***Starting population 1000 individuals, population decline average 38% / annum until local extinction after 12 years.***

Initial population of 1000 individuals declined at 65.11% until reaching density dependence of decline vector at three years, then slowing to 29% / annum until numbers fall below two after 12 years.

Population Model 2

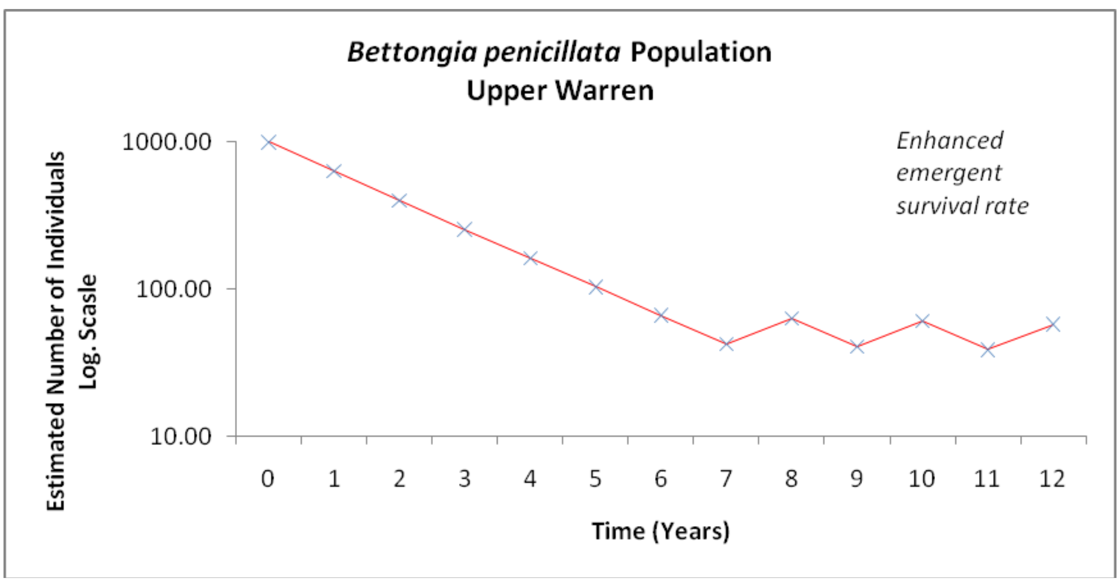

Figure 4 ***Starting population 1000 individuals, emergent survival rate enhanced to 100%***

Population declines at an average of 36.36% / annum until stabilising around decline vector density dependence after 6 years; average 53 individuals.

Population Model 3

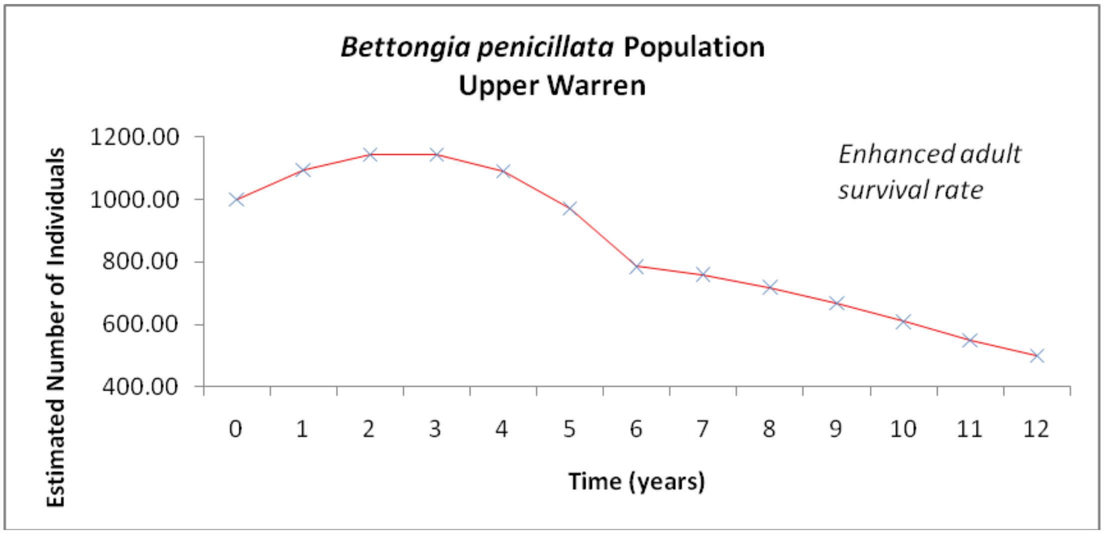

Figure 5 ***Starting population 1000 individuals, enhanced adult survival rate to 100%***

Population declines by an average of 9.2% / annum after 4 years and is less than 50% of start population after 12 years.

Population Model 4

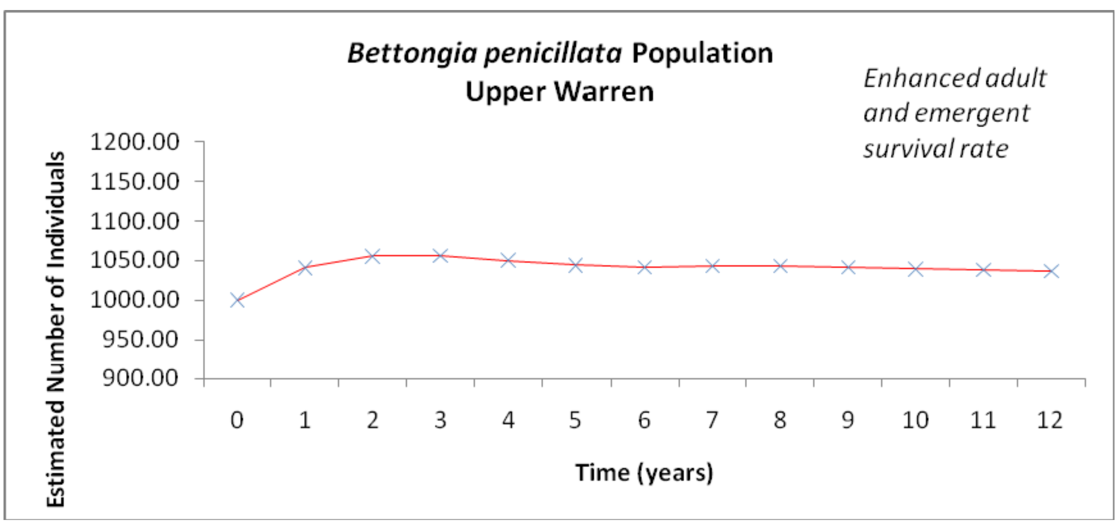

Figure 6 ***Combined minima for increased population after 12 years, adult survival = 77.78%, emergent survival = 39%***

With adult survival at an estimated rate independent of the acute decline pressures (77.78%), emergent survival needs to be raised to 39% to produce an increased population after 12 years. However, the population still shows a gradual decline of 0.13% / annum over the last five years.

Discussion

It must be remembered that these linear models are fairly simplistic in relation to real life scenarios. They rely entirely on assumptions based on research data, often incompletely available and, in the case of a species down to such low numbers, increasingly difficult to obtain. Age demographics have been particularly difficult to assess with the Woylies, time spent as sub-adults is very short and their lack of representation in the trapping data makes it difficult to ascertain trends in survivorship from emergence to maturity. Stochasticity is inherently difficult to incorporate into these models but, as populations fall towards minimum viability, this is likely to take on a higher significance in a population's continuation. With declines to such an extent as seen in these areas severe selection pressure is likely to have been imposed on these populations, coupled with greatly reduced migration opportunities; this is likely to lead to much reduced genetic diversity within populations and may seriously affect future population dynamics. However, given these limitations, these simple models may give a useful indication of future trends and could prove helpful in sensitivity analysis of variable factors.

In the first model current estimates are considered and local extinction (assuming no outside interference) is seen after 12 years, even though density effects on the decline vectors reduce the mortality rate after only three years; therefore, scenarios that would significantly affect this prognosis within those 12 years were considered. Since individuals remain fecund and have a high reproduction rate it seems likely that survivorship is likely to be the main consideration rather than recruitment numbers themselves. Two variables are considered for analysis, adult survival rate and survival from pouch emergence to maturity. In model 2, emergent survival rate is increased to 100% (all else as model 1), this is still seen to result in an acute decline, though at a lesser rate. The population is seen to stabilise at about 50 individuals, around the density dependence of the decline vectors. Whether these vectors would in fact immediately take effect again in the real world as populations recover beyond this point is unclear. Model 3, similarly, considers adult survival rate at 100%, in this scenario decline rates are far slower though the population is still less than 50% after the 12 years. Model 4 shows the most successful scenario for an increase over the time scale. In this a combination of the two variables is considered; the adult survival rate is enhanced to the estimated survival rate without the decline pressure (77.78%) and emergent survival is raised to the minimum required for an increased population after 12 years (39%).

These results would seem to agree broadly with the main conservation effort to exclude and control predators as these seem to have by far the biggest impacts on survivorship, both through the spread of disease and predation. The intention to continue and enhance Western Shield would certainly seem the most effective mechanism for Woylie recovery. Genetic bottlenecks must be considered and relocation programmes are proposed with thought given to genetic sources of introduction. More emphasis seems to have been placed in the report on adult survival though model 3 would suggest that this alone would not be enough. Survivorship to sexual maturity must also be enhanced considerably, though as we have seen this presents particular difficulties. Research is proposed to carry out more sophisticated data analysis and monitoring to gain a better understanding of age class demographics. The development of an inoculation programme, if possible, might be desirable, particularly if a vaccine that passed antibodies from the mother to the offspring were available. The unaffected population at Karakamia demonstrates that predator proof barriers do prove effective in preventing these acute declines but they also restrict movement of what would naturally be a highly mobile species; with implications for genetic management and the production of somewhat artificial populations. Recommendations for increasing public awareness and involvement are particularly important, especially where programmes to eradicate invading species are considered; public support and understanding is often essential to the success of any conservation effort.

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Appendix

Population Model 1

Current estimates of demographic survival rates
Average population decline = 38% / annum
Local extinction after 12 years

Age	Yr 0	Av. Adult survi	Breeding fema	Pouch Youn	Survl to Matu	Cmbnd Offs	Yr 1	Av. Adult survi	Breeding fema	Pouch Your	Survl to Matu	Cmbnd Offs	Yr 2	Av. Adult surv
1yr	305.57	101.85	50.92	45.32	5.07	12.41	40.63	13.54	6.77	6.03	0.67	1.65	14.82	4.94
2yr	250.00	83.33	41.66	37.08	4.15	10.16	101.85	33.95	16.97	15.11	1.69	4.14	13.54	4.51
3yr	194.45	64.81	32.41	28.84	3.22	7.90	83.33	27.77	13.89	12.36	1.38	3.39	33.95	11.31
4yr	138.90	46.30	23.15	20.60	2.30	5.64	64.81	21.60	10.80	9.61	1.07	2.63	27.77	9.26
5yr	83.35	27.78	13.89	12.36	1.38	3.39	46.30	15.43	7.72	6.87	0.77	1.88	21.60	7.20
6yr	27.76	9.25	4.63	4.12	0.46	1.13	27.78	9.26	4.63	4.12	0.46	1.13	15.43	5.14
Totals	**1000.03**	333.31	166.65	148.32	16.58	40.63	**364.68**	121.55	60.77	54.09	6.05	14.82	**127.11**	42.36

Breeding fem	Pouch Youn	Survl to Matu	Cmbnd Offs	Yr 3	Av. Adult surv	Breeding fem	Pouch Your	Survl to Matu	Cmbnd Offs	Yr 4	Av. Adult surv	Breeding fem	Pouch Your	Survl to Maturi
2.47	2.20	0.25	0.60	5.16	4.02	2.01	1.79	0.20	0.49	4.02	3.13	1.56	1.39	0.16
2.26	2.01	0.22	0.55	4.94	3.84	1.92	1.71	0.19	0.47	4.02	3.12	1.56	1.39	0.16
5.66	5.03	0.56	1.38	4.51	3.51	1.76	1.56	0.17	0.43	3.84	2.99	1.49	1.33	0.15
4.63	4.12	0.46	1.13	11.31	8.80	4.40	3.92	0.44	1.07	3.51	2.73	1.37	1.22	0.14
3.60	3.20	0.36	0.88	9.26	7.20	3.60	3.20	0.36	0.88	8.80	6.84	3.42	3.05	0.34
2.57	2.29	0.26	0.63	7.20	5.60	2.80	2.49	0.28	0.68	7.20	5.60	2.80	2.49	0.28
21.18	18.85	2.11	5.16	**42.39**	32.97	16.48	14.67	1.64	4.02	**31.39**	24.41	12.21	10.86	1.21

Cmbnd Offs	Yr 5	Av. Adult surv	Breeding fem	Pouch Youn	Survl to Matu	Cmbnd Offs	Yr 6	Av. Adult surv	Breeding fema	Pouch Your	Survl to Matu	Cmbnd Offs	Yr 7	Av. Adult surv
0.38	2.98	2.31	1.16	1.03	0.12	0.28	2.07	1.61	0.80	0.71	0.08	0.20	1.30	1.01
0.38	3.13	2.43	1.22	1.08	0.12	0.30	2.31	1.80	0.90	0.80	0.09	0.22	1.61	1.25
0.36	3.12	2.43	1.21	1.08	0.12	0.30	2.43	1.89	0.95	0.84	0.09	0.23	1.80	1.40
0.33	2.99	2.32	1.16	1.03	0.12	0.28	2.43	1.89	0.94	0.84	0.09	0.23	1.89	1.47
0.83	2.73	2.12	1.06	0.95	0.11	0.26	2.32	1.81	0.90	0.80	0.09	0.22	1.89	1.47
0.68	6.84	5.32	2.66	2.37	0.26	0.65	2.12	1.65	0.83	0.74	0.08	0.20	1.81	1.41
2.98	**21.79**	16.95	8.47	7.54	0.84	2.07	**13.69**	10.65	5.32	4.74	0.53	1.30	**10.29**	8.01

Breeding fem	Pouch Youn	Survl to Matu	Cmbnd Offs	Yr 8	Av. Adult survi	Breeding fem	Pouch Youn	Survl to Matu	Cmbnd Offs	Yr 9	Av. Adult surv	Breeding fem	Pouch Youn	Survl to Matu
0.50	0.45	0.05	0.12	0.98	0.76	0.38	0.34	0.04	0.09	0.72	0.56	0.28	0.25	0.03
0.62	0.56	0.06	0.15	1.01	0.79	0.39	0.35	0.04	0.10	0.76	0.59	0.30	0.26	0.03
0.70	0.62	0.07	0.17	1.25	0.97	0.49	0.43	0.05	0.12	0.79	0.61	0.31	0.27	0.03
0.74	0.65	0.07	0.18	1.40	1.09	0.54	0.48	0.05	0.13	0.97	0.76	0.38	0.34	0.04
0.73	0.65	0.07	0.18	1.47	1.14	0.57	0.51	0.06	0.14	1.09	0.85	0.42	0.38	0.04
0.70	0.63	0.07	0.17	1.47	1.14	0.57	0.51	0.06	0.14	1.14	0.89	0.44	0.40	0.04
4.00	3.56	0.40	0.98	**7.58**	5.89	2.95	2.62	0.29	0.72	**5.47**	4.25	2.13	1.89	0.21

Cmbnd Offs	Yr 10	Av. Adult surv	Breeding fem	Pouch Youn	Survl to Matu	Cmbnd Offs	Yr 11	Av. Adult surv	Breeding fem	Pouch Youn	Survl to Matu	Cmbnd Offs	Yr 12
0.07	0.52	0.40	0.20	0.18	0.02	0.05	0.37	0.29	0.14	0.13	0.01	0.03	0.26
0.07	0.56	0.43	0.22	0.19	0.02	0.05	0.40	0.31	0.16	0.14	0.02	0.04	0.29
0.07	0.59	0.46	0.23	0.20	0.02	0.06	0.43	0.34	0.17	0.15	0.02	0.04	0.31
0.09	0.61	0.47	0.24	0.21	0.02	0.06	0.46	0.36	0.18	0.16	0.02	0.04	0.34
0.10	0.76	0.59	0.29	0.26	0.03	0.07	0.47	0.37	0.18	0.16	0.02	0.05	0.36
0.11	0.85	0.66	0.33	0.29	0.03	0.08	0.59	0.46	0.23	0.20	0.02	0.06	0.37
0.52	**3.88**	3.02	1.51	1.34	0.15	0.37	**2.73**	2.12	1.06	0.94	0.11	0.26	**1.92**

Population Model 2

Emergent survival rate increased to 100%
Average decline of 36.36% / annum before stabilising around average of 53 individuals after 6 years

Age	Yr 0	Av. Adult surv	Breeding fema	Pouch Youn	Survl to Matu	Cmbnd Offs	Yr 1	Av. Adult surv	Breeding fema	Pouch Youn	Survl to Matu	Cmbnd Offs	Yr 2	Av. Adult surv
1yr	305.57	101.85	50.92	45.32	38.98	95.49	312.52	104.16	52.08	46.35	39.86	97.66	198.93	66.30
2yr	250.00	83.33	41.66	37.08	31.89	78.13	101.85	33.95	16.97	15.11	12.99	31.83	104.16	34.72
3yr	194.45	64.81	32.41	28.84	24.80	60.77	83.33	27.77	13.89	12.36	10.63	26.04	33.95	11.31
4yr	138.90	46.30	23.15	20.60	17.72	43.41	64.81	21.60	10.80	9.61	8.27	20.25	27.77	9.26
5yr	83.35	27.78	13.89	12.36	10.63	26.05	46.30	15.43	7.72	6.87	5.91	14.47	21.60	7.20
6yr	27.76	9.25	4.63	4.12	3.54	8.68	27.78	9.26	4.63	4.12	3.54	8.68	15.43	5.14
Totals	**1000.03**	333.31	166.65	148.32	127.56	312.52	**636.57**	212.17	106.09	94.42	81.20	198.93	**401.84**	133.93

Breeding fem	Pouch Youn	Survl to Matu	Cmbnd Offs	Yr 3	Av. Adult surv	Breeding fem	Pouch Your	Survl to Matu	Cmbnd Offs	Yr 4	Av. Adult surv	Breeding fem	Pouch Your	Survl to Matur
33.15	29.51	25.37	62.17	125.58	41.86	20.93	18.63	16.02	39.24	79.49	26.49	13.25	11.79	10.14
17.36	15.45	13.29	32.55	66.30	22.10	11.05	9.83	8.46	20.72	41.86	13.95	6.98	6.21	5.34
5.66	5.03	4.33	10.61	34.72	11.57	5.79	5.15	4.43	10.85	22.10	7.37	3.68	3.28	2.82
4.63	4.12	3.54	8.68	11.31	3.77	1.89	1.68	1.44	3.54	11.57	3.86	1.93	1.72	1.48
3.60	3.20	2.76	6.75	9.26	3.09	1.54	1.37	1.18	2.89	3.77	1.26	0.63	0.56	0.48
2.57	2.29	1.97	4.82	7.20	2.40	1.20	1.07	0.92	2.25	3.09	1.03	0.51	0.46	0.39
66.97	59.60	51.26	125.58	**254.37**	84.78	42.39	37.73	32.45	79.49	**161.88**	53.95	26.98	24.01	20.65

Cmbnd Offs	Yr 5	Av. Adult surv	Breeding fem	Pouch Youn	Survl to Matur	Cmbnd Offs	Yr 6	Av. Adult surv	Breeding fema	Pouch Your	Survl to Matu	Cmbnd Offs	Yr 7	Av. Adult surv
24.84	50.59	16.86	8.43	7.50	6.45	15.81	32.35	10.78	5.39	4.80	4.13	10.11	20.76	16.15
13.08	26.49	8.83	4.42	3.93	3.38	8.28	16.86	5.62	2.81	2.50	2.15	5.27	10.78	8.39
6.91	13.95	4.65	2.32	2.07	1.78	4.36	8.83	2.94	1.47	1.31	1.13	2.76	5.62	4.37
3.62	7.37	2.45	1.23	1.09	0.94	2.30	4.65	1.55	0.77	0.69	0.59	1.45	2.94	2.29
1.18	3.86	1.29	0.64	0.57	0.49	1.21	2.45	0.82	0.41	0.36	0.31	0.77	1.55	1.21
0.96	1.26	0.42	0.21	0.19	0.16	0.39	1.29	0.43	0.21	0.19	0.16	0.40	0.82	0.64
50.59	**103.51**	34.50	17.25	15.35	13.20	32.35	**66.43**	22.14	11.07	9.85	8.47	20.76	**42.47**	33.04

Breeding fem	Pouch Youn	Survl to Matu	Cmbnd Offs	Yr 8	Av. Adult surv	Breeding fem	Pouch Youn	Survl to Matu	Cmbnd Offs	Yr 9	Av. Adult surv	Breeding fem	Pouch Youn	Survl to Matu
8.07	7.19	6.18	15.14	30.97	10.32	5.16	4.59	3.95	9.68	19.80	15.40	7.70	6.85	5.90
4.19	3.73	3.21	7.86	16.15	5.38	2.69	2.39	2.06	5.05	10.32	8.03	4.01	3.57	3.07
2.19	1.95	1.67	4.10	8.39	2.80	1.40	1.24	1.07	2.62	5.38	4.19	2.09	1.86	1.60
1.14	1.02	0.88	2.15	4.37	1.46	0.73	0.65	0.56	1.37	2.80	2.17	1.09	0.97	0.83
0.60	0.54	0.46	1.13	2.29	0.76	0.38	0.34	0.29	0.72	1.46	1.13	0.57	0.50	0.43
0.32	0.28	0.24	0.60	1.21	0.40	0.20	0.18	0.15	0.38	0.76	0.59	0.30	0.26	0.23
16.52	14.70	12.64	30.97	**63.37**	21.12	10.56	9.40	8.08	19.80	**40.52**	31.52	15.76	14.03	12.06

Cmbnd Offs	Yr 10	Av. Adult surv	Breeding fem	Pouch Youn	Survl to Matu	Cmbnd Off	Yr 11	Av. Adult surv	Breeding fem	Pouch Youn	Survl to Matu	Cmbnd Offs	Yr 12
14.44	29.55	9.85	4.93	4.38	3.77	9.24	18.90	14.70	7.35	6.54	5.63	13.78	28.21
7.53	15.40	5.13	2.57	2.28	1.96	4.81	9.85	7.66	3.83	3.41	2.93	7.18	14.70
3.92	8.03	2.68	1.34	1.19	1.02	2.51	5.13	3.99	2.00	1.78	1.53	3.74	7.66
2.04	4.19	1.40	0.70	0.62	0.53	1.31	2.68	2.08	1.04	0.93	0.80	1.95	3.99
1.06	2.17	0.72	0.36	0.32	0.28	0.68	1.40	1.09	0.54	0.48	0.42	1.02	2.08
0.56	1.13	0.38	0.19	0.17	0.14	0.35	0.72	0.56	0.28	0.25	0.22	0.53	1.09
29.55	**60.48**	20.16	10.08	8.97	7.71	18.90	**38.68**	30.09	15.04	13.39	11.51	28.21	**57.73**

Population Model 3

Adult survival rate increased to 100%
Population declines by average 9.2% / annum after 4 years.

Age	Yr 0	Av. Adult surv	Breeding fema	Pouch Youn	Survl to Matu	Cmbnd Offs	Yr 1	Av. Adult surv	Breeding fema	Pouch Your	Survl to Matu	Cmbnd Offs	Yr 2	Av. Adult surv
1yr	305.57	305.57	152.79	135.98	15.20	37.25	121.89	121.89	60.95	54.24	6.06	14.86	133.37	133.37
2yr	250.00	250.00	125.00	111.25	12.44	30.47	305.57	305.57	152.79	135.98	15.20	37.25	121.89	121.89
3yr	194.45	194.45	97.23	86.53	9.67	23.70	250.00	250.00	125.00	111.25	12.44	30.47	305.57	305.57
4yr	138.90	138.90	69.45	61.81	6.91	16.93	194.45	194.45	97.23	86.53	9.67	23.70	250.00	250.00
5yr	83.35	83.35	41.68	37.09	4.15	10.16	138.90	138.90	69.45	61.81	6.91	16.93	194.45	194.45
6yr	27.76	27.76	13.88	12.35	1.38	3.38	83.35	83.35	41.68	37.09	4.15	10.16	138.90	138.90
Totals	**1000.03**	1000.03	500.02	445.01	49.75	121.89	**1094.16**	1094.16	547.08	486.90	54.44	133.37	**1144.18**	1144.18

Breeding fema	Pouch Youn	Survl to Matu	Cmbnd Offs	Yr 3	Av. Adult surv	Breeding fem	Pouch Your	Survl to Matu	Cmbnd Offs	Yr 4	Av. Adult surv	Breeding fem	Pouch Your	Survl to Maturi
66.68	59.35	6.64	16.26	139.46	139.46	69.73	62.06	6.94	17.00	139.53	139.53	69.77	62.09	6.94
60.95	54.24	6.06	14.86	133.37	133.37	66.68	59.35	6.64	16.26	139.46	139.46	69.73	62.06	6.94
152.79	135.98	15.20	37.25	121.89	121.89	60.95	54.24	6.06	14.86	133.37	133.37	66.68	59.35	6.64
125.00	111.25	12.44	30.47	305.57	305.57	152.79	135.98	15.20	37.25	121.89	121.89	60.95	54.24	6.06
97.23	86.53	9.67	23.70	250.00	250.00	125.00	111.25	12.44	30.47	305.57	305.57	152.79	135.98	15.20
69.45	61.81	6.91	16.93	194.45	194.45	97.23	86.53	9.67	23.70	250.00	250.00	125.00	111.25	12.44
572.09	509.16	56.92	139.46	**1144.75**	1144.75	572.37	509.41	56.95	139.53	**1089.83**	1089.83	544.91	484.97	54.22

Cmbnd Offs	Yr 5	Av. Adult surv	Breeding fem	Pouch Youn	Survl to Matu	Cmbnd Offs	Yr 6	Av. Adult surv	Breeding fema	Pouch Your	Survl to Matu	Cmbnd Offs	Yr 7	Av. Adult surv
17.01	132.84	132.84	66.42	59.11	6.61	16.19	118.56	118.56	59.28	52.76	5.90	14.45	95.76	95.76
17.00	139.53	139.53	69.77	62.09	6.94	17.01	132.84	132.84	66.42	59.11	6.61	16.19	118.56	118.56
16.26	139.46	139.46	69.73	62.06	6.94	17.00	139.53	139.53	69.77	62.09	6.94	17.01	132.84	132.84
14.86	133.37	133.37	66.68	59.35	6.64	16.26	139.46	139.46	69.73	62.06	6.94	17.00	139.53	139.53
37.25	121.89	121.89	60.95	54.24	6.06	14.86	133.37	133.37	66.68	59.35	6.64	16.26	139.46	139.46
30.47	305.57	305.57	152.79	135.98	15.20	37.25	121.89	121.89	60.95	54.24	6.06	14.86	133.37	133.37
132.84	**972.67**	972.67	486.33	432.84	48.39	118.56	**785.66**	785.66	392.83	349.62	39.09	95.76	**759.53**	759.53

Breeding fema	Pouch Youn	Survl to Matu	Cmbnd Offs	Yr 8	Av. Adult surv	Breeding fema	Pouch Youn	Survl to Matu	Cmbnd Offs	Yr 9	Av. Adult surv	Breeding fem	Pouch Youn	Survl to Matu
47.88	42.61	4.76	11.67	92.58	92.58	46.29	41.20	4.61	11.28	87.61	87.61	43.80	38.99	4.36
59.28	52.76	5.90	14.45	95.76	95.76	47.88	42.61	4.76	11.67	92.58	92.58	46.29	41.20	4.61
66.42	59.11	6.61	16.19	118.56	118.56	59.28	52.76	5.90	14.45	95.76	95.76	47.88	42.61	4.76
69.77	62.09	6.94	17.01	132.84	132.84	66.42	59.11	6.61	16.19	118.56	118.56	59.28	52.76	5.90
69.73	62.06	6.94	17.00	139.53	139.53	69.77	62.09	6.94	17.01	132.84	132.84	66.42	59.11	6.61
66.68	59.35	6.64	16.26	139.46	139.46	69.73	62.06	6.94	17.00	139.53	139.53	69.77	62.09	6.94
379.76	337.99	37.79	92.58	**718.74**	718.74	359.37	319.84	35.76	87.61	**666.88**	666.88	333.44	296.76	33.18

Cmbnd Offs	Yr 10	Av. Adult surv	Breeding fem	Pouch Youn	Survl to Matu	Cmbnd Off	Yr 11	Av. Adult surv	Breeding fem	Pouch Youn	Survl to Matu	Cmbnd Offs	Yr 12
10.68	81.29	81.29	40.64	36.17	4.04	9.91	74.19	74.19	37.09	33.01	3.69	9.04	67.04
11.28	87.61	87.61	43.80	38.99	4.36	10.68	81.29	81.29	40.64	36.17	4.04	9.91	74.19
11.67	92.58	92.58	46.29	41.20	4.61	11.28	87.61	87.61	43.80	38.99	4.36	10.68	81.29
14.45	95.76	95.76	47.88	42.61	4.76	11.67	92.58	92.58	46.29	41.20	4.61	11.28	87.61
16.19	118.56	118.56	59.28	52.76	5.90	14.45	95.76	95.76	47.88	42.61	4.76	11.67	92.58
17.01	132.84	132.84	66.42	59.11	6.61	16.19	118.56	118.56	59.28	52.76	5.90	14.45	95.76
81.29	**608.63**	608.63	304.32	270.84	30.28	74.19	**549.98**	549.98	274.99	244.74	27.36	67.04	**498.46**

Population model 4

Combined minima for increased population after 12 years
Adult survival rate = 77.78% (estimated survival rate with no decline pressure), emergent survival rate increased to 39% (currently 13%)

Age	Yr 0	Av. Adult surv	Breeding fema	Pouch Youn	Survl to Matu	Cmbnd Offs	Yr 1	Av. Adult surv	Breeding fema	Pouch Your	Survl to Matu	Cmbnd Offs	Yr 2	Av. Adult surv
1yr	305.57	237.67	118.84	105.76	35.47	86.91	284.43	221.23	110.61	98.45	33.02	80.90	295.98	230.21
2yr	250.00	194.45	97.23	86.53	29.02	71.10	237.67	184.86	92.43	82.26	27.59	67.60	221.23	172.07
3yr	194.45	151.24	75.62	67.30	22.57	55.31	194.45	151.24	75.62	67.30	22.57	55.31	184.86	143.79
4yr	138.90	108.04	54.02	48.08	16.12	39.51	151.24	117.64	58.82	52.35	17.56	43.02	151.24	117.64
5yr	83.35	64.83	32.41	28.85	9.68	23.71	108.04	84.03	42.02	37.39	12.54	30.73	117.64	91.50
6yr	27.76	21.59	10.80	9.61	3.22	7.90	64.83	50.42	25.21	22.44	7.53	18.44	84.03	65.36
Totals	**1000.03**	777.82	388.91	346.13	116.09	284.43	**1040.66**	809.42	404.71	360.19	120.81	295.98	**1054.98**	820.56

Breeding fem	Pouch Youn	Survl to Matu	Cmbnd Offs	Yr 3	Av. Adult surv	Breeding fem	Pouch Your	Survl to Matu	Cmbnd Offs	Yr 4	Av. Adult surv	Breeding fem	Pouch Your	Survl to Maturi
115.11	102.45	34.36	84.18	300.06	233.38	116.69	103.86	34.83	85.34	300.14	233.45	116.72	103.88	34.84
86.04	76.57	25.68	62.92	230.21	179.06	89.53	79.68	26.73	65.48	233.38	181.53	90.76	80.78	27.09
71.89	63.98	21.46	52.58	172.07	133.84	66.92	59.56	19.98	48.94	179.06	139.27	69.64	61.98	20.79
58.82	52.35	17.56	43.02	143.79	111.84	55.92	49.77	16.69	40.90	133.84	104.10	52.05	46.32	15.54
45.75	40.72	13.66	33.46	117.64	91.50	45.75	40.72	13.66	33.46	111.84	86.99	43.49	38.71	12.98
32.68	29.08	9.76	23.90	91.50	71.17	35.58	31.67	10.62	26.02	91.50	71.17	35.58	31.67	10.62
410.28	365.15	122.47	300.06	**1055.26**	820.78	410.39	365.25	122.50	300.14	**1049.75**	816.50	408.25	363.34	121.86

Cmbnd Offs	Yr 5	Av. Adult surv	Breeding fem	Pouch Youn	Survl to Matu	Cmbnd Offs	Yr 6	Av. Adult surv	Breeding fema	Pouch Your	Survl to Matu	Cmbnd Offs	Yr 7	Av. Adult surv
85.36	298.57	232.23	116.11	103.34	34.66	84.92	296.90	230.93	115.47	102.76	34.47	84.44	296.13	230.33
66.38	233.45	181.57	90.79	80.80	27.10	66.40	232.23	180.63	90.31	80.38	26.96	66.05	230.93	179.62
50.93	181.53	141.19	70.60	62.83	21.07	51.63	181.57	141.23	70.61	62.85	21.08	51.64	180.63	140.49
38.07	139.27	108.33	54.16	48.21	16.17	39.61	141.19	109.82	54.91	48.87	16.39	40.16	141.23	109.85
31.81	104.10	80.97	40.48	36.03	12.08	29.61	108.33	84.26	42.13	37.49	12.58	30.81	109.82	85.42
26.02	86.99	67.66	33.83	30.11	10.10	24.74	80.97	62.98	31.49	28.02	9.40	23.03	84.26	65.53
298.57	**1043.90**	811.94	405.97	361.31	121.18	296.90	**1041.19**	809.84	404.92	360.38	120.87	296.13	**1042.99**	811.24

Breeding fem	Pouch Youn	Survl to Matu	Cmbnd Offs	Yr 8	Av. Adult survi	Breeding fema	Pouch Youn	Survl to Matu	Cmbnd Offs	Yr 9	Av. Adult surv	Breeding fem	Pouch Youn	Survl to Matu
115.17	102.50	34.38	84.23	296.65	230.73	115.37	102.68	34.44	84.37	296.46	230.59	115.29	102.61	34.42
89.81	79.93	26.81	65.68	230.33	179.15	89.58	79.72	26.74	65.51	230.73	179.46	89.73	79.86	26.79
70.25	62.52	20.97	51.37	179.62	139.71	69.85	62.17	20.85	51.09	179.15	139.34	69.67	62.01	20.80
54.92	48.88	16.40	40.17	140.49	109.27	54.64	48.63	16.31	39.96	139.71	108.66	54.33	48.36	16.22
42.71	38.01	12.75	31.23	109.85	85.44	42.72	38.02	12.75	31.24	109.27	84.99	42.50	37.82	12.69
32.77	29.16	9.78	23.96	85.42	66.44	33.22	29.56	9.92	24.29	85.44	66.45	33.23	29.57	9.92
405.62	361.00	121.08	296.65	**1042.35**	810.74	405.37	360.78	121.01	296.46	**1040.77**	809.51	404.75	360.23	120.82

Cmbnd Offs	Yr 10	Av. Adult surv	Breeding fem	Pouch Youn	Survl to Matu	Cmbnd Off	Yr 11	Av. Adult surv	Breeding fem	Pouch Youn	Survl to Matu	Cmbnd Offs	Yr 12
84.32	296.01	230.24	115.12	102.46	34.36	84.19	295.53	229.86	114.93	102.29	34.31	84.05	295.11
65.62	230.59	179.35	89.68	79.81	26.77	65.58	230.24	179.08	89.54	79.69	26.73	65.48	229.86
50.95	179.46	139.59	69.79	62.12	20.83	51.04	179.35	139.50	69.75	62.08	20.82	51.01	179.08
39.74	139.34	108.38	54.19	48.23	16.18	39.63	139.59	108.57	54.28	48.31	16.20	39.70	139.50
31.08	108.66	84.52	42.26	37.61	12.61	30.91	108.38	84.30	42.15	37.51	12.58	30.83	108.57
24.30	84.99	66.11	33.05	29.42	9.87	24.17	84.52	65.74	32.87	29.25	9.81	24.04	84.30
296.01	**1039.07**	808.19	404.09	359.64	120.62	295.53	**1037.61**	807.05	403.53	359.14	120.45	295.11	**1036.43**

Terrestrial vertebrate assemblage and species level patterns between major habitat types inside and outside a fenced exclosure in south-western Australia.

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G.J. Yeatman, A.F. Wayne, and H. Mills

Understanding the processes that shape the distribution and abundance of species is necessary for effective wildlife conservation. This becomes more important for managers in areas where significant manipulation of the environment is undertaken. This study investigated patterns in the small vertebrate assemblage inside and outside a fully fenced exclosure in south-western Australia, and forms part of a larger monitoring project on the flora and fauna of the area. Three small mammal, thirteen reptile and nine frog species were recorded at 18 sites within three main habitat types. There were significant differences in the small vertebrate assemblage between habitat types. There was a decline in species richness and overall capture rates moving upwards in the landscape from creeks to ridge top sites. Eight of the small vertebrate species trapped accounted for 95% of the variation in the total species set. Creek sites were characterised by a greater relative abundance of frog species, particularly *Crinia glauerti*, *Pseudophryne guentheri* and *Heleioporus eyrei*. Slope sites had a greater abundance of some small mammal (*Sminthopsis griseoventer*) and reptile (*Morethia obscura*) species. Ridge sites were dominated by reptiles, in particular, *Lerista distinguenda*. The differences in the assemblage between habitats suggest that habitat level monitoring should continue as broader scale observation may lack sensitivity to changes within the different assemblages in the area. This study provides vital information for the management of this exclosure and as part of the wider monitoring project, has the potential to inform the management of other fenced areas.

Introduction

The use of exclusion fencing to conserve native fauna has become an increasingly necessary option for wildlife managers around the world (Whitehouse and Kerley, 2002, Ikuta and Blumstein, 2003, van Dyk and Slotow, 2003, Long and Robley, 2004, Hayward and Kerley, 2009, McGavin, 2009, Hameed *et al.*, 2012). The selective removal and exclusion of undesirable species and subsequent introduction of species of conservation concern is a radical form of ecological manipulation. A common objective of the managers of fenced areas is the conservation of focal species (Short *et al.*, 1994, Short and Turner, 2000, Whitehouse and Kerley, 2002, Richards and Short, 2003, Gross, 2009, de Tores and Marlow, 2012, Hayward, 2012). As a result of this type of single species management, it is often unclear whether human intervention has led to other profound ecosystem changes beyond those which were directly intended. Information about such changes has the potential to inform the managers of exclosures as well as broader species conservation. Particular areas of interest include identifying which species benefit and which are disadvantaged in fenced systems and whether exclosures can be self sustaining or require continued management intervention to prevent ecosystem collapse (Hayward and Kerley, 2009).

Currently in Australia there are 23 fenced exclosures designed to protect native fauna from the threat of introduced species (Long and Robley, 2004, Dickman, 2012). A common management aim for many of these exclosures is the conservation of medium-sized mammals (Moseby *et al.*, 2009, de Tores and Marlow, 2012, Dickman, 2012). As a result, where these areas are monitored, data collection is generally biased towards those target mammalian species (Moseby *et al.*, 2009, de Tores and Marlow, 2012). There is very little published literature regarding the potential impact that fencing may have on the ecology of vertebrate species in general, but a particular deficit in information relating to reptiles, amphibians and small, less mobile mammal species. Other than an increase in the density of medium-sized mammals in the absence of introduced predators (Kinnear *et al.*, 2002, Richards and Short, 2003, Dexter *et al.*, 2012), the consequences of establishing a fenced exclosure are not inherently obvious and the ecosystem-wide implications remain unclear.

Perup Sanctuary (PS) in the Upper Warren region of south-western Australia is one example of a fenced exclosure used as a management tool for the conservation of medium-sized mammals. The target species for conservation at Perup is the woylie (*Bettongia penicillata*) which has suffered a 95% decline in abundance in the Upper Warren region (Yeatman and Groom, 2012). The sanctuary was constructed with the aim of creating an insurance population of woylie which were protected from predation by introduced foxes (*Vulpes vulpes*) and cats (*Felis catus*). There are many elements to consider when investigating the potential impacts of the construction of a fenced area. These include, but are not limited to, the absence of introduced predators, restriction of movement of species for which the fence is a barrier, increased abundance of species limited by predation or competition with those species which have been removed, changed abundance of species (both plants and animals) which are responding to those released from predation/competition and changes in habitat structure in response to changes in the abundance of species which can modify habitats (Hayward and Kerley, 2009, Dickman, 2012, Hayward and Somers, 2012). At Perup it is also important to consider the additional removal of the native chuditch (*Dasyurus geoffroii*) which is a predator of small vertebrates, and the removal of large herbivorous macropods (*Macropus fuliginosus* and *Macropus irma*).

We set out to study the small terrestrial vertebrates as part of a wider initiative monitoring the flora and fauna in and outside the newly constructed PS. The aim of the wider project was to establish a baseline understanding of the ecology of the species in the area which could then be used as a reference point for future monitoring efforts. An essential foundation to the study of ecology is an understanding of the processes that shape the distribution and abundance of species (Odum, 1959, Krebs, 1985).With this in mind, this study investigated patterns in the small mammal, reptile and amphibian assemblage between three main habitat types. Specifically, we addressed the following questions:

1. Are there differences in the assemblage of small vertebrates found in each of the habitat types? In particular, are there differences in the structure of the assemblage and/or the abundance of species within the assemblage group?
2. Are there differences in the size of individuals of the same species that may relate to the habitat in which they are found? Are there characteristics of the habitats or the species themselves which may account for such differences?

Methods

Study area

Perup Sanctuary is a 423 ha fully fenced area approximately 50 km outside Manjimup (34.2506º S, 116.1425º E) in the Upper Warren region of south-western Australia (Figure 1). The sanctuary lies within Perup Nature Reserve in the southern jarrah forest and is managed by the Western Australian Department of Environment and Conservation (DEC). The region has a Mediterranean-type climate with warm dry summers and cool wet winters. The area is characterised by open dry sclerophyll forest in which the overstorey is dominated by a jarrah (*Eucalyptus marginata*) and marri (*Corymbia calophylla*), and in some places, wandoo (*Eucalyptus wandoo*).

The sanctuary fence was designed to withstand penetration by foxes, cats and rabbits (Oryctolagus cuniculus). Construction was completed in September 2010 and followed by an intensive program to completely remove foxes and cats. Western grey kangaroos (*Macropus fuliginosus*), brush wallabies (*Macropus irma*), all emu (*Dromaius novaehollandiae*), some brushtail possums (*Trichosurus vulpecula*) and all chuditch (*Dasyurus geoffroii*) were also removed as they were identified as potential problem species if left within the sanctuary. Forty one woylies were sourced from the surrounding Upper Warren population and released into the sanctuary in December 2010.

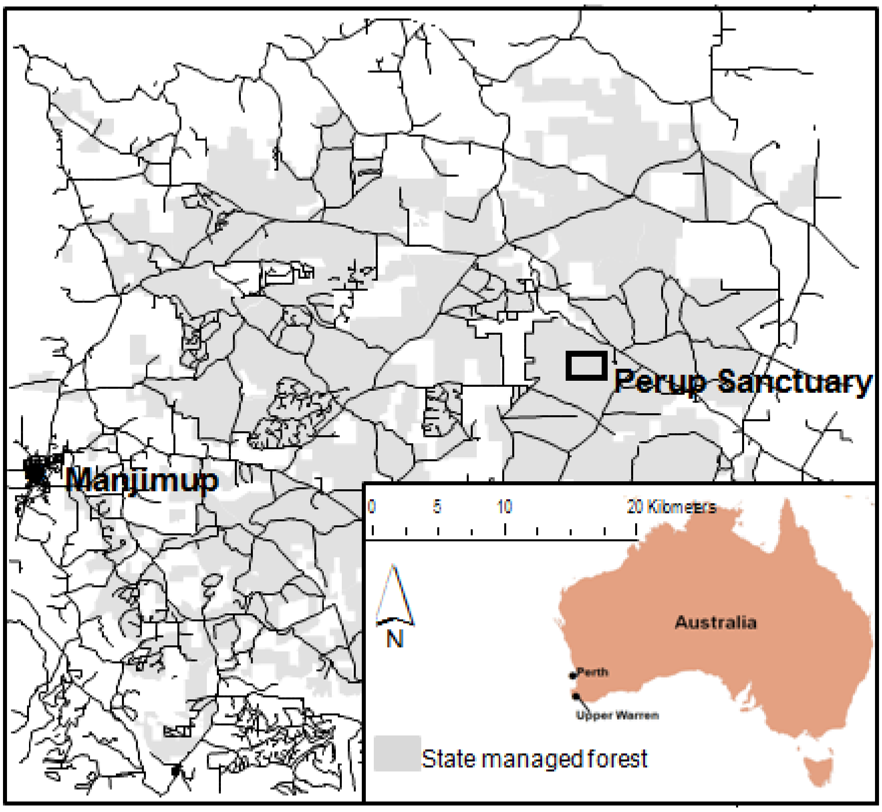

Figure 1: Location of Perup Sanctuary in relation to Manjimup and Perth in south-western Australia.

Small Vertebrate Trapping Surveys

Nine sites were selected inside the sanctuary and nine outside to the south west of PS (Figure 2). There were three replicates of each of three habitat types inside and outside the sanctuary. The three vegetation types were identified using Havel and Mattiske (2000) classifications. These were Yerraminnup flat, Yerraminnup and Bevan. These are broad and common vegetation types in Perup Nature Reserve and are the dominant types found within PS. Each of the types is characterised by position in the landscape, soil structure/hydrology and vegetation structure/composition.

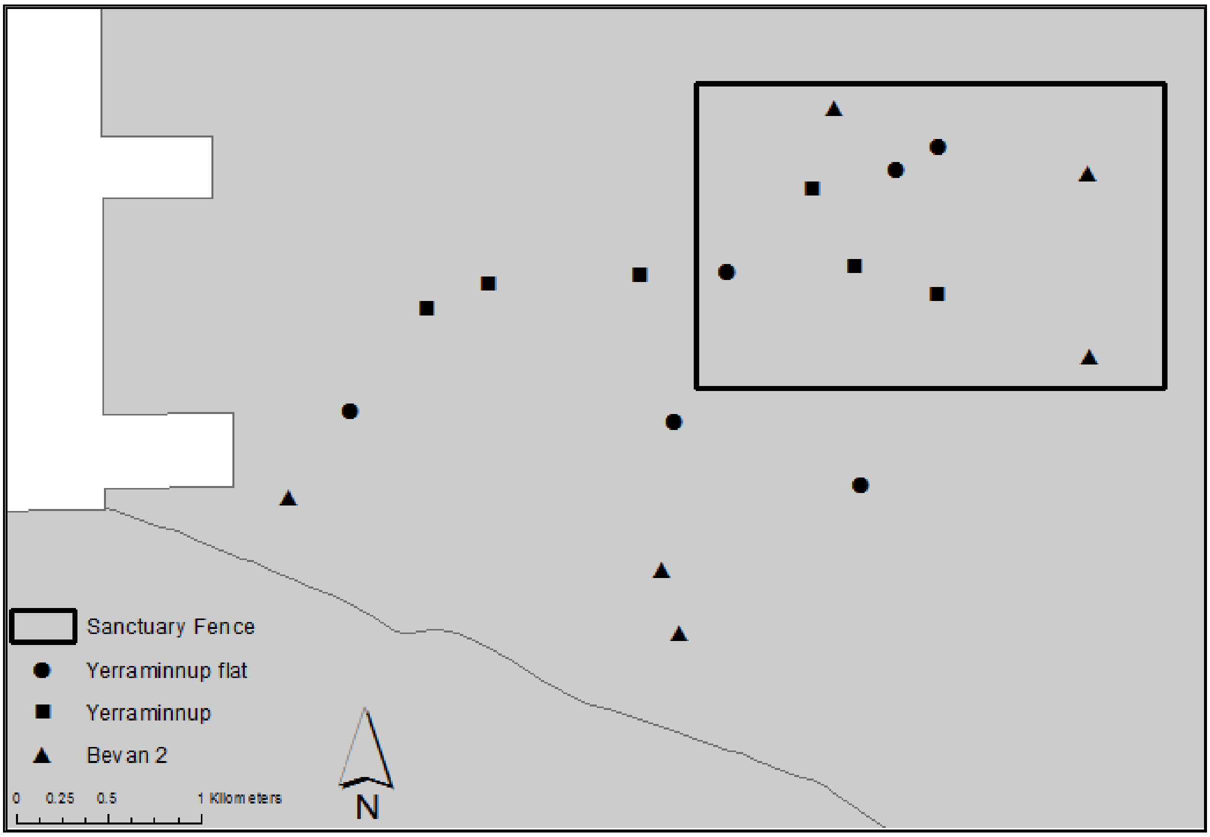

Figure 2: Map of 18 survey sites in Perup Nature Reserve. Symbols indicate habitat type of each site. There were three replicates of each of three habitat types inside and outside the sanctuary.

Yerraminnup flat describes the floor of a minor valley which has sandy loam topsoil over mottled clay. These sites are moderately fertile, water gaining areas which become seasonally waterlogged because of the lack of lateral drainage. There is less canopy cover at these sites and usually only scattered wandoo (*Eucalyptus wandoo*) and flooded gum (*Eucalyptus rudis*). The second storey is a mix of *Acacia* and *Hakea sp*. and the shrub/herb storey is dominated by thickets of *Melaleuca viminea* with some *Xanthorrhoea preissii*, *Hypocalymma angustifolium* and *Drosera bulbosa*. The six Yerraminnup flat study sites all had seasonal creeks flowing and were heavily water logged during winter months.

Yerraminnup describes the woodland slopes of a minor valley moving up away from the Yerraminnup flat sites. These areas are moderately fertile with gravelly sandy loam soil over sandy clay. Water is shed away from these sites and although the soil absorbs water well, it has a moderate to poor storage capacity. There is much greater canopy cover at Yerraminnup sites compared to Yerraminnup flat sites and the dominant species are marri and jarrah with some scattered wandoo. There is no clear second storey. The shrub/herb layer consists of *Acacia pulchella*, *Hakea lissocarpha*, *Hibbertia cunninghamii*, *Leucopogon capitelatus, Leucopogon propinqus, Macrozamia riedlei*, *Bossiaea linophylla* and *Trymalium ledifolium*.

Bevan describes the woodland and open forests of the ridges and upperslopes moving up further from the Yerraminnup sites. These areas have low fertility with gravelly sand topsoil and some lateritic duricrust. Water can infiltrate and be stored in these areas and there is some weak water shedding capacity. The overstorey is a mixture of jarrah and marri and the second storey is weakly developed with some *Persoonia longifolia* and *Banksia grandis*. The shrub and herb storey consists of *Bossiaea ornata*, *Astroloma pallidium*, *Macrozamia riedlei* and *Trymalium ledifolium*. For ease of reference, the site types will furthermore be referred to as creek (Yerraminnup flat), slope (Yerraminnup) and ridge (Bevan).

All sites except for PS7 and PS8 were >200 m apart with the average distance between one site and its nearest neighbour being >400 m. Sites PS7 and PS8 were 131 m apart. All sites except PS7 were at least 100 m away from tracks to reduce possible edge effects. Due to the reduced number of creek type habitats within the sanctuary at which sites could be established, site PS7 had to be established <50 m from the edge of a track. At each site, 25 trap points were arranged in a web formation (Figure 3). Traps were spaced 25 m apart along the eight arms of the web. Each trap point consisted of a 25 L bucket dug into the ground with a 7 m long, 30 cm high fly wire mesh running over the centre of the bucket. There were a total of 450 traps across the 18 sites.

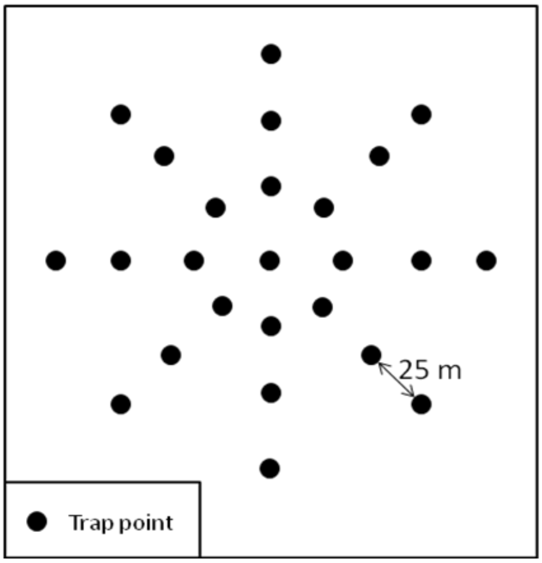

Figure 3: Web arrangement of the 25 trap points located at each of the 18 sites. Each trap point consisted of one 25 L pit trap and 7 m long drift fence erected over the centre of each pit. Trap points were spaced 25 m apart along each arm of the web.

A total of six trapping sessions were conducted in September and November 2011 and January/February, March, May and July of 2012. Each session consisted of four consecutive nights trapping. Traps were checked in both the morning and afternoon. When an animal was captured, it was weighed, marked (excluding frog species), sexed (if possible) and skeletal measurements were taken. Mammals were marked using individual ear notch numbers and reptiles were marked on the underside of the body with a non toxic permanent marker. The non toxic permanent marker allowed the identification of recaptures during that trapping session but would not last between sessions. Frogs were not marked.

Statistical Analyses

Small vertebrate assemblage

The number of captures at each site during each of the six sessions was combined to form a species by sample matrix. Each sample referred to a particular site and session, for example the animals captured at site PS1 in the September session were treated as one sample. Data were standardised according to trap effort. The September session had half the trap effort of the following five sessions as it was treated as a trial session. During each session there was slight variation in effort as some traps had to be closed due to water logging or ant activity. Data were fourth root transformed prior to analysis to ensure that sites with a particularly large number of captures during a session did not dominate the data (Clarke and Warwick, 2001). Pair-wise similarities between samples were estimated by the Bray-Curtis similarity coefficient. The data were then analysed using a permutational repeated measures analysis of variance (PERMANOVA; (Anderson, 2001). All data analysis was conducted using the PRIMER-E software package and the PERMANOVA+ add-on (Anderson *et al.*, 2008).

Habitat type, month (session) of trapping and whether the site was inside or outside the sanctuary were all treated as fixed factors (Table 1). As there was only one sanctuary, this factor was not able to be properly replicated. The 'Sanctuary' factor was included as a blocking factor in order to help account for any variation between the two areas. This paper will only report differences found between inside and outside the sanctuary. This research does not intend to conclude on any impact of the sanctuary itself as this survey is intended as a baseline dataset to which future surveys can be compared. The individual site was treated as a random factor nested within habitat and sanctuary. Average maximum temperature and average rainfall during each session were included as covariates in the PERMANOVA analysis. Average rainfall was taken from the Bureau of Meteorology records from Deeside rain station approximately 25 km from the study site (BOM, 2012). Maximum temperature records were taken from Manjimup weather station (BOM, 2012). Significant relationships were tested using 9999 randomisations. Terms in the analysis were pooled when $p > 0.25$ (Anderson *et al.*, 2008). Where factors were found to be significant, principal coordinates analysis (PCO) was conducted to visualise the separation of samples.

SIMPER analysis within the PRIMER-E package was used to identify which species accounted for the dissimilarity between and similarities within habitats. BEST analysis within the PRIMER-E package was used to identify which subset of species best explained the patterns occurring in the 24 species captured.

Table 1: PERMANOVA design table of factors included in the small vertebrate assemblage and species level analyses.

Factor	Nested within	Fixed or Random
Sanctuary	-	Fixed
Month	-	Fixed
Habitat	-	Fixed
Site	Habitat and Sanctuary	Random

Species richness
Species richness was an estimate of the number of species that would be captured when 25 individuals had been collected. This was calculated using rarefaction in the PRIMER-E package. These data were analysed as a univariate PERMANOVA. The factors and covariates used in the species richness analyses were as described above (Table 1).

Species level responses
Individual species analyses were conducted on the eight most abundant species. These were *Sminthopsis griseoventer* (Class Marsupialia), *Lerista distinguenda*, *Hemiergis peronii*, *Morethia obscura* (Class Sauropsida), *Heleioporus eyrei*, *Limnodynastes dorsalis*, *Crinia sp.* complex and *Pseudophryne guentheri* (Class Amphibia). Comparisons of the size of individuals of the same species were made between the different habitat types. Both body mass and a skeletal measurement were used to compare the size of animals between sites. Head length was used as the skeletal measurement for the mammal species and snout-vent length (SVL) was used for reptile and frog species. For each species, a PERMANOVA analysis with mass and the skeletal measurement as variables was conducted to determine if there was a difference in the size of individuals based on the habitat in which they were found. The structure of the design and factors included are described in Table 1. No covariates were included in this analysis.

Results

A total of 751 individuals from 25 species were captured over 9625 trap nights (Table 2). Three mammal, 13 reptile and nine frog species were identified. Due to the similarity in morphology of *C. subinsignifera* and *C. pseudinsignifera* (Roberts, 2010), these species were difficult to identify in the field. As both species are also very similar in their ecology, captures of these species were combined and will be referred to as *Crinia sp*. complex. Captures of the introduced mouse (*Mus musculus*) were not included in any data analysis. Creek sites had the greatest number of captures over the six sessions at 0.12 (SE = 0.028) captures per trap, with slope and ridge sites both at 0.05 (SE = 0.009 and 0.016 respectively). The total number of captures for reptiles was greatest in the ridges and slopes at 0.03 captures per trap (SE = 0.016 and 0.012 respectively; Figure 4). The total number of captures for frogs was greatest at creek sites at 0.10 (SE = 0.053) captures per trap. Mammal captures per pit were greatest on the slopes at 0.01 (SE = 0.002).

The average number of captures per trap for all taxa across all sessions was 0.07 (SE = 0.11). The greatest number of captures was in January (0.23, SE = 0.043) and fewest in July (0.007, SE = 0.0016; Figure 5). All three major taxa had their highest capture rates in January (0.15 (SE = 0.047), 0.01 (SE = 0.002) and 0.06 (SE = 0.011) for frogs, mammals and reptiles respectively). The lowest capture rates were in September for frogs (0.001, SE = 0.0011) and July for mammals (0.003, SE = 0.0010) and reptiles (0).

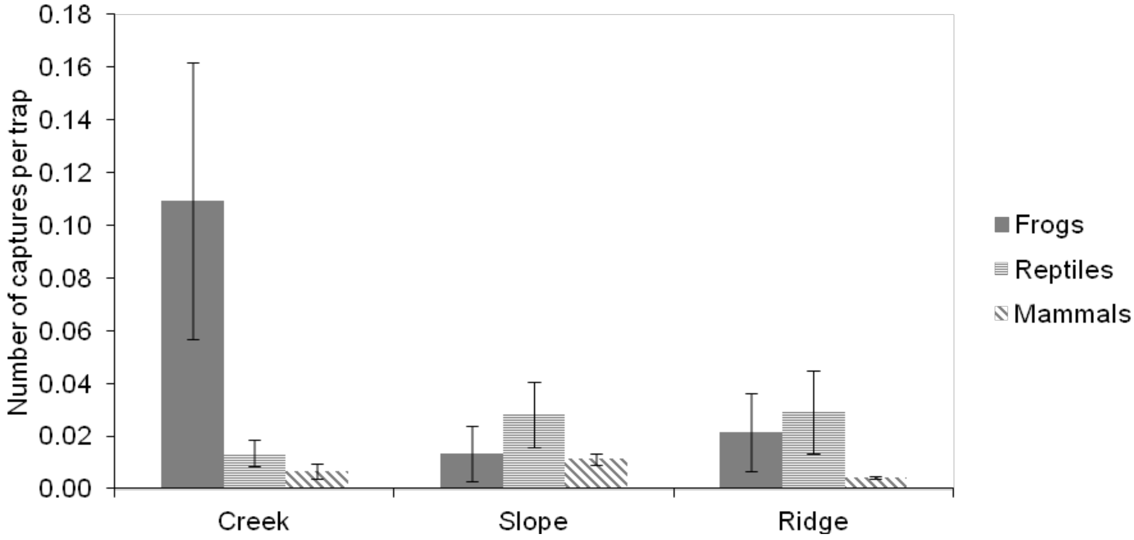

Figure 4: Total number of captures per trap during the six survey sessions according to habitat type. Bars indicate standard errors.

Table 2: Total number of amphibian, mammal and reptile captures and number of species trapped during the six survey sessions according to habitat. Number in brackets indicates the total number of species.

		Habitat		
Taxon	**Species**	**Creek**	**Slope**	**Ridge**
Amphibians	*Crinia georgiana*	6	7	-
	Crinia glauerti	18	3	-
	Crinia sp complex	79	10	4
	Heleioporus eyrei	75	15	5
	Heleioporus inornatus	-	-	1
	Heleioporus psammophilus	5	1	4
	Limnodynastes dorsalis	31	5	57
	Neobatrachus pelobatoides	2	-	-
	Pseudophryne guentheri	95	3	-
		311 (8)	**44 (7)**	**71 (5)**
Mammals	*Sminthopsis griseoventer*	16	37	13
	Mus musculus	17	3	1
	Cercartetus concinnus	4	1	1
		37 (3)	**41 (3)**	**15 (3)**
Reptiles	*Acritoscincus trilineatum*	3	1	2
	Christinus marmoratus	-	2	-
	Ctenotus catenifer	-	1	-
	Ctenotus labillardieri	-	1	3
	Egernia napoleonis	1	1	1
	Hemiergis peronii	9	19	6
	Lerista distinguenda	13	43	71
	Lerista microtis	-	1	-
	Menetia greyii	2	2	2
	Morethia lineoocellata	-	1	3
	Morethia obscura	11	23	6
	Parasuta gouldii	1	-	-
	Ramphotyphlops australis	1	-	2
		41 (8)	**95 (11)**	**96 (9)**

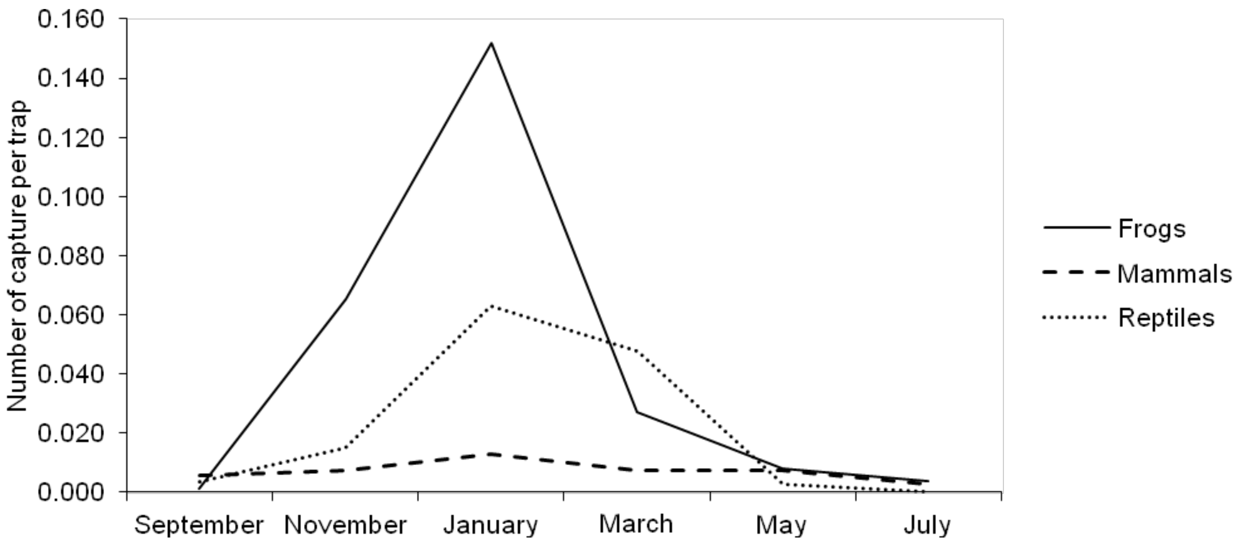

Figure 5: Total number of captures per trap of each taxonomic group according to month of survey. Bars indicate standard errors.

Small vertebrate assemblage responses

There was a significant difference in the composition of the small vertebrate assemblage between the three habitats (PERMANOVA, p=0.0001; Table 3). There was also a significant difference in the assemblage based on the month of sampling (p=0.0001), average rainfall (p=0.0001) and maximum temperature (p=0.0178). There was no evidence that there was a difference in the small vertebrate assemblage inside the sanctuary compared to outside (p=0.1027). There was a significant interaction between habitat and month (p = 0.0061). Habitat type accounted for 10.6% of the variation in the assemblage whereas month sampled, average rainfall and maximum temperature accounted for 28.8, 7.9 and 0.8 % of the variation respectively. The Habitat x Month interaction accounted for 5.4% of the variation in the assemblage. The terms Sanctuary x Habitat, Sanctuary x Month and Sanctuary x Habitat x Month were pooled for this analysis.

Table 3: PERMANOVA results of the effect of sanctuary, habitat, month and site on the structure of the small vertebrate assemblage at Perup. Maximum temperature and average rainfall were also included in the analysis as covariates. Stars indicate significant factors.

Source	**df**	**Mean Square**	**Pseudo-F**	**P value**	**# Permutations**
Maximum temperature	1	2885.2	2.9791	0.0178*	9959
Average rainfall	1	18925	19.539	0.0001*	9958
Sanctuary	1	2374.7	1.8441	0.1027	9950
Habitat	2	9478.9	6.6323	0.0001*	9941
Month	4	9534.5	9.7866	0.0001*	9908
HabitatxMonth	10	1666.8	1.721	0.0061*	9865
Pooled (Site(HabitatxSanctuary) + SanctuaryxHabitat +SanctuaryxMonth)	19	1080.5	1.1157	0.2504	9833
Pooled (Residual + SanctuaryxHabitatxMonth)	69	968.37			
Total	107				

Due to the significant Habitat x Month interaction, pair-wise comparisons were performed on the factor habitat within each month (Table 4a) and on the factor month within each habitat (Table 4b). There was a significant difference in the assemblage in the creek compared to the ridge and the creek compared to the slope in November, January and March (Table 4a). There was also a difference in the assemblage between the creek and the slope in May. There was no difference in between the slope and the ridge during any month.

Within creeks, the months of November, January and March had a significantly different assemblage to all other sampled months but not to each other (Table 4b). Within slopes, January had a significantly different assemblage to all sampled months except March. March had a significantly different assemblage to all months except January and November. Within ridges, January and March had a significantly different assemblage to all other sampled months but not to each other.

Table 4: Pair-wise tests comparing (a) habitat within month of sampling and (b) month within habitat. 'Yes' indicates a significant difference in the assemblage and 'No' indicates no evidence of a difference (PERMANOVA, a = 0.05).

(a)	**September**		**November**		**January**		**March**		**May**		**July**	
	Creek	**Slope**	**Creek**	**Slope**	**Creek**	**Slope**	**Creek**	**Slope**	**Creek**	**Slope**	**Creek**	**Slope**
Slope	No	-	Yes	-	Yes	-	Yes	-	Yes	-	No	-
Ridge	No	No	Yes	No	Yes	No	Yes	No	No	No	No	No

(b)	**Creek**					**Slope**					**Ridge**				
	Sept	**Nov**	**Jan**	**March**	**May**	**Sept**	**Nov**	**Jan**	**March**	**May**	**Sept**	**Nov**	**Jan**	**March**	**May**
Nov	Yes	-	-	-	-	No	-	-	-	-	No	-	-	-	-
Jan	Yes	No	-	-	-	Yes	Yes	-	-	-	Yes	Yes	-	-	-
March	Yes	No	No	-	-	Yes	No	No	-	-	Yes	Yes	No	-	-
May	No	Yes	Yes	Yes	-	No	Yes	Yes	Yes	-	No	No	Yes	Yes	-
July	No	Yes	Yes	Yes	No	No	Yes	Yes	Yes	No	No	No	Yes	Yes	No

CAP analysis based on the factor habitat (Figure 6a) correctly classified 65% of the samples into their correct habitat based on five principal coordinate axes. This was significant with a trace statistic of 0.51468 (p = 0.0001). The abundance of the *Crinia sp*. complex, *H. eyrei* and *P. guentheri* was positively correlated with the CAP1 axis but the abundance of *S. griseoventer* was negatively correlated with this axis. The CAP 2 axis was positively correlated with *S. griseoventer* and *M. obscura* abundance.

CAP analysis based on the factor Month (Figure 6b) correctly classified 55% of the samples into their month of sampling. This was significant, with a trace statistic of 1.30527 (p = 0.0001). The abundance of *H. eyrei*, *L. distinguenda* and *M. obscura* was positively correlated with the CAP 1 axis. The CAP 2 axis was negatively correlated with the abundance of *H. peronii*, *H. eyrei* and *M. obscura*.

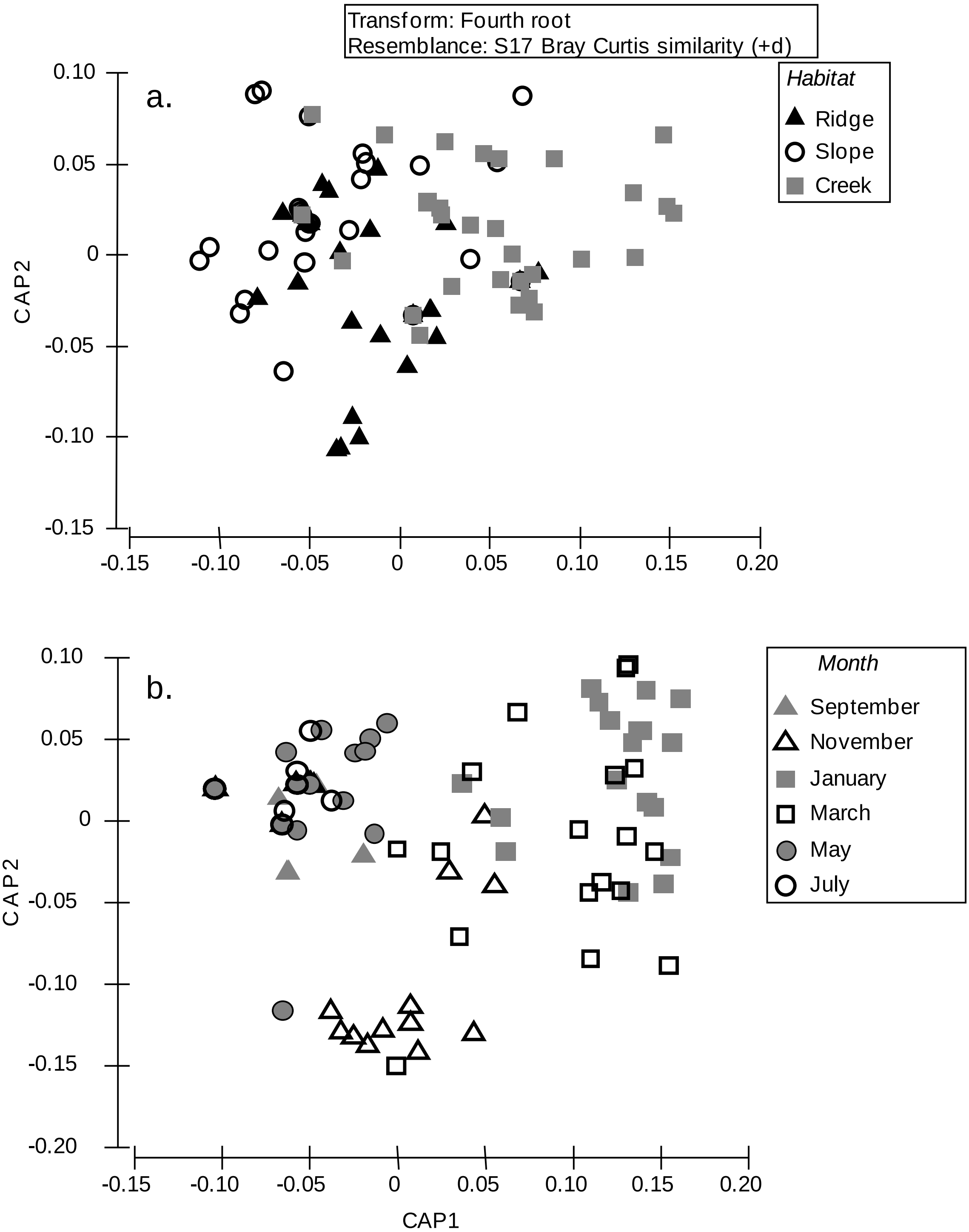

Figure 6: CAP analysis of the small vertebrate assemblage from 108 samples collected during six surveys based on (a) habitat type and (b) month of sampling. Data were fourth root transformed prior to analysis.

The distinctions between habitats in the ordination can be accounted for by differences in the relative abundances of individual species. SIMPER analysis reported an average dissimilarity of 83.8% between ridge and slope habitats, 91.5% between ridge and creek habitats and 87.6% between slope and creek habitats (Table 5). The dissimilarity between ridge and slope sites appeared to be the result of a higher average abundance of *S. griseoventer* at slope sites which contributed 28.8% to the dissimilarity between these habitat types. There was also a higher average abundance of *L. distinguenda* at ridge sites contributing to 15.0% difference, and greater abundance of *H. peronii*, *M. obscura* and *H. eyrei* at slope compared to ridge sites.

Creek sites had a greater average abundance of *H. eyrei* and *Crinia sp*. complex than ridge sites which contributed 12.3 and 11.0% to the dissimilarity between sites. Creek sites had a lower average abundance of *L. distinguenda* but higher average abundances for *P. guentheri* and *Crinia glauerti* when compared to ridge sites. Creek and ridge sites had similar average abundances for *L. dorsalis*.

Slope sites had a greater average abundance of *S. griseoventer*, *M. obscura*, *H. peronii* and *L. distinguenda* when compared to creek sites which contributed 22.7, 10.2, 8.8 and 7.8% respectively to the dissimilarity between these habitats. Creek sites had a greater average abundance of *H. eyrei*, *Crinia sp*. complex and *P. guentheri* contributing to 11.0, 9.3 and 9.2% of the dissimilarity between sites.

Within each habitat type, sites were not very similar. Ridge sites had an average similarity of 10.6%, slopes 30.5% and creeks 14.5%. The species contributing to the similarity of ridge sites were *L. distinguenda* and *S. griseoventer* at 36.0 and 35.8% contribution respectively. The species contributing to the similarity of slopes sites were *S. griseoventer*, *M. obscura* and *H. peronii* (77.1, 7.6 and 7.1% contribution respectively). The species contributing to the similarities between creek sites were *P. guentheri*, *H. eyrei* and *Crinia sp*. complex (24.3, 22.2 and 21.5% contribution respectively).

BEST analyses starting with a set of ten random species identified a subset of nine species sampled that best explained the patterns occurring in the overall small vertebrate assemblage (Spearman correlation coefficient = 0.95, $P<0.001$). These species were the *Crinia sp*. complex, *S. griseoventer*, *H. eyrei*, *H. psammophilus*, *H. peronii*, *L. distinguenda*, *L. dorsalis*, *M. obscura* and *P. guentheri*.

Table 5: Summary of the average similarity within and dissimilarity between habitats based on the small vertebrate assemblage. The shaded boxes contain the average similarity between sites of the same habitat type and the species which are common within that habitat. The unshaded boxes contain the average dissimilarity between sites of different habitat types and the species which contribute to those differences. Which habitat has the greater average abundance of the species is also described.

Habitat	Ridge	Slope	Creek
Ridge	**10.55** *Lerista distinguenda, Sminthopsis griseoventer*		
Slope	**83.79** *Sminthopsis griseoventer* Slope > Ridge *Lerista distinguenda* Ridge > Slope *Hemiergis peronii* Slope > Ridge *Morethia obscura* Slope > Ridge *Heleioporus eyrei* Slope > Ridge	**30.54** *Sminthopsis griseoventer, Morethia obscura, Hemiergis peronii*	
Creek	**91.51** *Heleioporus eyrei* Creek > Ridge *Crinia sp*. complex Creek > Ridge *Pseudophryne guentheri* Creek > Ridge *Lerista distinguenda* Ridge > Creek *Limnodynastes dorsalis* Creek ≈ Ridge	**87.59** *Sminthopsis griseoventer* Slope > Creek *Morethia obscura* Slope > Creek *Hemiergis peronii* Slope > Creek *Lerista distinguenda* Slope > Creek *Heleioporus eyrei* Creek > Slope *Crinia sp.* complex Creek > Slope *Pseudophryne guentheri* Creek > Slope	**14.45** *Pseudophryne guentheri, Heleioporus eyrei, Crinia sp*. complex

Species richness

PERMANOVA analysis showed a significant effect for habitat (p = 0.0066), month of sampling (p = 0.0001), maximum temperature (p = 0.0003) and average rainfall (p = 0.0001; Table 6). The terms Sanctuary, Sanctuary x Month and Habitat x Sanctuary x Month were pooled for this analysis. Pair-wise tests demonstrated a significant difference in species richness between ridges and slopes (p = 0.04) and ridges and creeks (p = 0.02). There was no evidence to suggest a difference in species richness between slopes and creeks (p = 0.11). Species richness was on average greatest at creek sites and lowest at ridge sites (Figure 8). Species richness was greatest in January and lowest in July (after accounting for the reduced trapping effort in September; Figure 8).

Table 6: PERMANOVA results of the effect of habitat, month and site on species richness at Perup. Maximum temperature and average rainfall were also included in the analysis as covariates. Stars indicate significant factors.

Source	df	MS	Pseudo-F	P(perm)	# Permutations
Maximum temperature	1	3317.2	11.015	0.0003*	9943
Average rainfall	1	17736	58.911	0.0001*	9955
Month	4	7434.6	25.105	0.0001*	9953
Habitat	2	1081.3	3.9848	0.0066*	9944
HabitatxSanctuary	2	205.27	No test		
HabitatxMonth	10	407.97	1.355	0.1656	9920
Pooled (1) Site(HabitatxSanctuary)+Sanctuary+SanctuaryxMonth	18	203.69	No test		
Pooled (2) Res + HabitatxSanctuaryxMonth	69	301.23			
Total	107				

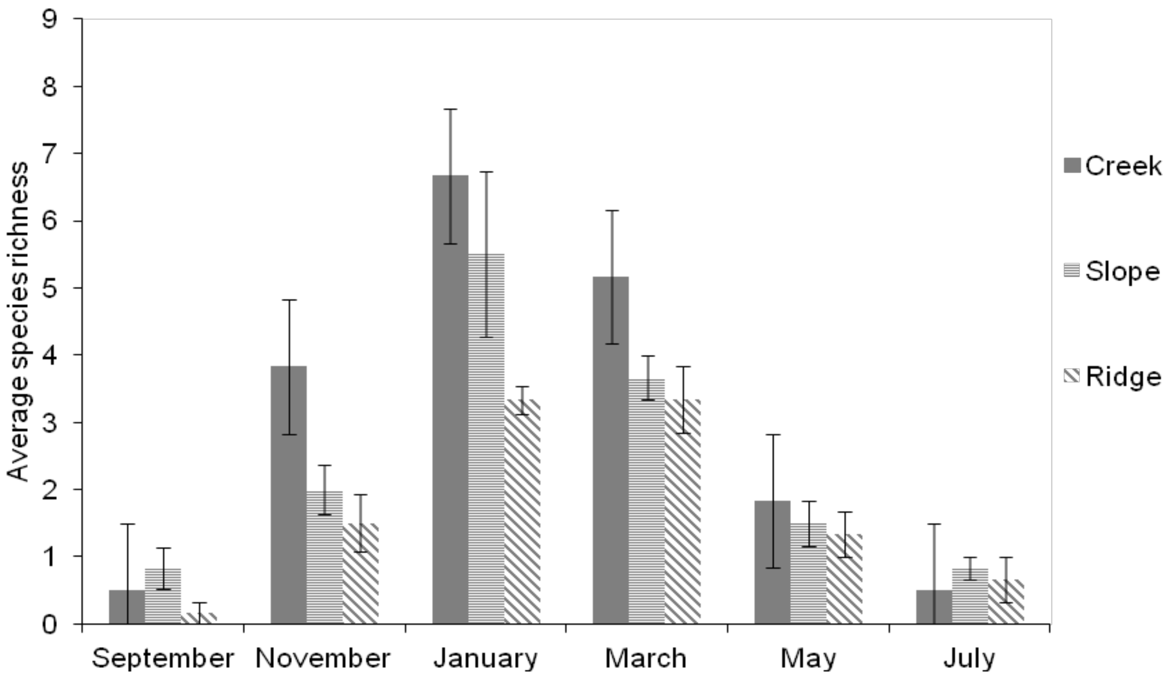

Figure 8: Average species richness of each habitat type according to month of sampling. Bars indicate standard errors.

Species level responses

Sminthopsis griseoventer

There was a significant difference in the size of *S. griseoventer* based on month of capture (PERMANOVA, $p = 0.0002$). The heaviest dunnarts were caught during July and September and the lightest during November. Head length was greatest in May and July and the smallest in November. On average, females were heavier than males and had shorter head lengths. All other terms were pooled in the analysis and there was no evidence to support a difference in size based on sanctuary, habitat or site.

Morethia obscura

There was a significant difference in the size of *M. obscura* based on month of capture (PERMANOVA, $p = 0.0163$). This species was captured during September, November, January and March. On average, the heaviest individuals were captured during November and the lightest in September. The greatest SVL on average was in November and January and smallest during March. All other terms were pooled during the analysis and there was no evidence to support a size difference based on habitat, sanctuary or site.

Hemiergis peronii

There was no evidence to support a difference in the size of *H. peronii* based on month, sanctuary, habitat or site. All terms except sanctuary were pooled during the analysis.

Lerista distinguenda

There was a significant difference in the size of *L. distinguenda* based on month (PERMANOVA, $p = 0.0001$). This species were captured during January and March. On average, individuals had a greater mass and SVL during January compared to March. There was no evidence to suggest a difference in the size of individual inside compared to outside the sanctuary. All other terms were pooled in the analysis and there was no evidence to support a difference in the size of individuals based on site or habitat.

Limnodynastes dorsalis

There was a significant difference in the size of *L. dorsalis* based on month (PERMANOVA, $p = 0.0001$) and sanctuary ($p = 0.0197$). On average the smallest individuals were captured during January. The largest individual was captured in July but there was only one capture during this month. Individuals were on average smaller outside the sanctuary compared to inside however there were only five captures inside the sanctuary compared to 88 captures outside the sanctuary. The data is heavily skewed because of one trapping night during January where 52 sub adult individuals were captured at a ridge site and another 27 at a creek site adjacent to the ridge site. There was a large movement of sub adults from the creek upwards towards the ridge site and this large activity event resulted in the highest capture rate outside the sanctuary and during the January session. There was also a significant interaction effect between month and sanctuary ($p = 0.0158$). There was a significant difference in the size of individuals between individual sites ($p = 0.0027$). All other terms were pooled in the analysis and there was no evidence to support a size difference based on habitat.

Heleioporus eyrei

There was a significant difference in the size of individuals based on month of capture (PERMANOVA, $p = 0.0159$). The smallest individuals on average were captured during November and the largest were captured during May. All other terms were pooled in the analysis and there was no evidence to support a size difference based on habitat, sanctuary or site.

Crinia sp. complex
There was a significant difference in the size of *Crinia sp*. complex based on month of sampling (PERMANOVA, p = 0.0259). The smallest individuals on average were captured during November and the largest in July. There was also a significant difference in the size of individuals based on site (p = 0.004). All other terms were pooled during analysis and there was no evidence to support a difference based on habitat or sanctuary.

Pseudophryne guentheri
There was a significant difference in the size of *P. guentheri* based on month of capture (PERMANOVA, p = 0.0079) and site (p = 0.0256). *P. guentheri* were captured in November, January, March and July (although there was only a single capture during July). There was an increase in the average body mass and SVL of individuals from November to March. All other terms were pooled during the analysis and there was no evidence to support a size difference based on sanctuary or habitat.

Discussion

There was a significant difference in the small vertebrate assemblage found in each of the three main habitat types. Creek habitats had the greatest number of captures and species richness overall but this was heavily influenced by the large seasonal influx of frogs after rainfall events in November, January and March. The creek habitat is unsurprisingly the most important site for the frog species in the area as it provides appropriate refuge and a breeding location for all frog species captured (Lee, 1967, Main, 1968, Silla, 2010). These sites were dominated by *P. guentheri*, *H. eyrei* and the *Crinia sp*. complex. The least mobile and the smaller of the frog species were found in the creek habitats only, with only a few records of those species higher up in the landscape. The more mobile and larger species such as *H. eyrei* and *L. dorsalis* were spread more evenly through the landscape. It was not uncommon to find *H. eyrei* in the sandy slope habitat as this is a suitable refuge location used after the breeding season (Lee, 1967, Main, 1968, Berry, 2001). *L. dorsalis* was as likely to be found in the ridge sites as the creek sites and did not show the apparent preference for areas lower in the landscape as with the other frog species. This species is the largest of the frog species captured and has a relatively smaller body surface from which evaporation takes place compared to smaller frogs (Bellis, 1962). This suggests that they may dehydrate at a slower rate and so are able to move away from the moister areas in the lower parts of the landscape (Bellis, 1962).

Slope sites were characterised by a greater presence of *S. griseoventer*, *H. peronii* and *M. obscura*. Slope locations appeared to be particularly important for *S. griseoventer* with over 56% of their captures in this habitat. *S. griseoventer* are opportunistic predators which feed on small arthropods which dwell in the leaf litter and coarse woody debris layer (Fisher and Dickman, 1993, McCaw, 2011). The slopes have greater leaf litter and debris cover as this habitat has the greatest canopy cover and lower storey vegetation density (Havel and Mattiske, 2000). This habitat may also provide better thermal insulation when *S. griseoventer* is resting and better cover from avian predators while foraging at night. The slope habitat also possesses characteristics suitable for *M. obscura* and *H. peronii* which are both terrestrial skinks which forage in dense leaf litter under low, dense shrubs (Smyth, 1974, Nichols and Bamford, 1985). Slope sites will also have patches of sunny and shady positions which reptiles can utilise for temperature regulation while maintaining cover from predators. The slopes sites may provide a more complex vegetation structure and therefore a greater number of microhabitats for these species to utilise.

Ridge sites were characterised by a greater relative abundance of *L. distinguenda* with over 55% of the captures of this species in this habitat. The association of this species with the ridge habitat supports the findings of other studies which show an association between *L. distinguenda* and areas with high leaf litter cover but a lower understorey density (Nichols and Bamford, 1985).

The patterns in seasonal activity and the significant effect of temperature and rainfall reflect the physiological and breeding characteristics of the species captured (Bellis, 1962, Main, 1968, Smyth, 1974, Friend, 1993). Frog species demonstrated the strongest seasonality with greatest relative abundance during January and March where it is likely that large rainfall events after extended periods of warm and dry conditions initiated mass breeding and/or feeding activities (Main, 1968). Reptiles also demonstrated seasonal patterns of activity with the greatest relative abundance during the warm summer months. Reptiles were less likely to be captured during or immediately after large rain events. Reptiles were not captured during the colder winter months as they are inactive during this time due to the cooler temperatures (Spellerberg, 1972). Female reptiles in temperate climates exhibit highly seasonal patterns in reproduction (Murphy *et al.*, 2006). Eggs are laid in late spring or early summer and offspring hatch or are born in summer (Murphy *et al.*, 2006). Summer is the only time of year where soil temperatures and insolation are high enough to permit rapid embryonic development (Murphy *et al.*, 2006). *S. griseoventer* which represented the majority of the mammal captures did have a slight increase in relative abundance during the warmer months but did not display a strong seasonal pattern of activity as in the frog and reptile species. This species is endothermic and is able to remain active year round (Friend, 1993), which probably explains the lack of a strong seasonal pattern in activity.

The size of the effect of habitat on the small vertebrate assemblage varied according to the month of sampling. This is shown by the significant interaction between month and habitat based on the relative abundances of each of the species captured. During months where fewer species were active, it was more difficult to distinguish the assemblages between each habitat. The seasonal change in the size of the effect of habitat highlights the importance of repeated sampling over many months (Mac Nally, 1997). Ideally this survey would have re-sampled for a following twelve months to verify the seasonal pattern in activity. The seasonal differences in the assemblage have implications for the monitoring of the area. Monitoring will need to be timed appropriately to ensure that a significant portion of the assemblage is detected. Ideally monitoring would occur every other month however this may be unachievable due to resource limitations. If sampling is restricted to only one part of the year, sampling in warmer conditions after rain will yield the most captures and therefore provide the best opportunity to survey species in the area.

Although species richness was different between habitats, this was not also reflected in species diversity. Creek sites had the greatest species richness but no greater diversity of species compared to the slope and ridge sites. This is because there were often one or two frog species which had very large relative abundances in the creek sites and so these species dominated the assemblage. Species diversity was however influenced by the month of sampling and this mirrored the pattern seen in species richness with the greatest diversity in the warm summer months and the least in the cooler winter months. This demonstrates again the seasonal behaviour of the species within the assemblage where many species become active and are breeding during the warmer months and after large rain events.

Of the 24 native species captured, a subset of nine species described over 95% of the variation in the complete assemblage (*Crinia sp*. complex, *S. griseoventer, H. eyrei, H. psammophilus, H. peronii*, *L. distinguenda*, *L. dorsalis*, *M. obscura* and *P. guentheri*). Most of these species are those which were shown to characterise each of the habitat types and this again demonstrates the distinction in the assemblage across the landscape. Each of these species (excluding *H. psammophilus*) are relatively abundant within the survey area and require less intensive trapping to be detected than rare or cryptic species. This survey used a relatively large number of pit traps (450) which required a significant effort in terms of labour by staff and volunteers as well as vehicle costs. If in the future only a portion of these traps are able to be monitored because of resource limitations, the smaller trap effort may not detect all 24 species. In this case, it is useful to know that data on just these more abundant and easily detectable species may aid in describing the patterns occurring in the wider assemblage.

There was no evidence to support a size difference of species between habitats. Of the eight most abundant species analysed, none of the species showed a significant difference in their body mass or skeletal length based on the habitat in which they were found. All of the species except *H. peronii* demonstrated an average size difference of individuals based on month of capture. This pattern is indicative of the life history and breeding biology of these species. With *S. griseoventer*, the largest individuals were captured in the winter months and the smallest in November. This reflects the winter breeding where the population is solely larger adults, followed by the emergence of juveniles from their natal nests in late October (Van Dyck and Strahan, 2008). *M. obscura* was largest in November as it moves into its breeding season and females are gravid and smallest in September when it becomes first active after the extended period of inactivity over the winter months (Chapman and Dell, 1985). The frog species *L. dorsalis*, *H. eyrei*, *C. pseudinsignifera* and *P. guentheri* are all winter breeders and the smallest individuals were captured during the spring and summer months. This is a result of juveniles and sub adult frogs entering the population following metamorphosis (Main, 1968).

The assemblage found inside the sanctuary was not significantly different to outside the sanctuary, however the p-value was borderline ($p=0.059$). When selecting survey sites we attempted to select similar sites outside the sanctuary as inside, but it became clear during trapping and as the season changed that one of the external creek sites (S9) was quite different to the other creek sites. This site was in a much broader valley than the other creek sites and held a greater amount of water. It also drained into a larger swamp area located on private land to the west of the survey area. This site had the greatest relative abundance of frog species and particularly the smaller *Crinia* sp. The characteristics of this site make it a particularly valuable breeding location for most frog species and also useful for the smaller species which require a moist environment year round. Smaller sub adult *L. dorsalis* captured at this site caused the significant size difference between inside and outside the sanctuary for this species. This was a result of one event where a large number of sub adult *L. dorsalis* (>70) moved out of the creek at site S9 and upwards toward the ridge line at site S8. As this site appears to be particularly valuable to many of the frog species in the area, it will be an important site to monitor and conserve in the future.

The association between species and habitat has important implications for how the sanctuary and surrounding area is managed. Sampling in only one habitat type or sampling without regard for the distinction between habitats is will compromise the monitoring of the species in the area. Future surveys should continue monitoring each of the habitats in order to be as sensitive as possible to changes in the assemblage. This approach is also relevant to the fire management of the sanctuary. As the movement of species across the landscape is potentially restricted because of the sanctuary fence, the use of prescribed burns has the potential to negatively impact populations. If a burn is conducted that impacts all of one particular habitat within the sanctuary, this could hinder the recovery of a fire sensitive species after the fire event if that habitat is of greater value than other areas in the sanctuary. One recommendation based on the results of this survey is the division of the sanctuary into smaller units based on habitat type which can then be monitored and burned at that scale. Any prescribed burn plan should ensure that if any one unit is burned, there are other units from the same habitat type which remain unburnt. This will assist in the recovery after fire of species which utilise that particular habitat.

Conclusion

The construction of fences can be viewed as a radical form of environmental manipulation particularly when the composition of species within the fenced area is also artificially modified. This strategy for the conservation of wildlife is becoming increasingly common in Australia and New Zealand as managers attempt to reduce the impact of introduced and invasive species (Burns *et al.*, 2012, de Tores and Marlow, 2012, Dickman, 2012). As the protection of vulnerable native species is time sensitive, few studies have been undertaken which deal with the potential unintended effects of fencing wildlife. The current study has established a baseline data set which can be used to monitor the changes in the extant small vertebrate population in Perup Nature Reserve in order to improve our knowledge of the processes occurring in a highly modified environment. In this situation, different habitats appear to hold different values for particular species and possess characteristics necessary for the completion of life history traits. The modification of the environment in the form of the construction of the fence, increasing medium sized mammal density and prescribed burning mean that close monitoring and consideration of the documented ecology of the small vertebrate species will be a necessary part of the management of the area if negative impacts are to be minimised. This information will be of interest to all managers of fenced exclosures as the success of management outcomes of target species within fenced areas may be linked to the health and viability of the wider assemblage.

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