4.5. Resources

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Abstract

The availability, abundance and suitability of resources such as food have been identified as a potential factor in woylie declines. While the woylie is known to be mycophagous (i.e. hypogeal and epigeal fungi are a major component of the diet), no comprehensive studies have been undertaken examining temporal and spatial variation in diet and food resource availability. The food resources component of the Woylie Conservation Research Project (WCRP) is being addressed through a collaborative PhD research project between Murdoch University and the Department of Environment and Conservation. Preliminary surveys investigating fungi availability and the methods for assessing sporocarp abundance have been conducted. Overall the preliminary results indicate that hypogeal fungi are not limiting at any of the Population Comparison Study sites where woylies have declined. However, more information regarding diet and feeding preferences is required. The aims, results so far and proposed future work are outlined in this report.

4.5.1. Introduction

An understanding of the biology and ecology of a threatened species is necessary for effective conservation and management (Chen *et al.,*. 1998) and the diagnosis of decline (Caughley 1994; Caughley and Gunn 1996; Peery *et al.,* 2004). Knowledge of the diet is an essential component of this understanding. The accessibility, abundance and suitability of resources, such as food, have been identified as a potential factor in the recent woylie declines (Wayne *et al.,* 2006). The woylie is mycophagous, excavating and ingesting large amounts of hypogeal (truffle-like) fungi and some epigeal fungi (i.e. mushrooms) throughout the year (Christensen, 1980; Davis, 2005; Garkaklis, 2001; Lamont *et al.*, 1985; Lee, 2003). However, no comprehensive studies have examined the temporal and spatial variation in diet and food resource availability.

The food resources component of the Woylie Conservation Research Project (WCRP) will be addressed by a collaborative PhD research project between Murdoch University and Department of Environment and Conservation (DEC). The aim of the PhD project is to determine if the dietary ecology of the woylie is related to current woylie population declines. Specifically the project aims to examine temporal and spatial variation in the diet of the woylie, examine changes in woylie diet in relation to population decline and to investigate food resource availability. In order to address these aims an analysis of dietary components will be conducted using scat samples and stomach contents. Food resource availability will be assessed by means of seasonal fungi and vegetation surveys. Data obtained from these studies will be interpreted in relation to climate change, land use history and the ecological role of woylies in ecosystem health and diversity. The PhD project commenced in January 2007 and is scheduled for completion early in 2010.

Preliminary studies were conducted by DEC researchers during 2006. The purpose of these studies was to develop the foundations for the collaborative PhD research project. In addition to collecting the scat and digesta samples for subsequent analysis, a pilot study was conducted to;

1. Survey hypogeal fungi at the WCRP Population Comparison Study (PCS) sites;
2. Conduct a preliminary assessment of whether there are any relationships between food resources and woylie abundances and population trends; and

3. Assess the feasibility and sampling requirements required to more rigorously test whether there are any significant differences in the hypogeal fungi food resources related to woylie abundances and trends.

The results of these studies are appended (Bougher 2006 and Robinson *et al.*. 2007) (Volume 2 Appendices 2 and 3). A summary of the key results from these studies as well as the results to date from the collaborative PhD project are detailed in this report.

4.5.2.1. Preliminary hypogeal fungi surveys

Preliminary surveys for hypogeous fungi were undertaken in June, August and September 2006 at five sites within the Upper Warren Region (Keninup, Balban, Warrup, Winnejup and Boyicup). A survey at Karakamia Wildlife Sanctuary, approximately 50 km east of Perth, was conducted on the 4th of October 2006. During each survey, three plots were established at each site – one lowerslope, midslope and upperslope location each. Plots were 20 m x 50 m with the long edge running along the slope. Advice regarding an appropriate plot size was sought from T. Lebel (Royal Botanic Gardens, Melbourne) prior to the study. Hypogeous sporocarps were collected by raking the litter and organic soil layer with a four-pronged Canterbury hoe to a depth of 10-15 cm. Each plot was raked for the equivalent of 100 person minutes. The aim was to cover approximately 25-40% of the total plot area, with an effort made to distribute the search evenly across each plot.

All fungal sporocarps were collected and assigned a collection number. Sporocarps were described, assigned a species number and photographed in the laboratory. Collections were then air dried and weighed. The "reference type collection" for each species was then microscopically examined, photographed and where possible identified.

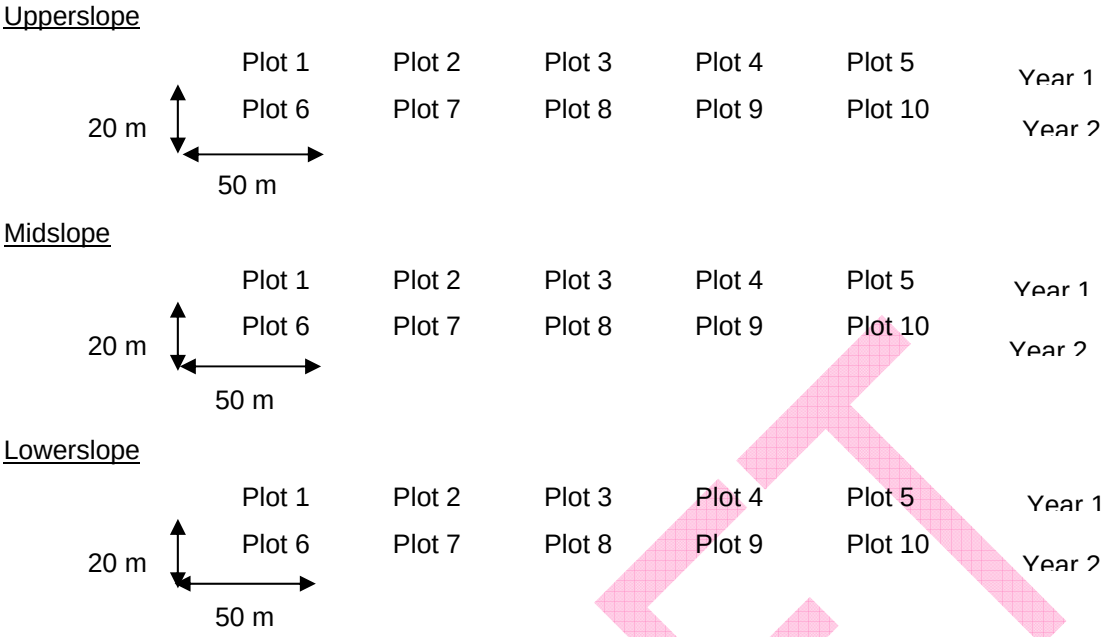
Vegetation structure and floristics data was collected during the preliminary assessment, but has not yet been analysed. Tree basal area, overstorey, midstorey and lowerstorey species cover and strata heights were recorded in each plot. All plots were assigned a vegetation health score and *Phytophthora cinnamomi* presence score. Soil colour and type were also noted along with the amount of litter in each plot. To aid in determining relationships between hypogeous fungal diversity and abundance, leaf litter and soil moisture data was collected during the September fungal survey.

For a detailed description of the methods used in these preliminary surveys please refer to the reports contained within Volume 2, Appendices 2 and 3

4.5.2.2. PhD project fungi surveys

The survey design for the PhD project builds on the design used for the preliminary surveys (Bougher, 2006; Robinson *et al.*, 2007). The same six woylie PCS sites will be surveyed during the PhD project, Keninup, Balban, Warrup, Winnejup, Boyicup and Karakamia Wildlife Sanctuary. As with the preliminary surveys, during each survey period, three plots will be surveyed in relation to topography – one upperslope, midslope and lowerslope plot at each site (Figure 4.5.1). Each plot will be 20 m x 50 m with the longest edge running across slope. A randomised-block design for repeat sampling within each topographic grouping is used to account for possible confounding by environmental factors (Figure 4.5.1). Over a two year period there will be a survey within each season, with extra surveys during winter as a greater number of fungi fruit at this time. A survey will be conducted in Winter 2007 (two surveys), Spring 2007, Summer 2007/08, Autumn 2008, Winter 2008 (two surveys), Spring 2008, Summer 2008/09 and Autumn 2009. Therefore, a total of 10 plots will be surveyed at each topographic position during 10 survey periods at each site. Each of the ten plots within each topographic position will be randomly assigned to a seasonal survey.

Fungi survey plots will be situated within 5 km of the population monitoring trapping grid at each site and will not be situated in recently burnt (< 6 months) areas, or near well used roads.



Note: Plots 1-10 within each topographic grouping will be randomly assigned to a seasonal survey.

Figure 4.5.1. Design of PhD research project fungi survey at each site.

Collection of hypogeal fungi sporocarps

Plots will be sampled using a time census method, developed by Claridge *et al.,* (2000). Based on the results of Robinson *et al.,* (2007) a 100 person minute sampling time will be used to survey each plot. Hypogeal fungi will be collected using a four-pronged hoe to rake the litter and soil layer to a depth of approximately 15 cm. Disturbed soil will be replaced immediately after the survey. All sporocarps will be collected. It is anticipated that the collections from the preliminary surveys and the PhD program will be joined and submitted to the Western Australian Museum upon completion of the project.

Processing and identification of sporocarps

In the laboratory, each sporocarp collection will be identified to species or morphospecies. Specimens will then be dried for 4-5 days in a drying cupboard or food dehydrator at 40°C. A dry weight for each specimen will be recorded. To confirm identifications a sample will be taken from dried specimens using a razor blade to cut a thin section of gleba and mounted on a glass slide. Specimens will be stained with KOH and in some cases Melzer's reagent. The spores will be examined under x1000 magnification using a compound microscope. A photograph will be taken to be used as a reference for the identification of fungal spores in the woylie faecal samples.

Surveying of epigeal fungi sporocarps

Bougher (2006) recommended that epigeal fungi are included in future surveys in response to evidence that mammals at Karakamia have been observed feeding on epigeal fungi. As a result epigeal fungi will also be surveyed as part of the PhD project. Each plot will be surveyed thoroughly for epigeal fungi, prior to commencement of the hypogeal sporocarp searches. All epigeal species present within the plot will be identified (where possible) and recorded. Due to the large number of epigeal species present within the plots, the numbers of species rather than fruiting bodies will be recorded.

The date, plot location, associated vascular plants, time since last burn, litter layer depth and soil characteristics (soil type, soil colour and soil moisture content) will also be recorded for each plot.

Robinson *et al.* (2007) recommended that portable weather stations be installed at each site in order to analyse relationships between climatic conditions and fungal species diversity and richness, as

rainfall patterns in the Upper Warren region appear to be variable and highly localized. Rainfall stations have now been installed at each of the five population comparison study sites within the Upper Warren Region.

At the time of this report, two surveys to assess the availability of epigeal and hypogeal fungal sporocarps (fruiting bodies) at each of the six PCS sites, Karakamia, Keninup, Balban, Warrup, Winnejup and Boyicup have been conducted as part of the PhD research project. The results from these two surveys are included in this report.

4.5.3. Results

4.5.3.1. Preliminary hypogeal fungi surveys

During the three surveys within the Upper Warren Region, 388 collections comprising 826 sporocarps of hypogeous fungi were made. Thirty-four species of fungi were recognized. Thirty-one species were Basidiomycetes; two were Glomeromycetes (*Endogone* spp.) and one was an Ascomycete (*Hydnoplicata convoluta*). Seven were identified to species level and 15 were identified to genus only. Full collection details are provided in Robinson *et al.* (2007) (Volume 2 Appendix 2).

Five species were represented among 11 collections from Karakamia Sanctuary during the survey in October 2006, three Basidiomycetes and two Zygomycetes. Two species, *Cytangium sessile* and *Dermocybe globuliformis* were collected from both Karakamia Sanctuary and the Upper Warren Region. Identifications, descriptive details, and images of the specimens are provided in Appendix 3 (Volume 2).

The results of the preliminary fungi surveys showed that the highest species diversity and abundance was recorded in August. Overall, species diversity was similar between the sites. However, species composition varied considerably for each of the sites, with only a few recorded species common to all sites. Sporocarp abundance varied between sites. Generally, abundance was found to be higher at sites with higher rainfall.

The lowest overall number of sporocarps was collected from upperslope sites. The only exception was Boyicup, where the most sporocarps were recorded from the upperslope site. Correspondingly, the upperslope sites also recorded the lowest overall total dry weight of sporocarps.

The total number and biomass of sporocarps tended to be inversely related to woylie abundance, which suggests that hypogeal fungi (and presumably food resources) were available at sites where woylies have declined, Analysis of the data from the preliminary work relating to vegetation structure and floristics and woylie nest and digging densities remain to be completed (Wayne unpublished data).

The full reports for the preliminary fungi surveys are included as Appendix 2 and Appendix 3 (Volume 2).

4.5.3.2. PhD project fungi surveys

Epigeal Fungi

During the two surveys in June and August 2007, 88 species were recorded from Boyicup, 77 species from Warrup, 52 species from Keninup, 64 species from Balban, 65 species from Winnejup and 59 species from Karakamia. Only some of these species are likely to be eaten by woylies. Although this will not be known until the results of the dietary scat analysis are available (see Section 4.5.5. Future Work).

Hypogeal Fungi

To date, 595 sporocarps have been collected; totalling 62.8 grams (dry weight). Up to 29 hypogeal fungi species from the first survey (Winter 1) and 27 hypogeal fungi species from the second survey (Winter 2) have been recorded. Approximately 45 species in total have so far been recorded. It is likely that a number of new species have already been discovered. Further taxonomic work is required to confirm identifications. In addition, reference slides are being produced from these samples to aid with the faecal analysis (see Section 4.5.5. Future Work).

A greater number of sporocarps were collected during the August survey (Winter 2), with 345 sporocarps collected compared with 250 sporocarps collected in June 2007 (Winter 1) (Figure 4.5.2). Soil moisture was generally higher during the second (Winter 2) survey (Figure 4.5.3). The highest number and dry weight of sporocarps was collected from plots at Boyicup in the Upper Warren Region (Figures 4.5.2 and 4.5.4). Soil moisture content was also the highest at Boyicup during both surveys (Figure 4.5.3). Conversely, the lowest number of sporocarps and total dry weight was recorded from Karakamia Sanctuary (Figures 4.5.2 and 4.5.4). A similar result was observed during the preliminary surveys (Volume 2 Appendices 2 and 3).

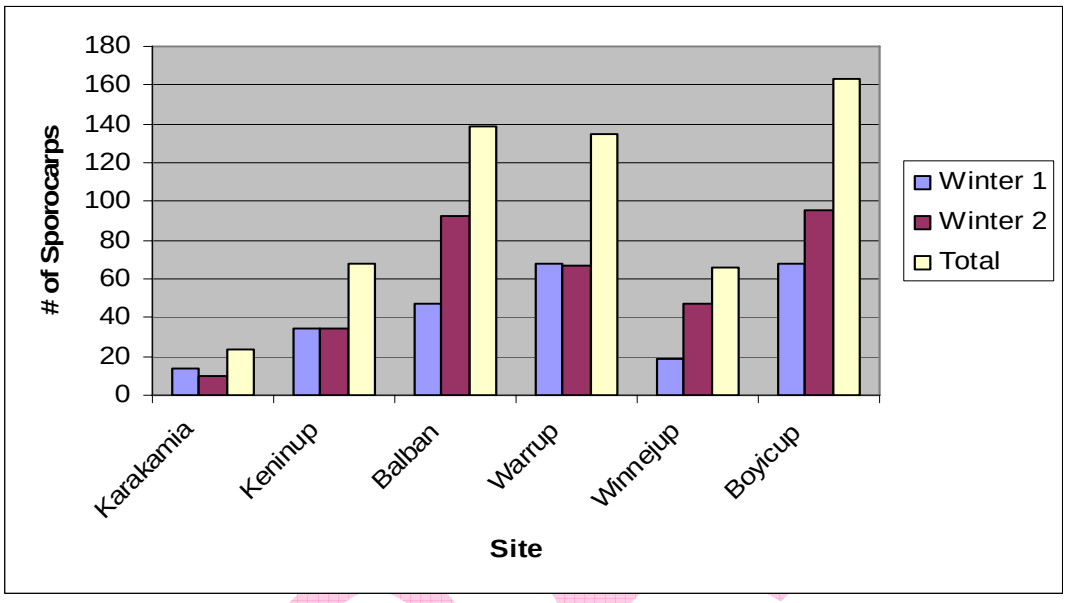


Figure 4.5.2. Number of hypogeal sporocarps collected during the two surveys at the six woylie PCS sites.

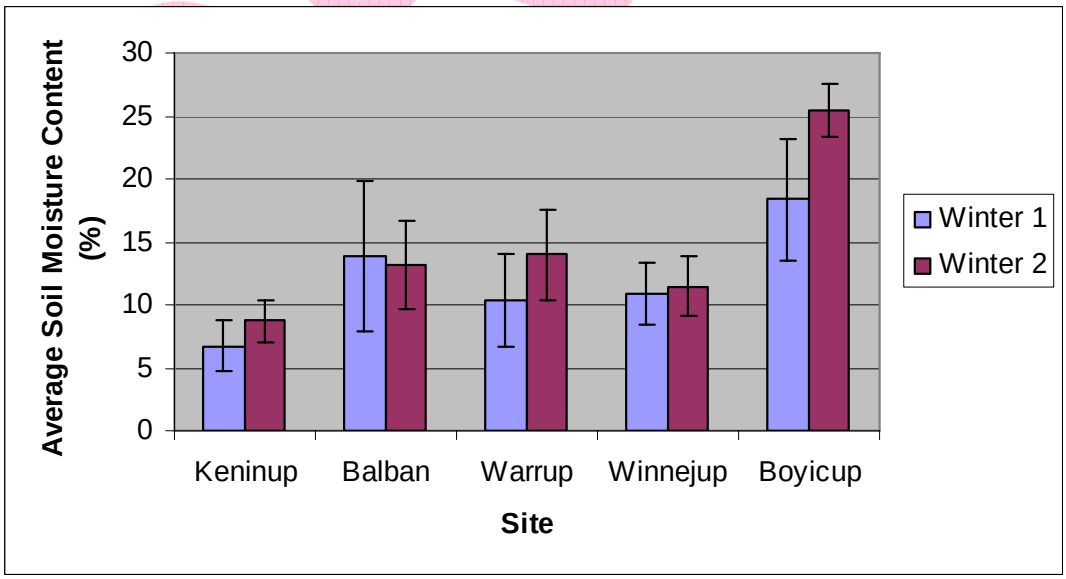


Figure 4.5.3. Average soil moisture content (%) recorded during the two winter surveys at each of the five woylie PCS sites in the Upper Warren.

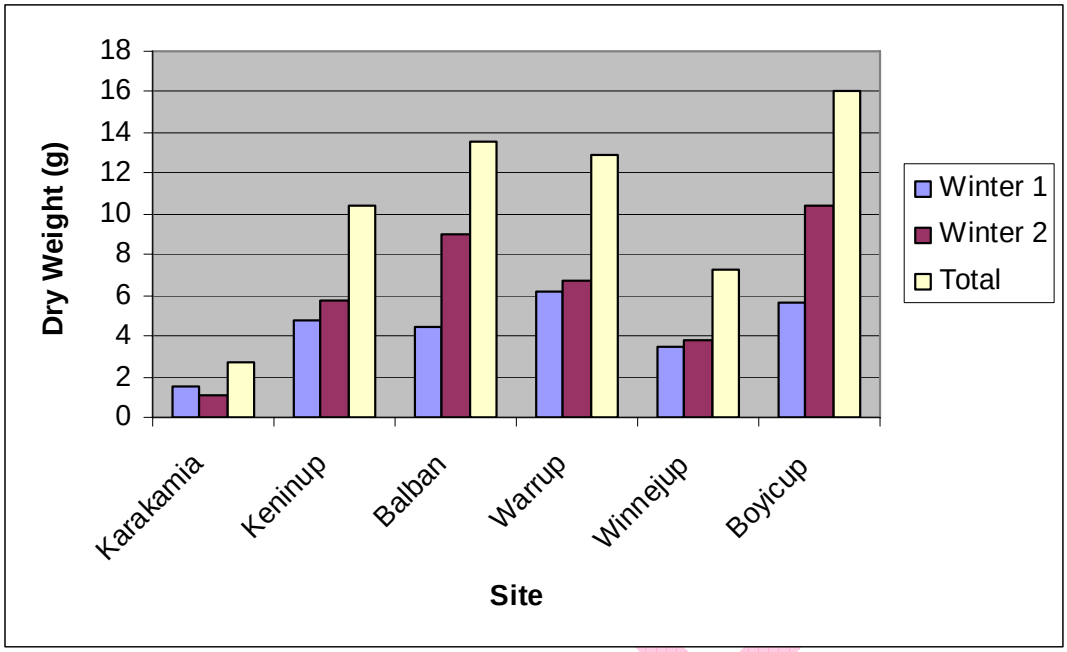


Figure 4.5.4. Total dry weight (grams) of sporocarps recorded during the two winter surveys at each of the six woylie PCS sites.

The majority of sporocarps collected to date during the PhD project have been from upperslope plots, followed by the midslope and lowerslope plots (Figure 4.5.5). This is in contrast to the results of the preliminary surveys where the fewest sporocarp collections were made from upperslope plots (Volume 2 Appendix 2). However, the relationship between the number of sporocarps collected and topographic position varies considerably at each site (Figure 4.5.6).

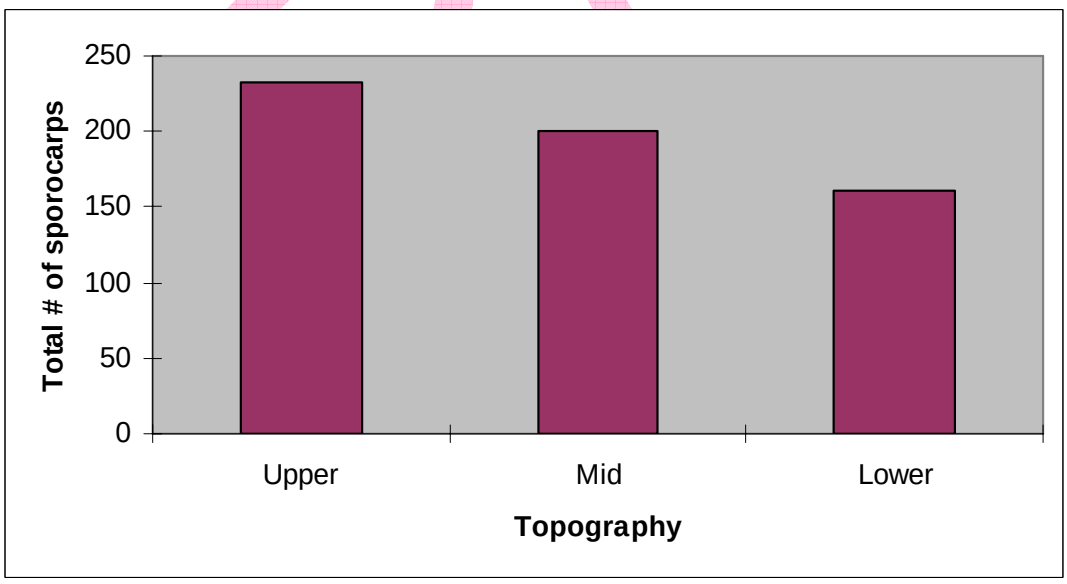


Figure 4.5.5. Total number of hypogeal sporocarps collected within each topographic grouping.

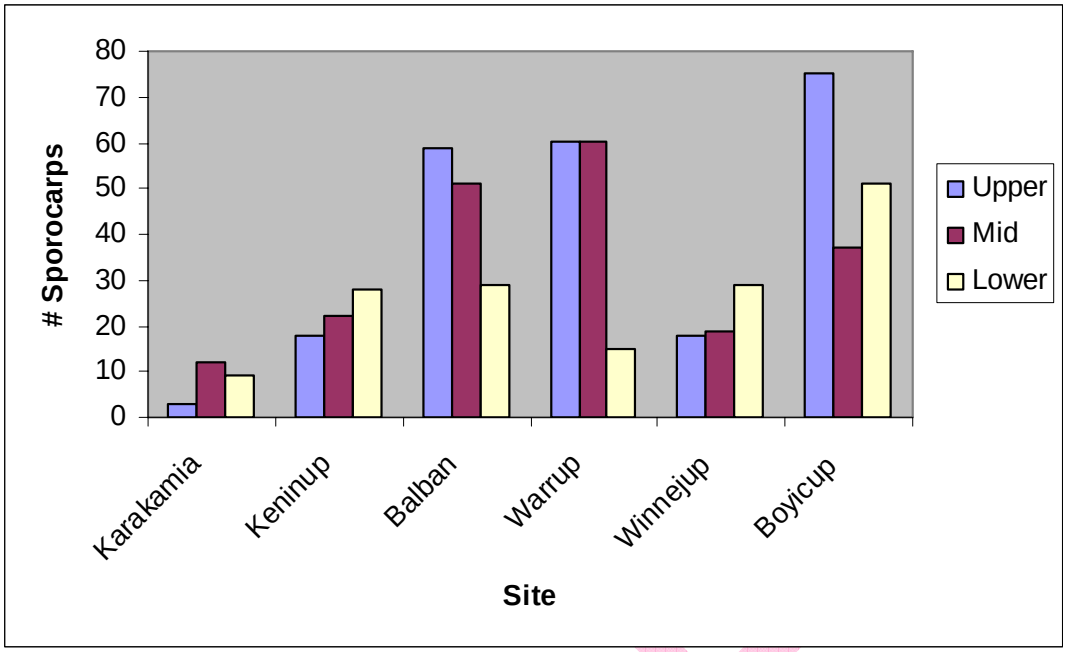


Figure 4.5.6. Number of hypogeal sporocarps collected within each topographic grouping at the six woylie PCS sites.

4.5.4. Discussion

The hypogeal habit, ephemeral nature and patchy distribution of sequestrate fungal sporocarps, means that their availability is difficult to measure (Bougher and Lebel, 2001). Previous studies have had difficulties due to a small number of collections or because a large number of plots was required. However, the results of the preliminary surveys and the two surveys conducted as part of the PhD project indicate that the methodology has so far been successful, with a large number of collections.

Previous studies have found seasonal differences in the abundance of fungi, where some species demonstrate peaks in abundance during wetter periods (Abell *et al.,* 2006; Claridge *et al.,*. 1993). While the studies conducted so far, as part of the WCRP have only involved sampling in winter and spring, it seems likely that there will be a significant seasonal trend in the number of sporocarps recorded. More sporocarp collections were made during August for both the PhD project and during the preliminary survey. This is likely to be the result of more appropriate conditions for fruiting, as soil moisture content was also found to be higher during the August surveys. Previous studies have also shown a relationship between soil moisture levels and fruiting, where those species with sporocarps of high moisture content fruited when soil moisture was also high (Johnson, 1994). A number of hypogeal species recorded during the Winter 1 survey were not recorded during the Winter 2 survey and vice versa.

Further research regarding dietary preferences of woylies is required before the consequences of seasonal variation in the number and abundance of fungi species for woylies can be determined. If the availability of hypogeal sporocarps is limiting at certain times of the year and woylies are reliant on other food sources, this may influence woylie survival and reproductive output.

No real trend with regard to topographic position and the number of collections made has been observed at this stage. However, this may become more prominent during the drier months, where lowerslope sites are more likely to retain soil moisture.

During the preliminary surveys and the two surveys for the PhD project, the highest number of sporocarp collections was made at sites where woylies are not present in any great numbers. Conversely, very few collections were made where woylies are present in high densities. Robinson *et al.*. (2007) suggested that this result could be related to the foraging activities of woylies, whereby

sporocarps are more difficult to unearth where researchers are in competition with woylies. While this is likely to be true, the preliminary results also indicate that hypogeal fungi are not limiting at any of the population comparison sites where woylies have declined. However, more information regarding diet and feeding preferences is required.

4.5.5. Future work

The collaborative PhD research project between Murdoch University and DEC is still in its early stages (i.e. started January 2006). Future work will involve continuing the fungi availability surveys and commencing the woylie dietary analysis.

Future work for DEC Science should also include an analysis of the vegetation structure and floristics data collected during the preliminary surveys and the completion of vegetation surveys within each of the PCS trapping grids. This data will be related to both food and woylie abundance.

Woylie Dietary Analysis

An analysis of dietary components will be conducted using faecal samples collected during population monitoring and opportunistically from the stomach contents of recovered woylie bodies. This approach has the advantage of providing a large quantity of information on the diet of the woylie while using non-invasive procedures.

A large amount of faecal material has been collected (and will continue to be collected) from woylies during trapping programmes run by the Department of Environment and Conservation, Australian Wildlife Conservancy and the South Australian Department of Environment and Heritage. Faecal material for dietary analysis has been collected from a number of locations within the Upper Warren region, Karakamia Wildlife Sanctuary, Tutanning Nature Reserve, Dryandra Woodland, Batalling State Forest and Venus Bay Conservation Park (SA).

Each faecal sample collected has been labelled with a unique code and information on sex, date of capture, location of capture and the health and reproductive condition of the individual has been recorded. The faecal analysis will be based upon the methodology used by Tory *et al.* (1997) and Green *et al.* (1999). Briefly, this will involve filtering the faecal material into a coarse and fine fraction and then examining a series of sub-samples of each fraction under a compound microscope. Food items will be identified to the lowest grouping possible and the relative percentage of each dietary category calculated by examining a set number of fields of view.

To investigate spatial variation in the diet of woylies, a selection of faecal samples from corresponding seasons will be assessed from each of the locations mentioned earlier. Temporal variation will be assessed by investigating the different components of woylie diets and examining any differences in the relative proportion of dietary components between seasons, over a two year period, from woylie populations at Karakamia Sanctuary and the Tone Perup Nature Reserve/Greater Kingston National Park, where sampling has been much more frequent. In addition, dietary comparisons will be made during the various stages of reproduction.

To examine changes in woylie diet in relation to population decline, three sets of woylie faecal samples will be analysed. Firstly, the dietary composition and proportions of dietary items will be compared for woylies in populations of high (Karakamia Sanctuary), moderate (Warrup and Keninup) and low (Boyicup and Winnejup) densities. Secondly, the composition and proportions of dietary items will be analysed for woylies from the Balban site, during the transition from a moderate density population to a low density population. Thirdly, the composition and proportions of dietary components will be compared for woylies pre decline (pre 2001) and post decline. Pre-decline samples are available from other studies conducted in the Upper Warren Region and Batalling before woylie populations started to decline in number (prior to 2001).

To compliment the results of the faecal sample analysis, stomach contents collected opportunistically from deceased woylies will be analysed. Stomach contents are generally accepted to be more reliable as the dietary items are less digested than those found in faecal samples. This will then provide a comparison for the faecal analysis, and allow a determination of the level of accuracy with which the faecal results may be interpreted.

As well as forming the basis for a PhD thesis, it is expected that a number of publications of articles in scientific journals will result from this research.

4.5.6. Conclusion

The results of the fungi availability surveys conducted to date indicate that food resources may not be limiting at any of the PCS sites where woylie numbers have substantially declined (Boyicup and Winnejup). In fact, fungal sporocarps are in lower abundance at Karakamia Wildlife Sanctuary and Keninup where woylie numbers are the highest. However, it is premature to draw conclusions and caution is required when interpreting the results because of the preliminary nature of the work. For example, it is imprudent to assume that all of the fungal species recorded are suitable to be consumed by woylies. More information regarding diet and feeding preferences will be determined once the faecal analysis has been conducted. Both food resource availability and dietary analysis need to be assessed to identify relationships between diet and seasonal changes in the abundance of food resources. It seems likely, however, that the number and abundance of fungi species and other dietary items will vary seasonally. As a consequence, there may be times of the year when preferred food resources are limiting and this may influence woylie survival and reproductive output.

Information obtained during the PhD research project will not only assist with the diagnosis of current woylie declines, but will also enable a better understanding of long-term conservation requirements. An understanding of when food resources may be limiting and temporal and spatial variations in sporocarp production will assist future recovery programmes, including re-introductions and translocations.

4.5.7. References

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