The Use of Artificial Refuges to Census Populations of the 'Threatened' Striped Legless Lizard, *Delma impar* in Western Victoria

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Declaration

I hereby declare that the work involved in this investigation was performed by the author and that no material presented herein has been submitted for any previous degree, diploma or publication, except where due reference has been made.

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Ethics Approval.

The research undertaken in connection with this thesis was approved by La Trobe University Animal Ethics Committee (Approval No: AEC05/14(L)) and Zoos Victoria Animal Ethics Committee (Approval No: ZV05008).

Wildlife Research

The use of wildlife in connection with this thesis was authorized by the Department of Sustainability and Environment under the *Wildlife Act 1975* (Research Permit No: 10003423).

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SUMMARY

The Striped Legless Lizard (*Delma impar*) is considered to be a flagship species for the conservation of native lowland grasslands. It is ‘endangered’ in Victoria and is listed as ‘threatened’ under several additional legislations. The factors influencing the presence and detectability of *Delma impar* in remnant grasslands in Western Victoria, surveyed using a passive survey technique (artificial refuges: roof tiles), were investigated. Habitat and microhabitat characteristics, environmental conditions, time of day, and other taxa influencing tile occupation by *D. impar,* were examined for twenty roof tile grid sites. Morphometrics of *D. impar* were investigated and the relationship of tile occupancy rate to abundance was considered. Refuge preference of *D. impar* was determined in a laboratory experiment.

Cloud cover, temperature and relative humidity (both under and outside a tile) appeared to influence tile occupation by *D. impar*. Occupation rates of tiles by *D. impar* were also associated with the percentage cover of kangaroo grass (*Themeda triandra)* and agricultural grass underneath tiles. A shorter maximum height of vegetation and an increased cover of agricultural grasses around tiles increased tile occupancy by *D. impar*. The degree of soil cracking under tiles negatively influenced the presence of *D. impar*, as did the current or past occupancy of tiles by snakes. *D. impar* showed a strong avoidance of terracotta tiles under the conditions of the laboratory experiment, but had a strong preference for grass tussocks.

D. impar was found to be sexually dimorphic, with females the larger of the two sexes. Sex ratios did not significantly differ from 1:1 over time or for each month surveyed. Further investigation is warranted to determine if a relationship exists between occupancy rates and abundance, in order to assess the effectiveness of roof tiles as an alternative census technique.

Chapter 1. INTRODUCTION

Lowland remnant grasslands have significantly declined in area since European settlement (Stuwe and Parsons 1977; Kutt 1993; Osborne *et al*. 1993; Smith and Robertson 1999; Lunt 2003). As a result, they are among the most threatened ecosystems in southern Australia (Osborne *et al*. 1993; Lunt 2003). Accordingly, fauna associated with grasslands have also rapidly declined (e.g. *Eulamprus tympanum marnieae* (Corangamite Water Skink), *Perameles gunnii* (Eastern Barred Bandicoot), *Burhinus frallarius* (bush stone-curlew), which are all listed as 'threatened' in Victoria) (Dufty 1994; Peterson 1997; Michael *et al*. 2004). Among such species is the Striped Legless Lizard (*Delma impar*), which is listed as 'endangered' in both Victoria (*Flora and Fauna Guarantee Act* 1988*)* and South Australia (*National Parks and Wildlife Act* 1972*)* and 'vulnerable' in New South Wales (*Threatened Species Conservation Act* 1995) and Canberra (*Nature Conservation Act* 1980) (Osborne *et al*. 1993; Dorrough 1995; Hadden 1995; Wilson and Swan 2003; O'Shea 2004). It is listed as 'vulnerable' internationally by the ANZECC and the IUCN and by the Australian Commonwealth under the *Environment Protection and Biodiversity Conservation Act* 1999 (Hadden 1995; Wilson and Swan 2003).

The main threatening process for endangerment of *D. impar* in Victoria is habitat destruction and degradation. Ploughing, pasture improvement and cropping, extended and intense grazing, inappropriate fire regimes (applied at inappropriate intensity, season and frequency), urban development and other heavy disturbances significantly contribute to the decline of this species (Kutt 1993; Hadden 1995; O'Shea 1996, 2004; Dorrough and Ash 1999; Rauhala and Andrew 1998; Smith and Robertson 1999; Koehler 2004).

D. impar is currently used as a flagship species for the conservation of grasslands (Smith and Robertson 1999). It has been acknowledged that considerable attention is attracted towards *D. impar* due to the species' 'novelty' (snake-like appearance) and public appeal (Smith and Robertson 1999). Hence, research into what may become a well-known and well-liked flagship species may be invaluable for conservation of its native grassland habitat and the species itself (Smith and Robertson 1999).

1.1. *Delma impar*

The Striped Legless Lizard (*Delma impar* Fischer 1882) is the most southerly distributed member of the Pygopodidae family, which are endemic to Australia and Papua New Guinea (Coulson 1990; Wilson and Swan 2003). *D. impar* is distinguished by broad olive to brown vertebral stripes that run from the tympanum to just beyond the vent, with dark and narrow lateral lines from the tympanum extending towards the end of the tail (Coulson 1990; Wilson and Swan 2003). Hatchlings have dark brown or black heads and lack the vertebral and lateral lines (Coulson 1990). Individuals may have yellow infralabial and adjacent gular scales that extend towards the tympanum (Coulson 1990). However, the extent of this yellow colouration varies, and can be absent in some individuals. Tail length is generally twice the length of the body, with total length not exceeding 306mm (Coulson 1990).

The pygopodids including *D. impar* are oviparous, laying a clutch size of two eggs in late spring-summer (usually December/January), with hatching occurring in January/February (Patchell and Shine 1986a; Smith and Robertson 1999). It has been estimated that age at first reproduction is at 3-4 years in females and 2-3 years in males, with adult females capable of breeding every year (Smith and Robertson 1999 from ARAZPA 1996). However, at present, this is only speculative and is based on growth rates and expected life-history patterns (Smith and Robertson 1999). Accordingly, additional aspects of the life-history (e.g. mortality, animal movement, egg oviposition sites) of *D. impar* remain largely unknown (Smith and Robertson 1999).

D. impar is semi-fossorial, retreating into the soil layer or grass tussocks during the night and active at surface level during the day (Coulson 1990; Hadden 1995; O'Shea 2004). They are thigmotherms (non-basking reptiles, maintaining body temperature by heat exchange with the substrate) (Rawlinson 1971; Heatwole and Taylor 1987; Coulson 1990), but have also been found to show some heliothermic behaviours (using radiant energy for body heat by basking (Heatwole and Taylor 1987)) (Hadden 1995). *D. impar* also predominantly inhabits cracking clay soils (Coulson 1990; Dorrough 1995), with cracks providing refuges during the summer months (G. Peterson *pers. comm.*) and torpor sites in winter (Smith and Robertson 1999). Rocks and grass-tussock bases are also used as over-wintering sites in this species (Smith and Roberstson 1999). Smith and Robertson (1999) suggested that the availability of these shelter/refuges may be a key to survival in rural areas during disturbance events (e.g. grazing, ploughing and perhaps prescribed burns).

Historically, *D. impar* was found only in primary grasslands. However, with the formation of secondary grasslands (due to the removal of trees and canopy cover in woodlands) since European settlement, *D. impar* has been recorded in these recently formed vegetation communities (Coulson 1990; Dorrough 1995; Dorrough and Ash 1999). Dorrough (1995), Hadden (1995), O'Shea (1996, 2004) and Koehler (2004) have focused on these broad habitat relationships with *D. impar*, but only Koehler (2004) looked at the habitat structure provided by the vegetation types. It has been suggested that a dense and continuous structure may be more important than the floristic composition of grassland in influencing the presence of *D. impar* (Smith and Robertson 1999). Smith and Robertson (1999) indicated that further research is required on the relationships between the structure and floristics of grasslands and the abundance of *D. impar*. It was recommended that existing known populations of *D. impar* be the focus of such a study.

Insufficient knowledge of the micro-habitat use of this species has also been documented (Smith and Robertson 1999). Kerr *et al.* (2003) maintain that, for lizards living in heterogeneous environments, the choice of microhabitat is dependent on many ecological processes (e.g. exposure to predators, conspecific or interspecific competition and temperature differences). For *D. impar* such predators may include elapid snakes and dunnarts, with competitors consisting of other lizard species. Therefore, *D. impar* may have quite specific microhabitat requirements (Warwick *et al.* 1998; Kerr *et al.* 2003) that are yet to be acknowledged. Within the microhabitat, they may even make finer choices regarding thermal properties (Schlesinger and Shine 1994).

1.2. Census Techniques for *D. impar*

To estimate abundances or the presence of reptiles, passive census techniques involving capture of individuals are generally employed (Brown and Nicholls 1993; Blomberg and Shine 1996). In the case of *D. impar*, previous census techniques have focused on pitfall trapping with drift fences (Coulson 1990; Dorrough 1995; O’Shea 1996, 2004) and rock-rolling (Beggs 1997). More recently, however, census techniques for *D. impar* have began to focus on the use of artificial refuges (roof tiles) (Koehler 2004; O’Shea 2004) as an alternative monitoring and survey method. It is suggested that cover items (artificial refuges) such as wood and tin increase the ease of capture of some elusive species (Houze and Chandler 2002; Michael *et al.* (2004; Ford and Hampton 2005). However, Kunz *et al.* (2005) found coverboards to be poor indicators of both population size and density for California Legless Lizards. Hence, the effectiveness of artificial refuges (roof tiles) to determine population size and density is currently enigmatic.

Use of various census techniques for monitoring *D. impar* requires careful consideration. Trapping techniques such as pitfall traps have been acknowledged as potentially affecting the behaviour of individuals (Sutherland 1996). Individuals such as *D. impar* have been found to become trap-shy with decreased capture rates over time (Coulson 1990; O'Shea 2004). They have also been seen to avoid drift-fences, by springing over them (Coulson 1990). Therefore, the trap-shyness of individuals to pitfall traps will provide underestimates of population size (Sutherland 1996). Alternatively, survival of *D. impar* may be compromised within such traps by predation and extreme weather conditions (Sutherland 1996; Houze and Chandler 2002). Conversely, roof tiles do not constrain individuals and survival is uncompromised (Koehler 2004; O'Shea 2004). Roof tiles therefore, do not need to be monitored as frequently as do pit-fall traps (O'Shea 2004).

Consideration must also be given to the time and labour required for installation of the census technique and also the costs involved (Ryan *et al.* 2001; Houze and Chandler 2002; O'Shea 2004). For rocky areas, such as the basalt plains or areas with clay soils, installation of pitfall buckets can be both difficult and time consuming (if they can be installed at all). Therefore, census techniques must be optimal for the habitat of study sites (Ford and Hampton 2005). It is also costly for the equipment needed, as apposed to artificial refuges, which can be brought for as little as $1 each (Ryan *et al.* 2001; O'Shea 2004). Furthermore, artificial refuges can be easily dispersed for broad geographic coverage (Rodda *et al.* 2001; G. Peterson *pers. comm.*).

Additionally, the impact that the given census technique has on the habitat must be considered. Pit-fall traps have been found to be destructive to native grasslands, removing vegetation and disturbing the soil (O'Shea 1996; Koehler 2004). Roof tiles on the other hand can be installed with minimal effort, and easily moved and hence have a substantially reduced impact on threatened grassland communities (O'Shea 1996, 2004; Koehler 2004). O'Shea (2004) commented that vegetation disturbance by tiles only occurred in the area directly under a tile.

Different census techniques have been found to vary in their effectiveness at detecting species (Brown and Nicholls 1993; Bailey *et al.* 2004; Ryan *et al.* 2002). Pit-fall traps have been found to have low capture and recapture rates of *D. impar* (Coulson 1990; O'Shea 2004). Koehler's study in 2004 also achieved limited success in capturing *D. impar* via the use of artificial refuges (roof tiles). This could have been attributed to time constraints, unseasonal weather prior to and during surveying and insufficient lag time within her study. Similarly, O'Shea (2004) only undertook brief studies on their effectiveness, but found slightly higher captures using roof tiles as apposed to pitfall traps with drift fences. However, both Koehler (2004) and O'Shea (2004) maintain that artificial refuges (roof tiles) may be a successful alternative census technique for *D. impar.* Additionally, other grassland species (small vertebrates) have been found using the artificial refuges (roof tiles) (Koehler 2004). An extensive monitoring study is therefore needed to provide insight and identify the factors influencing the use of roof tiles by *D. impar*. Further research may also confirm the validity of using roofing tiles as an alternative passive survey method.

1.2.1. Ecological factors influencing presence/absence and abundances using artificial refuges

The ability of artificial refuges (roof tiles) to detect the presence/absence or abundance of a species will depend upon several factors. These may include the activity of the species (which can be influenced by home range or weather conditions) and the size or habit of the species being censused (Blomberg and Shine 1996; Howard *et al*. 2003; Shah *et al*. 2004). Additionally, the use of artificial refuges (roof tiles) will be influenced by whether they are used as basking sites or as refuges during inactivity periods.

The abundance of a species using an artificial refuge may also be influenced by the quality of a refuge (e.g. vegetation cover under or around the artificial refuge and occurrence of other animals). Furthermore, it may be attributed to the refuge availability and refuge preference of individuals. If particular resources (refuges) are in limited supply, then an addition of these should increase the abundance of the species. Thus, the ability of artificial refuges to be able to determine abundances of *D. impar* and covariates affecting their use requires investigation.

Alternatively, if the artificial refuges used for censusing are not the preferred refuge of the species, an underestimation of abundance will occur. Shah *et al*. (2004) have suggested that laboratory experiments be used to gain further insight into the selection of refuges, which may remain ambiguous from field studies alone.

1.3. Aims

The general aim of this study was to determine the factors influencing the presence and detectability of *Delma impar* in remnant grasslands in Western Victoria when surveyed using a passive survey technique.

The specific aims were to:

1. Assess the effectiveness of roof tile grids as a census technique (i.e. determine presence/ absence and relative abundances) for *D. impar*
2. Describe sexual dimorphism and sex ratios of *D. impar*
3. Determine if occupancy rates of roof tiles by *D. impar* reflect their abundances at sites
4. Determine the habitat characteristics of sites that influence occupancy rates/abundances of *D. impar*
5. Determine optimal sampling conditions for monitoring of *D. impar* using roof tile grids
6. Determine some of the factors that influence individual tile use by *D. impar*
7. Determine microhabitat use by *D. impar*
8. Determine the refuge preferences of *D. impar* using a laboratory experiment

Chapter 2. MATERIALS AND METHODS

2.1. Study sites

Twenty sites located in Western Victoria (Figure 2.1) were selected on the basis that they all supported extant populations of *Delma impar* (G. Peterson, *pers. comm.*). The sites were generally long and narrow remnant grasslands, mainly occurring on roadsides or rail reserves. A few sites also occurred on agricultural land, which included sheep paddocks or conservation reserves (Table 2.1). In the case of the roadsides or rail reserves, the grassland management typically involved prescribed burns, whereas private paddocks were grazed by livestock (G. Peterson, *pers. comm.*) (Table 2.1).

Physiographically, the study sites were situated in the West Victorian Volcanic Plains division (basalt plains) (Kenley 1971) and were located within the warm temperate bassian zoogeographic sub-region (Hadden 1995). Average annual rainfall for the area is between 500-750mm (Hadden 1995), and elevation ranges between 0-300m above sea level. The majority of study sites were on level to gently undulating plains (Table 2.2) (Maher and Martin 1987). Soils were either hard pedal duplex or shallow friable loams, with basalt or sedimentary rock origins (Table 2.2) (Maher and Martin 1987).

The sites were characterized by either Plains Grassland, Plains Grassy Woodland or Plains Grassland/ Plains Grassy Woodland mosaic Ecological Vegetation Communities (EVC) (pre-1750s) (Table 2.1) (DSE 2003).

Figure 2.1: Location of study sites in Western Victoria. ■ indicates location of study sites. (Map taken from Church 1998).

Table 2.1: Descriptions of study sites used in the surveys for *D. impar*.

Site	Latitude & Longitude	Ecological Vegetation Class (EVC)*	Land Use	Management Activities
Blacks Creek	37°36'S 143°19'W	Plains Grassland/ Plains Grassy Woodland Mosaic	Sheep Paddock	Grazing
Bochara Station	37°42'S 141°56'W	Plains Grassy Woodland	Rail Reserve	Grazing
Boonderoo Nature Reserve	37°58'S 138°02'W	Plains Grassland	Conservation Reserve	Grazing
Camperdown-Foxhow Rd #1	38°05'S 143°22'W	Plains Grassland/ Plains Grassy Woodland Mosaic	Roadside	Prescribed Burns
Camperdown-Foxhow Rd #2	38°08'S 143°16'W	Plains Grassland/ Plains Grassy Woodland Mosaic	Roadside	Prescribed Burns
Caramut-Chatsworth Rd	37°54'S 142°35'W	Plains Grassland	Roadside	Prescribed Burns
Cavendish-Dunkeld Rd	37°36'S 142°12'W	Plains Grassland	Roadside	Prescribed Burns
“Challicum Park”	37°26'S 143°09'W	Plains Grassland/ Plains Grassy Woodland Mosaic	Sheep Paddock	Grazing
Chatsworth Rd	37°55'S 143°06'W	Plains Grassy Woodland	Roadside	Prescribed Burns
Cressy Trotting Track	38°01'S 143°37'W	Plains Grassy Woodland	Conservation Reserve	Prescribed Burns
Cressy-Shelford Rd	38°01'S 143°52'W	Plains Grassland/ Plains Grassy Woodland Mosaic	Roadside	Prescribed Burns
Forest Lane	37°43'S 142°15'W	Plains Grassland/ Plains Grassy Woodland Mosaic	Roadside	Prescribed Burns
Geggies Rd	38°00'S 143°44'W	Plains Grassland/ Plains Grassy Woodland Mosaic	Roadside	Prescribed Burns
Hamilton HWY-Tea-tree Creek	37°58'S 142°34'W	Plains Grassland/ Plains Grassy Woodland Mosaic	Roadside	Prescribed Burns
Lubra Creek	37°56'S 142°30'W	Plains Grassland/ Plains Grassy Woodland Mosaic	Roadside	Prescribed Burns
Old Ararat Rd	37°37'S 127°27'W	Plains Grassy Woodland	Roadside	Prescribed Burns
Rokewood Common	37°54'S 143°43'W	Plains Grassland/ Plains Grassy Woodland Mosaic	Conservation Reserve	Grazing
Rokewood-Shelford Rd	37°54'S 143°46'W	Plains Grassland/ Plains Grassy Woodland Mosaic	Roadside	Prescribed Burns
Rossbridge Flora Reserve	37°29'S 142°52'W	Plains Grassland/ Plains Grassy Woodland Mosaic	State Reserve	Grazing
Vite Vite Rail Reserve	37°52'S 143°10'W	Plains Grassy Woodland	Rail Reserve	Prescribed Burns

* Ecological Vegetation Class (EVC) as mapped in DSE (2003).

Table 2.2: Soils and geomorphology of the study sites used in the surveys for *D. impar*.

Site	Geomorphology*	Soil Type*
Blacks Creek	Undulating rises (basalt)	Shallow friable loams with rough-ped fabric
Bochara Station	Undulating low hills (basalt)	Hard pedal mottled-brown duplex & red smooth-ped earths
Boonderoo Nature Reserve	Level plains (basalt)	Hard pedal mottled-black duplex
Camperdown-Foxhow Rd #1	Steep high cones	Shallow friable loams with rough-ped fabric
Camperdown-Foxhow Rd #2	Level plains (sedimentary)	Hard pedal mottled-yellow duplex
Caramut-Chatsworth Rd	Gently undulating plain (basalt)	Hard pedal mottled-yellow duplex
Cavendish-Dunkeld Rd	Gently undulating plain (basalt)	Hard pedal mottled-yellow duplex
"Challicum Park"	Gently undulating rises (basalt)	Hard pedal mottled-yellow duplex
Chatsworth Rd	Gently undulating plain (basalt)	Hard pedal mottled-yellow duplex
Cressy Trotting Track	Gently undulating plain (sedimentary)	Hard pedal mottled-black duplex
Cressy-Shelford Rd	Level plains (basalt)	Hard pedal mottled-black duplex
Forest Lane	Gently undulating plain (basalt)	Hard pedal mottled-yellow duplex
Geggies Rd	Undulating rises (basalt)	Shallow friable loams with rough-ped fabric
Hamilton HWY-Tea-tree Creek	Gently undulating rises (basalt)	Hard pedal mottled-yellow duplex
Lubra Creek	Gently undulating rises (basalt)	Hard pedal mottled-yellow duplex
Old Ararat Rd	Gently undulating plain (basalt)	Hard pedal mottled-yellow duplex
Rokewood Common	Gently undulating rises (basalt)	Hard pedal mottled-brown duplex & red smooth-ped earths
Rokewood-Shelford Rd	Undulating rises (basalt)	Shallow friable loams with rough-ped fabric
Rossbridge Flora Reserve	Gently undulating rises (basalt)	Hard pedal mottled-yellow duplex
Vite Vite	Gently undulating plain (basalt)	Hard pedal mottled-yellow duplex

*Geomorphology and soil type as mapped and described in Maher and Martin (1987).

The sites used in this study were originally selected by the Department of Sustainability and Environment from South-west Biodiversity Maps (Koehler 2004) on the basis of their EVC. Coulson (1990) and Hadden (1995) previously identified plains grasslands and grassy woodlands as habitat for *D. impar*.

The dominant vegetation species at many of these sites was *Themeda triandra* (Kangaroo grass) or other short to medium tussock-forming grasses (e.g. *Poa* spp., *Stipa* spp.). However, agricultural grasses had become more wide-spread at some of the sites (e.g. Rossbridge Flora Reserve and Old Ararat Rd.) and only small patches of *T. triandra* remained.

2.2. Artificial refuge (roof tile) census

2.2.1. Tile grid arrangement

A grid consisting of fifty artificial refuges (roof tiles), which were either concrete (450 x 275mm) or terracotta (430 x 340mm) were used to survey *D. impar* at the sites. Tiles were arranged in five rows, which consisted of ten tiles in each row (Fig 2.2). Grids were established in July 2003 sixteen months prior to the study by the Department of Sustainability and Environment (DSE) and Koehler (2004) as part of a large scale survey for *D. impar* across South Western Victoria (G. Peterson *pers. comm.*.). Some tiles were replaced during the survey period due to damage sustained from either cattle, vehicles or machinery. Tiles were spaced 5m apart and covered an area of 900m^2 (refer to Koehler 2004). Individual tiles were also given reference numbers using the Cartesian system, with (0,1) being located at the bottom left-hand corner of a grid and (4,10) in the top right-hand corner (Koehler 2004). These reference numbers were then used to identify individual tile occupation by animals during the sampling period.

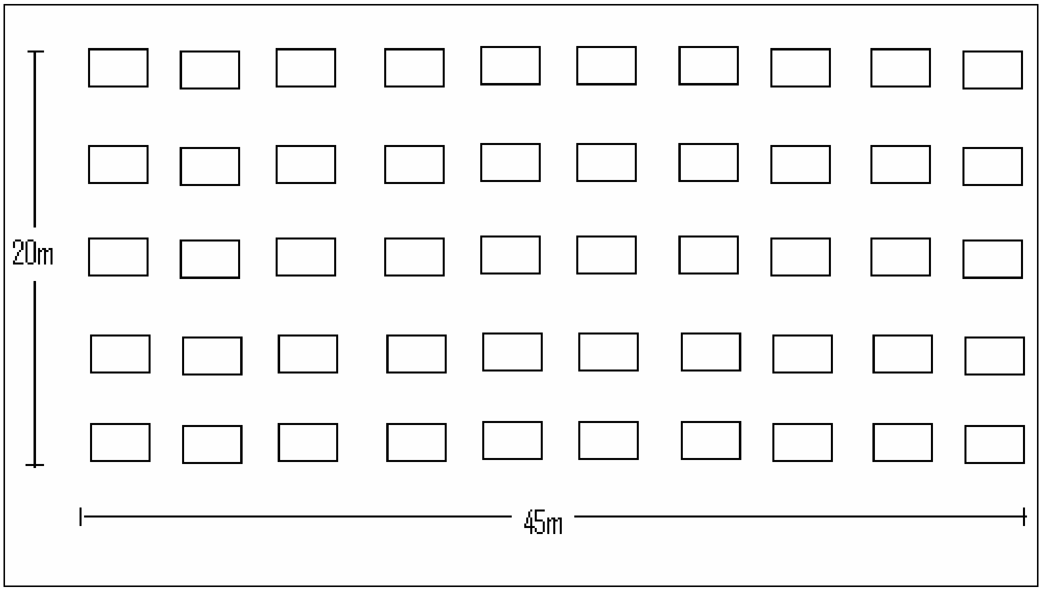

Figure 2.2: Arrangement of roof tiles in a sampling grid for *D. impar* at study sites. Each rectangle represents one tile. Total number of tiles in a grid =50.

2.2.2. Sampling Programme

Between nine and eighteen surveys of each site were conducted from 28 September 2005- 22 January 2006 to establish the occupation of tiles by small vertebrates on a monthly basis. Sites were surveyed for small vertebrates by lifting roofing tiles at least once per month. This occurred over a three-five day period (Appendix I), generally between 0800hrs and 1800hrs. The only exceptions were in January, where, due to extreme temperatures, surveys were restricted from sunrise until early afternoon. Ten sites in close proximity to each other were surveyed during the first week of each sampling period, followed by the other ten sites in the following week.

The order in which sites were checked was randomized (some constraints were imposed on the degree of randomization due to large travel distances between sites). It was intended that ten sites would be sampled per day, however, due to *D. impar* captures and travel distances, this was not always possible.

2.2.3. Time, weather condition, temperature and relative humidity under and outside tiles

For each day of sampling, all fifty roof tiles at a grid site were checked. Standard site variables were taken at the commencement and finish of each grid survey at tile (0,1). These variables included ambient air and substrate temperatures (°C) and ambient air and substrate relative humidities (%).Cloud cover (none, patchy-light, patchy-heavy or overcast), solar radiation (direct sun, breaks or filtered sun), wind speed and direction, and time of day were also recorded within each grid. Wind speed, air temperature and relative humidity were measured using a pocket weather meter (Kestrel® 3000), while substrate temperature and substrate relative humidity were measured using a thermo-hygrometer (AEMC Instruments® CA846) underneath tiles. The tiles were then systematically checked by lifting the tiles to observe if any small vertebrates were occupying the cavity beneath the tile. The tile was then placed back in its original position.

2.2.4. Capture and measurements

Small vertebrates (excluding elapid snakes), when discovered under a tile were caught by hand. Each individual was then identified by reference to standard field guides (Cogger 2000), sexed when possible (presence or absence of hemipenes for lizards and teste for small mammals) and classified as either adult or juvenile. The grid reference number of the tile under which they were found was also recorded. Once checked, each tile was placed back in its original position and the small animal was released immediately at the point of capture.

Each of the previously described weather and temperature measurements (refer to section 2.2.3) were taken on each occasion a *D. impar* was found under a tile. The temperature and humidity under the tile were also taken from the exact location the *D. impar* was found.

After capture, *D. impar* were measured. Measurements included snout-vent length (SVL), tail length (measured from cloaca to the undamaged tip of the tail), tail regeneration length, head length (measured from snout to anterior edge of ear opening), head width (across temporal jaw bulge) and head depth (measured at deepest point anterior to the posterior edge of the parietal scales) (Thorpe 1975; Peterson 1997). The last three were measured using digital calipers (Mitutoyo®) to the nearest 0.1mm, while all other measurements were taken using a metal ruler to the nearest 1mm. All individuals were weighed using electronic scales (A.N.D® HL-400) to the nearest 0.1g and sexed using a dissecting microscope. *D. impar* were sexed according to the presence or absence of cloacal spurs under the vestigial limb flaps (with cloacal spurs occurring only in males) (Rauhala and Andrew 1998). Digital photographs of dorsal and lateral colours and marking patterns were taken to describe individuals and to assist in the identification process. Individuals were then marked for recapture studies (refer to section 2.2.5).

2.2.5. Mark- recapture

Pyro-branding and freeze-branding have been the most commonly used methods for marking *D. impar* (Kutt 1992; Nunan 1995; O'Shea 1996, 2004; Hadden, 1995; Dorrough 1995). However, O'Shea 2004 found that pyro-brands were becoming less visible after eight months. For an endangered species such as *D. impar*, long-term monitoring is important, especially for the development of a recovery plan. For this reason, head scale pattern mapping was used to assess the recapture of individuals, as it has been found to be an adequate and effective method for identifying individuals (Coulson 1990; O'Shea 2004) and will also allow long-term monitoring of survival and estimations of population size.

Head scale pattern maps of *D. impar* consisted of a photograph taken in the field by a digital camera (Ricoh® Caplio R1v), which was optically zoomed in on the dorsal head scales of the individual. Photos were checked immediately for clarity and repeated if necessary. Images were later filed into directories corresponding to the dates on which they were taken and saved digitally. Records were kept to maintain the relationship between the images and individual animals.

Nail polish was used to identify recaptures within each five day sampling period to avoid repeating measurements on the same individual (Coulson 1990). A small dot was painted on the posterior lateral tip of the tail, which appeared to disappear by the end of the five day sampling period.

2.3. Habitat characteristics

In order to relate the abundances of *D. impar* to vegetation characteristics, a transect line was placed diagonally through the grid from one corner (at tile (0,1)) to the opposite corner (at tile (4,10)). A series of $1m^2$ quadrats were then placed at 5m intervals starting at 3.5m. Overall, a total of twenty quadrats were used at each site for every month of habitat monitoring (October, December and January). At each 5m mark, a quadrat was placed either side of the transect line. Subsequent measurements of maximum vegetation height (including seed heads), minimum vegetation height and the mean height of the dominant species were made using a 1m ruler. The dominant species within each quadrat was also recorded. Samples were sometimes collected for later identification. Additionally a photo-plot was taken of each quadrat using a digital camera (Ricoh® Caplio R1v) to estimate percentage cover of vegetation, bare ground and basalt rock. Microsoft Windows® Picture and Fax Viewer was used to view photos during recording of these data. The photo-plots are useful in time-constrained circumstances and enable long-term monitoring of sites (Koehler 2004).

The mean value of the variables of all twenty quadrats for a site, were taken for each of the five habitat variables to be used in the analysis. This was done for each of the three habitat survey months. This provided a representation of the habitat over the whole tile grid, rather than a single patch within the grid.

2.4. Microhabitat characteristics under and around tiles

To assess microhabitat use by *D. impar*, twenty tiles were photographed at each site for three sampling periods (November, December and January) using a digital camera (Ricoh® Caplio R1v). These tiles were selected using randomly generated numbers from Microsoft Excel®, regardless of whether *D. impar* had utilized them or not, with a different random number set used for each month. Two photos were taken at each tile. The first provided an image of the microhabitat directly under the tile, while the second provided an image of the microhabitat approximately 1m^2 around the tile. These were always taken from the same height (approx 140cm) and angle (approx. 45°). Photos were also taken at any tile under which a *D. impar* was found throughout the study period. These photos were used to record microhabitat characteristics influencing presence/absence (or presence/ non-detection) of *D. impar*. This was performed using Microsoft Windows® Picture and Fax Viewer. These variables included the number of soil cracks, basalt rock cover, tussock-forming grass cover and non-tussock-forming grass cover. A grid composed of 2cm squares (on overhead projector transparency) was used to score the degree of soil cracks present under and around tiles. All photos were enlarged to full screen size (15" @ 1024x768).

Other taxa present under the randomly selected tiles (namely spiders (redback and wolf), snakes, frogs, other lizard species, mammals, centipedes and ants) were also recorded.

2.5. Refuge preference of *Delma impar*

A laboratory experiment was undertaken in order to determine refuge preference by *D. impar*. Twenty-five *D. impar* were obtained from Melbourne Zoo, Parkville for use in the refuge preference experiment. The majority of adult animals came from building salvage sites in Victoria. Juveniles were bred from the captive colonies at the Zoo.

2.5.1. Captive maintenance

Animals were housed in individual plastic containers (29 x 21.5 x 9.5cm) with a hole cut out in the lid (approximately 8 x 15cm) which was covered with fly-wire mesh. These containers were kept on the shelves of a wooden rack. A thermal gradient (23°C-28°C) was created in the containers using heating strips placed under the containers at one end, from 0900hrs until 1700hrs. The heating strips were turned off during the night and containers were allowed to cool to room temperature (22°C). A natural day/night cycle was produced using light from two large nearby windows. Moist palm peat was used to line the bottom 3cm of the containers, with coconut fibre filling half of the remaining volume of the container. Water was available *ad libitum* and was changed on a daily basis. Approximately four small crickets (1cm long) were supplied every 2-3 days. The palm peat was also moistened every 2 days using a spray bottle.

2.5.2. Experimental design

Refuge preference experiments were conducted from 15 March- 22 March 2006. Four refuge types (2 natural and 2 artificial) were used in the choice experiments. Each refuge was placed into an arena (119 x 60 x 21cm) made from MDF wood. A total of four arenas (which were joined together) were used to test four individuals simultaneously. The artificial refuges provided in each arena consisted of a terracotta roof tile (430 x 340cm) and a concrete roof tile (450 x 275cm). Natural refuges consisted of a basalt rock which occurred at some sites (similar in size but ranging from (28 x 22 x 9cm) to (37 x 24x 12cm)) and a grass tussock (*Themeda triandra*, 8cm diameter, 15cm high). The positions of these were randomized in each successive trial. A small layer (approx 1cm in depth) of palm peat was used to line the base of each arena and mixed prior to each trial to destroy any chemical trails. The experiment was carried out at a constant temperature of 24°C, with an approximately natural photoperiod (daylight: 0700hrs- 2000hrs).

Single animals were placed in the centre of an arena at approximately 1000hrs and were checked for refuge use at approximately 0900hrs the following morning.

Substrate temperature (°C) and relative humidity (%) under tiles/rocks and at the base of the tussock was recorded using a thermo-hygrometer (AEMC Instruments® CA846).

Following each trial, all tiles and basalt rocks were soaked in hot water (containing hospital grade disinfectant) for at least 30 minutes in order to remove any chemical scents. These were then scrubbed, soaked again for about 10 minutes, rinsed and then air dried.

2.6. Statistical analysis

All data were analyzed using the statistical program SPSS for Windows® (Version 12). Normality and homoscedasticity tests were performed on all data sets (Dytham 1999; Quinn and Keough 2002; Pallant 2005) except for the presence/absence data. Normality was assessed using the Kolmogorov-Smirnov test, while homoscedasticity was assessed using Levene's Test (Pallant 2005). In regression analyses, residual plots were used to test the model assumptions of normality and homoscedasiticity.

Because most of the data sets were not homogeneous or normally distributed and transformations were unsuccessful, nonparametric statistical tests were used (Dytham 1999). An alpha level of significance was set at 5%, except for where Bonferonni adjustments were made to eliminate type 1 errors (Zar 1999; Quinn and Keough 2002).

A Friedman Test was used to determine if the occupancy rate differed seasonally. Wilcoxon signed pairs tests were then used as non-parametric post-hoc tests. Bonferroni corrections were made to the significance level according to the number of Wilcoxon tests performed.

Chi-square goodness of fit tests were used to determine sex biases in capture rates for each month. Chi-square contingency tests of independence were used to determine sex ratio change over time. Chi-square goodness of fit tests were also used to test for refuge preference.

Sexual dimorphism between body size (SVL) in males and females was analyzed using a student's t-test. Morphometric characteristics such as head length and head width were scaled allometrically with body size (SVL) by initially converting all metric measurements into natural logs. These were then regressed against SVL using the equation:

$$\ln(y) = a.\ln(x) + b$$

where x is the SVL and y is the allometric variable (Thorpe 1975; Shea 1995). The allometric characters were adjusted to values in which they would assume if uniform body size was apparent in all specimens using the equation:

$$y = e^{\ln\left(y_i\right) - a\left(\ln\left(x_i\right) - \ln(x)\right)}$$

where y is the adjusted variable, y_i is the unadjusted dependent variable, x_i is the SVL of an individual, x is the mean SVL (82.8mm) of the combined sample and a is the mean slope of the regression line (Thorpe 1975; Shea 1995). Sexual dimorphism in the metric characters (head width and head length) was then analyzed using analysis of covariance.

Multinomial logistic regression was used to analyze the influence of other taxa on the presence/ absence (non-detection) of *D. impar*. The multinomial logistic regression was used due to the categorical nature of the data for both other taxa and *D. impar*. For variables to be removed from the model, significance was set at 0.05.

Binary logistic regression was used to analyze microhabitat characteristics under tiles. It was also used to analyze microhabitat characteristics around (outside) tiles to determine the influence of microhabitat choice within the habitat on tile use by *D. impar*.

Multiple linear regression (backward stepwise) was used to determine the influence of habitat variables on the occupation rate of tiles by *D. impar*. This was undertaken for structural components (vegetation height and cover, basalt rock cover and bare ground). Scatterplots were used for floristic characteristics (vegetation cover of species).

Chapter 3. RESULTS

3.1. Census data

3.1.1. All Small terrestrial vertebrates

A total of 278 grid surveys (between nine and eighteen per site) were undertaken from 28 September 2005 - 22 January 2006. This resulted in a total of 13,900 roof tiles being checked over the survey period. A total of 964 animals were encountered under tiles over the entire survey period, with 14 different species recorded (Table 3.1). These comprised of 9 reptilian, 3 amphibian and 2 mammalian species (Table 3.1). The occupancy rate per grid site (or per 50 tiles) for all taxa was 3.04 individuals, with 2.71 reptiles, 0.18 amphibians and 0.15 mammals encountered for every 50 tiles surveyed.

Egernia whitii (White's skink) and *Pseudemoia pagenstecheri* (Tussock Skink) were the most commonly encountered species. These were followed by *Delma impar* (Table 3.1).

3.1.2. *Delma impar* across sites

Overall, a total of 156 *D. impar* were encountered under roof tiles, which resulted in an occupancy rate of 0.56 individuals per grid site (= 50 tiles) (Table 3.1). Twenty-six sloughed skins of *D. impar* were also recorded under tiles. Of the 156 individuals encountered, 92 were captured and measured. At least seventeen of these were recaptured over the three-five day period of each survey. The remainder either escaped down burrows located underneath tiles, down soil cracks or into the surrounding vegetation.

Due to time constraints, no mark-recapture analysis was undertaken. This may have allowed more individuals being recognized as recaptures.

Table 3.1: Fauna recorded under artificial refuges (roof tiles) from late September 2005 to late January 2006 in Western Victoria. Occupancy rate is the number of individuals (known recaptures included) per grid site (= 50 tiles).
(Total number of tiles turned = 13,900)

Species	Common Name	Total Number of Individuals	Occupancy Rate
Pygopodidae			
Delma impar	Striped Legless Lizard	156	0.56
Scincidae			
Egernia whitii	White's Skink	236	0.85
Pseudemoia pagenstecheri	Tussock Skink	187	0.67
Bassiana duperreyi	Eastern Three-lined Skink	24	0.09
Pseudemoia entrecasteauxii	Southern Grass Skink	3	0.01
Tiliqua nigrolutea	Blotched Blue-tongue	3	0.01
Tiliqua rugosa	Shingleback	2	0.01
Unidentified skinks		143	0.51
Elapidae			
Austrelaps superbus	Lowland Copperhead	67	0.24
Suta flagellum	Little Whip Snake	52	0.19
Myobatrachidae			
Limnodynastes tasmaniensis	Spotted Marsh Frog	34	0.12
Crinia signifera	Common Froglet	14	0.05
Hylidae			
Litoria ewingii	Southern Brown Tree Frog	1	0.004
Dasyuridae			
Sminthopsis crassicaudata	Fat-tailed Dunnart	4	0.01
Muridae			
Mus musculus	House Mouse	35	0.13
Unidentified small mammals		3	0.01
Total		964	3.47

3.2. Morphometrics of *Delma impar*

Of the 92 *D. impar* captured, 84 individuals were adults, comprising 40 males and 44 females. Juveniles (SVL < 70mm) were comprised of 7 males and 1 female. One male was assumed to be a first year juvenile (SVL 58mm), while the rest were assumed to be second year juveniles (SVL 60-69mm). Ninety-one percent of individuals captured underneath artificial refuges (roof tiles) were mature.

Snout-vent lengths of captured individuals ranged from 58mm to 100mm. When juveniles were excluded (SVL < 70mm), adult female SVL ranged from 72mm to 100mm and adult male SVL ranged from 70mm to 91mm (Figure 3.1).

SVL in females ($\overline{X}$ = 87.1mm) was found to be significantly longer than in males ($\overline{X}$ = 78.1mm) (t-test (equal variances not assumed) t=6.072, df=77.394, $p<0.001$). Therefore, because the SVL was found to be different between males and females, further tests were conducted on head width and head length of the two sexes. Sexual dimorphism was found in only one adjusted metric character. Head length was found to be significantly larger in females (ANCOVA; $F_{1,84}=8.028$, $p=0.006$), but head width was not significantly different (ANCOVA; $F_{1,84}=0.271$, $p=0.604$).

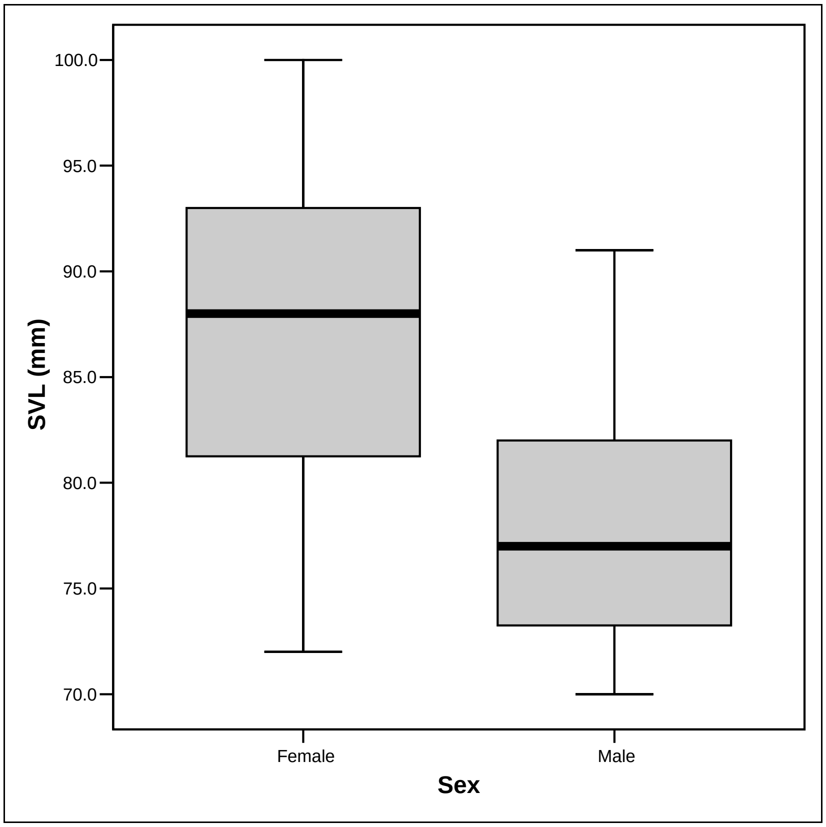

Figure 3.1: Box-plots of snout-vent lengths (SVL) for adult male and female *D. impar* captured throughout the survey (females n=44; males n=40). Horizontal bar = median; box = first quartile (lower edge) to third quartile (upper edge) and whiskers = range.

3.2.1 Sex Ratios of adult *D. impar*

An overall sex ratio of 1:1 was found over the survey period. The sex ratio of *D. impar* did not change significantly over time (χ^2=5.192, df=3, *p*=0.158) and was not significantly different from a 1:1 ratio in early October (χ^2=0.474, df=1, *p*=0.491), late October (χ^2=0.231, df=1, *p*=0.631) and November (χ^2=2.667, df=1, *p*=0.102) (Figure 3.2). December was excluded from this analysis due to the small sample size of *D. impar* caught within this period.

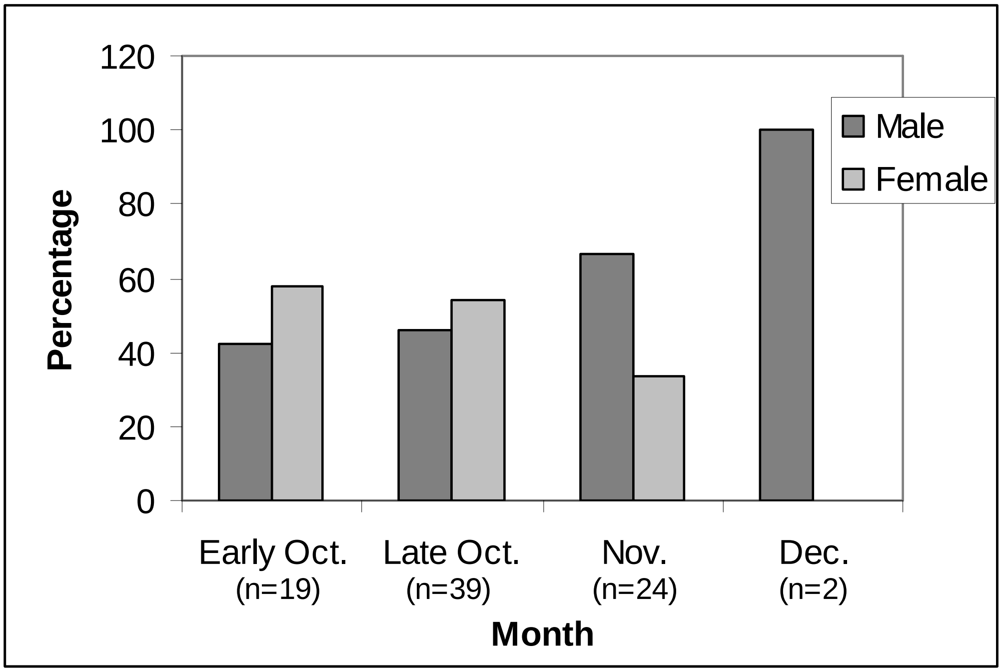

Figure 3.2: Sex ratios (%) of adult *D. impar* captured during surveys for all sites. Total number of individuals (n) are shown.

3.3. Habitat characteristics of sites and occupancy rates

3.3.1 Tile Occupancy Rates for *D. impar*

The occupancy rate of *D. impar* was found to be significantly different between sites (Kruskal-Wallis Chi-square χ^2=104.743, df=19, p<0.001), with occupancy rates ranging between 0.06-2.9 individuals (Table 3.2). Large differences in occupancy rates were also found between some sampling periods, within a single site (e.g. “Challicum Park”: 0.50-5.50) (Table 3.2). “Challicum Park” and Caramut-Chatsworth Rd. sites had the highest occupancy rate of *D. impar* throughout the study with mean occupancy rates of 2.90 and 2.08 individuals respectively (Table 3.2). These sites had relatively large numbers of *D. impar* recorded across all sampling periods (except December and January) (Table 3.2). Other sites (e.g. Lubra Creek, Blacks Creek and Rokewood-

Table 3.2: Tile occupancy rates of *D. impar* at survey sites for each sampling period. Occupancy rate is the mean number (±SD) of *D. impar* for every 50 tiles checked (=grid site). Occupancy rate includes recaptured individuals.

			Occupancy rate for each sampling period				
Site	**Total no. of tiles checked**	**Site occupancy rate**	**Early Oct.**	**Late Oct.**	**Nov.**	**Dec.**	**Jan.**
Blacks Creek	450	0.22 ± 0.44	1.00 ± 0.00	0.50 ± 0.71	0	0	0
Bochara Station	550	1.27 ± 1.01	1.00 ± 0.00	1.50 ± 0.71	2.00 ± 1.41	1.67 ± 1.16	0
Boonderoo Nature Reserve	750	0	0	0	0	0	0
Camperdown-Foxhow Rd #1	750	0.60 ± 0.91	1.25 ± 1.50	0.67 ± 0.58	0.50 ± 0.71	0.33 ± 0.58	0
Camperdown-Foxhow Rd #2	750	0	0	0	0	0	0
Caramut-Chatsworth Rd	650	2.08 ± 2.53	1.00 ± 0.00	5.50 ± 3.54	4.00 ± 1.73	0.67 ± 1.16	0
Cavendish-Dunkeld Rd	550	1.27 ± 1.19	1.50 ± 0.71	2.00 ± 1.41	2.50 ± 0.71	0.67 ± 1.16	0
“Challicum Park”	500	2.90 ± 3.67	5.50 ± 4.95	5.50 ± 6.36	3.00 ± 0.00	0	0.50 ± 0.71
Chatsworth Rd	850	0	0	0	0	0	0
Cressy Trotting Track	900	1.17 ± 1.34	0.25 ± 0.50	1.40 ± 0.89	2.33 ± 2.31	1.33 ± 1.53	0.67 ± 1.16
Cressy-Shelford Rd	800	0	0	0	0	0	0
Forest Lane	550	0.36 ± 0.50	0	1.00 ± 0.00	1.00 ± 0.00	0	0
Geggies Rd	900	0.22 ± 0.55	0.50 ± 1.00	0.20 ± 0.45	0.33 ± 0.58	0	0
Hamilton Hwy- Tea-tree Creek	600	0	0	0	0	0	0
Lubra Creek	650	0.23 ± 0.44	0	0.50 ± 0.71	0.67 ± 0.58	0	0
Old Ararat Rd	600	0.67 ± 0.78	0.50 ± 0.71	1.00 ± 0.00	1.50 ± 0.71	0.67 ± 1.16	0
Rokewood Common	900	0.22 ± 0.65	0	0.40 ± 0.89	0.67 ± 1.16	0	0
Rokewood-Shelford Rd	900	0.11 ± 0.32	0	0	0.33 ± 0.58	0	0.33 ± 0.58
Rossbridge Flora Reserve	500	1.30 ± 1.34	2.00 ± 0.00	3.00 ± 1.41	2.00 ± 0.00	0	0.33 ± 0.58
Vite Vite Rail Reserve	800	0.06 ± 0.25	0	0.25 ± 0.50	0	0	0
Total	13900	0.56 ± 1.28	0.56 ± 1.38	0.87 ± 1.76	1.02 ± 1.41	0.28 ± 0.73	0.09 ± 0.35

Shelford Rd.) had relatively small numbers recorded across all sampling periods (Table 3.2). At some sites (e.g. Rokewood-Shelford Rd, Blacks Creek and Vite Vite Rail Reserve), individuals were also recorded only during some sampling periods and not others (Table 3.2). Alternatively, *D. impar* was not recorded at five sites (25%) over the entire sampling period (Table 3.2). *D. impar* had been recorded at these sites but in low numbers only twelve months prior to the current study (G. Peterson p*ers. comm.*). Sites in which *D. impar* were not recorded included Boonderoo Nature Conservation Reserve, Cressy-Shelford Rd. #3, Camperdown-Foxhow Rd. #2 and Hamilton Hwy-Tea-tree Creek. *D. impa*r was also not encountered at Chatsworth Rd. in this study; however, a single sloughed skin was found underneath a tile.

3.3.2 Seasonal Occupancy rates

The occupancy rate of tiles by *D. impar* was found to increase during spring (October and November), but decline substantially in summer (December and January) (Figure 3.3). It was also significantly different among sampling periods (Friedman's test χ^2=32.7, df=4, p<0.001) (Figure 3.3). Significantly more individuals were recorded in November than in early October (Wilcoxon signed rank test Z=-2.894, p=0.004) and December (Z=-3.068, p<0.002). Therefore, in order to minimize the number of pairwise comparisons, the occupancy rate of tiles in January would also be significantly different to November. There was also no significant difference in occupancy rates between early October and late October (Wilcoxon signed rank test Z=-1.077, p=0.281) or early October and December (Z=-0.440, p=0.660).

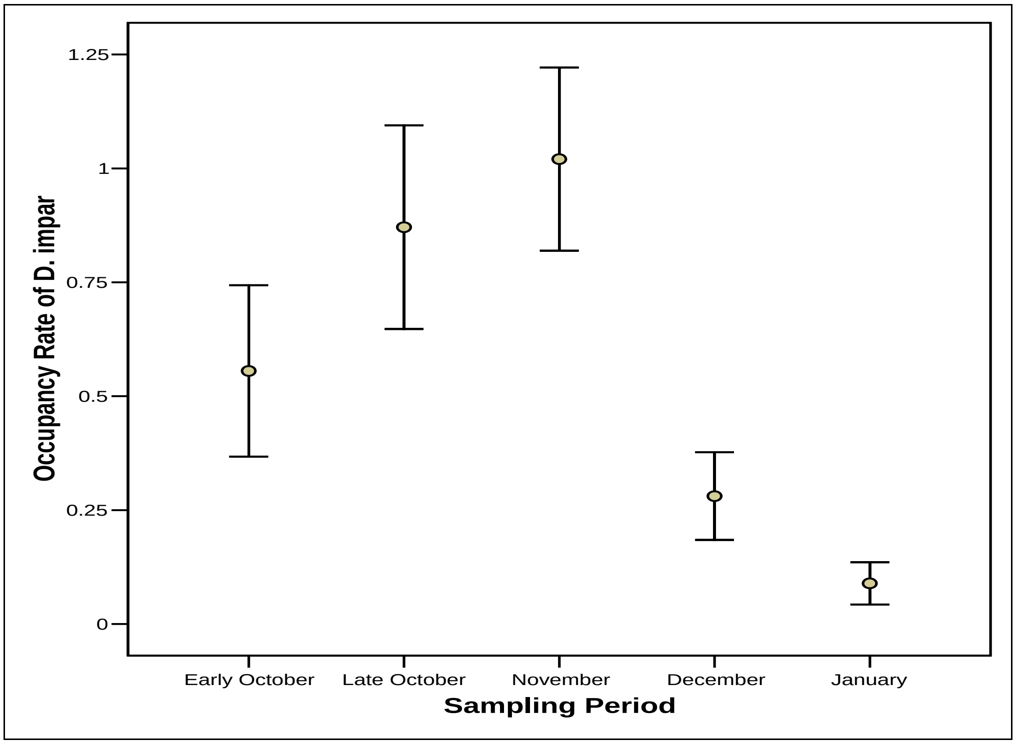

Figure 3.3: Seasonal variation in tile occupancy rate by *D. impar* at all survey sites. Occupancy rate is the mean number of individuals per grid site (= 50 tiles) (±SE).

3.3.3 Structural habitat characteristics

A total of 1200 quadrats were analyzed to assess the influence of structural attributes of the vegetation on occupation rates at sites.

Multiple regression analysis using the structural characteristics of the vegetation on site occupancy rate indicated that only the maximum vegetation height was significant (Table 3.3). The maximum vegetation height (cm) was negatively associated with *D. impar* occupancy rate (Figure 3.4). With increasing vegetation height at sites, the number of *D. impar* recorded under tiles declined ($y=2.463-0.031x$; $r^2=0.155$, $F_{1,55}=10.073$, *p*=0.002, Figure 3.4). However, maximum vegetation height explained only 14.1% of the variation in the occupancy rate of *D. impar* at a site.

Table 3.3: Results of multiple regression analysis testing for the influence of structural vegetation characteristics on tile occupation rate of *D. impar* at sites. t statistics indicate the contribution that the variable would make to the overall model (if included); bold values indicate *p*<0.05.

Independent Variable	*B*	t statistic	*P*
Maximum vegetation height (cm)	**-0.031**	**-3.174**	**0.002**
% Cover of bare ground	-0.078	-0.596	0.553
Dominant vegetation height (cm)	-0.068	0.435	0.665
Minimum vegetation height (cm)	-0.059	-0.417	0.678
% Cover of basalt rocks	0.030	0.240	0.811
Constant	**2.463**	**3.844**	**<0.001**

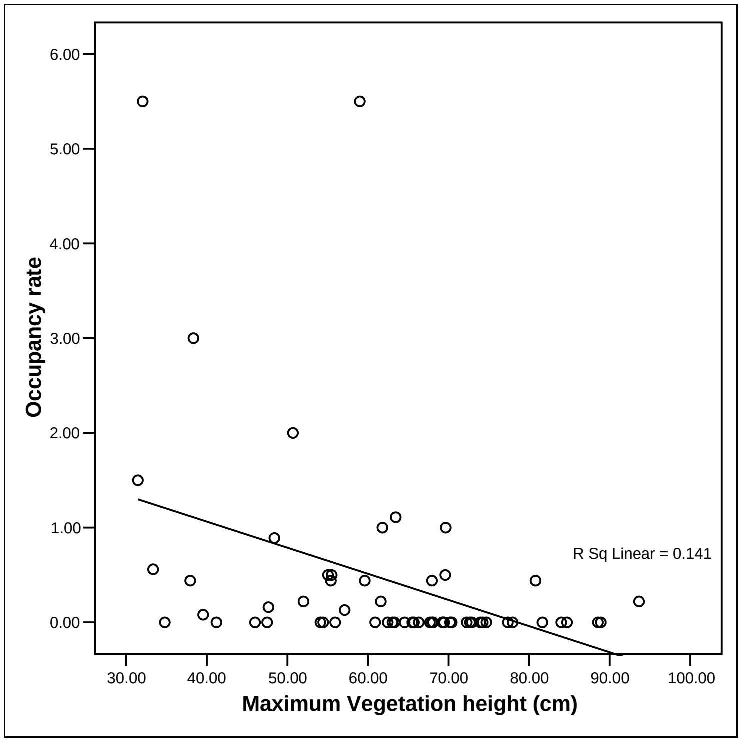

Figure 3.4: Relationship between maximum vegetation height (cm) and tile occupancy rate of *D. impar* at sites.

3.3.4. Floristic habitat characteristics

Scatterplots were initially used to explore relationships between the different species and the occupancy rate of tiles by *D. impar* (Appendix II). However, less than a 3% relationship was found between occupancy rates and each of the variables (Table 3.4). Subsequently, multiple regression analysis was not undertaken.

Table 3.4: Relationship between floristic variables and occupancy rate of tiles by *D. impar*. Variables represent percent cover of species.

Independent variable	R^2 Value
Agricultural grasses	0.021
Austrodanthonia spp.	0.003
Daisy species	0.004
Eryngium ovinum	0.001
Phalaris aquatica	0.019
Plantago spp.	0.014
Poa spp.	0.002
Stipa spp.	0.032
Taraxicum officinale	0.016
Themeda triandra	0.008

3.4. General tile use

3.4.1 Weather conditions and time of day

Fifty-seven percent of individuals were found to be occupying tiles between 1100hrs and 1500hrs (Figure 3.5). The number of individuals was found to increase during the morning, peak at midday (between 1100hrs and 1300hrs) and then decline again in the afternoon (first at 1300hrs, then further at 1500hrs) (Figure 3.5).

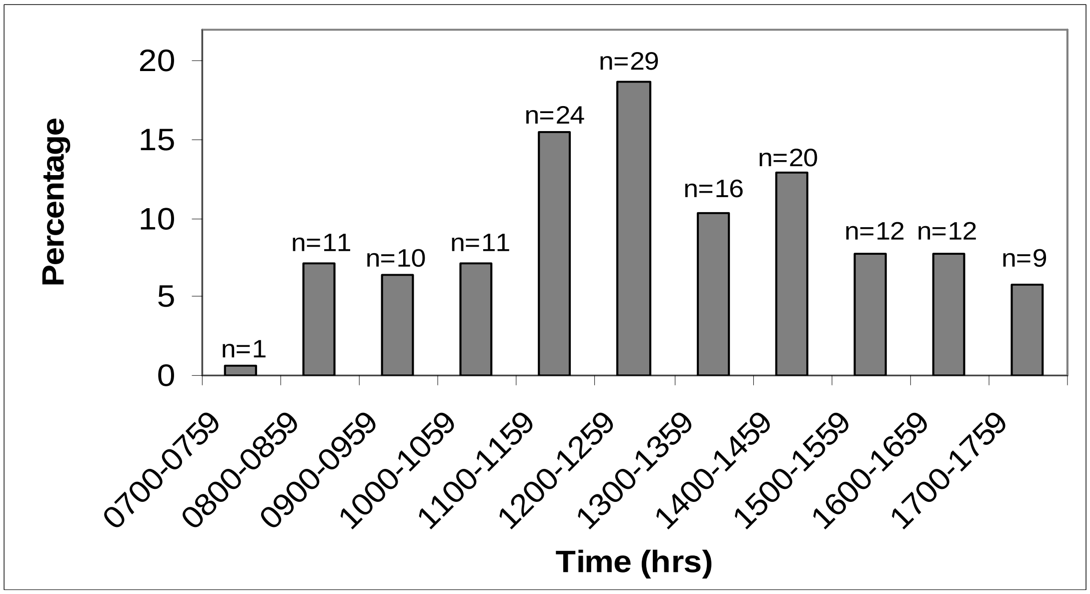

Figure 3.5: Proportion of individuals occupying tiles at different times of the day. Percentage and total number (n) of individuals are shown for each interval. Time is Australian Eastern Standard Time.

Figure 3.6 illustrates the environmental conditions under and outside tiles when tiles were occupied. When the temperature under tiles exceeded 15°C, the number of individuals increased substantially (Figure 3.6c). This was also the case for temperature outside of tiles (ambient air) (Figure 3.6a). A considerable increase was also recorded when temperature under tiles was between 20-24.9°C (Figure 3.6c), and a decline in the number of individuals was found to occur when temperature outside tiles exceeded 30°C (Figure 3.6a). However, temperatures over 30°C were only recorded in summer and observations within these temperatures were limited to only a few. The majority (71.6%) of tile occupations occurred when temperature outside tiles was between 15°C and 24.9°C (Figure 3.6a). Tile occupation also increased markedly when relative humidity outside tiles was between 40-49.9% (Figure 3.6b). Relative humidity under tiles appeared to have little influence on tile occupancy (Figure 3.6d).

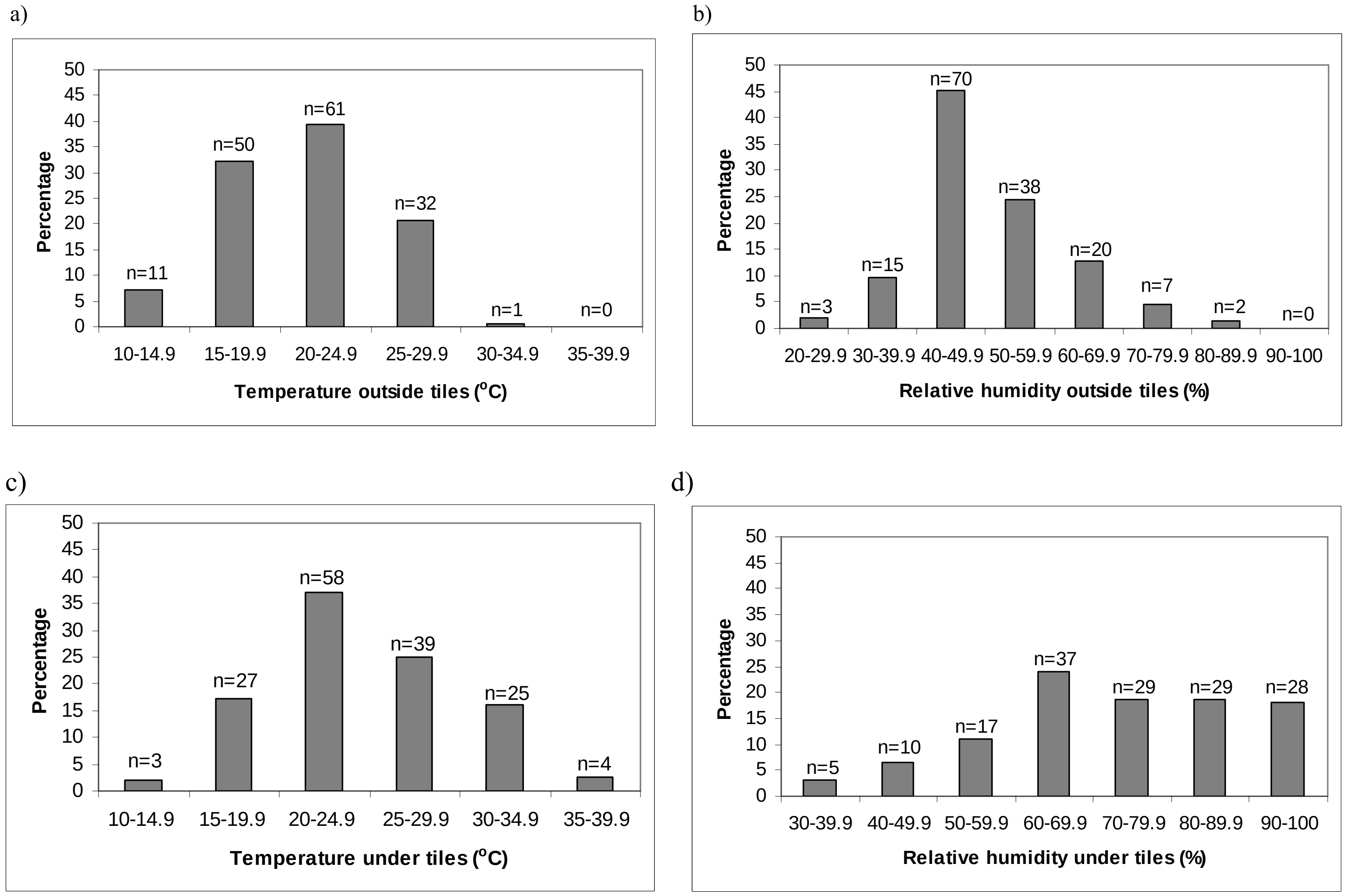

Figure 3.6: Temperature and relative humidity outside (a & b) and under (c & d) tiles occupied by *D. impar*. Percentage and total number (n) of individuals are shown for each interval.

Figures 3.7 and 3.8 show the regression analyses used to determine differences between temperatures (°C) and relative humidities (%) under and outside tiles. The temperature under a tile (°C) was significantly warmer than that of the temperature outside a tile (°C) by about 2-3°C ($y=1.938+1.055x$; $r^2=0.717$, $F_{1,154}=384.517$, $p<0.001$, Figure 3.7). However, when ambient air temperatures are below 16°C, substrate temperatures appear to be very similar to that of the ambient air (Figure 3.7). The relative humidity under a tile (%) was also significantly higher than the relative humidity outside of a tile (%) by about 25-26% ($y=26.527+0.916x$; $r^2=0.373$, $F_{1,154}=90.543$, $p<0.001$, Figure 3.8).

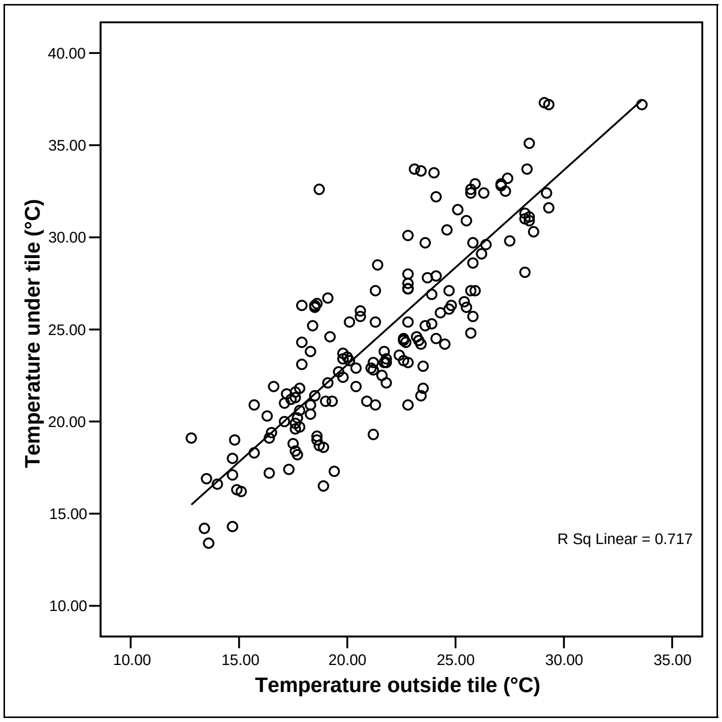

Figure 3.7: Relationship between temperature outside tile (°C) and temperature under tile (°C) when *D. impar* is found occupying a tile.

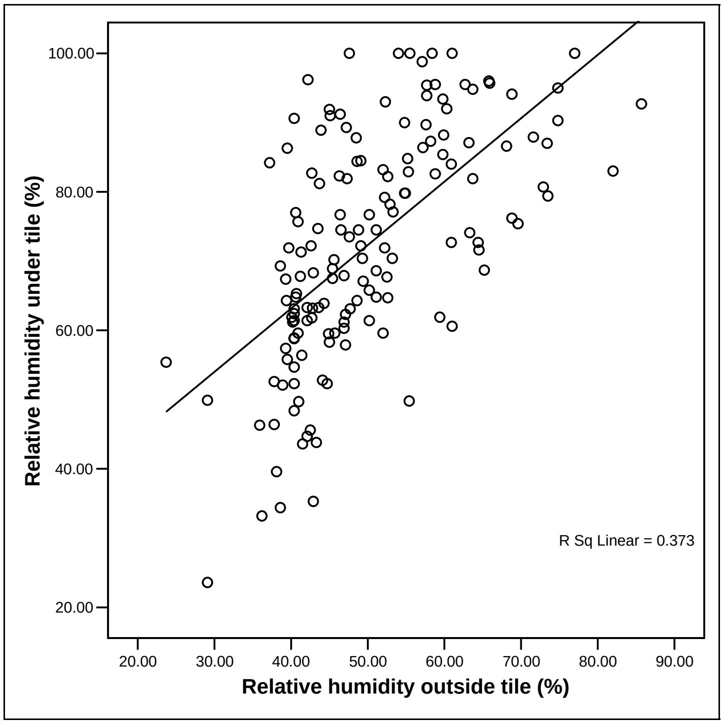

Figure 3.8: Relationship between relative humidity outside tile (%) and relative humidity under tile (%) when *D. impar* is found occupying a tile.

The amount of cloud cover appeared to influence observations of *D. impar* underneath tiles (Figure 3.9). Occupancy of tiles was approximately 53% higher on overcast days than when no cloud cover was present, with 54% of individuals recorded at this cloud cover (Figure 3.9). A further 29% of all *D. impar* were observed underneath tiles when cloud cover was patchy-heavy. These results indicate that *D. impar* are more likely to utilize tiles when more cloud cover is present and are seemingly avoiding tiles when there is no cloud cover (Figure 3.9).

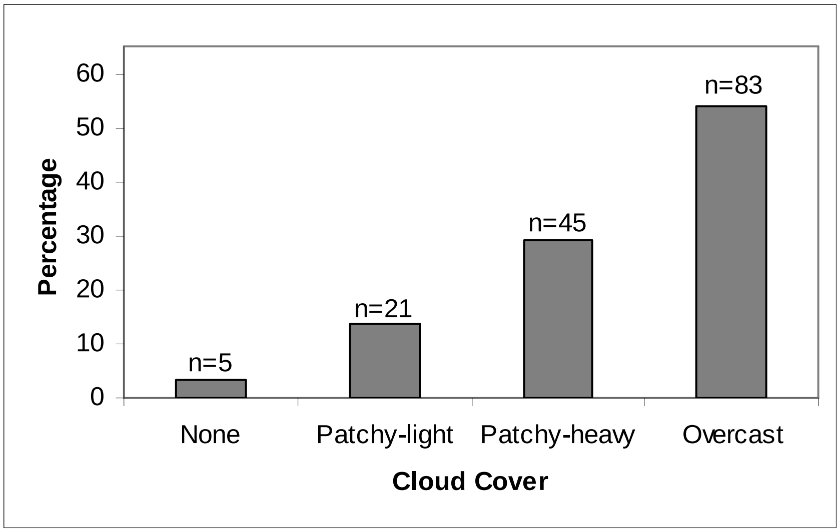

Figure 3.9: Proportion of individuals occupying tiles under different cloud covers. Percentage and total number (n) of individuals are shown for each interval.

3.5. Individual tile use

3.5.1. Influence of other animal taxa

In total, 1353 tiles were used to determine the influence of the presence or absence of other taxa on the presence or absence of *D. impar* under a tile. *D. impar* occurred under 290 or 21.4% of these tiles.

A stepwise backward Wald logistic regression was used and all taxa entered originally as both main effect and as two-way interactions. Because the two-way interactions produced no significant difference to the model, producing high *p* values, they were subsequently removed from the model.

Initially all potential predators or competitors that might influence utilization of tiles by *D. impar* were included in the analysis. However, after closer inspection, the fat-tailed dunnarts (*Sminthopsis crassicaudata*) were removed due to the extremely small numbers recorded.

The presence or absence of *D. impar* under an individual tile was found to be influenced by the utilization of the tile by snakes (*Austrelaps superbus* and *Suta flagellum*) and redback spiders (*Latrodectus hasselti*) (Table 3.5). Positive B values (Table 3.5) indicate that the taxa have a positive relationship with the presence of *D. impar*. The inverse of this applies to a negative B value (Table 3.5). Where the Exp(B) value is less than one, increasing numbers of that taxa will result in a decreased probability of *D. impar* being present. When the Exp(B) value is greater than one, increasing numbers of that taxa will result in an increased probability of *D. impar* being present. Therefore, snakes showed a negative relationship with *D. impar* (Table 3.5). Tiles which a snake currently or previously occupied were less likely to be used by *D. impar*. Additionally, if the number of snakes at a site increased, the probability of *D. impar* being present under tiles would decrease (Table 3.5).

D. impar and redback spiders were found to have a positive relationship, resulting in an increased likelihood of finding *D. impar* underneath a tile when redback spiders were present (Table 3.5). Other taxa that were found underneath tiles, did not significantly influence whether *D. impar* was present or absent from that tile (Table 3.5).

Ants were expected to have a negative influence on the presence of *D. impar*. However, the results showed no significant influence (Table 3.5). This result may be speculative, because all ant species were pooled in order to limit the number of variables. Thus, individual ant species and their numbers (i.e. colonies or individuals) were not distinguished due to the pooling process.

Table 3.5: Results of logistic regression analysis testing for the influence of presence/absence of other taxa and the presence/absence of *D. impar* under a tile. Chi-square values for each taxon initially included in the model are presented; bold values indicate *p*<0.05.

Taxa	*B*	Exp(B)	χ^2	df	*p*
Redback spiders	**1.236**	**3.443**	**12.099**	**1**	***<0.001***
Snakes	**-2.489**	**0.083**	**14.219**	**1**	**0.014**
Wolf spiders	-0.126	0.881	0.111	1	0.739
Centipedes	0.298	1.347	0.230	1	0.632
Lizards	0.159	1.172	1.173	1	0.279
Ants	0.177	1.193	1.190	1	0.275
Frogs	-0.651	0.522	1.658	1	0.198
Constant	**-1.302**	**0.272**	**362.457**	**1**	**<0.001**

3.5.2. Microhabitat characteristics under tiles

Photographs were taken of the area under a total of 1293 tiles and analyzed to determine whether microhabitat characteristics underneath tiles influenced their occupancy by *D. impar*. Of the 1293 roof tiles sampled, *D. impar* was found to be present at 18%, or 233 of these.

Degree of soil cracking was found to have a negative association with the presence of *D. impar* under tiles (Table 3.6). Therefore, the more soil cracks present, the less likely *D. impar* would be found under a tile. The logistic regression analysis also showed positive associations between the percent cover of both *Themeda triandra* and agricultural grass species and the occurrence of *D. impar* under tiles (Table 3.6). Therefore, the presence of these vegetation types under tiles resulted in an increased likelihood of *D. impar* occupying a tile. Thus, as percent cover of each of the vegetation types increases, the occupation by *D. impar* is also likely to increase. This suggests that tile choice is selective rather than random in regards to microhabitat characteristics under tiles.

Table 3.6: Results of logistic regression analysis testing for the influence of microhabitat characteristics underneath tiles and the presence/absence of *D. impar*. Chi-square values for each independent variable initially included in the model are presented; bold values indicate *p*<0.05.

Independent Variable	*B*	Exp (B)	Wald value (χ^2)	df	*p*
Degree of soil cracking	**-0.716**	**0.839**	**12.402**	**1**	**<0.001**
% Cover of *Themeda triandra*	**0.012**	**1.012**	**22.460**	**1**	**<0.001**
% Cover of agricultural grasses	**0.030**	**1.031**	**13.193**	**1**	**<0.001**
% Cover of *Poa* spp.	0.015	1.015	2.312	1	0.128
% Cover of *Austrodanthonia* spp.	0.006	1.006	0.214	1	0.624
Constant	**-1.515**		**438.410**	**1**	**<0.001**

3.5.3. Microhabitat characteristics around tiles

A total of 1293 tiles were sampled using photographs and analyzed to determine microhabitat characteristics 1m around tiles which may influence the presence or absence of *D. impar* under roof tiles. They were also used to determine whether microhabitat selection within the habitat influenced tile occupancy.

Logistic regression analysis of the microhabitat 1m around tiles showed that the percentage cover of agricultural grass species around a tile had a positive association with the presence of *D. impar* (Table 3.7). Increasing cover of agricultural grass species outside of a tile was found to increase the probability of tile occupation by individuals. All other variables entered into the model had no influence on the presence or absence of *D. impar* (Table 3.7).

Table 3.7: Results of logistic regression analysis testing for the influence of microhabitat 1m around tiles and the presence/absence of *D. impar* underneath tiles. Chi-square values for each independent variable initially included in the model are presented; bold values indicate *p*<0.05.

Independent Variable	*B*	Exp (B)	Wald value (χ^2)	df	*p*
% Cover of agricultural grasses	**0.011**	**1.011**	**32.828**	**1**	**<0.001**
%Cover of *Phalaris aquatica*	-0.072	0.931	3.087	1	0.079
% Cover of *Themeda triandra*	0.003	1.003	1.868	1	0.172
Degree of soil cracking	-0.157	0.855	0.654	1	0.419
% Cover of *Austrodanthonia* spp.	-0.001	0.999	0.156	1	0.693
% Cover of *Poa* spp.	0.000	0.997	0.000	1	0.997
Constant	**-1.519**		**439.35**	**1**	**<0.001**

3.6. Refuge preference in a laboratory experiment

Results of the laboratory experiment showed that individual *D. impar* were selective in their use of refuges, with a highly significant preference under the experimental conditions (air temperature 24.1°C, relative humidity of 82.7%, wind speed of 1.7mph and no heat source) towards certain refuge types (χ^2= 14.520, df=3, *p*=0.002). Fifty-six percent of the individuals showed a preference towards using grass tussocks, with this refuge being the most used type (Table 3.8). Concrete tiles and basalt rocks were preferred equally and were not that different from the expected value (Table 3.8). However, terracotta tiles were avoided by *D. impar*, with only 1 individual using this refuge (Table 3.8).

Table 3.8: Refuge preference by *D. impar* in laboratory trials. The observed and expected number of individuals for each refuge type are shown. The contribution of each refuge type to the χ^2 value is also presented.

Refuge Type	Observed	Expected	Percentage of χ^2
Terracotta Tile	1	6.3	30.71
Concrete Tile	5	6.3	1.85
Basalt Rock	5	6.3	1.85
Grass tussock	14	6.3	64.8

Chapter 4: DISCUSSION

4.1. Census data

Artificial refuges (roof tiles), were an effective method for censusing vertebrate fauna, with 14 different species recorded. Roof tile grids recorded *D. impar* in relatively large numbers (156) and nearly as often as two common grassland reptile species, White's Skink (*Egernia whitii*) and the Tussock Skink (*Pseudemoia pagenstecheri*). This is promising, considering *D. impar* is extremely cryptic and found in low numbers (Coulson 1990; O'Shea 2004). Nine times more *D. impar* were caught in this study compared to a study by Koehler (2004). This may however, be attributed to the use of sites where *D. impar* had been previously recorded, and to drier weather conditions. Additionally, more *D. impar* may have been found due to the effort of this study, which surveyed 2.3 times more tiles than Koehler (2004) (13,900 tiles compared to 6100 tiles). The present study was also undertaken over two seasons, Spring and Summer, whereas Koehler (2004) only surveyed for *D. impar* in Spring and over a shorter time frame (14 September- 4 October).

4.2. Morphometrics of D. *impar*

A number of pygopodids have been found to show sexual dimorphism in body size, with females growing larger than males (Kluge 1974; Patchell and Shine 1986). The results found in this study confirm that, like other pygopodids, *D. impar* is sexually dimorphic in regards to snout-vent length and head-length. Females were found to be larger, with longer heads than males. Sexual dimorphism in only ventral scale counts in *D. impar* has been reported by Kluge (1974) and Coulson (1990). Females were found to have a higher mean number of paired enlarged ventral scales than males, in their studies. Coulson (1990) also found no significant difference in snout-vent length (SVL) as a percentage of the total length. However, why Coulson (1990) analyzed the ratio is unknown. His result may have simply implied that males and females have different tail lengths in relation to SVL.

Several hypotheses have been proposed to explain larger body size in females. The most common of these focuses on fecundity advantages of attaining a larger size. Larger females often have increased clutch sizes and/or larger eggs due to increased abdominal space (Shine 1988a, 1992, 1994; Bonnet *et al.* 2000). Shine (1988a) noted that pygopodids usually have a defined clutch size of two eggs, although occasionally females produced one. Therefore, smaller females may consistently produce only one or no eggs, with larger females being able to produce two. Advantages obtained from a larger SVL may also include increased survival of individuals (Shine 1988a). Differential mortality may exist between males and females, with females needing to grow larger to survive. This may also explain the delayed age of reproductive maturity in females.

The increased head-length found in females may reflect the size or range of prey items consumed. This is however gape limited. It has been suggested that larger mouths are needed to overcome constraints imposed by larger body size (Patchell and Shine 1986b). Thus, longer head-lengths in females may occur to increase mouth size for prey items. Females may have different diets compared to males, especially during egg development periods when additional resources are required. Research is therefore needed on the individual diets of male and females, instead of only the general diet of this species (see Kutt *et al*. 1998).

4.2.1. Sex ratios of *D. impar*

Sex ratios throughout this study did not differ from 1:1. This is consistent with previous studies by Rauhala and Andrew (1998) and O'Shea (2004), who also found an overall 1:1 sex ratio of *D. impar*. Results also showed no significant change from a 1:1 ratio occurring over time in this study. O'Shea (2004) however, found that a swap over between the proportion of sexes occurred over time under artificial refuges (roof tiles). She found that males were more commonly observed between September and October following their emergence from brummation. Conversely, females were more commonly observed during the late breeding season (November and December) due to thermoregulatory needs for egg development and deposition (O'Shea 2004). Additionally, increased tile occupancy by females during this period may indicate that tiles are used as protective shelters, reducing predation (G. Peterson *pers. comm.*). As females would be gravid during this period, there would be a fitness cost associated with reproducing. Because all sites were pooled in the present study (due to small sample sizes at some sites), this was not observed. It is suggested that the different sites may have had differential sex ratios.

4.3 Habitat characteristics of sites and occupancy rates

4.3.1. Tile Occupancy rates for *D. impar*

D. impar were recorded at all sites the previous year, although in low numbers at some of the sites (G. Peterson *pers. comm.*). In this study, *D. impar* were recorded at fifteen of the twenty sites sampled, with evidence of occupation at an additional site (Chatsworth Rd.) by way of a single sloughed skin. The absence of *D. impar* at the remaining four sites throughout the duration of the study raises concern. This is concerning for two reasons: 1) roof tiles as a census technique for *D. impar* may not always detect their presence or 2) a local extinction may have occurred.

MacKenzie (2005a) commented that it is generally impossible to confirm that a species is absent in an area. Absence may be due to the failure of the survey method in detecting the species (Mackenzie *et al*. 2003; MacKenzie and Nichols 2004; MacKenzie 2005a, 2005b). Koehler (2004) also suggested that roof tiles may have created false-negative errors in her study, but attributed this to her short time frame (14 September- 4 October 2004), the weather conditions (unusually high rainfall) and single season (spring) of her census. The failure to detect the presence of *D. impar* at four of the sites where they had been recorded the previous year is unlikely to be due to small sampling effort or influences of weather. These sites were sampled over a five month period and at a wide range of weather conditions. At least fifteen grid surveys were undertaken at these sites.

If a local extinction has occurred, it may indicate that the habitat has changed (Brown and Lomolino 1998). It may also indicate that, by chance, a local extinction at that site has occurred due to an originally small population size. However, if local extinction has occurred, management and research of this species must be increased and must occur at a more rapid pace than at present.

The observed occupancy rates of tiles by *D. impar* may be related to the local abundances at sites. MacKenzie *et al*. (2004), McDonald (2004) and Pollock *et al*. (2004) commented that to be able to draw inferences about abundance, an estimation of the detection probability of *D. impar* is required (i.e. the probability that *D. impar* appears in a count for the survey area, if present). It has been suggested that a higher detection probability (presence/absence) will occur at sites where there is greater local abundance (MacKenzie 2005a). When there is a lower abundance of *D. impar* at a site, the presence of this species may be harder to detect (MacKenzie 2005a). Therefore, the sites in which *D. impar* was not recorded could be due to low abundance and a low detection probability. However, at present it cannot be confidently said that occupancy of tiles is related to relative abundances of *D. impar*.

Differences in occupancy rates may also be due to the heterogeneous landscapes in Western Victoria. It may also be due to the quality of the site (McDonald 2004). Thus, within a species geographic range, patchy or aggregated distributions of individuals may occur (Brown and Lomolino 1998). Consequently, *D. impar* populations may have few individuals in some areas and many more in other areas according to the extent of niche requirements being met at particular sites (Brown and Lomolino 1998). Sites such as “Challicum Park” and Caramut-Chatsworth Rd. may be seen as sites in which the majority of niche requirements of *D. impar* are being met. As a result, many more individuals will be occupying these sites compared to others. The reasons for low occupancy rates at sites such as Blacks Creek, however, are less straight forward and remain enigmatic. Additionally, the aggregation of individuals may have severe consequences for *D. impar* populations, especially if habitat modification and fragmentation continues. With the aggregation of populations, most individuals of this species will therefore only occur in a small area within its known range (Brown and Lomolino 1998). If the grassland habitat sustaining these aggregated populations is removed or poorly managed, then extinction for these populations may be inevitable.

4.3.2. Seasonal tile occupancy

Results showed that tile occupancy rates increased in spring but declined in summer, with a significant difference in occupancy rates among sampling periods. This is supported by O'Shea (2004), who also found occupancy rates of tiles to increase in spring (October). Increased occupancy rates in spring may coincide with the breeding season, with increased activity in November (Smith and Robertson 1999). This also supports results found within the present study. O'Shea (2004) suggested that more individuals may be surface active during spring because of thermoregulation requirements. This would additionally support tile use if they are used for thermoregulation. It was also suggested that during summer, *D. impar* is able to maintain a semi-fossorial habit due to high surface temperatures and thus will be less surface active (O'Shea 2004). This also coincides with soil cracks forming and therefore an increased availability of alternative refuges. Additionally, decreased tile occupancy rates may be due to tiles reaching temperatures of 70°C during summer (G. Peterson *pers. comm.*).

4.3.3. Habitat characteristics

If occupancy rate reflects abundance, then the habitat characteristics may be determining the abundance of *D. impar* at a site. However, site habitat characteristics may also determine occupancy rates independent of abundance. If so, then *D. impar* will be more likely to occupy tiles in some habitats, because of the structural features. However, it is unknown whether or not there is a relationship between occupancy rates and abundance and the strength of that relationship.

The height and structure of vegetation at a site often reflects management practices (Hadden 1995). These may include grazing by livestock (predominantly sheep in Western Victoria) and prescribed burning. Shorter maximum vegetation heights are expected at sites which have prescribed burns either annually or every few years, as well as sites that are grazed at regular intervals (Koehler 2004). Results showed that a shorter maximum height of vegetation increased tile occupancy by *D. impar*. Koehler (2004) also found that presence of *D. impar* was determined by a reduced height of the dominant vegetation and when the tallest vegetation height was lower. These results suggest that processes that have these effects, such as prescribed burns and grazing have a positive influence on the occupancy rates of this species. However, this may only apply if these management types are applied at an intensity and frequency that is non-detrimental to this species (Russell *et al*. 1999). They should also not lie outside ecological or physiological tolerances of *D. impar* and should allow sufficient refuges (grass-tussocks in particular) to remain (Irvin *et al.* 2003; D.S.E 2005). Thus, because *D. impar* is threatened, management such as prescribed burning should be undertaken during seasons where suitable soil cracks are available (refuges to escape fires) and when *D. impar* may be less active on the surface (Koehler 2004; O'Shea 2004).

Koehler (2004) indicated that the height of *Phalaris aquatica* influenced whether *D. impar* was present at a site. She also indicated that the presence of *P. aquatica* could control the distribution of *D. impar*. The present study did not find that the height of *P. aquatica* significantly influenced the use of tiles by *D. impar*. This may correspond to only small patchy areas of *P. aquatica* at some sites and the fact that these sites were selected because of the presence of *D. impar*.

4.4 General tile use

4.4.1. The influence of time of day and environmental conditions

Results of this study indicated that higher occupation of tiles occurred in the middle of the day (1100hrs-1500hrs), when the incidence of solar radiation striking tiles was at its maximum. This suggests that tiles may be primarily used as concealed sites for thermoregulation. The decrease in tile use between 1300-1359hrs indicates that tiles may have become too hot for *D. impar* and individuals have moved away. It may also indicate that individuals are undertaking alternative activities to thermoregulation during this period. Tiles also appear to be used to some extent as overnight refuges. This is supported by observations of tile use both early in the morning (0700-1059hrs) and late afternoon (1500-1759hrs).

Results showed that tile occupation by *D. impar* occurred more often when cloud cover was overcast or patchy-heavy. When cloud cover is high, tiles would not heat up as much, as less solar radiation would be striking the tiles. Therefore, tiles will not be reaching temperatures that are uncomfortable or reaching the critical thermal maximum of *D. impar*. However, cloud cover may not be acting independently in determining tile use by individuals. Air temperature will also influence tile use under these cloud conditions.

D. impar might also be using grass tussocks for thermoregulation using direct sunlight (covert basking), when there is little or no cloud cover present. Therefore, fewer individuals will be using tiles under these conditions. This supports previous studies that maintain *D. impar* may also be heliothermic (refer to Hadden 1995). However, it remains unknown as to whether *D. impar* is strictly thigmothermic or is also heliothermic. It has been suggested that many lizards are not strictly heliothermic, but may also be thigmothermic in some sense (Heatwole and Taylor 1987). This may be dependent on the environmental conditions and may result in a species alternating between the two (Heatwole and Taylor 1987). *D. impar* was never observed basking in the open (*pers. obs.*), which is a condition of being heliothermic (i.e. rely on radiant energy for body heat) (Heatwole and Taylor 1987).

Thermal constraints exist for ectotherms in the natural environment, and as such *D. impar* may occupy certain thermal microclimates within its habitat (Grant and Dunham 1988). It has also been suggested that thigmotherms will exhibit behavioural selection of the thermal environment in which they are found, changing locations to maintain preferred body temperatures (Heatwole and Taylor 1987). Individuals will therefore move from under an artificial refuge (roof tile) when temperatures become too hot, so that body temperature does not exceed the critical thermal maximum (Grant and Dunham 1988; Huey *et al*. 1989). The ability to select microclimatic conditions within the habitat is particularly important for *D. impar* which has a low thermal inertia caused by its small size and shape (Grant and Dunham 1988). It is also important where exceeding the critical thermal maximum could result in death or temperature impairment for the individual (Grant and Dunham 1988; Huey *et al*. 1989). The avoidance by *D. impar* of roof tiles at both high air and substrate temperatures in this study, exemplifies its ability to select appropriate microclimatic conditions within its preferred body temperature (Adolph 1990). It may also explain the decreased occupancy rates of tiles in late summer when air temperatures exceed 30°C.

Temperature both under and outside a tile appear to play an important role in the use of tiles by *D. impar*. Decreased occupancy rates of roof tiles by *D. impar* during the afternoon may correspond with increased levels of solar radiation heating tiles to uninhabitable levels. Huey *et al*. (1989) found that temperatures exceeding lethal levels occurred underneath thin rocks in full sun during the afternoon. In their study, garter snakes (*Thamnophis elegans*) were found to retreat into burrows at depths greater than 2.5cm or retreat under rocks that were thicker than 20cm at such temperatures. Because tiles used in the present study are significantly thinner than 20cm (approx. 5cm), it is assumed that many *D. impar* retreated from underneath tiles in the afternoon when substrate temperatures exceeded 35°C and sought alternative refuges that provided cooler microclimates (Heatwole and Taylor 1987). The apparent disappearance of *D. impar* from under tiles may additionally support the apparent use of soil cracks as refuges in the summer months (December and January) (*pers. obs.* and T. Rohr and G. Peterson *pers. comm.*).

The warmest part of the thermal environment of a reptile has often been found to be the soil surface (Heatwole and Taylor 1987), with substrate temperatures higher than that of the ambient air. Substrate temperatures were generally 2-3°C higher in this study. When the sun's rays strike the ground, the air directly above the soil surface is heated (Heatwole and Taylor 1987). Consequently, warmer substrate temperatures underneath tiles will correspond to hot air becoming trapped between the soil surface and the roof tile above it. This is extremely beneficial to thigmothermic reptiles such as *D. impar* that rely upon heat exchange with the substrate to maintain body temperatures (Heatwole and Taylor 1987).

Relative humidity both under and outside a tile also appear to influence the utilization of tiles by *D. impar*. Results indicated that a high relative humidity underneath tiles and a low relative humidity outside tiles increased tile occupation by *D. impar*.

Koehler (2004) also found that tile use by this species occurred at lower ambient humdities. A higher humidity underneath tiles may be beneficial to *D. impar*, as it reduces evaporation rates which cool body temperatures (Heatwole and Taylor 1987). This is particularly important for *D. impar* because of their small size. In small animals, higher evaporation rates are known to occur due to larger surface area to volume ratios (i.e. size and shape of *D. impar*) (Heatwole and Taylor 1987). The evaporation rate is also related to the substrate temperature, with evaporation rate rising with increasing temperatures. This may explain the decreased occupancy rate of artificial refuges (roof tiles) by *D. impar* above substrate temperatures of 25°C. Water evaporation rate underneath tiles will also be greatly reduced because there is less air movement under tiles as apposed to exposed substrates (Heatwole and Taylor 1987).

4.5 Individual tile use

4.5.1. Influences of other animal taxa

Small lizards have been observed to reduce the risks of potential predation by avoiding tiles where snakes were or are by the detection of their scent (e.g. Downes and Shine 1998a, 1998b; Amo *et al*. 2004). The results of this study showed that the current or past occurrence of a snake under a specific tile had a negative influence on the subsequent occupancy of that tile by *D. impar*. Elapids such as *Austrelaps superbus* (Lowland Copperhead) and *Suta flagellum* (Little Whip snake) are known predators of small lizards (Shine 1977, 1987, 1988b) and were observed to often occupy tiles at some sites. This would have influenced the overall occupancy rates of tiles by *D. impar* at some sites. This suggests that at sites, in which snake abundance was high, fewer *D. impar* were detected than their real abundances would have otherwise allowed. It has also been suggested that individuals will immigrate into less favourable habitat or refuges to avoid these predators (Martin and Lopez 1998; Sabo 2003).

Positive associations between *D. impar* and redback spiders (*Latrodectus hasselti*) found in this study were unexpected. Redback spiders have not been recorded as a component of *D. impar*s' diet (Kutt *et al*. 1998). Although, the diet of *D. impar* does contain some spiders, they are mainly of the families Salticidae (jumping spiders) and Miturgidae (wolf spiders) (Coulson 1990; Kutt *et al*. 1998). Redback spider venom could easily kill a small lizard such as *D. impar* and it is possible that small lizards become prey of this species. Explanations for the positive association of *D. impar* with redback spiders therefore tend towards similar microhabitat use and/or microhabitat structure. This may include factors such as relative humidity and temperature underneath tiles (Goldsbrough *et al*. 2004). It may also involve potential prey of both species being located underneath these tiles (Webb and Shine 1998; Goldsbrough *et al*. 2004). However, the present study did not determine the abundance of prey species under the tiles, but merely the presence or absence of selected invertebrates and arachnids. It is suggested that future studies are needed to determine whether potential prey species are influencing the presence or absence of *D. impar* under tiles and thus whether tiles are also acting as foraging sites.

4.5.2. Microhabitat characteristics under and outside tiles

Results showed that occupation of individual tiles by *D. impar* was associated with kangaroo grass (*Themeda triandra)* and agricultural grass cover underneath tiles. This vegetation cover may be beneficial to *D. impar*, by creating a more complex microhabitat underneath the tile. It is suggested that vegetative cover underneath a tile may raise the position of *D. impar* from the substrate, allowing a thermal gradient to exist (Hadden 1995). Individuals would, therefore, be able to move closer to the tile to warm up and away from the tile in order to cool down. Additionally, the grass cover may act as insulation at cooler temperatures, or provide more suitable microhabitat for prey species.

An increased percent cover of agricultural grasses 1m around tiles was also found to be associated with the occupation of an individual tile by *D. impar*. Therefore, agricultural grass cover may be influencing microhabitat use of this species within the habitat. These results suggest that *D. impar* may be still able to persist in agricultural grasslands that were historically composed of native tussock-forming grasses. It may also be inferred that, as long as some tussock-forming grasses remain within agricultural land, then *D. impar* may still be present.

Tile use by *D. impar* also appears to be influenced by the placement of tiles within the habitat, with tiles occupied more often when they were surrounded by agricultural grasses. This may also imply that the vegetation underneath a tile will be correlated with the vegetation around a tile. It is suspected that the significance of agricultural grass cover in determining the presence of *D. impar* under a tile may be due to the pooling of all sites. For sites such as "Challicum Park" and Chatsworth-Caramut Rd., tiles were surrounded by native *T. triandra*. These sites had the largest tile occupancy rates of all sites. Therefore, the percent cover of agricultural grasses around tiles in influencing the presence of *D. impar* at these sites is not applicable and the results should be treated with caution. However, it may indicate that in degraded habitats where there is little available cover and/or refuges, an increased usage of tiles will be observed.

Results also indicated that the degree of soil cracking under tiles negatively influenced the presence of *D. impar*. This may be explained in several ways: 1) *D. impar* were seeking refuge in soil cracks when they formed and were not found under tiles; 2) the larger the soil cracks the more exposed *D. impar* may have been to predators and therefore, these tiles were avoided or 3) the more soil cracks that were present, the drier the conditions underneath the tile were (causing increased evaporative loss in *D. impar*) and hence these tiles were not utilized.

4.6. Refuge Preference in a laboratory experiment

D. impar showed a strong preference for grass-tussocks under the conditions of the experiment (air temperature 24°C, wind speed 1.7mph, relative humidity 82.7% and no radiant heat). Hadden (1995) suggested that the tussocks provide important microhabitat for *D. impar*, providing both shelter and basking sites. Concrete tiles and basalt rocks were also used equally and were chosen close to the expected frequency. This suggests that concrete tiles are used on the basis that they are encountered by *D. impar*. It also suggests that the physical and thermal properties of concrete tiles are similar to that of the natural basalt rocks. This was also found by Webb and Shine (2000) in velvet geckoes (*Oedura lesueurii*) using concrete pavers as artificial refuges. Conversely, *D. impar* showed a strong avoidance of terracotta tiles under the conditions of the experiment. Therefore, if conditions are similar in the field to the laboratory experiment, *D. impar* will be expected to occupy terracotta tiles less often. Alternatively, it has been found that *D. impar* exhibited no preference for any refuge type, when refuges were heated (B. Malone *pers. comm.*). These results suggest that thermoregulation is a major factor influencing tile utilization by *D. impar*.

Bias may also be caused from an insufficient lag time provided for individuals to get used to the artificial refuges (concrete and terracotta roof tiles). This has previously been found to influence the use of tiles by *D. impar* (Koehler 2004; O'Shea 2004). These issues should be investigated for future studies. It is suggested that laboratory experiments be undertaken on the influence of conspecific scent on refuge utilization by this species.

There were some limitations imposed on the laboratory experiment in testing for the refuge preference of *D. impar*. These included refuges being essentially only monitored and used as overnight refuges. Thermoregulatory use of refuges was thus not investigated due to the time of day in which tiles were checked. It is suggested that for future studies, refuge preference should be recorded on more than one occasion, with individuals left in arenas for longer periods of time.

Conclusions

Roof tile grids as a census technique are effective at providing information for a wide range of fauna. However, their effectiveness at detecting the presence of *D. impar* remains enigmatic as 25% of sites recorded no individuals using this census technique. The effectiveness of roof tiles as a population monitoring technique is also uncertain. Mark-recapture studies may indicate 'trap-shyness' of individuals, with a decline in the number of individuals recaptured with continued monitoring.

D. impar was found to be sexually dimorphic, with females the larger of the two sexes. Females had both a longer snout-vent length and longer head-length than males. The advantage of an increased snout-vent-length appeared to support increased egg size.

It still remains elusive as to whether occupancy rates of roof tiles by *D. impar* reflect their abundance at a site. There may be a relationship between abundances and occupancy rates, but the strength of this relationship is undetermined. However, occupancy rates have been found to be highly seasonal and vary over time of day and with environmental conditions. Until further analysis is undertaken, tile occupancy rate instead of relative abundance should continue to be used.

Tile occupation is influenced by many factors and is non-random. These include, but may not be limited to, microhabitat characteristics (percent cover of *Themeda triandra* and agricultural grasses underneath tiles), the degree of soil cracking, and occupation by snakes (*Austrelaps superbus* and *Suta flagellum*) and redback spiders. Tile occupation also appears to be related to the thermal environment (temperature and humidity), cloud cover, time of day and season. Percent cover of agricultural grasses around tiles also appears to influence microhabitat use of *D. impar* within the habitat.

Variation in occupancy rates among sites was determined by the maximum height of vegetation, with a reduction in height resulting in increased occupancy rates of tiles. Structural characteristics of the habitat were more important than floristic characteristics in influencing the occupancy rate of *D. impar*. However, at this stage, it is unknown whether or not vegetation characteristics reflect local abundances.

Under some environmental conditions, *D. impar* will show a preference for grass tussock refuges and avoid terracotta tiles for over night refuge. Concrete tiles also appear to be occupied at rates in which they are encountered by this species.

Results of this study indicate that roof tile grid monitoring will be optimal in late October and November (when increased tile occupation by *D. impar* occurs). Optimal sampling conditions for *D. impar* were also found to be between 1100-1500hrs, particularly during overcast or heavily clouded days, and when air temperature is below 25°C and air relative humidity is less than 50%.

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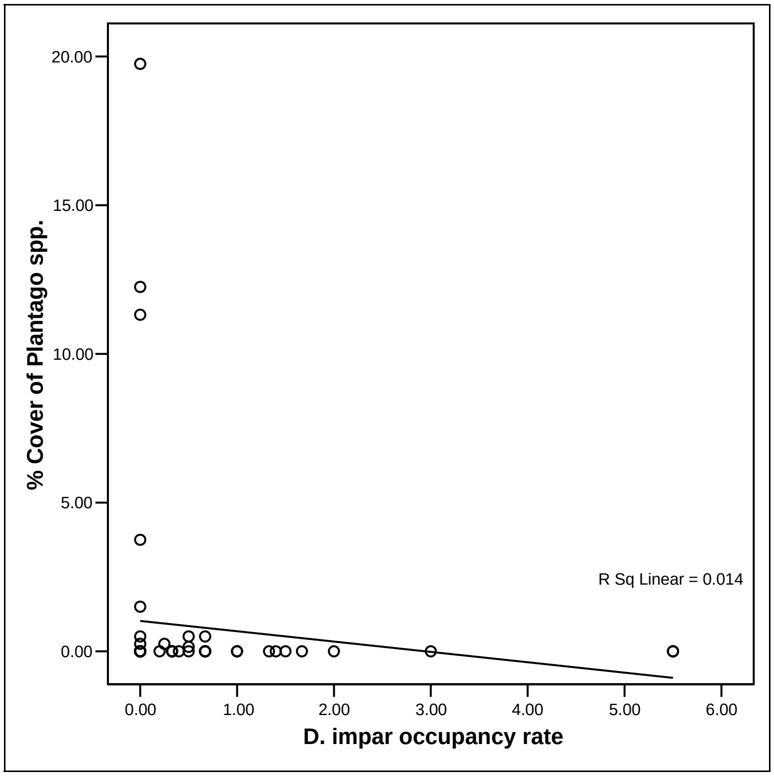
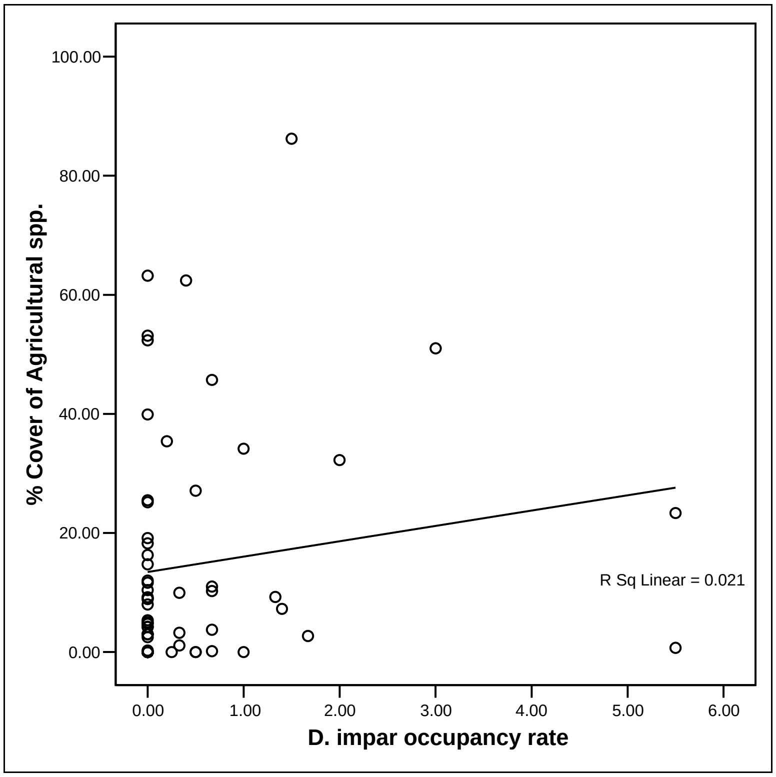
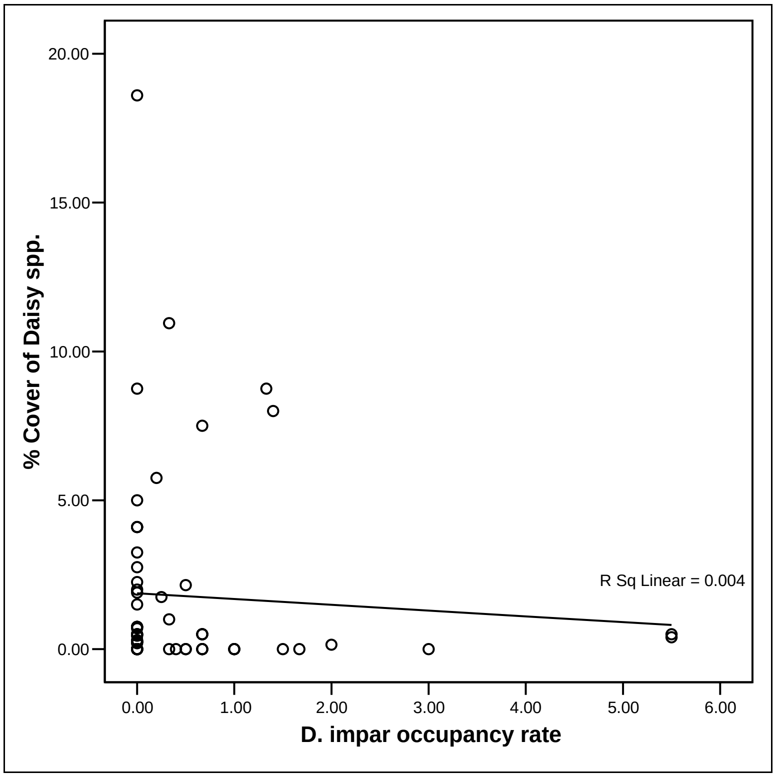
Appendix I: Survey dates of all sites from late September 2005- late January 2006.

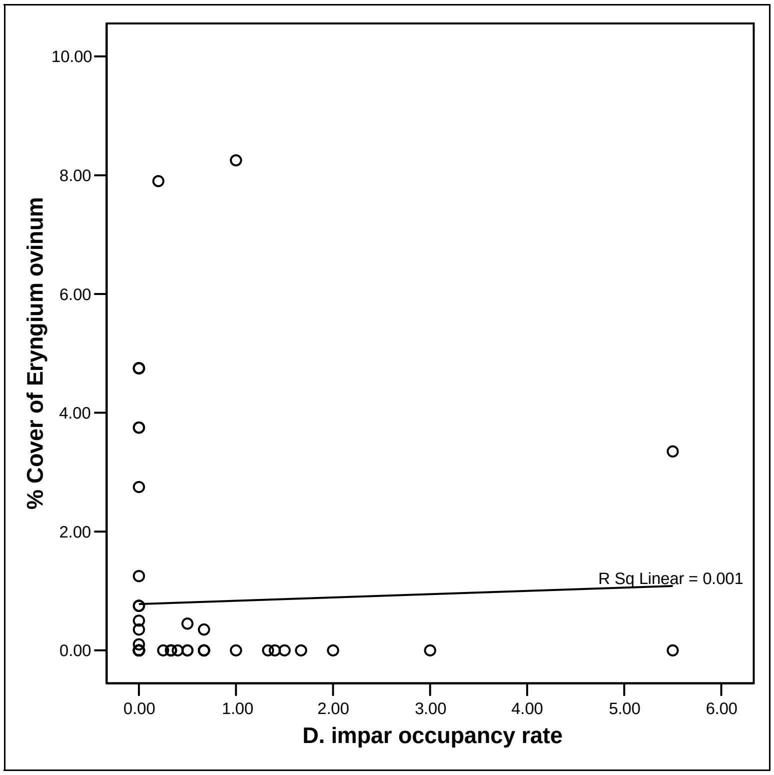
	Survey period				
Sites	**Early Oct. 2005**	**Late Oct. 2005**	**Nov. 2005**	**Dec. 2005**	**Jan. 2006**
Blacks Creek	4/10 - 7/10	26/10 - 30/10	9/11 - 13/11	10/12 - 13/12	18/01 - 22/01
Bochara Station	4/10 - 7/10	26/10 - 30/10	9/11 - 13/11	10/12 - 13/12	18/01 - 22/01
Boonderoo Nature Reserve	28/09 - 02/10	19/10 - 23/10	18/11 - 20/11	07/12 - 10/12	14/01 - 16/01
Camperdown-Foxhow Rd #1	28/09 - 02/10	19/10 - 23/10	18/11 - 20/11	07/12 - 10/12	14/01 - 16/01
Camperdown-Foxhow Rd #2	28/09 - 02/10	19/10 - 23/10	18/11 - 20/11	07/12 - 10/12	14/01 - 16/01
Caramut-Chatsworth Rd	4/10 - 7/10	26/10 - 30/10	9/11 - 13/11	10/12 - 13/12	18/01 - 22/01
Cavendish-Dunkeld Rd	4/10 - 7/10	26/10 - 30/10	9/11 - 13/11	10/12 - 13/12	18/01 - 22/01
“Challicum Park”	4/10 - 7/10	26/10 - 30/10	9/11 - 13/11	10/12 - 13/12	18/01 - 22/01
Chatsworth Rd	28/09 - 02/10	19/10 - 23/10	18/11 - 20/11	07/12 - 10/12	14/01 - 16/01
Cressy Trotting Track	28/09 - 02/10	19/10 - 23/10	18/11 - 20/11	07/12 - 10/12	14/01 - 16/01
Cressy-Shelford Rd	28/09 - 02/10	19/10 - 23/10	18/11 - 20/11	07/12 - 10/12	14/01 - 16/01
Forest Lane	4/10 - 7/10	26/10 - 30/10	9/11 - 13/11	10/12 - 13/12	18/01 - 22/01
Geggies Rd	28/09 - 02/10	19/10 - 23/10	18/11 - 20/11	07/12 - 10/12	14/01 - 16/01
Hamilton HWY-Tea-tree Creek	4/10 - 7/10	26/10 - 30/10	9/11 - 13/11	10/12 - 13/12	18/01 - 22/01
Lubra Creek	4/10 - 7/10	26/10 - 30/10	9/11 - 13/11	10/12 - 13/12	18/01 - 22/01
Old Ararat Rd	4/10 - 7/10	26/10 - 30/10	9/11 - 13/11	10/12 - 13/12	18/01 - 22/01
Rokewood Common	28/09 - 02/10	19/10 - 23/10	18/11 - 20/11	07/12 - 10/12	14/01 - 16/01
Rokewood-Shelford Rd	28/09 - 02/10	19/10 - 23/10	18/11 - 20/11	07/12 - 10/12	14/01 - 16/01
Rossbridge Flora Reserve	4/10 - 7/10	26/10 - 30/10	9/11 - 13/11	10/12 - 13/12	18/01 - 22/01
Vite Vite Rail Reserve	28/09 - 02/10	19/10 - 23/10	18/11 - 20/11	07/12 - 10/12	14/01 - 16/01

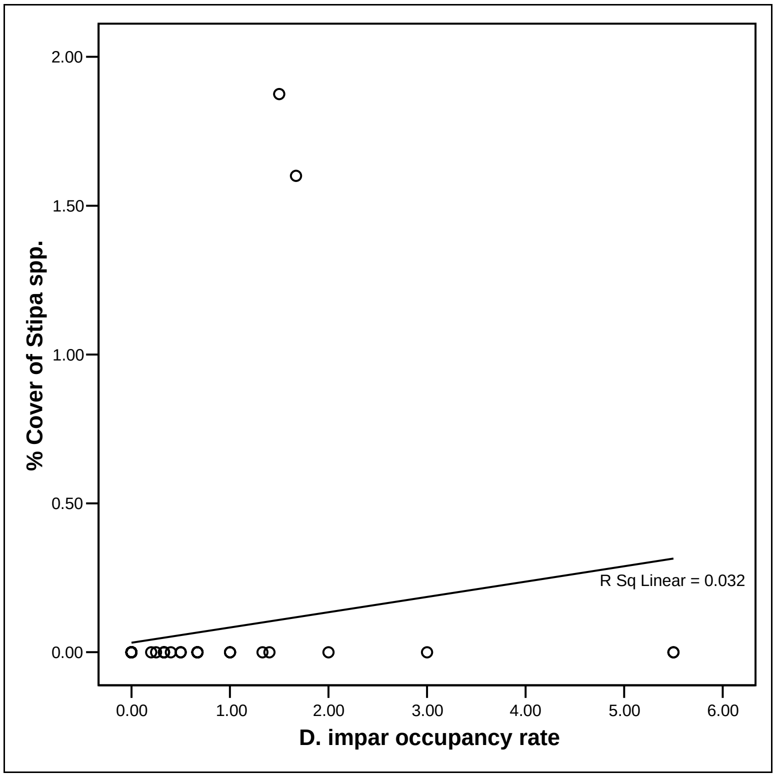
Appendix II: The relationships between mean percentage cover of vegetative species and occupancy rate of *D. impar* at sites.

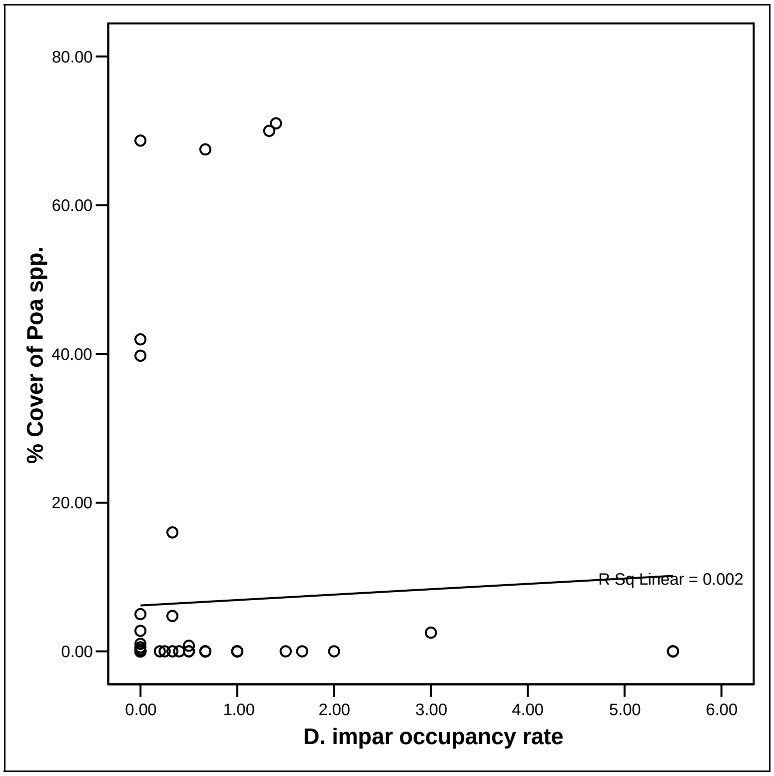


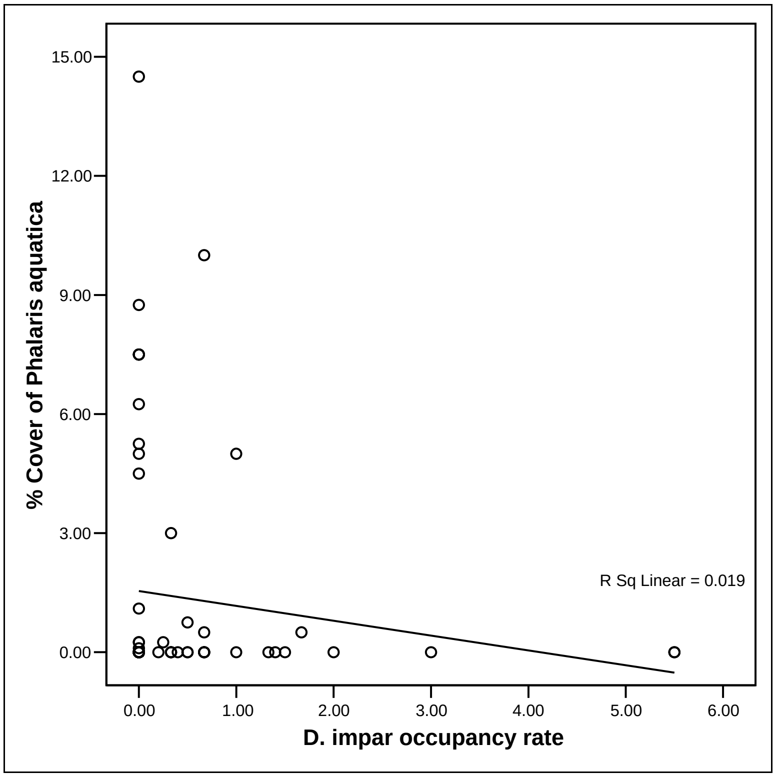


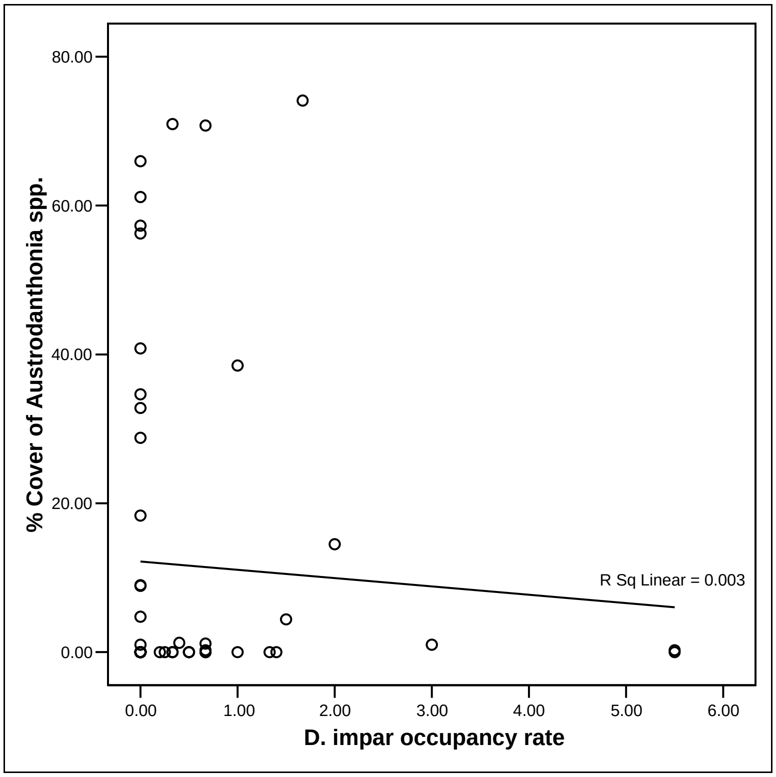

20.00
15.00
10.00
5.00
0.00
% Cover of Daisy spp.
R Sq Linear = 0.004
0.00
1.00
2.00
3.00
4.00
5.00
6.00
D. impar occupancy rate

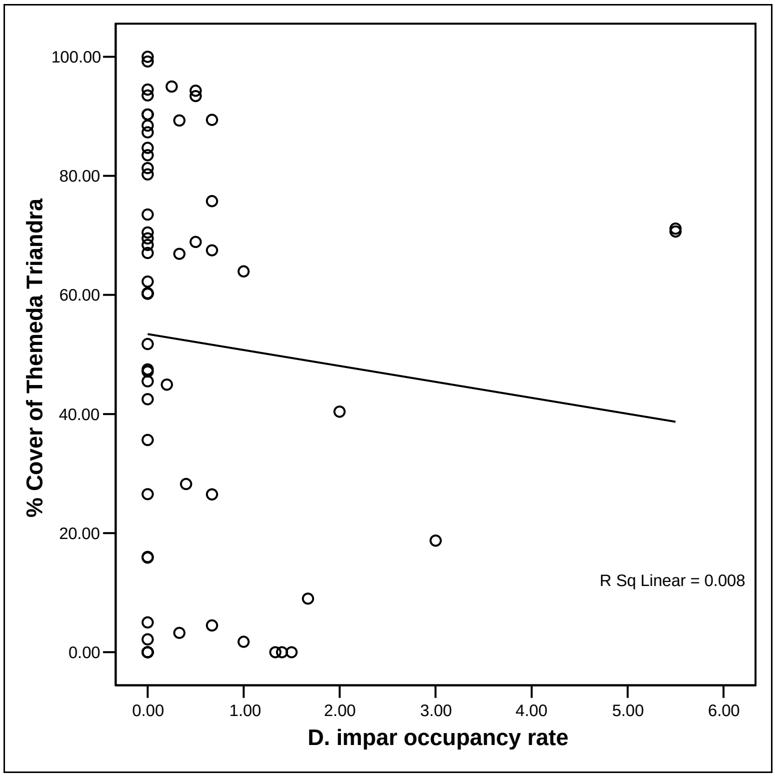

10.00
8.00
6.00
4.00
2.00
0.00
% Cover of Eryngium ovinum
R Sq Linear = 0.001
0.00
1.00
2.00
3.00
4.00
5.00
6.00
D. impar occupancy rate

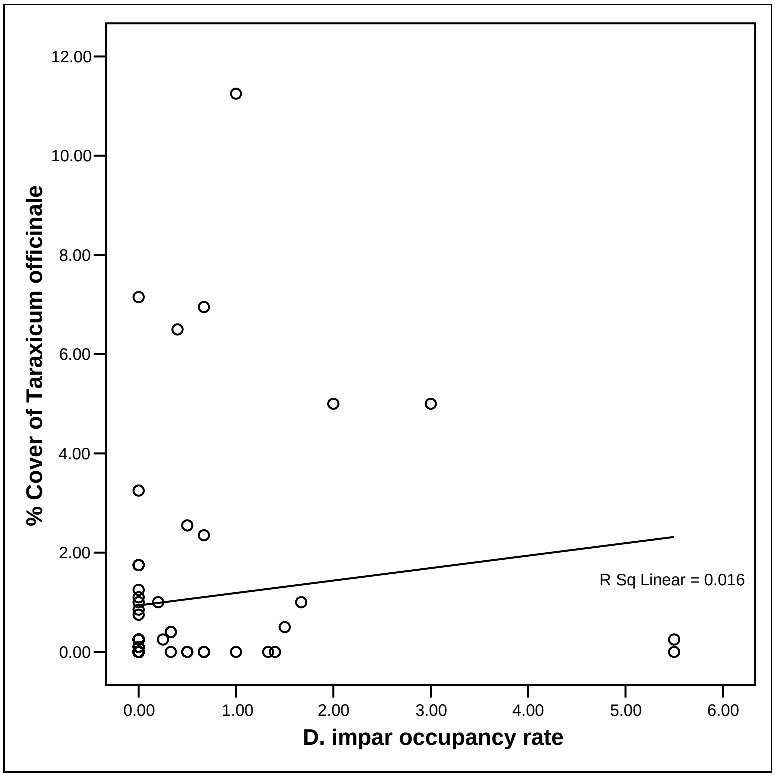

2.00
1.50
1.00
0.50
0.00
% Cover of Stipa spp.
R Sq Linear = 0.032
0.00
1.00
2.00
3.00
4.00
5.00
6.00
D. impar occupancy rate


80.00
60.00
40.00
20.00
0.00
% Cover of Poa spp.
R Sq Linear = 0.002
0.00
1.00
2.00
3.00
4.00
5.00
6.00
D. impar occupancy rate


15.00
12.00
9.00
6.00
3.00
0.00
% Cover of Phalaris aquatica
R Sq Linear = 0.019
0.00
1.00
2.00
3.00
4.00
5.00
6.00
D. impar occupancy rate


80.00
60.00
40.00
20.00
0.00
% Cover of Austrodanthonia spp.
R Sq Linear = 0.003
0.00
1.00
2.00
3.00
4.00
5.00
6.00
D. impar occupancy rate


% Cover of Themeda Triandra
100.00
80.00
60.00
40.00
20.00
0.00
R Sq Linear = 0.008
0.00
1.00
2.00
3.00
4.00
5.00
6.00
D. impar occupancy rate


% Cover of Taraxicum officinale
12.00
10.00
8.00
6.00
4.00
2.00
0.00
R Sq Linear = 0.016
0.00
1.00
2.00
3.00
4.00
5.00
6.00
D. impar occupancy rate