



PRODUCTION PERENNIALS FOR BIODIVERSITY

Can saltbush plantings improve biodiversity in South Australian Murray Mallee agro-ecological cropping systems?



Future Farms Industries Technical Report

Anita K. Smyth (Project Leader), Talat H. Ansari, Dave Armstrong, Robert Brandle, Stuart Collard, Mike Crowther, Andrew Fisher, Alison Fitch, Mike Gardner, Stephanie Godfrey, Melanie Lancaster, David Phalen, Elizabeth Smees, Patrick Smith

February 2012



For more information, please contact:

Dr Anita Smyth (Principal Investigator)

P (08) 8313 1270 **M** 0427 619 140

E anita.smyth@adelaide.edu.au or

neets@iinet.net.au

Author: Anita Smyth

Document Version: V2.0

Status: Final

Date: 23 March 2012



BioScience Solutions
Dr Anita Smyth (Principal Investigator)
Hawthorndene SA 5051 Australia

T: +61 8 8370 0160

M: +61 (0)427 619 140

E: anita.smyth@adelaide.edu.au or neets@iinet.net.au

W: http://www.aekos.org.au/eco-informatics_facility

W: <http://www.csiro.au/Organisation-Structure/Divisions/Ecosystem-Sciences/AnitaSmyth.aspx>

Table of Contents

Executive Summary	1
Recommendations	2
Chapter 1: Do woody perennial farming systems provide resources that enhance the persistence of native vertebrate fauna in Australian agricultural landscapes?	4
Introduction	5
Methodology.....	8
Identifying and sorting vertebrate species.....	10
Defining resource needs of vertebrate species	10
Resource use decisions.....	10
Data analyses.....	10
Results	13
Discussion.....	17
Conclusions	18
References	19
Chapter 2: The potential of planted saltbush (<i>Atriplex nummularia</i>) to support avian biodiversity in the Murray Mallee of South Australia	26
Chapter 1 of PhD Thesis.....	26
References	27
Chapter 3: A direct biodiversity benefit of saltbush-based farming for the sleepy lizard, <i>Tiliqua rugosa</i>, in southern Australia	29
Abstract	30
Introduction	30
Methods	31
Study site and sample collection	31
DNA extraction and microsatellite genotyping.....	32
Population genetic structure analysis	33
Parentage analysis	33
Results	33
Presence of <i>T. rugosa</i> in saltbush plantations	33
Hardy-Weinberg equilibrium proportions and null alleles and probability of identity	35
Population genetic structure analysis	33
Evidence of breeding in saltbush plantations.....	35

Spatial relationship between parents and offspring.....	36
Discussion.....	36
Acknowledgements	38
References	38
Chapter 4: Chronically ill wild sleepy lizards <i>T. rugosa</i>, in one of Australia’s food bowl.....	40
Abstract	41
Introduction	41
Methods	43
Study sites	43
Sample design and collection for assessing wild lizard health	45
Body condition	46
RCI	46
Ecoparasite presence	46
Post mortem examination.....	46
Statistical analysis	46
Results	47
Lizard health in Baseline and Severe sites	47
Body condition	47
Haematology	48
Differential haematology.....	50
Influence of habitat connectivity on lizard health in Severe sites	50
Body condition	50
Haematology	51
Post Mortem findings	53
Elapsed sampling time since brumation (CUMDAYS)	53
Discussion.....	53
Acknowledgements	58
References	58
Supplementary matrials	61

Executive Summary

Native biodiversity in Australian agricultural landscapes continues to decline in response to a range of threatening processes. Woody perennial farming systems are being established across southern Australia as land managers strive to achieve more sustainable and profitable farming practices. Woody perennial farming systems can improve the persistence of biota either directly, for example, by providing additional food resources; or indirectly by ameliorating risk factors, such as altered hydrological processes, that are threatening biota. The focus of the work reported here is on the food, shelter and breeding resources that perennial farming systems can provide for native biota and thus potentially contribute to the persistence of a range of native species in agricultural landscapes. Although woody perennial farming systems will rarely provide all of the resources required by a species adapted to complex natural ecosystems, they may provide significant quantities of some limiting resources or supplementary resources at key points of an organism's life cycle.

In this report, we have presented the results of three subprojects in the form of near-final manuscripts and make recommendations at the end of the summary.

In Chapter 1, we present a review of the resource requirements of all terrestrial vertebrate species found in the temperate agricultural landscapes of South Australia and Western Australia. We seek to answer the following questions:

- What resources do native vertebrate fauna require?
- What resources are provided by woody perennial farming systems?
- What resources are required by 'at risk' vertebrate fauna species?
- Can woody perennial farming systems provide the resources required by 'at risk' vertebrate fauna species?

We document the food, shelter and nesting requirements of native terrestrial vertebrate fauna, considered species by species, in the temperate agricultural landscapes of southern Australia, based upon literature review and expert opinion. By comparing the resources required by listed threatened species with the resources provided by commercial woody perennial farming systems, we assessed the extent to which these systems may contribute to the persistence of 'at risk' species.

Woody perennial farming systems offer to varying degrees the resources required by threatened vertebrate fauna. Low foliage and woody infrastructure are available in most woody perennial farming systems, as is some form of woody debris and leaf litter. Fodder shrubs in particular provide vegetative structure close to the ground. None of the woody perennial farming systems considered offer the tree hollows and crevices required by threatened mammals and birds.

Woody perennial farming systems could be modified to incorporate these key resources, enhancing their suitability for 'at risk' vertebrate species at the local scale and potentially improving their persistence within agricultural landscapes.

This review has highlighted the lack of basic knowledge on the life histories of our native biota and their ability to persist in fragmented, multiple-use landscapes. Significantly increased

research is required to resolve this knowledge gap and address our currently inadequate knowledge of the role of woody perennial farming systems in biodiversity conservation.

Chapter 2 present the summary results of the first of a set of studies being undertaken as a part of a doctorate on the potential of planted saltbush (*Atriplex nummularia*) to support avian biodiversity in the Murray Mallee of South Australia. This study examined bird abundance and species richness trends between remnant vegetation with adjacent saltbush plantings, isolated remnant vegetation, isolated saltbush plantings, and cleared land cropping/pasture use to understand the influence of adjacency to remnant vegetation on avian use of saltbush plantings and identify influential factors driving responses. We found saltbush plantings has potential to provide improved habitat and biodiversity conservation value for birds over conventionally managed agricultural land. However, the simple structured monoculture design of these plantings means they supported a significantly reduced suite of species compared to that present in remnant vegetation. Saltbush plantings supported several bird species not found at agricultural sites, suggesting these species would be otherwise absent from conventional cropping/pasture landscapes. Like similar studies elsewhere, it has shown that adjacency to remnant vegetation can increase the avian abundance and richness in saltbush plantings, suggesting remnant vegetation holds the greatest biodiversity value and is important for biodiversity conservation in cereal cropping landscapes.

In Chapter 3, we explored the use of native perennial (saltbush) plantations *Atriplex nummularia nummularia* by the sleepy lizard, *Tiliqua rugosa*, an endemic Australian species common in the South Australian Murray Mallee region (SAMM). We visited the same saltbush plantations multiple times throughout 2010 and 2011 and collected samples for genetic analysis from adults (n = 55) and juveniles (n = 25). Using 8 microsatellite loci, parents were assigned to over half of all juveniles with high statistical confidence. We found that parents were sampled in the same patch of saltbush as their offspring, thus supporting the observation that juvenile sleepy lizards remain within the home range of their parents prior to dispersal. Most importantly, our results indicate that saltbush provides important habitat for *T. rugosa* at significant life stages – prior to and during breeding for adults, and post-birth but before dispersal for juveniles. Analysis of population structure also indicated that gene flow appears to be uninhibited across the whole SAMM, suggesting habitat connectivity throughout. The long lived nature of this species may mean that the effects of reduced connectivity are yet to manifest in these data. We can conclude that revegetation using simple, monoculture plantations is beneficial in preserving native biodiversity in human-altered agricultural landscapes and that the cereal cropping landscapes offer structural connectivity at least for this species.

In Chapter 3, we assess the body condition and hematology of the sleepy lizard in grazed rangelands (native vegetation relatively intact) and severely modified cropping landscapes (remnants of native vegetation surrounded by cereal crops) of the SAMM to assess lizard health and make inferences about the vulnerability of habitats in these extremes of habitat modification and discuss implications for their persistence and farming systems management. We found that the health of lizards when measured indirectly by body condition and confirmed by an hematological assessment in cropping landscapes was chronically poor with haemolytic anaemia. Although body weights of adults and ‘body condition’ was lower in the cropping landscapes, the

differences in the percentage of polychromatophilic red blood cells , PCV and absolute and differential blood cell counts clearly show the health of lizards was alarmingly poor for almost 50% of our study animals. This widespread decline in health wasn't due to degraded habitat complexity (measured by habitat type and structural connectivity). Haemolytic anaemia can have a number of causes. A potential cause in the SAMM cropping system is exposure to a variety of agrochemicals that may be toxic. Our findings raise a number of questions about the ecosystem health of cropping systems for wildlife and possibly humans. We conclude that the health of the sleepy lizard population in the SAMM cropping landscape is a serious concern and that research into the impact of agrochemicals on ecosystem health of cropping systems is urgently needed to identify whether action is required to preserve biodiversity and to protect human health. The charismatic sleepy lizard, which is a favorite among farmers, could play a sentinel role in cropping systems for biodiversity, ecosystem and human health assessment.

In conclusion, saltbush plantings can improve biodiversity by providing additional habitat and adding to the connectivity of native and vegetated habitats. We have direct evidence that monocultural plantings provide a direct benefit as a breeding resource for a ground-dwelling species that needs shelter from predators, especially during semi-hibernation over winter. However, in terms of farming systems management, there's another 'elephant in the room' that has implications for farm pest management, biodiversity health, ecosystem health and perhaps human health. As a precaution to human health, it is important that the impacts of pest toxins in cropping systems are studied.

Recommendations

- Significantly increased research is required to improve the basic lack of knowledge on the life histories of our native biota and their ability to persist in fragmented, multiple-use landscapes.
- Remnant native vegetation in the cereal cropping landscapes holds the greatest biodiversity value and is especially important for the avian communities.
- Revegetation using simple, monoculture plantations is beneficial to the sleepy lizard and bird communities inhabiting cereal cropping landscapes as it offers structural connectivity.
- Conservation physiology is an important tool to directly assess the health of animal populations in addition to behavioural and landscape genetic studies.
- Better understanding of the impacts of pesticides and other agro-chemicals on the health of humans and wildlife is important for pest management on farms.
- Sleepy lizard physiological health should be used as a sentinel in cropping systems to assess biodiversity, ecosystem and human health.

CHAPTER 1

Do woody perennial farming systems provide resources that enhance the persistence of native vertebrate fauna in Australian agricultural landscapes?

S. J. Collard¹, A. M. Fisher², F. P. Smith³, R. Brandle², D. Armstrong²

¹*Rural Solutions SA, Level 8, 101 Grenfell St, Adelaide South Australia 5001,* ²*Department of Environment and Natural Resources, GPO Box 1047, Adelaide South Australia 5001,* ³*CSIRO Ecosystem Science, Private Bag 5, Wembley Western Australia 6931*

Abstract

Native biodiversity in Australian agricultural landscapes continues to decline in response to a range of threatening processes. Woody perennial farming systems are being established across southern Australia, as land managers strive to achieve more sustainable and profitable farming practices. Woody perennial farming systems can offer food, shelter and breeding resources for native biota and thus have the potential to contribute to the persistence of a range of native species in agricultural landscapes. Although woody perennial farming systems will rarely provide all of the resources required by a species adapted to complex natural ecosystems, they may provide significant quantities of some limiting resources or supplementary resources at key points of an organism's life cycle.

Here we present a review of the resource requirements of all terrestrial vertebrate species found in the temperate agricultural landscapes of South Australia and Western Australia. We seek to answer the following questions:

- What resources do native vertebrate fauna require?
- What resources are provided by woody perennial farming systems?
- What resources are required by 'at risk' vertebrate fauna species?
- Can woody perennial farming systems provide the resources required by 'at risk' vertebrate fauna species?

We document the food, shelter and nesting requirements of native terrestrial vertebrate fauna, considered species by species, in the temperate agricultural landscapes of southern Australia, based upon literature review and expert opinion. By comparing the resources required by listed threatened species with the resources provided by commercial woody perennial farming systems, we assessed the extent to which these systems may contribute to the persistence of 'at risk' species.

Woody perennial farming systems offer to varying degrees the resources required by threatened vertebrate fauna. Low foliage and woody infrastructure are available in most woody perennial farming systems, as is some form of woody debris and leaf litter. Fodder shrubs in particular provide vegetative structure close to the ground. None of the woody perennial farming systems considered offer the tree hollows and crevices required by threatened mammals and birds.

Woody perennial farming systems could be modified to incorporate these key resources, enhancing their suitability for ‘at risk’ vertebrate species at the local scale and potentially improving their persistence within agricultural landscapes.

This review has highlighted the lack of basic knowledge on the life histories of our native biota and their ability to persist in fragmented multiple-use landscapes. Significant research is required to resolve this knowledge gap. Such work could significantly add to our currently inadequate knowledge of the role of woody perennial farming systems in biodiversity conservation.

Introduction

Native biodiversity in Australian agricultural landscapes continues to decline in response to a range of threatening processes (Hobbs & Saunders 1991; Lindenmeyer 2007; Szabo *et al.* 2010; Ford 2011). Historically native vegetation was cleared for arable agriculture, which directly destroyed both native flora and fauna. The removal of native vegetation reduced the total amount of suitable habitat and its accessibility for native fauna (McIntyre & Hobbs 1999). The resultant fragmentation has exposed the remaining patches to degradation from increased edge effects leading to microclimatic and vegetation changes and competition and predation from abundant species (Fischer & Lindenmeyer 2007), depending on the nature of the surrounding landscape.

Conservation biology has undergone a paradigm shift over the past 30 years. The habitat fragmentation model, derived from island biogeography theory, focussed on reserves for the conservation of native biota. Remnants of native vegetation were viewed as “islands” amidst a “sea” of inhospitable land use. As the importance of scale in conservation ecology became apparent, large-scale landscape linkages were promoted to address issues relating to connectivity (e.g. WildLands in the USA, Gondwanalink in WA, NatureLinks in SA; Wyborn 2011). This has led to a reappraisal of “off-park” areas and the recognition of the importance of the matrix between patches of native vegetation. Where once non-reserve areas were viewed as basically homogeneous and hostile for the bulk of native species, the matrix is now regarded as an important part of integrated landscape conservation (McIntyre & Barrett 1992). The type, extent and location of the constituent land uses within a landscape can have a marked influence on the habitat value of the vegetation and the persistence of native fauna within that landscape (Haslem & Bennett 2008). Furthermore, ‘novel ecosystems’ (*sensu* Hobbs *et al.* 2008), where previously disassociated species are brought together, as a result of human activities or deliberate introductions of species to new locations, have the potential to play an important role in contributing to conservation, particularly in production landscapes (Prober & Hobbs 2008).

Throughout southern Australia, where native vegetation was historically cleared for agriculture, farming systems based on perennial plants are increasingly being adopted to improve productivity and sustainability in the face of a variable and changing climate. Many of these systems are based on Australian native woody plant species (Bennell *et al.* 2008; Table 1). Substantial areas of previously cleared farming land are being replanted to native woody vegetation, typically in the form of monocultures or simple polycultures. More of these plantings are likely under emerging landscape restoration and carbon farming initiatives.

Basic ecological theory states that biota require a range of available resources to survive and reproduce (e.g. Andrewartha & Birch 1954). The ongoing existence of a species is termed its persistence, and this would normally be specified in a management goal that is constrained in time and space. Persistence captures not only the requirement for a species to remain extant within a given study area, but also that the processes which allow for the ongoing replacement of that species are operating, so that viable populations of the species continue to exist.

Woody perennial farming systems (WPFS) can offer food, shelter and breeding resources for native biota and thus have the potential to contribute to the persistence of a range of native species in agricultural landscapes. Whilst it is recognised that WPFS would rarely provide all of the resources required by a species adapted to complex natural ecosystems, they may provide significant quantities of some limiting resources or supplementary resources at key points of an organism's life cycle. This can be represented as shown in Figure 1. Species can be categorised as being 'safe' (presently have viable populations and are considered safe from local extinction), 'at risk' (presently vulnerable to local extinction but could be made safe by new resources provided by WPFS), or 'departing' (species that will go locally extinct regardless of intervention). Some species that are presently locally extinct may be 'returned' as a result of new resources provided by WPFS. The goal of using WPFS from a biodiversity perspective is to see species moved from being 'at risk' to 'safe' within agricultural production landscapes.

Table 1. Woody perennial species that are currently used or have the greatest potential across southern Australia (Source: Bennell *et al.* 2008)

Species grouped by commercial purpose	Common Name	Height (m)
Pulp, Fibre/Particleboards		
<i>Eucalyptus viminalis</i> ssp. <i>cygnetensis</i>	Rough-barked Manna Gum	>2
<i>Eucalyptus globulus</i> ssp. <i>bicostata</i>	Victorian Blue Gum	>2
<i>Eucalyptus ovate</i>	Swamp Gum	>2
<i>Eucalyptus bridgesiana</i>	Apple Box	>2
<i>Eucalyptus porosa</i>	Mallee Box	>2
<i>Codonocarpus cotinifolius</i>	Native Poplar	>2
<i>Eucalyptus goniocalyx</i>	Long-leafed Box	>2
<i>Eucalyptus botryoides</i>	Southern Mahogany	>2
Fibre/Particleboards, Pulp, Fodder		
<i>Acacia retinodes</i>	Wirilda	>2
<i>Acacia salicina</i>	Willow Wattle	>2
<i>Acacia saligna</i>	Golden Wreath Wattle	>2
Bioenergy		
<i>Eucalyptus cladocalyx</i>	Sugar Gum	>2
<i>Acacia retinodes</i>		
<i>Eucalyptus leucoxydon</i>	South Australian Blue Gum	>2
<i>Eucalyptus chloroclada</i>	Dirty Gum	>2
<i>Eucalyptus globulus</i> ssp. <i>bicostata</i>		
<i>Eucalyptus viminalis</i> ssp. <i>cygnetensis</i>		
<i>Eucalyptus camaldulensis</i>	River Red Gum	>2
Oil/Bioenergy		
<i>Eucalyptus porosa</i>		
<i>Eucalyptus incrassata</i>	Ridge-fruited mallee	>2
<i>Eucalyptus aromaphloia</i> ssp. <i>sabulosa</i>	Scentbark	>2
<i>Eucalyptus dives</i>	Broad-leafed Peppermint	>2
<i>Eucalyptus polybractea</i>	Blue-leafed Mallee	>2
<i>Eucalyptus loxophleba</i>	York Gum	>2
Fodder Only		
<i>Atriplex nummularia</i>	Old man Saltbush	<2
<i>Eremophila longifolia</i>	Weeping Emubush	1-6m
<i>Chenopodium nitrariaceum</i>	Nitre Goosefoot	<2
<i>Indigofera australis</i>	Austral Indigo	<2
<i>Atriplex vesicaria</i>	Bladder Saltbush	<2
<i>Maireana pyramidata</i>	Black Bluebush	<2
<i>Rhagodia spinescens</i>	Spiny Saltbush	<2
Other		
<i>Melaleuca uncinata</i>	Broombush	2-4m
<i>Santalum album/lanceolatum/spicatum</i>	Sandalwood	>2

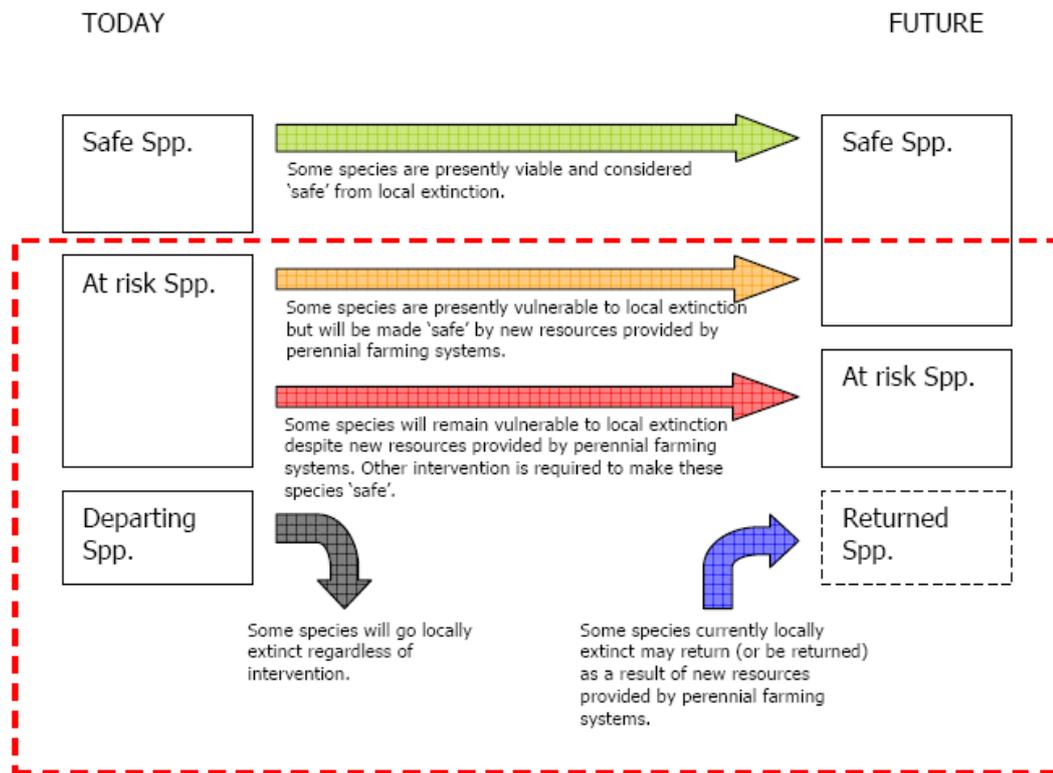


Figure 1. Woody perennial farming systems cannot 'save' or return all biodiversity in agricultural landscapes, but they can potentially make a substantial contribution. The orange arrow represents the potential for new perennial-based farming systems to convert some species at risk today into relatively safe species in the future.

It is acknowledged that the use of WPFS is only one approach to enhancing the persistence of native biota within the agricultural landscapes of southern Australia. Other approaches (e.g. large-scale revegetation with a mix of native species) have proved challenging because of the major shift in land use required and the associated socio-economic consequences as well as difficulties with establishment in ex-agricultural lands (Ward *et al.* 2005). In contrast, WPFS require modification to existing practices which are not so extreme as to be initially prohibitive to land managers, and offer tangible production benefits.

A number of studies have recorded the use by native biota of planted woody perennial vegetation. Plantation forestry provides the most obvious examples (Lindenmeyer & Hobbs 2004). Commercial and agro-forestry plantings using native (but non-indigenous species) have been shown to be used by a range of vertebrate species, in particular birds (Kavanagh *et al.* 2007; Loyn *et al.* 2007). Munro *et al.* (2007) review the use of revegetation sites by fauna. The increased number of bird species using saltbush fodder shrub plantings compared with conventional pasture has been documented in both NSW and SA (Seddon *et al.* 2009; Collard *et al.* 2011). However few studies have gone beyond recording which species occur in planted systems and their relative abundance compared with remnant and conventional farming systems. Collard & Fisher (2010) outline the types of resources that fodder shrub plantings can provide for fauna. Within the farming landscape of the WA wheatbelt, mallees planted as belts integrated with agriculture have been shown to provide a major food source for the Western Pygmy Possum (Short *et al.* 2009). Bird activity documented by Smith (2009a) in

mallee plantings in WA showed that native bird species utilised a range of food resources including blossoms, fruits, seeds and invertebrates. Smith (2009b) found that mallee plantations lacked the structural and floristic diversity of natural woodlands, which may explain the lower diversity of native vertebrates found in these systems. Whilst sound information exists on the types of fauna using planted woody vegetation, these studies highlight the need for a more comprehensive analysis of the resource requirements of native fauna. This will enable land managers to move beyond general statements about the biodiversity benefits of woody perennial farming systems to specific management advice that can be used to inform regional biodiversity conservation.

Here we review the resources required by terrestrial vertebrate fauna in the temperate agricultural landscapes of South Australia and Western Australia. We seek to answer the following questions:

- What resources do native vertebrate fauna require?
- What resources are provided by woody perennial farming systems?
- What resources are required by ‘at risk’ vertebrate fauna species?
- Can woody perennial farming systems provide the resources required by ‘at risk’ vertebrate fauna species?

This review has of necessity been restricted to terrestrial vertebrate fauna. Whilst invertebrates are recognised as being a pivotal component of biodiversity both in terms of abundance and function (Wilson 1987), little information is available on their distribution and resource requirements. In addition, the extensive taxonomic difficulties that exist contribute to the lack of useful data on invertebrates. By contrast, there is extensive and robust information on vertebrate fauna distributions available within state government databases and extensive literature and expert knowledge on vertebrate taxonomy and behaviour. As the focus of this work was on agricultural landscapes, obligate marine and aquatic species were excluded. This resulted in a review comprising an analysis of the resource requirements of all terrestrial species of Aves, Mammalia and Reptilia found within the wheat-sheep belt of Western Australia and South Australia.

Woody perennial farming systems were based on the enterprises within the remit of the Future Farm Industries Cooperative Research Centre (CRC). These included biomass plantings for fibre and pulp products, bioenergy plantings, fodder shrubs and plantings for other assorted plant products, as summarised in Table 1.

It is acknowledged that WPFS may improve the persistence of native vertebrates through means other than resource provision. For example, the use of such systems in revegetation is often aimed at managing altered hydrology where this threatens important habitats. For example, woody revegetation is often used as part of a package of technologies to mitigate the impacts of secondary salinisation. Such uses of WPFS may, in fact, ultimately prove to be the most important contribution by these systems to improving the persistence of native biota, however, they are not considered here.

Methodology

The study area was defined as the wheat and sheep belts of Western Australia and South Australia (Figure 2). These regions have been the focus of recent investigations on biodiversity values of planted woody perennial vegetation (Smith 2008; Smith 2009; Collard *et al.* 2011). Both regions have similar annual rainfall (250-450 mm) and land management systems and the degree of fragmentation and faunal assemblages are similar to those in agricultural landscapes across southern Australia. Significant planting of woody crops has been undertaken and priority areas for future plantings have been identified (Hobbs 2009) across these landscapes.

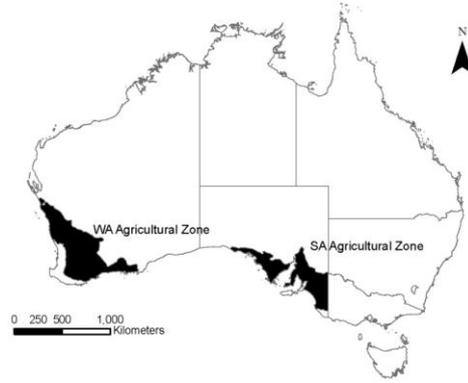


Figure 2. Map of the study area showing the agricultural zones of South Australia and Western Australia

An overview showing the logic of the methodology used in this study is presented in Figure 3.

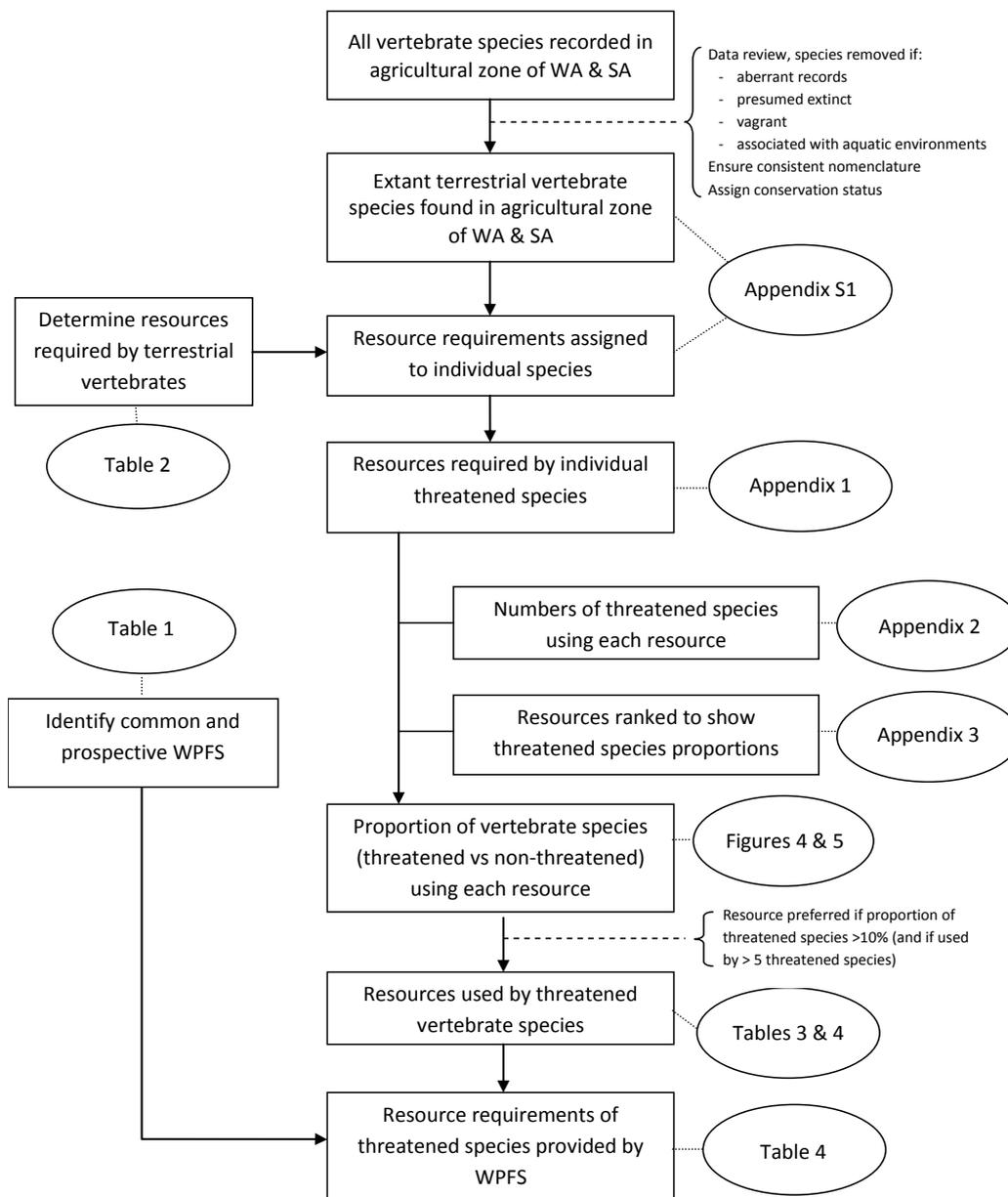


Figure 3. Overview of the methodology with links to relevant Figures, Tables and Appendices.

Identifying and sorting vertebrate species

To determine which vertebrate species to include in the analysis, we used a GIS to query records from the biological databases held by the Western Australian and South Australian State Government Departments, clipped to a layer of the agricultural zone of these states. The species lists were reviewed and aberrant/unlikely records were omitted. These included species that are presumed extinct from the area, and records of vagrants whose known distributions and habitats fall well outside the study area. Species associated with aquatic environments (including amphibians, waterbirds) were excluded from the analyses as woody vegetation is seldom planted in permanent or ephemeral aquatic environments. Burrowing frogs of the genus *Neobatrachus* (seven species) were included in the analyses because of their occurrence in terrestrial environments. Given this small number of frogs compared with the 207 reptile species included in the analysis, these amphibian species are grouped within the broad “reptile” classification for ease of analysis and data presentation. Nomenclature was adjusted to conform with the latest taxonomic classifications for birds (Christidis & Boles 2008), mammals (Van Dyke & Strahan 2008) and reptiles (Cronin 2001; Wilson & Swan 2003). Conservation status was defined according to the South Australian *National Parks and Wildlife Act 1972* (Schedule update 2000) and the Western Australian *Wildlife Conservation Act 1950* (updated 2010).

Defining resource needs of vertebrate species

A list was derived of the specific food, shelter, nesting and other resources that may be obtained by vertebrates from the most commonly planted perennial farming systems across southern Australia (Table 2). The resources represent a summary of a list compiled for vertebrate fauna in WA and SA as part of earlier research undertaken for the Future Farm Industries CRC. Where possible, resources were selected from existing habitat classification systems and vegetation condition metrics (e.g. Smith 2009; Catling & Burt 1995; Croft *et al.* 2005), ensuring that the metrics were relevant to planted and native woody systems and consistent across reptilian, mammalian and avian taxonomic groups.

It is recognised that other resources may be used by native biota, for example, rubbing posts to remove parasites, gizzard stones and solar radiation, however these were excluded from the formal analyses because they were largely captured by other resource requirements.

Resource use decisions

Assessments of resource requirements of mammals, reptiles and birds were made by separate reviewers (technical experts with respect to these taxa). Decisions regarding the use of different resources by individual species were made using a combination of documented behaviour in the literature and expert opinion. Species known to use each resource were scored using a binary system where 1 = yes (the species has been documented or observed regularly using the resource or in the absence of documented evidence could be reasonably expected to use the resource, based on expert opinion) and 0 = no (the species has not been recorded or observed using the resource). Where unusual behaviour was documented or observed for a species (e.g. a specialist insectivore feeding on plant material), a score of 0 was assigned. Where there was no documented information for a certain species, decisions were based on the behaviour of similar species and/or field-based knowledge of the species by the expert reviewer. Where there was a complete absence of documented evidence or expert knowledge of the species' behaviour, it was assumed not to use the resource (i.e. assigned a score of 0), a conservative approach which ultimately ensured that the species would not be incorrectly predicted to use resources provided by woody plantings.

There was some ambiguity about the use by fauna of some specific resources. For example, species that are known to require a daily source of water (e.g. parrots, pigeons) were considered to use “open water” as a food resource. Species that have been observed drinking from open water but do not

require this resource for their survival scored a 0 for their use of this resource. A weighted system of scoring was considered (i.e. to capture the greater dependence of some species on particular resources) but decision-making at this level was limited by knowledge of the specific resource requirements and/or preferences for the majority of vertebrate species. This deficiency in basic natural history data highlights the need for more detailed knowledge of vertebrate fauna across the agricultural landscapes of southern Australia.

Decisions regarding the provision of resources by different planted woody systems were made based on the nature of the planting at maturity. For example, foliage in the 0-1 m and 1-2 m height ranges would be present in recently established eucalypt plantings but would not be present at maturity. In eucalypt systems, it was assumed that trees would be harvested before hollows or crevices could form. The likely presence of resources not provided directly by different plantation types (e.g. invertebrates, seeds) was informed by previous ecological surveys in these or similar systems (e.g. Hobbs *et al.* 2003; Cunningham *et al.* 2005; Norman *et al.* 2008; Collard *et al.* 2009).

In fodder shrub systems (e.g. *Atriplex nummularia* and *Eremophila longifolia*) or in other systems used for grazing, plants were assumed to be grazed at intermediate levels of intensity, maintaining the woody infrastructure and foliage of a mature plant throughout the year. For plantings that are periodically harvested as part of on-going management and production practices (e.g. coppicing of broombush or mallees) the form and height of the foliage and woody infrastructure were assumed to be typical of the plants at maturity.

Data analyses

Resource use data for the different taxa were compiled from expert reviewers and summarised in MS Excel spreadsheets using the binary scoring system described above.

Initially, multivariate analysis routines in PRIMER (version 6) (Clarke & Gorley 2006) were used in an attempt to identify groups of species with similar resource requirements (i.e. guilds). The results of these analyses are not presented here because species groupings were too weak to interpret with any confidence. Instead, summary tables and charts were prepared from the raw resource use data.

To address the question regarding persistence in the landscape, we assigned each species to “threatened” or “non-threatened” groups. Threatened species correspond to “at risk” species in Figure 1. Species were considered to be threatened if they were listed under the *Environment Protection and Biodiversity Conservation Act 1999*, the *South Australian National Parks and Wildlife Act 1972* (Schedule updated 2000) or the *Western Australian Wildlife Conservation Act 1950* (updated 2010). The importance of resources to threatened species was determined by ranking each resource according to the proportion of threatened species using that resource. Resources were deemed to be of greater importance to threatened (at risk) species if the proportion of threatened species using the resource exceeded 10%. Confidence in the results was considered to be low if the resource was used by fewer than five threatened species.

The most commonly used and prospective woody perennial plantings (adapted from Bennell *et al.* 2008) were evaluated for their potential to provide the resources listed in Table 3. Plant species were grouped according to commercial purpose, with very little variation between resources provided within groups. Only those resources provided directly to vertebrates by the plants were considered in this analysis. For example, where a particular plant species provides good habitat to invertebrates that in turn provide a useful food resource to vertebrates, these benefits were not scored.

Table 2. List of resources (and codes used for analyses) used to assess the food, shelter and nesting requirements of vertebrate fauna

Food	Shelter	Nesting
Invertebrates		Natural substrate (nat)
Aquatic (fiag)	Free Water (sfwa)	In soil (natural cracks, depressions, embankments etc) (nnis)
Terrestrial (fite)	Open Ground (sogr)	Open ground (incl stones?) (nnog)
Arboreal (fiar)	Foliage 0-1m (sf01)	Open water (nnow)
Aerial (fiaa)	Foliage 1-2m (sf12)	Foliage 0-1 m (nnf1)
Vertebrates	Foliage 2+m (sf2+)	Foliage 1-2 m (nnf2)
Small (<250g) (fvsm)	Woody Infrastructure 0-1m (sw01)	Foliage 2+ m (nnf+)
Medium (>250g) (fvme)	Woody Infrastructure 1-2m (sw12)	Woody Infrastructure 0-1m (nnw1)
Carion (fcar)	Woody Infrastructure 2+m (sw2+)	Woody Infrastructure 1-2m (nnw2)
Plants	Hollows & crevices 0-1m (sh01)	Woody Infrastructure 2+m (nnw+)
Fruits (fpfr)	Hollows & crevices 1-2m (sh12)	Tree hollows & crevices 0-1m (nnh1)
Seeds (fpse)	Hollows & crevices 2+m (sh2+)	Tree hollows & crevices 1-2m (nnh2)
Foliage/ vegetation (fpfo)	Woody debris and/or litter (swdl)	Tree hollows & crevices 2+m (nnh+)
Flowers & buds (fpfl)	Rocks/ boulders (srob)	Other bird's nests (nnbn)
Nectar (fpne)	Burrows (sbur)	Amongst/ under rocks (nnur)
Pollen (fppo)	Cracks (scra)	Rock ledges and crevices (includes built infrastructure) (nnlc)
Roots (fpro)		Woody debris and/or litter (nnwd)
Bark (fpba)		Nesting construction material (con)
Sap (fpsa)		In soil (burrows) - include termite mounds (ncis)
Fungi		Open ground (scrape) (ncog)
Subterranean (ffst)		Sticks (>5mm diameter) (ncsl)
Terrestrial (ffte)		Twigs/Stems (<5mm diameter) (ncss)
Arboreal (ffar)		Feathers/down (ncfd)
Free water (ffwa)		Wool/fur/hair (ncwf)
		Leaves (ncle)
		Grass/Reeds (ncgr)
		Moss/Lichen (ncml)
		Bark (ncbk)
		Rocks/Stones/Pebbles (ncrs)
		Mud (ncmu)
		Wood shavings/chips/dust (ncws)
		Spider webs (ncsw)
		Rootlets (ncro)

Foliage includes leaves, phylloides and new plant growth. Woody infrastructure includes the woody components of shrubs and trees e.g. stems and trunks. 0-1m = resource available between ground level and 1 metre, 1-2m = resource available between 1 and 2 metres, 2+m = resources available at heights above 2 metres

Results

A total of 476 extant vertebrate species were identified as being likely to occur within the terrestrial environments of the agricultural landscapes of southern and western Australia (Appendix S1), comprising 214 reptile species, 56 mammal species and 206 bird species. Of these, 26 reptiles, 12 mammals and 56 birds were threatened (Figure 4).

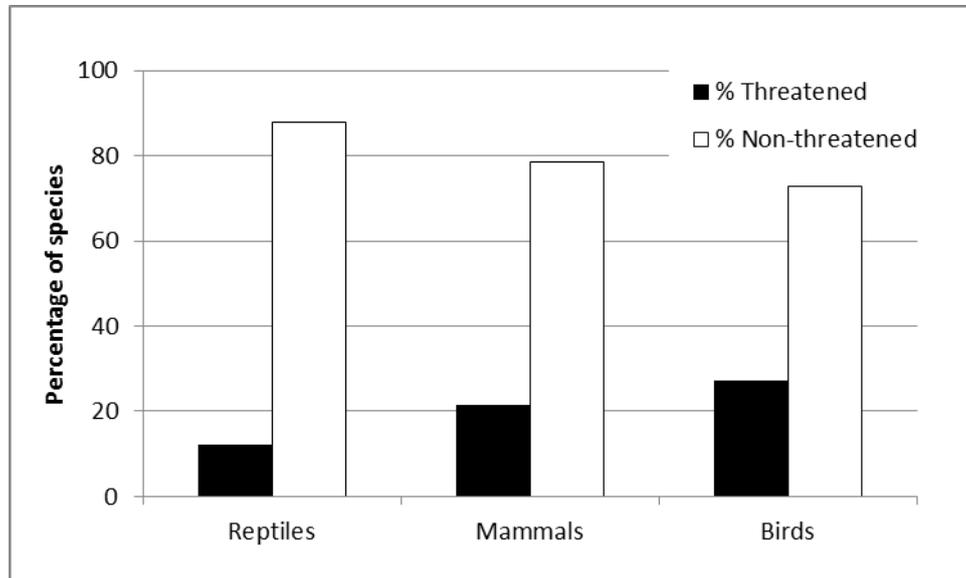


Figure 4. Proportion of threatened and non-threatened reptile, mammal and bird species considered in the analyses.

The most commonly used food resources across all taxa were terrestrial invertebrates, arboreal invertebrates and seeds. The most commonly used shelter resources were woody infrastructure less than 1 m in height, foliage less than 1 m in height and woody infrastructure greater than 2 m in height. Woody debris, hollows greater than 2 m in height and foliage greater than 1 m in height were the most commonly used nesting substrates and the most commonly used nesting materials were grass, soil, and bark. A summary table showing the number of reptile, mammal and bird species using each resource is provided at Appendix 1.

Detail on the resource requirements for individual threatened species is provided at Appendix 2.

Certain resources were found to be used by a higher proportion of threatened species. Figure 5 shows ranked proportions for threatened and non-threatened reptile, mammal and bird species using the different foraging, shelter and nesting resources. Rank proportions across all resources showed that threatened reptiles were most strongly associated with foraging and shelter resources, mammals with foraging and nesting resources and bird species with nesting resources (Appendix 3).

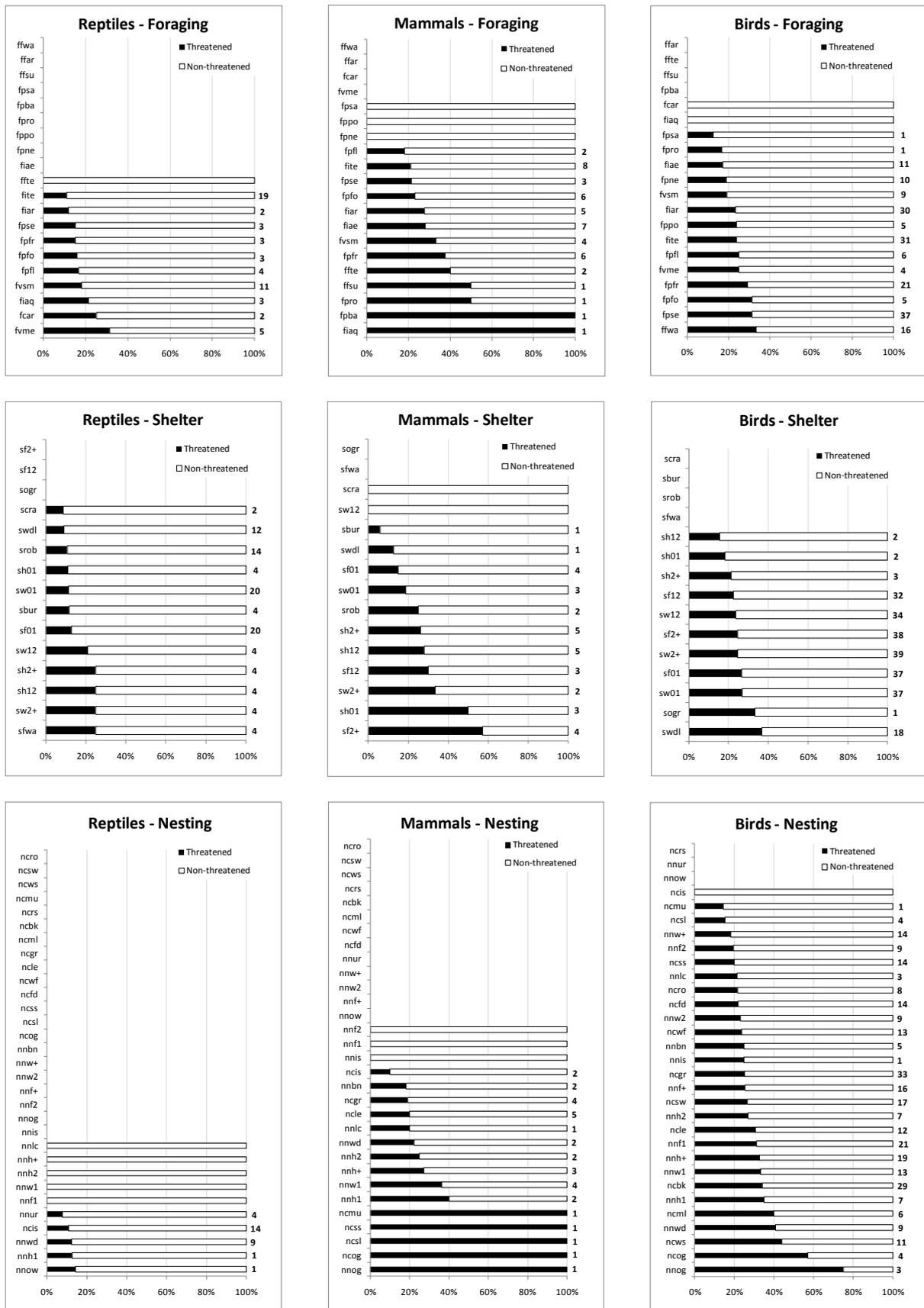


Figure 5. Proportion of threatened and non-threatened reptile, mammal and bird species using each foraging, shelter and nesting resource. Values to the right of each chart indicate the number of species that contributed to the percentage value for threatened species. Resources used by fewer than five threatened species were not considered in subsequent analyses. Resource codes are provided in Table 2.

Table 3 summarises the resources that were used by a high (>10%) proportion of threatened species. In addition to free water, threatened birds in the study area required a range of plant products and invertebrates for food. Shelter at all levels was used by threatened birds along with a suite of nesting materials. Threatened reptiles fed on small vertebrates and terrestrial invertebrates, required shelter and used nesting materials in, on or near the ground. Threatened mammals preferentially used fruits and foliage for food, and required hollows for shelter.

Table 3. Food, shelter and nesting resources used by threatened (“at risk”) reptile, mammal and bird fauna in the temperate agricultural zones of South Australia and Western Australia. Species were considered threatened if listed under relevant Commonwealth or State-level legislation in South Australia and/or Western Australia.

Food	Shelter	Nesting*
<i>Threatened reptile species</i>		
Small vertebrates	Foliage 0-1m	Woody debris and/or litter (nat)
Terrestrial invertebrates	Woody infrastructure 0-1m Rocks and boulders	In soil (including termitaria) (con)
<i>Threatened mammal species</i>		
Fruits	Hollows and crevices 1-2m	-
Aerial invertebrates	Hollows and crevices 2+m	
Foliage/vegetation		
Terrestrial invertebrates		
<i>Threatened bird species</i>		
Free water	Woody debris/litter	Woody debris and/or litter (nat)
Seeds	Woody infrastructure 0-1m	Moss/lichen (con)
Fruits	Foliage 0-1m	Tree hollows and crevices 0-1m (nat)
Flowers/buds	Woody infrastructure 2+m	Bark (con)
Terrestrial invertebrates	Foliage 2+m	Woody infrastructure 0-1m (nat)
Arboreal invertebrates	Woody infrastructure 1-2m	Tree hollows and crevices 2+m (nat)
Small vertebrates	Foliage 1-2m	Foliage 0-1m (nat)
Nectar		Leaves (con)
Aerial invertebrates		Tree hollows and crevices 1-2m (nat)
		Spider webs (con)
		Foliage 2+m (nat)
		Grass/ reeds (con)
		Other birds' nests (nat)
		Wool/fur/hair (con)
		Woody infrastructure 1-2m (nat)
		Feathers/ down (con)
		Rootlets (con)
		Twigs/stems (<5mm) (con)
		Foliage 1-2m (nat)
		Woody infrastructure 2+m (nat)

*nat = natural substrate, con = construction material

Table 4. Identified resource requirements of threatened reptiles, mammals and birds and resources provided by the different woody perennial farming systems, grouped by commercial purpose. Constituent plant species in each system are listed in Table 1.

Resource		Pulp, Fibre/ Particleboards	Fibre/Particleboards, Pulp, Fodder	Bioenergy	Oil/ Bioenergy	Fodder Only	Other	
Reptiles								
Shelter	Foliage 0-1m					+	+	
	Woody infrastructure 0-1m	+	+	+	+	+	+	
Nesting	Woody debris and/or litter	+	+	+	+	+	+	
	In soil (including termitaria)	+	+	+	+	+	+	
Mammals								
Foraging	Fruits	+	+	+	+	+	+	
	Foliage/vegetation	+	+	+	+	+	+	
Shelter	Hollows and crevices 1-2m							
	Hollows and crevices 2+m							
Birds								
Foraging	Seeds	+	+	+	+	+	+	
	Fruits	+	+	+	+	+	?	
	Flowers/buds	+	+	+	+	+	+	
	Nectar	+	+	+	+	+	+	
Shelter	Woody debris/litter	+	+	+	+	+	+	
	Woody infrastructure 0-1m	+	+	+	+	+	+	
	Foliage 0-1m					+	+	
	Woody infrastructure 2+m	+	+	+	+	?	?	
	Foliage 2+m	+	+	+	+	?	?	
	Woody infrastructure 1-2m	+	+	+	+	+	+	
	Foliage 1-2m					+	+	
	Nesting	Woody debris and/or litter (nat)	+	+	+	+	+	+
		Tree hollows and crevices 0-1m (nat)						
		Bark (con)	+	+	+	+	?	+
Woody infrastructure 0-1m (nat)		+	+	+	+	+	+	
Tree hollows and crevices 2+m (nat)								
Foliage 0-1m (nat)						+	+	
Leaves (con)		+	+	+	+	+	+	
Tree hollows and crevices 1-2m (nat)								
Foliage 2+m (nat)		+	+	+	+	?	?	
Woody infrastructure 1-2m (nat)		+	+	+	+	+	+	
Nesting	Rootlets (con)	+	+	+	+	+	+	
	Twigs/stems (<5mm) (con)	+	+	+	+	+	+	
	Foliage 1-2m (nat)					+	+	
	Woody infrastructure 2+m (nat)	+	+	+	+	?	?	

? indicates uncertainty due to variation among species within the same WPFS group

A range of different planted perennial vegetation systems provide resources that were identified as being important for threatened vertebrate fauna (Table 4). With the exception of low foliage (at 0-1 m in height), the shelter and nesting requirements of threatened reptiles were broadly met by each of the planted perennial vegetation systems. The foraging requirements of threatened mammals were met by all woody systems; however shelter requirements (in the form of hollows and crevices) were unlikely to be provided by any of these systems. Threatened bird species used a greater range of foraging, shelter and nesting resources than both mammals and reptiles. The majority of these resource requirements were met by the different planted systems. However, only “Fodder and “Other” systems provided foliage below two metres for shelter and/or nesting. None of the systems provided tree hollows and crevices required by a high proportion of threatened bird species for nesting.

Discussion

This study provides a review of the resource requirements of extant terrestrial vertebrate fauna of the temperate agricultural zones of southern and western Australia. Although demonstrating the breadth of resources used by terrestrial vertebrate fauna, there are some general patterns which emerge that indicate how WPFS may contribute to their persistence, some of which may also be relevant to fragmented multiple-use agricultural landscapes elsewhere in Australia.

The analysis presented here highlights the importance of low vegetation, litter and woody debris, along with hollows and crevices as important resources for terrestrial vertebrate fauna in the agricultural landscapes of southern and western Australia. This is supported by a number of studies on birds (Ford 2011; Reid 2009), reptiles (Brown *et al.* 2008) and mammals in the agricultural landscapes of southern Australia.

WPFS offer to varying degrees the resources required by threatened vertebrate fauna. Low foliage and woody infrastructure are available in most WPFS, as is some form of woody debris and leaf litter. Fodder shrubs in particular provide vegetative structure close to the ground (Collard & Fisher 2010). None of the WPFS considered offer the combination of resources required by threatened mammals and birds, in particular tree hollows and crevices. This is consistent with other studies of fauna in plantations in southern Australia which have shown that hollows and fallen wood resources are not provided by plantations (Vesk *et al.* 2008; Kavanagh *et al.* 2010).

Clearly the management of WPFS determines the extent to which resources are available for native fauna. If a site is completely and/or repeatedly harvested, critical resources for vertebrate fauna such as tree hollows will never be available. Similarly fodder systems are often heavily grazed, resulting in the structure of the vegetation being significantly altered. Whilst the production impetus for establishing WPFS is recognised, triple bottom line accounting provides the opportunity to implement more sustainable management options. This could take the form of deliberate provision of key resources at a given site for fauna species of local significance (e.g. retained trees, nest boxes, logs, boulders) or staged harvesting/grazing at the site or property level. This is consistent with established commercial forestry practices (Dickerson 2008) and leading grazing enterprises (Dorrrough *et al.* 2008). This also highlights the benefits of integrating the management of a site and property into a regional landscape planning approach to ensure that the actions in one location do not undermine those in another, especially with regard to the conservation of threatened species.

The extent to which resources at a given site can be accessed by vertebrate fauna will be heavily influenced by a number of factors, including landscape context. A WPFS site adjacent to remnant native vegetation is likely to be of more use to native reptiles, mammals and birds than a similar site that is surrounded by cleared cropping and grazing land. Diversification of land uses at the property and regional scales, which may be driven by a production imperative, allows for a ‘softening’ of the

matrix between native vegetation remnants when the landscape is considered from an animal's perspective (Watling *et al.* 2011). Resources may also be used opportunistically by different species, particularly by highly mobile vertebrate fauna such as birds. This may take the form of occasional visits to planted systems and/or temporary seasonal residence when resources are available. The extent to which planted systems are used is likely to be highly dependent on their proximity and level of connectedness to other suitable habitats.

This review has summarised information based on the premise that resources are a key determinant of the persistence of a species. It is however recognised that factors other than resource limitation (e.g. habitat modification resulting from salinisation, competition and predation from invasive and generalist native species) may be the reason for a given species being considered as threatened. This review has concentrated on eliciting major trends from an extensive dataset, but it is acknowledged that the behavioural idiosyncrasies and preferences of different species, including co-dependencies may become apparent if the data are investigated further. Species are also able to adapt their behaviour to changing environmental conditions. Such phenotypic plasticity has not been explicitly considered in this review (outside documented behaviours for each species), but may become important as the impacts of climate variability become more pronounced (Steffen *et al.* 2009).

The literature relating to resource requirements of birds is considerably more extensive than the documentation available for mammals and reptiles. Whilst there are various guild/ functional group classifications for birds which relate to resources (e.g. Attwood *et al.* 2009; Recher & Holmes 1985), similar representations of mammal and reptile species are not currently available. For birds, it has been consistently shown that species at risk or considered decliners in agricultural landscapes are those which forage on or near the ground (Ford 2011). WPFS offer some ground level and low-stratum resources which can supplement those on offer in remnant native vegetation for such species.

Being constrained by available information, this review has focussed on only vertebrate fauna with the resource requirements of individual species being systematically considered. WPFS are recognised to support terrestrial, arboreal and aerial invertebrates and these are important components of the biodiversity and overall ecosystem function at any given site (Cunningham *et al.* 2005, Norman *et al.* 2008; Collard *et al.* 2009), which warrant further investigation.

In order to address the issue of persistence, this review develops an understanding of the resources needed by 'at risk' species from an overall consideration of resource requirements by vertebrate fauna. This provides the background for more detailed work on selected species as part of a larger program of work on improving the persistence of the native biota of southern Australia.

Conclusions

Woody perennial farming systems are an increasingly common component of agricultural landscapes across southern Australia, as land managers strive to achieve more sustainable and profitable farming practices. We have demonstrated that these systems have the potential to provide foraging, shelter and nesting resources for a range of threatened and non-threatened vertebrate fauna. However, a number of key resources needed by threatened species are typically not provided by the majority of these planted systems, including foliage below two metres for shelter and hollows and crevices at different heights for both nesting and shelter. Plantings could be modified to incorporate these resources, enhancing their suitability for 'at risk' vertebrate species at the local scale and potentially improving their persistence across the landscape.

This review has further highlighted the lack of basic knowledge on the life histories of our native biota and their ability to persist in fragmented multiple-use landscapes. Significant research is

required to resolve this knowledge gap, including collecting, collating and analysing of data on the specific resource use preferences and behaviours of native fauna. Such work could significantly add to our current, clearly inadequate, knowledge of the role of woody perennial farming systems in biodiversity conservation.

References

- Andrewartha, H. G. and Birch, L. C. (1954). *The distribution and abundance of animals*. University of Chicago Press, Chicago.
- Attwood, S. J., Park, S. E., Maron, M., Collard, S. J., Robinson, D., Reardon-Smith, K. M. and Cockfield, G. (2009). Declining birds in Australian agricultural landscapes may benefit from aspects of the European agri-environment model. *Biological Conservation* **142**, 1981-1991.
- Bennell M., Hobbs, T. J. and Ellis, M. (2008). *Evaluating agroforestry species and industries for lower rainfall regions of southeastern Australia: FloraSearch1a*. Report to the Joint Venture Agroforestry Program (JVAP) and the Future Farm Industries CRC. RIRDC, Canberra, ACT.
- Brown, G. W., Bennett, A. F. and Potts, J. M. (2008). Regional faunal decline – reptile occurrence in fragmented rural landscapes of south-eastern Australia. *Wildlife Research* **35**, 8-18.
- Catling, P.C. and Burt, R.J. (1995). Studies of the ground-dwelling mammals of eucalypt forests in south-eastern New South Wales: the effect of habitat variables on distribution and abundance. *Wildlife Research* **22**, 271-288.
- Christidis, L. and Boles, W.E. (2008). *Systematics and Taxonomy of Australian Birds*. CSIRO Publishing, Collingwood, Vic.
- Clarke K. R. and Gorley R. N. (2006). *PRIMER v6: User Manual/Tutorial*. PRIMER-E, Plymouth, UK.
- Collard, S.J., Fisher, A.M. and McKenna, D.J. (2009). *Biodiversity value of saltbush (Atriplex nummularia) plantings in mixed farming landscapes of the Southern Mallee, South Australia*. DWLBC Technical Report 2009/27, Government of South Australia, through Department of Water, Land and Biodiversity Conservation, Adelaide, SA.
- Collard, S.J., Fisher, A.M. and McKenna, D.J. (2011). Planted saltbush (*Atriplex nummularia*) and its value for birds in farming landscapes of the South Australian Murray Mallee. *Ecological Management and Restoration* **12**, 38-46.
- Collard, S.J. and Fisher, A.M. (2010). Shrub-based plantings of woody perennial vegetation in temperate Australian agricultural landscapes: What benefits for native biodiversity? *Ecological Management and Restoration* **11**, 31-35.
- Croft, S.J., Pedler, J.A. and Milne, T.I. (2005). *Bushland Condition Monitoring Manual: Southern Mount Lofty Ranges*. Nature Conservation Society of South Australia, Adelaide, SA.
- Cronin, L. (2001). *Australian reptiles and amphibians*. Envirobook, Annandale, NSW.
- Cunningham, S.A., Floyd, R.B. and Weir, T.A. (2005). Do *Eucalyptus* plantations host an insect community similar to remnant *Eucalyptus* forest? *Austral Ecology* **30**, 103-117.
- Dickenson, I. (2008). Balancing the three-legged stool: a case study of forest conversion and conservation. Pp. 45-54 in T. Lefroy, K. Bailey, G. Unwin & T. Norton (eds) *Biodiversity: Integrating Conservation and Production*. CSIRO Publishing, Collingwood, Vic.
- Dorrough, J., Stol, J. & McIntyre, S. (2008). *Biodiversity in the paddock: a land managers guide*. Future Farm Industries CRC.
- Ford, H. A. (2011). The causes of decline of birds in eucalypt woodlands: advances in our knowledge over the last 10 years. *Emu* **111**, 1-9.
- Fischer, J. and Lindenmayer, D. B. (2007). Landscape modification and habitat fragmentation: a synthesis. *Global Ecology and Biogeography* **16**, 265-280.

- Hobbs, R.J. and Saunders, D.A. (1991). Re-integrating fragmented landscapes –a preliminary framework for the western Australian wheatbelt. *Journal of Environmental Management* **33**,161-167.
- Hobbs, R., Catling, P.C., Wombey, J.C., Clayton, M., Atkins, L. and Reid, A. (2003). Faunal use of bluegum (*Eucalyptus globulus*) plantations in southwestern Australia. *Agroforestry Systems* **58**, 195–212.
- Hobbs, R. J., Arico, S., Aronson, J., Baron, J. S., Bridgewater, P., Cramer, V. A., Epstein, P. R., Ewel, J. J., Klink, C. A., Lugo, A. E., Norton, D., Ojima, D., Richardson, D. M., Sanderson, E. W., Valladares, F., Vilà, M., Zamora, R. and Zobel, M. (2008). Novel ecosystems: theoretical and management aspects of the new ecological world order. *Global Ecology and Biogeography* **15**, 1-7.
- Hobbs, T. (ed) (2009). *Regional Industry Potential for Woody Biomass Crops in lower rainfall southern Australia - FloraSearch 3c*. RIRDC Publication No. 09/045. Rural Industries Research and Development Corporation, Barton, ACT.
- Kavanagh, R. P., Stanton, M. A. and Herring, M. W. (2007). Eucalypt plantings on farms benefit woodland birds in south-eastern Australia. *Austral Ecology* **32**, 635-650.
- Lindenmayer, D. B. and Hobbs, R. J. (2004). Fauna conservation in Australian plantation forests – a review. *Biological Conservation* **119**, 151-168.
- Lindenmayer, D.B. (2007). *On borrowed time : Australia's environmental crisis and what we must do about it*. Penguin Books and CSIRO Publishing, Camberwell, Vic.
- Loyn, R. H., McNabb, E. G., Macak, P. and Noble, P. (2007). Eucalypt plantations as habitat for birds on previously cleared farmland in south-eastern Australia. *Biological Conservation* **137**, 533-548.
- McIntyre, S. and Barrett, G.W. (1992). Habitat variegation, an alternative to fragmentation. *Conservation Biology* **6**,146-147.
- McIntyre, S. and Hobbs, R.J. (1999). A framework for conceptualizing human effects on landscapes and its relevance to management and research models. *Conservation Biology* **13**,1282-1292.
- Munro, N.T., Lindenmayer, D.B. and Fischer, J. (2007) Fauna response to revegetation in agricultural areas of Australia: A review. *Ecological Management & Restoration* **8**, 199-207.
- Norman, H. C., Masters, D. G., Silberstein, R., Byrne, F., Nichols, P. , Young, J., Atkins, L. , Wilmot, M. G., Rintoul, A. J., Lambert, T., McClements, D., Raper, P., Ward, P., Walton, C. and York, T. (2008) *Sustainable Grazing on Saline Lands WA 1 research project – final report*. Available from URL: <http://www.landwaterwool.gov.au/products/pk071404>
- Prober, S. M. and Hobbs, R. J. (2008). Temperate eucalypt woodlands. Pp. 19 – 25 in *Ten Commitments: Reshaping the Lucky Country's Environment* edited by D. Lindenmayer, S. Dovers, M. H. Olson & S. Morton. CSIRO Publishing, Collingwood, Vic.
- Recher, H. F., Holmes, R. T., Schulz, M., Shields, J. and Kavanagh, R. (1985). Foraging patterns of breeding birds in eucalypt forest and woodland of south-eastern Australia. *Australian Journal of Ecology* **10**, 399-420.
- Reid, J.R.W. (1999). *Threatened and declining birds in the New South Wales Sheep-Wheat Belt: I. Diagnosis, characteristics and management*. Consultancy report to NSW National Parks and Wildlife Service. CSIRO Wildlife and Ecology, Canberra, ACT.
- Robinson, A.C., Caspersen, K.D. and Hutchinson, M.N. (2000). *A List of the Vertebrates of South Australia*. Department for Environment and Heritage, Adelaide, SA.
- Seddon, J., Doyle, S., Bourne, M., Maccallum, R. and Briggs, S. (2009) Biodiversity benefits of alley farming with old man saltbush in central western New South Wales. *Animal Production Science* **49**, 860-868.

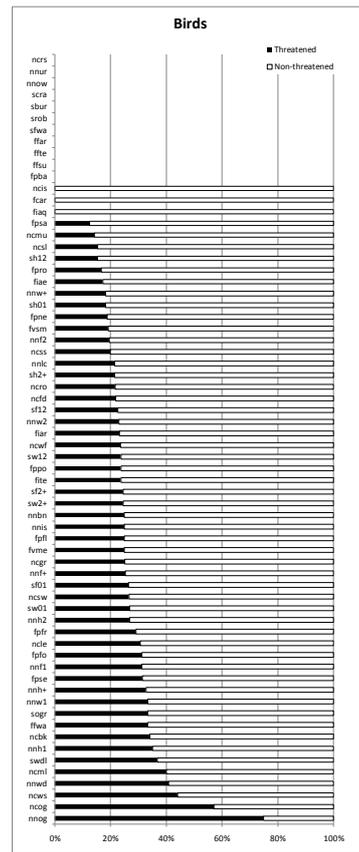
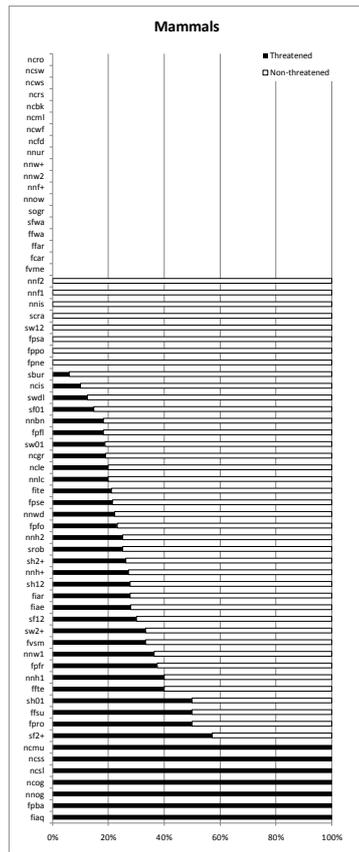
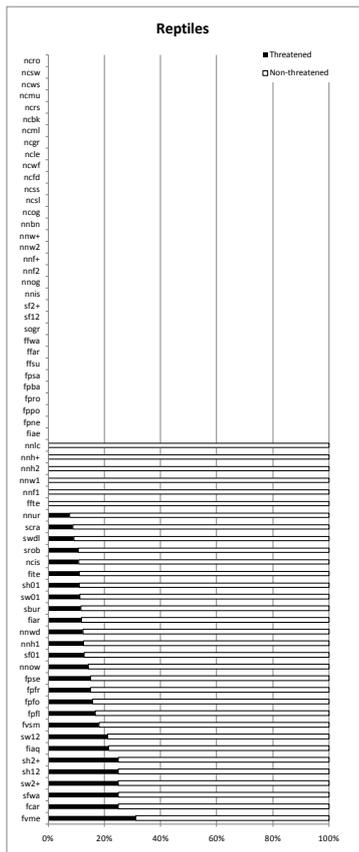
- Short, M., Smith, F. P. and van Etten, E. (2009). Oil mallees provide foraging habitat for the Western Pygmy Possum (*Cercartetus concinnus*) in the wheatbelt of Western Australia. *Ecological Management & Restoration* **10**, 233-236.
- Smith, F.P. (2009a). Bird activity in oil mallee plantings in the wheatbelt of Western Australia. *Ecological Management & Restoration* **10**, 236–238.
- Smith, F.P. (2009b). Assessing the habitat quality of oil mallees and other planted farmland vegetation with reference to natural woodland. *Ecological Management and Restoration* **10**, 217-227.
- Steffen, W., Burbidge, A.A., Hughes, L., Kitching, R., Lindenmayer, D., Musgrave, W., Stafford Smith, M. and Werner, P.A. (2009). *Australia's biodiversity and climate change: A strategic assessment of the vulnerability of Australia's biodiversity to climate change*. A report to the Natural Resources Management Ministerial Council commissioned by the Australian Government. CSIRO Publishing, Collingwood, Vic.
- Szabo, J. K., Vesk, P. A., Baxter, P. W. J. and Possingham, H. P. (2010). Paying the extinction debt: Woodland birds in the Mount Lofty Ranges, South Australia. *Emu* **111**, 59-70.
- Van Dyke, S and Strahan, R. 2008. *The Mammals of Australia*, Third Edition. Reed New Holland, Sydney.
- Vesk, P.A., Nolan, R., Thomson, J.R., Dorrough, J.W. and Mac Nally, R. (2008). Time lags in provision of habitat resources through revegetation. *Biological Conservation* **141**, 174-186.
- Ward, J., Bryan, B., Gale, G. and Hobbs, T.J. (2005). *Market-based instrument approaches to implementing priority revegetation in the South Australian Murray-Darling Basin*. CSIRO Land and Water, Adelaide, SA.
- Watling, J. I., Nowakowski, A. J., Donnelly, M. A. and Orrock, J. L. (2011). Meta-analysis reveals the importance of matrix composition for animals in fragmented habitat. *Global Ecology and Biogeography* **20**, 209-217.
- Wilson, E. O. (1987). The little things that run the world (the importance and conservation of invertebrates). *Conservation Biology* **1**, 344-346.
- Wilson, S. and Swan, G. (2003). *A complete guide to the reptiles of Australia*. New Holland Publishers, Chatswood, NSW.
- Wyborn, C. (2011). Landscape scale ecological connectivity: Australian survey and rehearsals. *Pacific Conservation Biology* **17**, 121-131.

Appendix 1. Total number of non-threatened (NT) and threatened (T) reptile, mammal and bird species using each resource. Full descriptions for resource codes are provided in Table 2.

Resource	Reptiles		Mammals		Birds		All species		
	NT	T	NT	T	NT	T	NT	T	Total
fiaq	14	3	1	1	8		23	4	27
fite	173	19	38	8	130	31	341	58	399
fiar	17	2	18	5	129	30	164	37	201
fae			25	7	64	11	89	18	107
fvsm	61	11	12	4	47	9	120	24	144
fvme	16	5			16	4	32	9	41
fcar	8	2			14		22	2	24
fpfr	20	3	16	6	72	21	108	30	138
fpse	20	3	14	3	118	37	152	43	195
fpfo	19	3	26	6	16	5	61	14	75
fpfl	24	4	11	2	24	6	59	12	71
fpne			6		53	10	59	10	69
fppo			3		21	5	24	5	29
fpro			2	1	6	1	8	2	10
fpba			1	1			1	1	2
fpsa			2		8	1	10	1	11
ffsu			2	1			2	1	3
ffte	5		5	2			10	2	12
ffar									
ffwa					48	16	48	16	64
sfwa	16	4					16	4	20
sogr					3	1	3	1	4
sf01	156	20	27	4	140	37	323	61	384
sf12			10	3	142	32	152	35	187
sf2+			7	4	155	38	162	42	204
sw01	177	20	16	3	138	37	331	60	391
sw12	19	4	4		143	34	166	38	204
sw2+	16	4	6	2	159	39	181	45	226
sh01	145	16	6	3	11	2	162	21	183
sh12	16	4	18	5	13	2	47	11	58
sh2+	16	4	19	5	14	3	49	12	61
swdl	132	12	8	1	49	18	189	31	220
srob	131	14	8	2			139	16	155
sbur	78	9	17	1			95	10	105
scra	23	2	2				25	2	27
nnis			4		4	1	8	1	9
nnog			1	1	4	3	5	4	9
nnow	7	1					7	1	8
nnf1	1		3		67	21	71	21	92
nnf2			1		46	9	47	9	56
nnf+					63	16	63	16	79
nnw1	2		11	4	39	13	52	17	69
nnw2					39	9	39	9	48
nnw+					77	14	77	14	91

nnh1	8	1	5	2	20	7	33	10	43
nnh2	6		8	2	26	7	40	9	49
nnh+	6		11	3	58	19	75	22	97
nnur	52	4					52	4	56
nnbn			11	2	20	5	31	7	38
nnlc	1		5	1	14	3	20	4	24
nnwd	73	9	9	2	22	9	104	20	124
<hr/>									
ncis	129	14	20	2	6		155	16	171
ncog			1	1	7	4	8	5	13
ncsl			1	1	26	4	27	5	32
ncss			1	1	70	14	71	15	86
ncfd					64	14	64	14	78
ncwf					55	13	55	13	68
ncle			25	5	39	12	64	17	81
ncgr			21	4	131	33	152	37	189
ncml					15	6	15	6	21
ncbk					85	29	85	29	114
ncrs									
ncmu			1	1	7	1	8	2	10
ncws					25	11	25	11	36
ncsw					64	17	64	17	81
ncro					37	8	37	8	45
<hr/>									
Total	33	27	47	39	55	52	64	64	64

Appendix 3. Rank proportions for threatened and non-threatened reptile, mammal and bird species across all resources.



CHAPTER 2

The potential of planted saltbush (*Atriplex nummularia*) to support avian biodiversity in the Murray Mallee of South Australia.

*Tim Richards*¹, *S. J. Collard*², *A. M. Fisher*³, *David Paton*¹

¹*School of Evolution and Environmental Science, The University of Adelaide – North Terrace, Adelaide, South Australia, 5005,* ²*Rural Solutions SA, Level 8, 101 Grenfell St, Adelaide South Australia 5001,* ³*Department of Environment and Natural Resources, GPO Box 1047, Adelaide South Australia 5001*

Chapter 1 of PhD Thesis:

A summary of a comparison of bird abundance and diversity among native mallee remnants with adjacent saltbush plantings, isolated native remnant, isolated saltbush, and isolated agricultural land.

The aim of this study was to examine bird abundance and species richness trends between remnant vegetation with adjacent saltbush plantings, isolated remnant vegetation, isolated saltbush plantings, and cleared land cropping/pasture use. In addition, I aimed to determine the influence adjacency to remnant vegetation on avian use of saltbush plantings and identify additional influencing factors.

The need to extend conservation and management efforts beyond reserve systems to encompass private production land is becoming ever greater (Law and Dickman 1998; Lindenmayer, *et al.* 2010a). Saltbush plantings offer an opportunity to increase perennial vegetation cover in fragmented landscapes, thereby potentially improving landscape connectivity and heterogeneity, providing refuges for transient species, and offering increased foraging resources. Birds represent an ideal taxon for initial studies of saltbush plantings as they are highly mobile and therefore capable of exploiting newly available habitat, are the most conspicuous and numerous vertebrate fauna, and data can be obtained more easily than for other taxa (Hobbs, *et al.* 2003; Loyn, *et al.* 2007; Mac Nally 2007; Munro, *et al.* 2011).

This study took place at 16 sites (four replicates of each described above) in the South Australian Murray Mallee during spring 2010 and autumn 2011. Birds were surveyed along transects within 500 x 500 m quadrats four times at each site during spring and again during autumn. Records included bird species, GPS location, activity, and plant species used (among others). Environmental surveys followed at each site examining the plant species richness and foliage density.

Remnant vegetation supported the greatest abundance and species richness while isolated saltbush sites supported mainly generalists and shrub land specialists. Bird communities were significantly different across treatments and abundances changed seasonally. When adjacent to remnants, saltbush plantings boosted overall bird species diversity.

This study highlights the potential of saltbush plantings to provide improved habitat and biodiversity conservation value for birds over conventionally managed agricultural land. However, the simple structured monoculture design of these plantings means they supported a significantly reduced suite of species compared to that present in remnant vegetation. Saltbush plantings supported several bird species not found at agricultural sites, suggesting these species would be otherwise absent from conventional cropping/pasture landscapes.

The stark differences in bird communities between saltbush and conventional agricultural land echo those of (Collard, *et al.* 2011) and (Seddon, *et al.* 2009), who found more diverse bird communities in saltbush plantings compared to cleared agricultural land. Indeed a number studies illustrate several forms of production perennials support biodiversity levels markedly improved upon conventional land uses but below those of remnant vegetation (e.g. (Arnold 2003; Martin, *et al.* 2004; Smith 2009). This study complements others supporting the notion that plantings of saltbush can contribute in part toward improving landscape scale biodiversity conservation (Lefroy, *et al.* 2005; Lefroy and Smith 2004). In addition this study has illustrated adjacency to remnant vegetation can increase the avian abundance and richness in saltbush plantings but highlights remnant vegetation as holding the greatest biodiversity value and the need for its conservation.

References

- Arnold G. W. (2003) Bird species richness and abundance in wandoo woodland and in tree plantations on farmland at Baker's Hill, Western Australia. *Emu* **103**, 259-269.
- Collard S., Fisher A. and McKenna D. (2011) Planted saltbush (*Atriplex nummularia*) and its value for birds in farming landscapes of the South Australian Murray Mallee. *Ecological Management & Restoration* **12**, 37-45.
- Hobbs R., Catling P. C., Wombey J. C., Clayton M., Atkins L. and Reid A. (2003) Faunal use of bluegum (*Eucalyptus globulus*) plantations in southwestern Australia. *Agroforestry Systems* **58**, 195-212.
- Law B. S. and Dickman C. R. (1998) The use of habitat mosaics by terrestrial vertebrate fauna: Implications for conservation and management. *Biodiversity and Conservation* **7**, 323-333.
- Lefroy E. C., Flugge F., Avery A. and Hume I. (2005) Potential of current perennial plant-based farming systems to deliver salinity management outcomes and improve prospects for native biodiversity: a review. *Australian Journal of Experimental Agriculture* **45**, 1357-1367.
- Lefroy E. C. and Smith F. P. (2004) The biodiversity values of farming systems and agricultural landscapes. *Pacific Conservation Biology* **10**, 80-87.
- Lindenmayer D., Bennett A. and Hobbs R. J. (2010a) An overview of the ecology, management and conservation of Australia's temperate woodlands. *Ecological Management & Restoration* **11**, 201-209.
- Lindenmayer D. B., Cunningham R. B., Crane M. J., Michael D. R., Montague-Drake R., Macgregor C. and Muntz R. (2010b) Revegetation and birds: insights from large-scale, long-term work on the South West Slopes of New South Wales. In: *The State of Australia's Birds 2009, Supplement to Wingspan Vol. 20, No. 1* The State of Australia's Birds 2009, Supplement to Wingspan Vol. 20, No. 1.
- Loyn R. H., McNabb E. G., Macak P. and Noble P. (2007) Eucalypt plantations as habitat for birds on previously cleared farmland in south-eastern Australia. *Biological Conservation* **137**, 533-548.
- Mac Nally R. (2007) Consensus weightings of evidence for inferring breeding success in broad-scale bird studies. *Austral Ecology* **32**, 479-484.
- Martin W., Eyars-Chaddock M., Wilson B. and Lemon J. (2004) The value of habitat reconstruction to birds at Gunnedah, New South Wales. *Emu* **101**, 177-189.
- Munro N. T., Fischer J., Barrett G. W., Wood J., Leavesley A. and Lindenmayer D. B. (2011) Bird's Response to Revegetation of Different Structure and Floristics—Are “Restoration Plantings” Restoring Bird Communities? *Restoration Ecology* **19**, 223-235.

- Seddon J., Doyle S., Bourne M., Maccallum R. and Briggs S. (2009) Biodiversity benefits of alley farming with old man saltbush in central western New South Wales. *Animal Production Science* **49**, 860-868.
- Smith P. F. (2009) Bird activity in oil mallee plantings in the wheatbelt of Western Australia. *Ecological Management & Restoration* **10**, 236-238.

CHAPTER 3

A direct biodiversity benefit of saltbush-based farming for the sleepy lizard, *Tiliqua rugosa*, in southern Australia

Melanie L. Lancaster^{1, 2,3}, Michael G. Gardner^{1,2,4a}, Alison J. Fitch^{2,4}, Talat H. Ansari^{2,4}, Anita K. Smyth^{5,6,7}

¹ School of Earth and Environmental Sciences, The University of Adelaide, Adelaide, South Australia, 5005, Australia. . ² South Australian Regional Facility for Molecular Ecology and Evolution, South Australian Museum, Adelaide, South Australia, 5000, Australia ³ Healesville Sanctuary, Badger Creek Road, Healesville, Victoria, 3777 ⁴ School of Biological Sciences, Flinders University, Adelaide 5001, South Australia, Australia. ⁵ CSIRO, PMB 2, Glen Osmond, South Australia, 5064, Australia. ⁶ Terrestrial Ecosystem Research Network – Adelaide Node, The University of Adelaide, Adelaide, South Australia 5005, Australia. ⁷ Future Farm Industries Cooperative Research Centre, The University of Western Australia, 35 Stirling Hwy, Crawley, WA 6009, Australia

Keywords: Agriculture, biodiversity, microsatellite, parentage, reptile, revegetation, saltbush, *Tiliqua rugosa*.

Running title: Biodiversity value of saltbush for *T. Rugosa*

^a Corresponding author: michael.gardner@flinders.edu.au

Abstract

Land alteration for intensive agriculture has been a major cause of species decline and extinction globally. In marginal grazing regions of temperate southern Australia, perennial shrubs are increasingly being planted to supplement pasture feeding of stock. Such plantations have the additional benefits of reducing erosion and salinity, and importantly, the potential provision of habitat for native fauna. We explored the use of saltbush plantations by the sleepy lizard, *Tiliqua rugosa*, an endemic Australian species common in the region. We visited the same saltbush plantations multiple times throughout 2010 and 2011 and collected samples for genetic analysis from adults (n = 55) and juveniles (n = 25). Using 8 microsatellite loci, parents were assigned to over half of all juveniles with high statistical confidence. We found that parents were sampled in the same patch of saltbush as their offspring, thus supporting the observation that juvenile sleepy lizards remain within the home range of their parents prior to dispersal. Most importantly, our results indicate that saltbush provides important habitat for *T. rugosa* at significant life stages – prior to and during breeding for adults, and post-birth but before dispersal for juveniles. We can conclude that revegetation using simple, monoculture plantations is beneficial in preserving native biodiversity in human-altered agricultural landscapes and that the cereal cropping landscapes offer structural connectivity at least for this species.

Introduction

Agro-ecological landscapes are essential for our food security (Stokes & Howden 2007). Much is already known about the loss of biodiversity through agricultural land use and the associated destruction and degradation of species habitats (Saunders et al. 1993; McIntyre et al. 2002; Lindenmayer & Fischer 2006). Despite an acute awareness of the need for guiding principles, the design of landscape networks to mitigate biodiversity loss is poorly understood (Menninger & Palmer 2006). Given also the uncertainty of climate change impacts on food production and biodiversity, an understanding of how agro-ecological landscapes can be productive and manipulated to maintain biodiversity persistence is an important ecological question in Australia (Morton et al. 2009).

In recent times, broad-scale revegetation has been implemented in an attempt to rehabilitate agricultural landscapes and improve their biodiversity value (Munro *et al.* 2007). Such actions can have indirect and direct benefits for native species. Plantings can protect native vegetation from wind and water erosion and incursion of ground water and nutrients. They can also benefit fauna directly if they provide cover, food sources and habitat (Lefroy *et al.* 2005). Whilst the replication or recreation of complex indigenous habitats that existed prior to land clearing is desirable, simple revegetation in managed agricultural landscapes is more feasible and has the potential to contribute to biodiversity conservation (Collard & Fisher 2010).

In Mediterranean climates the planting of halophytic shrubs such as saltbush (*Atriplex* spp.) has been demonstrated to be one of the most effective ways of restoring land to production (Le Houérou 1992). Increasingly, land managers in marginal farming areas of Australia are also using perennial saltbush plantings as fodder to supplement their pastures during summer and autumn months. This setup offers direct economic benefits to landholders by reducing the costs of

supplementary feeding, allowing higher stocking rates and ultimately improving animal production per hectare (Monjardino *et al.* 2010). In effect, saltbush plantations halt the spread of salinity and reduce soil erosion while allowing grazing on previously degraded areas (Norman *et al.* 2008).

As of 2002, approximately 454,000 ha of Australia was occupied by plantings of salt tolerant perennials (Lefroy *et al.* 2005) and in South Australia, saltbush (mostly *A. nummularia*) comprises approximately 7,000 ha of agricultural land. In the South Australian Murray Mallee (SAMM) region it has been proposed that saltbush has the potential to provide shrub layer vegetation and associated resources for biodiversity, as well as supplementing understorey structure in existing stands of native vegetation (Collard & Fisher 2010). If this is the case, these agricultural systems could be highly important for the conservation of South Australia's native fauna, which has experienced local and landscape-scale range contractions and extinctions since the 1800s as a result of the conversion of native vegetation to farmland (Hobbs 1993).

Our study explores the direct biodiversity value of saltbush plantations for the sleepy lizard, *Tiliqua rugosa*, in the South Australian Murray Mallee (SAMM) region. *T. rugosa* is a medium-sized, long-lived scincid lizard that is broadly distributed across arid, semi-arid and temperate landscapes in Southern Australia (Cogger 2000). Adults are monogamous and have small, stable home range sizes (200 – 1000 m²) that overlap during the breeding season (Bull 1988). Young are born annually but generally do not disperse in their first year of life, instead remaining within the same home range as their mothers (Bull & Baghurst 1997). *Tiliqua rugosa* shelters under shrubs and leaf litter (Kerr *et al.* 2003) and is mainly herbivorous (Dubas & Bull 1991). Population studies conducted over 30 years on this species at Mt. Mary in the extensive rangelands about 60 km northwest of our study area show it is not a species of conservation concern (Bull 1987, 1994, 1995). However, anecdotal observations by local farmers suggest this species may have declined in the past 70 years in the SAMM. As adults, they have few predators but many individuals are killed on roads by vehicles and some may die due to rabbit and fox baiting in late summer and early autumn. Juveniles are highly predated (61-86% mortality, Bull 1987) by large venomous snakes, foxes and cats. We predict that saltbush could have a conservation value for *T. rugosa* if there is evidence that the species uses the plantations as habitat.

Methods

Study site and sample collection

This study took place in the fragmented landscapes of the South Australian Murray Mallee (SAMM) wheatbelt in southern Australia (Fig. 1). The wheatbelt extends south from Waikerie to Narkat Conservation Park and east from Murray Bridge to the Victorian border (latitude and longitudes). Land use in the region is predominantly cereals and sheep broad acre farming interspersed with discontinuous, linear roadside or patch-like remnants of mallee woodland (dominated by *Eucalyptus socialis*) and saltbush (*Atriplex nummularia nummularia*) on unproductive cropping areas. Native vegetation has been intensively cleared in the region since the 19th century (20% remains, Willoughby 2006) to make way for farming and saltbush has been planted in the region as a fodder reserve since the late 1990s (5% of SAMM).

Samples of blood (see below) were taken from adult *T. rugosa* for marker evaluation and population genetic structure analysis from across the SAMM. Blood was collected for parentage analyses from *T. rugosa* adults and juveniles at a site within a replicate of severely modified landscape (LSS3) used to assess the species' physiological health (Smyth et al. unpubl). The site was chosen for a number of reasons. The maturity (10-year old) and vigour of two nearby saltbush plantings provided complex habitat for shelter from predators and summer heat, food and basking. The site consisted of two small saltbush plantings (A - 6.9 ha and B - 12.7 ha) and a nearby native remnant patch (C - 3.6 ha) and a small strip of planted *Acacia* spp. (D - 0.3 ha) which made it practical to resample intensively until blood samples were obtained from just about all of the individuals at the site for parentage analysis. Most importantly, the site was surrounded by cereal crops on all sides and isolated from any linear strips of roadside native vegetation – a spatial arrangement that we predicted would limit the flow of new migrants and thus improve our chances of saturation sampling of lizards.

Lizards were surveyed by employing a single reptile visual encounter survey (Manley et al. 2005) using a randomised line transect method in all saltbush plantings and small remnants. Teams of two observers systematically searched neighbouring rows of monocultures of saltbush (approximately 8-10m apart) and 10m wide strip between observers in remnants at a consistent slow pace ensuring that both sides of individual saltbush rows and 10m-wide path in the remnant were surveyed thoroughly for lizards. Multiple sampling occurred over a 5-day period for 10 weeks between November 2010 and February 2011 until new captures were minimal. Site density was estimated at 3.9 individuals per ha.

Immediately after capture, blood was taken followed by morphometrics and microchipping (PIT tag, TROVAN® ID 100). A sample of 0.4mL-0.6mL blood was collected from each individual using the caudal tail vein venipuncture with pre-heparinised disposable Terumo® 23G needle/1 mL syringe for adult/subadults (≥ 20 cm) or Terumo® 21G needle /1 mL syringe for juveniles (≤ 20 cm) (Jacobson 1993a). Blood was stored on Whatman's® FTA elute cards at room temperature with silica until required for DNA extraction.

DNA extraction and microsatellite genotyping

DNA was extracted from 3 mm blood samples taken from 55 adult and 25 juvenile *T. rugosa* (for parentage analysis with a further 219 adult *T. rugosa*, totalling 274 adults, for population genetic structure and marker suitability) in accordance with the Whatman™ FTA™ Elute card procedure (GE Healthcare, Buckinghamshire, UK). Following the final wash, DNA was eluted in 50 μ l of sterile distilled water and stored at -20 °C. All individuals were genotyped at a panel of 10 microsatellite loci previously isolated from *T. rugosa* (Gardner *et al.* 2008). Loci used were Est1, TrL1, TrL3, TrL10, TrL14, TrL16, TrL21, TrL27, TrL30, TrL32. PCR conditions and visualisation followed Gardner *et al.* (2008). One negative control per PCR was run and 8.3% of individuals were amplified and scored blindly twice to generate genotyping error rates for subsequent use in parentage analysis.

To test the performance of all microsatellites as population genetic markers, we used the expanded dataset of 274 individuals. . All microsatellites were checked for deviations from Hardy-Weinberg Equilibrium (HWE) proportions and genotypic disequilibrium using GENEPOP

v. 3.4 (Raymond & Rousset 1995). Null allele frequencies (r) were estimated using MICRO-CHECKER v. 2.2.3 (Van Oosterhout *et al.* 2004).

We tested the strength of our microsatellite panel for parentage analysis by calculating the probability of identity using genotypes from all adult and juvenile lizards. This approach estimates the average probability that two unrelated individuals drawn from the same population have the same genotype.

Population genetic structure analysis

We used genotypes from all loci except TrL16 and TrL30, from the 274 adult *T. rugosa* as input in the program STRUCTURE using the admixture model, infer alpha, correlated allele frequencies, lambda = 1.0. We set K from 1-5 with 100,000 burn-in, 900,000 repetitions post burn-in and performed 10 iterations. Results were examined using STRUCTURE HARVESTER v. 0.6.8 (Dent & vonHoldt 2011)

Parentage analysis

Parentage was inferred using the software program CERVUS v. 3.0.3 (Marshall *et al.* 1998), which uses a likelihood-based approach to assign parentage to individuals based on co-dominant markers and a number of user-defined variables. For all analyses, allele frequencies were generated from the larger data set of *T. rugosa* individuals ($n = 274$) described above. A simulation of parent-pair analysis with neither parent nor sex known was run to generate log likelihood (LOD) ratios of true parents to arbitrarily assigned parents with statistical confidence. We assumed that 60 % of all candidate parents were sampled and we simulated parentage for 10000 offspring with 1000 parents, 0.86 of individuals genotyped and a 0.005 error rate (based on our calculated error rates). This simulation was then used in our analysis of parentage. Only trios (offspring plus both parents) that were assigned with statistical confidence (80 % and 95 %) and with no allele mismatches were included.

Results

*Presence of *T. rugosa* in saltbush plantations*

We sampled 61 adults and 30 juvenile lizards over ~700 hours. Six juveniles belonged to the 2011 cohort. Densities were highest in saltbush B (5 ha^{-1}), followed by remnant C (3.6 ha^{-1}) and saltbush planting A and the planted strip D (1.3 and 1.5 ha^{-1}) (Fig. 1). Juvenile density was higher in saltbush plantings (A and B combined). One adult moved from remnant C to the southeast of saltbush B and another adult moved from saltbush B into the eastern edge of saltbush A. Recaptures didn't show individuals moving from saltbush A, B or the planted strip D into remnant C during sampling. Most individuals appeared resident in saltbush B during the study. A juvenile was observed once on a track near D about 300 m northwest of saltbush B (Fig. 1). Successful captures of adults and juveniles within saltbush plantations and from the native remnant into saltbush plantings indicate that this habitat is important to multiple life stages of the species and from one breeding season to next for juveniles.

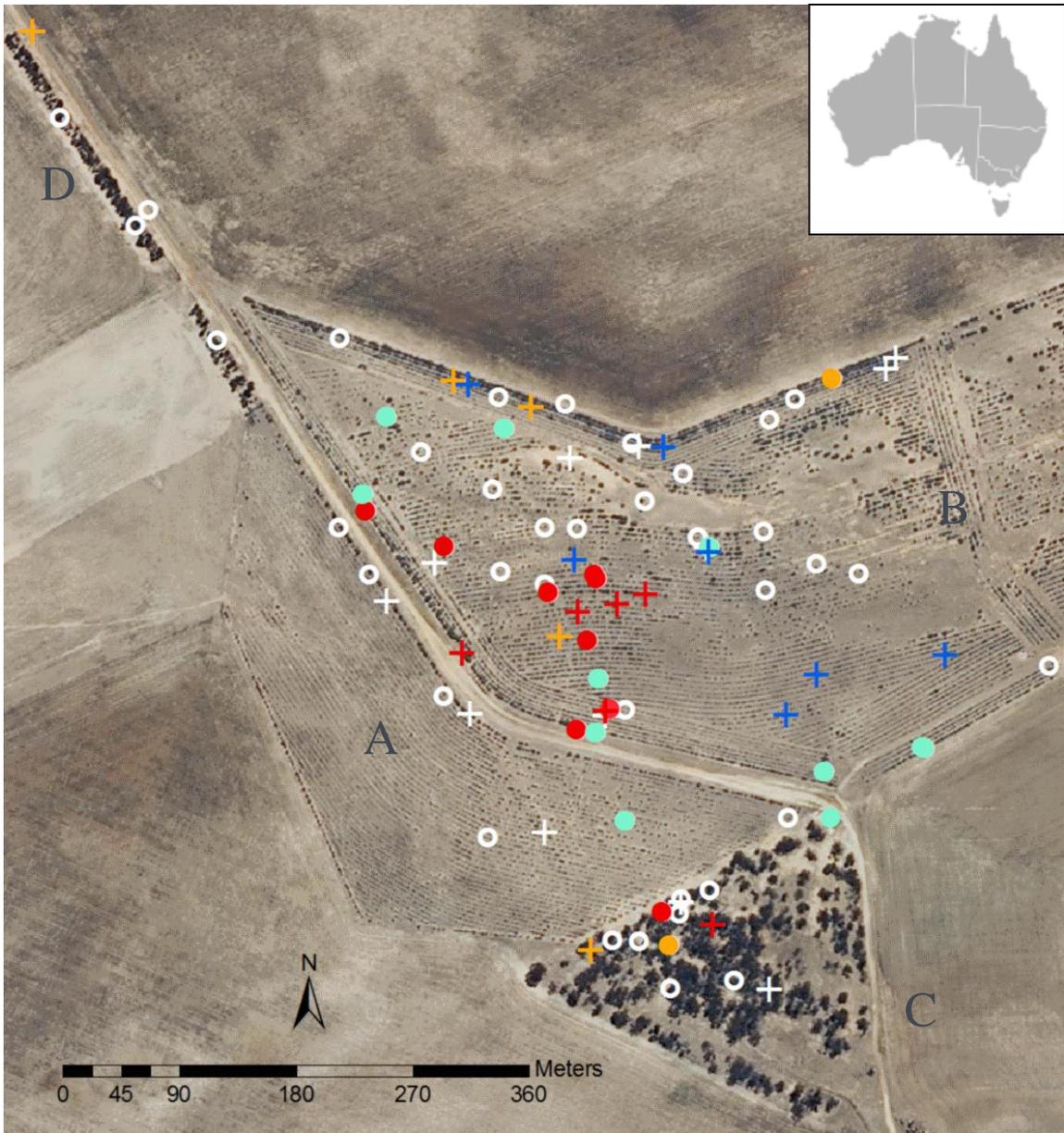


Figure 1. Location of adult (circle) and juvenile (cross) *Tiliqua rugosa* in saltbush plantings A (6.9 ha), B (12.7 ha), native mallee remnant C (3.6 ha) and planted *Acacia* spp. D (0.3 ha) surrounded by cereal crops, South Australian Murray Mallee region, Australia. (Parentage: white – no parentage, red – 95% confidence, blue – 80% confidence, orange – ‘most likely’ related)

Hardy-Weinberg equilibrium proportions and null alleles and probability of identity

All 10 microsatellites were highly polymorphic (8-49 alleles, mean =25.9, Table 1). All except two PCR-amplified reliably and showed no evidence of null alleles. TrL16 and TrL30 deviated significantly from HWE proportions as a result of homozygous excess, most likely due to null alleles (Table 1). These loci were removed from further analyses. The average probability that two unrelated individuals drawn from the same population have the same genotype in our samples was low (3.86×10^{-14} for unrelated individuals and 1.27×10^{-4} for siblings (GenAlEx 6.0, Peakall & Smouse 2006), indicating that our locus panel was sufficient to assign unique genotypes to even closely-related individuals.

Table 1. Microsatellite variation in *Tiliqua rugosa*, where N_a denotes number of alleles, N_e , effective number of alleles, H_e , expected heterozygosity and H_o , observed heterozygosity. *** denotes statistical significance at the 99.9 % confidence interval following Hardy-Weinberg exact test.

Locus	N_a	N_e	H_e	H_o
Est1	45	23.5	0.96	0.89
TrL1	49	31.4	0.97	0.93
TrL10	8	2.9	0.65	0.63
TrL3	29	16	0.94	0.93
TrL14	22	11.1	0.91	0.95
TrL16	17	8.1	0.88	0.64***
TrL21	20	9	0.89	0.85
TrL27	13	5.6	0.82	0.82
TrL30	53	9.56	0.9	0.86***
TrL32	21	10.3	0.9	0.88

Population genetic structure

Examination of the STRUCTURE results indicated a single genetic cluster existed across the sampling area.

Evidence of breeding in saltbush plantations

Of the 25 *T. rugosa* juveniles sampled from saltbush plantations, 13 were assigned a parent pair (mother and father) with statistical confidence. Of the remaining 12, five were assigned parent pairs with high LOD scores (3.53 – 7.37) but low delta scores (difference in LOD between the first and second most likely parent pair). These low delta scores were due to the second most-likely parent pair also having a high probability of being true parents. As our loci had sufficient power for parentage exclusion (3.9×10^{-11} for parent pairs), it is likely that at least one of these second most-likely parents was genuinely related to at least one true parent. This result may indicate limited dispersal in this fragmented landscape and subsequently, high relatedness among adults. Alternatively, it may be the result of high natal philopatry of one or both sexes of the species. The remaining eight juvenile *T.*

rugosa had negative LOD scores for assignment to their most likely parent pair. This implies that for these individuals the field-assigned parents were less likely to be the true parents than a pair of arbitrary unrelated parents. This proportion of unresolved parentage assignments is not surprising given that only 60 % of parents in the region were likely to have been sampled.

Spatial relationship between parents and offspring

Most parental pairs were located within proximity of offspring in saltbush planting B. One parent of both juveniles captured in remnant C were first captured in B and continued to move around in B although the last captured was in November 2010 and the second parent and juvenile was first sighted in February 2011. Both parents of the juvenile near D remained within B while their ‘most likely’ offspring was captured near D during November 2010. Offspring of parents in B may have dispersed from a natal site into B although it is more likely that saltbush planting B is the natal site of the February 2010 and 2011 cohorts. Remnant C also appears to be a natal site for the February 2011 cohort.

Discussion

Planting of perennial monocultures in agricultural landscapes can improve agricultural production and reduce overall environmental impacts of native vegetation clearance. This study has shown that saltbush planted in the South Australian Murray Mallee (SAMM) region for stock fodder also provides habitat for the endemic Australian sleepy lizard, *T. rugosa*. Results of field sampling and molecular analysis of parentage indicate that saltbush plantations are used by adult lizards prior to and during the breeding season, and by their young prior to dispersal. While complexity and structure of revegetation have been flagged as key indicators of biodiversity retention, our findings suggest that even simple plantations can have direct conservation benefits for native fauna.

Using molecular genetic data, we assigned parentage to 52 % (13/25) of all *T. rugosa* juveniles sampled in saltbush plantations. An additional 20 % (n = 5) were assigned parents with high likelihood scores and no allele mismatches but low statistical confidence, suggesting that relatedness between some adult lizards in the area was likely to be high. This is potentially explained by aspects of the species’ behavioural ecology: *T. rugosa* takes 3 – 5 years to reach maturity and has low juvenile dispersal and high adult survivorship (80-90%) and are long lived (up to 50 years) (Bull 1995). A previous study of *T. rugosa* in South Australia noted that in their first year, juveniles establish small home ranges largely within the home range of their mother (Bull & Baghurst 1997). Contact between parents and their adult offspring may therefore be quite common (Bull & Cooper 1999). If we include these five additional assignments in our results, over two-thirds of juveniles sampled in the saltbush were born to adults found in the same plantations. Although we did not track these lizards individually, we can conclude that saltbush plantations form at least part of the home ranges of breeding adult and juvenile sleepy lizards in the SAMM region.

Many of the saltbush plantations in the SAMM region lie adjacent to roadside remnants of native vegetation, which presumably provide important habitat for *T. rugosa*. A previous study found bird abundance and species diversity to be significantly lower in saltbush than in native vegetation sites, although some bird species used saltbush for nesting (Collard *et al.* 2011; Seddon *et al.* 2009). Based on our finding that sleepy lizards use saltbush plantations, it must be assumed that plantations provide them with resources. In agricultural landscapes, planting of saltbush effectively re-establishes mid-storey vegetation in habitats that are otherwise devoid of complexity (Seddon *et al.* 2009). Further, in eastern Australia, a wide variety of native grasses and forbs grow under and around saltbush plants,

thereby improving structure and composition of the habitat and enhancing the biodiversity value of the area (Seddon *et al.* 2009). Although further study into fine-scale habitat use and diet of *T. rugosa* is required, it is probable that saltbush plantations provide direct resources in the form of food and shelter to both adult and juvenile sleepy lizards. Certainly, individuals were observed foraging on plant species (flowers, leaves) that have been reported in *T. rugosa* diet about 60 km away at Mt. Mary (Bull pers comm)

The success of revegetation efforts in halting species declines across Australia has been mixed and appears to be linked to the level of ecological specialisation of different species (e.g., Kanowski *et al.* 2006; Collard *et al.* 2011). Overwhelmingly, remnant vegetation contains the highest abundance and species diversity when compared to plantations or cleared land for birds, mammals and reptiles (Cunningham *et al.* 2007; Munro *et al.* 2007; Collard *et al.* 2011). However, plantations are not without their benefits. A previous study on the effects of revegetation on reptiles found three-toed skinks (*Hemiergis decresiensis talbingoensis*), olive legless lizards (*Delma inornata*) and eastern blue-tongue lizards (*Tiliqua scincoides*) to be approximately three times more abundant in farms with plantings than farms without plantings (Cunningham *et al.* 2007). Furthermore, in the same region as used in this study, threatened bird species were observed in saltbush plantations but not in adjacent farmland sites (Collard *et al.* 2011). These findings suggest that although revegetation in the form of simple plantations is unlikely to be equivalent for remnant vegetation in terms of its biodiversity benefit, for many species it may be a better option than no revegetation at all.

In intensively farmed regions, such as the South Australian Murray Mallee region, revegetating large blocks of land is likely to compromise farm productivity through the loss of land for farming. Economically, planting ten percent of total farm area with perennial shrubs has been shown to increase farm profitability by an average of 24 percent in some regions; however planting beyond this threshold results in reduced profitability (Monjardino *et al.* 2010). In these cases, planting fodder shrubs such as saltbush may be the only means of reintroducing structural complexity into the landscape. Our most significant finding from this study was that saltbush is used by sleepy lizards in multiple life stages – as adults, before, during and after the breeding season, and by juveniles, prior to dispersal. Based on this, we put forward the following recommendations for managing saltbush plantations in this and comparable regions: Firstly, it may be beneficial for landholders to manage the timing and intensity of saltbush grazing by livestock in order to maintain a mosaic of saltbush habitats for *T. rugosa*. This may be achieved by having multiple plantations that are grazed at different times of the year, or by planting multiple species of saltbush that vary in their tolerance to grazing; Secondly, the configuration of saltbush plantations is likely to be important on both a broad scale; for establishing functional connectivity (dispersal and gene flow) across the landscape, and on a fine scale; to ensure that lizards have access to saltbush plantations as well as remnant vegetation within their home ranges. While there are few large reserves of remnant woodland left in the SAMM region, there are many stretches of remnant roadside vegetation that could potentially act as corridors and/or suitable habitat for *T. rugosa*. Given the lack of population genetic structure across the area studied, gene flow appears to be uninhibited at the scale measured. However, the long lived nature of this species may mean that the effects of reduced connectivity are yet to manifest in these data. Therefore, planting saltbush adjacent to these remnants would increase connectivity and the overall area of habitat that could be utilised by lizards. Finally, we acknowledge the potential biodiversity benefits of alley farming, an alternative to conventional crop-pasture rotation systems that has been shown to improve the structure, function and composition of vegetation at the site and paddock scale (Seddon *et al.* 2009). While this method of farming has the potential to integrate successful agricultural practices with conservation, further study is required to determine the biodiversity value of strips of saltbush

(alley farming) compared with larger, contiguous patches of saltbush (currently used in the SAMM region). For now we can conclude that revegetation using simple, monoculture plantations is beneficial in preserving native biodiversity in human-altered agricultural landscapes.

Acknowledgements

We thank Liz Smee and the many volunteers for helping with lizard sampling and the Proud family for allowing access to their property during sampling. Dr. Ken Wallace of the Future Farms Industry CRC provided helpful comments on the manuscript. The work was funded by the Future Farms Industry CRC and CSIRO. The University of Adelaide, Flinders University and the South Australian Regional Facility for Molecular Ecology and Evolution were contracted by CSIRO to undertake the genetic analyses.

References

- Bull CM (1988) Mate fidelity in an Australian lizard *Trachydosaurus rugosus*. *Behavioral Ecology and Sociobiology* 23, 45-49.
- Bull, C. M. (1995) Population Ecology of the Sleepy Lizard, *Tiliqua rugosa*, at Mt- Mary, South-Australia. *Australian Journal of Ecology* 20(3): 393-402.
- Bull CM, Baghurst BC (1997) Home range overlap of mothers and their offspring in the sleepy lizard, *Tiliqua rugosa*. *Behavioral Ecology and Sociobiology* 42, 357-362.
- Bull CM, Cooper SJB (1999) Relatedness and avoidance of inbreeding in the lizard, *Tiliqua rugosa*. *Behavioral Ecology and Sociobiology* 46, 367-372
- Collard SJ, Fisher AM, McKenna DJ (2011) Planted saltbush (*Atriplex nummularia*) and its value for birds in farming landscapes of the South Australian Murray Mallee. *Ecological Management and Restoration* 12, 37-45.
- Collard SJ, Fisher AM (2010) Shrub-based plantings of woody perennial vegetation in temperate Australian agricultural landscapes: what benefits for biodiversity? *Ecological Management and Restoration* 11, 31-35.
- Cunningham RB, Lindenmayer DB, Crane M, Michael D, MacGregor C (2007) Reptile and arboreal marsupial response to replanted vegetation in agricultural landscapes. *Ecological Applications* 17, 609-619.
- Earl, Dent A. and vonHoldt, Bridgett M. (2011). STRUCTURE HARVESTER: a website program for visualising STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources DOI: 10.1007/s12686-01109548-7 Version: v0.6.8 Oct 2001.
- Dubas G, Bull CM, 1991. Diet choice and food availability in the omnivorous lizard, *Trachydosaurus rugosus*. *Wildl Res* 18:147-155.
- Fahrig L (2002) Effect of habitat fragmentation on the extinction threshold: a synthesis. *Ecological Applications* 12, 346-353.
- Gardner M.G, Sanchez J.J, Dudaniec R.Y, Rheinberger L, Smith A.L, Saint K.M (2008). *Tiliqua rugosa* microsatellites: isolation via enrichment and characterisation of loci for multiplex PCR in *T. rugosa* and the endangered *T. adelaidensis*. *Conservation Genetics* 9, 233-237.
- Hobbs RJ (1993) Effects of landscape fragmentation on ecosystem processes in the Western Australian wheatbelt. *Biological Conservation* 64, 193-201.
- Kanowski JJ, Reis TM, Catterall CP, Piper SD (2006) Factors affecting the use of reforested sites by reptiles in cleared rainforest landscapes in tropical and subtropical Australia. *Restoration Ecology* 14, 67-76.

- Kerr, G.D., Bull, C.M. & Burzacott, D. (2003). Refuge sites used by the scincid lizard *Tiliqua rugosa*. *Aust. Ecol.* 28, 152–161.
- Lefroy EC, Flugge F, Avery A, Hume I (2005) Potential of current perennial plant-based farming systems to deliver salinity management outcomes and improve prospects for native biodiversity: a review. *Australian Journal of Experimental Agriculture* 45, 1357-1367.
- Le Houérou HN (1992) The role of saltbushes (*Atriplex* spp.) in arid land rehabilitation in the Mediterranean Basin: a review. *Agroforestry Systems* 18, 107-148.
- Manley, P.N, Van Horne, B., Roth, J.K., Zielinski, W.K., McKenzie, M.M., Weller, T.J., Weckerly, F.W. and C Vojta. (2005). *Multiple Species Inventory and Monitoring Technical Guide*. Last accessed 22/10/2010.
http://www.fs.fed.us/psw/programs/snrc/featured_topics/msim/documents/msim_preprint.pdf
- Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology* 7, 639-655.
- Monjardino M, Revell D, Pannell DJ (2010) The potential contribution of forage shrubs to economic returns and environmental management in Australian dryland agricultural systems. *Agricultural Systems* 103, 187-197.
- Munro NT, Lindenmayer DB, Fischer J (2007) Faunal response to revegetation in agricultural areas of Australia: a review. *Ecological Management and Restoration* 8, 199-207.
- Norman HC, Masters DG, Silberstein R *et al.* (2008) Sustainable grazing on saline lands WA 1 research project – final report. Available from URL:
<http://www.landwaterwo.gov.au/products/pk071404.hing> Asia
- Peakall R, Smouse PE (2006) GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6, 288-295.
- Raymond M, Rousset F (1995) GENEPOP (Version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* 86, 248-249.
- Sala OE, Chapin Iii FS, Armesto JJ, *et al.* (2000) Global biodiversity scenarios for the year 2100. *Science* 287, 1770.
- Seddon J, Doyle S, Bourne M, Maccallum R, Briggs S (2009) Biodiversity benefits of alley farming with old man saltbush in central western New South Wales. *Animal Production Science* 49, 860-868.
- Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* 4, 535-538.

CHAPTER 4

Chronically ill wild sleepy lizards, *Tiliqua rugosa* in one of Australia's food bowl

Anita K. Smyth^{1,2*}, Elizabeth Smee³, Stephanie S. Godfrey⁴, Mathew Crowther³ and David Phalen^{5†}

¹CSIRO, PMB 2, Glen Osmond SA 5064 Australia

²Future Farm Industries Cooperative Research Centre, The University of Western Australia, 35 Stirling Hwy, Crawley, WA 6009, Australia

³Wildlife Health and Population Management, School of Biological Sciences, The University of Sydney NSW 2006 Australia

⁴School of Biological Sciences, Flinders University, GPO 2010, Adelaide 5000 SA Australia

⁵Faculty of Veterinary Science, The University of Sydney NSW 2570 Australia

Keywords: Haemolytic anaemic, haematological assay, body condition, polychromasia, pesticides, agricultural landscapes, *Tiliqua rugosa*

Running Title: Physiological health of lizards in agro-ecological landscapes

*Present Address: Eco-informatics Facility, Tern Terrestrial Research Network, The University of Adelaide, North Terrace, Adelaide SA 5005 Australia

† Corresponding author: david.phalen@sydney.edu.au

Abstract

Assessing the relative ‘vulnerability of habitats required by all farmland taxa’ (termed habitat vulnerability) although scientifically important is too costly and very difficult to implement. Instead, ‘ecosystem level’ surrogates such as the structural connectivity of native vegetation have become the convenient way to measure habitat vulnerability. At the population level, measures such as density, mean survival, reproductive output replace the ecosystem level surrogates. These conventions have stood the test of time as surrogates of population persistence. In humans, societal health is routinely assessed using a combination of body condition, haematology and other physiochemical measures. Pet health is also assessed using similar techniques yet the same reliable techniques have rarely if ever been applied by ecologists to assess the health of wild animal populations at a landscape scale. We assess the body condition and haematology of the charismatic sleepy lizard *Tiliqua rugosa* in grazed rangelands (native vegetation relatively intact) and severely modified cropland landscapes (remnants of native vegetation surrounded by cereal crops) to assess lizard health and make inferences about the habitat vulnerability. We found that the body condition and haematology of lizards in the cropping landscapes were chronically poor, showing haemolytic anaemic. Although body weights of adults and ‘body condition’ was lower in the cropping landscapes, the differences in the percentage of polychromatophilic red blood cells, packed cell volume and absolute and differential blood cell counts clearly showed the health of lizards was alarmingly poor for almost 50% of our study animals (n = 78). This landscape scale decline in health wasn’t due to degraded habitat complexity, measured by habitat type and structural connectivity. Instead, it was due to haemolytic anaemic which can have a number of causes but is most likely due to exposure to pesticides (and possibly other agro-chemicals) that potentially act as toxins. Our findings have serious implications for pest management on farms and its effects on wildlife possibly humans in cereal cropping landscapes.

Introduction

Agro-ecological landscapes are essential for our food security (Stokes & Howden 2007). Much is already known about the destruction and degradation of species habitats on biodiversity loss for agricultural productivity (Saunders et al. 1993; McIntyre et al. 2002; Lindenmayer & Fischer 2006). The specific design of landscape networks to mitigate biodiversity loss remains poorly known despite general guiding principles being well understood (Menninger & Palmer 2006). Given also the uncertainty of climate change impacts on food production and biodiversity and the lack of a coherent biodiversity planning framework (Wallace 2012), an understanding of how agro-ecological landscapes can be productive and managed to maintain biodiversity persistence is an important ecological question in Australia (Morton et al. 2009).

To mitigate biodiversity loss in agro-ecological landscapes, land managers must be able to reduce or remove some of the immediate threats to biodiversity. Information on threatening processes and their collective impact on the relative vulnerability of species habitats (natural or anthropogenic) is crucial for managing biodiversity persistence (Wilson et al. 2005; Prober & Smith 2009). However, not all taxa (animals especially) require exactly the same habitats for survival; the composition, structure and amount of habitat required varies from taxon to taxon (Van Horne 1983; Hall et al. 1998; Fischer & Lindenmayer 2007). As a consequence, assessing the ‘vulnerability of habitats for all taxa’ (hereafter termed habitat vulnerability) using farmland, although desirable, is too costly and very difficult to implement. Instead, ‘ecosystem level’ surrogates such as the structural connectivity of native vegetation are often used to assess the vulnerability of habitats required by taxa (Doerr et al. 2010). At the population level, mean survival, reproductive output and density replace ecosystem level surrogates. These measures have stood the test of time but in agro-ecological landscapes where habitat

modification by people is extreme, studies are questioning whether species density is a valid measure of the vulnerability of habitats for multiple taxa (e.g., Bock & Jones 2004).

An approach for obtaining information on the impacts of threatening processes on habitat vulnerability in agro-ecological landscapes is to study an organism's physiological health as it influences population responses (Wikelski & Cooke 2006). In this context, 'physiological health' includes the functional systems of an organism such as the metabolic, endocrine, cardiovascular, and fat storage systems (Homyack, 2010). Information about the physiological health of populations in agro-ecological landscapes can indicate population fitness directly (Young et al. 2006) and directly relates to assessments of 'habitat vulnerability'.

Severe stress affects physiological health. It results in mortality or at the very least compromises physiological and behavioural functions, leading to a dysfunctional immune system, decreased disease resistance, growth rate and reproduction (Davis et al. 2008). Stressors encountered by ground animals in agro-ecological landscapes include food deterioration and unpredictability, habitat loss and alteration (e.g., clearing, wild fire), predation, parasites, other infectious agents and pesticides. Over time, stressors such as these develop into sustained overstimulation of an organism's coping functions, including physiological and behavioural responses, which leads to chronic stress or serious sub-lethal stress in wild populations (Romero 2004). It differs from acute stress which occurs in short bursts of physiological stress (e.g., "fright and flight" responses) and is generally less harmful.

Throughout southern Australia, native vegetation was historically cleared for agriculture. The South Australian Murray Mallee (SAMM) region is typical of the agro-ecological landscapes that remain (Lancaster et al. submitted). The natural vegetation of the region is typified by a mosaic of mallee woodlands with a changing understorey of chenopod shrubs, perennial grasses and heaths influenced by rainfall, soil type and fire. Land use north of the Murray River since the 1850s is mostly livestock grazing (rangelands) and with the introduction of rabbits and goats, the vegetative structure, composition and ground cover have been modified, especially at areas of high use such as water points (Tiver & Andrew 1997). South of the Murray River (cropping landscapes), much of the mallee was completely removed in the 1900s for cereal cropping and only 20% (3,660 km²) remains (Willoughby 2006) as discontinuous roadside or small patches of remnant vegetation (mean area = 2.3 ha). In the 1960s, the remaining mallee on the tops of sand dunes was removed to control rabbits but was replanted with monocultures of old man saltbush (*Atriplex numularia numularia*) in the late 1990s to stabilise the sand dunes from wind erosion and provide a late 'dry season' fodder reserve. These plantings are small (mean area = 5.92 ha) but provide habitat for wildlife including feral rabbits, foxes and cats. They represent around 5% (397 ha) of the total area of cropping landscapes in the SAMM. Poisoned baits are used to control rabbits and foxes annually and occasional plagues of house mice which similar to locusts erupt in response to good rains. Chemical spray is used to control locusts. State governments are responsible for pest management on public lands whereas farmers are responsible for managing pests on their farms using government guidelines.

A charismatic species which inhabits Australia's rangeland and agro-ecological landscapes is the sleepy (shingleback or bobtail) lizard (*Tiliqua rugosa*). Much is known about its ecology because of studies that have been undertaken at Mt. Mary in the SAMM rangelands over the past 30 years, including demographic research (Bull 1987, 1994, 1995). It is a very common, indigenous, diurnal heliothermic species liked by farmers as it 'freezes when disturbed' making it easy to handle. It is a large (snout-vent length: ~295 mm, weight: ~548g) species that lives for about 20 years but some make it to 50 years. Males and females (heavier sex) have a small overlapping home range (200 m² - 1 km²) but during the breeding season (September-December), monogamous pairs remain close to

each other within 1m². During this time, individuals emerge from “hibernation” after winter to bask from about 10:00 hrs until the ground temperature reaches about 23°C after which time they forage mostly on annual forbs (new growth and flowers) and seeds until about 15:00 to 16:00 hrs, depending on temperatures (Kerr & Bull 2004). If ground temperature exceeds 30°C, lizards seek shelter in ground cavities (rabbit burrows, fallen debris such as logs), under dense ground cover buried near the central root system or bury deep into leaf litter well into the sandy substrate (Kerr et al. 2003). The species has flexible habitat requirements, occurring in many vegetation types from dry sclerophyll forest to mallee woodlands, shrublands and coastal sand dunes most of which are modified by livestock grazing or vegetation clearance for cereal cropping. Anecdotal observations by some farmers however suggest this species may have declined in the past 70 years in South Australia’s Murray mallee cereal cropping landscapes. As adults, they have few predators while their young are highly predated (61-86% mortality, Bull 1987). Many are killed on roads by vehicles.

The aim of our study is to examine whether sustained exposure to severe habitat alteration in intensively farmed agro-ecological landscapes can affect the environmental health of wild sleepy lizards and thereby infer habitat vulnerability and inform farm management. As far as we are aware no physiological studies with conservation aims have been conducted on the health of wild animals in agro-ecological landscapes at a landscape scale. We investigate the health of wild sleepy lizards within replicate(s) of two types of agro-ecological landscapes representing baseline and severe habitat modification: (1) baseline – no cereal cropping, low livestock grazing density with mallee vegetation largely intact and (2) severe - intensively farmed for cereal crops. We use body condition, haematology and ectoparasite load to test whether animals inhabiting native vegetation in cereal cropping landscapes will be less healthy than those using native perennial plantings and remnant native vegetation. If our predictions are not upheld, then it is likely other farming practices and not vegetation management affects lizard health in cereal cropping landscapes.

Methods

Study sites

The study took place in a NW-SE transect (168 km long x 60 km wide) of the South Australian Murray Mallee (SAMM) region of southern Australia (Figure 1). The region has cold wet winters - early spring, and hot dry summers - early autumn. Mean annual monthly rainfall over 37 years ranges from 23.8 mm (November) to 48.8 mm (September) with the mean annual monthly maximum temperature of the study period varies from 17.0°C (September) to 24.4°C (November). The south-eastern area of the transect is marginally drier and warmer than the north-western parts.

Sampling to assess environmental health took place in six landscape-scaled sites (LS) during September to November 2010 after lizard brumation (semi-hibernation over winter). ‘Baseline (B) samples’ from animals exposed to base levels of habitat modification were collected from one unreplicated location (BS0 -Mt. Mary) in the rangelands during November 2010. This site is located in the NW rangelands of the transect (139° 21’E, 33 ° 55’ S) and is a 120 km² area of semi-arid, continuous mosaic of mallee woodland and shrubland (Petney & Bull, 1984). It is occasionally grazed by sheep but largely intact. ‘Severe(S) samples’ from animals exposed to severe habitat modification were collected from the other three replicated sites in the cropping landscapes. These sites were self-selected based on the existing spatial configuration of sleepy lizard habitat resulting from historical clearing and contemporary revegetation with saltbush. Three sites contained combinations of the main habitats of the sleepy lizard – saltbush plantings, roadside and remnant mallee vegetation where the remnant vegetation was often small disconnected linear strips or patches with lower habitat

complexity. These sites varied in size (LS1 – 730 km², LS2 – 625 km², LS3 – 540 km²). All landscape sites captured the widespread differences in structural connectivity (derived using ARCGIS 10 spatial analysis tool) of lizard habitats in the cropping landscapes.

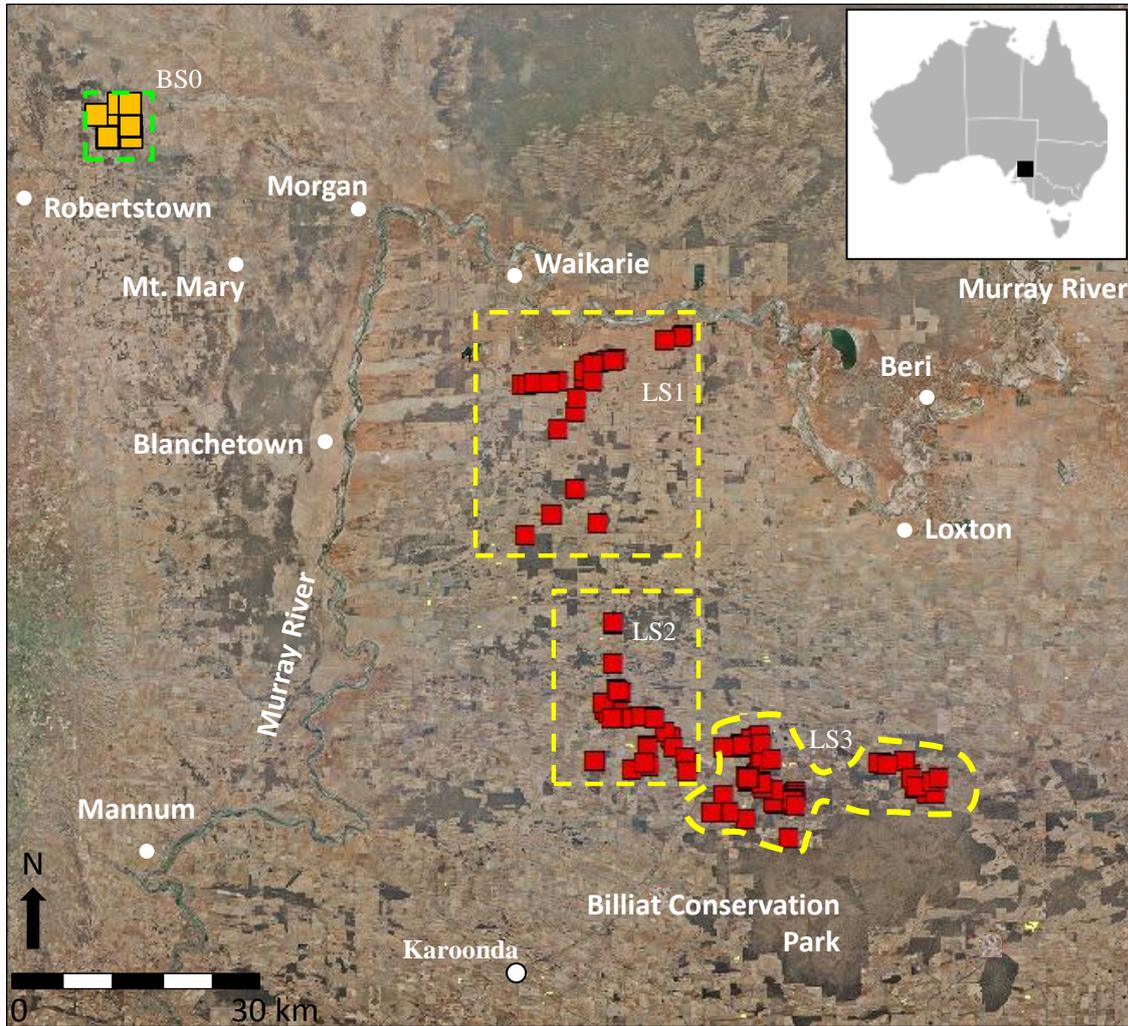


Figure 1. Location of the Baseline study site (orange) in the rangelands (BS0) and Severe study sites (red) in the cereal cropland landscapes (LS1, LS2, LS3) in the South Australian Murray Mallee region of southern Australia. (Dark grey-green – native mallee vegetation, pale browns south of Murray River – cropping landscapes)

Sample design and collection for assessing wild lizard health

Three sampling designs were used to collect data on body condition, haematology and ectoparasite load. Design 1 (Baseline vs Severe) investigated regional differences in the health of wild lizards using a single treatment (fixed, 2 levels: baseline habitat modification, $n = 30$; severe habitat modification, $n = 30$) and one covariate for body condition (3 measures: residual condition index, body mass index and ectoparasite load where $n = 30$ for each). Design 2 (Severe only) examined the influence of habitat complexity on lizard health in cereal cropping landscapes using differences in two factors: (1) habitat type (fixed, 3 levels: remnant native mallee vegetation where remnants were either small disconnected linear strips or patches with reduced habitat complexity, $n = 27$ replicates; saltbush plantings, $n = 28$ replicates; cereal/rested fields, $n = 23$ replicates) and (2) structural connectivity (fixed 4 levels: low - $< 63\%$ connectedness, $n = 24$ replicates; medium - $63-88\%$ connectedness, $n = 25$ replicates; high - $> 88\%$ but not connected, $n = 19$ replicates; connected to native mallee vegetation, $n = 10$). Both factors were nested within three replicated sites (LS1, LS2, LS3) within the severely modified cropping landscapes. All blood samples were identified by age (following Smallridge & Bull 2000) but not by sex. Despite considerable analyses with 10 known males (unreliable eversion of hemipenes), consistent sex determination based on morphometrics of 120 adults of the Severe sites remained elusive. Brumation is another factor known to affect reptile haematological data interpretation (Saggese 2009). To assess its effect on haematological data in the cropping landscapes, we recorded data on the 'cumulative days since the first day of sampling' (CUMDAYS). Ideally, repeated blood samples from the same individual throughout the study would have been a more thorough investigation of Brumation. However, only three individuals were recaptured once during the study which was insufficient repeated sampling.

Lizards were surveyed by employing a single reptile visual encounter survey (Manley et al. 2005) using a 'fixed 2-hr maximum time limit' randomised line transect method in all saltbush plantings and patches and roadside strips of remnant vegetation. Two observers systematically searched neighbouring rows of monocultures of saltbush (approximately 8-10m apart) and 10-m apart transects in roadside and remnant patches at a consistent slow pace in each habitat type, ensuring that both sides of individual saltbushes rows and 10m wide strip between observers in remnants were surveyed thoroughly for lizards. Whilst this method is the most effective at providing unbiased statistical estimates for observable individuals, individuals hidden from view were not sampled. When encountered, blood was processed followed by morphometrics, ectoparasite search and microchipping (PIT tag, TROVAN® ID 100).

Haematological processing

Immediately after capture, blood was taken. For 'severe samples', 0.4mL-0.6mL blood was collected from each individual blood using the caudal tail vein venipuncture with pre-heparinised disposable Terumo® 23G needle/1 mL syringe for adult/subadults (≥ 20 cm) or Terumo® 21G needle /1 mL syringe for juveniles (≤ 20 cm) (Jacobson 1993a) (Department of Environment and Natural Resources Ethics Committee and The University of Sydney Ethics Committee (Approval no. 32/2010). Immediate duplicate blood smears were also prepared and later stained back in the laboratory with Wright-Giesma® stain. Heparinised blood was transferred to a capillary tube and the percent of the blood that was composed of red blood cells or packed cell volume (PCV) was determined using a microcentrifuge ($n=85$) 'Baseline' blood smears were collected by Flinders University staff as part of a 30 year population study at Mt. Mary (BS0) via toe-clipping and prepared using the same technique (Goodfrey, pers. comm. 2010). PCV was not determined for these animals.

Total White Blood Cells counts, differentials and H (heterophil):L (lymphocyte) ratio were enumerated using Carl Zeiss Axio® Imager 2 microscopy. The total number of heterophils was

estimated by counting the number of heterophils present in each of five fields using the 40X objective of the microscope in an area of the blood smear where the red cells first became overlapping. The number of cells per field was then averaged and the average was multiplied by 2,000 to give an estimated number of cells per microlitre. Differential counts were determined by counting 100 white blood cells under 100 X magnification and recording the percentage of heterophils, lymphocytes, monocytes, and other granulocytes. The fraction of heterophils was then divided into the estimated total heterophil count to give the total white blood cell count and this was used to calculate the total monocyte, lymphocyte and other granulocyte total counts. The other granulocyte count included cells that matched the description of basophils seen in other species of reptiles, another cell type that contained clear round vacuoles that appeared to have degranulated, and large round cells with fine basophilic granules. The percentage of polychromatophilic red blood cells was determined by counting the number of polychromatophilic red blood cells per 200 red blood cells. All blood measurements in this study are collectively termed haematology.

Body condition

We used two indices of lizard 'body condition': (1) residual condition index (RCI) and (2) tick load. RCI of body condition has been used previously for sleepy lizards (Smallridge and Bull, 2000), and is widely used for other reptiles (Platenberg and Griffiths, 1999; Lebas and Marshall, 2001; Hoare *et al.* 2006; Moore *et al.* 2007; Connolly and Cree, 2008). RCI has restricted use in this study as calibration curves for sex and other structural characteristics of *T. rugosa* (muscle, bone and fat mass) from different populations throughout its distribution haven't been determined. As indicators of general body condition influencing selection pressure, they are misleading (Green 2001) but in our study do have comparative value for assessing lizard health. Ecoparasitic ticks *Aponomma hydrosauri* and *Amblyomma libatum* infest *T. rugosa* in the study region. High tick loads are known to decrease body condition but not necessarily survival in *T. rugosa* at the Baseline site (Bull & Brzacott 1993).

RCI

RCI was calculated from the residuals of a ranged major axis (RMA) regression between log-transformed body mass and log-transformed body length. It meets the assumptions of linearity ($r = 0.73$) and independence (Green 2001). Lizards were weighed in a vertical position with a cord secured under the forelimb as a harness using a hand-held Pesola scale to the nearest 0.1g. Length was measured with a ruler to the nearest 1mm.

Ecoparasite presence

The presence or absence of ticks for each individual was recorded by searching all surface areas of a lizard's body. Both species of ticks present are highly visible and are commonly found in the ear, the forelimb axillary regions and occasionally on the dorsal surface. Tick load was measured as the number of ticks per individual.

Post mortem examination

During the course of this study, two freshly dead sleepy lizards were found on the road in the Severe study site. These were dissected the same day and representative tissues were fixed in neutral-buffered formalin. Fixed tissues were paraffin-embedded and 4 μm sections were routinely stained with haematoxylin and eosin.

Statistical analyses

Data were explored for normality (Shapiro-Wilks W test), homogeneity of variances (Levene test) and multicollinearity (correlation matrix, Persons r). Fourth root (absolute counts), arcSin (differentials) or \log_{10} (body mass and length) transformations were used to achieve normality and homodescasticity,

when necessary. All exploratory data analyses and tests of differences in means between Baseline and Severe variables were applied using Statistica V7.1 (Statsoft 1985-2005).

Differences in adult environmental health (absolute and differential counts) were examined using a multivariate, one-way nested Type I PERMANCOVA (analysis of covariance) (Anderson 2001, McArdle & Anderson 2001, Anderson & ter Braack 2003) with RCI or tick load as a covariate. We used this approach as the environmental health data were interdependent for each animal. Type I (sequential) analyses was chosen because the covariate(s) are fitted first and the sums of squares are no longer independent of one another. PERMANOVA and not MANOVA analyses were used for reasons published in Anderson and McArdle papers. The influence of habitat type and structural connectivity of the severe sites on body condition and environmental health (absolute counts and differentials) were examined using multivariate, one-way nested Type I PERMANOVA (analysis of variance) as the slope of each covariate was homogeneous ($p > 0.05$). We ran two separate analyses for habitat type and connectivity factors because the nature of historical vegetation clearance meant some levels of the structural connectivity were not represented in each of the levels of habitat type; some cells were empty with no replicates. At the time of publication, the absence of a multivariate Type IV PERMAN(C)OVA also influenced this decision. All analyses were conducted using Primer-E® V6 PERMOVA® add-on (Primer-E Ltd 2009).

Results

Data were collected from a total of 30 adults at the Baseline site in the grazed rangelands and 75 adults and 14 young (juveniles and sub-adults) at the Severe sites in the severely modified cropping landscapes. Two animals in the Baseline site had exceptionally high total white blood cell counts of 39,184 and 74,667 above the normal counts of 3,000 – 15,000 expected for reptiles (Campbell & Ellis 2007; Nevarez 2010). We treated these animals as outliers and they were not included in the study. To maintain orthogonal analyses between Baseline and Severe sites, 28 animals were randomly selected from the Severe site for statistical analysis.

Lizard Health in Baseline and Severe sites

Body condition

Transformed body mass of adults was related to transformed body length ($r = 0.73$, RMA regression $F_{1,54} = 20.94$, $n = 56$, $p \leq 0.001$) (Figure 2). Twenty-three residuals (82%) were within 2 standard deviations of zero and between 2.5 and 3 standard deviations. In the Baseline site, one animal was 295 mm in length but weighed 910 g, exceeding the nearest animal of the same similar size by 85 g possibly due to pregnancy. At the other extreme, a larger animal at 310 mm long and weighed only 525 g which was approximately 200 g lighter than the three individuals of similar size in the Baseline site. Nevertheless, Baseline adults were larger and heavier than those in Severe sites whether the outliers were included or omitted in analyses (Student $t_{length} = 6.11$, d.f. = 54, $n = 56$, $p < 0.0001$; Student $t_{mass} = 8.8$, d.f. = 54, $n = 58$, $p \leq 0.001$). Twenty-one percent (12) of animals weighed > 800 g, 38% (21) between 640 g and 775 g and 23 between 460 g to 600 g.

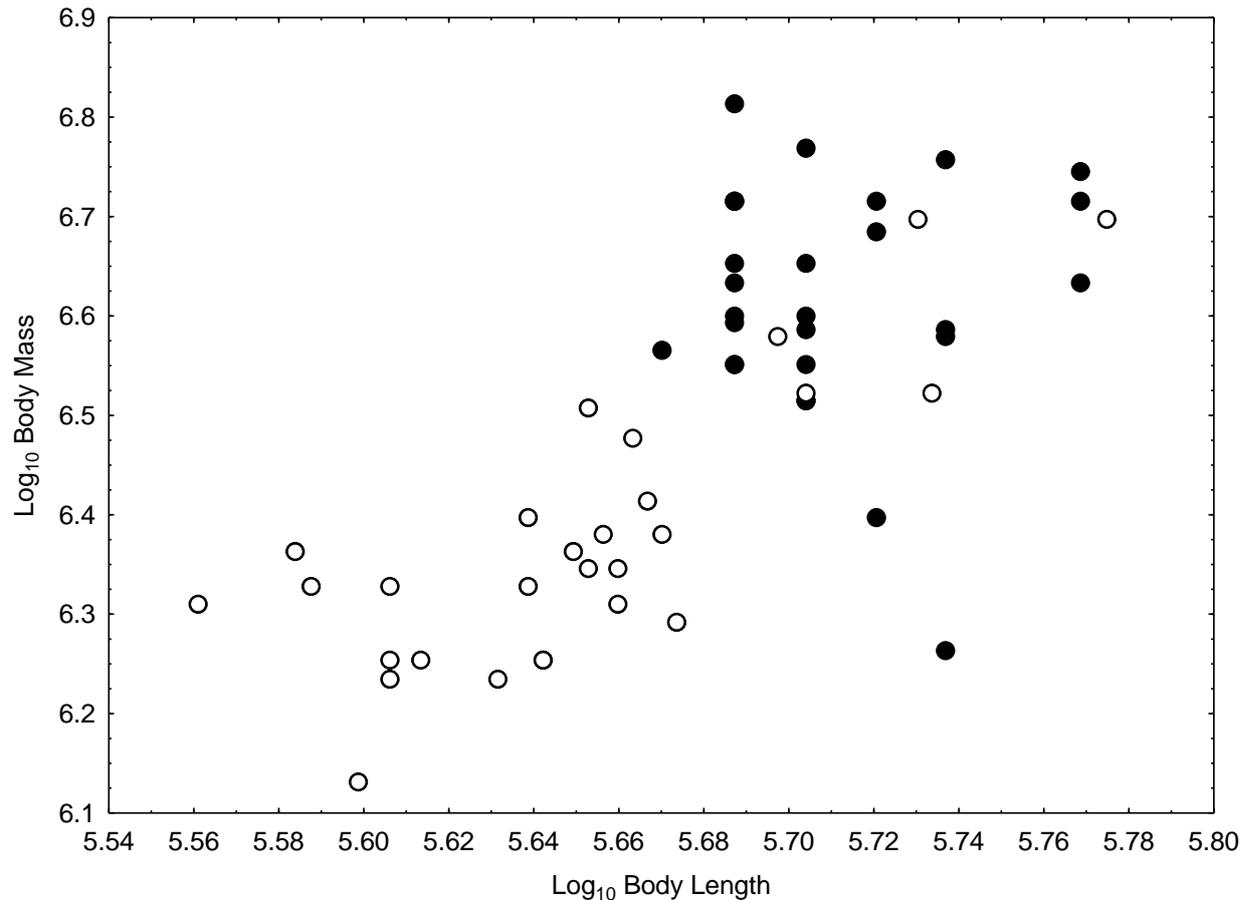


Figure 2. Relationship between $\log_{10}(\text{body mass}+0.1)$ and $\log_{10}(\text{body length}+0.1)$ for wild sleepy lizard *Tiliqua rugosa* in Baseline (solid circles) and Severe (open circles) sites of rangeland and cropland landscapes in southern Australia ($r=0.73$, RMA regression $F_{1,56} = 20.94$, $n=56$, $p \leq 0.001$)

Tick load had a disproportionate influence on adult RCI. At Baseline sites, tick load was noticeably higher and as a consequence RCI significantly decreased as transformed tick load increased ($r = -0.52$; $F_{1,26} = 9.89$, $n = 28$, $p \leq 0.004$). Conversely, adults at Severe sites had a lower tick load and didn't influence RCI (Supplementary Table 1).

Haematology

In all lizards where the percentage of polychromatophilic red blood cells was considered to be abnormally high (>10%), the red blood cell maturation sequence was normal. A consistent gradient of cells similar to what would be present in bone marrow was found. Additionally, cytoplasm staining of the polychromatophilic red blood cells was uniform. Examination of mature red blood cells revealed that the majority of the sleepy lizards from the Severe sites had at least some red blood cells that had circular defects in their cytoplasm. These defects in the cytoplasm varied in diameter, but the diameter was never more than 20% of the length of the cell. Similar defects were not found in the red blood cells collected from animals from the Baseline site.

Average absolute cell counts for all white blood cell categories of adult lizards were greater in the Baseline sites (Table 1) but all values were right skewed (bulk of distribution to the left and stragglers to the right) and indices interdependent (Supplementary Fig. 1).

Table 1. Health indices summarized for individuals of the sleepy lizard *Tiliqua rugosa* at Baseline (and Severe sites located in rangeland and cropland landscapes respectively of the Murray Mallee region in southern Australia.

Health Index	Baseline Mean±SE (N = 28)	Severe Mean±SE (N = 28)
Body Condition		
Residual Condition	0.56±0.2	-0.53±0.1
Body Mass (g)	757.5±16.3	591.8±9.8
Body Length (mm)	301.8±1.4	284.1±1.7
Number of ticks	13.1±6.8	6.5±2.2
Absolute count		
Heterophils	7873.3±1817.4	2360.0±169.8
Lymphocytes	1927.4±376.0	1393.4±110.8
Monocytes	718.2±135.1	543.4±86.3
Other granulocytes	2863.8±417.7	518.6±84.5
Total Blood Count	13735.0±2503.0	4819.8±327.2
Differential counts		
Heterophils (%)	54.7±2.1	49.9±1.8
Lymphocytes (%)	15.8±1.7	29.2±1.3
Monocytes (%)	6.6±1.5	10.7±1.4
Other granulocytes (%)	21.8±1.7	10.2±1.1
H:L ratio	5.6±0.9	1.9±0.2
Polychromasia (%)	1.7±0.4	13.2±1.5

Overall, RCI was related to absolute counts of heterophils and granulocytes; lymphocytes to heterophils and monocytes, granulocytes to monocytes and heterophils (Supplementary Table 1a). These relationships altered for the Baseline site and changed again for the Severe sites (Supplementary Table 1b,c). There was no relationship between H:L ratio (an index of environmental stress) with either tick load (Figure 3a) or RCI (Figure 3b).

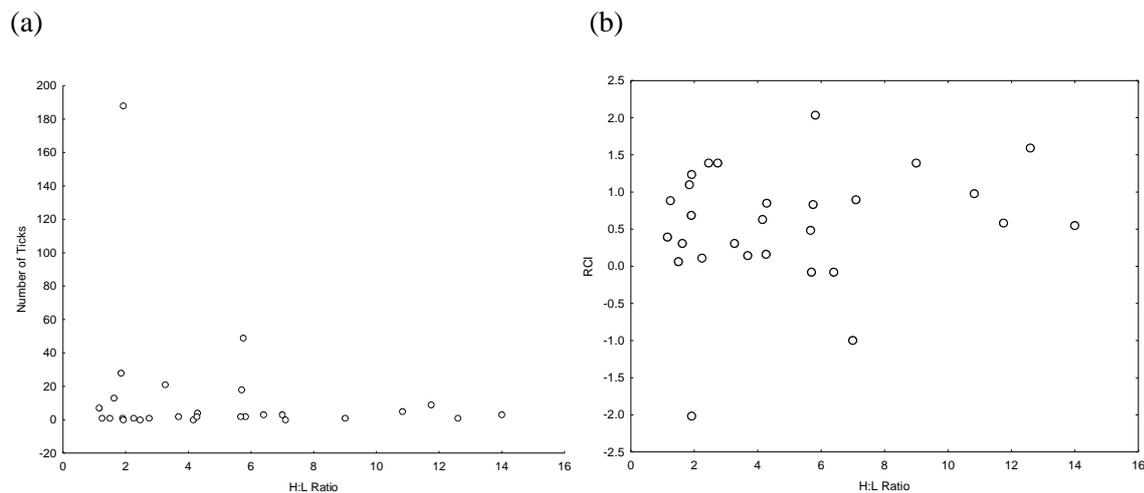


Figure 3. Relationship of heterophil:lymphocyte (H:L) ratio with (a) tick load (Pearson $r = 0.039$, $P > 0.89$, $n = 28$) and Regression Condition Index (RCI) (Pearson $r = -0.16$, $p > 0.40$, $n = 28$) of individuals from Baseline sites in the Murray Mallee region, southern Australia.

Using RCI as the only covariate because unlike that for tick load, individuals were structured into two groups (Supplementary Fig. 2), preliminary analysis indicated there was no interaction between Baseline and Severe sites. The health of lizards (as measured by fourth root transformations of absolute counts of heterophils, lymphophils, monocytes and other granulocytes) differ significantly between Baseline and Severe sites (Pseudo- $F_{1,52} = 15.44$, $p_{(9,970 \text{ permutations})} \leq 0.001$, $n = 56$) despite differences in RCI (Pseudo- $F_{1,52} = 6.18$, $P = 0.003$, $n = 56$). The multivariate dispersion of health indices for adult lizards in Baseline and Severe sites (deviations from each centroid) was not significantly different (PERMDISP $F_{1,54} = 1.67$, $p \leq 0.23$, $n = 56$) indicating that absolute white blood cell values within and between treatments wasn't due to locational differences. Principle components analysis explained the underlying drivers of white blood cell differences. PC1 accounted for much of the variability (56%) with PC2 and PC3 explaining 27 and 14%, i.e. 96% which is atypically high. PC1 was equally weighted by the absolute counts of heterophils and other granulocytes and not lymphocytes and monocytes. PC2 and PC3 were largely weighted by monocytes and lymphocytes absolute counts respectively.

Differential haematology

Average percentage of heterophils was higher in the Baseline than Severe sites whereas the percentage of monocytes, lymphocytes, and other granulocytes were higher in the Severe sites (Table 1).

Overall, tick load decreased RCI and differential monocyte counts (Supplementary Table 2a). H:L ratio was negatively correlated to monocytes while heterophils were negatively correlated to lymphocytes. In the Baseline site, tick load was negatively related to RCI and monocyte differential count, monocytes were related negatively to H:L ratio and heterophils negatively to lymphocytes differentials (Supplementary Table 2b). In Severe sites, RCI weakly increased as the H:L ratio increased and as monocytes increased heterophil different counts decreased (Supplementary Table 2c).

Excluding the differentials of two 'outlier animals', values of H:L ratio, monocytes and other granulocytes were transformed due to right-hand skewness and all except heterophil and polychromasia differentials were interdependent (Supplementary Fig. 3). Exploratory data analyses again indicated that RCI and not tick load was structuring different haematology (Supplementary Fig. 4). Despite there being a significant effect of RCI structuring haematology of lizards in the Baseline and Severe sites (PERMCOVA Pseudo $F_{1,52} = 10.08$, $p_{(9,948 \text{ permutations})} \leq 0.001$, $n = 56$), the differential haematology of adult lizards (measured by interactive percentages of heterophils, lymphocytes, monocytes, other granulocytes and H:L ratio) remained consistently different between Baseline and Severe sites (Pseudo $F_{1,52} = 12.60$, $p_{(9,960 \text{ permutations})} \leq 0.001$, $n = 56$) and this was not due to dispersion in distances from the centroids of the treatment levels, i.e. locational differences ($F_{1,54} = 0.02$, $p \geq 0.8148$, $n = 56$). There was no interaction with RCI and treatment (Pseudo $F_{1,52} = 1.34$, $p_{(9,960 \text{ permutations})} \geq 0.253$, $n = 56$). PC1, PC2 and PC3 principle component axes explained 49%, 28% and 14% (total = 92%) which is also atypically high. Differences among the differential counts of animals were mostly weighted by lymphocyte percentage and no other haematological measures (PC1). PC2 was largely weighted by heterophil percentage and PC3 by polychromasia and monocyte percentages.

Influence of Habitat Complexity on Lizard Health in the Severe Sites

Body Condition

Adult RCI, body mass, body length and transformed adult tick load was statistically similar among habitat types (Table 2, $F_{2,54} = 0.60$, $p \leq 0.5517$, $n = 75$) and structural connectivity levels (Table 2, $F_{3,74} = 1.22$, $P \leq 0.3069$, $n = 75$). The same results were observed for juveniles.

Haematology

PCV was determined for 85 sleepy lizards in the Severe sites. The PCV ranged from 7% to 41% with an average of 25%. Forty two lizards (49%) had PCVs of less than 25%, 16 of these (19% of the total) had PCVs of less than 20% and three animals had PCVs of less than 10%. PCV had no relationship to body condition, other haematological measures or elapsed sampling days since brumation ($p \geq 0.05$). In particular, there was no correlation between haemolytic polychromasia (%) and H:L ratio (Fig. 4).

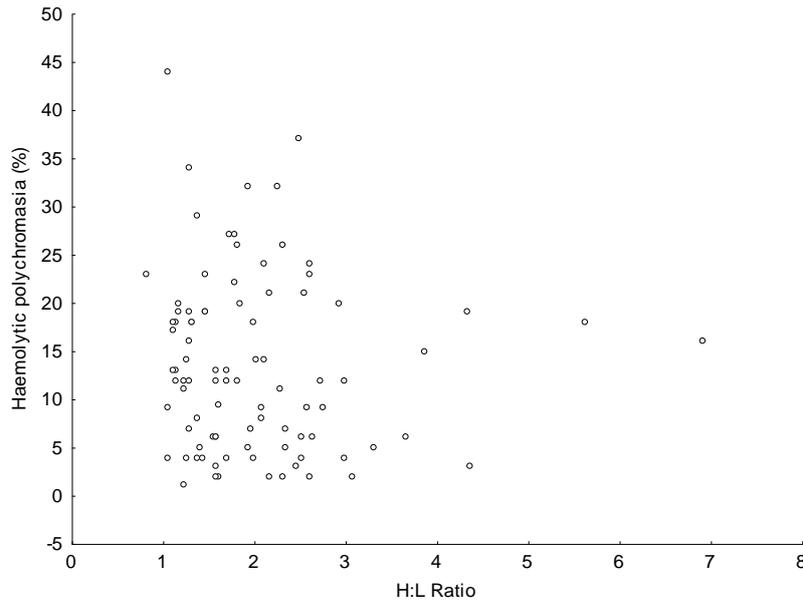


Figure 5. Relationship of heterophil:lymphocyte (H:L) ratio and haemolytic polychromasia (%) of individuals from Severe sites in the Murray Mallee region, southern Australia (Pearson's $r = -0.01$, $P > 0.34$, $n = 75$)

Average absolute blood cell counts and differentials of adult lizards were variable among habitat types and also among connectivity levels, showing no distinct pattern related to habitat complexity as measured by habitat type and structural connectivity (Table 2). Statistical results further confirmed no difference among absolute counts of white blood cells and differential haematology in different habitats (Absolute counts: Pseudo- $F_{2,66} = 0.82$, $p_{(9,685 \text{ permutations})} = 0.6223$, $n = 75$; Differentials: Pseudo- $F_{2,66} = 0.82$, $p_{(9,707 \text{ permutations})} = 0.3366$, $n = 75$) or in sites with different connectivity (Absolute counts: Pseudo- $F_{2,66} = 1.23$, $p_{(9,707 \text{ permutations})} = 0.3367$, $n = 75$; Differentials: Pseudo- $F_{2,66} = 0.70$, $p_{(9,945 \text{ permutations})} = 0.6492$, $n = 75$). Both habitat types and structural connectivity were not influenced by location in the landscape.

With habitat type and structural connectivity combined, absolute counts of heterophils (PC1 - 32%), lymphocytes (PC2 - 26%) and H:L ratio (PC3 - 17%) principle components explained 74% of the total absolute white blood count 'explained variation' among animals. In the case of differentials, heterophil proportion (PC1 - 51%), polychromasia (PC2 - 36%) and lymphocyte proportion (PC3 - 12%) explained 99.5% of the total variation in animal health in the severely modified landscapes.

Table 2. Summary of health indices for factors (habitat use type, structural connectivity, cumulative days since winter ‘hibernation’ and age class) recorded for the severe modification the sleepy lizard *T. rugosa* for the Murray Mallee region, South Australia.

	Adult Habitat Use Type			Adult Structural Connectivity Class			Age Class		
	Native Mallee Remnant mean±SE (N = 27)	Saltbush Planting mean±SE (N = 28)	Cereal /rested field mean±SE (N = 23)	Low mean±SE (N = 24)	Medium mean±SE (N = 25)	High mean±SE (N = 19)	Connected mean±SE (N =10)	Juvenile/ Subadult mean±SE (N = 14)	Adult mean±SE (N = 75)
Body Condition									
RCI	-0.52±0.2	-0.21±0.2	-0.07±0.1	-0.34±0.2	-0.33±0.1	-0.15±0.2	-0.15±0.2	0.26±0.1	7.3±0.2
Body mass (g)	460.78±38.4	519.2±35.1	538.9±28.2	510.0±40.6	483.0±32.5	524.74±40.3	518.7±50.9	225.4±16.5	6.5±2.2
Body length (mm)	257.0±9.0	269.1±6.7	270.9±6.7	267.2±9.2	262.72±7.9	266.3±7.8	267.1±11.3	204.5±4.8	
Number of ticks	1.4±0.5	1.2±0.4	1.2±0.4	1.4±0.6	1.2±0.1	1.1±0.2	1.6±0.1	1.8±0.6	
WBC count									
Heterophils	2800.0±398.3	2400.0±239.9	2400.0 ±231.9	3269.6±466.8	2333.3±230.5	2294.7±253.8	2000.- ±221.1	2142.9±253.7	2538.5±175.5
Lymphocytes	1527.5±193.1	1286.6±111.8	1460.8±154.7	1742.6±222.8	1286.2±119.7	1360±137.1	10180±309.3	956.9±107.5	1416.1±85.8
Monocytes	376.3±79.3	535.4±97.3	368.9±51.6	472.6±87.5	371.1±84.0	480.3±83.4	309.3±74.9	348.4±107.5	423.8±42.7
Other granulocytes	520.3±99.1	477.8±77.7	621.2±231.8	670.8±237.8	467.6±84.4	534.3±106.6	377.4±73.6	92.0±18.0	180.0±42.1
Total	5215.4±588.6	4533.4±363.3	5023.1±568.4	6063.3±759.6	4413.2±347.9	4669.9±438.9	3870.3±263.1		
Differential count									
Heterophils (%)	53.6±2.0	52.9±1.8	49.6±2.1	53.3±2.0	52.4±2.1	50.1±2.2	52.6±3.6	55.3±2.8	51.8±1.0
Lymphocytes (%)	29.1±1.3	28.9±1.3	29.4±1.2	28.7±1.3	29.7±1.5	28.9±1.4	28.9±1.8	25.6±2.3	29.3±0.7
Monocytes (%)	7.2±1.0	8.1±1.1	10.9±1.8	8.3±1.2	7.9±1.5	10.5±1.6	8.1±2.0	8.4±2.0	8.8±0.7
Other granulocytes (%)	10.0±1.1	10.0±2.0	10.1±1.5	9.5±1.4	10.0±1.4	10.5±1.4	10.4±2.3	2.4±0.5	3.1±0.3
H:L ratio	2.0±0.1	2.0±0.2	1.8±0.1	2.0±0.1	2.0±0.2	1.8±0.2	1.9±0.2	2.6±0.4	1.9±0.1
Polychromasia (%)	13.1±2.0	11.4±1.7	15.9±2.0	13.2±2.4	15.0±1.9	13.4±2.2	9.3±2.6	12.9±1.8	13.5±1.1
CUMDAYS	36.2±1.5	36.0±2.3	36.9±1.9	35.5±2.1	36.6±1.6	38.5±2.0	33.9±3.7	31.5±2.7	37.2±1.1

Post Mortem Findings

Microscopic examination of the tissues from two sleepy lizards that had died as the result of road trauma was done. In both animals, the majority of the liver sinusoidal macrophages contained haemosiderin granules that distended their cytoplasm. A locally extensive chronic active steatitis was present in a section of the intracoelomic fat pad from one animal.

Elapsed sampling time since brumation (CUMDAYS)

Sampling duration (CUMDAYS) was not correlated with any of the WBC health indices. However, percent polychromasia of adults decreased significantly with CUMDAYS (Pearson $r = -0.46$, $P = 0.0002$, $n = 75$). All other differential indices had no consistent relationship with sampling duration.

Discussion

Many factors can influence the persistence of animals in severely modified landscapes. Researchers have frequently studied the effects of habitat loss and its degradation using species occurrences, species numbers and genetic measures (Scoble & Lowe 2010). In parallel, but to date in different literature, there have been advances in conservation physiology leading to improvements in the effectiveness of species conservation and management at local scales (Young et al. 2006).

Our study has shown that phenotypically the adult sleepy lizards south of the Murray River barrier are smaller in size (body length) and that their haematological health is compromised in cereal cropping landscapes as shown by the exceptionally high levels of haemolytic anaemia. Although body weights of adults and 'body condition' was lower in the cropping landscapes, the differences in the percentage of polychromatophilic red blood cells, PCV and absolute and differential blood cell counts clearly show the health of lizards in the cereal cropping landscapes was chronically poor for at almost 50% of our study animals. Our findings suggest that the habitat quality of cereal cropping landscapes is degraded and, contrary to our predictions, habitat complexity doesn't appear to influence lizard health. Despite remnant vegetation being retained and native perennial shrubs planted in unproductive cropping parts of fields, it seems such management isn't enough to change the health of wild sleepy lizards in cropping landscapes of our study area. At the time of our study, the relative poor and widespread environmental health of habitats used by wild sleepy lizards in the cropping landscapes of the SAMM was discernible suggestively in 'body condition' but confirmed haematologically. These findings raise a number of questions about the poor health of wildlife living in cereal cropping landscapes. There may also be implications for human health.

The RCI has often been used as an indication of overall physical condition in reptiles (Platenberg and Griffiths 1999; Hoare et al. 2006; Moore et al. 2007; Connelly & Cree 2008). In wild reptiles, an increased value is often inferred as evidence of improved condition. In our study, RCI decreased as tick load increased but increased as heterophils and other granulocyte absolute counts increased. It was however positively related to H:L ratio which is often regarded as a measure of environmental stress (Davis et al. 2009). This result is counterintuitive as 'body condition' is expected to decrease as ecosystem stress increases. This raises doubts about the reliability of the index (*sensu* Green 2001).

Haematological parameters, including red and white blood cell indices, and protein concentrations have been used for decades to assess the health of individual animals including humans. More recently, they have been used to assess the health of populations of domestic and wild animals. Red blood cell indices are used to determine if an animal has a low, normal or high red blood cell volume. Animals with low red cell volumes (anaemia) can be anaemic because of decreased blood production,

blood loss, or red blood cell destruction (haemolysis). In animals with haemolysis or acute blood loss, red cell production is increased and the number of immature red blood cells increases in the circulation. In general, animals experiencing more severe blood loss or haemolysis will have proportionately more immature cells (e.g., regeneration) (Neville 2009). These cells, called polychromatophilic cells, have a cytoplasm that stains with both the basophilic and eosinophilic dyes used in haematological stains and thus the cytoplasm is slightly more blue than that of a fully mature red blood cell.

In this study, we provide strong evidence that many of the sleepy lizards collected in the cereal cropping landscapes were experiencing either a blood loss or red blood cell destructive (haemolytic) anaemia. The percentage of polychromatophilic red blood cells in the lizards from these landscapes was significantly higher on average than the percentages in the less disturbed rangeland landscape and very high values were seen in individual animals in the cropping landscapes, but not the rangeland landscape, indicating that these animals were producing new red blood cells at a greater rate in response to a decrease in red blood cell mass (Saggese 2009). The PCV values obtained in this study also support the conclusion that the animals in the cropping landscapes were experiencing a decrease in red blood cell mass (Nevarez 2010). PCV values were not obtained from rangelands, but recent work with captive sleepy lizards suggests that the normal range for their PCV is between 25 and 35% (Cheryl Moller, pers. Com 2012). This means that just under 50% of the lizards sampled in the severe site were at least mildly anaemic and 16% were moderately anaemic. Three animals had PCVs of 10% or less and these values were approaching those that would be considered lethal. Lizards with elevated percentages of polychromasia and those with PCVs less than 25% were identified across the entire collection time, indicating that the cause of the anaemia seen in these animals was ongoing throughout this period.

Blood loss was initially considered as a cause of the anaemia seen in these lizards. Ectoparasites, such as ticks which were found on these lizards and mosquitoes which would have been present in the environment can cause anaemia in their hosts. The tick burden found on animals from the cropping landscapes, however, was less than that found on the animals from the Baseline site and the animals there were not experiencing anaemia. Mosquito counts in the area during the study period were low, arguing against mosquitoes as a cause of blood loss (Waikarie-Loxton Council Annual Report 2010). Internal parasites can also cause blood loss. However, these were not identified in two lizards that were necropsied. The anticoagulant Pindone 2-(2,2-Dimethyl-1-oxopropyl)indane-1,3-dione is a rodenticide used to bait rabbits with poisoned oats in the cropping landscapes and the sleepy lizards might eat baits containing it. This bait, however, is relatively expensive and is used locally around towns and would not have been used across the entire study area. Likewise, it is typically distributed in the summer (December to March) and not in the spring when this study was conducted (Anonymous 2011).

Three lines of evidence support haemolysis and not chronic blood loss as the cause of the anaemia detected in these lizards. The first is the robust and orderly nature of the regenerative response seen in these lizards. Polychromatophilic red blood cells were generally of normal shape and size and their cytoplasm stained evenly. This type of response is expected in both red cell destructive anaemias and acute blood loss. Chronic blood loss, which would have been more likely in these animals, given the presence of the anaemia over the study period, would have resulted in a different haematological picture (Campbell & Ellis, 2007). With chronic blood loss the lizards would have become iron deficient and the polychromatophilic cells would have exhibited poor or variable cytoplasmic staining, as well as, variation in size and shape. Chronic haemolysis, however, does not result in iron

loss and normal red blood cell regeneration would be expected as was seen in these lizards. The second line of evidence supporting haemolysis as the cause of the anaemia seen in these animals is the data obtained from the post mortems done on two road-killed animals collected during the study. Hemosiderin, a type of iron based pigment that is formed as the result of red blood cell destruction, was found in unusually high concentrations in the liver of these animals. This is the type of physiologic response that would occur if red blood cells were being destroyed and their haemoglobin being recycled. Lastly, red cell membrane defects were observed in animals from the cropland landscapes, but not the rangeland site and steatitis was found in a necropsy specimen from the cropping landscapes. Both these lesions can be caused by the increased presence of free radicals resulting in membrane damage and in the case of red blood cells increased cell destruction.

The causes of haemolytic anaemia are many. They can be immune-mediated where the body produces auto antibodies against an epitope on the surface of the cell. The epitope can be a normal cellular component or the epitope could be a viral, bacterial or parasitic protein, a drug or a toxin that is on the surface of the cell. Blood parasites can induce haemolytic anaemia in reptiles but were not found in either of the study sites (Campbell 2000). Changing climate might also indirectly affect animal health although the rangelands and cropland sites were within 60 km apart in the same rainfall zone. Toxins can also damage the red blood cell making it less flexible resulting in its premature destruction. Immune mediated haemolytic anaemias are generally isolated incidents and it would be very unusual to see them occurring in animals across an entire ecosystem, therefore it is most likely that these animals were experiencing a haemolytic anaemia that was secondary to exposure to a toxin.

The lizards in the cropping landscapes are exposed to a variety of agrochemicals that could potentially act as toxins. Fox and rabbit baiting with the poison 1080 is commonly used in this area and can be applied at any time during the year. The frequency of baiting has not changed significantly in recent years (A. Growdon, NRM Officer Mallee Coorong NRM Group, Department of Environment and Resource Management, South Australia). Mouse baiting with wheat coated with zinc phosphide or wheat pellets containing zinc phosphide, in contrast, increased significantly in 2010 and 2011 because of mouse plagues and these were distributed in the cropping landscapes and not the Baseline site (K. Haebich, Team Leader, Rangelands and Rivers NRM Group, Department of Environment and Resource Management, South Australia). Because of the herbivorous nature of sleepy lizards, it is possible that they would consume these poisoned baits.

Locust plagues also occurred during the study period. Aerial spraying with Green Guard[®] ULV (Becker Underwood Pty. Ltd) was done in November and December of 2010 east of the Baseline site. Green Guard contains fungal spores that kill locust and would not be expected to impact reptiles. Green Guard[®] was not used in the cropland sites, instead ground spraying with the organophosphate chlorpyrifos (Lorsban*500 EC, Dow Agrosiences) was done. Local spraying by landholders with carbaryl, diazinon, fenitrothion, fipronil and various synthetic pyrethroids may have also occurred as indicated in South Australian government advisory fact sheets provided to farmers http://www.pir.sa.gov.au/_data/assets/pdf_file/0014/131054/FS_-_horticulture_and_locusts_2010_Aug.pdf.

Detailed studies on how each of these toxins would affect reptiles individually or in combination are not known. Reptiles are generally considered to be less sensitive to the effects of 1080 as compared to mammals (McIlroy et al. 1985). However, sleepy lizards from South Australia have been found to be more susceptible to the 1080 than sleepy lizards from Western Australia where this compound is found in the natural vegetation (Twigg et al. 1988). Reptiles have also been shown to be susceptible

to zinc phosphate (Aggarwal et al. 1999) and haemolytic anaemia has been demonstrated in a human intoxicated with similar toxin (aluminium phosphate). Reptiles are also susceptible to intoxication with organophosphates, e.g., chlorpyrifos, diazinon, and fenitrothion, and carbamates, e.g. carbaryl. Acute effects would be on the central nervous system, but the effects of low level or chronic exposure are not known. There is, however, a report of a horse developing a haemolytic anaemia after treatment with chlorpyrifos (Steckle et al.1983). Ultimately, the effect of these chemicals on sleepy lizards will require controlled exposure trials.

Absolute and differential white blood cell counts can reveal important information about the health of an animal. Significant increases in the absolute heterophil counts often occur when a reptile is experiencing a sub-acute or chronic inflammatory disease and sudden drops in heterophils can be associated with viral infections and overwhelming bacterial infections. Elevations in monocyte counts occur in reptiles that are experiencing chronic inflammatory disease (Campbell and Ellis, 2007). Two Baseline animals were found to have marked increases in their total white blood cell counts which were largely driven by high total heterophil counts. It is highly likely that these two animals were experiencing a chronic inflammatory, possibly infectious disease. Researchers at the Baseline site have reported signs of infection for some animals over the past 20 years. Similar high total white blood cell counts and high heterophil counts were not observed in the cropland sites.

Heterophil (reptiles, birds and some mammals) to lymphocytes ratios (H:L) and neutrophil (mammal) to lymphocyte ratios have been used to assess stress in individual animals and populations of domestic and wild animals (reviewed in Davis et al.2008). Heterophils increase and lymphocytes decrease resulting in an increased H:L ratio value with the release of glucocorticoid hormones from the adrenals as the result of acute and chronic stressors. Many factors, including concurrent inflammatory diseases and the impact of handling prior to sample collection, however, can impact the H:L ratio in some circumstances making it a less useful measure of long-term environment stress. An example of this is the herring gull that appears to be severely stressed by capture and handling resulting in a rapid increase in the H:L ratio within minutes of capture (Leighton 1984). To date, there are only a very few studies that have looked at H:L ratios in reptiles in relationship to environmental stressors and the results of these studies are inconclusive (reviewed in Davis et al.2008).

Many sleepy lizards in the cropping landscapes study area were experiencing significant stressors, including red blood cell destruction, in some cases a moderate to severe anaemia and possibly loss of condition. It was expected that the H:L ratio would be higher in animals experiencing a decrease in red blood cell mass and animals experiencing poorer body condition than those that were not. This, however, was not the case. H:L ratios were compared as a function of RCI, PCV and percent polychromasia and no reliable correlation could be found. The only evidence at all suggesting the presence of a stress impact on the H:L ratio was the inverse correlation between heterophil percentages and lymphocytes percentages. Given that there was no evidence of inflammatory disease in the animals from the cropping landscapes sites and that the two animals from the rangelands site with evidence of inflammation were removed from the study we must conclude that H:L ratios in this species under these circumstances are not a good measure of environmental stress. Further studies comparing blood corticosterone levels to H:L ratios in this species will be necessary before the value of H:L ratios can be determined.

In contrast to the expected increase in H:L ratio in samples collected from the animals from the cropland sites, H:L ratios were significantly higher in the animals collected from the rangeland site. This was due largely to an absolute increase (and thus greater percentage) of heterophils in the

animals in the rangeland site. Absolute lymphocyte numbers in the two populations did not differ. Absolute monocyte counts did not differ either, although the absolute “other granulocyte” counts were also elevated in the baseline group. Possible explanations for these differences are many and they include a greater prevalence of inflammatory disease in the rangeland population or some sort of suppressive influence on the heterophil and other granulocyte production in the cropland sites. The later is unlikely as substances suppressing granulocyte production would also suppress lymphoid production as their precursors are both rapidly dividing cells. Whether these findings are results of differences in the prevalence of inflammation in these populations or an effect of a toxin, or some other cause will require additional studies.

The analysis of haematological parameters in this study proved valuable. We were able to show that that a population of lizards within a single ecosystem, characterized by its intensive agricultural management, were experiencing a significant impact to their red blood cell mass and this was reflected in body condition. It is most likely that this impact was the result of one or a combination of toxins to which these lizards were exposed. Red blood cell physiology in reptiles is similar to that found in fish, amphibians, birds, and mammals. Therefore it is likely that the cause of the anaemia in the lizards could also be affecting other wildlife in this ecosystem, farm animals and possibly humans. These findings suggest that sleepy lizards may prove important sentinels for assessing ecosystem health.

Future studies can be used to build on these findings. Additional haematological values, such as total plasma protein values those determined by protein electrophoresis, can be used to provide evidence of blood loss and inflammation in these populations. Including plasma biochemistries in the study would provide additional evidence as to whether toxins in the environment were muscle, liver, kidney or pancreatic disease. Collecting samples over the entire period (spring, summer, and autumn) when sleepy lizards are active and correlating this with the timing of application of agricultural chemicals would help to identify the possible causes of the loss in red blood cell mass seen in these animals in this study. Lastly, increased post mortem sampling of sleepy lizards killed on the road, including a broader range of tissues, including bone marrow will help to better identify the disease processes that are occurring in these animals. Tissues from necropsy specimens could also be screened for at least some of the toxins that the sleepy lizards have been exposed.

With over 80 studies clearly indicating that structural connectivity provides additional habitat (Doerr et al. 2010), it was surprising that diminished habitat complexity of the cropping landscapes didn't affect lizard health. Tentative evidence suggests that greater structural connectivity enables movement and gene flow between large patches of habitat and perhaps the cropping landscapes provide sufficient structural connectivity as another study found that there was no genetic population structure in the cropping landscapes (Lancaster et al. in this report). Nevertheless, ‘biodiversity friendly’ farmers are actively planting native perennials for livestock grazing to augment crop productivity and improve structural connectivity for biodiversity (e.g., native perennial grass pastures, Marsh 2008; oil mallee plantings, Short et al. 2009, Smith 2009a, b; native perennial shrub plantings, Collard et al. 2011). An important conclusion from our study that has serious implications for cereal farming is that pest management on farms may be affecting the health of both humans and wildlife. If this is the case, then maintaining the health of humans and wildlife is linked to better management of pesticides. The outputs from this research underline the value of retaining a diversity of wildlife in rural areas as indicators of human health, and as a repository of knowledge concerning the potential impacts of changes in land use.

Acknowledgements

We thank Scott Kuhlmann, Leonie White, KB and other volunteers for helping with lizard sampling and the many landholders who gave us access to their farms. Dr Ken Wallace and reviewers made helpful comments on the manuscript. The work was funded by the Future Farms Industry CRC and the CSIRO.

References

- Aggarwal, P., Handa, R., Wig, N., Biswas, A., Saxena, R., and Y. P. Wali. (1999) Intravascular hemolysis in aluminium phosphate poisoning. *American Journal of Emerging Medicine*. 17:488-489.
- Anonymous (2011) Mallee Baitlayer Operating Instructions. Update **13** issue 3
<http://www.malleefutures.org.au/files/update14.pdf>
- Anderson, M.J. 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology* 26: 32-46.
- Anderson, M.J. and ter Braak, C.J.F. (2003). Permutation tests for multi-factorial analysis of variance. *Journal of Statistical Computation and Simulation* 73: 85-113.
- Bock, C.E. and Z.F. Jones. (2004). Avian habitat evaluation: should counting birds count?
- Bull, C. M. (1987). A Population Study of the Viviparous Australian Lizard, *Trachydosaurus rugosus* (Scincidae). *Copeia*, 1987:749-757.
- Bull, C.M. (1994). 'Population dynamics and pair fidelity in Sleepy Lizards', in *Lizard ecology: historical and Experimental Perspectives*, eds LJ Vitt & ER Plankal, Princeton University Press, Princeton, pp. 159-164.
- Bull, C.M. (1995). Population ecology of the Sleepy Lizard, *Tiliqua rugosa*, at My Mary, South Australia', *Australian Journal of Ecology* 20: 393-402.
- Bull, CM & Burzacott, D. (1993). 'The impact of tick load on the fitness of their lizard hosts', *Oecologia* 96:415-419.
- Campbell TW. (2000) Hemoparasites. In Reptile Medicine and Surgery (ed 2) (Ed. Mader D. R). St Louis, MO, Saunders/Elsevier, pp 601-805, 2000
- Campbell TW and Ellis C. 2007. Hematology of Reptiles. In Avian & Exotic Animal Haematology & Cytology. Backwell Publishing Ames Iowa USA pp 51-82.
- Collard, S.J., Fisher, A.M. and D.J. McKenna. (2011). Planted saltbush (*Atriplex nummularia*) and its value for birds in farming landscapes of the South Australian Murray Mallee. *Ecological Management and Restoration* 12, 38-46.
- Connolly, J.D., Cree, A. (2008). Risks of a late start to captive management for conservation: Phenotypic differences between wild and captive individuals of a viviparous endangered skink (*Oligosoma otagense*). *Biological Conservation* 141: 1283-1292
- Davis, A.K., Maney, D.L. and J.C. Maerz. (2008), The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. *Functional Ecology*, 22:760-772.
- Doerr, V.A.J., Doerr, E.D., Davis, M.J. (2010). Does structural connectivity facilitate dispersal of native species in Australia's fragmented terrestrial landscapes? Systematic Review No. 44, Collaboration for Environmental Evidence, pp.71.
- Fischer, J. and D. B. Lindenmayer (2007). Landscape modification and habitat fragmentation: a synthesis. *Global Ecology and Biogeography* 16: 265-280.
- Green, A.J. (2001). Mass/length residuals: measures of body condition or generators of spurious results? *Ecology* 82:1473-1483.
- Hall, L.S., Krausman, P.R. and M.L. Morrison. (1997). The Habitat Concept and a Plea for Standard Terminology. *Wildlife Society Bulletin*, 25: 173-182.
- Hoare, J.M., Pledger, S., Keall, S.N., Nelson, N.J., Mitchell, N.J. and Daugherty, C.H. (2006). Conservation implications of a long-term decline in body condition of the Brothers Island tuatara (*Sphenodon punctatus*). *Animal Conservation* 9, 456-462.

- Homyack, J.A. (2010). Evaluating habitat quality of vertebrates using conservation physiology tools. *Wildlife Research* 37: 332-342
- Jacobson, E.R. (1993). 'Blood collection techniques in reptiles: laboratory investigations', in *Zoo and Wild Animal Medicine: Current Therapy 3*, ed Fowler ME, WB Saunders, Philadelphia.
- Kerr, G.D. and C.M. Bull. (2004). Microhabitat use by the Scincid Lizard *Tiliqua rugosa*: exploiting natural temperature gradients beneath plant canopies. *Journal of Herpetology*, 38: 536-545.
- Kerr, G.D., Bull, C.M., and D.A. Burzacott. (2003). Refuge sites used by the scincid lizard *Tiliqua rugosa*. *Austral Ecology*, 28: 152-160.
- Lebas, N.R. and Marshall, N.J. (2001). No evidence of female choice for a condition-dependent trait in the agamid lizard, *Ctenophorus ornatus*. *Behaviour* 138, 965-980.
- Leighton FA (1984) The toxicity of ingested crude petroleum oil to marine birds: pathology and pathogenesis. PhD thesis, Cornell University, Ithaca, NY
- Lindenmayer, D.B. and J. Fischer. (2006) *Habitat Fragmentation and Landscape Change: an Ecological and Conservation Synthesis*. Island Press, Washington, D.C.
- Manley, P.N, Van Horne, B., Roth, J.K., Zielinski, W.K., McKenzie, M.M., Weller, T.J., Weckerly, F.W. and C Vojta. (2005). *Multiple Species Inventory and Monitoring Technical Guide*. Last accessed 22/10/2010.
http://www.fs.fed.us/psw/programs/snrc/featured_topics/msim/documents/msim_preprint.pdf
- Marsh, D. (2008). Farming from first principles. In: *Integrating Conservation and Production* (eds. Lefroy E.C., Bailey, K., Unwin, G., & Norton, .T.W.) pp. 55-62. CSIRO Publishing, Collingwood.
- McArdle, B.H. & M.J. Anderson. (2001). Fitting multivariate models to community data: a comment on distance-based redundancy analysis. *Ecology* 82: 290-297.
- McIlroy, J. C., King, D. R. and A. J. Oliver. (1985) The sensitivity of Australian animals to 1080 poison VIII.* Amphibians and reptiles. *Australian Wildlife Research*, 12, 113-18.
- McIntyre, S., McIvor, J.G. and K.M. Heard. (eds) (2002) *Managing and Conserving Grassy Woodlands*. CSIRO Publishing, Melbourne.
- Menninger, H.L. and M.A. Palmer. (2006) Restoring ecological communities: from theory to practice. In: *Foundations of Restoration Ecology* (eds D.A. Falk, M.A. Palmer & J.B.Zedler) pp. 88-112. Island Press, Washington DC.
- Moore, J.A., Hoare, J.M., Daugherty, C.H. and Nelson, N.J. (2007). Waiting reveals waning weight: Monitoring over 54 years shows a decline in body condition of a long-lived reptile (tuatara, *Sphenodon punctatus*). *Biological Conservation* 135, 181-188.
- Morton, S.R., Hoegh-Guldberg, O., Lindenmayer, D.B., Harriss Olson, M., Hughes, M.T., McCulloch, M.T., McIntyre, S., Nix, H.A., Prober, S.M., Saunders, D.A., Anderson, A.N., Burgman, M.A., Lefroy, E.C., Lonsdale, W.M., Lowe, I., McMichael, A.J, Parslow, J.S, Steffen, W., Williams, J.E., Woinarski, J.C.Z. (2009) The Big Ecological Questions Inhibiting Effective Environmental Management in Australia. *Austral Ecology* 34, 1–9.
- Nevarez, J.G. (2010). I got blood – now what? Proceedings of North American Veterinary Conference, 10-16 January 2010, Orlando, Florida.
- Neville, H. (2009). Diagnosis of nontraumatic blood loss in birds and reptiles.
<http://doi:10.1053/j.jepm.2009.04.011>
- Petney, T.N, Bull C.M. (1984). 'Microhabitat selection by two reptile ticks at their parapatric boundary' *Australian Journal of Ecology*, vol 9, pp. 233-239.
- Platenberg, R.J. and Griffiths, R.A. (1999). Translocation of slow-worms (*Anguis fragilis*) as a mitigation strategy: a case study from south-east England. *Biological Conservation* 90, 125-132.
- Prober, S.M. and F.P. Smith. (2009). Enhancing biodiversity persistence in intensively-used agricultural landscapes: a synthesis of 30 years of research in the Western Australian wheatbelt. *Agriculture Ecosystems and Environment* 132, 173-91.
- Romero, L.M. (2004). Physiological stress in ecology: lessons from biomedical research. *Trends in Ecology & Evolution*, 19: 249–255.

- Saggese, M.D.(2009). 'Clinical Approach to the Anaemic reptile', *Journal of Exotic Pet Medicine* 18:98-111.
- Saunders, D. A., Hobbs, R. J. and P.R. Ehrlich. (eds) (1993) *Nature Conservation 3: Reconstruction of Fragmented Ecosystems, Global and Regional Perspectives*. Surrey Beatty & Sons, Sydney.
- Scoble, J., Lowe, A.J. (2010). A case for incorporating landscape genetics into species distribution modelling to improve climate adaptation and conservation planning . *Diversity and Distributions* 16:343-353.
- Short, M., Smith, F. P. and E. van Etten. (2009). Oil mallees provide foraging habitat for the Western Pygmy Possum (*Cercartetus concinnus*) in the wheatbelt of Western Australia. *Ecological Management & Restoration* 10, 233-236.
- Smallridge, C.J. and Bull C.M. (2000). Prevalence and intensity of the blood parasite *Hemolivia mariae* in a field population of the skink *Tiliqua rugosa*. *Parasitology Research* 86, 655 – 660.
- Smith, F.P. (2009a). Bird activity in oil mallee plantings in the wheatbelt of Western Australia. *Ecological Management & Restoration* 10, 236–238.
- Smith, F.P. (2009b). Assessing the habitat quality of oil mallees and other planted farmland vegetation with reference to natural woodland. *Ecological Management and Restoration* 10, 217-227.
- Steckel R. R.; Kazacos K. R.; Harrington D. D.; et al. (1983) Equine pulmonary habronemiasis with acute hemolytic anemia resulting from organophosphate treatment. *Equine Practice* 5, 35-39
- Stokes C.J. and S.M. Howden. (eds) (2008). *An overview of climate change adaptation in Australian primary industries - impacts, options and priorities*. Prepared for Land and Water Australia by the CSIRO Climate Adaptation National Research Flagship. CSIRO, Canberra.
- Tiver, F. and M.H. Andrew. (1997). Relative effects of herbivory by sheep, rabbits, goats and kangaroos on recruitment and regeneration of shrubs and trees in eastern South Australia. *Journal of Applied Ecology* 34:903-914.
- Twigg, L. E., King, D. R., and A. J. Bradley. (1988) The effect of sodium monofluoroacetate on plasma testosterone concentration in *Tiliqua rogersi* (Gray). *Compendium Biochemistry and Physiology*. 91C: 343-347.
- Van Horne, B. (1983). Density as a misleading indicator of habitat quality. *Journal of Wildlife Management*, 47:893-901.
- Wallace, K.J. (2012). Values: drivers for planning biodiversity management. *Environmental Science & Policy* 17:1-11.
- Wikelski, M., Cooke, S.J. (2006). Conservation physiology. *Trends in Ecology & Evolution*, 21:38-46.
- Willoughby, N.(2006). A Vegetation Strategy for the Murray Mallee. Towards a Habitat Restoration Strategy for the Murray Mallee. South Australia.
- Wilson, K., Wilson K, Pressey RL, Newton A, Burgman M, Possingham H, Weston C. (2005). Measuring and incorporating vulnerability into conservation planning. *Environmental Management* 35:527-43.
- Young, J.L., Bornik, Z.B., Marcotte, M.L., Charlie, K.N., Wagner, G.N., Hinch, S.G. & Cooke, S.J. (2006). Integrating physiology and life history to improve fisheries management and conservation. *Fish and Fisheries* 7:262–283.

(a) All animals (n = 56)							
Variable	Monocytes	Granulocytes	Heterophils	Lymphocytes	Total WBC	RCI	Tick Load
Monocytes	1.0000						
	p= ---						
Granulocytes	.3115	1.0000					
	p=.019	p= ---					
Heterophils	.1603	.8145	1.0000				
	p=.238	p=.000	p= ---				
Lymphocytes	.4681	.3107	.3256	1.0000			
	p=.000	p=.020	p=.014	p= ---			
Total WBC	.2915	.8019	.9035	.4953	1.0000		
	p=.029	p=.000	p=0.000	p=.000	p= ---		
RCI	.0453	.4012	.3742	-.1637	.3261	1.0000	
	p=.740	p=.002	p=.004	p=.228	p=.014	p= ---	
Tick Load	-.0889	-.0133	-.0696	.0041	-.0812	-.3205	1.0000
	p=.514	p=.922	p=.610	p=.976	p=.552	p=.016	p= ---

(b) Baseline site (n = 28)							
Variable	Monocytes	Granulocytes	Heterophils	Lymphocytes	Total WBC	RCI	Tick Load
Monocytes	1.0000						
	p= ---						
Granulocytes	.4507	1.0000					
	p=.016	p= ---					
Heterophils	.2325	.7256	1.0000				
	p=.234	p=.000	p= ---				
Lymphocytes	.5326	.4580	.4297	1.0000			
	p=.004	p=.014	p=.022	p= ---			
Total WBC	.2737	.6859	.8404	.5660	1.0000		
	p=.159	p=.000	p=.000	p=.002	p= ---		
RCI	.1317	-.0392	-.2312	-.2909	-.2062	1.0000	
	p=.504	p=.843	p=.236	p=.133	p=.292	p= ---	
Tick Load	-.1871	.0197	-.0907	-.0048	-.1141	-.5159	1.0000
	p=.341	p=.921	p=.646	p=.981	p=.563	p=.005	p= ---

(c) Severe sites (n=28)							
Variable	Monocytes	Granulocytes	Heterophils	Lymphocytes	Total WBC	RCI	Tick Load
Monocytes	1.0000						
	p= ---						
Granulocytes	.2875	1.0000					
	p=.130	p= ---					
Heterophils	.1231	.7204	1.0000				
	p=.525	p=.000	p= ---				
Lymphocytes	.4063	.3021	.2938	1.0000			
	p=.029	p=.111	p=.122	p= ---			
Total WBC	.3806	.7822	.9310	.5562	1.0000		
	p=.042	p=.000	p=.000	p=.002	p= ---		
RCI	-.1448	.0473	.3514	-.0596	.2722	1.0000	
	p=.454	p=.808	p=.062	p=.759	p=.153	p= ---	
Tick Load	.0043	-.2057	-.1957	.0153	-.1800	-.3447	1.0000
	p=.982	p=.284	p=.309	p=.937	p=.350	p=.067	p= ---

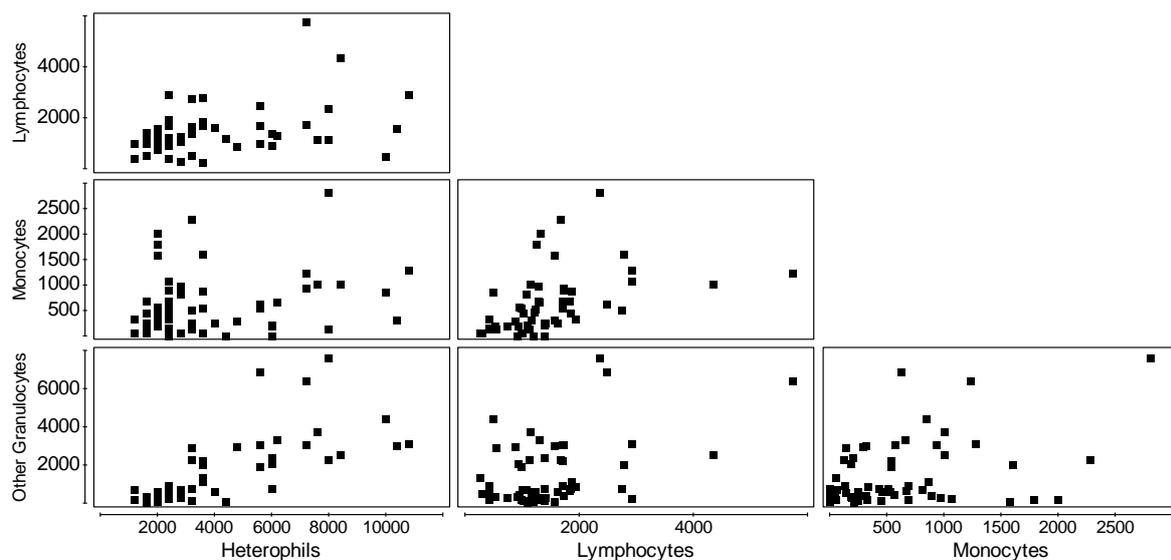
Supplementary Table 1. Relationships among transformed 'body condition and tick load and absolute counts haematological indices for (a) all sites combined, (b) the Baseline and (c) Severe and. Highlighted correlations are significant at $p \leq 0.05$. WBC – white blood cells, RCI -residual condition index.

Supplementary Table 2. Relationships among transformed body condition and tick load and differential counts of haematological indices for (a) all sites combined, (b) the Baseline and (c) Severe and. Highlighted correlations are significant at $p \leq 0.05$. WBC – white blood cells, RCI -residual condition index.

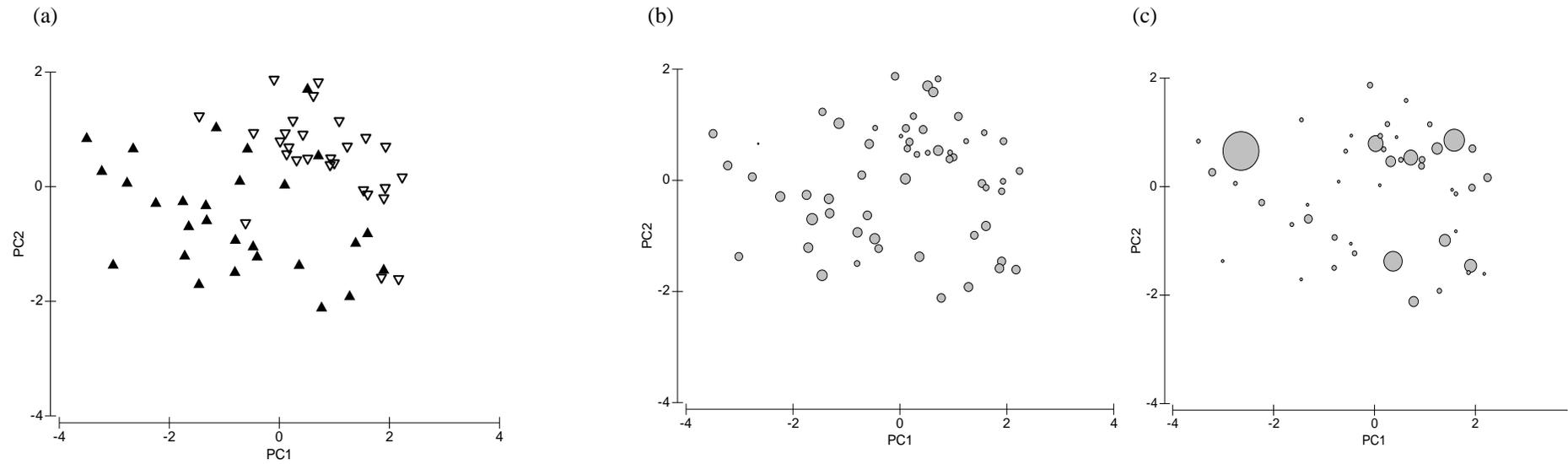
(a) All animals (n =56)								
Variable	Heterophils	Lymphocytes	Monocytes	Granulocytes	H:L ratio	Polychromasia	Tick Load	RCI
Heterophils	1.0000							
	p= ---							
Lymphocytes	-.5302	1.0000						
	p=.004	p= ---						
Monocytes	-.0261	.4312	1.0000					
	p=.895	p=.022	p= ---					
Granulocytes	.0189	-.1582	-.1928	1.0000				
	p=.924	p=.421	p=.326	p= ---				
H:L ratio	.0137	-.1864	-.5316	.1087	1.0000			
	p=.945	p=.342	p=.004	p=.582	p= ---			
Polychromasia	.0280	-.1931	-.1086	.2424	-.2302	1.0000		
	p=.887	p=.325	p=.582	p=.214	p=.239	p= ---		
Tick Load	-.2524	.0315	-.3981	.0812	.1645	.2107	1.0000	
	p=.195	p=.873	p=.036	p=.681	p=.403	p=.282	p= ---	
RCI	.0995	-.1631	.2781	.1928	.0312	-.0620	-.5250	1.0000
	p=.615	p=.407	p=.152	p=.326	p=.875	p=.754	p=.004	p= ---
(b) Baseline site (n = 28)								
Variable	Heterophils	Lymphocytes	Monocytes	Granulocytes	H:L ratio	Polychromasia	Tick Load	RCI
Heterophils	1.0000							
	p= ---							
Lymphocytes	-.5302	1.0000						
	p=.004	p= ---						
Monocytes	-.0261	.4312	1.0000					
	p=.895	p=.022	p= ---					
Granulocytes	.0189	-.1582	-.1928	1.0000				
	p=.924	p=.421	p=.326	p= ---				
H:L ratio	.0137	-.1864	-.5316	.1087	1.0000			
	p=.945	p=.342	p=.004	p=.582	p= ---			
Polychromasia	.0280	-.1931	-.1086	.2424	-.2302	1.0000		
	p=.887	p=.325	p=.582	p=.214	p=.239	p= ---		
Tick Load	-.2524	.0315	-.3981	.0812	.1645	.2107	1.0000	
	p=.195	p=.873	p=.036	p=.681	p=.403	p=.282	p= ---	
RCI	.0995	-.1631	.2781	.1928	.0312	-.0620	-.5250	1.0000
	p=.615	p=.407	p=.152	p=.326	p=.875	p=.754	p=.004	p= ---

Variable	(c) Severe sites (n =28)							
	Heterophils	Lymphocytes	Monocytes	Granulocytes	H:L ratio	Polychromasia	Tick Load	RCI
Heterophils	1.0000							
	p= ---							
Lymphocytes	-0.5647	1.0000						
	p=.002	p= ---						
Monocytes	-0.5900	-0.0373	1.0000					
	p=.001	p=.851	p= ---					
Granulocytes	-0.2096	-0.2144	-0.2892	1.0000				
	p=.284	p=.273	p=.135	p= ---				
H:L ratio	0.7540	-0.8532	-0.2870	0.1586	1.0000			
	p=.000	p=.000	p=.139	p=.420	p= ---			
Polychromasia	-0.0519	0.2467	-0.2696	0.1353	-0.0753	1.0000		
	p=.793	p=.206	p=.165	p=.492	p=.703	p= ---		
Tick Load	-0.0915	0.2618	0.0241	-0.2127	-0.2980	-0.0881	1.0000	
	p=.643	p=.178	p=.903	p=.277	p=.124	p=.656	p= ---	
RCI	0.3076	-0.3472	0.0983	-0.1834	0.4038	0.0294	-0.3408	1.0000
	p=.111	p=.070	p=.619	p=.350	p=.033	p=.882	p=.076	p= ---

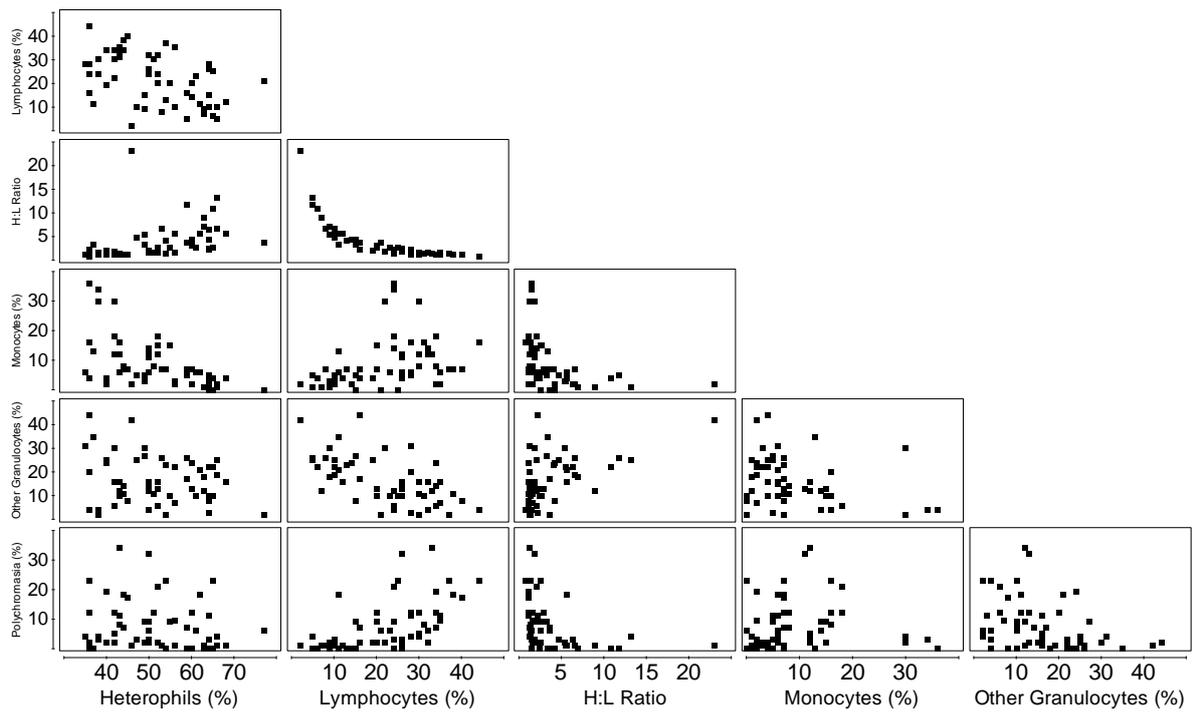
Supplementary Table 2. cont.



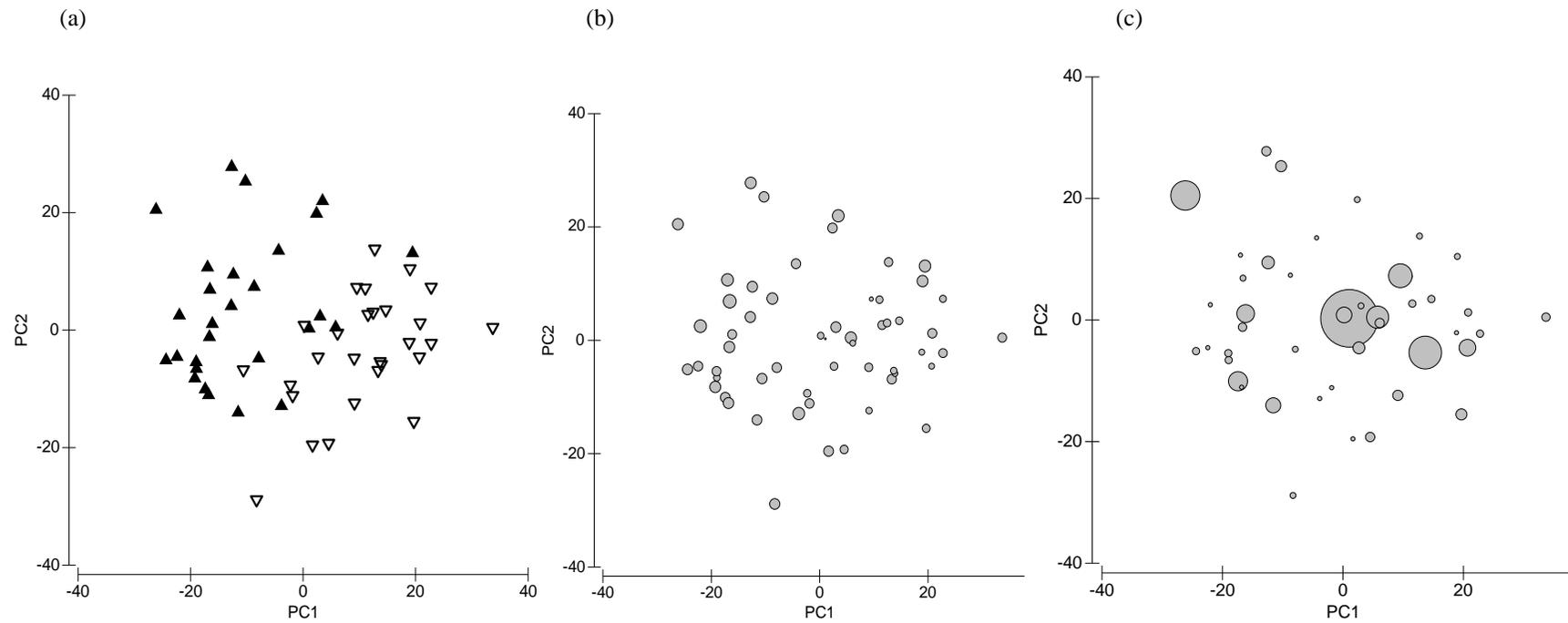
Supplementary Figure 1. Absolute counts of white blood cells of adult *Tiliqua rugosa* (n = 56) combined for both Baseline and Severe sites in the Murray Mallee region, southern Australia.



Supplementary Figure 2. (a) Principal Components ordination of absolute count of white blood cells with (b) regression condition index (RCI) and (c) tick load superimposed, showing separate groupings for RCI and not for tick load on *Tiliqua rugosa* individuals. (PC1 – 55.6% of total variation explained, PC2 – 26.7% of total variation explained. Baseline sites – filled triangles in (a), mostly medium and large bubbles in (b) and Severe sites – unfilled triangles in (a), mostly tiny and small bubbles in (b). Treatments not distinguishable in (c).



Supplementary Figure 3. Differential counts of white blood cells of adult *Tiliqua rugosa* (n = 56) combined for both Baseline and Severe sites in the Murray Mallee region, southern Australia.



Supplementary Figure 4. (a) Principal Components ordination of differential count of white blood cells with (b) regression condition index (RCI) and (c) tick load superimposed, showing separate groupings for RCI and not for tick load on *Tiliqua rugosa* individuals. (PC1 – 55.6% of total variation explained, PC2 – 26.7% of total variation explained. Baseline sites – filled triangles in (a), mostly medium and large bubbles in (b) and Severe sites – unfilled triangles in (a), mostly tiny and small bubbles in (b). Treatments not distinguishable in (c).

