The behavioural ecology of the thick-billed grasswren

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(MSc Biology)

A thesis submitted in fulfilment of the requirements for the

Degree of Doctor of Philosophy

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Cover image: Typical thick-billed grasswren habitat with chenopod shrubs at Witchelina Nature Reserve in South Australia, and (inset) a thick-billed grasswren (*Amytornis modestus raglessi)* in the hand. Photos by Marina Louter.

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Thesis Summary

This study uses the conservation behaviour framework to understand the ecological context of nesting behaviour and fledging success in a previously unstudied threatened songbird: the thick-billed grasswren (*Amytornis modestus raglessi*). Data were collected from 2012 to 2015 at Witchelina Nature Reserve, a former pastoral sheep station, located in the arid rangelands of central South Australia. In 2010, the Nature Foundation of South Australia purchased the 124,000 ha reserve and removed all livestock. The re-vegetation and recovery of overgrazed land to 'natural' habitat is an opportunity for flora and fauna conservation. This study provides a test of the efficacy of habitat restoration from grazing as a conservation tool for the persistence of a threatened songbird species.

In this thesis I address four main issues in relation to vegetation cover: (1) grazing history and habitat use, (2) predation risk and predation outcome, (3) insect abundance and parental care (including prey size), and (4) genetic relatedness within groups. I radio-tracked adult males to show that the average home range size of thick-billed grasswrens is large (8 ha) and did not differ across the study site. I found that active grasswren territories were more likely located at sites with low prior grazing intensity; vegetation cover and insect abundance were significantly associated with grazing history. I measured predation risk at Witchelina Nature Reserve using artificial nests: rodent predation risk was ~10 %, avian predation risk was ~12 %, and observed predation at natural nests was ~13 %. Exposed artificial nests with little vegetation concealment were depredated by avian predators more often; at natural grasswren nests, active nests had dense vegetation cover and vegetation concealment did not predict predation (suggesting snakes and rodents as main predators). Predation at natural nests was low; of 47 thick-billed grasswren nests, most (74 %) produced fledglings. Vegetation cover predicted parental feeding at nests; parents at sites with dense cover fed nestlings more frequently. Video recordings of feeding at nest showed that grasshoppers and caterpillars were commonly fed to developing nestlings. Finally, this study shows that thick-billed grasswrens are cooperative breeders, with up to two helpers observed feeding at 50 % of video-monitored nests. Using ddRAD-seq genetic analysis we established that helpers were related to the attending pair. We did not find evidence for extra-pair paternity in offspring of five thick-billed grasswren groups.

In conclusion, vegetation cover was significantly associated with grazing history: areas with high previous grazing history had little vegetation cover and few arthropods. Vegetation cover was related to key parameters of nesting success of the thick-billed grasswren, including presence of active territories, parental feeding activity, and avian nest predation. Collectively, the results of this research can be directly implemented in focused recovery plans for the vulnerable thick-billed grasswren. Conservation of habitat with dense vegetation cover and abundance of preferred chenopod nesting shrubs *Maireana pyramidata* and *Maireana astrotricha* throughout the range of the thick-billed grasswren is recommended as a key management approach to conserve this previously unstudied arid zone species under threat.

Declaration

'I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.'

Louter

Maria Carolina Johanna Louter

03.07.2016

Acknowledgements

This thesis could not have been written without the encouraging help and guidance of my main supervisor Sonia Kleindorfer. She not only gave me the opportunity to join her Birdlab at Flinders University, but also guided me throughout my entire PhD journey. Her contagious enthusiasm and her positive outlook on life never failed to inspire me, and she kick-started me back into action when needed. Her patience, reviews and keenness to exchange knowledge greatly increased the quality of this work and more importantly, contributed so very much to my professional development. Sonia’s way of translating scientific findings into a captivating and clear story, plus her talent to then passionately communicate science, has made a lasting mark on my way of thinking about science and communication, and the role we can play in this ourselves. Undoubtedly she has made a lasting mark on my curriculum vitae for which I am immensely grateful.

I would also like to recognize Jeremy Robertson, who introduced me to the world of bird song, taught me how to use acoustic field gear, and explained how to analyse bird song data digitally using complicated software. Mike Gardner and Tessa Bradford were of invaluable help with genetic work in the lab.

For the financial assistance of this project I am greatly indebted to the Nature Foundation of South Australia, who not only secured the main funding for my research, but also permitted use of Witchelina Nature Reserve as a research site, and provided lodging during the many visits we made to this beautiful property in the middle of the stunning arid rangeland of South Australia. I am very thankful to BirdsSA, Birdlife Australia, and the Field Naturalists Society of South Australia for funding various parts

of my research. I also gratefully acknowledge the financial support of a Flinders University Research Scholarship, of which without I would now certainly be broke.

It goes without saying that I could have not done this project alone. There are so many people that helped me in all kind of different ways, and without them I would definitely have not been able to finish my (at times painfully slow) PhD journey. It is my intension to sincerely thank each and every one of you, and my sincere apologies to those of you that I unintentionally forgot to mention.

A big thank you to NFSA and all the managers of Witchelina Nature Reserve, for their assistance and logistical support during the numerous field trips we undertook over the past 4 years; It was always a pleasure to catch up with you and you made me feel like Witchelina was my second home. I can safely say that without your help I would probably still be out there in the desert somewhere, on a wet muddy dirt track, the Flinders University 4-WD car bogged belly-deep into the red-coloured mud, having only a very little shovel to dig it out. You came to the rescue more than once, and situations like these made my PhD really feel like an adventure, moments I will not forget.

I was very fortunate to have many helpers in the field, to assist me with all the stuff I needed (or perhaps just wanted to?) collect. A special thank you to Valeria, Wikke, Madeline, Petra, Simone, Paul, Sara, Andrea, Bianca, and Georgie, who all helped out with various fieldwork tasks. All of these people made my time at Witchelina a very special and an unforgettable experience.

Three fellow Birdlabbers were always there for support: Katharina, Christine and Amy. Our chat sessions, discussions, phone calls, coffees, lunch breaks, get-togethers after work, were (and still are) so much fun. Thank you also very much for listening to me, for giving me advice throughout my project, for cheering me up when things got tough, and for putting up with me in general. I can't wait to find out where our PhD's will take us, but wherever it may be, I sincerely hope we can remain close friends!

Last, but not least I would like to sincerely thank Scott. You have been nothing but supportive throughout my entire PhD, never doubting my capacity to conduct research and "be a scientist", instead always re-affirming your belief in me, and in my abilities to succeed. Your incredible patience, your assistance in the field, your everlasting tolerance of my long hours at work, and your shoulder to lean on when things got me down have made this all possible. I'm sure the finishing of this thesis is a huge relief to you, just as much as it is for me.

Statement of Authorship/Contribution and Acknowledgment

This thesis is a series of four papers (Chapters 2-5), preceded by an introductory chapter (Chapter 1) and concluded with a general discussion (Chapter 6). The papers presented in this thesis arising from my research conducted for the degree of Doctor of Philosophy are in draft form for publication. They are presented here as self-contained papers, but have been slightly modified to satisfy the style of this thesis.

Much of the fieldwork was conducted with the assistance of volunteers working under my supervision. These volunteers contributed labour in the field and played no part in the writing of manuscripts, data analysis, or interpretation of the results. In particular, volunteers helped with insect fauna sampling and insect identification (Chapter 2), with collection of predation data (Chapter 3), with nest searching and monitoring (Chapter 4 & 5), and the collection of vegetation measurements, where data collection required two or more people for efficiency (roles of observers and recorders; Chapter 2 & 4). Volunteers also assisted with bird capture by helping set-up mist-nets, and with processing by recording morphological data.

All research procedures reported in this thesis follow the guidelines for the use of animals in research (Flinders University) and were approved by the Animal Welfare Committee of Flinders University (permits: E325, E312 and E385), and by the Australian Bird and Bat Banding Scheme (ABBBS authority: 2601 and 3100).

Chapters 1 & 6: ML

Chapter 2:

Data collection: ML, ALS

Statistical analyses: ML, SK

Manuscript writing: ML, SL, SK

Chapter 3:

Data collection: ML

Statistical analyses: ML, SK

Manuscript writing: ML, SK

Chapter 4:

Data collection: ML, ALS

Statistical analyses: ML, SK

Manuscript writing: ML, SK

Chapter 5:

Data collection: ML

Laboratory analysis of DNA samples: ML, ALS, MGG

Statistical analyses: ML, SK

Manuscript writing: ML, SK

ALS : Amy L. Slender
MGG : Michael G. Gardner
ML : Marina Louter
SK : Sonia Kleindorfer
SL : Stephan T. Leu

Chapter 1 General introduction

Behavioural conservation framework

In order to counteract population declines and to protect and conserve threatened and endangered species, we need to have knowledge of species-specific ecological requirements (Berger-Tal *et al.*, 2011). A comprehensive understanding of the ecology and behavioural domains is a prerequisite for successful conservation of a species (Araujo *et al.*, 2002, Austin *et al.*, 1996, Buchholz, 2007, Clemmons and Buchholz, 1997, Luck, 2002, Sutherland, 1998), because without this information it remains challenging to provide appropriate conservation strategies with targeted restoration directions. Detailed information on specific habitat use, breeding biology, mating system, and the reproductive performance of threatened and endangered species allows us to examine how factors, whether anthropogenic or not, impact on these habits and hence on the success and persistence of populations. This knowledge in turn will help inform conservation efforts, and allow for careful assessment of vegetation and landscape features associated with the habits of species.

Maluridae as conservation models

The Maluridae, a family of terrestrial passerines endemic to Australasia and Papua New Guinea, consists of three genera: *Amytornis* (grasswrens), *Stipiturus* (emu-wrens) and *Malurus* (fairy-wrens). A review on the conservation status within this family revealed that grasswrens (*Amytornis*) contain the highest number of species prone to extinction: twelve of the nineteen (68%) extant (sub)species are at risk (Table 1.1), and five species have already become extinct (reviewed in Skroblin and Murphy, 2013).

Table 1.1 The conservation status, extrinsic threats and population trends of *Amytornis* based on Garnett *et al.* (2010) and IUCN Red list of threathened species (V3.1). Subspecies are based on Schodde and Mason (1999); Black *et al.* (2010); Black (2016), Christidis *et al.* (2010); Garnett *et al.* (2011); Lee *et al.* (2012).

Common name	Scientific name	Conservation status and extrinsic threats based on Garnett *et al.* (2010)	IUCN Conservation status and population trend (V3.1)
Kalkadoon Grasswren	*Amytornis ballarae*	Endangered, grazing	Least concern, increasing
Grey Grasswren	*A. barbatus*		Least concern, decreasing
	A. barbatus barbatus		
	A. barbatus diamantina		
Carpentarian Grasswren	*A. dorotheae*	Near threatened, fire	Vulnerable, decreasing
Eyrean Grasswren	*A. goyderi*		Least concern, stable
Black Grasswren	*A. housei*	Near threatened, fire	Near threatened, stable
Short-tailed Grasswren	*A. merrotsyi*		Near threatened, stable
	A. merrotsyi merrotsyi	Vulnerable, fire	
	A. merrotsyi pedleri	Vulnerable, fire	
Thick-billed Grasswren	*A. modestus*		Not assessed
	A. modestus curnamona	Near threatened, fire	
	A. modestus indulkanna	Vulnerable, grazing & drought	
	A. modestus inexpectatus	Extinct, grazing & drought	
	A. modestus modestus	Extinct, grazing & drought	
	A. modestus obscurior	Critically endangered, grazing & drought	
	A. modestus raglessi	**Vulnerable, grazing**	
	A. modestus eyre	Vulnerable	
Dusky Grasswren	*A. purnelli*		Least concern, increasing
Striated Grasswren	*A. striatus*		Least concern, decreasing
	A. striatus rowleyi		
	A. striatus striatus	Near threatened, fire	
	A. striatus whitei		
Western Grasswren	*A. textilis*		Least concern, decreasing
	A. textilis myall	Vulnerable, grazing	
	A. textilis textilis		
	A. textilis carteri	Extinct, grazing & feral predators	
	A. textilis gigantus	Extinct	
	A. textilis macrourus	Extinct	
White-throated Grasswren	*A. woodwardi*	Vulnerable, fire	Vulnerable, decreasing

In contrast, among fairy-wrens (*Malurus*) species there is a much lower proportion of species at risk of extinction: only four of twenty-five (16.0 %) species are currently at risk (Skroblin and Murphy, 2013). Since the Maluridae family has a wide distribution and has been exposed to varying anthropogenic factors that operate in diverse habitats across Australia, it is considered a useful model system to understand conservation and land-management issues in Australia (Skroblin and Murphy, 2013).

Conservation status of grasswrens (*Amytornis*)

Grasswrens are terrestrial passerines endemic to Australia. Unlike fairy-wrens, which are considered one of the most well studied avian groups within Australia, grasswrens are among the rarest, most elusive and least studied Australian birds. The lack of knowledge about their ecology and natural history is most likely due to the extremely shy and cryptic nature, as well as inconspicuous behavioural characteristics of most grasswren species, making observations and scientific studies challenging (Rowley and Russell, 1997, Serventy, 1982). Additionally, grasswrens generally occur in the most arid, remote and inhospitable regions of Australia, characterized by a lack of infrastructure and low human occupancy (Rowley and Russell, 1997, Serventy, 1982). They are widely considered to have poor dispersal ability (Christidis *et al.*, 2010, Higgins *et al.*, 2001) and restricted flight capabilities, factors which may contribute to isolation of populations in a fragmented landscape, poor gene flow and an increased vulnerability to catastrophic events. Consequently, populations of different grasswren species have been declining and *Amytornis* contains the highest proportion of threatened species within the Maluridae family, as well as the only extinct infrataxa (reviewed in Skroblin and Murphy, 2013). Threatening anthropogenic factors such as overgrazing by livestock and feral herbivores, introduced feral predators such as Domestic cats (*Felis catus*) and Red foxes (*Vulpes vulpes*), altered fires regimes, and habitat fragmentation and destruction are likely contributing factors (Garnett *et al.*, 2011, Reid and Fleming, 1992, Schodde, 1982b). However, the identification of the causal mechanisms underlying grasswren population declines continues to be a difficult task, especially when our knowledge on the behavioural ecology of most grasswren species is still very limited.

Reproductive behaviour of grasswrens Amytornis

One of the most important factors influencing the decline (or growth) of populations is the reproductive behaviour of individuals (Martin, 1992). Natural selection favours individuals that choose resources to enhance breeding success, thus the identification of factors that influence reproductive success are critical to understand why certain species are thriving and others are threatened. Aside from brief descriptions of eggs, nests, nesting habits, and clutch size (Black and Longmore, 2009, Freeman, 1970, Johnstone and Kolichis, 1999, North, 1910, Rowley and Russell, 1997, Schodde and Mason, 1975, Serventy, 1982), little work specific to breeding biology and vegetation characteristics at *Amytornis* nests have been published (but see Brooker, 1998a, Schodde and Mason, 1975). To our knowledge the reproductive success of only two grasswrens species has been studied in more detail: the western grasswren, *Amytornis textilis textilis* (previously known as the thick-billed grasswren) (Brooker, 1998a, b, 2000, 2003, Brooker, 1988) and the striated grasswren, *Amytornis striatus* (Karubian, 2001, Wood, 2014a, b). Information about specific breeding biology and habitat requirements, such as nesting shrub species and nest site characteristics could have direct and quantifiable implications for issues such as the management of vegetation and landscape features within reserves, to enhance nest survival and productivity, which will likely have strong influences on population growth rates and species persistence.

The thick-billed grasswren *Amytornis modestus* (North, 1902) is one of 11 currently recognised species within the genus *Amytornis* (Black *et al.*, 2010, Christidis *et al.*, 2010). Previous research identified seven thick-billed grasswren subspecies based on the mitochondrial DNA (ND2) network (Austin *et al.*, 2013) (Figure 1.1) and recent research identified the eighth subspecies, based on morphological data (Black, 2016).

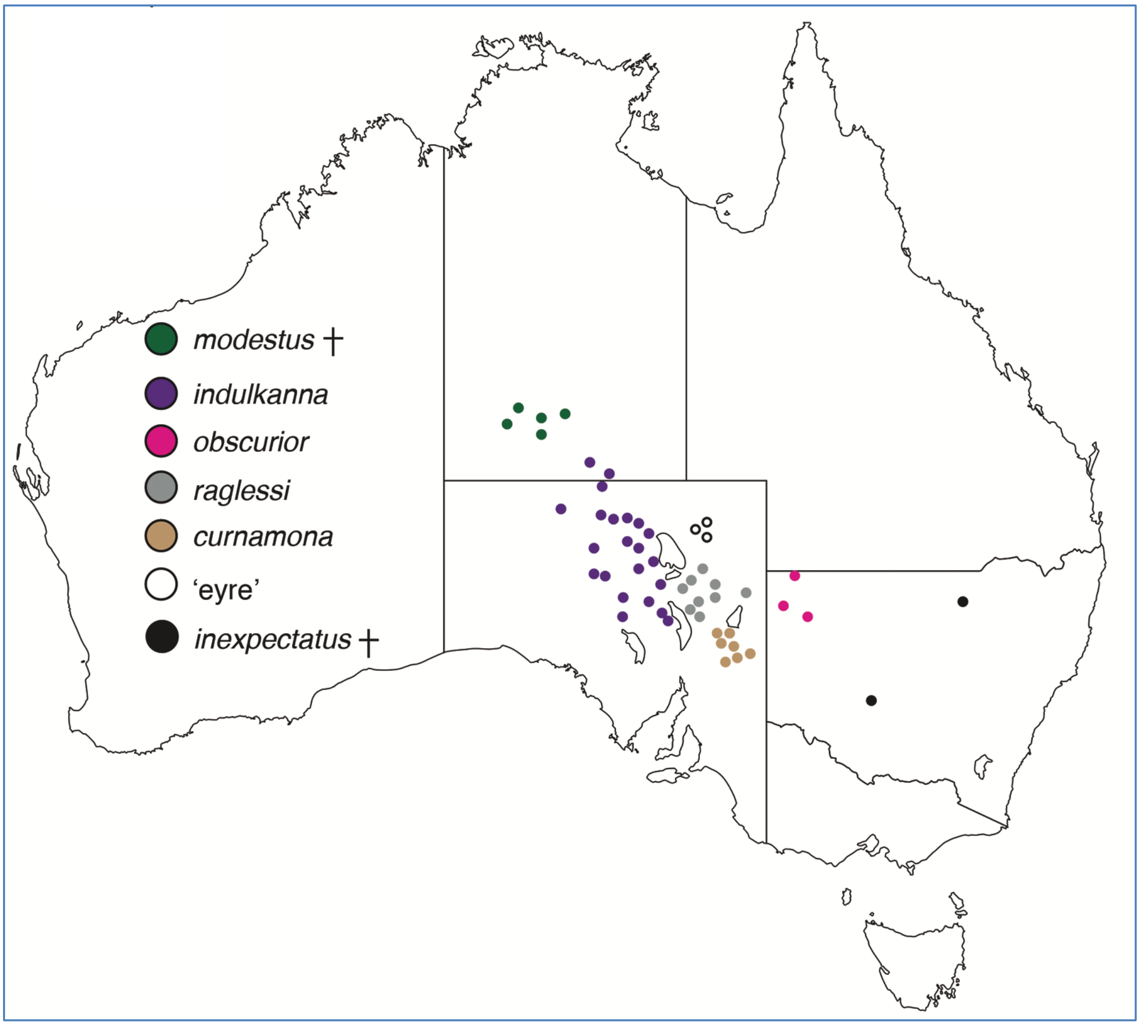

Figure 1.1. Map of Australia, showing occurrence of seven thick-billed grasswren *Amytornis modestus* subspecies, based on the mitochondrial DNA (ND2) network, after Austin *et al.* (2013). † indicate extinct taxa.

In this study I investigate the ecological context of nesting behaviour and fledging success in the previously unstudied vulnerable thick-billed grasswren (subspecies *Amytornis modestus raglessi*), which occurs in Witchelina Nature Reserve (30º01’340” S, 138º02’772” E) in South Australia (Figure 1.2).

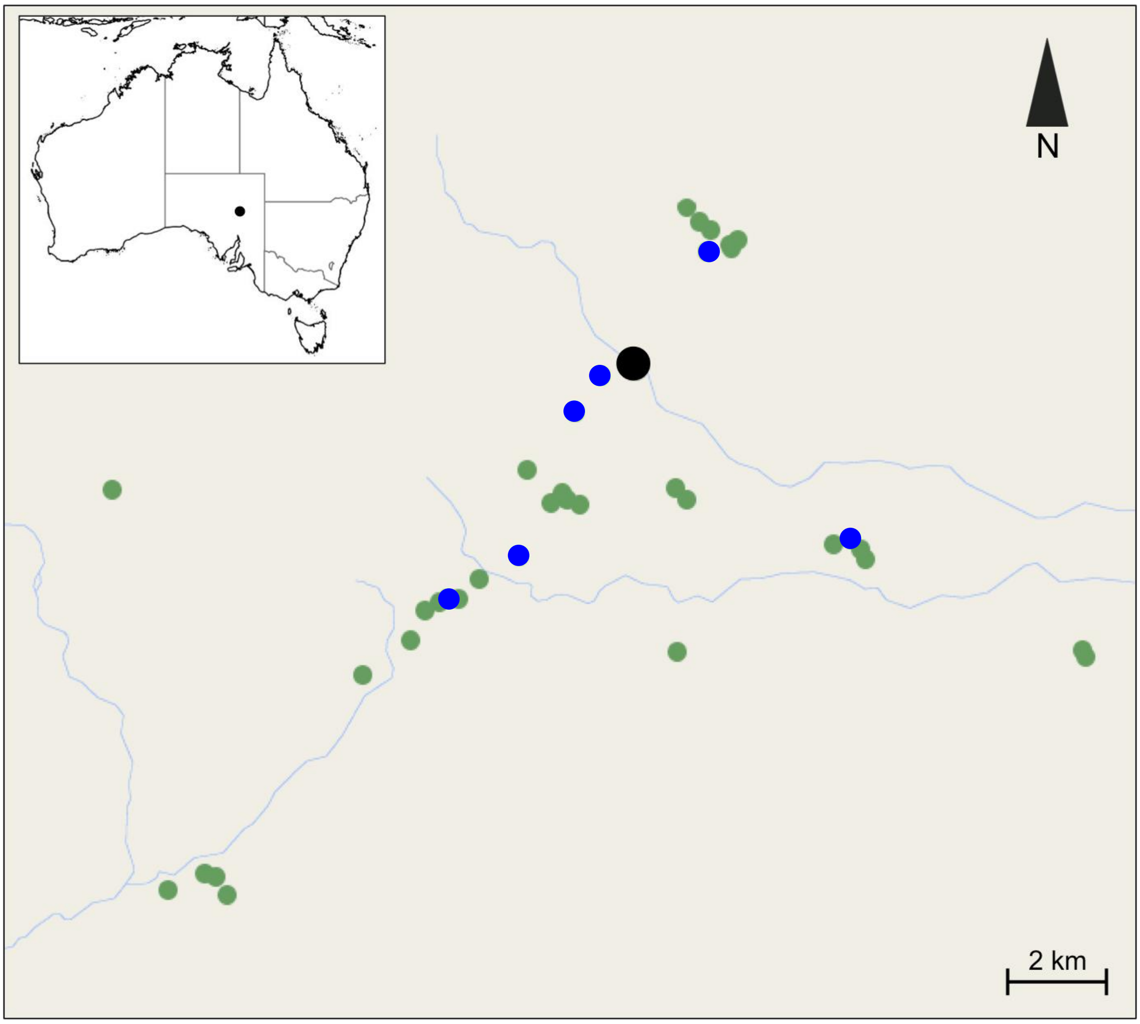

Figure 1.2 Map of the location of Witchelina nature reserve in South Australia (inset) and the distribution of 42 thick-billed grasswren (*Amytornis modestus raglessi*) territories (green and blue circles) within Witchelina Nature Reserve (30º01’340” S, 138º02’772” E). Blue circles represent territories that were used for extra-pair paternity (EPP) analysis (see Chapter 5). Black circles in both maps represent the location of Witchelina homestead.

Thick-billed grasswrens generally have a stout dark bill, dull brown feathers, which are white-streaked on the head and back, and carry their tails cocked up, in the wren manner (Figure 1.3). Both males and females are largely plumage-monomorphic. Recent research revealed slight morphological differences between sexes of *A. m. raglessi* (Slender *et al.*, b, *in prep*). However, males are only slightly larger than females in all their morphological features, thus in the field females can only be distinguished from males by the presence of a small rufous flank-patch underneath the wings, which is acquired by females at an early age (1-2 months) (Schodde, 1982a) (Figure 1.4)

Thick-billed grasswrens generally live in communities of saltbush (*Rhagodia* sp.), blue bush (*Maireana* sp.) (Figure 5.1A, B), cotton bush (*Maireana* sp.), samphire (*Crithmum* sp.) and grasses, on watercourses and among flood debris (Rowley and Russell, 1997, Schodde, 1982a, Serventy, 1982) (Figure 1.6). Once, thick-billed grasswrens were widely distributed across arid and semi-arid Southern and Central Australia, but at present they have a patchy distribution in the arid rangelands of South Australia and New South Wales (Black and Baxter, 2003, Black *et al.*, 2011a, Black *et al.*, 2010, Garnett *et al.*, 2011). Consequently, the species is currently listed as vulnerable under the Australian Government (EPBC Act 1999). The cause for their decline is not well understood, but habitat loss, fragmentation, and degradation through grazing of livestock and feral herbivores have been suggested as main contributing factors (Ford, 1987, Garnett *et al.*, 2011, McAllan, 1987).

Figure 1.3 An adult breeder thick-billed grasswren (*Amytornis modestus raglessi*) male, with a grasshopper in his bill, on his way to an active nest with one fully feathered nestling (~12 days old). Photo taken by Peter Owen at Witchelina Nature Reserve in May 2016.

Figure 1.4. A thick-billed grasswren (*Amytornis modestus raglessi*) adult female (left) perched on thorny saltbush (*Rhagodia spinescens*), and a thick-billed grasswren adult male (right) perched on acacia (*Acacia carneomorum*). In the field, females can be identified by the presence of rufous flanks, visible underneath the wings (arrow), which are absent in the male. Photos taken by Peter Gower at Witchelina Nature Reserve in August 2013.

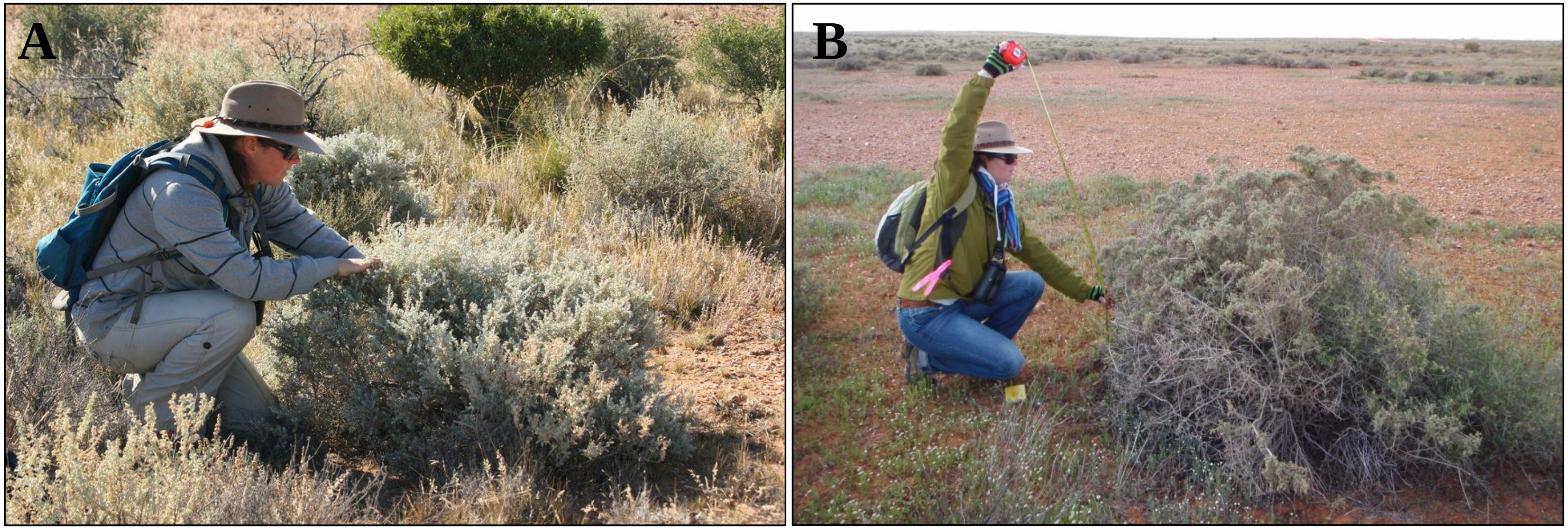

Figure 1.5 **A**) M. Louter checking the content of a thick-billed grasswren nest, located in a chenopod shrub (low bluebush - *Maireana astrotricha*) at Witchelina Nature Reserve, South Australia (Photo by Petra Hanke); **B**) M. Louter measuring the height of a chenopod shrub (blackbush - *Maireana pyramidata*) containing a thick-billed grasswren nest at Witchelina Nature Reserve, South Australia (Photo by Amy L. Slender).

Figure 1.6 Typical open chenopod shrubland with low vegetation (<1.5 m) at Witchelina Nature Reserve, in South Australia (Photo by M. Louter).

Thesis scope and objective

The chapters of this thesis examine the efficacy of habitat restoration from grazing as a conservation tool for the persistence of a vulnerable arid songbird species, the thick-billed grasswren (*Amytornis modestus*). This information is essential for the development of targeted conservation for bird species under threat.

Specifically the aims of this study are to:

1. Identify predictors of thick-billed grasswren occurrence and quantify home range
2. Identify factors affecting thick-billed grasswren nesting success
3. Investigate factors affecting nest predation
4. Describe breeding behaviour of thick-billed grasswrens
5. Determine the social and genetic mating system of thick-billed grasswrens

Organization of this thesis

The thesis is organised in manuscript format with each data chapter representing a potential peer-reviewed publication. The chapters are:

1. General introduction
2. Louter *et al.* a (*in prep*): Habitat recovery from grazing predicts presence but not home range size in thick-billed grasswrens (*Amytornis modestus raglessi). Austral Ecology*.
3. Louter *et al.* b (*in prep*): What predicts nest predation in thick-billed grasswrens (*Amytornis modestus raglessi*)? *Biological Conservation*.
4. Louter *et al.* c (*in prep*): Vegetation cover predicts parental feeding rates in thick-billed grasswrens (*Amytornis modestus raglessi*). *PeerJ*.
5. Louter *et al.* d (*in prep*): Lack of extra-pair paternity (EPP) in the vulnerable thick-billed grasswren (*Amytornis modestus raglessi*). *Conservation Genetics*.
6. Discussion and general conclusion

Chapter 2 Habitat recovery from grazing predicts presence but not home range size in thick-billed grasswrens (*Amytornis modestus raglessi*).

Marina Louter, Amy L. Slender, Stephan T. Leu and Sonia Kleindorfer

In preparation to be submitted to *Austral Ecology*

Abstract

The movement of organisms through their environment is an intrinsic component of an animal's ecology and lies at the heart of ecological field research. Understanding the effects of human disturbance on a species' presence/absence and home range size is important for successful biodiversity management. Our study system is the thick-billed grasswren (*Amytornis modestus raglessi*), an Australian bird of conservation concern that occurs in arid zone habitat that is recovering from intensive livestock grazing. We measured vegetation parameters of long-lived perennial (chenopod shrubs (% cover), abundance and diversity) and sampled arthropods (abundance and diversity) to assess habitat quality at sites (n = 62) of varying grazing impact. We used 20 min/ha bird surveys to detect presence/absence of thick-billed grasswrens at sites. In 2014 we conducted a radio telemetry study to record and compare home range size of thick-billed grasswren males during the breeding season size in areas with varying grazing impact in Witchelina Nature Reserve, South Australia. There were significantly fewer thick-billed grasswrens present in areas recovering from heavy grazing. Areas recovering from heavy grazing intensity differed significantly in ecological variables: sites recovering from heavy grazing had less vegetation cover and fewer arthropods. The average home-range size of breeding males, calculated using 95 % fixed kernel density estimate, was 8.0 ha ± 1.7 SE (n = 8). Grasswren males had small core activity areas (50 % fixed kernel density estimate) covering 31 ± 4 % SE of their total home ranges. In conclusion, thick-billed grasswrens were more likely to occur in areas recovering from moderate grazing impact, but home range size was comparable across all birds sampled with most birds radio-tracked in areas with moderate grazing impact.

Introduction

Theory predicts that animals should occupy the smallest possible areas within which they can maximize resource acquisition and minimize time and energy for territory defence (Maynard Smith, 1974). Studies of home range size provide an insight into the spatial requirements of a species and can identify particular habitat features that affect their spatial distribution and movement behaviour (Anich *et al.*, 2010, Kerr and Bull, 2006, Pickens and King, 2013), which in turn can increase our understanding of how animals respond to anthropogenic changes in study habitats (Buij *et al.*, 2015, Cohen *et al.*, 2004). Research on spatial behaviour and home range size is therefore of clear value to conservationists for any reintroduction plan and for management of wild populations. Home range size is defined as “that area traversed by an individual in its normal activities of food gathering, mating, and caring for young” (Burt, 1943) and has been shown to be influenced by the availability and distribution of critical resources such as food (Tufto *et al.*, 1996, Village, 1982), mates and vegetation cover (Kerr and Bull, 2006). There is robust empirical support across taxa that home range size decreases when food availability or when vegetation cover increases (Getz *et al.*, 2005, Hayes *et al.*, 2007, Ims, 1987, Schoepf *et al.*, 2015). Given ecological characteristics of home range size, one can apply these insights to guide conservation management of declining species. In particular, home range size in human-altered habitat should be considered when designing conservation management plans.

Human impact may alter home range use of animals directly and indirectly (Körtner *et al.*, 2007, Loft *et al.*, 1993, Schulz and Guthery, 1987). Direct impacts include urbanisation, barriers caused by roads, and noise and light pollution. Indirect effects include land use modification such as grazing by domestic livestock and feral grazers (Schulz and Guthery, 1987). Overgrazing by livestock and feral grazers can alter native shrub and grass composition and change the structure of native vegetation as well as vegetation cover (Adler *et al.*, 2001), thereby decreasing invertebrate abundance and diversity (Hutchinson and King, 1980, Kruess and Tscharntke, 2002). Species depending on insects and vegetation for food and shelter are especially vulnerable to impacts of grazing, with declines in richness and abundance observed in reptiles, mammals and birds (Cardoni *et al.*, 2015, Gonnet, 2001). Bird communities are negatively affected by the reduction in food and shelter induced by grazing, particularly for ground foraging and nesting species (Davies *et al.*, 2010, Lusk and Koper, 2013, Martin and Possingham, 2005). Home ranges of birds have been found to be negatively affected by grazing impact, with observed increases in home range size as result of decreased resource abundance (Schulz and Guthery, 1987). Birds are therefore commonly used as grazing indicator species, to better understand animal responses to human altered environments (Bock and Webb, 1984, Read *et al.*, 2000).

Chenopodiaceae is a prominent plant family in deserts worldwide with a distribution extending across temperate and subtropical arid zones of Australia, as well as central Asia, Africa, and the Americas (Barlow, 1981). Chenopods shrublands (<1.5m tall) cover 8% of the Australian arid zone. These shrublands mainly consist of a mixture of different species of *Chenopodiaceae* (saltbushes, bluebushes and copperburrs). Bird species that inhabit chenopod shrublands have been declining due to clearing and fragmentation of native habitat, as well as habitat degradation caused by the grazing of domestic and feral herbivores (Garnett *et al.*, 2011). Ungulate livestock have grazed Australia's arid rangelands since European settlement in the late 1800's (Harrington *et al.*, 1984, Wilson, 1990), and the effects of grazing on environmental attributes have been studied extensively, in particular in South Australia (Landsberg *et al.*, 2002, Landsberg *et al.*, 2003, Tiver and Andrew, 1997). Selective grazing by ungulate livestock has significantly altered the composition and biomass of native vegetation (McIvor and Gardener, 1990, Pettit *et al.*, 1995). Overall, native plant species in natural ecosystems such as chenopod shrublands evolved in the absence of large ungulate species and are characterised by low recruitment rates and slow regeneration in long-lived perennial chenopod shrubs and trees. These life history traits make them particularly vulnerable to impacts of grazing, with declines observed in long-live perennial chenopod shrubs, such as saltbush (*Atriplex vesicaria*), black bush (*Maireana pyramidata*) and low blue bush (*M. astrotricha*) (e.g. Eldridge *et al.*, 1990, Hunt, 2001, Hunt, 2010).

The Maluridae, a passerine family endemic to Australasia and Papua New Guinea, are widespread and occur in nearly all sclerophyllous and semi-mesic habitats in tropical, temperate and arid zones. Given their wide distribution, different populations of Maluridae have been exposed to varying degrees of grazing pressure in various habitats across Australia. Thus, Maluridae are a good model system to study the effect of grazing on various response parameters important for land management (Skroblin and Murphy, 2013). The thick-billed grasswren (*Amytornis modestus*) (North, 1902, Parker, 1972), a member of the Maluridae family, is particularly suitable to investigate grazing impact in arid regions of Australia, as it is endemic to this region and gregarious. Once, thick-billed grasswrens were widely distributed across arid and semi-arid Southern and Central Australia, but they have disappeared from areas where they were once abundant, and are now confined in small isolated populations across Northern South Australia (Black and Baxter, 2003, Black *et al.*, 2011a, Black *et al.*, 2010, Garnett *et al.*, 2011). As a consequence, the species is currently listed as vulnerable under the (E. P. B. C. Act 1999). The cause for its decline is not well understood, but habitat loss, fragmentation, and degradation through grazing of livestock and feral herbivores have been suggested to contribute to their decline (Ford, 1987, Garnett *et al.*, 2011, McAllan, 1987).

In this study we investigate the effects of grazing on the presence/absence of thick-billed grasswrens *Amytornis modestus* at sites with varying grazing impact. Further we investigate whether grazing affects home range size in thick-billed grasswrens as a consequence of differential vegetation characteristics and arthropod communities. The study area Witchelina Nature Reserve had been grazed for ~160 years before being converted to a conservation area in 2010, when grazing stopped. Prior to becoming a nature reserve, Witchelina was intensively used to graze sheep and cattle from the 1850's to 2010. We predict (1) less vegetation cover, lower chenopod shrub abundance and diversity, and fewer arthropods in areas with heavy grazing impact. Based on the optimal foraging theory we predict (2) larger home range size of thick-billed grasswrens in areas with heavy grazing impact. We sampled vegetation parameters along transects and sampled arthropod abundance and diversity using pitfall traps. In addition to testing basic theory, the study aims to obtain base-line data on foraging behaviour, habitat use and home range size estimates to inform conservation management for the recovery and future protection of this declining subspecies.

Methods

Study area

This study was conducted at Witchelina Nature Reserve (30º01’340” S, 138º02’772” E), (Figure 1.2, p.6), one of the largest conservation reserves in arid heartland of South Australia, covering 4219 sq. km (421.000 ha). Witchelina Nature Reserve is owned and managed by the Nature Foundation of South Australia (Nature Foundation of South Australia Inc, 2016) and lies within the range of the thick-billed grasswren (*Amytornis modestus raglessi*) (Black *et al.*, 2011a, Rowley and Russell, 1997). Previous bird surveys conducted in 2011, recorded thick-billed grasswrens as common in the study area (Black *et al.*, 2011b, Wurst, 2011). The reserve consists of 25 paddocks containing a total of 82 artificial dams, bores and wells. Stock numbers from 1955 to 1998 usually ranged from 3-4 sheep/sq. km, and were highest in 1990, when a total of 19391 sheep and around 500 cattle grazed the property (Gould and Halliday, 1999). All livestock were removed from Witchelina in 2010 to reduce grazing pressure, and to restore and conserve native vegetation such as chenopod shrublands. Witchelina Nature Reserve spans three bioregions, Willouran, Mulgarie and Marree, these are divided into ten land major systems: 1) Mumpie (undulating gibber tableland country); 2) Myrtle (complex dunes with mulga); 3) Oodnadatta (undulating gibber tableland with gilgai depressions); 4) Paradise (channels, floodplains, low stony hills and flats); 5) Saltia (alluvial foot slopes and plains of stony red soils); 6) Stuarts Creek (sandy and clay flats); 7) Torrens (lake Torrens salina and shoreline); 8) Umberatana (hills and low hills with skeletal fine-textured soils); 9) Willouran (slate hills and stony valleys of the Willouran Ranges); 10) Wirringina (red sand plains, dunes and sand accumulations). All these land systems are dominated by chenopod shrublands and long-lived perennial chenopod shrubs.

Study species

We studied the thick-billed grasswren (subspecies *Amytornis modestus raglessi* (Black, 2011a) from July to September in 2013 and 2014, plus one 2-week period in 2015 (August), spanning a total of 18 weeks. This period coincides with the presumed main breeding season of the species, which is defined as August to September (Black *et al.*, 2011a, Rowley and Russell, 1997). The thick-billed grasswren is a small (~19 g) ground-dwelling passerine that is found mainly in low-growing chenopod shrublands, dominated by drought and salt-tolerant chenopod shrub species from the Genus *Atriplex* (salt bush) and *Maireana* (blue bush, black bush and cotton bush) (Black *et al.*, 2011a, Rowley and Russell, 1997). Like most other grasswren species, the thick-billed grasswren is shy and elusive with cryptic plumage and secretive behaviour, making behavioural studies challenging and time-consuming. For this reason, baseline ecological data are lacking for most of the eleven currently recognized grasswren species (but see Brooker, 1998a, 2000, Brooker, 1988, Karubian, 2001).

TBGW occurrence

To determine thick-billed grasswren presence/absence, we used 62 sites randomly selected. Due to the remoteness of the study site and limitations to site accessibility, coordinates were plotted alongside roads, within an area of ~50 sq. km in Witchelina Nature Reserve (Figure 1.2, p.6). During August-September 2013 we used a standard timed area of search of 20 min/1 ha (Watson, 2003) at each site to determine presence/absence of grasswrens. We used the GPS coordinates for the central location from which we walked 50 m in opposite directions. For each site, we recorded the presence of grasswrens, the number of individuals observed and the detection method (seen or heard). Surveys were conducted on calm days (< 15 knots) between 6.30 hrs to 12:00, and from 14:00 hrs to 18:00, excluding hot midday hours.

Grazing impact – distance to dam

Grazing impact refers to previous grazing impact in Witchelina Nature Reserve, prior to stock removal in 2010. We did not measure grazing impact directly, but instead used distance from water dams as a measure for grazing impact, which is a well-documented, reliable, and generalizable proxy for impact of grazing on Australian arid rangelands (Andrew, 1988, Fensham and Fairfax, 2008, James *et al.*, 1999, Lange, 1969). We estimated grazing impact as the distance (km) to the nearest water dam at 62 survey sites within the study area. For analysis we used distance to dam as well as grazing categories. We categorized grazing impact based on Fensham and Fairfax (2008): heavy grazing = 0 to 2 km from dam; moderate grazing = 2 to 5 km from dam; no/little grazing ≥ 5 km from dam.

Vegetation sampling – Jessup transects

We sampled vegetation parameters at each survey site during August – September 2014 and August 2015, using Jessup transects (Jessup, 1951). Jessup transects were 100 m in length, divided into 10 m x 2 m blocks (sampling squares) on either side of the transect line (total sampling area = 400 sq. m) and ran from North to South, starting at one of 62 random GPS locations. Vegetation was measured across a large area to account for the spatial heterogeneity of arid-zone vegetation, which occurs due to patchy soil types and variable rainfall (Morton *et al.*, 2011b, Williams, 1982).

Vegetation surveys at sites using Jessup transects took approximately 60 to 90 minutes, depending on the density and complexity of vegetation. At each transect we recorded all adult woody chenopod species, defined as plants larger than 10 cm in height (predominantly shrubs, but also some tree species, henceforth termed chenopod shrubs, that typically live for at least 5–10 years. Most chenopod species are slow growing, even in the absence of grazing, and have life spans measured in decades, e.g. *Atriplex vesicaria* (15-30 yrs) (Crisp, 1978, Crisp and Lange, 1976, Osborne *et al.*, 1935). The impact of seasonal conditions on chenopod vegetation dynamics is therefore limited and we do not expect an effect of year. Based on a review of the literature (Black *et al.*, 2011a, Black *et al.*, 2011b, Garnett *et al.*, 2011, Rowley and Russell, 1997), vegetation features that we considered important for TBGW spatial ecology and behaviour were (1) overall vegetation cover (2) chenopod shrub cover, (3) chenopod shrub abundance, and (4) number of chenopod shrub species (chenopod diversity). We calculated overall vegetation cover as a percentage of all vegetation measured per transect. Chenopod shrub cover was measured as a percentage of cover of all chenopod shrub species belonging to the genus *Rhagodia*, *Atriplex*, *Maireana*, *Eremophila* and *Acacia* per transect. We calculated the frequency of chenopod shrub species per transect, as a measure of chenopod shrub abundance. Chenopod shrub diversity was calculated as the total occurrence of shrubs belonging to the above-described genus per transect. This sampling strategy was designed to detect varying grazing impact on chenopod communities, and did not attempt to be comprehensive with respect to the total flora at each site. Nomenclature for plants follows Kutsche *et al.* (2003). At each site data were summarized per transect for further analysis.

Arthropod sampling Pitfalls

Arthropod sampling was done in September of 2013 and 2014. Due to the remoteness and accessibility of sites, we used rapid assessment techniques to sample arthropod abundance. We used 1-5 pitfall traps per site at a subset of 43 sites out of 62 survey sites: at 23 sites we placed 5 pitfall traps in a row, with 10 m between adjacent traps, in the centre of Jessup transects we used for the vegetation sampling. At 20 sites, we placed one pitfall trap in the centre of Jessup transects. Pitfall traps were 35 mm in diameter (200 ml volume); they were dug into the ground with the top of the trap flush with the soil surface and were left closed for approximately 24 hours to account for effects of digging disturbance, for example of ants (Greenslade, 1973). Pitfall traps were opened the following morning (between 7 :00 am and 9:00 am), filled to approximately 50 % capacity with ethanol (75 %) and a drop of odourless TWEEN ® 80 (Polyoxyethylene Sorbitan Monooleate) was added to decrease surface tension. All pitfall traps were collected after ~24 hours. In the lab, specimens were identified to the order level (Orthoptera, Lepidoptera (adult and larvae), Coleoptera, Aranae, Phasmatodea, Hemiptera, Formicidae, Diptera, Blattodea). Arthropod diversity and arthropod abundance data were averaged per site for further analysis.

Radio tracking of grasswrens

In 2014 we radio-tracked fourteen adult TBGW males at various distances from water dam (S1 Table 2.1) Of these, eleven had nests with eggs or chicks and three had fledglings. TBGWs were captured using mist-nets (9-12 m long, 30 mm-mesh). Within a territory two to four mist-nest were set up in straight lanes or in a u-shape across drainage lines and creek lines, placing the bottom shelf string on the ground and securing it with rocks to prevent grasswrens passing underneath the bottom shelf of mist-nets. Individuals were herded towards the nets by 2-3 observers. Thick-billed grasswrens tend to run or scurry along the ground when moving between chenopod vegetation and consequently almost all birds were captured in the bottom shelf of nets. Once caught, each bird was fitted with an aluminium band (ABBBS) and a unique combination of three coloured leg bands. All birds captured were measured for morphology (Slender *et al.*, b, *in prep*) and sex was assigned in the field according to the presence (females) or absence (males) of a rufous flank-patch of feathers underneath the wing (Schodde, 1982a). Sex was later confirmed (100 % accuracy) from blood samples obtained at the time of capture using a modified version of the methods described in Griffiths *et al.* (1998), Smith and Burgoyne (2004). Radio tracking was restricted to adult males because they are heavier (19.62 g ± 0.19 SE, n = 42) than adult females (18.05 g ± 0.27 SE, n = 29), allowing us to attach a radio transmitter. A 0.50-g VHF radio-transmitter (Model BD-2N, Holohil Systems, Carp, Ontario, Canada) was attached with fast-setting cyanoacrylate glue to the feathers and skin of the bird, within the inter-scapular region. Combined transmitter and attachment weight did not exceed the recommended 3 % (Kenward, 2001) of adult body-weight of TBGW males. Tagged individuals were released 15 minutes after attachment at the point of capture. Radio tracking commenced 24 hours after the release time, to allow birds to acclimate to their transmitters.

Tracking was conducted using an R-1000 radio receiver with headphones, a 3-element (folding) hand-held yagi antenna (Sirtrack Ltd., Goddards Lane, Havelock North, New Zealand) and a hand-held Global Positioning System (GPSAMP 62s Garmin Inc. Wichita KS) with a tracking resolution (accuracy of ± 3 m). We used the homing method (Kenward, 2001, Millspaugh and Marzluff, 2001, White and Garrott, 1990) and approached birds until we could visually locate the individual with binoculars. We avoided approaching a bird too closely to reduce the effect on the birds' behaviour and movement (mean distance of observer: 30.0 m ± 1.36 SE, n = 231). Once the focal bird left the location, a GPS reading was taken at the point of initial siting. On occasions when the focal individual was not visible (e.g. due to dense vegetation or a creek line) the location (accuracy of ± 3 m) of the bird was determined based on either the strength of the signal (approached the shrub/creek line with the antenna from several angles to confirm the bird's presence), or the presence of conspecifics nearby (either from sighting of other group members, or audible contact calls). We located each bird at least once a day (1.60 ± 0.05 SE locations/day, range 1 - 6 locations/day), alternating mornings and afternoons. Sample regime was comparable between all fourteen radio-tracked birds. Tagged males were radio tracked for up to 29 days (mean: 17 days; range: 4 - 29 days, n =14). We recorded a total of 394 location points (fixes) for the fourteen males during the breeding season of 2014 (mean: 28.1 range: 14 - 42) (S1 Table 2.1).

Home range estimates are sensitive to sampling regime and serial autocorrelation (Börger *et al.*, 2006, Fieberg, 2007, Swihart and Slade, 1985a, b), thus we excluded periods 30 min after sunrise and 30 min before sunset to avoid repeatedly recording the same overnight roosting sites (Kenward, 2001). Based on field observations of movement speed, an interval of minimal 30 minutes min was considered sufficient for a male TBGW to cross its home range, ensuring independence of each fix (following Kenward 2001). We further minimized effects of autocorrelation on home range determination by excluding fixes that were sampled less than 30 minutes apart.

Home Range Estimation

We calculated TBGW home range size and core activity area size using kernel density (KDE) estimates (Worton, 1989) in the programme Anatrack Ltd Ranges8 v2.13

(Kenward *et al.*, 2008). We nominated 95 % fixed KDE (Seaman and Powell, 1996) as "outer home range" and 50 % KDE as "core activity area". To facilitate comparison with other studies as suggested by Harris et al (1990) and Goldingay and Kavanagh (1993), we also calculated 100 % Minimum Convex Polygon (MCP) home range sizes. The Minimum Convex Polygon (Mohr, 1947) is a classic and widely used method of home range estimation (Laver and Kelly, 2008, Seaman *et al.*, 1999), which includes all location records, and creates a convex polygon around the outermost points in a location data set (i.e. its 'total range': (Linn and Key, 1996).

As home range estimates are highly sensitive to the number of location points (fixes) (e.g. Börger *et al.*, 2006, Boyle *et al.*, 2009, Seaman *et al.*, 1999), data for each radio-tracked individual bird were subjected to incremental area analysis, and based on visual inspection of incremental plot we determined if a sufficient number of fixes had been collected for an accurate home range estimate following Harris *et al.* (1990). Only those birds whose incremental area curves reached an asymptote (8 out of 14 birds), indicating a stable home range (Fielding and Bell, 1997), were included in analysis examining home range size and core activity area of intense use. Eight birds had a minimum of 27 sampling locations and although lower than the recommended minimum number of 30 fixes needed to use Kernel estimates in home range estimation as suggested by Seaman *et al.* (1999) – this was considered a valid sample size as the number of fixes was not correlated to home range area (95 % KDE: $r = -3.19$, $n = 8$, $P = 0.44$).

Group size, habitat use and foraging behaviour of grasswrens

To record information on TBGW group size, as well as fundamental information on TBGW habitat use, we estimated group size, recorded behavioural data, and recorded the species of plants used by birds with each locational fix. Behavioural data was categorized as follows: (1) hiding: individual was stationary, hiding in vegetation, most commonly at the base of a shrub; (2) perching on shrub; (3) running on ground; (4) flying; or (5) incubating/feeding at nest. Additionally, we collected foraging data by recording so-called "first foraging observations”; each time we observed an individual foraging we recorded the first foraging behaviour that was observed (glean, sally, or probe), to ensure independence of samples.

Statistical analysis

All statistical analyses were performed in SPSS Statistics version 22 for Windows (SPSS Inc., Chicago, USA). Before conducting statistical analyses, we examined the data to determine if they conformed to assumptions of normality and homogeneity of variance. To avoid issues with (multi)collinearity among vegetation and insect predictor variables, correlating variables ($r \geq 0.5$) were removed using Pearson correlation co-efficient test. Chenopod vegetation cover was highly correlated to chenopod shrub diversity ($r = 0.58$), chenopod shrub abundance ($r = 0.71$), and overall vegetation cover ($r = 0.71$) therefore chenopod vegetation cover was used in further analysis. Most insect orders were relatively infrequent in occurrence, except ants (Formicidae) and arthropod abundance and ant abundance were therefore included as separate variables in our analysis. We used Ordinary Least Square (OLS) Linear Regression analysis to test if grazing impact is a predictor for ecological parameters (chenopod vegetation cover and arthropod abundance), with chenopod vegetation and arthropod parameters as dependent variables and distance to water dam (in km) as independent variable. We used non-paired Binomial Logistic Regression analysis to test the directional effects of grazing and our a-priori identified ecological parameters (chenopod vegetation cover, arthropod abundance) as predictor variables on TBGW presence (0,1) as the binary outcome, to test the idea that habitats with little grazing impact, more vegetation cover and more arthropods can better sustain TBGWs. To assess ecological predictor variables for TBGW home range size we tested chenopod vegetation cover and arthropod abundance using (OLS) Linear Regression with 100 % MCP, 95 % KDE, and 50 % KDE as our response variable in separate analysis.

Results

Grazing impact and ecological predictor variables

A total of 5521 chenopod shrubs were recorded at 62 survey sites. The most abundant chenopod shrub species at sites was black bush (*Maireana pyramidata)* (n = 1898), followed by bladder saltbush (*Atriplex vesicaria*) (n = 1220), and low blue bush (*Maireana astrotricha)* (n = 1171). Vegetation parameters were variable between survey sites with varying grazing impact (Table 2.1) Chenopod vegetation cover was significantly associated with grazing impact and was higher at sites with no/little grazing impact (Linear regression r = 0.33, t = 2.68, n = 62, $P = 0.010$).

Pitfall sampling at 43 sites collected a total of 2017 individuals (hymenoptera: n = 1120) of 12 different insect orders. Arthropod abundance and ant abundance were significantly associated with grazing impact: arthropod abundance (Linear regression, r = 0.38, t = 2.62, n = 43, $P = 0.012$) and ant abundance (Linear regression, r = 0.31, t = 2.07, n = 43, $P = 0.045$) were higher at sites with little grazing impact.

TBGW occurrence

TBGWs were present in 36 out of 62 survey sites (Figure 2.1). TBGW presence was significantly associated with grazing impact: grasswrens were more likely to occur at sites with little grazing impact (χ^2 (1) = 14.87, $P < 0.001$, Nagelkerke $R^2 = 0.29$, n = 62). There was a significant association between TBGW presence and chenopod vegetation cover; grasswrens were more likely to occur at sites with more chenopod vegetation cover (χ^2 (1) = 15.79, $P < 0.001$, Nagelkerke $R^2 = 0.30$, n = 62) (Table 2.2). Neither arthropod abundance (χ^2 (1) = 0.62, $P = 0.43$, Nagelkerke $R^2 = 0.02$, n = 44) nor ant abundance (χ^2 (1) = 0.98, $P = 0.32$, Nagelkerke $R^2 = 0.03$, n = 44) was significantly associated with grasswrens occurrence and were comparable between sites with and without grasswrens present (Table 2.2).

Habitat use & foraging behaviour

Behavioural observations (at the initial moment of detection) taken with each locational fix (n = 416) showed that TBGWs were most often hiding (30.3 %), followed by perching (22.9 %), running between vegetation (18.9 %), foraging on the ground (7.9 %), flying at ground level (6.6 %), or incubating or feeding nestlings (1.4 %). In 60.8 % of all 416 observations, the radio-tracked focal male was in the visual or audible vicinity of other group members.

The most commonly used substrate by radio-tracked TBGW males was black bush (*Maireana pyramidata*) (n =105, 43 %), followed by *Acacia sp* (n = 59, 24 %), cotton bush (*Maireana aphylla*) (n = 26, 11 %), thorny saltbush (*Rhagodia spinescens*) (n = 23, 9 %), emu bush (*Eremophila sp*) (n = 12, 5 %), low blue bush (*Maireana astrotricha*) (n = 6, 2 %), *Senna sp* (n = 4, 2 %), and other (n = 2, 1 %). We collected a total of 93 independent first foraging observations: TBGWs were most commonly gleaning food items of the ground (n = 84, 90 %), followed by sallying (catching flying insects) (n = 4, 4 %), snatching insects of vegetation (n = 3. 3 %), and probing in ground litter (n = 2, 2 %).

Home range size and ecological predictor variables

For eight of the fourteen radio-tracked males a sufficient number of fixes was collected (S1 Table 2.1) and these eight birds were used to estimate home range and core areas size. The mean home range size of TBGW males was large (8.0 ha ± 1.7, n = 8), and males occupied large core activity areas (2.5 ha ± 0.58, n = 8), representing 31.0 ± 1.3 % of their estimated home range (Table 2.3). Group size ranged from 2 to 5 individuals and was independent of home range size (Linear Regression, r = 0.57, t = 1.71, n = 8, *P* = 0.139). Grazing impact was not significantly associated with TBGW home range size (95 % KDE: Linear regression, r = -0.16, t = -0.41, n = 8, *P* = 0.699), or core activity area (50 % KDE: Linear regression, r = -0.21, t = -0.54, n = 8, *P* = 0.611). We found no significant associations between vegetation or insect parameters and home range size in this study: neither chenopod vegetation cover, arthropod abundance, nor ant abundance predicted TBGW male home range size (100 % MCP, 95 % KDE: Linear Regression, *all P* > 0.4) nor size of the core activity area (50 % KDE: Linear Regression, *all P* > 0.5).

Discussion

Summary of results

The main results of this study showed a negative association between grazing impact (distance to water dam in km) and TBGW occurrence; sites with heavy grazing impact did not contain TBGWs. Additionally we found a negative association between grazing impact and long-lived perennial shrub vegetation cover: chenopod vegetation cover decreased with increasing grazing impact. Similarly, we found that arthropod abundance and ant abundance were lower in areas with heavier grazing impact, compared to areas furthest away from water dams with least grazing impact.

A decline in vegetation and arthropods has the potential to negatively affect bird species that depend on vegetation for cover and insects for food (Dennis *et al.*, 2005). In Witchelina Nature Reserve, TBGW mostly occurred in areas with little grazing and less in areas of heavier grazing impact. While male TBGW home range size and core area size were variable between individual males, they were comparable between areas of variable grazing impact. Acknowledging the small sample size (n = 8), we did not find a relationship between grazing and home range size of TBGW in this study. However, the absence of grasswrens observed closer to water dams suggests that grazing may have an indirect adverse impact on TBGWs through a reduction in available chenopod vegetation cover and arthropods, which are essential resources for TBGWs.

Impacts of grazing on vegetation

Grazing is one of the most important disturbances of vegetation worldwide, changing plant community structure and composition in a global range of environments (Huntly, 1991, Milchunas and Lauenroth, 1993, but see Silcock and Fensham, 2013). In Witchelina Nature Reserve we found that chenopod shrub cover was significantly lower in areas of heavy grazing. The negative association between chenopod vegetation cover and our proxy for grazing pressure suggests that grazing negatively impacts on vegetation, which is in congruence with well-documented detrimental impacts of long-term water-centred grazing on vegetation. For example, in the arid rangelands of South Australia grazing pressure on vegetation close to artificial water bodies resulted in an overall decline in plant species richness and plant composition with increasing proximity to water (Landsberg *et al.*, 2002, Landsberg *et al.*, 2003). Native chenopod shrub species that are common to arid rangelands such as *Atriplex* (salt bush) and *Eremophila (*emu bush*),* are highly palatable for livestock and heavy grazing pressure can therefore cause severe declines (Landsberg *et al.*, 2003, Watson *et al.*, 1997), and subsequent local extinctions of these species close to water dams (e.g. Hunt, 2001). Changes in vegetation structure, composition, and species diversity have been known to affect bird communities, especially ground foraging species, which depend on vegetation for food and shelter (Cardoni *et al.*, 2015, Gonnet, 2001).

Impact of grazing on TBGWs

At Witchelina Nature Reserve, TBGWs are found mainly in chenopod shrublands, dominated by low slow-growing long-lived perennial shrubs, such as *Atriplex* and *Maireana* (Slender *et al.*, a, *in prep*). We found that, in these habitats, TBGWs commonly used the chenopod shrub black bush (*Maireana pyramidata*), and that they forage mainly on the ground, around bases of shrubs, gleaning food items from the ground. Because TBGWs in Witchelina Nature Reserve specifically use chenopod shrub species for foraging, shelter, and as a nesting substrate (Louter et al. b, *in prep*; Chapter 3), a reduction in the cover of chenopod shrubs not only reduces the amount of substrate available for foraging and shelter, but likely also reproductive success. For example, bird nests may be trampled by livestock (Chaiyarat and Eiam-Ampai, 2014, Mandema *et al.*, 2013, Sharps *et al.*, 2015). Furthermore, grazing can increase predation risk in more open habitats (Cardoni *et al.*, 2012, Little *et al.*, 2015, Sharps *et al.*, 2015), reduce the amount of available nesting substrate (Harrison *et al.*, 2011), and lower prey availability (Sutter and Ritchison, 2005). Removal of livestock showed the recovery of vegetation structure and subsequently increased avian diversity (Earnst *et al.*, 2012, Nelson *et al.*, 2011).

Most *Amytornis* species area gregarious: the majority of their diet consists of seeds and a wide range of insects, including ants, beetles, grasshoppers, moths and termites

(Rowley and Russell, 1997). A quantitative study on the diet of the western grasswren (*Amytornis textilis textilis*) in Western Australia, found that scats of *A. t. textilis* contained some seeds, but mainly ants, beetles and termites (Brooker, 1998a). Similarly, feeding observations at TBGW nests in Witchelina Nature Reserve showed that adults fed a variety of different insects species to their offspring, including ants, beetles, spiders, caterpillars and grasshoppers (Louter et al. c *in prep*; Chapter 4). A reduction in arthropod and ant abundance as a result of grazing may therefore negatively impact food availability of TBGWs, which in turn could affect their foraging behaviour, home range and core area size.

Amytornis are notorious for their secretive behaviour and as a result baseline ecological data of most grasswren species are still lacking. Yet, effective wildlife management is underpinned by knowledge of species resource requirements, as well as their behaviour (Caughley and Sinclair, 1994). This study is the first to use radio tracking to estimate home range size of TBGWs. Few other studies described territory size, not home range size, of grasswrens. Western grasswrens (*Amytornis textilis textilis*) have a territory of 1.2 to 2.0 ha, based on observations and re-sightings of colour-banded birds (Brooker, 1998a, b, Brooker, 1988); the striated grasswren *A. striatus* occupies territories of 3.0 ha ± 1.1 SE, based on observations and re-sightings of colour-banded birds (Karubian, 2001), and territory size of the white-throated grasswren *A. woodwardi* is described as 10 ha (Noske, 1992). Thick-billed grasswrens are known to be extremely shy and consequently difficult to observe, but radio-telemetry provided an unbiased set of bird locations and allowed us to estimate home range size for this species. There was no significant association between TBGW home range size and grazing in this study, nor did we detect a relationship between home range size and vegetation or insect parameters. Based on the optimal foraging theory we hypothesized larger home range size of TBGW in areas with heavy grazing impact. However, our sample size was too small to sufficiently test this.

Conclusions

Grazing is the most abundant form of land use in arid South Australia, but it is only recently that the maintenance of biodiversity and ecosystem function have emerged as substantial concerns for grazing management. Within the Maluridae family, *Amytornis* has the highest number of threatened and extinct species, and the perceived threats to grasswrens were summarized by Skroblin and Murphy (2013). Many grasswren species occur in areas that are being altered through anthropogenic disturbances, including habitat fragmentation, habitat destruction and livestock grazing (Garnett *et al.*, 2011, Skroblin and Murphy, 2013). In this study we identified changes in chenopod vegetation cover and insect abundance due to grazing pressure in an arid rangeland wildlife reserve recovering from grazing pressure by livestock. We established that TBGWs are mostly absent from areas that are close to artificial water dams, which contain little chenopod vegetation cover, suggesting that TBGWs are vulnerable to effects of grazing. The home range size (~8 ha) of TBGW is relatively large given the birds' size and life history traits as a primarily ground-dwelling gregarious bird. The results of this study provide natural history field information for a hitherto unstudied endemic member of an Australasian bird family. It also provides empirical evidence for the importance of livestock removal to preserve avian species affected by livestock grazing. The preservation and habitat restoration of chenopod shrubland throughout the range of the thick-billed grasswren *A. m. raglessi* is crucial and should therefore be a high priority conservation tool for the persistence of the species.

Acknowledgements

We thank the Nature Foundation of South Australia for allowing access to Witchelina Nature Reserve and for providing accommodation on the property. We thank all the management staff of Witchelina Nature Reserve for assistance and logistical support. We thank Simone Dalgairns for expert advice on radio tracking in the 2014 field season. Scott Kinlock, Wikke Popken, Madeline Thomson, Sara Criddle, Petra Hanke, Andrea Robinson and Bianca Staker helped with catching birds, field observations and nest searching. The Australian Bird and Bat Banding Scheme (ABBBS) provided bands. All procedures conducted in this study complied with the laws of the country in which they were performed, followed the guidelines for the Australian Code of Practice for the use of animals for scientific research, and were approved by the Flinders University Animal Welfare Committee (permits: E325, E312 and E385). We are immensely grateful to the Nature Foundation of South Australia Inc. for their generous financial support. BirdsSA and Birdlife Australia also funded this study.

Table 2.1 Overview of ecological parameters (mean ± SE) at survey sites with varying grazing impact (heavy, moderate, little) in Witchelina Nature Reserve, South Australia. Significant differences between sites with varying grazing impact are bold and shaded in grey.

Parameter	*Heavy grazing (0-2 km from dam)*	*Moderate grazing (2-5 km from dam)*	*No/little grazing (> 5 km from dam)*
Overall vegetation cover (%)	25.13 ± 2.83 (n = 20)	27.39 ± 2.03 (n = 36)	40.40 ± 9.26 (n = 6)
Chenopod vegetation cover (%)	**6.35 ± 1.28 (n = 20)**	**9.25 ± 1.20 (n = 36)**	**10.18 ± 3.31 (n = 6)**
Chenopod shrub abundance	78.65 ± 18.48 (n = 20)	114.25 ± 15.89 (n = 36)	104.0 ± 32.58 (n = 6)
Chenopod shrub diversity	3.90 ± 0.40 (n = 20)	4.72 ± 0.19 (n = 36)	5.17 ± 0.48 (n = 6)
Arthropod abundance (minus ants)	**4.51 ± 1.65 (n = 18)**	**9.92 ± 1.89 (n = 21)**	**4.85 ± 1.67 (n = 4)**
Ant abundance	**3.74 ± 1.83 (n = 18)**	**5.53 ± 1.56 (n = 21)**	**14.55 ± 6.45 (n = 4)**
Arthropod diversity	1.44 ± 0.25 (n = 18)	2.04 ± 0.24 (n = 21)	2.07 ± 0.29 (n = 4)

Table 2.2 Overview of ecological parameters (mean ± SE) at survey sites with TBGW present or absent in Witchelina Nature Reserve, South Australia. Significantly differences between TBGW presence/absence sites are bold and shaded in grey.

Parameter	*TBGW absent*	*TBGW present*
Grazing impact (distance to dam in km)	**1.61 ± 0.28 (n = 26)**	**3.13 ± 0.26 (n = 36)**
Overall vegetation cover (%)	24.30 ± 2.86 (n = 26)	30.54 ± 2.18 (n = 36)
Chenopod vegetation cover (%)	**4.73 ± 0.96 (n = 26)**	**11.06 ± 1.16 (n = 36)**
Chenopod shrub abundance	74.31 ± 15.81 (n = 26)	135.33 ± 15.08 (n = 36)
Chenopod shrub diversity	3.58 ± 0.34 (n = 26)	4.72 ± 0.19 (n = 26)
Arthropod abundance (minus ants)	6.45 ± 1.88 (n = 20)	7.83 ± 1.60 (n = 23)
Ant abundance	6.03 ± 2.24 (n = 20)	5.27 ± 1.42 (n = 23)
Arthropod diversity	2.07 ± 0.29 (n = 20)	1.40 ± 0.15 (n = 23)

Table 2.3 Summary table of home range estimates and core activity area estimates of eight adult male thick-billed grasswrens (*Amytornis modestus raglessi*) at Witchelina Nature Reserve, South Australia. Home range estimates are derived from the Kernel Density estimates (KDE) at fixed 95 % utilisation isopleths and core activity area estimates are derived from fixed KDE at fixed 50 % utilisation isopleths. Minimum Convex Polygon (MCP) 100 % home range estimates are provided for comparison purposes.

Bird ID	**No of GPS fixes**	**Distance to dam (in km)**	**KDE 95 % (ha)**	**Core activity area (ha)**	**% Core activity area of total 95 % KDE**	**MCP 100 % (ha)**
1	42	5.7	7.7	2.4	31 %	8.0
2A	38	3.4	3.2	1.0	31 %	9.2
31	37	1.3	5.1	1.4	27 %	7.4
50	36	0.7	4.5	1.7	38 %	5.3
25	35	0.8	18.2	6.1	34 %	18.2
18	40	5.8	8.0	2.4	30 %	12.6
4	33	3.2	6.2	1.6	26 %	5.5
35	27	3.4	11.4	3.4	30 %	6.6
Mean ± SE	36.5 ± 1.3	5.1 ± 3.0	8.0 ± 1.7	2.5 ± 0.6	30.8 % ± 1.3	9.1 ± 1.54

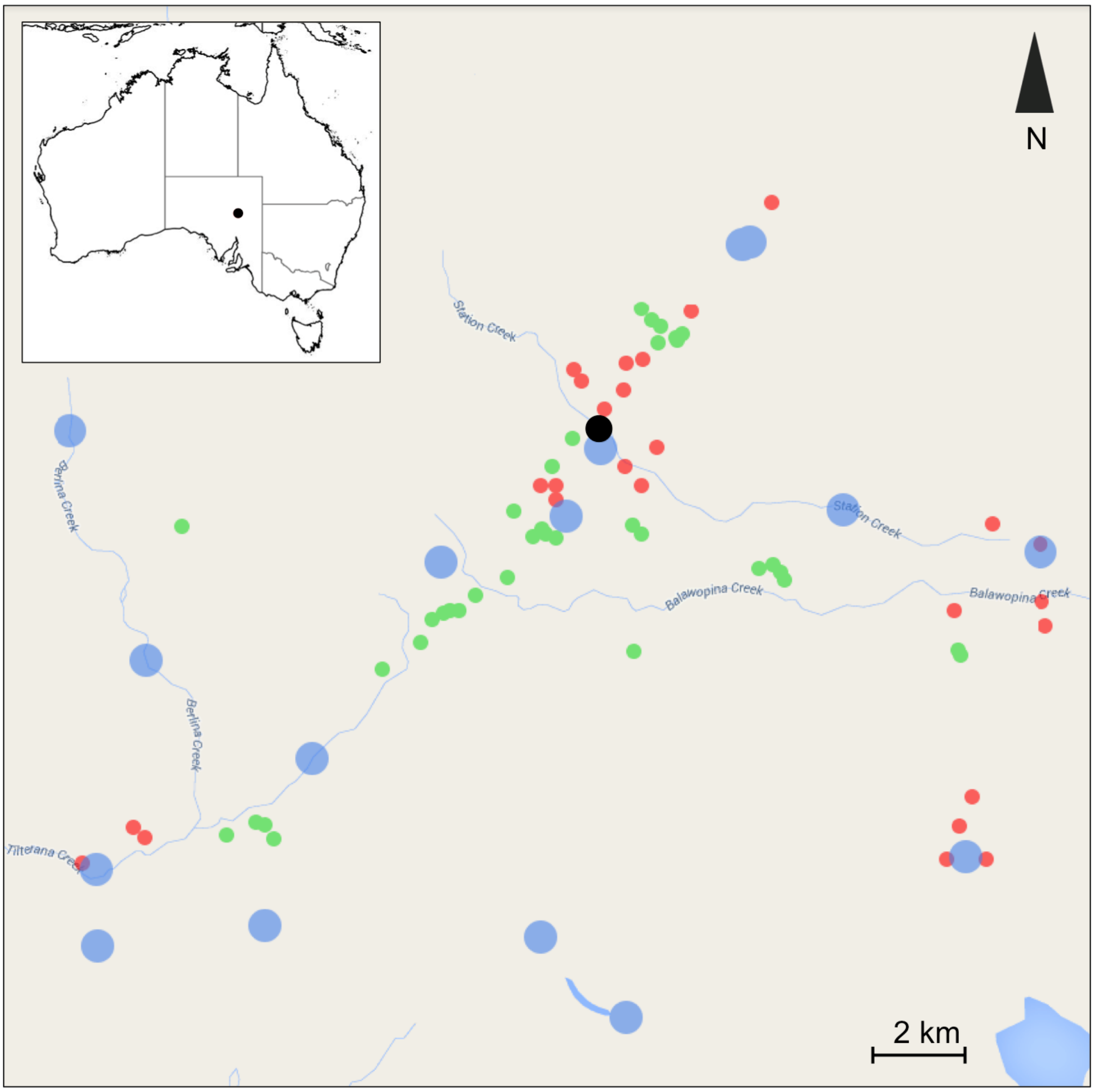

Figure 2.1 Map showing the location of Witchelina Nature Reserve (inset), and Witchelina homestead (black circle) with the distribution of artificial water dams (blue circle), sampling sites with thick-billed grasswrens (*Amytornis modestus raglessi*) present (green circle), and sampling sites without TBGWs present (red circle).

S1 Table 2.1 Overview table of radio tracking parameters: body mass, tagging date, transmitter battery life, and tracking duration (no of days observed) of 14 radio-tagged adult male thick-billed grasswrens (*Amytornis modestus raglessi*) at Witchelina Nature Reserve, South Australia.

Bird ID	Group size	No of fixes	Distance to nearest water dam (km)	Body mass (g)	Transmitter weight % body mass	Tagging date	Battery life (days)	No of days observed
1	5	42	5.7	17.9	2.79	18/8/14	29	29
2	3	27*	3.2	19.1	2.62	19/8/14		13 (fell off)
2A	2	35	3.4	21.3	2.35	19/8/14		20 (fell off)
31	2	35	1.3	21.2	2.36	20/8/14	29	29
50A	2	14*	0.7	19.3	2.59	21/8/14		6 (fell off)
34	2	17*	3.5	22.2	2.25	22/9/14		6
50	3	33	0.7	18.8	2.66	21/8/14	25	25
25	5	35	0.8	19.7	2.54	24/8/14	21	21
49	2	23*	0.6	20.1	2.49	25/8/14		23
56	2	16*	3.6	17.5	2.86	24/9/14		4
18	2	35	5.8	18.9	2.65	27/8/14	23	23
4	3	33	3.2	19.4	2.58	10/9/14		17
35	3	27	3.4	19.9	2.51	15/9/14		12
18A	2	22*	5.4	19.6	2.55	13/9/14		15
Mean ± SE	2.7± 0.3	28.1 ± 2.3	3.0 ± 0.5	19.6 ± 0.3	2.6 ± 0.04	-	25.4 ± 1.6	17.4 ± 2.2

* Individuals excluded from the KDE home range estimation due to low number of fixes.

Chapter 3 What predicts nest predation in thick-billed grasswrens (*Amytornis modestus raglessi*)?

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In preparation to be submitted to *Biological Conservation*

Abstract

Nest predation is the primary cause of avian nesting failure. In order to manage vulnerable and threatened bird populations, it is central to identify key factors affecting predation risk. We conducted the first field study of the vulnerable thick-billed grasswren (*Amytornis modestus raglessi*), which we studied at Witchelina Nature Reserve in the arid rangelands of South Australia, with the aim to assess the role of predation as a threatening process for population persistence. We used a combination of experimental and observational approaches to (1) identify predator type, (2) assess avian and rodent predator activity, (3) quantify predation outcome at natural grasswren nests, and (4) identify nesting site variables that covary with predation outcome in natural and artificial nests. Using 404 artificial nests (open-cupped, domed) across two years, we found evidence for rodent (10 %) and avian (12 %) predators; there were significant differences in predator type across years. Rodent activity (assessed by 189 footprint tracking tunnels) differed across years and predicted predation at artificial nests. Avian predator activity (assessed by point count bird surveys) did not differ across years nor did it predict predation at artificial nests. We examined nesting outcome at 47 natural grasswren nests and continuously video-recorded at 12 nests; total nest predation was 11.5 %, whereby no filmed nest was depredated. We examined the effects of nesting site variables (vegetation concealment, nesting height) on predation outcome at artificial and natural nests; nest concealment predicted avian and rodent predation at artificial nests but not

at natural nests. We conclude that nest predation may not be the main factor contributing to the decline of the vulnerable thick-billed grasswren and discuss possible impacts of local conservation management such as predator control at Witchelina Nature Reserve.

Introduction

Low nesting success, and consequent low offspring recruitment into the breeding population, is a significant contributing factor for population declines in terrestrial songbird species (Martin, 1992). Predation is the main cause of low nesting success in most avian species, resulting in 30-95 % nesting failure (Martin, 1988, McGuire and Kleindorfer, 2007, Nice, 1957, Ricklefs, 1969). Nest predation may therefore be important for birds of conservation concern (Remeš *et al.*, 2012a). Predation risk varies within and between species depending on numerous factors such as nest type (Martin, 1993b), nest concealment (Lambert and Kleindorfer, 2006, Martin, 1993a, Martin and Roper, 1988), and the behaviour of both prey and predator (DeGregorio *et al.*, 2016, Martin *et al.*, 2000, Thompson III and Burhans, 2003). Bird species with open-cup nests have higher predation risk compared with species with domed nests (Galligan and Kleindorfer, 2008, Ricklefs, 1969); the underlying explanation for this pattern is higher nest content detectability by visually hunting predators. Studies have shown that visually conspicuous nests (e.g. open-cup) are more likely to be depredated by visually hunting predators such as birds (Kleindorfer *et al.*, 2003, Peak, 2003, Remeš, 2005b, Stokes and Boersma, 1998, but see Howlett and Stutchbury, 1996,), while concealed nests (e.g. domed) are more likely to be depredated by olfactory predators such as rodents and snakes (Colombelli-Négrel and Kleindorfer, 2009). Identifying predator type is a key component for identifying predation risk for a given species. Therefore, in order to manage declining populations affected by predation risk, conservation managers need to address the likelihood of different predator types in relation to nest site characteristics of the focal avian species of conservation concern.

Nesting site vegetation is a key component of avian nesting success in areas affected by visually hunting predators (Batary *et al.*, 2004, Best and Stauffer, 1980) or when nesting site vegetation provides climbing structures for predator access (e.g. Galligan and Kleindorfer, 2008). The nest concealment hypothesis predicts that birds that select a nesting site with dense vegetation characteristics (i.e. high vegetation concealment) should have a lowered predation risk because inconspicuous nests are less likely to transmit cues to predators and/or can be inaccessible to potential predators (Chalfoun and Martin, 2009, Filliater *et al.*, 1994, Flaspohler *et al.*, 2000, Lambert and Kleindorfer, 2006, Martin and Roper, 1988, Prokop and Trnka, 2011). Previous studies have found associations between vegetation concealment of avian nests and predator type; visually conspicuous nests were more likely to be depredated by visually hunting predators, and visually concealed nests were more likely to be depredated by predators using olfactory or thermal cues to locate their prey (Colombelli-Négrel and Kleindorfer, 2009, Rangen *et al.*, 2000, Remeš, 2005a). Therefore, there are well-supported associations between vegetation concealment at nests and risk of predation by different predator types. Understanding the characteristics of habitats in regards to both factors (vegetation concealment, predator type) are important variables to manage predation risk.

Human activities, such as pastoralism (e.g. grazing) and the introduction of feral predators, can significantly affect flora abundance and diversity (Adler *et al.*, 2001, Landsberg *et al.*, 1999, Wilson, 1990), as well as fauna abundance and diversity (Rambo and Faeth, 1999, Read and Cunningham, 2010). Because habitat characteristics have a strong influence on avian nest predation (Bowman and Harris, 1980, Martin, 1993a, Noske *et al.*, 2008), changes in the structure and composition of vegetation communities can have cascading effects for altering predation risk (e.g. Newmark and Stanley, 2011). Evidence for increased nest predation in grazed and fragmented landscapes is well documented for ground-nesting birds (Beja *et al.*, 2014, Cardoni *et al.*, 2012, Chaiyarat and Eiam-Ampai, 2014, Little *et al.*, 2015, Lusk and Koper, 2013, Sutter and Ritchison, 2005). The introduction of (feral) predators can further reduce avian nesting success (Owens and Bennett, 2000, Remeš *et al.*, 2012b). Understanding local associations between nesting site attributes, predator type, and nest predation patterns is an important first step to direct conservation management efforts of vulnerable and declining avian populations.

The use of artificial nests baited with plasticine eggs is an effective way to collect information on predator type because predator marks can be left on the soft plasticine eggs tethered to the nest (Colombelli-Négrel and Kleindorfer, 2009, Major, 1991, Møller, 1989). Artificial nests can also be used to estimate predation rate (reviewed in Moore and Robinson, 2004, Wilson and Cooper, 1998), though the findings on predation rate may not accurately reflect trends at natural nests (Burke *et al.*, 2004, Major and Kendal, 1996). Other effective approaches to identify predator type involve nest monitoring, such as audio-video surveillance systems with miniature cameras, at both artificial (Patterson *et al.*, 2016, Weidinger, 2001) and natural avian nests (e.g. Pietz and Granfors, 2000, Stake *et al.*, 2004, Thompson III *et al.*, 1999). The presence (or absence) of specific predator types in a given study area can be inferred from artificial nests studies, which is useful information to predict the cause of nesting failure of natural nest in threatened species (Benson *et al.*, 2010).

The thick-billed grasswren *Amytornis modestus* (North, 1902, Parker, 1972) is a small terrestrial songbird that is endemic to the arid regions of inland Australia. Formerly, thick-billed grasswrens had a broad distribution across arid and semi-arid Southern and Central Australia (Baird, 1990, Black, 2004, 2011b, 2012), but their current distribution is restricted to small isolated populations across Northern South Australia (Black *et al.*, 2011a). Consequently, the thick-billed grasswren is listed as nationally vulnerable under the EPBC act (1999) (Garnett *et al.*, 2011). Anthropogenic factors such as habitat loss and habitat alteration through pastoralism and overstocking have been attributed as a major reason for a reduction in their distribution (Garnett *et al.*, 2011). Another factor that may threaten the thick-billed grasswren and other grasswren species is increased nest predation by introduced feral predators such as Domestic cats (*Felis catus*) and Red foxes (*Vulpes vulpes*) (Garnett *et al.*, 2011), although this is yet to be confirmed as there is no direct evidence of the identity of grasswren nest predators. Currently, the ecology and breeding biology of thick-billed grasswrens is poorly known, because detailed studies for this species are lacking (but see Chapter 2). Aside from brief descriptions of nests, nesting habits, eggs and clutch size (Chapman, 1996, Rowley and Russell, 1997, Serventy, 1982, Whitlock, 1924), no studies on reproductive success and factors that influence nesting outcome for thick-billed grasswrens have been published to date. Without information on nesting biology and nesting failure, the causes of decline remain speculative for thick-billed grasswren populations.

In this study we examined nest predation at artificial nests and natural thick-billed grasswren (subspecies *Amytornis modestus raglessi*) (Black, 2011a) nests in Witchelina Nature Reserve, studied in the arid pastoral rangelands of South Australia. We aim to identify the most common predator types of open-cup and domed nests, and to assess the role of predation risk as a threatening process for population persistence in this nationally vulnerable bird species. Firstly, we conducted nest-predation experiments to quantify predation rates and identify predators. We use artificial nests baited with plasticine eggs to identify predator type (avian, rodent) in relation to nest type (open-cupped, domed). While there are known drawbacks, such as underestimation of predation risk at natural nests when using artificial nests (Faarborg, 2004, Major and Kendal, 1996, Moore and Robinson, 2004, Thompson and Burhans, 2004, Weidinger, 2001, Zanette, 2002), they offer several advantages for a rapid assessment of predation risk including: (a) the ability to implement controlled experiments across different treatments even when the species of interest is absent (i.e. tests of habitat condition) (Bayne and Hobson, 1997, Major and Kendal, 1996); (b) increased sample size and hence statistical power (Faarborg, 2004); and (c) information on predator type from marks left on eggs (Møller, 1989). Secondly, we measure rodent activity (using footprint tracking tunnels) and avian activity (using point count bird surveys) to assess activity levels of potential predators. We tested if predator activity was associated with predation outcome at artificial nests (survived, depredated). Finally, we test if nest type (open-cup, partly domed, domed) and nest site characteristics (vegetation cover, nest height, nest placement, shrub height) are associated with predation outcome at artificial nests and natural thick-billed grasswren nests.

We test the following predictions about nest type and nesting site characteristics for predation outcome: (1) higher nest predation at open-cup than domed nests, due to differences in accessibility and concealment of nest contents, (2) higher avian predation at visually conspicuous nests with low vegetation concealment and no association between rodent predation and vegetation concealment, (3) an effect of nest height on predation outcome with more rodent predation at lower nests (natural nests, artificial nests), due to nest accessibility, (4) a statistical association between predator activity (rodent, avian) and nest predation, and (5) effects of nesting site attributes (nest height, nest concealment) on predation type and therefore nesting outcome in thick-billed grasswren nests.

Methods

Study site

This study was conducted during the years 2012 to 2015 with field trips during August to September spanning a total of 18 weeks. The study site was Witchelina Nature Reserve (30º01’340” S, 138º02’772” E), located in the arid rangelands of inland South Australia (Figure 1.2, p.6). Witchelina is approximately 4200 sq. km in size, and is owned and managed as a nature reserve by the Nature Foundation of South Australia (NFSA). Before its purchase in 2010, Witchelina was a pastoral sheep station, but since then all livestock has been removed from the property. Since 2010 there is an active feral predator control program in use, with removal of over 500 feral Domestic cats (*Felis catus*) and over 150 Red foxes (*Vulpes vulpes*) (NFSA, unpublished data). The region has an arid climate, characterized by hot, dry summers and mild winters. Maximum temperatures average around 28.8 °C, but can reach up to 49.5 °C in summer, and minimum temperatures average around 13.3 °C, but can drop to -2.8 °C in winter (Australian Bureau of Meteorology, location: Marree, period 1939-2015). Average annual rainfall is 163.9 mm (period 1939 2014), but year-to-year variation can be very high: during the years of this study total annual rainfall was as follows: 2012: 172 mm, 2013: 99 mm, 2014: 208 mm (Australian Bureau of Meteorology, location: Marree (Farina), period 1939-2015). In 2011, the year prior to the commencement if this study, total annual rainfall was extremely high (total annual rainfall: 305 mm), which was associated with one of the strongest La Niña events on record (Australian Bureau of Meteorology). Witchelina inhabits ten major habitat types; including gibber plains, red river gum and coolibah woodlands, saltbush plains, acacia dunefields and blue bush shrublands. Bird surveys conducted on Witchelina Nature Reserve confirmed that thick-billed grasswrens occur in saltbush plains and blue bush shrublands within the reserve (Black *et al.*, 2011b, Wurst, 2011). Within an area of ~50 sq. km in Witchelina Nature Reserve we found and mapped out a least 42 grasswren territories located in these two habitat types on the plains of the reserve (Figure 1.2, p.6).

Study species

The thick-billed grasswren (*Amytornis modestus raglessi*) (Black, 2011a) is a small (~19 g) ground-dwelling passerine that occurs in the arid zone of South Australia and New South Wales. Thick-billed grasswrens are sedentary and their distribution is associated with low slow-growing chenopod shrublands, dominated by drought and salt tolerant long-lived perennial shrub species such as *Maireana* (blue bush, black blue bush and cotton bush) and *Atriplex* (saltbush) (Black, 2012, Black *et al.*, 2011a, Rowley and Russell, 1997). They have a stout dark coloured bill, cryptic plumage with brown and white streaked feathers (striations) on their head and neck, and females can be distinguished from males by presence of a red rufous flank-patch under the wing (Figure 1.4, p.9). The main breeding season is defined as August to September (Black *et al.*, 2011a, Rowley and Russell, 1997), but thick-billed grasswrens are also known to breed opportunistically after rainfall events, thus breeding can occur throughout the year. This is the first field study of nesting behaviour of this species.

Natural nest searching, nest monitoring, and nesting outcome

We found and monitored a total of 47 active thick-billed grasswren nests at Witchelina Nature Reserve across the three sampling years. Nests were primarily located via intensive systematic searches in chenopod vegetation within known thick-billed grasswren territories. When a nest was found, its vegetation characteristics (see below) were measured and its location was recorded using a handheld GPS. Flagging tape was inconspicuously placed in a shrub located 15 meters northerly of the nest location (Martin and Geupel, 1993). We visited active nests every 1–2 days to monitor predation outcome. To identify nest predators at natural nests, a digital user-built video recording (DVR) system (described in Louter et al. c, *in prep*; Chapter 4) was deployed at a sample of active thick-billed grasswren nests with nestlings (n = 5 in 2013, n = 7 in 2014), which recorded continuously (range 1-14 days) at each nest until the fate of the nest was known (abandoned, predated, or fledged). Nest fate was ascertained following Martin and Geupel (1993) and Martin et al. (1997), defining successful nests as those that fledged at least one young. Around suspected fledge dates, we noted a nest to be successful if any fledglings were present near nests, either by directly observing fledglings or observing adults carrying food or calling near nests. Some thick-billed grasswren nests (n = 9 in 2013; n = 1 in 2014) were discovered just after the young had fledged, but these nests were confidently aged to the breeding season of that year by examining the appearance of the nest (e.g. fresh lining and/or fresh faecal samples in the nest) or by observing fledglings in the vicinity (within 10 meters) of the nest location. When a nest failed, we attempted to determine the cause of failure. If nests/eggs were destroyed or eggs/chicks were missing, the nest outcome was classified as depredated. At depredated nests, the state of the nest (nest intact, lining outside of nest opening, nest destroyed) and the presence of any remains (eggs, eggs shells, dead chicks) were recorded. If the clutch was cold on three consecutive visits (n = 3) or nestlings were found dead (n = 1), the nest was classified as abandoned. Nests with eggs or nestlings that were abandoned by adults in the absence of a partial predation event, and nests that had an unknown fate (e.g. nesting outcome could not be established due to time constraints) were excluded from predation analyses.

Nest-predation experiment

A total of 202 open cup-shaped nests and 202 domed-shaped nests baited with plasticine eggs were used to assess egg predation rates and identify predator type at 202 sites (2012: n = 179, at 90 sites; 2013: n = 225 at 113 sites). Methods of artificial nests and artificial egg preparation are described in detail in Colombelli-Négrel and Kleindorfer (2009) and Galligan and Kleindorfer (2008). Nests contained two artificial plasticine eggs that were shaped and sized to approximate natural size egg size of thick-billed grasswrens (length x width: 20.7 mm ± 0.14 SE x 15.48 mm ± 0.08 SE, n = 53, this study). Artificial nests were placed at sites using random GPS coordinates, across a ~50 km^2 area within Witchelina Nature Reserve. We alternated the position of the two artificial nests per site so that one cupped and one domed nest were positioned approximately 50 metres apart, with each pair of nests separated by 1 to 50 km. At sites, artificial nests were placed in habitat that appeared to resemble grasswren habitat (determined by the presence of chenopod shrubs and bushes) in typical grasswren nesting sites, such as chenopod shrubs (pers. obs.). Since the natural height of grasswren nests was only determined at the end of this field study, we used the range in nest height from other species of Maluridae for the placement of artificial nests (0.1 m to 1.5 m, dependent on nature of the vegetation structure). Nests were left for 14 days, which approximates the incubation period for many passerine species (Yom-Tov, 1987), and removed after assessment. A predation event was recorded if a potential nest predator marked any of the plasticine eggs (Colombelli-Négrel and Kleindorfer, 2009, McGuire and Kleindorfer, 2007, Remeš, 2005a). Predator type was inferred from the shape of marks on the eggs as follows: avian predation was inferred if we found triangular marks or a single entry circular mark (Thorington and Bowman, 2003); mammalian predation was inferred if we found parallel/rectangular marks that were indicative of tooth marks, or otherwise "gnashed eggs" with multiple serrations (Colombelli-Négrel and Kleindorfer, 2009, Fulton and Ford, 2003, Major and Kendal, 1996, McGuire and Kleindorfer, 2007, Rangen *et al.*, 2000). We inferred nest survival if there were no marks on the plasticine eggs. Artificial nests with an unknown predation outcome (e.g. when a nest was found on the ground, or a nest was lost) were excluded from analysis.

Rodent predator activity

Black footprint tracking tunnels (500 × 100 × 100 mm; Gotcha Traps, 2 Young Street, RD2, Warkworth, NZ) were used to detect rodent activity in parallel with the artificial nest experiment. Preparation of tracking tunnels and baits are described in detail elsewhere (see Colombelli-Négrel and Kleindorfer 2009). A total of 189 footprint tracking tunnels (2012: n = 76; 2013 n = 113) were placed approximately 20 m from each pair of artificial nests (open cup, domed) and left in the field for five days. Rodent footprints in tracking tunnels were identified as by Hasler *et al.* (2004). Rodent activity was scored based on the presence or absence of rodent footprints on the inkpad.

Avian predator activity

To survey avian predator activity, we used point counts bird surveys with a count period of ten minutes and with an unlimited count radius (Ralph *et al.*, 1995, Ralph *et al.*, 1993). All 202 sites were sampled on three consecutive days at different times of day, allowing for a range of weather condition and times of day to be sampled at each site. During the survey we recorded the species of all birds seen or heard and the detection method per bird (sight or sound). We classified a bird species as a potential avian predator, or potential brood parasite based on previous published studies for similar-sized songbirds (e.g. Berry, 2002, Conner *et al.*, 2010, Hethcoat and Chalfoun, 2015). All point count surveys were conducted on wind-still days (< 15 knots) between 6.30 am (sunrise) and 6:00 pm, excluding the hotter hours of the middle of the day. The count of the number of potential avian predators at each artificial nest site was converted to presence or absence of avian predators, irrespective of the avian predator species or abundance.

Nesting site characteristics (natural and artificial)

The following nesting site characteristics of both natural and artificial nests were measured: (1) nesting height (measured in cm from the ground to the bottom of the rim of the nest opening), (2) nest concealment (% vegetation cover above, in front, and on two sides of the nest, measured at 1 m from the nest at nesting height level; artificial cup-shaped nests were placed in dense chenopod shrubs in such a way that there was an ‘entry point’ after nest placement, which was determined to be the front of the nest), (3) nesting shrub height, and (4) nest placement (nesting height in relation to the substrate height calculated as nesting height/substrate height; ratio values ranging from 0-1). For analyses, side concealment was taken as a mean of the two side measures, and overhead concealment and front concealment were treated as separate variables. Pearson correlation tests were used to measure the strength and direction of association between the explanatory nesting site variables. To avoid issues with collinearity among predictor variables, correlated variables were removed ($r \geq 0.5$) using Pearson correlation co-efficient test. Shrub height and nesting height of artificial nests were highly correlated ($r = 0.81$) therefore the former was removed from further analysis. The variables nesting height ratio and nesting height were highly correlated for artificial and natural nests ($r = 0.60$, $r = 0.55$, respectively), therefore the former was removed from further analysis. Eventually, four nesting site characteristics (nesting height, vegetation cover above nest, in front, and on the sides of the nest) were retained for modelling effects on predation in artificial nests. Five nesting site characteristics (nesting height, vegetation cover above nest, in front, and on the sides of the nest, shrub height) were retained for modelling effects on predation in natural grasswren nests. The effects of these nesting site characteristics on nesting outcome were evaluated by comparing variables in successful and depredated natural and artificial nests.

Statistics

The statistical program SPSS (version 23) was used to complete all statistical analyses. Before conducting statistical analyses, we examined the data to determine if they conformed to assumptions of normality and homogeneity of variance. We used Chi-squared tests to test for the difference in proportion of nesting outcome (survived, rodent predation; survived bird predation) in relation to nest type (open-cup, domed) within years and across years. We used Binary Logistic Regression to test for the directional effects of nest type, nesting site characteristics (vegetation cover, nesting height), and predator activity (avian and rodent) as predictor variables on the following outcome variables: survived (0,1), rodent predation (0,1), and avian predation (0,1).

Results

Nesting biology

We found a total of 47 thick-billed grasswren nests at Witchelina Nature Reserve across three sampling years (2013: n = 18; 2014: n = 18; 2015: n = 11), in various stages of the breeding cycle (Table Grasswren nests were usually built close to the ground (nesting height: 23.07 cm ± 0.9 SE) and the majority of nests (98 %) were built in live dense woody chenopod long-lived perennial shrubs (S1 table 3.1). The most common nesting shrub species was black bush (*Maireana pyramidata*, n = 30), followed by low blue bush (*M. astrotricha*, n = 7), thorny saltbush (*Rhagodia spinescens*, n = 5), cotton bush (*M. aphylla*, n = 4), and bladder saltbush (*Atriplex vesicaria*, n = 1). The mean height of the nesting substrates measured 64.1 cm ± 13.4 (range 29 – 97 cm, n = 47), with an average crown spread of 130.1 cm ± 31.8 (n = 47). Nest-site fidelity was high, both within and between years, thick-billed grasswrens built nests within 100 meters of their previous nest (41.5 m ± 29.5 SD, range 3 – 90 m, n = 11 territories). Thick-billed grasswren nests in Witchelina Nature Reserve were solid structures, composed of densely weaved dry grass and/or twigs; the lining was entirely of plant origin. The shape of thick-billed grasswren nests varied, ranging from open cup-shaped nests (n = 3), cups with an elevated rim forming partly domed nests (n = 35), and nests with a fully constructed hood forming completely domed nests (n = 9) (Figure 3.1). Nests were oriented toward the east (38 %) or south (30 %), with fewer facing north (18 %) or west (17 %). Nests were well hidden (total nest concealment: 79 ± 19 % cover, range 82-100 %) and situated below the centre of the substrate height (mean nest height ratio 0.37 m ± 0.11 SE, n = 46 (S1 table 3.1).

Predation outcome grasswren nests

We established the nesting outcome for 35 out of 47 monitored nests of the thick-billed grasswren (Table 3.1). Due to time constraints we were unable to observe the outcome of 12 active nests, because these nests were still active when the fieldwork period ended. Of the 35 grasswren nests with a known nesting outcome, 26 nests (74.3 %) successfully fledged ≥ 1 offspring, one nest (2.9 %) contained an infertile clutch of two eggs and was abandoned after 25 days of incubation, and four grasswren nests (11.5 %) were depredated (Table 3.1). Predation rate was similar in both 2013 and 2014 (11.8 % both years). Four grasswren nests (2 in 2013, 2 in 2014) were abandoned for unknown reasons (3 with eggs, 1 with nestlings) and were excluded from further predation analysis.

Predator identity natural grasswren nests

Natural depredated thick-billed grasswren nests (2 open-cup, 2 domed) were found entirely intact, without any detectable signs of predator activity at nests, or in the vegetation surrounding the nest. Predation events were not observed directly, nor were they recorded on video. Eleven out of the twelve video-recorded nests (see Louter et al. c, *in prep*; Chapter 4) successfully fledged ≥ 1 offspring (one nest was abandoned when nestlings were nine days of age). Consequently, the identity of thick-billed grasswren nest predators remained unknown.

Nest-predation experiment

A total of 22 % (n = 88) of the 404 artificial nests were depredated. Marks on plasticine eggs showed evidence of rodent (11.6 % 47/404) and avian (10.1 % 41/404) predation. At Witchelina Nature Reserve predation rate varied significantly across years ($\chi^2 = 17.04$, df = 1, $P < 0.001$), with more nest predation in 2012 (27.7 %) than in 2013 (15.8 %). Predator type (rodent or avian) also varied across years: rodent predation at artificial nests was significantly higher in 2012 than in 2013 ($\chi^2 = 34.99$, df = 1, P < 0.001), whereas avian predation at artificial nests was comparable between the two sampling years (χ^2 =1.45, df = 1, P = 0.139, Figure 3.2). As predicted, nest type (open-cup, domed) was associated with predator type (avian, rodent): comparing artificial open-cup and domed nests, we found a significantly higher proportion of avian marks in open-cup nests (χ^2 = 12.74, df = 1, $P <$ 0.001, Figure 3.3), and a significantly higher proportion of rodent marks in domed nests (χ^2 = 4.59, df = 1, $P <$ 0.023, Figure 3.3).

Rodent activity and rodent nest predation

We assessed the presence or absence of rodents (rodent activity) across years (2012, 2013) using a total of 189 footprint tracking tunnels, and examined if rodent activity was associated with rodent predation (survived, rodent predation) at artificial nests. Based on the evidence of rodent footprints in tracking tunnels, rodent activity was significantly higher in 2012 (48 % of tunnels had marks) than in 2013 (6 % of tunnels had marks) (χ^2 = 88.879, df = 1, $P <$ 0.001). Rodent activity was associated with rodent predation in artificial nests in 2012 (Pearson Correlation, r = 0.184, P = 0.024), but not in 2013 (Pearson Correlation, r = 0.086, P = 0.199).

Avian predator activity and avian nest predation

We assessed the presence or absence of avian predators (avian predator activity) across years (2012, 2013) using three point count surveys per site and examined if avian activity was associated with avian predation (survived, avian predation) at artificial nests. Point count surveys identified nine potential avian predators at the study site: Australian Raven (*Corvus coronoides*), Australian Magpie (*Gymnorhina tibicen*), Black Kite (*Milvus migrans*), Black-faced Cuckoo-shrike (*Coracina novaehollandiae*), Brown Falcon (*Falco berigora*), Crested Bellbird (*Oreoica gutturalis*), Grey Butcherbird (*Cracticus torquatus*), Nankeen Kestrel (*Falco cenchroides*), and Whistling Kite (*Haliastur sphenurus*). Avian predator activity varied across years and was significantly lower in 2012 (7 %) than in 2013 (36 %) (χ^2 = 49.408, df = 1, P < 0.001). There was no statistical association between avian predator activity and avian predation at artificial nests in 2012 (Pearson Correlation, r = -0.99, P = 0.186) or 2013 (Pearson Correlation, r = -0.17, P = 0.798). Point count surveys identified the presence of two species of potential brood parasites at our study site: the Horsfield's Bronze-Cuckoo (*Chrysococcyx lucidus*) and Black-eared Cuckoo (*Chrysococcyx osculans*), although they were infrequently observed (n = 8).

Nesting site characteristics and nest predation at artificial and natural nests

The effect of nesting site characteristics on predation type and outcome was evaluated by comparing these variables in successful and depredated artificial and natural nests. At artificial nests, rodent predation was predicted by high vegetation concealment (Logistic Binomial Regression, B = -0.019, P = 0.038, (Table 3.2). Avian predation was also predicted by vegetation concealment, but in an opposite direction: artificial nests with low vegetation cover in front of the nest were more likely to be depredated by avian predators (Logistic Binomial Regression, B = 0.027, P = 0.003, Table 3.3). At natural thick-billed grasswren nests, none of the measured nest-site characteristics (nest type, vegetation cover, nesting height, nest placement, nesting shrub height) predicted nesting outcome (predated, survived) (Logistic Binomial Regression, *all* P > 0.3). Thick-billed grasswren nests in Witchelina Nature Reserve were well concealed (S1 Table 3.1); the few depredated nests occurred in smaller shrubs (S1 table 3.2).

Discussion

Thick-billed grasswren nesting biology

This is the first study on the nesting biology of the vulnerable thick-billed grasswren (subspecies *Amytornis modestus raglessi*) in the wild, in South Australia. At Witchelina Nature Reserve, thick-billed grasswrens seem specialize in their use of nesting substrate, as 63 % of all nests were found in the chenopod shrub black bush (*Maireana pyramidata*). Thick-billed grasswren nests showed variation in type; we found open-cup, partly-domed and fully domed nests (Figure 3.1), which is unusual as most bird species generally build a particular nest type. In general, avian nests are considered to be adapted and optimized for high nesting success suited to their breeding environment (e.g. Heenan *et al.*, 2015). Currently there are eleven recognized grasswren species (Black, 2004, Black *et al.*, 2010, Christidis *et al.*, 2013, Schodde and Mason, 1999). The nests of most grasswren species have been described to some degree (Brooker, 1998a, Rowley and Russell, 1997, Schodde, 1982a, Serventy, 1982), and show great variation in nest types both within and across species (Table 3.4). A study on breeding biology of the western grasswren (*Amytornis textilis textilis*), which is the most closely related taxon to the thick-billed grasswren (Christidis *et al.*, 2010), found that western grasswren nests vary in shape, from open to fully domed nests (Brooker, 1998) similar to the nest type variation we observed in thick-billed grasswrens. The variation in western grasswren nest type was linked to vegetation characteristics around their nest (Brooker 1998): when vegetation growth was prolific, five out of 13 (38 %) western grasswren nests lacked a hood (i.e. were cup-shaped), whereas when vegetation cover was sparse, all 24 nests had some degree of a hood structure. We observed a similar trend at thick-billed grasswrens nests in Witchelina Nature Reserve, where open-cup nests tended to have higher vegetation cover above the nest than did domed or semi-domed nests (S1 table 3.1).

Predation outcome thick-billed grasswren

Results of this study show that predation at natural thick-billed grasswren nests in Witchelina Nature Reserve was low; 13 % (4/31) of nests with known nesting outcome were depredated and the predation pattern was comparable between 2013 and 2014 (11.8 %, Table 3.1). We did not observe partial depredation at any of the depredated nests and the frequency of nest desertion was low (4/35 nests, 11.4 %). Although effort was made to find and monitor all active nests in the 42 monitored grasswren territories at our study, grasswren nests are generally extremely hard to find. For some territories we were unable to observe breeding activity and therefore nests could not be located. Unsuccessful nesting attempts (e.g. depredated/abandoned nests) might therefore not have been discovered prior to their failure, which might have resulted in an underestimation of predation events. Additionally, breeding activity and active nests of thick-billed grasswrens at Witchelina Nature Reserve was monitored during the proposed breeding season of the species (August to September) (Black *et al.*, 2011a), but breeding activity could have persisted after the fieldwork period ended. This, together with the knowledge that grasswrens can breed opportunistically after rainfall events, might have resulted in an underestimation of overall nest predation.

Brood parasitism

Two cuckoo species occurred at Witchelina Nature Reserve, the Horsfield’s Bronze-Cuckoo (*Chrysococcyx lucidus*) and the Black-eared Cuckoo (*Chrysococcyx osculans).* Although the Horsfield's Bronze-Cuckoo is known to parasitize grasswren and fairy-wren nests (Brooker and Brooker, 1989, Rowley and Russell, 1997), no brood parasitism was observed at 47 thick-billed grasswren nesting attempts, indicating that brood parasitism by cuckoos is relatively uncommon in the population of the thick-billed grasswren occurring at Witchelina

Nature Reserve. Concurrent opportunistic monitoring of breeding activity of white-winged fairy-wrens (*Malurus leucoptera*), which co-occur in grasswren territories at our study sites (pers. observation), revealed brood parasitism of 3 out of 18 (16.7 %) active nests (unpublished data), indicating that cuckoos are active within our study area.

Predator type at grasswren nests

Despite continuous monitoring at twelve natural thick-billed grasswren nests (n = 5 in 2013, n = 7 in 2014), we were unable to identify thick-billed grasswren nest predators, because eleven out of twelve nests successfully fledged and one nest was abandoned when the nestlings were nine days old. Although avian predators more frequently predated artificial nests with low vegetation concealment, there was no statistical association between nest concealment and predation at natural grasswren nests. Due to the low variation in nesting site characteristics (S1 Table 3.1), our modest sample size, and the observed low predation rate, none of the measured nesting site characteristics (vegetation concealment, nest type and nesting height) predicted predation outcome of natural thick-billed grasswren nests. Most natural thick-billed grasswren nests at Witchelina Nature Reserve were extremely well concealed (vegetation concealment range 82 - 100 %) and as such visually searching predators such as birds of prey may not have been able to successfully detect grasswren nests. Likewise, since thick-billed grasswrens have well-camouflaged plumage, soft high-pitched songs and calls, and generally behave very secretively, by keeping to low cover in shrubs and drainage lines, it is unlikely that visual predators receive clues of the nest location from adult birds attending nests. We therefore conclude that visually hunting birds of prey were not the main predators of natural thick-billed grasswren nests at Witchelina Nature Reserve. The low position of thick-billed grasswren nests at our study site makes them a more likely candidate for predation by ground-dwelling olfactory predators such as snakes and rodents, as low nests are more accessible to these predators (Best and Stauffer, 1980, Martin, 1993b, Söderström *et* *al.*, 1998). None of the depredated thick-billed grasswren nests at our study site showed any detectable signs of predator activity; nests were found intact and vegetation around the nest was undisturbed. This lack of nest disturbance is in line with results from studies examining snake predation at bird nests. In Australia, snakes are one of the most common nest predator of shrub-nesting songbirds (Conner *et al.*, 2010, Maguire and Mulder, 2004), and snakes are known to leave nests undisturbed after a predation event (Thompson III and Burhans, 2003). At our study site we infrequently observed eastern brown snakes (*Pseudonaja textilis*) and feral Domestic cats (*Felis catus*) in thick-billed grasswren territories, but we did not detect any direct evidence linking these two potential nest predators to nest predation of thick-billed grasswrens.

Nest-predation experiment

The identification of potential nest predators is critical for the development and testing of alternative hypotheses for nest predation patterns (Benson *et al.*, 2010, Ribic *et al.*, 2012). Artificial nests baited with plasticine eggs provided useful information about predator type and annual changes in predation risk at our study site in Witchelina Nature Reserve. By assessing bite marks on artificial eggs we were able to identify avian and rodent nest predators at our study site, which are both commonly known predators of songbirds (Söderström *et al.*, 1998). At our study site predator type and predator activity differed significantly across two sampling years, being higher for rodents in 2012, when annual rainfall was high (172 mm), and slightly higher for birds in 2013, when annual rainfall was lower (99 mm). Rainfall and consequent pulses of productivity are highly unpredictable in arid and semi-arid ecosystems (Morton *et al.*, 2011a), especially in central and northern Australia (Van Etten, 2009). It is known that high rainfall events can trigger irruptions or “booms” of rodents (Letnic and Dickman, 2006), whereas during prolonged dry periods the rodent numbers may plummet and go “bust” (Dickman *et al.*, 2010). The difference in annual predator type could have been the result of these differences in annual precipitation. In 2011 (the year prior to the commencement of this study) the mean annual rainfall was extremely high (305 mm), which was associated with a La Niña year. The observed high rodent activity at Witchelina Nature reserve in 2012 could have been the result of a rodent eruption, triggered by the high annual precipitation in 2011 and the subsequent high rainfall in 2012 (172 mm). When rodent activity was high, rodent predation at artificial nests was correlated with rodent activity in the area, whereas there was no pattern of association between avian activity and avian predation.

Nest predation in other grasswren species

One other study examined fledging success and causes of nesting failure in *Amytornis*. In western grasswrens (*A. textilis textilis*), 69 % (22/36) of active nests successfully fledged one or more young (Brooker, 2000, Brooker, 1988). Overall nest predation of western grasswren nests was 33 % (12/36 nests): nest predation varied across years from 22 % to 41 % (1994: 30 %, 1995: 41 %, 1996: 22 %) (Brooker, 1998a). Therefore, depredation of western grasswren nests was at least two-fold higher than the thick-billed grasswrens predation observed rates at Witchelina Nature Reserve. Although nest concealment was not measured in western grasswrens, their nests were more successful in years with heavier rainfall and prolific growth of climber vegetation, which suggests that the degree of nesting cover was correlated with nesting success (Brooker 2000). It is possible that parental care may compensate for nest concealment in the thick-billed grasswren as greater parental care has been shown to decrease predation in other songbird species (Martin *et al.*, 2000, Remeš, 2005b), but this remains to be tested.

Conclusion and management implications

There is strong evidence to suggest that the removal of nest predators in combination with habitat management can increase nesting success in songbirds (White *et al.*, 2014). However, predator control needs to be done with care, as removal of introduced predators can result in a compensatory shift in predator type (Ellis- Felege *et al.*, 2012). This study did not find high predation outcome in thick-billed grasswrens, and therefore we tentatively conclude that nest predation is not the primary current threatening process for the persistence of this species. A possible explanation for the infrequent predation events could be improved nesting conditions given land management approaches, such as a feral predator control scheme. A combination of high rainfall in 2011 and 2012, plus the implemented vegetation restoration program at Witchelina Nature Reserve, which removed all feral grazers from the reserve in 2010, could have increased vegetation growth and hence cover at thick-billed grasswren nesting substrates, which favoured nesting success. *Amytornis* are a species group that occur in stony (gibber) deserts (Ford, 1973). Their nests are inconspicuous, their plumage is drab, and their behaviour is highly secretive with scurrying, low flight, and low song rate. We conclude that thick-billed grasswrens would be most at risk from ground-dwelling olfactory or thermally hunting predators rather than visual or auditory predators. Specialist species such as grasswrens appear to be more limited by habitat availability, which would be further impaired by destruction and fragmentation of suitable habitat. Even when overall predation rates are low, habitat degradation (i.e. grazing) may make species more vulnerable to predation because of interactions between habitat and predation pressure (Evans, 2004, Posadas-Leal *et al.*, 2010, Whittingham and Evans, 2004). Hence, when managing reclaimed agricultural habitat such as Witchelina Nature Reserve predator monitoring should be considered (Little *et al.*, 2015).

Acknowledgements

We thank the Nature Foundation of South Australia Inc. for their generous research and logistical support, for allowing access to Witchelina Nature Reserve and for accommodation and excellent human resource support while on the property. We extend sincere thanks to the management staff and volunteers of Witchelina Nature Reserve for their assistance throughout this project. We thank Scott Kinlock, Wikke Popken, Madeline Thomson, Sara Criddle, Petra Hanke, and Valeria Zanollo for help with various fieldwork tasks. All procedures conducted in this study complied with the laws of the country in which they were performed, followed the guidelines for the Australian Code of Practice for the use of animals for scientific research, and were approved by the Flinders University Animal Welfare Committee (permits: E325, E312 and E385). We thank the Nature Foundation of South Australia Inc. for financial support, as well as BirdsSA and Birdlife Australia. We are grateful to Belinda Cale for helpful suggestions that improved this manuscript.

Table 3.1 Summary table of the nesting stage and nesting outcome of thick-billed grasswren (*Amytornis modestus raglessi*) nests at Witchelina Nature Reserve, South Australia, monitored over three breeding seasons (2013-2015).

	2013	***2014***	***2015***	***TOTAL***
Period monitored	1 Aug - 29 Sept	27 July 29 Sept	10-22 Aug	**18 weeks**
Total no. of nests found	**18**	**18**	**11**	**47**
No. of nests fate undetermined	1	1	10	12
No. of nests with known outcome	17	17	1	35
Nest found during:				
Nest-building	0	0	2	2
Incubating	6	14	7	27
Nestling	5	2	2	9
Post-fledging	7	2	0	9
Fate of nests with known outcome	**17**	**17**	**1**	**35**
Nest fate: fledged	13 (76.5 %)	12 (67 %)	1	26 (74.3 %)
Nest fate: abandoned				
- *eggs – infertile clutch*	0	1 (5.9 %)	-	1 (2.9 %)
Nest fate: unclear				
- *abandoned eggs – reason unclear*	1 (5.9 %)	2 (11.8 %)	-	3* (8.6 %)
- *nestlings dead – reason unclear*	1 (5.9 %)	0	-	1* (2.9 %)
Nest fate: depredated				
- *eggs – nest intact*	1 (5.9 %)	0	-	1 (2.9 %)
- *nestlings – nest intact*	1 (5.9 %)	2 (11.8 %)	-	3 (8.6 %)

* Excluded from predation analysis.

Table 3.2 Nesting site and nest concealment effects on rodent predation at artificial nests (0 = no rodent predation, 1 = rodent predation); data from 404 artificial nests baited with 2 plasticine eggs each at Witchelina Nature Reserve, South Australia. Statistical results from Binary Logistic Regression analysis; significant correlations (P < 0.05) are shaded.

	B	***S.E.***	***Wald***	***df***	***P-value***	***Exp(B)***	***Lower 95 % CI***	***Upper 95 % CI***
Nest height	0.004	0.011	0.168	1	0.682	1.004	0.984	1.026
% Nest cover above	0.001	0.009	0.031	1	0.861	1.001	0.985	1.018
% Nest cover side	-0.018	0.011	2.880	1	0.090	0.982	0.961	1.003
% Nest cover front	-0.019	0.009	4.291	1	**0.038**	0.981	0.964	0.999
Constant	-0.382	0.928	0.169	1	0.681	0.683		

Table 3.3 Nesting site and nest concealment effects on avian predation at artificial nests (0 = no avian predation, 1 = avian predation); data from 404 artificial nests baited with 2 plasticine eggs each at Witchelina Nature Reserve, South Australia. Statistical results from Binary Logistic Regression analysis; significant correlations (P < 0.05) are shaded.

	B	***S.E.***	***Wald***	***df***	***P-value***	***Exp(B)***	***Lower 95 % CI***	***Upper 95 % CI***
Nest height	0.000	0.010	0.002	1	0.965	1.000	0.980	1.021
% Nest cover above	-0.007	0.007	1.110	1	0.292	0.993	0.980	1.006
% Nest cover side	0.011	0.012	0.953	1	0.329	1.011	0.989	1.035
% Nest cover front	0.027	0.009	9.012	1	**0.003**	1.028	1.010	1.046
Constant	-4.471	1.050	18.127	1	0.000	0.011		

Table 3.4 Summary of the different nest types as described for the currently recognized eleven *Amytornis* species.

Species	***Scientific name***	***Nest types***
Kalkadoon grasswren	*A. ballarae*	*Not described*
Grey grasswren[1,3]	*A. barbatus*	Semi-domed, domed*
Carpenterian grasswren[1,2,3]	*A. dorotheae*	Domed*
Eyrean grasswren[1,2,3]	*A. goyderi*	Cupped, semi-domed*
Black grasswren[2]	*A. housei*	Domed (n = 11)
Short-tailed grasswren	*A. merrotsyi*	*Not described*
Thick-billed grasswren[6]	*A. modestus*	Cupped, semi-domed, domed (n = 47)
Dusky grasswren[1,2,3]	*A. purnelli*	Semi-domed*
Striated grasswren[1,2,3]	*A. striatus*	Party-domed or domed*
Western grasswren[1,2,3,4]	*A. textilis*	Cupped, semi-domed, generally domed (n = 37)
White-throated grasswren[1,3]	*A. woodwardi*	Domed*

Source: [1]Russell and Rowley (1997), [2]Serventy (1982), [3]Schodde (1982), [4]Brooker (1998), [5]Johnstone and Kolichis (1999), [6]**this study**.
*Sample size not listed by authors.

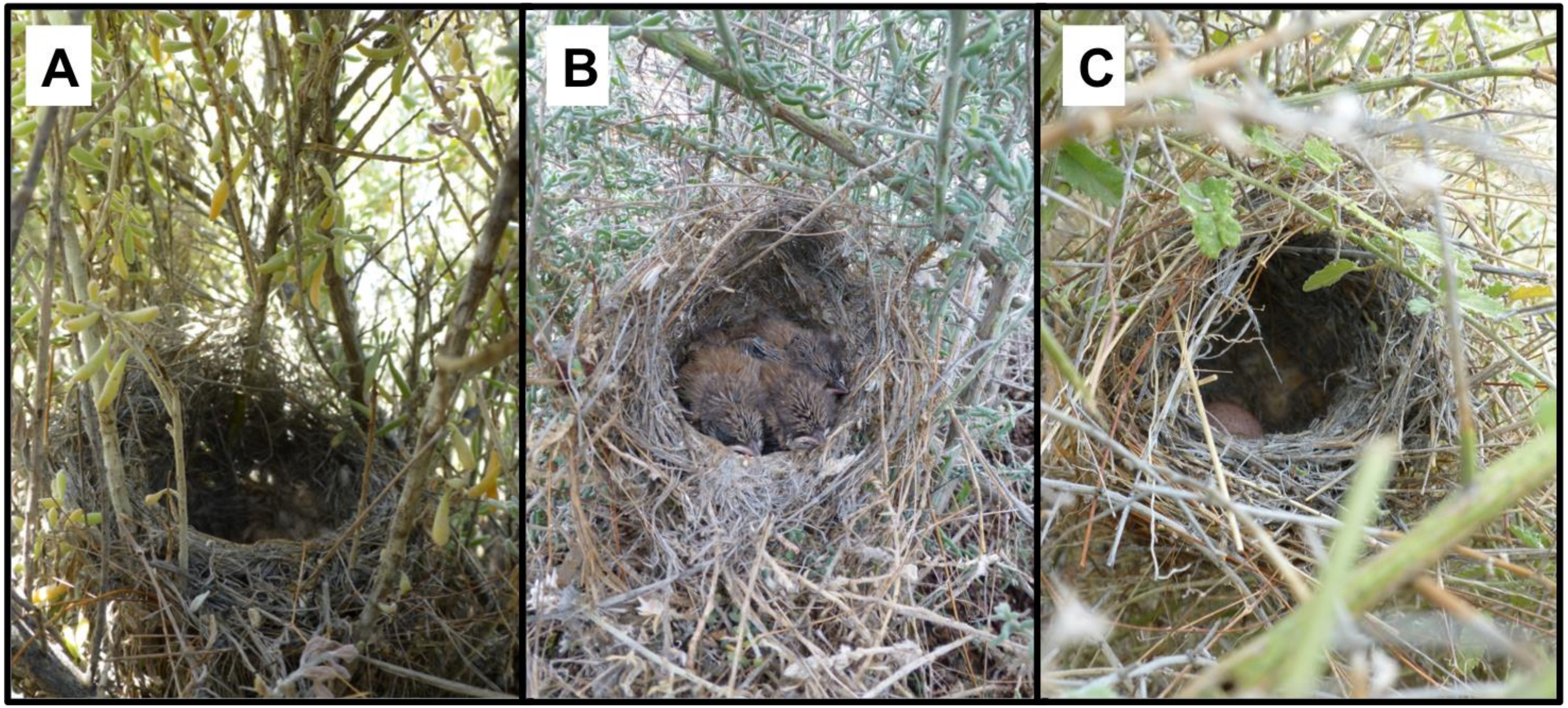

Figure 3.1 Examples of different nest types of thick-billed grasswrens (*Amytornis modestus raglessi*) observed at our study site Witchelina Nature Reserve, in South Australia. a) an open-cup nest, b) a party-domed nest (with elevated rim) containing three nestlings of eight days old, c) a fully domed nest with two eggs (only one egg is visible). Photos by Marina Louter.

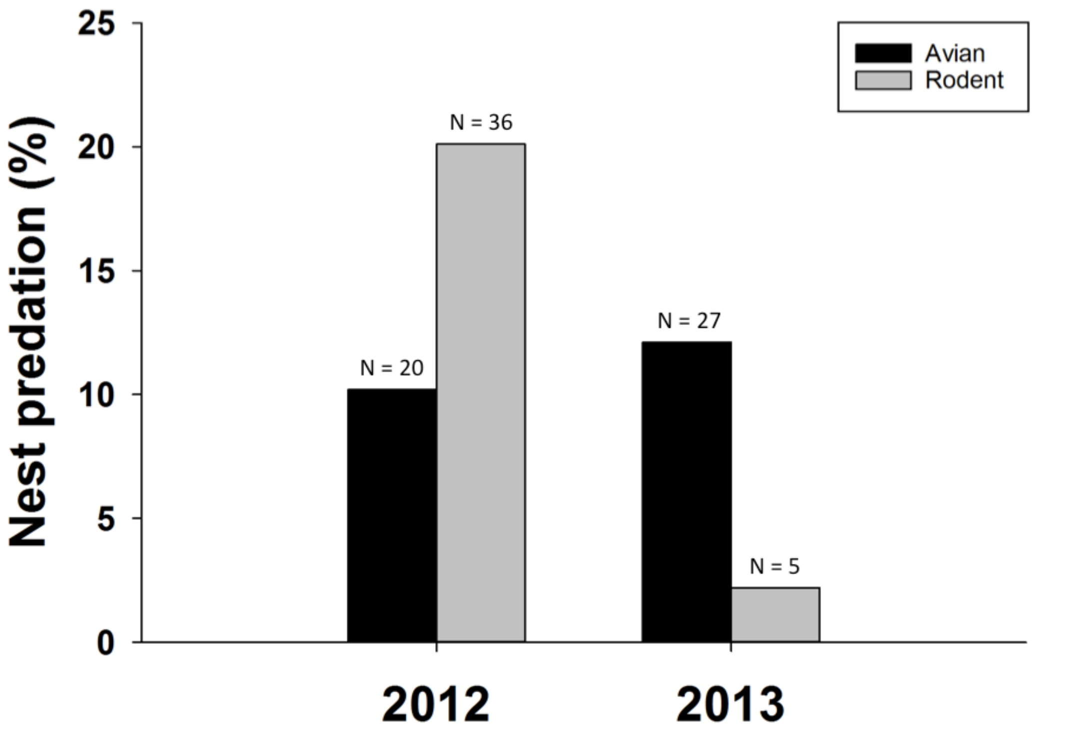

Figure 3.2 The percentage of artificial nests (n = 179 in 2012, n = 225 in 2013) depredated by avian and rodent predators, as assessed from marks on plasticine eggs, at Witchelina Nature Reserve, South Australia. Predator type varied across two years, with higher rodent predation in 2012 and higher avian predation in 2013.

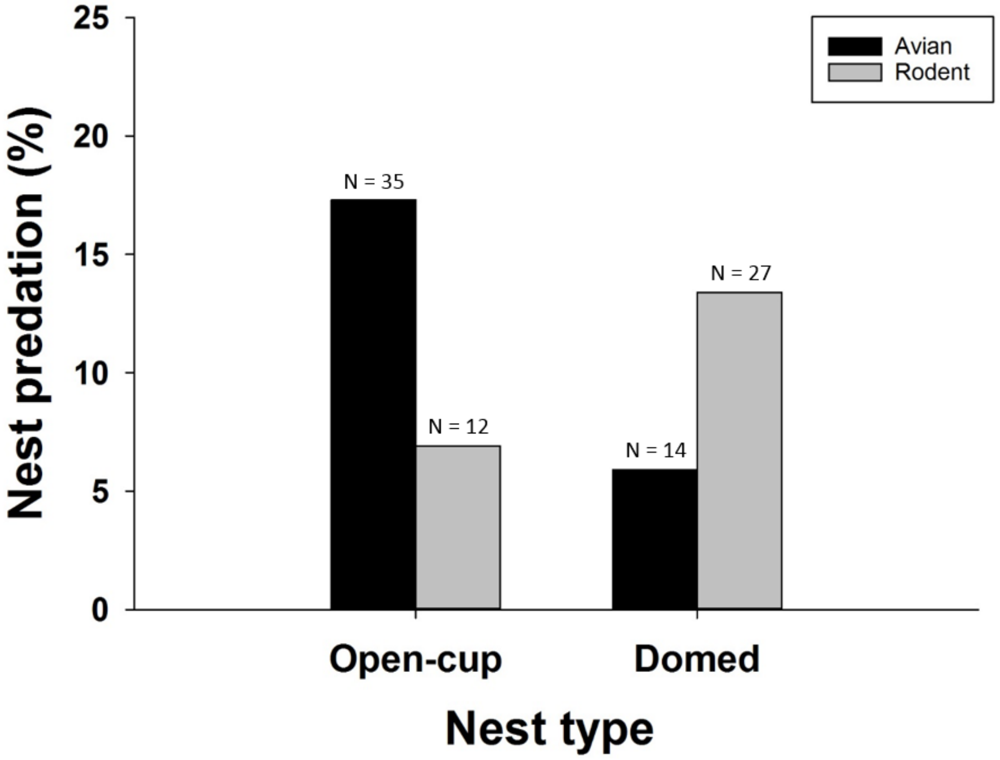

Figure 3.3 The percentage of artificial open-cup (n = 202) and domed (n = 202) nest depredated by avian and rodent predators, as assessed from marks on plasticine eggs, at Witchelina Nature Reserve, South Australia. Predation rates were similar for open-cupped and domed nests. Avian predation was higher in open-cup nests and rodent predation was higher in domed nests.

S1 Table 3.1 Nesting site characteristics (Mean ± SD) of natural thick-billed grasswren (*Amytornis modestus raglessi*) nest (per nest type) at Witchelina Nature Reserve, in the arid regions of South Australia.

Parameters	***Open-cup***	***Partly-domed***	***Domed***	***All nests***
	n = 3	***n = 35***	***n = 9***	***n = 47***
Nesting height (cm)	28.0 ± 2.1	22.3 ± 1.0	24.4 ± 2.4	23.1 ± 0.9
Nesting shrub height (cm)	63.0 ± 9.2	65.4 ± 2.3	59.2 ± 4.7	64.1 ± 2.0
Nest height ratio	0.47 ± 0.08	0.35 ± 0.01	0.43 ± 0.06	0.37 ± 0.02
% Vegetation cover above nest	88.3 ± 9.3	85.6 ± 4.0	70.0 ± 11.8	82.8 ± 3.8
% Vegetation cover side nest	100.0 ± 0.0	88.7 ± 2.5	84.4 ± 7.1	88.6 ± 2.3
% Vegetation cover front nest	88.3 ± 9.3	79.4 ± 4.7	84.4 ± 9.7	81.0 ± 3.9

S1 Table 3.2 Nesting site characteristics (Mean ± SD) of successful and predated thick-billed grasswren (*Amytornis modestus raglessi*) nest at Witchelina Nature Reserve, in the arid regions of South Australia.

Parameters	***Successful nests*** ***n = 26***	***Predated nests*** ***n = 4***
Nesting height (cm)	22.19 ± 1.1	20.0 ± 3.4
Nesting shrub height (cm)	64.0 ± 2.6	61.0 ± 11.8
Nest height ratio	0.35 ± 0.17	0.41 ± 0.15
% Vegetation cover above nest	80.4 ± 5.2	90.0 ± 10.0
% Vegetation cover side nest	88.3 ± 2.9	87.5 ± 12.5
% Vegetation cover front nest	81.7 ± 5.6	81.3 ± 11.3

Chapter 4 Vegetation cover predicts parental feeding rates in thick-billed grasswrens (*Amytornis modestus raglessi*)

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In preparation to be submitted to *PeerJ*

Abstract

Populations of the vulnerable thick-billed grasswren (*Amytornis modestus raglessi*), an Australian arid zone songbird, have been declining and anthropogenic factors such as grazing by ungulate domestic livestock, are likely contributing factors. Grazing can alter the composition and structure of vegetation and influence prey availability, and such changes can impact the reproductive performance and nesting success of birds inhabiting grazed landscapes. Here, we ask if vegetation characteristics in previously grazed thick-billed grasswren territories are associated with prey abundance and parental care behaviour. We examine the association between vegetation cover at thick-billed grasswren nest sites (using vegetation transects) with insect abundance (using pitfall traps), and assess how these habitat parameters affect their reproductive performance. We measured parental feeding rate and nest attendance (using 295 hours of video footage of twelve grasswren nests) and monitored nesting success. Mean vegetation cover (31.9 % ± 18.5 SD) was not correlated with insect abundance at grasswren nest sites, but was positively correlated with parental care behaviour: sites with greater vegetation cover had increased feeding rates. Neither habitat (vegetation cover and insect abundance) nor parental care (feeding rate and nest attendance) predicted nestling body condition or nesting success. Vegetation cover did not predict thick-billed grasswren group size, but group size predicted parental feeding rate. Grasshoppers (62 %) and caterpillars (22 %) made up the majority of identified dietary items fed to grasswren nestlings. These findings will inform the conservation management of this species of conservation concern.

Introduction

Pastoralism and livestock grazing is one of the most extensive forms of land use worldwide, with more than 70 % of the world's grasslands devoted to agriculture and other human use (Hannah *et al.*, 1995). The loss and degradation of grassland habitats from livestock grazing has been considered a primary cause of severe population declines of avian species occupying agricultural habitats (Buckingham *et al.*, 2006, Pearce-Higgins and Grant, 2002, Schaub *et al.*, 2010) and has been considered a primary cause of severe global population declines in grassland birds (Fuller and Gough, 1999, Vickery *et al.*, 1999). In Australia, ungulate livestock have grazed the arid rangelands since European settlement in the late 1800's (Harrington *et al.*, 1984, Wilson, 1990) and habitat degradation caused by selective grazing of domestic and feral herbivores has significantly altered the structure, composition and biomass of native vegetation (Landsberg *et al.*, 2003, McIvor and Gardener, 1990, Pettit *et al.*, 1995). The negative impact of grazing pressure on vegetation has been implicated in widespread population declines of avian populations across Australia (reviewed in Ford *et al.*, 2001, Martin *et al.*, 2005, Martin and McIntyre, 2007, Martin and Possingham, 2005), particularly in ground-foraging and ground-nesting insectivorous songbirds (Davies *et al.*, 2010, Martin and Possingham, 2005, Perlut *et al.*, 2008, but see Lusk and Koper, 2013).

Bird communities are especially vulnerable to impacts of grazing, because vegetation cover is important for feeding and nesting, which are both key parameters for recruitment into populations and hence persistence of species (Perlut *et al.*, 2008). Livestock grazing severely alters vegetation structure in landscapes (Adler *et al.*, 2001, Díaz *et al.*, 2007, Fleischner, 1994, Milchunas and Lauenroth, 1993), which may influence survival as it can affect predation risk and foraging efficiency (Butler and Gillings, 2004, Douglas *et al.*, 2008, Murray *et al.*, 2016, Schaub *et al.*, 2010, Whittingham and Evans, 2004). Additionally, vegetation cover is closely linked to invertebrate abundance and diversity (Di Giulio *et al.*, 2001, Hutchinson and King, 1980, Kruess and Tscharntke, 2002, Marini *et al.*, 2008). Insectivorous songbird populations in habitats with little vegetation cover may have few available invertebrate prey (Dennis *et al.*, 2008, Devereux *et al.*, 2006, Gibson *et al.*, 1992) and may need to spend more time foraging (Bruun and Smith, 2003, Morrison *et al.*, 2010, Sutter and Ritchison, 2005, but see Douglas *et al.*, 2008), thereby increasing their exposure to predators (Evans, 2004). Declines in avian species richness and abundance have been documented in heavily grazed habitats as a result of the reduction in food availability (Evans *et al.*, 2005, Vickery *et al.*, 2001) as well as a reduction in shelter and nesting sites (Cardoni *et al.*, 2012, Cardoni *et al.*, 2015, Gonnet, 2001) caused by changes in vegetation structure.

Vegetation cover at nesting sites provides protection against potential nest predators (Borgmann and Conway, 2015, Burhans and Thompson III, 1998, Howlett and Stutchbury, 1996, Little *et al.*, 2015) and a reduction in vegetation cover at nests can have direct negative impacts on nesting success (Sharps *et al.*, 2015, Sutter and Ritchison, 2005). Overgrazing by livestock has been found to affect egg size (Evans *et al.*, 2005) and offspring sex ratio (Prior *et al.*, 2011) of a common upland passerine, the meadow pipit *Anthus pratensisa*. Given the negative impacts from grazing on vegetation cover and food availability, and the high energetic costs of parental care (Bryant, 1988, Drent and Daan, 1980), there is a need to monitor parental care, fledgling development, and nesting success in avian species threatened by impacts of grazing (Douglas *et al.*, 2008).

We documented nesting outcome and parental care for the thick-billed grasswren (*Amytornis modestus*) (North, 1902, Parker, 1972), a ground-dwelling terrestrial songbird that was once widely distributed across the rangelands of arid and semi-arid Southern and Central Australia. Previous research showed that the thick-billed grasswren has disappeared from areas where it was once abundant (Black and Baxter, 2003, Black *et al.*, 2011a, Black *et al.*, 2010, Garnett *et al.*, 2011), and as a result it is currently listed as vulnerable under the E.P.B.C. Act (1999). The cause for their decline is not well understood, but has been linked to habitat loss and fragmentation, and degradation of habitat through grazing of domestic livestock and feral herbivores (Ford, 1987, McAllan, 1987), as well as introduced feral predators (Garnett *et al.*, 2011). Information on particular habitat requirements contributes to our understanding of how anthropogenic habitat changes, such as grazing, may affect the persistence of thick-billed grasswren populations. Recent research on the thick-billed grasswren (subspecies *A. m. raglessi*) (Black, 2011a) showed that this subspecies is absent from previously extensively grazed areas that contain little vegetation cover and few chenopod shrubs (Louter *et al*. a, in prep; Chapter 2). Chenopod shrubs such as *Maireana pyramidata* and *Maireana astrotricha* were found to be the main nesting substrate for the species with infrequent predation of thick-billed grasswren nests (Louter *et al*. c, in prep Chapter 4). However, currently little is known about how differences in the characteristics of vegetation might affect parental feeding, fledgling quality and nesting success in thick-billed grasswrens.

With the aim to identify possible conservation measures, this study examines the association between vegetation cover and insect abundance at previously grazed thick-billed grasswren nest sites in relation to parental care and fledgling quality. Specifically, we assess the role of vegetation cover and insect abundance for parameters of breeding biology (nestling body condition, nesting success) and reproductive performance (feeding rate, nest attendance). We use in-nest video monitoring to quantify parental food delivery at thick-billed grasswren nests.

We test the following predictions: (1) a positive correlation between vegetation cover and insect abundance, (2) a positive correlation between insect abundance and provisioning (feeding rate, nest attendance), (3) a positive correlation between insect abundance and nestling body condition and nesting success, and (4) alternatively, there might be no effect of vegetation cover or insect abundance since studies have shown that parent birds may compensate for poor habitat quality with increased parental care, including increased nest attendance and nest defence (Burhans, 2000, Burhans and Thompson III, 1998, Cresswell, 1997). Since the thick-billed grasswren is a species of conservation concern (Garnett *et al.*, 2011), this project had two other aims: (5) to ascertain the identity and size of main prey items delivered to nestlings, and (6) to identify the number of adult birds provisioning offspring. This information is crucial for the development of targeted conservation management plans, which are essential for the recovery of vulnerable species affected by grazing, such as the thick-billed grasswren.

Methods

Study site

The study was conducted at Witchelina Nature Reserve (30º01’340” S, 138º02’772” E), a former pastoral property located in the arid rangelands of inland South Australia (Figure 1.2, p.6). Witchelina Nature Reserve was grazed by sheep and feral goats for ~160 years before it was bought by the Nature Foundation of South Australia in 2010, who converted it into a conservation reserve and removed all feral grazers. The reserve currently comprises of ten major habitat types, all of which contain various degrees of low chenopod shrubs such as saltbush (*Atriplex* sp) and blue bush (*Maireana* sp). For more complete descriptions of the vegetation in the area, see Slender *et al.* (a, *in prep*). Within an area of ~50 sq. km in Witchelina Nature Reserve we located and mapped out 42 thick-billed grasswren (*Amytornis modestus raglessi*) territories (Figure 1.2, p.6). Coinciding with the proposed main breeding season of the species (Black *et al.*, 2011a), we monitored thick-billed grasswren breeding behaviour from August to September 2013-2014, plus an additional 2-week trip in August 2015, spanning a total of 18 sampling weeks.

Vegetation cover, shrub abundance and grazing

Vegetation parameters at thick-billed grasswren sites were measured using Jessup transects of 100 m by 4 m (Jessup, 1951). Transects ran from North to South and along each transect we measured the abundance, diversity and cover of 17 common chenopod shrubs, as well as the total cover of all flora present, recorded as percentage vegetation cover . Grazing impact was measured indirectly, as the distance to nearest water dam (in km), which is a well-known proxy for impact of grazing on Australian arid rangelands (Andrew, 1988, Fensham and Fairfax, 2008, James *et al.*, 1999, Lange, 1969). For more detailed description of vegetation and grazing sampling methods see Louter *et al.* a, in prep; Chapter 2).

Arthropod sampling

Arthropod sampling was performed to estimate the relative abundance and availability of potential thick-billed grasswren prey per territory. Due to remoteness and accessibility of thick-billed grasswren sites we employed a rapid assessment technique using five pitfall traps: in each tick-billed grasswren territory, five pitfall traps were placed along the centre of the Jessup transect, with 10m between each pitfall trap. Pitfall traps were filled with ethanol (75 %) and were collected after ~24 hours. In the lab, insect specimens were identified to the order level (Orthoptera, Lepidoptera (adult and larvae), Coleoptera, Aranae, Phasmatodea, Hemiptera, Formicidae, Diptera, Blattodea). Due to their clumped occurrence (i.e. large numbers caught close to anthills), ants (Formicidae) were deducted from arthropod abundance. Because ants were an infrequent food source for grasswren nestlings (results below), this did not bias our arthropod estimates in relation to parental provisioning. To quantify prey abundance per thick-billed grasswren nest site, arthropod abundance was averaged.

Group size and composition

To determine group size and group composition of thick-billed grasswrens (*Amytornis modestus raglessi*), we captured grasswrens using mist-nets (9 or 12 m, 30 mm-mesh). Two to four mist-nets were set up in a straight line or u-shape in creek or drainage lines within a territory, or in close proximity of a nest location. Individuals were first observed with binoculars from a distance (~50-100 m) and then carefully herded towards the nets by 2-3 observers. Each captured bird was fitted with an aluminium ABBBS band (Australian Bird and Bat Banding Scheme) and a unique combination of three coloured leg bands, one above aluminium and two on the opposite tarsus, to enable individual identification in the field. All captured birds were measured (Slender *et al.*, b, *in prep*), sexed and aged based on morphology (presence of a yellow gape and feather condition) and on their behaviour, and for each adult we scored the presence or absence of a brood patch and/or cloacal protuberance. The sex of each individual was confirmed (100 % accuracy) from blood samples obtained at the time of capture, by using modified methods described in Griffiths *et al.* (1998), and Smith and Burgoyne (2004) (see Louter *et al.* in prep, Chapter 5 for detailed description of methods). Effort was made to capture entire groups within territories, but if this was not achieved we estimated group size and composition based on repeated monitoring of banded and unbanded individuals in the field and at active nests each year.

Nest monitoring and nestling measuring

Grasswren territories were visited at least once a week to record group size, determine breeding activity, and to search for active nests. Nests were found by intensive systematic searches in vegetation in thick-billed grasswren territories. Once a nest was discovered its location was recorded using a hand held GPS, and a small flag was inconspicuously placed in vegetation located 15 meters northerly of the nest location, which is considered enough distance to minimize nest predation (Martin and Geupel, 1993). All active nests were visited every 1-2 days to record the number of eggs, dates of hatching and fledging, and to band and measure nestlings when they were 6-12 days old. Nestling mass was determined using portable electronic scales (± 0.01 g accuracy), and nestling tarsus was measured with dual calipers (0.1 mm accuracy). Residuals (unstandardized) from mass–size ordinary least squares (OLS) regression, were used as an indices of nestling body condition (Schulte-Hostedde *et al.*, 2005), with tarsus length used as a linear measure of size. Nestling condition was averaged per nest.

Digital video recording (DVR) system design

To monitor parental care behaviour at thick-billed grasswren nests and to identify prey items, we deployed a user-built battery-powered digital video recording (DVR) that recorded 24-hr (continuous) at a subset of active thick-billed grasswren nests. Cameras were not deployed during the nest building or egg-laying phase to reduce disturbance at the nest and subsequent risk of nest desertion (Schaefer, 2004). We employed a similar video-monitoring set-up as the system described in (Cox *et al.*, 2012), but we attached an external solar panel to the system to enable battery recharge during the day (battery-life was ~ 3 days). A DVR unit (*TECHview, 4 Channel H.264DVR, model No QV-3029*), designed for the security industry, was used to store video recordings. This unit (D x W x H: 21 x 30 x 5 cm) had 500GB of internal storage, enabling us to store up to 300 hours of continuous full D1 resolution video recordings. Video recordings could be downloaded using a flash drive that connected directly to the USB port of the DVR unit. A miniature Jaycar monochrome CCD security camera (40 x 40 mm) with a 12 mm lens (*model Electus QC3474*) was used to record video (1.32 W power consumption at 12 V), which was connected to the DVR unit by means of a BNC power/video extension cable (~15 m). The camera was positioned in the nest shrub at approximately 0.2 – 0.5 m away from the nest (this proximity was necessary for identification of adult birds and prey items) facing towards the nest entrance. We took precautions not to alter the nest concealment during camera placement. A hand-held monochrome monitor was used to guide the positioning of the camera lens during deployment and to later check the status of nests without having to approach them. The equipment was powered by three rechargeable 12 V 12 Ah deep-cycle sealed lead acid batteries. The DVR unit and batteries were housed in a camouflaged waterproof container, which was placed ~15 m away from the nest, thereby allowing batteries to be changed while minimizing disturbance. We concealed all components of the system with natural vegetation to prevent predators from being affected by the equipment (Herranz *et al.*, 2002, Richardson *et al.*, 2009). On person did the camera set-up in approximately 15 minutes. Daily nests visits were made to each camera system to check nest status (using a portable monitor), check battery power and replace batteries (every three days). At each video-monitored nest we recorded continuously until the fate of the nest was known (abandoned, predated, or fledged). Nest fate was ascertained following Martin and Geupel (1993) and Martin et al. (1997), defining successful nests as those that fledged at least one young. Video recordings were viewed and analysed in our lab after each field season, with the software VLC Media Player V2.1.4. Rincewind (VLC authors and VideoLAN).

Effects of cameras on nest survival

We found 18 active nests in 2013 and 18 in 2014; we installed cameras at 7 nests in both years. Of the 14 camera-monitored nests, eleven were found during incubation and three nests were found during the early nestling phase (day 1–6 post-hatching). Due to time constraints in 2013 we removed a camera at one nest prior to egg hatching (unknown nest fate). None of the 12 nests with known nesting outcome were predated, thus thick-billed grasswren nest predation rates were not influenced by the presence of cameras. Nest abandonment was similar at nests with cameras (n = 2 of 14 nests) and nests without cameras (n = 2 of 22 nests) (Fisher’s exact probability test, P = 0.50). In 2013 one video-monitored nest with eggs was abandoned, and another nest containing two nestlings of nine days old was deserted 9 days after camera setup. For the latter, the extended period between camera installation and the abandonment of the nest suggests this was not caused by our activities. In 2014, two active nests without cameras were abandoned during incubation phase.

Prey size and prey items

Video footage was analysed and scored to estimates the size of prey items fed to grasswren nestlings (n = 12 nests, n = 295 hours of video footage, recorded over a total of 73 days). The size of each prey item was estimated based on the mean bill length of adult male and female grasswrens (mean bill length = 11.2 ± 0.8 SE mm, n = 71), and grouped into five categories (1): very small (≤ 6 mm, half of the bill length), (2) small (7-12 mm, 1 x bill length), (3) medium (13-24 mm, 2 x the bill length), (4) large (25-48 mm, 3-4 x bill length), and (5) very large (≥ 49 mm, ≥ 4 x bill length). Additionally, when possible, we identified the taxonomic order of prey items. Prey items were divided into invertebrate orders: Arachnida, Blattodea, Diptera, Coleoptera, Formicidae, Hemiptera, Lepidoptera adults, Lepidoptera larvae, Phasmatodea, and "unknown" (if the prey was observed, but could not be identified). This method probably resulted in a bias towards identification of larger prey items, which are more easily identifiable due to unique and more prominent visible features.

Feeding rate with nestling age and time of day

Video footage was scored in the lab for feeding rate during the nestling phase. Two nests were scored over the course of the entire nestling period (day 1-15) to identify if feeding rates vary with nestling age. Feeding rates increased with nestling age and were lowest in younger nestlings and highest in oldest nestlings (Figure 4.1). Five nests were analysed for an entire day (8:00-18:00) to identify variation in feeding rate over the course of a day. Since nests were analysed on different days of the nestling phase we calculated the proportion of feeds per hour (no feeds per hour/total number of feeds per day). We found that feeding rates at nests were lower in the earlier hours of the day (8:00-12:00) and higher in the later hours of the afternoon (13:00-18:00) (Figure 4.2).

Parental care behaviour

Since feeding rate was higher in the afternoon, we selected three afternoon hours of video footage (from 15:00 to 18:00) of nestlings aged 8-11 days old to analyse and score nestling parental care behaviour. Sex and status (dominant breeder, subordinate helper) of individuals attending the nest were scored through the identification of different combinations of coloured leg bands. Individuals usually perched on the rim of the nest when provisioning nestlings/leaving the nest, making leg bands clearly detectable on the video footage (Figure 4.3). We identified four parental care behaviours at grasswren nests: (a) brooding of nestlings, (b) nest guarding, (c) feeding, and (d) removing/consumption of faecal sack (Figure 4.4). The number of feeding-trips (i.e. feeding rate) to the nest has been found to be a good predictor of food delivery (Nolan *et al.*, 2001), and nestling growth (Lyon *et al.*, 1987, MacColl *et al.*, 2003). For each brood we recorded a) average number of feeding-trips per hour (i.e. feeding rate per hour), and b) average nest attendance per hour (time brooding + nest guarding + time spend feeding (nest attendance per hour). The total number of removal/consumption of faecal sacs over the course of three hours was also scored. For nine (4 breeder pairs, 5 breeder pairs with helpers) out of twelve video-monitored nests we were able to score individual provisioning behaviour, which was calculated per adult as the proportion of feeds at the nest per three hours.

Statistical Analysis

All data were analysed using SPPS 13.0 for Windows (SPPS, Chicago, USA). All means are presented as mean ± SD. We examined data for normality before using parametric tests. To satisfy conditions of normality, mean insect abundance and nest attendance were Log10 transformed. To avoid issues with collinearity among vegetation predictor variables, correlating variables ($r \geq 0.5$) were removed using Pearson correlation co-efficient test. Vegetation cover was highly correlated to chenopod shrub cover ($r = 0.89$), chenopod shrub diversity ($r = 0.58$), and chenopod shrub abundance ($r = 0.71$), and distance to dam (grazing) ($r = 0.72$), therefore vegetation cover was used in further analysis. Because feeding rate per hour, was significantly correlated with brood size (Linear Regression, $r = 0.73$, $t = 3.33$, $n = 12$, $P = 0.008$; nests with a larger brood had a significantly higher feeding rate), we controlled for brood size by calculating the per-nestling feeding rate for each nest as follows: feeding rate per hour/ number of nestlings per nest, which was used in further analysis. We used non-paired (OLS) Regression to test if habitat quality (vegetation cover, insect abundance) is a predictor for (1) feeding rate, (2) nest attendance, (3) brood size, (4) nestling body condition, and (5) group size. We used non-paired Binary Logistic Regression to test for the directional effects of habitat quality (vegetation cover, insect abundance) as predictor variables on helper presence (0,1) to test the idea that habitats with more vegetation cover and more prey can sustain larger group size including helpers.

Results

Vegetation cover and insect abundance

Table 4.1 presents nest site vegetation characteristics of thick-billed grasswren territories. The percentage of vegetation cover ranged from 14.3 % to 73.4 %; therefore there was variation in nest site vegetation cover between territories. Per territory there was also variation in chenopod shrub cover (range 1.7 % – 24.7 %), chenopod shrub abundance (range 10 – 239 shrubs), but not in chenopod shrub diversity (range 4 – 7 species) (Table 4.1). The mean number of insects per pitfall trap was 10.4 ± 10.8, and ranged from 1.2 to 36.0 across twelve grasswren territories (Table 4.1). We found no statistically significant association between vegetation cover and insect abundance at thick-billed grasswren nesting sites (Linear Regression, r = 0.255, t = -0.792, n = 11, P = 0.449).

Prey identity and prey size

We analysed 295 hours of video and recorded 2408 food deliveries (n = 2251 prey items) to thick-billed grasswren nestlings (Table 4.2). Thick-billed grasswrens mostly fed a single prey item per food delivery event (1.10 ± 0.35, n = 2408, range 1 - 4 prey items). We were able to estimate the size of 1707 prey items (76 %); most prey items were small (36 %) or large (16 %) (Table 4.2). We identified 639 (28.4 %) prey items, belonging to ten different insect orders (Table 4.3). The most commonly delivered prey items were large to very large sized grasshoppers (n = 395, 61.8 %, see Figure 1.3, p.8) and medium to large sized caterpillars (n = 143, 22.4 %) (Table 4.3).

Parental care and nest site ecology

There was a high variance in feeding rate across the twelve thick-billed grasswren nests, which ranged from 4 to 29 feeds per hour, with an average of 10.3 ± 6.2 feeds per hour (Table 4.4). Feeding rate was significantly associated with nest site vegetation cover; nests in areas with greater vegetation cover had significantly higher feeding rate (Linear Regression, $r = 0.73$, $t = 3.44$, $n = 12$, $P = 0.007$). Vegetation cover did not impact on nest attendance or brood size (Linear regression, *both* $P > 0.1$). Insect abundance did not impact on feeding rate, nest attendance, or brood size (Linear regression, *all* $P > 0.2$).

Nesting success of twelve monitored thick-billed grasswren nests was high; eleven out of the twelve video-recorded nests successfully fledged ≥ 1 offspring (Table 4.4). Consequently, none of the measured habitat or parental care parameters predicted fledgling success (*all* $P > 0.7$).

Cooperative breeding

Using 295 hours of video from colour-marked and molecularly sexed individuals at grasswren nests, we observed a total of 34 adult birds provisioning nestlings at the twelve nests (Table 4.5). At six thick-billed grasswren nests a single breeding pair provided biparental care; the female and male we observed brooding, nest guarding, feeding, and removing/consuming faecal sacks. At six nests we observed cooperative breeding; one to two individuals other than the breeding pair were observed feeding nestlings, and were therefore considered to be helpers (Table 4.5). In groups with helpers present, all but one group member shared parental care duties. Two breeding pairs were assisted in parental care by one helper, and four breeding pairs had two helpers assisting in parental care duties. One group consisted of a total of 5 adult members, but one individual did not assist in any parental care duties, and was therefore not considered to be a helper. Faecal sacs of offspring ($n = 172$) were either removed (21.5 %, $n = 37$), but mostly consumed (78.5 %, $n = 135$) by attending birds.

Group size

At Witchelina Nature Reserve, thick-billed grasswren group size varied across years (range: 2.1 – 3.6) and was highest in 2014 (Table 4.6). Across the sampling period, 30 % of the territories (28 out of 92) had one or more additional group members, and on average, 27 % of these additional group members were female (Table 4.7).

Parental care and helpers

At video-monitored nests (n = 12) the number of helpers was not associated with feeding rate, brood size, nestling body condition, or nest attendance (Linear Regression, *all P* > 0.1). For nine nests (n = 5 nests with helpers, n = 4 nests without helpers), we were able to accurately score individual feeding behaviour per individual: in breeder pairs, parental care was evenly divided between the male and female (Table 4.5). In breeder pairs, parental care was evenly divided between the male and female. The number of helpers was significantly associated with the feeding rate of the breeding male (Linear Regression, r = 0.70, t = -2.60 n = 9, *P* = 0.035); in groups with more helpers, the breeding male fed less prey items to nestlings. A similar trend was observed for the number of helpers and breeding female feeding rate (Linear Regression, r = 0.72, t = -2.72, n = 9, *P* = 0.030); breeding females fed nestlings less in groups with more helpers present.

Nestling body condition

Nestling body condition varied per nest (Table 4.4). There was no association between nestling body condition and vegetation cover or insect abundance (Linear regression, *both P* > 0.1). Feeding rate, nest attendance, the size of delivered prey items, and the number of helpers did not impact on nestling body condition (Linear Regression, *all P* > 0.1).

Discussion

Summary of results

This is the first study on parental care behaviour in the vulnerable thick-billed grasswren (*Amytornis modestus raglessi*) in the wild. Vegetation cover, which has been shown to be lower with high grazing impact, predicted parental feeding in thick-billed grasswrens at Witchelina Nature Reserve; in areas with less vegetation cover nestlings received fewer feeds per hour. Video-recordings of prey items delivered to nestlings, showed that thick-billed grasswren most commonly fed their nestlings small (6-12 mm) or large (25-48 mm) prey items. Large grasshoppers and caterpillars made up the largest proportion (84 %) of identified prey items. Insect abundance at nest sites did not predict food delivery, and there was no effect of habitat (vegetation cover and insect abundance) or parental care (feeding rate and nest attendance) on nestling body condition or nesting success.

Vegetation cover, insects and birds

Reduced arthropod food availability due to grazing impacts is considered a major causative factor for declining insectivorous bird populations in grazed landscapes (Buchanan *et al.*, 2006, Dennis *et al.*, 2005, Pearce-Higgins and Grant, 2002, Pearce-Higgins and Grant, 2006). Several studies have found associations between avian population decline and reduced abundance of key arthropod prey (Benton *et al.*, 2002, Dennis *et al.*, 2008, Evans *et al.*, 2015, Vickery *et al.*, 2001). This study did not find a significant association between vegetation cover and insect abundance at thick-billed grasswren nest sites, probably for two reasons. First, in Witchelina Nature Reserve thick-billed grasswren nests only occur in areas with moderate grazing impact and are absent from areas with heavy grazing history (Louter et al. a, *in prep*; Chapter 2). Our study design was not aimed to test the well-established association between grazing, vegetation cover and arthropod abundance. Rather, we aimed to examine grasswren nest site behaviour in relation to local vegetation cover and arthropod abundance in a study site recovering from grazing. Second, our rapid assessment of arthropod abundance was likely too crude to detect subtle changes that may have occurred in relation to vegetation cover. The lack of a change in insect abundance was most likely due to the moderate number of five pitfall traps, and the placement of pitfall traps close to thick-billed grasswren nests, which may not have been used for foraging by parent birds provisioning offspring. Additionally, pitfall traps mainly target ground-dwelling insects, but not leaf dwelling and flying insects. Our sampling strategy may have not properly captured insect abundance at sites. We nonetheless opted for the pitfall trap approach as we expected to find large differences in arthropod community, which was not supported by the findings in areas with thick-billed grasswrens present.

Vegetation cover and parental care

While there was no statistical association between vegetation cover and arthropod abundance, there was a significant positive association between vegetation cover and parental feeding rate, but not nest attendance at thick-billed grasswren nests. The increased feeding rate at concealed nests may reflect lower predation risk of attending parents (Eggers *et al.*, 2008, Fontaine and Martin, 2006, Lima, 2009).

Cooperative breeding

This study provides novel insights into the social mating system of thick-billed grasswrens. Video analysis of parental care at grasswren nests revealed that thick-billed grasswrens at Witchelina Nature Reserve are cooperative breeders; up to two helpers per group were observed helping at the nest. With the exception of one adult bird, we found that all group members provided parental care at nests. Both breeders and helpers were observed brooding and feed nestlings. We quantified, for the first time in *Amytornis*, the relative parental investment by each individual (breeder, helper) during the late stages of the nesting cycle. In groups with helpers present we observed load-lightening; the dominant breeder pair reduced their parental effort in response to the work done by additional carers, which is a phenomenon commonly observed in cooperatively breeding species (e.g. Hatchwell and Russell, 1996, Wright and Dingemanse, 1999).

The presence of helpers, and thus cooperative breeding, is suspected for many grasswrens, but has been confirmed for only one other grasswren species (S1 Table 4.1). A study by Brooker (1988) on a banded population of western grasswrens *A. textilis textilis* reported cooperative breeding in one out of 15 nests; at one active nest with nestlings, three individuals (the banded breeder pair, plus an unbanded adult male) were observed feeding. Although accounts of “helpers” have been reported anecdotally for other grasswren species, specific parental care behaviour of additional group members is often not documented and without this information the extent of cooperative breeding behaviour remains inconclusive (S1 Table 4.1). For example, Noske (1992) observed white-throated grasswrens *Amytornis woodwardi* and recorded that a group of three adult birds was attending one nestling and another group of four adult individuals attended two fledglings. Unfortunately, there is no record of parental care behaviour of the attending birds, thus cooperative breeding remains unclear. At Witchelina Nature Reserve, 30 % of monitored thick-billed grasswren territories had one or more additional group members (Table 4.6), which were mostly male (Table 4.7). Although parental care was not observed directly in all groups and the status of additional members other than the breeding pair thus remained unknown, it seems likely that most of these additional group members were helpers, given that all but one additional member in closely monitored groups were a helper.

Few studies of *Amytornis* have documented male and female investment in a clutch and the degree of involvement of males in incubation and provisioning has only been confirmed in the western grasswren *Amytornis textilis textilis* (Brooker, 1998a, b), and in the striated grasswren *Amytornis striatus* (Karubian, 2001). Thick-billed grasswren males and females at Witchelina Nature Reserve shared in incubation load (unpublished data). In 2013, we observed male incubation behaviour in three different groups, in 2014 we observed male incubation behaviour in six different groups, two of which had three individuals incubating the one clutch, suggesting cooperative incubation behaviour in this species.

Conclusion

Vegetation cover predicted per-nestling feeding rate; offspring in territories with more chenopod vegetation cover were fed more often. Therefore, vegetation cover plays an important role in thick-billed grasswren breeding behaviour. From previous study we know that active grasswren nests did not occur in areas with sparse chenopod vegetation cover associated with prior heavy grazing. Our finding that vegetation cover predicts food delivery rate supports the idea that thick-billed grasswrens have habitat requirements linked with grazing history, and that restoring habitat from grazing is a successful approach for parental care and nesting occurrence in this system.

Acknowledgements

We are immensely grateful to the Nature Foundation of South Australia Inc. for their generous financial support and for allowing access to Witchelina Nature Reserve, and for providing accommodation on the property. We sincerely thank the management staff of Witchelina Nature Reserve for their assistance and logistical support. We thank Scott Kinlock, Wikke Popken, Madeline Thomson, Sara Criddle, Andrea Robinson, Bianca Staker, and Petra Hanke for their help with various fieldwork tasks. All procedures conducted in this study complied with the laws of the country in which they were performed, followed the guidelines for the Australian Code of Practice for the use of animals for scientific, and were approved by the Flinders University Animal Welfare Committee (permits: E325, E312 and E385). Bands were provided by the Australian Bird and Bat Banding Scheme (ABBBS). BirdsSA, Birdlife Australia, and the Field Naturalists Society of South Australia also provided funds for this study.

Table 4.1 Summary table of vegetation characteristics and mean insect abundance in thick-billed grasswren territories with video-monitored nests (n = 12) at Witchelina Nature Reserve, South Australia.

Year	**Territory ID**	***Total Vegetation cover (%)***	***Chenopod shrub cover (%)***	***Chenopod shrub abundance***	***Chenopod shrub diversity***	***Mean number of insects***
2013	12	14.3	5.3	109	5.0	20.0
	21	18.0	8.6	83	3.0	10.0
	25	14.9	1.7	19	3.0	8.0
	33	26.8	12.4	116	4.0	36.0
	39	38.3	13.8	123	5.0	-
2014	12	14.3	5.3	109	5.0	20.0
	1	57.5	12.9	132	6.0	1.2
	2	43.9	12.9	111	7.0	1.8
	18	73.4	24.7	239	6.0	5.8
	31	29.4	7.1	10	5.0	2.8
	49	23.7	8.0	88	4.0	2.8
	2A	28.6	11.9	54	7.0	5.6
	Mean ± SD	**31.9 ± 18.5**	**10.4 ± 5.9**	**99.4 ± 59.3**	**5.0 ± 1.3**	**10.4 ± 10.8**

Table 4.2 The size of prey items fed at thick-billed grasswren (*Amytornis modestus raglessi*) nests at Witchelina Nature Reserve, South Australia. The most common prey sizes are in bold and shaded in grey.

Prey size	***No of prey items***	***Percentage***
Very small (≤ 6 mm)	207	*9 %*
Small (7-12 mm)	819	***36 %***
Medium (13 to 24 mm)	127	*6 %*
Large (25 to 48 mm)	359	***16 %***
Very large (≥ 49 mm)	195	*9 %*
Unknown size	544	*24 %*
TOTAL	2251	*100 %*

Table 4.3 The number and percentage (%) of prey items of known size and identity (n = 639), fed at thick-billed grasswren (*Amytornis modestus raglessi*) nests at Witchelina Nature Reserve, South Australia. Most common prey items are in bold and shaded in grey.

Size Category	*Orthoptera (grasshoppers)*	*Lepidoptera (moths) larvae*	*Lepidoptera (moths) adult*	*Coleoptera (beetles)*	*Aranae (spiders)*	*Phasmatodea (stick insects)*	*Hemiptera (true bugs)*	*Formicidae (ants)*	*Diptera (flies)*	*Blattodea (cockroaches)*
Very small (≤ 6 mm)	0	1	0	0	0	0	0	0	0	0
Small (7-12 mm)	6	5	5	24	7	0	3	3	3	0
Medium (13 to 24 mm)	21	**38**	6	0	4	0	4	0	0	0
Large (25 to 48 mm)	**193**	**85**	11	0	3	4	0	0	0	1
Very large (≥ 49 mm)	**172**	3	2	0	0	10	1	0	0	0
Unknown size	3	11	6	0	4	0	0	0	0	0
TOTAL	**395 (61.8 %)**	**143 (22.4 %)**	**30 (4.7 %)**	**24 (3.8 %)**	**18 (2.8 %)**	**14 (2.2 %)**	**8 (1.3 %)**	**3 (0.5 %)**	**3 (0.5 %)**	**1 (0.2 %)**

Table 4.4 Summary of provisioning data of video-monitored thick-billed grasswren (*Amytornis modestus raglessi*) nests (n = 12) in Witchelina Nature Reserve, South Australia. All twelve nests were analysed for 3 hrs in the afternoon (15:00-18:00), when nestlings were 8-10 days of age (nestling age). Nests with helpers present are shaded in grey.

Year	***Nest ID***	***Group size***	***No. helpers***	***Clutch size***	***Brood size***	***No. fledglings***	***Nestling Age (days)***	***Feeds/ 3hrs***	***Feeds/ h***	***Per-nestling feeding rate/h***	***mean prey size (mm)***	***Mean Nestling body condition***
2013	12	3	1	*	2	2	10	17	5.7	2.8	26.8 (n = 17)	-1.70
	21	2	0	3	1	1	10	13	4.3	4.3	30.0 (n = 7)	-0.43
	25	2	0	*	2	2	10	17	5.7	2.8	32.8 (n = 15)	-0.42
	33	2	0	2	2	2	11	28	9.3	4.7	19.6 (n = 26)	-1.38
	39	2	0	3	2	0**	8	17	5.7	2.8	38.5 (n = 12)	-0.90
2014	1	5	2 or 3	3	3	2	11	87	29.0	9	16.6 (n = 86)	0.65
	2	3	1	3	3	3	10	39	13.0	4.3	29.8 (n = 39)	0.65
	2A	2	0	2	2	2	11	26	8.7	4.3	16.2 (n = 25)	0.22
	12	≥4	≥2	2	2	2	10	20	6.7	3.3	37.5 (n = 8)	1.66
	18	4	2	*	2	2	13	44	14.5	7.3	28.3 (n = 18)	0.80
	31	2	0	2	2	2	10	34	11.3	5.7	26.4 (n = 23)	1.21
	49	≥4	2	2	2	2	10	34	11.3	5.7	18.9 (n = 26)	-0.36
Mean ± SD		**2.7 ± 1.1**	**0.6 ± 0.8**	**2.4 ± 0.5**	**2.1 ± 0.5**	**1.8 ± 0.7**	**10.3 ± 1.2**	**31.3 ± 20.1**	**10.3 ± 6.2**	**4.8 ± 1.9**	**26.8 ± 7.3**	-

*Nests found in nestling phase

**Nest abandoned when nestlings were 9 days of age

Table 4.5 Summary of parental care behaviour at nine monitored nests of thick-billed grasswren (*Amytornis modestus raglessi*) in Witchelina Nature Reserve, South Australia. Nests were analysed for 1 to 3 hrs, when nestlings were 10-15 days of age. Provisioning is shown as the total number of feeds (%), BM = breeder male; BF = breeder female; (m) = male; (f) = female.

	Nest ID	**Time of day**	**No of hours analysed**	**Nestling age (days)**	**Total No feeds**	**Total no feeds BM**	**Total no feeds BF**	**Total no feeds Helper 1**	**Total no feeds Helper 2**
Helpers absent	25	15:00-18:00	3	10	15	7 (46.7 %)	8 (53.3 %)	-	-
	33	15:00-18:00	3	11	28	15 (53.6 %)	13 (46.4 %)	-	-
	2A	15:00-18:00	3	11	26	12 (46.2 %)	14 (53.8 %)	-	-
	31	7:00-10:00	3	14	26	9 (34.6 %)	17 (65.4 %)	-	-
Helpers present	12 (2013)	15:00-18:00	3	13	45	18 (40.0 %)	10 (22.2 %)	17 (38.8 %) (m)	-
	2	8:0011:00	3	10	27	15 (55.6 %)	8 (29.6 %)	4 (14.8 %) (m)	-
	1	8:00-9:00	1	15	27	6 (22.2 %)	11 (40.7 %)	5 (18.5 %) (m)	5 (18.5 %) (f)
	12 (2014)	15:00-18:00	3	10	21	7 (33.3 %)	6 (28.6 %)	2 (9.5 %) (m)	6 (28.6 %) (m)
	18	15:00-18:00	3	13	38	12 (21.1 %)	18 (31.6 %)	9 (15.8 %) (m)	18 (31.6 %) (f)

Table 4.6 Group size of thick-billed grasswren (*Amytornis modestus raglessi*) groups, monitored from 2013 to 2015 in Witchelina Nature Reserve, South Australia.

Group size	***2013***	***2014***	***2015***	***Total***
Group size 2: breeding pair	36	16	12	64 (70 %)
Group size 3: breeding pair + 1 male “helper”	2	5	5	12 (13 %)
Group size 3: breeding pair + 1 female “helper”	0	1	1	2 (2 %)
Group size 4: breeding pair + 2 male “helpers”	2	1	0	3 (3 %)
Group size 4: breeding pair + 1 male and 1 female ”helper”	0	7	1	8 (9 %)
Group size 5: breeding pair + 2 male “helpers” and 1 female “helper”	0	2	0	2 (2 %)
Group size 5: breeding pair + 3 male “helpers”	0	1	0	1 (1 %)
Mean group size ± SD	**2.7 ± 1.1**	**3.6 ± 1.7**	**2.1 ± 0.9**	

Table 4.7 Group size and the number of male and female helpers of thick-billed grasswren (*Amytornis modestus raglessi*) groups in Witchelina Nature Reserve, South Australia.

Group size	***No of groups***	***No of male "helpers"***	***No of female "helpers"***
Group size 2: breeding pair	64	0	0
Group size 3: breeding pair + 1 male "helper"	12	12	0
Group size 3: breeding pair + 1 female "helper"	2	0	2
Group size 4: breeding pair + 2 male "helpers"	3	6	0
Group size 4: breeding pair + 1 male and 1 female "helper"	8	8	8
Group size 5: breeding pair + 2 male "helpers" and 1 female "helper"	2	4	2
Group size 5: breeding pair + 3 male "helpers"	1	3	0
Total	92	33 (73 %)	12 (27 %)

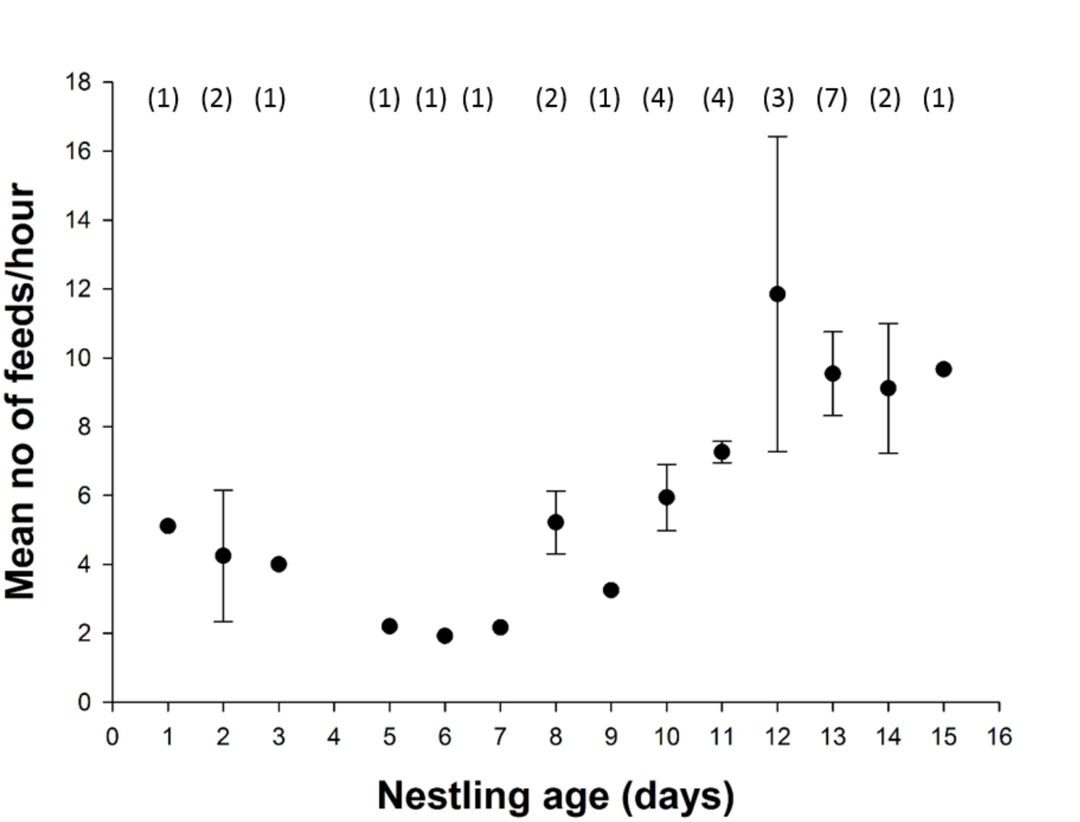

Figure 4.1 The number (mean ± SE) of feeds per hour at Thick-billed Grasswrens (*Amytornis modestus raglessi*) nests in relation to nestling age in days. The mean number of feeds per hour increases with nestling age. Sample size is given above each data point.

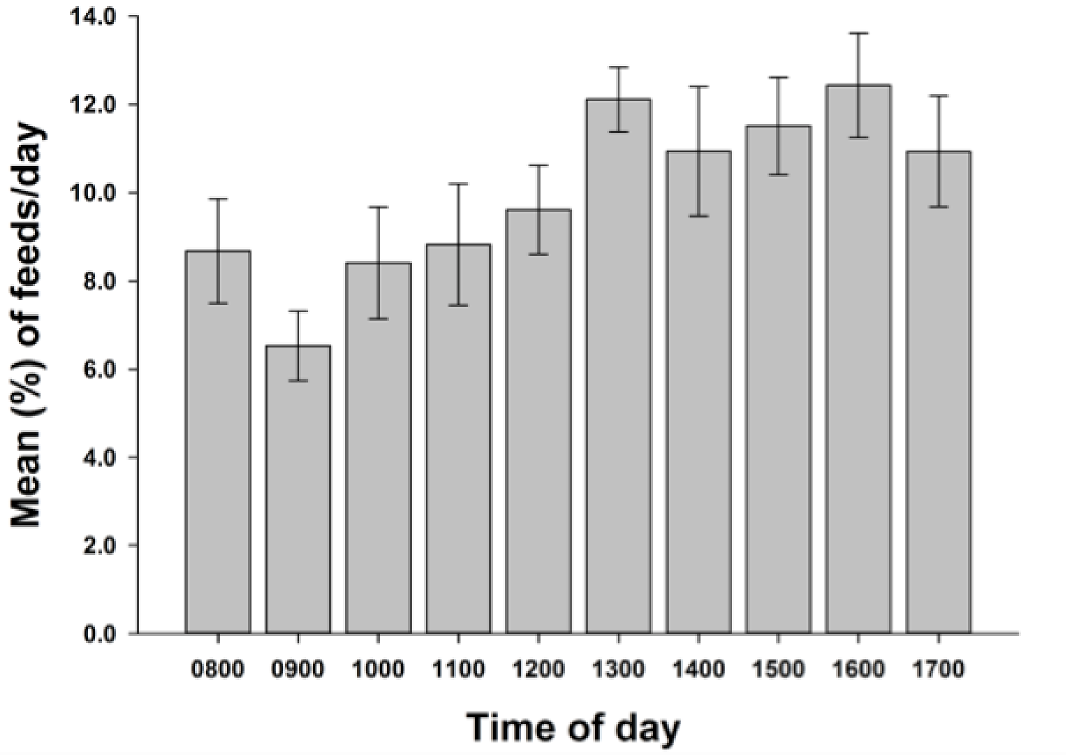

Figure 4.2 The percentage (mean ± SE) of feeds per hour at Thick-billed Grasswrens (*Amytornis modestus raglessi*) nests (n = 5) in relation to time of day. The percentage of feeds per hour increases during the day and is highest in the afternoon hours (13:00-17:00).

Figure 4.3 The identity of birds provisioning at the nest could be determined through identification of unique combinations of coloured leg bands (blue arrows); (A) and adult female and (B) an adult male thick-billed grasswren (*Amytornis modestus raglessi*) leaving the nest after provisioning.

Figure 4.4. Images of parental care behaviour identified at thick-billed grasswren (*Amytornis modestus raglessi*) nests in Witchelina reserve using a 12-mm, 90° lens-angle lens camera positioned ~0.2 m from nests: A) incubating/brooding nestlings, B) nest guarding, C) feeding (nestling of 10 days old), D) removal of faecal sack.

S1 Table 4.1 Summary table of observed cooperative breeding as described for the currently recognized eleven *Amytornis* species.

Species	***Scientific name***	***Cooperative breeding***	***Details of observations***	***Source***
Kalkadoon grasswren	*A. ballarae*	*Not described*	-	-
Grey grasswren	*A. barbatus*	Suspected	3 adult birds at nest (n = 1)*	Hardy (2010)
Carpenterian grasswren	*A. dorotheae*	Suspected	3-4 adult birds at nest**	Russell & Rowley (1997)
Eyrean grasswren	*A. goyderi*	*Not described*	-	-
Black grasswren	*A. housei*	Suspected	3 adult birds seen with 1 juvenile (n = 1)*	Freeman (1970), Russell & Rowley (1997)
Short-tailed grasswren	*A. merrotsyi*	*Not described*	-	-
Thick-billed grasswren	***A. modestus raglessi***	**Yes**	**Up to 2 helpers feeding nestlings (n = 6) (6 out of 12 groups)**	**This study**
Dusky grasswren	*A. purnelli*	Suspected	3 adult birds at nest (n = 1)*	Russell & Rowley (1997)
Striated grasswren	*A. striatus*	Suspected	3 adult birds at nest (n = 1)*	Karubian (2001)
Western grasswren	***A. textilis***	**Yes**	**3 adult birds feeding nestlings (n = 1) (1 out of 15 groups)**	**Brooker (1998, 1988), Russell & Rowley (1997)**
White-throated grasswren	*A. woodwardi*	Suspected	3 to 4 adult birds attending nestlings/fledglings (n = 2)*	Noske (1992), Russell & Rowley (1997)

* No behaviour recorded

** No sample size recorded, no behaviour recorded

Chapter 5 Lack of extra-pair paternity (EPP) in the vulnerable thick-billed grasswren (*Amytornis modestus raglessi*)

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In preparation to be submitted to *Conservation Genetics*

Abstract

The development and application of molecular genetic tools has revealed that behavioural observations of mating systems were not able to accurately assess the genetic mating systems in natural populations of songbirds. In contrast to earlier beliefs, we now know that birds are only very rarely sexually monogamous, with extra-pair offspring observed in approximately 90 % of all avian species. The highest proportion of extra-pair paternity (EPP) has been observed in several species of cooperatively breeding fairy-wrens (*Malurus*), which are part of the Australian Maluridae family. Our capacity to detect extra-pair paternity is now even greater with the advent of powerful molecular genomics. Here we use double-digest, restriction-site associated DNA sequencing (ddRAD-seq), to examine the genetic mating system of the thick-billed grasswren (*Amytornis modestus raglessi*), a close relative of fairy-wrens. We first assessed relatedness among 80 birds (38 adult males, 24 adult females, 12 male offspring, 6 female offspring), representing 21 breeding pairs, and among breeders and helpers of three cooperative groups. We estimated parentage and the level of EPP in the offspring of six broods (three breeder pairs, three cooperative groups). Overall, relatedness between breeder pairs was low ($r = 0.03 \pm 0.07$ SD), and in groups with helpers present, subordinate helpers were related to either one ($n = 1$) or both members of the breeder pair (n = 1), indicating that thick-billed grasswren juvenile offspring delay dispersal and become helpers in groups. There was no evidence of EPP in 12 offspring of 5 broods, irrespective of the presence of helpers. Despite the small samples sizes, our study provides insights into the mating system of this secretive member of the Australian Maluridae family.

Introduction

Although the majority of bird species form socially monogamous pair bonds (Lack, 1968), in approximately 90 % of avian species females copulate with extra-pair males with whom they often sire offspring (Griffith *et al.*, 2002, Petrie and Kempenaers, 1998, Westneat and Stewart, 2003, Wink and Dyrcz, 1999). Extra-pair paternity (EPP) has been studied extensively within the Maluridae, an Australian family of songbirds that includes emu-wrens (*Stipiturus*), grasswrens *(Amytornis)*, and fairy-wrens (*Malurus).* Although most fairy-wren species are socially monogamous, examination of the genetic mating system of *Malurus* revealed that many species are extremely sexually promiscuous, with some of the highest known frequencies of EPP (up to 76 % of all young can be sired by extra-pair males) reported among birds (Brooker *et al.*, 1990, Cockburn *et al.*, 2013, Double and Cockburn, 2003, Karubian, 2002, Mulder *et al.*, 1994, Webster *et al.*, 2004). Although extra-pair matings are proposed to have potential costs (e.g. reduced nest defence, reduced male parental care), and benefits (e.g. increased genetic quality of offspring), the overall benefits of EPP are not well understood (Arnqvist and Kirkpatrick, 2005, Griffith *et al.*, 2002, Jennions and Petrie, 2000, Petrie and Kempenaers, 1998, Schmoll, 2011). Additionally, within genera there are no general patterns in EPP levels. Among *Malurus*, for example, low levels of EPP have been observed in one fairy-wren species, the purple-crowned fairy-wren (*M. coronatus*) (Kingma *et al.*, 2009), which is in contrast to the generally high EPP occurrence observed in this family. This lack of consistency in general EPP patterns within taxonomic levels, plus the fact that the occurrence and levels of EPP can vary between populations of the same species and across years in single populations, highlights the need to analyse single-species genetic mating systems.

In contrast to the well-studied fairy-wrens, the genetic mating system of emu-wrens (*Stipiturus*) and grasswrens *(Amytornis)* remains largely unknown. The genetic mating system of only one emu-wren species has been examined in detail, the Southern emu-wren (*Stipiturus malachurus*), which displays low levels of cloacal protuberance, low levels of cooperative breeding, and low levels of EPP (Maguire and Mulder, 2004, Maguire and Mulder, 2008). To our knowledge there are no studies on EPP in grasswren species to date, despite the insights this knowledge can contribute to understanding patterns of extra-pair paternity within the Maluridae. Since *Amytornis* are considered among the most shy and elusive of avian species within Australia (Chapman, 1996, Rowley and Russell, 1997, Schodde, 1982a, Serventy, 1982), with cryptic plumage and extremely secretive behaviour, behavioural studies are challenging and time-consuming. In particular, studies using colour-marked and sexed individuals remain rare within *Amytornis*, limiting our understanding of parental care roles and mating systems within this genus. The dull plumage and relatively small testes of males of some grasswren species (Karubian, 2001, Rowe *et al.*, 2008, Rowe and Pruett-Jones, 2008, Rowley and Russell, 1997) suggests limited mating competition and hence relatively low levels of sexual promiscuity within this group, although there have been no studies conducted to confirm these ideas. The social mating system of two grasswren species, the western grasswren (*Amytornis textilis)* and the striated grasswren (*A. striatus*) has been previously described. Observations of parental care behaviour at nests revealed that cooperative breeding is uncommon in both these species (Brooker, 1998a, Brooker, 1988, Karubian, 2001). Unfortunately, these studies did not examine the genetic mating system of the species, and levels of EPP were therefore not assessed.

In this study we use a molecular genomics technique, double-digest, restriction-site associated DNA sequencing (ddRAD-seq), to examine the genetic mating system of a population of thick-billed grasswrens (*Amytornis modestus raglessi*) (Black, 2011a) at Witchelina Nature Reserve in South Australia. The thick-billed grasswren (*Amytornis modestus raglessi*) is a small (~19 g) ground-dwelling passerine which is endemic to the arid rangelands of South Australia and New South Wales (Black, 2011a). Populations of thick-billed grasswrens have been declining locally due to habitat degradation as a result of livestock grazing (Garnett *et al.*, 2011) and as a consequence, the species are currently recognized as nationally vulnerable under the 1999 Environment Protection and Biodiversity Conservation (EPBC) Act. Video-recording analysis of colour-marked and molecularly sexed individuals at twelve nests in 2013 and 2014 confirmed, for the first time in this species, that thick-billed grasswrens are facultative cooperative breeders (Louter et al. *in prep* c; Chapter 4); up to two helpers were present at 6 of 12 nests, and both male and female helpers were observed providing parental care.

Here, we use single-nucleotide polymorphisms (SNPs) acquired from ddRAD-seq, to assess levels of EPP in this vulnerable arid zone species. First, we aim to estimate relatedness of individuals within social breeder pairs, and between breeders and helpers, since highly related social pairs may be more likely to have extra-group offspring (e.g. Brouwer *et al.*, 2011, Kingma *et al.*, 2013, Varian-Ramos *et al.*, 2012). Second, we assess the level of EPP in thick-billed grasswren broods, and identify whether male and/or female helpers share in reproduction. Finally, we investigate whether males have an enlarged cloacal protuberance, since this morphological adaptation is known to be important for EP advertisement and mating competition in species within the Maluridae. Based on the behavioural (low levels of cooperative breeding) and morphological characteristics (small testes and cloacal protuberance) described in several other grasswren species (Brooker, 1998a, Karubian, 2001, Rowe and Pruett-Jones, 2006), we predict low levels of EPP, and limited cloacal protuberance in the thick-billed grasswren. Furthermore, because the number of subordinate helpers has been found to increase EPP levels in cooperative breeding fairy-wrens (Dunn and Cockburn, 1999, Mulder *et al.*, 1994, Webster *et al.*, 2004, but see Colombelli-Négrel *et al.*, 2010), we predict that breeding pairs with helpers present will have a higher probability of EP offspring. The findings of this study will shed light on the life history strategies of this vulnerable arid songbird and contribute to our understanding of the extra-pair paternity within the Maluridae family.

Methods

Study population

Data were collected from August to September 2013-2015 (spanning a total of 18 sampling weeks), at Witchelina Nature Reserve (30º01’340” S, 138º02’772” E), which is owned by the Nature Foundation of South Australia (NFSA) (described in Louter et al. a, *in prep* a; Chapter 2). The 4200 sq. km reserve is located in the arid rangelands of inland South Australia (Figure 1.2, p.6), and consists of ten major habitat types, including gibber plains, red river gum and coolibah woodlands, saltbush plains, acacia dunefields and blue bush shrubland. All these habitat types contain various degrees of low shrublands, dominated by chenopod shrub species such as saltbush (*Atriplex sp.*) and blue bush (*Maireana sp.*) (Slender *et al.*, a, *in prep*), which are typical of thick-billed grasswren habitat (Black *et al.*, 2011a). Within an area of ~50 sq. km in

Witchelina Nature Reserve, we located and mapped out 42 thick-billed grasswren territories (Figure 1.2, p.6). Effort was made to capture and band all thick-billed grasswrens in each territory each year, by using two to four mist nets (9-12 m, 30 mm-mesh), erected across drainage and creek lines. All captured birds were fitted with one aluminium band (provided by ABBBS) and a unique combination of three coloured leg bands, to enable individual identification of birds in the field. Each territory was checked at least weekly for group composition and breeding activity throughout the breeding season, and to band and sample nestlings when they were ≥ 8 days old.

Behavioural adaptations to EPP

Each grasswren territory was visited at least once a week, for the entire length of the study, to monitor nests, determine group size, and to assess the status (breeder or helper) of individuals (nest monitoring also described in Louter et al. *in prep* b, c; Chapters 3 and 4). Behavioural observations of individuals were recorded when territories were visited to record if thick-billed grasswrens display behavioural traits that typically co-occur with EPP in other fairy-wren species (e.g. extra-territorial intrusions, extra-pair copulations, extra-pair courtship displays, presentation of flower petals), as well as to document general behaviours (perching, hiding, foraging) and reproductive behaviour (incubating, brooding, feeding nestlings) of individuals.

Morphological adaptations to EPP – cloacal protuberance

All thick-billed grasswrens were measured for common morphological features at the time of capture (Slender *et al.*, b, *in prep*). Additionally, we measured the cloacal protuberance (CP) of all captured males (dominant and subordinates) at our study site. A high incidence of extra-pair paternity (which has been shown to correlate with sperm competition) is commonly associated with a large CP in birds (Birkhead, 2000, Birkhead *et al.*, 1992). A cloacal protuberance is an external swelling of the male reproductive organs that develops when males breed (Birkhead and Møller, 1998) and is the site of sperm storage. The CP has been found to be a good indicator of sperm competition and EPP rate in fairy-wrens (Kingma *et al.*, 2012, Rowe *et al.*, 2008, Rowe and Pruett-Jones, 2006, Tuttle *et al.*, 1996) and in emu-wrens (Maguire and Mulder, 2008). The CP of grasswren males was measured as width (w) and length (l) (perpendicular to each other; lateral and antero-posterior axes, respectively), using dual callipers (to the nearest 0.1 mm). The 'CP size' was calculated using the formula $(w/2 + l/2)^2 \times \pi$ (following Kingma *et al.*, 2012). We also noted the presence or absence of a brood patch for females and males. All birds were aged based on morphology (gape and feather condition) at the time of capture, and subsequent behaviour in the field and at active thick-billed grasswren nests.

DNA sampling and DNA extraction

A blood sample was taken at the time of capture/banding; for each individual we took a 5-µL blood sample from the brachial vein using a capillary tube which was stored on Whatman FTA® paper (Sigma-Aldrich) or in salt solution (Seutin *et al.*, 1991) and stored at 4 °C to prevent DNA degradation. Because blood samples were stored in different forms, two methods were used to extract DNA; (1) Blood stored in salt solution was extracted using a DNeasy Blood and Tissue kit (QIAGEN) and a Gentra Puregene Blood Kit (QIAGEN); (2) DNA was extracted from blood stored on Whatman FTA paper using a modified version from Smith and Burgoyne (2004). Specifically, a small disc (2 x 2 mm) from the FTA card sample was washed in 200 µl of FTA lysis buffer (100 mM Tris, 0.1 % SDS) for 30 mins before being washed in 200 µl of DNAzol© for 10 mins. Next, the discs were washed for 5 mins with 200 µl of H_2O (repeated 3 times), and washed for 10 mins with 200 µl of ethanol (95 %), whilst the residue was discarded after each wash. Samples were dried at room temperature for 15 mins before being eluted with 50 µl of TLE buffer and incubated at 90 °C for 5 mins. Extracted DNA samples were stored at 4 °C to prevent DNA degradation.

Sex determination

Adult thick-billed grasswrens are largely plumage-monomorphic: in the field, females can only be distinguished from males by the presence of a small rufous flank-patch underneath the wings (

Figure 1.4, p.9), which is acquired by females at an early age (1-2 months) (Schodde, 1982a), but juvenile males also sometimes have rufous feathering underneath wings (personal observation). Therefore the sex of all individuals (breeder, helper, offspring) was determined in the lab by DNA analysis, using a modified version of the method described in Griffiths *et al.* (1998), incorporating the primers P8 (5'-CTCCCAAGGATGAGRAAYTG-3') and P2 (5'-TCTGCATCGCTAAATCCTTT-3'), which were used to target the Chromo-Helicase-DNA-binding genes (CHD; Griffiths and Tiwari, 1996). The sexes differ in the size of an intron within these genes located on the allosome. PCR reactions were performed in 24 µl volumes containing 10-100 ng of extracted DNA, with a final concentration of 1 x MRT buffer (1 x Immolase buffer, 10 mM each dNTP, 2.5 mg/ml BSA), 0.2 µM of each primer and 0.5 units Immolase (Bioline). In order to detect DNA contamination in the mastermix, we included one sample without DNA as a negative control for each PCR. PCRs were performed in a Mastercycler pro (Eppendorf) with the following PCR cycling conditions: initial denaturation at 94 °C for 10 mins, followed by 35 cycles of 94 °C for 45 sec, 48 °C for 45 sec, 72 °C for 45 sec, with a final extension of 72 °C for 5 min and 25 °C for 2 mins. In order to visualise the size of PCR products, which were expected to differ by only a few base pairs (bp) between sexes, PCR reactions were separated by electrophoresis in a

3 % agarose gel. We used the PCR ladder hyperladder IV (Bioline) to estimate band size and gels were stained with GelRed stain (Biotium, Inc. Hayward, CA) to visualise the bands. The size and number of bands on the gel was used to assign the sex of each individual; samples with two bands at ~260 and 290 bp were assigned as female and those with one band at ~260 bp were assigned as males (Figure 5.1). Analysis of DNA samples of known-sex adults (individuals whose sex was determined in the field by plumage characteristics and behaviour; males: n = 42 and females: n = 31) was used to test the reliability of the P2 and P8 sexing method for the thick-billed grasswren, which successfully confirmed the sex of these individuals (100 % accuracy).

Restriction-site Associated DNA (RAD) sequencing

To provide genetic markers for our analyses, we used single-nucleotide polymorphisms (SNPs) from 81 individual birds generated using an efficient genotyping approach based on RAD sequencing (Baird *et al.*, 2008, Etter *et al.*, 2011, Miller *et al.*, 2007). We first quantified extracted DNA fluormetrically using a Qubit DNA assay, assessed DNA quality using gel electrophoresis, and a UV-spectrophotometer was used to check for protein and RNA contamination. Unique barcodes were attached to each sample following Poland *et al.* (2012). Genomic libraries were prepared after digestion with restriction enzymes PstI and MspI and we incorporated a control sample that did not contain any DNA, as well as duplicate samples (Honsdorf *et al.*, 2014). RAD libraries were sequenced at the Australian Genome Research Facility (AGRF) in Adelaide on an Illumina Nextseq, which produced 75 basepair (bp) single-end reads. We removed Illumina adapters and RAD barcodes and performed SNP calling using the Universal Network Enabled Analysis Kit (UNEAK) pipeline (Lu *et al.*, 2013) implemented in the program Trait Analysis by aSSociation, Evolution and Linkage (TASSEL) version 3.0 (Bradbury *et al.*, 2007, Glaubitz *et al.*, 2014). Reciprocal pairs of tags (sequencing error rate set to 0.03), were considered true SNPs based on the following parameters: read depth per tag > 10 reads, minimum call rate > 0.8, minimum minor allele frequency > 0.05. The average SNP error rate across 8 duplicate samples was 0.01 %, following Pompanon *et al.* (2005). Overrepresented SNPs (> 7000 reads/sample), SNPs that had a mean heterozygosity ratio less than 0.2, and SNPs with < 75 % coverage across individuals and individuals with < 40 % coverage of SNPs were removed from the data set, leaving a total of 1740 SNP loci. We used genotype data from 80 birds (38 adult males, 24 adult females, 18 offspring) to analyse allele frequencies for 1740 loci. Hardy–Weinberg (HW) and linkage equilibrium were tested for each locus using CERVUS version 3.0.3 (Field Genetics Ltd) (Kalinowski *et al.*, 2007, Marshall *et al.*, 1998), which detected 17 sex-linked loci that deviated from Hardy-Weinberg equilibrium. These loci were excluded from further analyses, resulting in a total of 1723 loci to use in relatedness and parentage analysis.

Relatedness

Prevuous studies indicate that closely related breeding pairs are more likely to seek out extra-pair copulations and hence EPP (Brouwer *et al.*, 2011, Kingma *et al.*, 2013, Varian-Ramos *et al.*, 2012), we determined the relatedness of members of 21 thick-billed grasswren breeding pairs. Relatedness measures the proportion of alleles shared between individuals that are identical by descent. We used COANCESTRY version 1.0.1.5 (Wang, 2011) to calculate maximum likelihood estimates of pair-wise relatedness (r) between breeder pairs with a maximum-likelihood estimator (Milligan, 2003). We chose the maximum-likelihood estimator because it is generally more accurate and tends to estimate certain values of relatedness with less bias and error than other estimators (Milligan, 2003).

Parentage analysis

We assessed EPP in the offspring of six thick-billed grasswren broods (three breeder pairs, three cooperative groups) using two commonly used parentage assignment packages, COLONY version 2.0.4.4 (Jones and Wang, 2010, Wang and Santure, 2009) and CERVUS version 3.0.3 (Field Genetics Ltd) (Kalinowski *et al.*, 2007, Marshall *et al.*, 1998), which have been shown to accurately assign parentage using SNP data (Hauser *et al.*, 2011). COLONY uses a full maximum-likelihood method to simultaneously infer parentage and sibship, which was developed to be applied to cases in which both sexes can be polygamous. The program can be used to infer parentage jointly as fullships or paternal and maternal half-sibships, based on the Mendelian law of inheritance, allele frequencies, and Hardy-Weinberg equilibrium (Jones and Wang, 2010). Using 1723 loci, parentage and sibship inference was performed using the full likelihood method in COLONY with the following parameters: long length run with high likelihood precision; genotyping error rates of 0.01 per SNP site with no allelic dropout; assuming a dioecious, diploid population with male and female polygamy; without inbreeding (appropriate when analysing parentages for offspring from several breeding seasons; Jones and Wang 2010). Samples were assigned in offspring and paternal cohorts based on age and genetic sex determination. The probability that fathers and mothers were included in the candidate samples was estimated to be 80 % for males and 90 % for females based on the proportion of sampled individuals of all marked (sampled) and unmarked (unsampled) individuals observed during the study period. Two helper males were included in both the offspring and paternal cohorts. Only parental assignments with a probability of > 95 % were retained.

CERVUS incorporates the occurrence of null alleles and mutations as well as incomplete sampling into likelihood calculations to decrease the probability of false exclusions, through a simulation module. The natural logarithm of the likelihood ratio is termed the LOD score. We used the parent-pair (sexes known) simulation program within CERVUS to estimate the required critical difference in LOD scores between the first and second most likely candidate parent-pair (sexes known) for assignment at a level of > 95 % and of > 80 % confidence.

Results

Behavioural observations

We collected a total of 827 (~130 hours) behavioural observations of individual adult thick-billed grasswrens over the course of three years (Table 5.1). Most observations were of grasswrens undertaking general activities (77.0 %), but in 10.4 % of observations grasswrens were engaged in breeding activities, such as brooding or feeding nestlings. In 12.6 % of all observations thick-billed grasswren were heard broadcasting song, a behaviour that may be associated with EPP. Most commonly one song was repeated 1-3 times (n = 93), but a series of up to 10 songs could be heard at one time (n = 11).

In 41 % of behavioural observations (n = 341) we were able to positively identify the sex and status of individuals (Table 5.1); we did not observe any individuals intruding conspecific territories, neither did we observe any males engaged in extra-pair copulations, extra-pair courtship displays, or presentation of flower petals.

Cloacal protuberance and brood patch

The CP of 41 adult thick-billed grasswren males varied in size (range 28.3 – 75.4 mm^2). Breeder males (52.3 mm^2 ± 14.1 SD, n = 38) as well as subordinate helper males (41.1 mm^2 ± 9.6 SD, n = 3) had an enlarged CP. A small portion of breeder males (16 %, n =

6) had a fully developed brood patch, whilst all three subordinate males had a fully developed brood patch.

Pairwise relatedness

Average pairwise relatedness (mean ± SD) of males and females of 21 thick-billed grasswren breeding pairs was $r = 0.03 \pm 0.07$ (Table 5.2). Cooperative breeders with helpers at the nest are typically expected to have helpers that are related to at least one member of the breeding pair (Riehl, 2013). In one cooperative group, for which we were able to obtain DNA from all individuals (the breeding pair, a male helper, and all offspring), the male helper was identified as offspring of the breeding pair (Figure 5.2, Table 5.3). In another cooperative group with one male helper, we were able to obtain DNA from all individuals, and found that the male helper in this group was related to the breeder male, but not to the breeder female (Figure 5.2). The third cooperative group consisted of a breeder pair and 2-3 helpers (2 males, 1 female). Although we have genetic samples for these individuals, we were unable to include these samples in our analysis due to financial constraints. Despite the latter the social male was assigned as the true father and sired all three offspring in this group.

Parentages and sibling relationships

Parentage analysis for six thick-billed grasswren broods consisting of 15 offspring as well as two helper males attending a brood (Table 5.2) was performed in COLONY and CERVUS (results not shown here, supplementary material (**S1 Table 5.1**). Here, we present results from COLONY only, as there was no discrepancy in the parental assignment of both programs, but COLONY uses more information in the data and is expected to be more powerful under most circumstances (Walling *et al.*, 2010). We assigned maternity for 80 % of genotyped nestlings (12 out of 15) and for one of two helper males (Table 5.3, Figure 5.1). The analysis indicates that egg dumping by helper or extra-group females did not occur at our study site. We were able to verify and ascertain correctness of assignments as we worked with samples of colour-marked molecularly sexed individuals with known social status (e.g. breeder male, breeder female, helper (fe)male). Comparing the assignment of the genetic mother with the identity of the known social mother that attended the nest showed that assignments were correct, as there were no mismatches between offspring and the social mother. Maternity could not be assigned for three offspring of one brood, as we were unable to trap and sample the social female of this group that probably sired these three individuals. One helper male for which maternity could not be assigned was already present at the beginning of the respective observation period (2013), thus an unsampled female from the previous cohort probably sired this helper male.

Paternity was assigned to 80 % of the nestlings and for both helpers (Table 5.3, Figure 5.1). For three nestlings, paternity could not be assigned, but the data are consistent with all three nestlings having the same father. A single female attended the nestlings of this brood alone, and a male was never observed in the territory or at the nest, and thus was not sampled. We did not find evidence of EPP in the population of thick-billed grasswren (*Amytornis modestus raglessi*) at Witchelina Nature Reserve (Table 5.3); the social male of the pair sired the offspring of three unassisted breeding pairs, and in three groups with helpers present there was no evidence to suggest that male helpers sired any offspring in the brood.

Discussion

Summary of results

This is the first study to genetically examine the mating system within the *Amytornis* genus. The outcomes contribute novel insights into the breeding ecology and genetic mating system of the thick-billed grasswren (*Amytornis modestus raglessi*). The aim of this study was to assess relatedness and EPP levels in thick-billed grasswren groups, using a powerful molecular genomics tool, ddRAD-seq. We used 1723 SNP loci to assess paternity with two commonly used software packages (COLONY and CERVUS) and did not detect any direct evidence for extra-pair paternity in the offspring of five thick-billed grasswren groups, suggesting that EPP levels are low in thick-billed grasswrens. We found that members of breeder pairs were unrelated to each other, and therefore EPP would not necessarily function to avoid inbreeding in this system. With further assessment of relatedness within cooperative thick-billed grasswren groups, we found that helpers were related to one or both individuals of the breeder pair. This suggests that breeders tolerate young from previous separate broods, at least until the next breeding attempt or breeding season. The cloacal protuberance size of 41 adult thick-billed grasswren males (38 breeder males, and 3 subordinate helper males) was relatively small compared to promiscuous species and grasswren males did not seem to display any of the behavioural traits that have been associated extra pair-matings in promiscuous species. Together, these findings support our conclusion that EPP is uncommon in the population of thick-billed grasswrens at Witchelina Nature Reserve.

EPP in other Maluridae

Our study found no evidence for EPP within the thick-billed grasswren. Although EPP is a defining feature of the mating system of *Malurus*, and high levels of EPP have been assumed to occur in all fairy-wren species (Brooker *et al.*, 1990, Brouwer *et al.*, 2011, Colombelli-Négrel *et al.*, 2010, Karubian, 2002, Mulder *et al.*, 1994, Rowe and Pruett-Jones, 2006, Webster *et al.*, 2004), not all members of the Maluridae family display high levels of EPP.. For example, the purple-crowned fairy-wren (*M. coronatus*) has low levels of EPP, with only 4.4 % of offspring sired by extra-pair males (Kingma *et al.*, 2009). The authors speculated that *M. coronatus* has lost its extreme mating system due to habitat constraints, but the habitat-constraint hypothesis does not seem to be limiting EPP in other fairy-wren species (Brouwer *et al.*, 2014). A study by Maguire and Mulder (2008) on the genetic mating system of Southern emu-wrens (*S. malachurus*) revealed that 12.0 % of offspring were sired by extra-pair males. This low EPP could be the result of limited opportunities for female extra-pair copulations, caused by a high dependence on paternal care for offspring survival (Maguire and Mulder, 2004, Maguire and Mulder, 2008). The findings of low levels of EPP in these studies were based on a considerably larger sample size (*S. malachurus*: 50 offspring of 27 broods; *M. coronatus*: 227 offspring from 104 broods) than the small sample size obtained in this study (12 offspring of five broods) and therefore it is possible that EPP was not detected. However, if EPP had been common in our study population, we would likely have detected it.

Behaviour and EPP

Consistent with the absence of extra-pair paternity, we did not observe any behaviour associated with extra-pair paternity, such as foraying behaviour or extra-pair song and courtship displays. Such behaviours are commonly performed by *Malurus* and can involve the presentation of a flower petal (Bradley and Bradley, 1958, Mulder, 1997, Rowley, 1991), although the latter occurs much less frequently and is usually difficult to observe. Moreover, actual extra-pair copulations are extremely cryptic in *Malurus*, and therefore rarely observed directly. In superb fairy-wrens (*M. cyaneus*) for example, they take place just before dawn, when females visit territories of extra-pair males (Double and Cockburn, 2000). Admittedly, it is possible that thick-billed grasswrens at our study site display such behaviours, but that their extremely shy and elusive nature inhibited us from observing these directly. However, it seems unlikely that thick-billed grasswrens would perform elaborate courtship behaviour, since over 400 behavioural observations of individual grasswrens did not detect any evidence for such behaviour. While for 61 % of observations we could not identify the sex and status of individual grasswrens, there was no indication of intruder behaviour (e.g. chase, alarm, etc.), indicative of low levels of territory intrusions and territorial displays. Additionally, a radio telemetry study of 14 adult males in 2014 revealed that thick-billed grasswren males have relatively large home ranges averaging 8 hectares (Louter et al. *in prep* a; Chapter 2). Individuals seem to only very occasionally enter neighbouring territories, and when they do cross a territory border, are typically in the company of their mate and/or other group members (personal observation). These findings further suggest that extra-pair fertilisations and extra pair paternity seem to be uncommon in the population of thick-billed grasswrens at our study site.

Morphological adaptations to EPP

The presence of an enlarged cloaca is typical of species with intense sperm competition (Birkhead and Møller, 1998). A cloacal protuberance has been described for many *Malurus* species (e.g. Mulder and Cockburn, 1993, Tuttle *et al.*, 1996) and an enlarged CP is generally correlated to high levels of EPP in fairy-wrens (Rowe *et al.*, 2008, Tuttle *et al.*, 1996). Even in fairy-wren species with low levels of extra-pair paternity, such as the purple-crowned fairy-wren (Kingma *et al.*, 2009), the enlarged CP size of non-breeding and pre-breeding males was positively correlated with the number of breeding females in the population, suggesting that CP size is sensitive to extra-pair mating opportunities (Kingma *et al.*, 2012). Unlike fairy-wrens, male emu-wrens lack a cloacal protuberance (Maguire and Mulder, 2004), which is consistent with the observed low levels of extra-pair paternity in this species (Maguire and Mulder, 2008). Within *Amytornis*, cloacal protuberance has only been measured in the striated grasswren (*A. striatus*) (Karubian, 2001, Rowe *et al.*, 2008, Rowe and Pruett-Jones, 2008), and in the western grasswren (*A. textilis*) (Rowley and Russell, 1997). These studies did not examine the genetic mating of these species and for this reason knowledge on the relationship between cloacal protuberance and extra-pair paternity levels is unknown. The marginally enlarged cloacal protuberance of thick-billed grasswren males observed in our study indicates that sperm competition, and thus extra-pair copulations, may be present in this species, despite the lack of evidence in the parentage analysis.

Conclusion

The use of SNPs from ddRAD-seq provided extremely high power to determine the relatedness and paternity in the thick-billed grasswren. Acknowledging our small sample size for the EPP analysis, we did not find any genetic, morphological or behaviour evidence for extra-pair paternity in the thick-billed grasswren (*Amytornis modestus raglessi*), which suggests that the population of thick-billed grasswrens at our study site is genetically monogamous. These findings, plus the fact that grasswren males do not have obvious male signalling traits (no investment in brightly coloured plumage, no enlarged testes, no elaborate song or courtship display), suggest that if EPP occurs in our study population, it is probably infrequent and does not seem play a major role in the mating behaviour of grasswrens. Perhaps because of the resource-limited arid environment in which grasswrens breed, coordinated biparental care appears essential to the reproductive success of male and female thick-billed grasswrens. Thick-billed grasswren males and females at Witchelina Nature Reserve have been observed to take turns incubating eggs (personal obs) and the occurrence of a developed brood patch in a large proportion of captured males (see Chapter 3) in this study suggest that thick-billed grasswrens generally share incubation load. Previous work has shown that feeding rates are equally divided between breeder pairs (Louter et al. *in prep* c; Chapter 4). Together, these findings suggest that grasswrens are constrained in their mate choice because males and females are highly co-dependent on their partner for parental care duties and hence offspring survival.

Acknowledgments

We are very grateful to the Nature Foundation of South Australia Inc. (NFSA) for their substantial financial support. BirdsSA, Birdlife Australia, and the Field Naturalists Society of South Australia also funded this research. We thank the NFSA for allowing us to work in Witchelina Nature Reserve and for providing accommodation on site. We sincerely thank the Witchelina Nature Reserve management for their assistance and logistical support during fieldwork. We also thank the following volunteers for their invaluable help in the field: Scott Kinlock, Wikke Popken, Madeline Thomson, Sara Criddle, Andrea Robinson, Bianca Staker, and Petra Hanke. All fieldwork procedures conducted in this study complied with the laws of the country in which they were performed, followed the guidelines for the Australian Code of Practice for the use of animals for scientific research, and were approved by the Flinders University Animal Welfare Committee (permits: E325, E312 and E385), and by the Australian Bird and Bat Banding Scheme (authority 2601 and 3100). We thank Tessa Bradford for valuable comments on the ddRAD-seq paragraph and are grateful to Belinda Cale for helpful suggestions that improved this manuscript.

Table 5.1 Summary table of behavioural observations (EPP related, reproductive, general) of unidentified and identified (sex, breeder, helper) thick-billed grasswrens (*Amytornis modestus raglessi*) at Witchelina Nature Reserve, South Australia, observed in 2013-2015.

Behaviour		**Identity unknown**	**Breeder female**	**Breeder male**	**Helper male**	**Helper female**	**Total n**
EPP related behaviour	*Intrusions*	-	-	-	-	-	**0**
	EP display	-	-	-	-	-	**0**
	Petal presentations	-	-	-	-	-	**0**
	Solo song	73	1	28	2	0	**104** (12.6 %)
Reproductive behaviour	*Incubating eggs*	55	2	10	0	1	**68** (8.2 %)
	*Dodgem car display**	0	1*	0	0	0	**1** (0.1 %)
	Brooding nestlings	12	0	0	0	0	**12** (1.5 %)
	Feeding nestlings	4	0	1	0	0	**5** (0.6 %)
General behaviour	*Perching on shrub*	173	4	93	12	1	**283** (34.2 %)
	Hiding in shrub	54	3	116	8	0	**181** (21.9 %)
	Foraging	115	7	44	6	1	**173** (20.9 %)
	TOTAL	**486**	**18**	**292**	**28**	**3**	**827**

* In many Maluridae species this defence behaviour is commonly displayed close to active nests, as a distraction for predators.

Table 5.2 Summary table of group composition and pairwise relatedness of 21 thick-billed grasswrens (*Amytornis modestus raglessi*) families, sampled in 2013 and 2014 in Witchelina Nature Reserve, South Australia. The six thick-billed grasswren families for which EPP was determined are shaded in grey. BM = breeder male, BF = breeder female, HM = helper male, HF = helper female, UB HM = unbanded helper male). Individuals in brackets are genetically sampled individuals that were not included in relatedness or EPP analysis.

Group ID	*Year*	*Breeder female*	*Breeder male*	*Pairwise relatedness (r)*	*Helper 1*	*Helper 2*	*Helper 3*	*Offspring 1*	*Offspring 2*	*Offspring 3*
1	2014	*(BF 1)*	BM 1	-	*(HM 1)*	*(HF 1)*		Nestling 1	Nestling 1	-
2A	2014	BF 2A	BM 2A	0.0000	-	-	-	*(Nestling 2A)*	*(Nestling 2A)*	-
4	2014	BF 4	BM 4	0.0000	HM 4	-	-	Nestling 4	Nestling 4	Nestling 4
9	2013	BF 9	BM 9	0.0518	-	-	-	*(Fledgling 9)*	*(Fledgling 9)*	
12	2013	BF 12	BM 12	0.0000	HM 12	-	-	Nestling 12	Nestling 12	Nestling 12
13	2013	BF 13	BM 13	0.0000	-	-	-	*(Fledgling 13)*	-	-
15	2013	BF 15	BM 15	0.0000	-	-	-	*(Fledgling 15)*	*(Fledgling 15)*	-
18	2013	BF 18	BM 18	0.0000	-	--	-	*(Fledgling 18)*	*(Fledgling 18)*	-
21	2013	BF 21	BM 21	0.0042	-	-	-	Nestling 21	Nestling 21	Nestling 21
25	2013	BF 25	BM 25	0.0000	-	-	-	*(Nestling 25)*	*(Nestling 25)*	-
26	2013	BF 26	BM 26	0.0595	-	-	-	*(Nestling 26)*	-	-
31	2013	BF 31	BM 31	0.0173	-	-	-	Nestling 31	Nestling 31	-
33	2013	BF 33	BM 33	0.0000	-	-	-	*(Nestling 33)*	*(Nestling 33)*	-
34	2014	BF 34	BM 34	0.0000			-	Nestling 34	Nestling 34	-
37	2013	BF 37	BM 37	0.0000	-	-	-	*(Nestling 37)*	-	-
45	2013	BF 45	BM 45	0.0000	-	-	-	*(Fledgling 45)*	-	-
49	2014	BF 49	BM 49	0.0031	UB HM	-	-	*(Nestling 49)*	*(Nestling 49)*	-
50	2014	BF 50	BM 50	0.0570	-	-	-	*(Nestling 50)*	*(Nestling 50)*	-
50A	2014	BF 50A	BM 50A	0.0231	*(HM 1)*	-	-	*(Nestling 50A)*	*(Nestling 50A)*	-
51	2013	BF 51	BM 51	0.2834	-	-	-	*(Fledgling 51)*	-	-
52	2013	BF 52	BM 52	0.1717	-	-	-	*(Nestling 52)*	*(Nestling 52)*	-
			Mean ± SD	**0.0336 ± 0.07**						

Table 5.3 Genetic parent-pair analysis as assigned by COLONY for 15 offspring and two helpers of six thick-billed grasswrens (*Amytornis modestus raglessi*) families, sampled in 2013 and 2014 in Witchelina Nature Reserve, South Australia.

Group ID	*Year*	*Offspring ID*	*Genetic mother*	*Behavioural father*	*Genetic father*	*Paternity*
1	2014	Helper male				Not included[b]
1	2014	Helper female				Not included[b]
1	2014	Nestling 1	unassigned	BM 1	BM 1*	No EPP
1	2014	Nestling 2	unassigned	BM 1	BM 1*	No EPP

1	2014	Nestling 3	unassigned	BM 1	BM 1*	No EPP
4	2014	Helper male	BF 4*	Undetermined	BM 4*	No EPP
4	2014	Nestling 1	BF 4*	BM 4[a]	BM 4*	No EPP
4	2014	Nestling 2	BF 4*	BM 4[a]	BM 4*	No EPP
4	2014	Nestling 3	BF 4*	BM 4[a]	BM 4*	No EPP
12	2013	Helper male	unassigned	Undetermined	BM 12*	No EPP
12	2013	Nestling 1	BF 12*	BM 12	BM 12*	No EPP
12	2013	Nestling 2	BF 12*	BM 12	BM 12*	No EPP
21	2013	Nestling 1	BF 21*	undetermined	unassigned	No EPP
21	2013	Nestling 2	BF 21*	undetermined	unassigned	No EPP
21	2013	Nestling 3	BF 21*	undetermined	unassigned	No EPP
31	2014	Nestling 1	BF 31*	BM 31	BM 31*	No EPP
31	2014	Nestling 2	BF 31*	BM 31	BM 31*	No EPP
34	2014	Nestling 1	BF 34*	BM 34[a]	BM 34*	No EPP
34	2014	Nestling 2	BF 34*	BM 34[a]	BM 34*	No EPP

* COLONY assignment probability = 1.0

[a] Behavioural father designation based on trapping data

[b] Due to financial limitations these samples were not included in ddRAD-seq and were therefore not included in EPP analysis

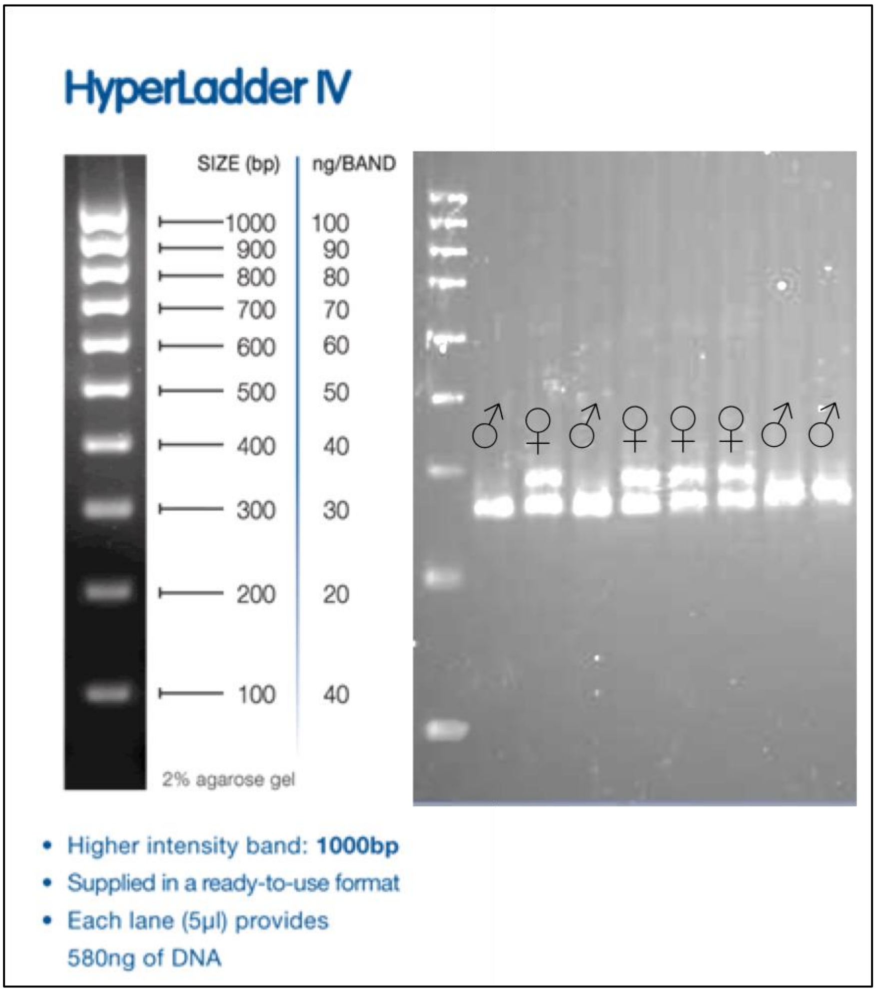

Figure 5.1 DNA sex identification of thick-billed grasswrens (*Amytornis modestus raglessi*), using PCR with a single set of primers P2 and P8. The known sex of each individual (n = 8) is indicated; those birds with two bands are females and those with one band are males. Photo of gel by Marina Louter.

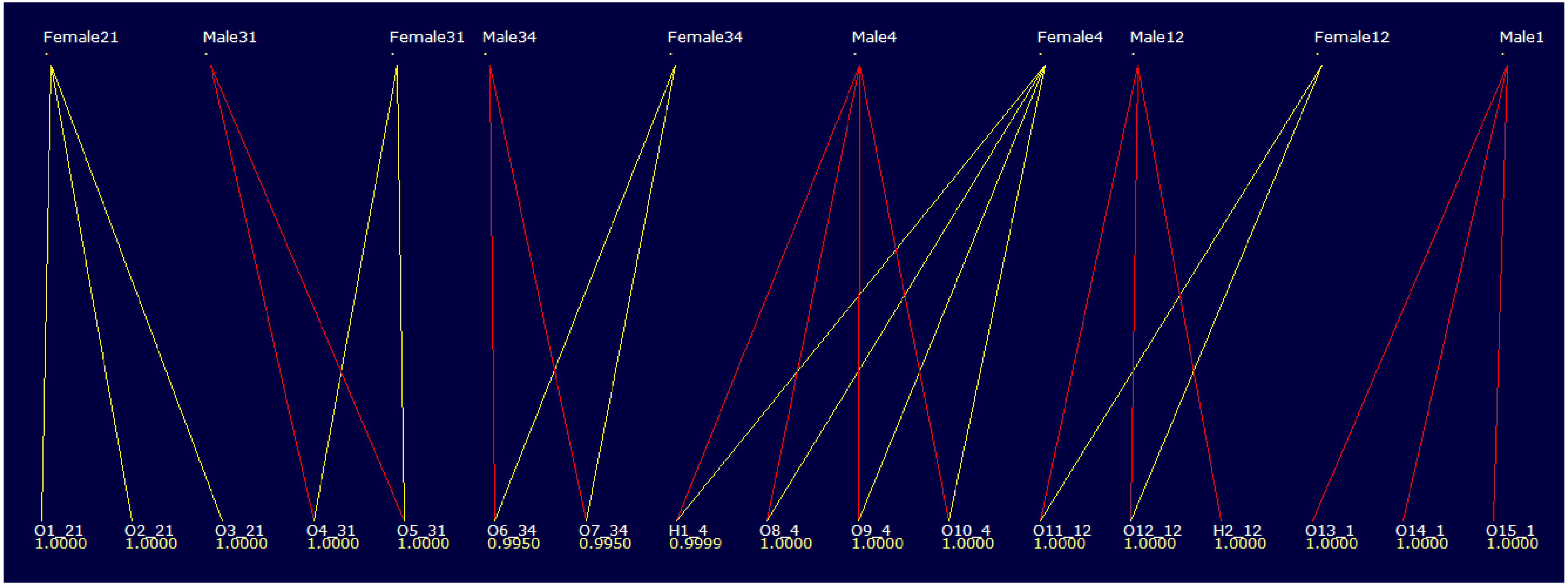

Figure 5.2 A pedigree drawn by Pedigree viewer (http://bkinghor.une.edu.au/pedigree.htm) from the ParentPair configuration file of COLONY.

S1 Table 5.1 Genetic parent-pair analysis (sexes known) as assigned by CERVUS for 15 offspring and two helpers of six thick-billed grasswrens (*Amytornis modestus raglessi*) groups, sampled in 2013 and 2014 in Witchelina Nature Reserve, South Australia.

Group ID	*Year*	*Offspring ID*	*Genetic mother*	*Behavioural father*	*Genetic father*	*Paternity*
1	2014	Helper 1				Not included[c]
1	2014	Helper 2				Not included[c]
1	2014	Nestling 1	unassigned	BM 1	BM 1**	No EPP
1	2014	Nestling 2	unassigned	BM 1	BM 1**	No EPP
1	2014	Nestling 3	unassigned	BM 1	BM 1**	No EPP
4	2014	Helper male	BF 4**	undetermined	BM 4**	No EPP
4	2014	Nestling 1	BF 4**	BM 4[a]	BM 4**	No EPP
4	2014	Nestling 2	BF 4**	BM 4[a]	BM 4**	No EPP
4	2014	Nestling 3	BF 4**	BM 4[a]	BM 4**	No EPP
12	2013	Helper male	unassigned	undetermined	BM 12*	No EPP
12	2013	Nestling 1	BF 12**	BM 12	BM 12**	No EPP
12	2013	Nestling 2	BF 12**	BM 12	BM 12**	No EPP
21	2013	Nestling 1	BF 21**	undetermined	unassigned	No EPP[b]
21	2013	Nestling 2	BF 21**	undetermined	unassigned	No EPP[b]
21	2013	Nestling 3	BF 21**	undetermined	unassigned	No EPP[b]
31	2013	Nestling 1	BF 31**	BM 31	BM 31**	No EPP
31	2013	Nestling 2	BF 31**	BM 31	BM 31**	No EPP
34	2014	Nestling 1	BF 34**	BM 34[a]	BM 34**	No EPP
34	2014	Nestling 2	BF 34**	BM 34[a]	BM 34**	No EPP

Paternity was designated as either no extra-pair paternity (EPP) or unassigned, based on the number of loci excluding paternity of the behavioural father and a maximum likelihood analysis of paternity. The likelihood analysis was based on a simulation with the following conservative parameters: proportion of typed of loci 88 %, number of candidate mothers = 30, proportion of females sampled = 80 %; number of candidate fathers = 50, proportion of males sampled = 74 %, error rate of 1 % to allow for mistyping; assignment at a 80 % or 95 % confidence level.

a Behavioural father designation based on trapping data

b Suspected parentage, i.e. one parent unassigned

c Due to financial limitations these samples were not included in ddRAD-seq and were therefore not included in EPP analysis

* Parentage assigned with loose confidence level 80 % likelihood

** Parentage assigned with strict confidence level: 95 % likelihood

Chapter 6 General Discussion

My thesis addresses the need for a detailed understanding of the ecology and behavioural domains of species as a prerequisite for their successful conservation, especially when species are threatened by anthropogenic changes. The findings of this thesis contribute specifically to our understanding of the habitat requirements (Chapter 2), nest predation threats (Chapter 3), the breeding biology (Chapter 4), and the genetic mating system (Chapter 5) of the previously unstudied vulnerable thick-billed grasswren (*Amytornis modestus raglessi*), and more generally to the role of habitat protection and vegetation recovery in avian conservation management. Below I highlight the most important findings of my research and offer suggestions for future research and conservation effort.

Impact of grazing on thick-billed grasswrens

Habitat loss and fragmentation due to anthropogenic factors such as grazing by livestock, as well as degradation of native habitat, are thought to be responsible for the decline of many Australian bird species, including the thick-billed grasswren *Amytornis modestus* (Garnett *et al.*, 2011, Skroblin and Murphy, 2013). A significant finding of this thesis is that habitat degradation through livestock grazing affects a population of thick-billed grasswrens (*Amytornis modestus*, subspecies *A. m. raglessi*) at our study site. In Witchelina Nature Reserve thick-billed grasswrens were absent from areas that were previously heavily grazed by domestic livestock, whereas grasswrens were present in areas with little to moderate historical grazing impact (Chapter 2), where they had a large home range (~8 ha). Larger home range sizes are often attributed to lower habitat quality; because this study occurred on previously grazed habitat, which may have lower primary productivity and food resources relative to other sites, the finding of large territory size may be explained by habitat still recovering from grazing impacts.

Those areas that were heavily grazed in the past had fewer native chenopod shrubs and little vegetation cover, which are both key habitat characteristics for grasswren nesting success (Chapter 3) and parental care (Chapter 4). Even though all livestock was removed from Witchelina Nature Reserve in 2010 and since then vegetation at the study site has had the chance to recover from intensive grazing pressures, the long-term effects of intensive grazing pressures still influence the distribution of the thick-billed grasswrens in Witchelina Nature Reserve today.

Impact of predation at grasswren nests

Nest predation is the principal cause of avian nesting failure, especially in passerines (Martin, 1992, Ricklefs, 1969), and much research has focussed on what ecological factors impact on nest predation rates (Angelstam, 1986, Vickery *et al.*, 2001). In order to manage and conserve vulnerable bird populations, such as the thick-billed grasswren, it is essential to identify natural and anthropogenic drivers of nest predation. Depredated artificial nests provided evidence for avian and rodent nest predators at our study site, and also identified yearly variation in predator activity at Witchelina Nature Reserve across years. This thesis is the first to examine breeding behaviour and nesting success of thick-billed grasswren nests. Surprisingly, thick-billed grasswren nests had a low predation rate; at Witchelina Nature Reserve 11.5 % of 47 monitored nests were depredated, suggesting that nest predation may not be the main factor contributing to the decline of vulnerable thick-billed grasswrens. It must be noted that, in this thesis, egg and nestling mortality due to nest predation was probably underestimated. Due to the shy nature of grasswrens, plus the fact that nests are generally well-hidden and placed in low dense chenopod shrubs with high concealment, it is likely that some nest predation events would have occurred at unmonitored nests prior to their discovery.

Studies have shown that many songbird species suffer from high (nest) predation rates caused by exotic predators such as feral Domestic cats (*Felis catus*) (e.g. Read and Bowen, 2001). Ground-nesting birds are particularly vulnerable to predation by cats (Sanders and Maloney, 2002). Since most grasswren species are ground-dwelling and generally build their nest close to the ground, they are potentially susceptible to predation by feral cats, which have been suggested to be a principal threat to many grasswren species (Garnett *et al.*, 2011). Nest predation events in this study were not observed directly, nor were they captured on video cameras, thus the identity of thick-billed grasswren nest predators remains unknown. However, the lack of nest disturbance observed at depredated thick-billed grasswren nests is suggestive of snake predation, since snakes are common songbird nest predators and they are known to leave nests undisturbed (Thompson III and Burhans, 2003).

Amytornis compared to other Maluridae

Unlike well-studied fairy-wrens (*Malurus*), the life histories of grasswrens (*Amytornis*) have not been studied in much detail. One of the aims of my thesis was to collect baseline natural history data for the previously unstudied vulnerable thick-billed grasswren (*Amytornis modestus raglessi*). Like fairy-wrens, thick-billed grasswrens at Witchelina Nature Reserve seem to be sedentary and occupy territories year round (unpublished data). Thick-billed grasswrens territories contain chenopod shrubs, which they use as a main nesting substrate, and they have a large home range (~8ha) (Chapter 2) compared to the home range size of other Malurids (Rowley and Russell, 1997). Studies on fairy-wrens provided insights into some of their life history strategies, such as cooperative breeding behaviour; in many fairy-wren species additional group members (called 'helpers') often assist a breeding pair in parental care duties. Grasswrens have been observed in groups of up to 7 (Gould, 1865, Rowley and Russell, 1997, Wood, 2014b, pers. comm. A. Black), but the role and status of group members remained unknown. Video analysis of parental care at nests revealed that, like fairy-wrens, thick-billed grasswrens at Witchelina Nature Reserve are cooperative breeders, with up to two helpers assisting in parental care duties (Chapter 5). In contrast to fairy-wrens, which generally experience high rates of nest predation (e.g. Colombelli-Négrel and Kleindorfer, 2009), thick-billed grasswren nesting success was high (Chapter 3).

Mating system of thick-billed grasswrens

Levels of extra-pair paternity (EPP) are high in some *Malurus* (fairy-wrens). The current study is the first to genetically examine the mating system within the genus *Amytornis*, which is the second genus of Maluridae outside *Malurus* for which parentage analyses have now been completed. The other examined genus is *Stipiturus* (emu wrens); a study on the genetic mating system of the southern emu-wren (*Stipiturus malachurus*) showed that EPP levels were low in this species (Maguire and Mulder, 2008). Acknowledging our small sample size for EPP analysis, the findings of this thesis suggest that if EPP occurs in thick-billed grasswrens, the frequency is very low, or perhaps even absent (Chapter 5). Several behavioural factors appear to contribute to the low frequency of EPP occurring in the thick-billed grasswren; males do not have obvious male signalling traits (no brightly coloured plumage, no enlarged cloacal protuberance, no elaborate song or courtship display).

The conclusions of my thesis are constrained by a few limitations, which are summarized here. Firstly, this study was conducted at a single site at Witchelina where thick-billed grasswrens have persisted, with findings limited to one site over three years. This is a brief window through which to study the biology of a species likely to be long-lived; and in a region where unpredictable and irregular rainfall events may mean that there are no typical years or typical ecological responses. Secondly, Witchelina Nature Reserve has been a protected area since 2010, when all feral grazers were removed and an active feral predator control program was put in place, and it is likely that these management strategies could have promoted vegetation growth and caused a reduction in the amount of feral predators (cats and foxes). The observed low nest predation rates at thick-billed grasswren nests at our study site (Chapter 3) might therefore be a result of improved nesting habitat and a reduction in the number of predators as a result of these management strategies. The findings of the present study should therefore be understood as reflecting the local conditions and not applicable to the species range. Thirdly, the shy and secretive nature of *Amytornis* in general, and thick-billed grasswrens in particular, makes behavioural studies on this species difficult. In order to record habitat use and home range behaviour we used radio-telemetry (Chapter 2), and to enable observations of parental care behaviour at nests we had to revert to a custom built video-monitoring system (Chapter 4). These constraints resulted in small sample sizes that limit confidence of certain analyses (Chapter 2, 4). Although we collected genetic samples from 80 offspring, financial constraints limited the amount of individuals that we could include for the EPP analysis (Chapter 5). Despite these constraints, the data I collected can significantly contribute to the conservation of thick-billed grasswrens.

Direction of future conservation work

On-going long-term population monitoring is crucial to determine the viability of populations, and is an intrinsic part of species conservation management, especially for highly specialized species that have small and range-restricted populations. We did not monitor thick-billed grasswren dispersal or population recruitment in this study, but few successfully fledged offspring remained in their natal territory and were observed in the following breeding season (n = 3, unpublished data), suggesting that dispersal may be limited in this species. Dispersal, recruitment and gene flow of populations increase their genetic diversity, which ultimately improves the long-term survival and persistence of species. Factors affecting population recruitment, dispersal and gene flow must therefore be identified and considered in the future conservation management of the vulnerable thick-billed grasswren.

Modern Australia has undergone extensive changes in landscape since European settlement in the late 1800's (Ford *et al.*, 2001), and predictions state that up to fifty per cent of Australia's terrestrial birds will be lost unless land management practices are changed (Kirkwood and O'Connor, 2010, Recher, 1999, Szabo *et al.*, 2012). The chapters of this thesis provide a crucial assessment of the proposed threats to the previously unstudied thick-billed grasswren (*Amytornis modestus raglessi*), an arid songbird of conservation concern. Most of the arid rangelands of inland and South Australia are used for pastoralism, and are grazed extensively by domestic cattle and sheep, and feral goats. These current agricultural practices are responsible for much of the loss or degradation of arid chenopod shrublands, which are critical habitat for thick-billed grasswrens as well as many other native arid fauna. The findings of this thesis emphasize the long-term detrimental effects of intensive livestock grazing on native flora and fauna, and highlight the need for a change in land-management practices, as well as the importance of conservation reserves such as Witchelina Nature Reserve. Witchelina Nature Reserve is one of only a few places where land has been purchased for habitat protection and vegetation recovery purposes. The joint involvement and collaboration of managers and researchers at Witchelina Nature Reserve is a successful conservation tool.

Future research should focus on the control of feral predators, but particularly on reducing the negative impacts of livestock grazing on long-lived slow growing chenopod shrublands that are present in most rangelands of inland and South Australia. The conservation and restoration of this critical habitat is key for on-going conservation management of the thick-billed grasswren. I recommend that land managers reduce grazing pressure on chenopod shrubland habitat, with the aim to restore vegetation cover and abundance of preferred chenopod nesting shrubs *Maireana pyramidata* and *Maireana astrotricha* if they want to provide thick-billed grasswrens with suitable habitat within their management area. Altered grazing regimes and targeted broad-scale habitat restoration of the heavily grazed degraded arid rangelands of Australia will not only benefit populations of the vulnerable thick-billed grasswren, but will also have a positive effect on the many other Australian flora and fauna that occupy this habitat (Read and Cunningham, 2010).

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