Ecology and conservation of the regent honeyeater

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CANDIDATE'S DECLARATION

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university. To the best of the author's knowledge, it contains no material previously published or written by another person, except where due reference is made in the text.

A handwritten signature in blue ink on a blue rectangular background. The signature appears to read 'R. A. Crates' with a stylized flourish at the end.

Ross A Crates

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ABSTRACT

In the age of the Anthropocene, avian diversity loss is occurring at an unprecedented rate. Australia is not immune to the Global extinction crisis, given pervasive threats from habitat loss, climate change and introduced species. High variability in Australia's climatic conditions has led many birds to evolve mobile life-histories, presenting unique challenges for their conservation. The nomadic, critically endangered regent honeyeater *Anthochaera phrygia* has suffered a severe population decline since the mid-19th century. The contemporary population is estimated to consist of 350-500 individuals, distributed across 600,000 km² of woodland in south-east Australia. The species tracks nectar resources at large spatial scales. Small population size, vast range and irregular movement patterns of the regent honeyeater have hampered understanding of the drivers of ongoing population decline. Lack of ecological data has prevented efforts to implement targeted management actions to conserve the wild population. This thesis aims to obtain contemporary ecological data to inform efforts to prevent extinction of the regent honeyeater. In chapter 2, we develop a monitoring strategy to locate breeding regent honeyeaters using a survey protocol that accounts for their rarity and mobility. Although regent honeyeaters are rare, they are not cryptic. In chapter 3, we review the literature on Allee effects to evaluate, based on life-history traits, the susceptibility of Australia's critically endangered birds to inverse density dependent population growth. We use the regent honeyeater to show how a lack of empirical evidence of Allee effects need not preclude efforts to account for their existence through precautionary conservation. In chapter 4, we present the contemporary breeding biology of regent honeyeaters. We provide evidence that nest success and productivity have declined over recent decades, nest success is highly spatially variable, predation is the main cause of nest failure and there is a male bias to the adult sex ratio. In chapter 5, we experimentally removed noisy miners, a major competitor and known cause of nesting failure, from a regent honeyeater breeding site. We monitored recolonisation of noisy miners following their removal, the co-occurrence of noisy miners and regent honeyeaters during nesting, and the response of the songbird community to miner removal. We significantly decreased noisy miner abundance at a time and location to benefit breeding regent honeyeaters. Abundance and species richness of the songbird community also increased. In chapter 6, we evaluate the genomic impact of severe population decline in regent honeyeaters. We find very weak population structure in the population prior to its rapid decline, that the population comprises a single conservation unit, and that some genetic diversity loss has occurred over the past 3 decades. In combination, effort and effective sampling can generate crucial population data to inform better conservation of rare and highly mobile species that may otherwise be dismissed as too challenging to study in detail.

‘Although it is very generally distributed, it’s presence appears to be dependent upon the state of the Eucalypti, upon whose blossoms the bird mainly depends for subsistence; and it is, consequently, only to be found in any particular locality during the season when those trees are in full bloom. It generally resorts to the loftiest and most fully-flowered trees, where it frequently reigns supreme, buffeting and driving every other bird away from its immediate neighbourhood; it is in fact, the most pugnacious bird I ever saw, evincing particular hostility to the smaller Meliphagidae, and even to others of its own species that may venture to approach the trees upon which two or three have taken station. While in Adelaide, in South Australia, I observed two pairs that had possessed themselves of one of the high trees that had been left standing in the middle of the city, which tree, during the whole period of my stay, they kept sole possession of, sallying forth and beating off every bird that came near. I met with it in great abundance among the brushes of New South Wales, and also found it breeding in the low apple-tree flats of the Upper Hunter. I have occasionally seen flocks of fifty to a hundred in number passing from tree to tree, as if engaged in a partial migration from one part of the country to another, probably in search of a more abundant supply of food.’

John Gould

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DECLARATION OF AUTHOR CONTRIBUTIONS

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Author contribution: RC and MW designed the study, RC collected and analysed data, and wrote the manuscript. AT and MW assisted with data analysis. All authors contributed to the manuscript.

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Author contribution: RC conceived the study, collected the samples, analysed the data and wrote the manuscript. GO conducted lab work, bioninformatics, analysed the data and wrote the manuscript. TS devised the lab protocol and assisted with data analysis. MA assisted with bioninformatics, NA assisted with lab work, LRa and SB contributed to data analysis. DI assisted with sample collection. All authors contributed to the manuscript.

CHAPTER 1: INTRODUCTION

In the age of the Anthropocene, biodiversity loss is occurring at an unprecedented rate (Dirzo et al. 2014; Ceballos et al. 2015). Habitat loss, disease, climate change and invasive species are primary drivers of a global extinction crisis (Sala et al. 2000; Millennium ecosystem assessment 2005), underwritten by interacting effects of human population growth and industrialisation (Wittemyer et al. 2008). There is mounting evidence that continued species loss will negatively impact ecosystem functioning and processes, such as pollination and nutrient cycling, upon which sustainable human life depends (Hooper et al. 2012). From philosophical and anthropocentric perspectives, society therefore has a duty to minimise the biodiversity impacts of its actions (Sala et al. 2000).

Highly mobile species are amongst the most susceptible to population decline, particularly as a consequence of habitat loss (Runge et al. 2014). Whether they be migratory, irruptive, nomadic or semi-nomadic, mobile species depend on a spatially discrete network of habitats that they occupy at different times across seasons and years (Runge et al. 2014). If critical habitat components, such as migratory bottlenecks, staging posts or breeding sites are lost or degraded, population growth rates of mobile species can be disproportionately impacted (Runge et al. 2014).

Whilst habitat loss is the declining population paradigm for most mobile species (Caughley 1994), it can trigger additional, interacting effects that magnify negative population growth (Berec et al. 2007; Sala et al. 2000). For example, mobile species may be less competitive than resident, generalist, invasive or larger-bodied species that gain competitive priority to limiting resources (Connell 1983; Mac Nally et al. 2012). Inverse density-dependent effects of small population size, known as Allee effects, can also drive population decline (Stephens and Sutherland, 1999). Survival or reproduction may decrease in small or sparse populations through less efficient movement (Simons 2004), increased predation (Gascoigne & Lipcius, 2004), foraging inefficiency (Grünbaum & Veit 2003), or suboptimal habitat selection (Schmidt, Johansson, and Betts 2015). In small or sparse populations, individuals may struggle to locate mates (Gascoigne et al. 2009; Gilroy & Lockwood 2012), particularly if population decline leads to sex ratio bias (Donald 2007). Genetic impacts of small effective population size can compromise population persistence and future recovery (Frankham 2005). For species with life-history traits that may predispose their populations to decline and associated feedbacks, preventing extinction may be extremely challenging once the population size or growth rate crosses below a critical threshold (Berec et al. 2007).

To prevent extinction of the most vulnerable species, a thorough knowledge of their ecology is critical (Cottee-Jones et al. 2015). Robust ecological knowledge of habitat requirements, movement patterns, dynamic distributions, population parameters and novel threats can inform cost-effective conservation actions that are targeted in space and time (Heinsohn et al. 2015; Stojanovic et al. 2014; Webb et al. 2014). This information is disproportionately lacking for mobile species, meaning they are currently under-conserved globally (Cottee-Jones et al. 2015).

Developing effective sampling regimes is a prerequisite for obtaining ecological knowledge to conserve rare and mobile species (Mackenzie et al. 2005). Whilst advanced modelling approaches can inform community-level conservation at macro-ecological scales (Martin et al. 2007; Runge et al. 2016), their capacity to inform urgent, targeted conservation action for rare species, whose threats may be complex or species-specific (Stojanovic et al. 2014, 2018), is limited. Similarly, traditional field survey techniques for sampling whole communities invariably fail to deliver sufficient data for the most threatened species (Rayner et al. 2014). Novel tracking techniques are revolutionising population monitoring, but are expensive (Hewson et al. 2016; Jönsson et al. 2016). Small sample sizes limit capacity to infer population-level processes of small (< 50 g), rare and mobile species from tracking data. Consequently, monitoring rare and mobile species requires a targeted, extensive field sampling regime that accounts for their irregular settlement patterns, low occupancy rates and specific habitat requirements (Webb et al. 2014, 2017).

Collecting standardised, spatially-extensive presence / absence data and associated habitat covariates is critical for robust analysis of population trends, explaining dynamic distributions and predicting where mobile species may occur in future (MacKenzie et al. 2005; Webb et al. 2017). Yet, locating rare and mobile species is just one component of a robust monitoring program. Follow-up searches to monitor breeding activity can provide key reproductive data, including sex ratio estimates, breeding participation, breeding success, productivity and the causes of breeding failure (Sutherland et al. 2002; Schmidt et al. 2008; Stojanovic et al. 2014). Over time, monitoring can also identify spatio-temporal variation in these key breeding parameters (Paradis et al. 2000). Together, this information can enable modelling of population trajectories (Heinsohn et al. 2015), identify limits to population recovery (Wedekind 2002) and inform how, where and when intervention measures could have the greatest conservation benefit (McDonald-Madden et al. 2010).

Although a critical means of maximising the cost-benefit of targeted conservation actions, field-based monitoring regimes for rare and mobile species are inherently expensive (McDonald-

Madden et al. 2010). At a time when threatened species conservation is severely under-funded, conservation programs that explicitly target the most vulnerable species have drawn criticism as inefficient use of limited resources (Bottrill et al. 2008, 2009; McDonald-Madden et al. 2010). To others, perceptions that certain species are too expensive or challenging to conserve sends a dangerous political message that extinction is acceptable and unpreventable (Woinarski et al. 2017). In reality, species conservation is indeed a political process (Woinarski et al. 2017). In combination with public donations, existing legal and policy frameworks such as biodiversity offsetting mean that the most vulnerable and high profile ‘flagship’ species attract disproportionate conservation funds (Maron et al. 2010). In many nations, biodiversity offsets currently represent a significant funding source for threatened species conservation (Miller et al. 2015). Species-specific offset funds come with conditions stipulating how they can be spent (Miller et al. 2015), but there is debate as to whether biodiversity offsetting can deliver conservation benefits or, at worst, ‘no net loss’ (Maron et al. 2010). The conservation challenge is to determine how broader biodiversity benefits can be gleaned from species-specific conservation programs (Bennett et al. 2014). For instance, how can monitoring programs tailored to a single species be used to monitor the broader community, including other, co-occurring threatened species? Or, how can species-specific conservation actions most benefit the broader community?

Habitat restoration and suppression of despotic competitors are two complementary ways that targeted, species-specific conservation actions could lead to community-level conservation benefits (Didham et al. 2007; Norton & Warburton 2015). Because flagship species are invariably habitat specialists, they tend to occupy high quality habitat patches, where they co-occur with a suite of other threatened taxa (Higa et al. 2016). Through spatially-extensive monitoring programs, it is possible to identify critical locations, where habitat restoration and competitor suppression can be implemented to most effectively benefit the most vulnerable species. Implementing competitor suppression in a precautionary, rather than a reactionary manner (i.e. before despotic competitors are abundant and widespread, but are otherwise likely to be so in future), increases the probability that competitor suppression will be successful, reduces financial and ethical costs, whilst also helping prevent the local extinction of threatened species (Leung et al. 2002; Davitt et al. 2018).

Population decline and local extinction of threatened species unavoidably leads to range contraction and / or fragmentation (Runge et al. 2015). Altered distribution patterns can have significant impacts on the genetic makeup of declining populations, depending on how range changes affect gene flow within the population (Frankham 2005). Population fragmentation can result in genetic fragmentation of populations, if dispersal between putative subpopulations

becomes restricted (Banks et al. 2013). Flow-on effects of inbreeding and genetic drift can then erode genetic diversity, with major implications for individual fitness, population persistence and capacity for population recovery (Frankham 2005). Knowledge of the genetic impact of population decline and range contraction is therefore crucial for informing effective conservation, for example through genetic management of captive populations (Kvistad et al. 2015) or genetic rescue of wild populations through translocations (Ralls et al. 2017). For rare and highly mobile species, however, the genetic impact of rapid population decline and range contraction is less clear-cut, given the potential for long-distance dispersal (Kvistad et al. 2015; Stojanovic et al. 2018). Thus, an understanding of the genetic impact of severe population decline in highly mobile species is also of great interest from a theoretical perspective (Latch et al. 2014). Advances in next-generation sequencing, particularly of museum samples, offers new capacity to increase the spatial, temporal and genetic resolution of population genetic analyses (Suchan et al. 2016; Schmid et al. 2018).

Thesis structure and rationale

The aim of this thesis is to improve understanding of the ecology and population biology of the critically endangered, nomadic regent honeyeater to enhance conservation of the wild population. This aim is achieved in a number of ways, based on a framework used to monitor and inform conservation of an ecologically similar species; the critically endangered swift parrot *Lathamus discolor*. First, we develop a monitoring program that accounts for the regent honeyeater's unusual life-history traits to locate breeding birds throughout their range. Second, we review the literature to determine how life-history traits of the regent honeyeater may explain the species' disproportionate population decline, and how these life-history traits could inform enhanced conservation action. Third, we intensively monitor regent honeyeaters over 3 years to uncover their contemporary breeding biology; we identify the causes of nesting failure and obtain robust estimates of breeding parameters, to determine whether breeding limitations may inhibit population recovery. We then experimentally implement targeted conservation action in the form of competitor suppression to enhance breeding success at a critical breeding site identified through the monitoring programme. Finally, we implement a comprehensive population genomic analysis to inform current and future genetic management of both the wild and captive populations.

Chapters 2-6 are written as self-contained scientific papers. Chapters 2, 3, and 4 are published in The Journal of Wildlife Management, EMU, and IBIS, respectively. Chapter 5 is in revision at Biological Conservation and chapter 6 is in preparation for submission.

Study species

The family *Meliphagidae*, or honeyeaters, consists of 157 species distributed primarily throughout Australia, New Guinea and the Pacific islands (Driskell and Christidis 2004). As the name suggests, honeyeaters are predominantly nectar feeders, but the remarkable range of niches they have evolved to fill makes them a model family for the study of adaptive radiation (Driskell & Christidis 2004; Normal et al. 2007). The regent honeyeater, a medium-sized (35 - 46 g) member of the genus *Anthochaera* ('wattlebirds', Driskell & Christidis 2004), was abundant and widespread prior to the mid-20th century, with an historic range extending in a broad swathe from the Adelaide Hills in the South-West to coastal southern Queensland in the north (Franklin et al. 1989). Regent honeyeaters inhabit a range of habitat types, including swamp mahogany / spotted gum-ironbark forest (Roderick et al. 2014), coastal heath and riparian gallery forest (Franklin et al. 1989; Oliver 2000). However, their preferred food tree species are those *Eucalypts* of the temperate box-gum-ironbark woodlands (Franklin et al. 1989). Of particular importance appears to be yellow box *Eucalyptus melliodora* and mugga ironbark *Eucalyptus sideroxylon* (Geering & French 1998; Oliver 1998), with which the regent honeyeater's historical range overlaps extensively (Franklin et al. 1989). Insects, lerp and manna also form a component of the regent honeyeater's diet (Franklin et al. 1989; Oliver, 1998).

Observations and colour marking studies support a view that regent honeyeaters have evolved a highly mobile (nomadic or semi-nomadic) life-history, facilitating the tracking of spatio-temporal variation in nectar resources at large scales (Franklin et al. 1989; Commonwealth of Australia 2016). Nesting appears to coincide with flowering events (Franklin et al. 1989; Geering & French 1998). Nesting traditionally occurs in loose aggregations, but regent honeyeaters do not breed cooperatively. (Franklin et al. 1989; Oliver, 1998; Geering & French, 1998). Females incubate a clutch of 2 -3 eggs for 12 days in an open cup nest comprised of bark, grass and spider web, typically located in the outer fork or limb of large trees (Geering & French 1998; Oliver 1998). During nesting, the male aggressively defends the nest from all species in proximity (Ford et al. 1993). Both sexes provision young, which fledge approximately 16-18 days after hatching (Geering & French 1998; Crates et al. In press). Juveniles become independent of their parents approximately 3 weeks post-fledging (Geering et al. 1998). During the non-breeding season, regent honeyeaters form flocks, historically containing > 100 individuals (Franklin et al. 1989). Regent honeyeaters commence breeding activity at one year of age, maximum known lifespan is 11 years 3 months and mean lifespan is an estimated 5-6 years (Commonwealth of Australia 2016).

The regent honeyeater's preferred food tree species, particularly those with which nesting is associated, tend to grow on the fertile soils of alluvial river flats (Ford 2011). Consequently, these tree species have been disproportionately cleared following European settlement, primarily for agriculture (Ford 2011). Land clearing has led to the loss of >90 % of the regent honeyeater's historical breeding habitat (Commonwealth of Australia 2016), with remaining breeding habitat being highly fragmented (Ford et al. 2001). It is without doubt that severe habitat loss is the principle driver of regent honeyeater population decline; the declining population paradigm (Caughley 1994, Ford et al. 2001; Commonwealth of Australia 2016). First detected in the 1970s (Peters 1979), the regent honeyeater population declined to approximately 1500 – 2000 individuals by the end of the 20th century, and to 350 – 500 individuals by 2015 (Menkhorst et al. 1998; Kvistad et al 2015). Given the challenges associated with monitoring the wild population, however, uncertainty surrounding the accuracy of population estimates is high (Clarke et al. 2003). Nevertheless, records of regent honeyeaters have diminished in space and time. Since 2010, regent honeyeaters have largely disappeared from Victoria, southern NSW and the Australian Capital Territory (Commonwealth of Australia 2016). Contemporary breeding records are exceedingly rare in traditional breeding sites in the Pilliga / Warrumbungles district and in southern Queensland (BirdLife Australia, unpubl.). It appears that the remaining wild population is largely restricted to breeding sites in two regions; the Bundarra-Barraba-Severn River district of the NSW northern Tablelands and the greater Blue Mountains, encompassing the Caperee, Wolgan, Lower Hunter and Burratorang Valleys, as well as the Goulburn River and Mudgee-Wollar regions of the Upper Hunter catchment (Crates et al., in press; Commonwealth of Australia 2016). The regent honeyeater was listed as critically endangered under federal legislation in 2015 (Department of Environment 2015). Current recovery actions focus on the release of captive-bred birds and small-scale protection and restoration of breeding habitat, but there is little evidence that these actions are contributing to population recovery (Commonwealth of Australia 2016). Based on expert elicitation, there is a 57% probability that the regent honeyeater will be extinct within two decades (Geyle et al. 2018). The regent honeyeater is therefore an umbrella species for a suite of threatened woodland birds (Ford et al. 2001; Kalinkat et al. 2017).

Despite severe habitat loss, however, large areas of potential breeding habitat remain (Commonwealth of Australia 2016, Rayner in prep). Although many co-occurring honeyeater species have declined, none have declined to the extent of the regent honeyeater (Ford 2011; Ford et al. 2001). Together, this suggests that additional factors are interacting with habitat loss and life-history traits specific to the regent honeyeater to drive the species' rapid and seemingly ongoing

decline (Ford et al. 2001; Crates et al. 2017). These additional factors include competition with larger nectarivores for remaining nectar resources, increased abundance and distribution of the hyper-aggressive noisy miner, habitat degradation and drought (Ford et al. 1993; Ford et al. 2011; Commonwealth of Australia 2016). The first aim of this thesis to identify ecological and life-history traits of the regent honeyeater that make the species particularly susceptible to population decline as an initial consequence of habitat loss. The second aim is to use this information in combination with contemporary monitoring data to develop novel and targeted conservation measures to try to prevent imminent extinction of the regent honeyeater.

CONTEXT STATEMENT

Chapter 1: The introduction discuss the challenges associated with the conservation of rare and highly mobile species. It outlines current knowledge of the ecology of the regent honeyeater and identifies critical knowledge gaps that hinder effective conservation.

Chapter 2: An ongoing population decline and range contraction have severely limited the capacity of traditional census techniques (i.e. 2 hectare, 20-minute transects and public sightings) to provide robust population data for the regent honeyeater (Clarke et al. 2003). In turn, data paucity severely limits understanding of regent honeyeater's fine-scale breeding habitat requirements, the current drivers of population decline and, subsequently, the effectiveness of conservation efforts. In chapter 2, we trial a novel occupancy monitoring design to locate breeding regent honeyeaters in the species' core breeding range. The sampling regime was spatially extensive, identifying habitat covariates that influence the probability of regent honeyeater site occupancy and detectability. We quantify the detectability of regent honeyeaters given the survey design. The capacity to confidently distinguish absence from non-detection is critical when sampling for rare species. The monitoring design forms the methodological basis for locating and monitoring regent honeyeaters throughout their range. Observations of regent honeyeaters detected via this sampling design stimulated the writing of chapter 3, contributed all breeding data presented in chapter 4, identified a critical breeding site to implement experimental competitor suppression in chapter 5 and allowed the collection of contemporary DNA samples analysed in chapter 6. Work is currently being undertaken by the regent honeyeater recovery team to adopt an occupancy approach to monitoring as the national standard for regent honeyeater population monitoring.

Chapter 3: This chapter was stimulated following extensive observations of regent honeyeaters during breeding in 2015. These observations rapidly and starkly highlighted how current conservation actions such as captive breeding and release could better implemented to facilitate population recovery. We explored the possibility that regent honeyeaters may be particularly susceptible to population decline via Allee effects (Stephens & Sutherland 1999). However, a lack of existing population data and challenges / time constraints associated with collecting the necessary population data means that empirical evidence for the existence of Allee effects is lacking and challenging to obtain. As an alternative, we conducted a literature review to identify component Allee effects (CAEs) in birds and life-history traits that may make species susceptible to each CAE. We then evaluate the relative susceptibility of Australia's critically endangered birds to Allee effects. Using the regent honeyeater as a case study, we show how conservation actions could better account for the potential presence of Allee effects. This paper was published as part of the Rowley review series in *EMU* and is the journal's most-read article. The findings will inform future release of captive-bred regent honeyeaters and targeted measures to increase breeding success.

Chapter 4: Despite the regent honeyeaters' imperilled population status, no standardised nest monitoring has been undertaken for over 20 years. The reasons for this are unconfirmed, but likely two-fold: First, previous studies suggested regent honeyeater breeding success was similar to other honeyeater species and was therefore not a driver of population decline. Second, perceived challenges associated with locating and monitoring nests given the species' small population size, vast range and irregular settlement may have discouraged efforts to monitor breeding activity in recent times. Thus, in chapter 4 we attempt to overcome these challenges to summarise the contemporary breeding biology of the wild regent honeyeater population. We obtain robust, range-wide estimates of population size, adult sex ratio and breeding participation. We quantify nest survival and identify factors contributing to variation in nest success. We also identify the causes of nest failure and quantify the short-term, post-fledging survival of juveniles. We show that it is entirely possible to locate and monitor contemporary breeding activity in a substantial proportion of the wild population. The results of this study will be used to inform targeted, spatially-explicit and urgent measures to increase regent honeyeater breeding success. The study also highlights the need to remain vigilant of temporal changes in critical breeding parameters in declining populations.

Chapter 5: In light of the results of chapters 3 and 4, we seized an opportunity to experimentally implement competitor suppression at a critical regent honeyeater breeding site, located using the survey design established in Chapter 1. We successfully reduced the abundance of hyperaggressive noisy miners, a source of nesting failure identified in chapter 4, for the duration of a 3-month breeding season. Six pairs of regent honeyeaters nested in the treatment area during this period. In addition, songbird abundance and species richness increased in the treatment site, relative to the control site. This study provides crucial evidence, in contrast to other recent studies (Davitt et al. 2018; Beggs et al. In press), that culling of noisy miners can be successful and of conservation benefit to regent honeyeaters and other threatened species if it is implemented in the right place and at the right time. The study will inform future noisy miner management to reduce their impact on regent honeyeaters at their mutual breeding sites.

Chapter 6: Population decline and associated fragmentation and contraction of species' ranges can have significant impacts on their population genetics. Through genetic drift and inbreeding, population decline can lead to the loss of population-level genetic diversity and the emergence of genetic differentiation between subpopulations (Frankham 2005). Accumulation of deleterious alleles and loss of adaptive potential can severely limit a species' capacity for population recovery (Frankham 2005). Thus, a comprehensive understanding of the population genetic impact of severe population decline can provide valuable information to aid genetic management of wild and captive populations (Harrison et al. 2014). The value of this genetic information can be enhanced by placing current genetic patterns in a temporal context (Diez-del-Molino et al. 2018). In chapter 6 we used recently-developed genomic techniques to obtain genomic data from a large sample of museum and contemporary samples (Suchan et al. 2016). Sample dates spanned beyond the period of the regent honeyeaters' severe population decline, providing a rare opportunity to evaluate the impact of population decline on levels of genetic diversity and genetic differentiation within the population over time. We found minimal genetic differentiation within the regent honeyeater population before and the species' decline. We also find evidence for the loss of genetic diversity since the 1980s. This suggests that potential for genetic management of the remaining population, for example through translocation (Ralls et al. 2017), is limited. The methods and results of this study should help increase the value of museum specimens in population genomics studies- a rapidly expanding area of research in conservation and evolutionary biology (Hung et al. 2014; Suchan et al. 2016; Stronen et al. 2018).

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CHAPTER 2: An occupancy approach to monitoring regent honeyeaters

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ABSTRACT

Conservation of rare and highly mobile species is frequently limited by a lack of monitoring data. Critically endangered regent honeyeaters (*Anthochaera phrygia*, population = 350–400) pose a substantial conservation challenge because of their high mobility and irregular settlement throughout their estimated 600,000 km² range. Given an ongoing population decline, enhanced monitoring efforts to inform population management are needed. We conducted an occupancy survey of regent honeyeaters and other nectarivores over 880 km² of the species' core range in New South Wales, Australia, during spring 2015. We located approximately 70 regent honeyeaters, potentially representing 20% of the population. Presence of regent honeyeaters was best predicted by high local nectar abundance. Detectability of regent honeyeaters when breeding (0.59) was similar to common, co-occurring nectarivores and was sufficient to distinguish absence from non-detection. For rare and highly mobile species, monitoring approaches that prioritize sampling extent over site visit duration and explicitly accommodate their life-history attributes can provide valuable population data, with subsequent benefits for conservation.

KEY WORDS *Anthochaera phrygia*, Australia, bird, conservation, detectability, monitoring, nomadic, spatial simultaneous autoregressive lag model, specialist, species distribution model.

INTRODUCTION

Monitoring is fundamental to identifying trends in population size, evaluating the success of conservation actions, and informing future management (Lovett et al. 2007; Martin et al. 2007; Tulloch et al. 2013). Despite its importance, few threatened species are monitored in a scientifically robust way (Martin et al. 2007; Butchart et al. 2011). Life-history traits are key determinants of the feasibility of implementing robust monitoring strategies. For instance, species that are sedentary (Olson et al. 2005), highly detectable (Martin et al. 2010), philopatric (Both & Visser 2001), or have a small geographic range (Chambers et al. 2008) provide few logistical challenges to detailed study. In contrast, sampling rare, cryptic, or highly mobile species is problematic (MacKenzie et al. 2005; Wintle et al. 2005; Runge et al. 2014; Hayes and Monfils 2015). Knowledge of the ecological requirements of these species is frequently inadequate (Cottee-Jones et al. 2015), which limits accurate assessments of their population status and the effectiveness of subsequent conservation actions (Clarke et al. 2003; Cunningham & Lindenmyer 2005).

Information on species' abundance, demographics, and vital rates is ideally required to establish their conservation status (Bakker et al. 2009). However, for rare and highly mobile species, even estimating their population size, status, or distribution can be challenging, leading to a dependence on data that are not collected using a systematic sampling design (Runge et al. 2015). Because high mobility is frequently linked to habitat specialism (Stojanovic et al. 2015), modeling the distribution of mobile species can also be limited by a lack of data-rich spatial layers that can otherwise provide important information relating to a species' ecological requirements (Osborne et al. 2001). Data paucity means that conservation planning for rare and highly mobile species is usually informed by presence-only modeling techniques or expert opinion (Martin et al. 2012; Morais et al. 2013; Rayner et al. 2014). However, modeling approaches incorporating presence and absence data that also account for imperfect detection perform better than presence-only techniques (Royle et al. 2007; Webb et al. 2014). Such techniques can improve monitoring strategies, assisting conservation planning efforts and avoiding the misallocation of scarce resources (MacKenzie 2005; Martin et al. 2005).

Quantifying detectability (the probability of detection given presence) is of particular importance when monitoring rare and mobile species, for whom occupancy is likely to be low and highly variable over time (Thompson 2002; MacKenzie et al. 2006). In addition, specialist species that form breeding aggregations often show spatial autocorrelation in their occupancy data, where monitoring sites are not spatially independent in terms of their habitat attributes or probability of occupancy

(Bardos et al. 2015; Webb et al. 2014). Modeling approaches must therefore account for imperfect detection and spatial dependence to make robust inferences about a species' ecology from occupancy data (Hui et al. 2006; Rota et al. 2016). Failure to account for either phenomena can bias occupancy estimates (Olson et al. 2005; Banks-Leite et al. 2014) and false absences (failing to detect a species when present), may inhibit capacity to identify changes in the size of small populations (Jones 2011; Ferguson et al. 2015) and compromise understanding of habitat selection (Gu & Swihart 2004), potentially limiting the effectiveness of management actions (Baxter & Possingham 2011; Gilroy et al. 2012).

We considered the case of a highly mobile bird species that poses a substantial challenge to monitoring, making the development of effective conservation actions very difficult (Clarke et al. 2003; Cottee-Jones et al. 2015). The critically endangered regent honeyeater (*Anthochaera phrygia*; International Union for Conservation of Nature 2016) undertakes semi-nomadic movements in response to flowering events in a small but historically widespread number of eucalyptus (*Eucalyptus* spp.) species throughout the species' large geographic range (Franklin et al. 1989; Garnett et al. 2011). The regent honeyeater's small population size ($N = 350\text{--}400$) and dynamic movements have severely constrained attempts to accurately determine spatio-temporal changes in population size and distribution (Clarke et al. 2003). The key predictors of regent honeyeater occurrence, the factors influencing population change, and the magnitude of the population decline are poorly understood (Clarke et al. 2003). Consequently, the effectiveness of targeted recovery actions (e.g., to increase nesting success) is unknown and current capacity to undertake adaptive management is limited. Developing a robust population monitoring program is therefore a management priority for the regent honeyeater (Commonwealth of Australia 2016).

Our objective was to develop an effective sampling protocol that allows cost-effective population monitoring at an appropriate sampling scale and intensity. Specifically, we assessed the suitability of a landscape-scale occupancy survey to identify the presence (or absence) of regent honeyeaters in their core range during the breeding season, developed a sampling protocol to maximize the detectability of regent honeyeaters while minimizing the time required for a single site visit, and identified the key environmental predictors of regent honeyeater occurrence. We predicted that nectar abundance would be an important factor determining regent honeyeater occupancy (Franklin et al. 1989; Geering & French 1998; Oliver et al. 1998).

STUDY AREA

We focused on a key region known to be frequently occupied by regent honeyeaters: the Capertee Valley subregion of the New South Wales south west slopes bioregion (Australian Department of the Environment and Energy 2016). The study area covered 880 km² of the southern Capertee River sub-catchment (bounded by -32.89° , 149.94° and -32.23° , 150.31°) from where regent honeyeaters are most frequently reported (Fig. 1).

Figure 1. Capertee Valley study area, New South Wales, Australia. Circles represent location of survey sites where regent honeyeaters were (black) and were not (grey) detected in spring 2015. White areas represent cleared or severely disturbed land, shaded areas are vegetated (though not necessarily suitable regent honeyeater habitat). Riparian areas are in dark grey. Inset: location of study area within the regent honeyeater's 600,000 km² range (dark grey).

METHODS

Survey Design

We generated 600 random survey points within the study area using ArcMap v10.2 (Environmental Systems Research Institute, Inc., Redlands, CA, USA). Survey sites contained ≥ 1 documented regent honeyeater food tree species (Table S1, available online in Supporting Information). We defined a site as a 50 m radius surrounding each point. We attempted to access as many survey points as possible, subsequently excluding points where land access was not possible. We moved sites up to 200 m from the original random point to locations that supported a higher abundance of food tree species. We ensured a minimum distance of 150 m between sites so that, when individuals were present and nesting, the chances of detecting the same individuals at adjacent sites were minimized. At sites where we detected regent honeyeaters, we used an adaptive sampling approach (Smith et al. 2004) to add further survey sites 150 m from occupied sites or in the closest area with food tree species beyond this distance. Using this approach, we surveyed 321 sites across the study area (Fig. 1).

We conducted repeated 5 minute point-counts to record detection or non-detection data for regent honeyeaters and other nectarivores. To minimize violation of the assumption of closure (a change in the occupancy status of each site during the survey period; MacKenzie et al. 2006), we surveyed each site 3 times (MacKenzie & Royle 2005) in a 1-month window starting on 26 September 2015. We considered a site to be occupied if a bird was present and using the site (i.e., not flying through it). The same observer (RC) conducted surveys at all times throughout the day but not in weather conditions that were likely to compromise detectability (e.g., rain, winds > 30 km/hr or temperatures $> 35^{\circ}\text{C}$). Because regent honeyeaters are responsive to song broadcast when breeding (Geering 1998), we broadcast regent honeyeater vocalizations (Pizzey & Knight 2014) using portable speakers for the first minute of each site visit. Based on known nest territory sizes (Geering & French 1998) and speaker volume, we assumed 50 m as a maximum distance that regent honeyeaters, given presence, would initiate a detectable response to playback. We recorded detections as one of a sighting, passive vocal detection, or response to playback. If we located a nest at a site during previous surveys, we specifically did not focus effort on the nest location and relied solely on the detection methods outlined above.

We divided site and visit-level covariates into those affecting occupancy and detectability, respectively (Table 1). To calculate an index of estimated nectar abundance at each site (nectar

score), we recorded a flowering index at every flowering tree within each site as 1–5, representing < 10%, 11–30%, 31–60%, 61–90%, and > 90% of crown area in flower, respectively. We scored the crown size of each flowering tree as 1, 3, or 9 to account for the relative crown volume of small (< 40 cm trunk diameter at breast height; DBH), medium (40–100 cm DBH), and large (> 100 cm DBH) trees, respectively. We accounted for variability in nectar concentration and volume among tree species (Oliver 2000; Law & Chidel 2008) by applying a correction factor to each tree species according to their nectar productivity (Oliver 2000; Table S1). We calculated site-level nectar scores for each visit as the sum of the nectar score (flowering index $\times$ crown size score $\times$ nectar productivity) for all flowering trees on site and used the mean of these values across the 3 visits as an overall site nectar score. Our nectar score was highly variable across sites and had a highly skewed distribution (range = 0–40.11, mean = 3.9, inter-quartile range = 3.06). To allow the inclusion of the nectar covariate as a factor in the models, we grouped the mean site values into scores from 0 to 4 (0, 0.01–1, 1.01–3, 3.01–10, > 10, respectively). We used this coarse index of nectar because of the need to account for variation in nectar productivity across eucalyptus species (and their relative importance to regent honeyeaters). We assumed our index of overall nectar productivity was a more accurate reflection of actual nectar abundance than if we ignored tree species and size (Oliver et al. 1999; Oliver 2000). We conducted research under an Australian National University animal ethics license (no. A2015/28), NSW scientific license (no. 101603), and Australian Bird and Bat Banding Service banding licenses (no. 2633, no. 3192).

Table 1. Description of covariates tested in single-season occupancy models of the regent honeyeater and other nectarivores in the Capertee Valley, New South Wales, Australia, spring 2015. We grouped covariates by site-level or visit-level and according to their input in the model (i.e., predicted to affect detectability or occupancy).

Level, input ^a	Covariate	Description	Justification
Site, detectability	Canopy	Estimated % canopy cover in survey area (50-m radius around survey point) to nearest 20%.	Detectability of nectarivores likely correlated with vegetation cover (Thompson 2002).
	Density	Vegetation density. Categorical value based on < 50%, 51–75%, or > 75% density.	Detectability of regent honeyeaters likely negatively correlated with vegetation density (Thompson 2002).
Site, occupancy	Location	X-Y point location. Triangulated as a spatial weights autocovariate.	Explains substantial proportion of variation in occupancy in aggregating species (Webb et al. 2014). Necessary to account for spatial autocorrelation.
	Water	Linear distance to the nearest water source, grouped by <50 m, 51–100 m, 101–300 m, 301–500 m, > 500 m.	Regent honeyeaters often associated with riparian zones (Geering & French 1998) and bird baths (BirdLife Australia, unpublished data).
	Mistletoe	No. clumps of live or dead mistletoe in survey area grouped into none (0), 1–5 plants (1), or > 5 plants (2).	Mistletoe abundance positively influences local bird diversity (Watson & Herring 2012). Often used by regent honeyeaters as a nesting substrate (Oliver et al. 1998).
	Competitor presence	Detection or non-detection of species larger than regent honeyeaters: noisy miner, noisy friarbird, red wattlebird, and musk lorikeet.	Presence of competitors likely to negatively affect occupancy through interspecific competition or aggressive displacement (Ford 1979; Mac Nally et al. 2012).
	Large flowering trees	No. large flowering trees of the key species (yellow box, mugga ironbark, Blakely's red gum [<i>E. blakelyi</i>]) > 50 < 150 m of survey site.	Large flowering trees likely positively related with regent honeyeater site occupancy (Oliver et al. 1999). Habitat occupancy likely determined by floral attributes at local scale beyond point count area.

	Nectar	Nectar score from 0–4 based on equation (1), averaged across 3 site visits.	Temporal stability of nectar may be important for occupancy related to breeding. Nectar abundance likely to govern occupancy by nectarivores (Mac Nally & McGoldrick 1996; Bennett et al. 2014).
Visit, detectability	Time	2-level factor, early morning-evening or mid-day.	Likely quadratic relationship with detectability, with peaks in morning and evening, and a decrease during mid-day (Field et al. 2002).
	Week	Week of survey season in which each survey was undertaken. Ordinal value from 1–4.	May affect (either positively or negatively) site occupancy or detectability due to change in breeding stage or status. Interval between repeat visits likely to affect closure assumption.
	Noise	Background noise (e.g., wind, other bird species) 3-level categorical, none, low, moderate	Background noise may affect aural detectability.

^a Some covariates may influence both occupancy and detectability. Because of the limited number of sites where we detected regent honeyeaters, we only tested an effect on either occupancy or detectability, based on our predictions.

Statistical Analysis

For each nectarivore species, we fitted single-season zero-inflated binomial (ZIB) occupancy models based on a robust survey design (Mackenzie et al. 2006) in the program PRESENCE v10.2 (MacKenzie et al. 2002; Hines 2006). We first fitted models with constant occupancy including only detectability covariates (Table 1) to compare the fit of a constant or a site-specific estimate of detectability for each species. Based on lowest quasi Akaike information criterion (QAIC_c) scores, with significant model improvement indicated by $\Delta\text{QAIC}_c > 2$, the most parsimonious models were chosen as the best models. Model assessment was supplemented by 500 bootstrap simulations, accounting for overdispersion by adjusting $\hat{c}$ if the model estimate of $\hat{c}$ was > 1 (MacKenzie et al. 2006). Once the best covariates for constant occupancy models were established using this process, covariates were added to the occupancy component of the models and the model selection process was repeated, again with the most parsimonious models selected based on ΔQAIC_c scores > 2 . Distance from water was highly correlated with large flowering trees ($r = 0.75$), so we did not include both of these covariates in the same model.

For the remaining analyses, we focused solely on the regent honeyeater, with a primary objective of clarifying factors influencing occupancy for this species. First we assessed the degree of spatial autocorrelation in regent honeyeater detection or non-detection data using correlograms (based on Moran's I statistic; Tiefelsdorf 2000) implemented in R (R Version 3.2.3, <https://cran.r-project.org>, accessed 27 Apr 2016), using the package *ncf* (Bjørnstad 2015). Because the correlograms indicated significant spatial autocorrelation in the data (see Results), we explored modeling approaches that complemented the PRESENCE analyses by explicitly accounting for spatial autocorrelation. We first tried fitting models that simultaneously accounted for spatial autocorrelation and imperfect detection using zero-inflated binomial models implemented using the EM Algorithm (Webb et al. 2014). However, the bivariate smooth spatial covariate in the occupancy component of these ZIBs caused overfitting (as indicated by an adjusted R^2 of 1 and 100% of deviance explained in the occupancy component of the model), and was clearly not an appropriate way to deal with the spatial autocorrelation present in these data.

We therefore tried alternative modeling approaches that account for spatial autocorrelation but which assume detection = 1. These approaches were generalized linear mixed models with site or region as random effects, generalized additive models with latitude and longitude as a smooth bivariate term, and spatial simultaneous autoregressive lag models (SARs), including a triangulated weights matrix as a spatial autocovariate. We considered these approaches appropriate and justified for 2 reasons. First, the importance of accounting for spatial autocorrelation in similar studies has been demonstrated (Koenig 1999; Webb et al. 2014). Second, detectability in the PRESENCE models was sufficiently high (and constant) to assume detectability = 1 (see Results and Fig. S1; Garrard et al. 2008). Of these alternative approaches, model diagnostics indicated SARs were the most appropriate choice. We implemented SARs using the *spdep* package (Bivand 2015) in R, with the weights matrix calculated using the *tri2nb* function in the *deldir* package (Turner 2016). We ranked SARs and chose the best model based on lowest AIC values.

RESULTS

We detected regent honeyeaters at 27 of the 321 sites (Fig. 1); 19% of detections were visual sightings, 50% were passive vocal detections, and 31% were direct responses to song broadcast. The median number of birds detected/visit at occupied sites was 2 (range = 1–7). Thus, reducing abundance data to presence-absence in analyses resulted in minimal loss of information.

Correlograms indicated regent honeyeater detection or non-detection data were positively spatially autocorrelated in the distance classes of 0–1 km and 2–3.5 km (Fig. 2). The variable ‘week’ was the only covariate that influenced detectability (Table 2), though including week offered only a marginal improvement on constant detectability ($\Delta\text{QAIC}_c = 1.43$). Once occupancy covariates were added (Table 1) to the occupancy component of the models, the effect of week on detectability was further reduced ($\Delta\text{QAIC}_c = 0.57$; Table 3). We therefore assumed constant detectability to be appropriate for all subsequent analyses. Under this assumption of constant detection, we estimated detectability of regent honeyeaters to be 0.59 ± 0.07 (SE) in the best model. This was in the mid-range of estimates for the wider nectarivore community and considerably higher than estimates for some other common nectarivores (Fig. 3). Given estimated detectability of 0.59 and that sites were visited on 3 occasions, the absence of regent honeyeaters from a site could be inferred with a high degree of confidence (Fig. S1; Garrard et al. 2008).

Figure 2. Spatial autocorrelation (Moran's I) of regent honeyeater detection or non-detection during a single-season occupancy survey in the Capertee Valley, New South Wales, Australia, spring 2015.

Black points represent significant spatial autocorrelation ($P < 0.05$) and grey dots represent non-significant spatial autocorrelation.

Figure 3. Estimated constant detectability ($\pm 95\%$ CI) of nectarivores surveyed in the Capertee Valley, New South Wales, Australia, spring 2015, from zero-inflated binomial models fit in PRESENCE. Species abbreviations (with sample sizes): ML, musk lorikeet (28); LL, little lorikeet (36); DW, dusky woodswallow (*Artamus cyanopterus*, 50); RW, red wattlebird (*Anthochaera carunculata*, 57); WN, white-naped honeyeater (*Melithreptus lunatus*, 46); R., regent honeyeater (27); NF, noisy friarbird (*Philemon corniculatus*, 180); YF, yellow-faced honeyeater (*Lichenostomus chrysops*, 166); F. fuscous honeyeater (*Lichenostomus fuscus*, 71); WP, white-plumed honeyeater (167); NM, noisy miner (126).

Table 2. Importance of individual covariates in determining regent honeyeater habitat occupancy in the Capertee Valley, New South Wales, Australia, spring 2015. Covariates grouped by category. Covariates are ranked by quasi Akaike information criteria (QAIC_c) within categories, but are comparable across categories.

Covariate category	Model*	QAIC _c	ΔQAIC _c	Overall QAIC _c rank
Nectar	Ψ(nectar)· <i>p</i> (constant)	96.34	-	1
	Ψ(large FT)· <i>p</i> (constant)	98.03	1.69	2
Habitat	Ψ(NF)· <i>p</i> (constant)	102.74	6.4	3
	Ψ(water)· <i>p</i> (constant)	107.32	10.98	4
	Ψ(RW)· <i>p</i> (constant)	111.99	15.65	6
	Ψ(ML)· <i>p</i> (constant)	113.31	16.97	8
	Ψ(mistletoe)· <i>p</i> (constant)	113.42	17.08	9
	Ψ(density)· <i>p</i> (constant)	113.42	17.08	10
	Ψ(canopy)· <i>p</i> (constant)	113.60	17.26	13
	Ψ(NM)· <i>p</i> (constant)	114.69	18.35	14
Detectability	Ψ(1)· <i>p</i> (week)	110.87	14.53	5
	Ψ(1)· <i>p</i> (constant)	112.30	15.96	7
	Ψ(1)· <i>p</i> (noise)	113.71	17.37	11
	Ψ(1)· <i>p</i> (time)	114.44	18.10	12
	Ψ(1)· <i>p</i> (survey-specific)	116.06	19.72	15

*large FT, large flowering trees; NF, noisy friarbird; RW, red wattlebird; ML, musk lorikeet; NM, noisy miner. See table 1 for definition of other covariates.

Occupancy models without a spatial autocovariate (ZIB implemented in PRESENCE) indicated that large flowering trees and mean nectar abundance were the strongest predictors of site occupancy by regent honeyeaters (Tables 2 and 3). The best SAR models (as indicated by ΔQAIC_c < 2) also included the covariates large flowering trees ($B = 0.057 \pm 0.014$ [SE], $Z = 4.224$, $P = < 0.001$) and nectar score ($B = 0.034 \pm 0.012$, $Z = 2.822$, $P = 0.005$), similarly indicating their importance as drivers of regent honeyeater occupancy. Site-level occupancy predictions from ZIB and SAR models were significantly correlated (Pearson 2-sided test $r = 0.57$, 95% CI = 0.46–0.66, $P < 0.001$). However, the SARs predicted many more sites with low occupancy probabilities (i.e., < 0.1), and more sites with higher probabilities of being occupied (i.e., 0.6–0.8; Fig. S2).

Table 3. Top (Δ quasi Akaike information criteria > 2) occupancy (Ψ) models (zero-inflated binomials) of regent honeyeater detection or non-detection data in the Capertee Valley, New South Wales, Australia, spring 2015. Models account for imperfect detection (p) but not spatial autocorrelation and are ranked by Akaike weight (w_i).

Model ^a	QAIC _c	Δ QAIC _c	w_i
$\Psi(\text{nectar}) \cdot p(\text{week})$	69.86		0.216
$\Psi(\text{nectar}) \cdot p(\text{constant})$	70.43	0.57	0.201
$\Psi(\text{nectar} + \text{large FT}) \cdot p(\text{week})$	70.50	0.64	0.127
$\Psi(\text{nectar} + \text{large FT}) \cdot p(\text{constant})$	70.78	0.92	0.098
$\Psi(\text{large FT}) \cdot p(\text{week})$	70.97	1.11	0.097

^a Large FT, large flowering trees. See Table 1 for definition of other covariates.

In addition to insights provided by the modeling analyses, our observations showed that habitat at monitoring sites occupied by regent honeyeaters were characterized by riparian corridors with adjacent flowering yellow box (*Eucalyptus melliodora*) and high abundance of flowering needle-leaf mistletoe (*Amyaema cambageii*), narrow strips of remnant valley floor vegetation (yellow box and mugga ironbark [*E. sideroxylon*]) on the lower slopes of hillsides, or small clusters of large yellow box paddock trees in highly degraded agricultural land (Fig. 1 and S3). Although regent honeyeaters occupied sites with high estimated nectar abundance, they were not detected at survey sites estimated to have the very highest abundance of nectar at the landscape scale (Fig. 4). Occupancy surveys led to the subsequent identification of 32 nesting attempts, all but one of which were located within 120 m of a survey site at which regent honeyeaters were detected (Fig. S3).

Figure 4: Frequency distribution of mean estimated nectar abundance of survey sites in the Capertee Valley, New South Wales, Australia, spring 2015, that were (grey bars) or were not (white bars) occupied by regent honeyeaters.

DISCUSSION

Collecting meaningful presence-absence data to estimate the geographic distribution and occupancy rates of regent honeyeaters during their breeding season is possible, despite the challenges posed by their mobile life history and small population size. Even though site occupancy was low, regent honeyeaters were sufficiently detectable when nesting to enable a rapid, regional-scale, intensive survey. By increasing detectability (e.g., use of song broadcast, small area of each individual monitoring site, surveyed early in nesting season) and minimizing the duration of site visits, future monitoring efforts can increase the spatial coverage and intensity of sampling and refine site location based on predictors of occurrence, without introducing bias caused by false absences.

Sampling intensively and extensively is critical for monitoring rare and mobile species because it improves capacity to detect spatiotemporal changes in occupancy patterns (Koenig et al. 1996) and population trends (Clarke et al. 2003). These survey attributes are particularly important when a substantial proportion of the population may aggregate in relatively small areas (Smith et al. 2004; Webb et al 2014). For instance, we located approximately 70 regent honeyeaters, potentially

representing 20% of the entire population (Garnett et al. 2011; Commonwealth of Australia 2016), through our monitoring efforts in a single season. A significant new breeding site (comprising a third of occupied sites) was also identified in a region that has previously been subject to long-term survey effort (Fig. S3). Furthermore, our sampling design provided a spatially explicit guide to the location of breeding activity; 94% of all wild regent honeyeater nests recorded in 2015 (BirdLife Australia, unpublished data) were subsequently found near occupied sites (Fig. S3).

Given the survey protocol, the detectability of regent honeyeaters was much higher than expected for such a rare species. Indeed, detectability was similar to or greater than that of some other common nectarivores such as the little (*Glossopsitta pusilla*) and musk lorikeet (*G. conchinna*). In contrast, resident and abundant species such as the noisy miner (*Manorina melanocephala*) and white-plumed honeyeater (*Lichenostomus penicillatus*) were highly detectable. We obtained a relatively high detectability estimate for regent honeyeaters by commencing surveys during the early stages of their breeding season. During this period, regent honeyeaters are largely sedentary, highly vocal, and aggressive while establishing and defending small breeding territories (Ford et al. 1993). Thus, difficulties associated with their mobile life history are largely negated during this period as nesting birds become central place foragers. Although detectability may decrease during incubation and the early nestling stage as birds become less vocal, the use of song broadcast serves as a means to maintain high detectability throughout the nesting period. Time of day had no effect on detectability, indicating surveys are viable throughout the day. This not only facilitates increased spatial survey coverage but also allow surveys to be conducted within as short a time window as possible, minimizing closure violations (Rota et al. 2009).

Although some violation of the closure assumption is unavoidable when monitoring highly mobile species (Hayes & Monfils 2015), we attempted to maximize closure with our survey design (i.e., short survey season coinciding with early nesting period, small sites located in core habitat). Regent honeyeaters that are unsuccessful breeders often disperse from nesting sites shortly after nest failure (Geering & French 1998). Because repeated site visits after an initial detection, but following nest failure and dispersal, are likely to decrease detectability estimates, the detectability of nesting regent honeyeaters (excluding transient non-breeders) may well be even higher than our estimate of 0.59. Timing surveys to coincide with the early stages of breeding is also critical because the cumulative probability of nesting failure increases with time (Dinsmore et al. 2002). Failing to locate regent honeyeaters during the early stages of their breeding period may therefore result in underestimated occupancy rates and overestimates of nesting success (Kidd et al. 2015).

The significant positive spatial autocorrelation we found in the distribution of regent honeyeater presence or absence data in the distance classes of 0–1 km and 2–3.5 km represents the distances between small aggregations of nesting regent honeyeaters detected across the study area (Fig. 1 and S3). Despite the low number of sites at which regent honeyeaters were detected, we were able to fit relatively simple occupancy models that accounted for spatial autocorrelation. Although occupancy predictions from these spatial models indicated similar predictors to the zero-inflated models (from *PRESENCE*), the frequency distribution of site occupancy probabilities differed between the approaches (Fig. S2). This difference highlights the importance of accounting for spatial autocorrelation in species that aggregate and is likely attributable to unmeasured variables or conspecific attraction (Webb et al 2014).

As predicted, regent honeyeater occupancy was largely determined by the abundant flowering of their food trees. However, we did not detect regent honeyeaters at the richest flowering sites in the landscape, possibly because of competitive exclusion by larger nectarivores (Ford 1979; Rota et al. 2016). Throughout the range of the regent honeyeater, negative associations between the noisy miner, a hyper-aggressive native honeyeater, and small-bodied birds have been documented (Piper & Catterall 2003; Mac Nally et al. 2012). However, too few data were available to confidently assess the effect of competitors on regent honeyeater site occupancy. Given their rarity, multiple seasons of monitoring data are likely required to help clarify the influence of aggressive competitors on regent honeyeater settlement decisions. While there was some evidence that distance to water may also influence regent honeyeater occupancy (Table 2), the best models did not include this variable, most likely attributable to the positive correlation with large flowering trees. Nevertheless, we rarely detected regent honeyeaters more than 150 m from a water source. Gaining a better understanding of the potential importance of distance to water for breeding regent honeyeaters may further increase the efficiency of future monitoring and enable better targeting of conservation actions.

MANAGEMENT IMPLICATIONS

When devising monitoring approaches for rare and highly mobile species, maximizing detectability during short site visits allows much greater spatial coverage without compromising data quality (e.g., false absences). Spatial autoregressive lag models offer a promising means of accounting for spatial autocorrelation when modeling the occurrence of rare species with sparse data, providing more realistic occurrence probabilities. Such an approach provides spatially comprehensive estimates of population distributions that can greatly enhance the efficiency of conservation planning and future population monitoring. Our findings reinforce that mobility and scarcity do not necessarily impede

the collection of highly valuable data for species that might otherwise be dismissed as too challenging to study in detail.

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CHAPTER 3: Undetected Allee effects in Australia's threatened birds: implications for conservation

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ABSTRACT

Allee effects occur when survival or reproductive success declines with decreasing population size or density. Species most severely impacted by Allee effects may be the very species for which these effects will be hardest to detect and overcome. This impedes effective conservation through a lack of evidence to drive management actions. We review the literature to identify (1) component Allee effects (components of fitness) which could lead to a demographic Allee effect (effect of all components on the population growth rate) in bird populations; and (2) traits that make species susceptible to component Allee effects. Concurrently, we assess the potential for undetected Allee effects to negatively influence the population growth rate of 14 critically endangered Australian bird species or subspecies. Whilst some (e.g. Helmeted Honeyeater) are unlikely to suffer from a demographic Allee effect, several (e.g. Great Knot, Orange-Bellied Parrot) are susceptible to a number of component Allee effects and, hence, a demographic Allee effect. However traits of the Regent honeyeater suggests this species' decline in particular is accelerated by an undetected demographic Allee effect. For this species and others, an inability to detect Allee effects need not preclude efforts to account for their potential presence through precautionary conservation management.

Key words: Allee effect; *Anthochaera phrygia*; Australia; endangered bird species; conservation; management; precautionary principle; regent honeyeater.

INTRODUCTION

Individuals of many species increase their fitness by associating with conspecifics (Allee 1931; Odum & Allee 1954; Krause & Ruxton 2002). The concept of dependence on group living was formalized as the Allee effect: a positive relationship between components of individual fitness and the number or density of conspecifics (Allee 1931; Stephens et al. 1999). In a conservation context, the Allee effect describes how population growth rates decrease with decreasing population size or density, accelerating the decline and extinction of small or sparse populations (Stephens et al. 1999; Stephens & Sutherland 1999). Component Allee effects (hereafter CAEs) influence components of individual fitness, but do not necessarily affect population growth rates. Rather, one or more CAEs can contribute to the existence of a demographic Allee effect (hereafter DAE): the overall effect of reduced population size or density on the population growth rate (Figure 1).

Figure 1: Simplified schematic of two component Allee effects (A and B) that give rise to a demographic Allee effect (C). Once population size or density decreases below the Allee threshold, population growth is negative and the population declines to extinction. Figure adapted from Berec et al. (2007). For a comprehensive summary of component and demographic Allee effects, see Figures 1 and 2 in Stephens et al. (1999) and Box 1 in Berec et al. (2007).

The detrimental impact of Allee effects on threatened species is widely acknowledged in theory (Courchamp et al. 2008), and as global biodiversity declines (Butchart et al. 2010), Allee effects are likely to increase in both frequency and magnitude (Courchamp et al. 2008; Gascoigne et al. 2009). However, Allee effects pose a challenge for conservation. Despite their potential role as a driver of extinction (Halliday 1980; Hung et al. 2014), empirical evidence of Allee effects in threatened species remains limited (Gregory et al. 2010; Kramer et al. 2009), and so too are the strategies for

redressing them (Gilroy et al. 2012). For example, despite ongoing declines in Australian avifauna (Cresswell & Murphy 2017), CAEs have been implicated in just three species (Cuthbert 2002; Grünbaum & Veit 2003; Gardner 2004) and in no Australian species has a DAE been demonstrated to negatively influence population growth rates.

Substantial conservation resources are invested in preserving small populations, but measuring the trajectory of fitness as a function of population size or density in such populations is extremely challenging (Gilroy et al. 2012). Traits that make a species difficult to monitor, such as high mobility and small population size, may also make them susceptible to a DAE (Courchamp et al. 2008). Time spent proving the existence of an Allee effect may see a declining population pass the ‘Allee threshold’, beyond which extinction may be unavoidable (Berec et al. 2007, Figure 1). Thus, Allee effects can hinder conservation success because their impact on population growth rates may be greatest in species for which they are least likely to be detected and most difficult to overcome.

The presence, strength and potential to overcome a DAE should therefore inform optimal management decisions (McDonald-Madden et al. 2010; Armstrong and Wittmer 2011). The trend-detection approach to management has been criticised for delaying management action (Nichols & Williams 2006), as undetected DAEs may already inhibit population recovery. Consequently, applying the precautionary principle and taking immediate management action when there is a strong case for the existence of a DAE (Cooney 2004; Courchamp et al. 2008) may prove most effective in achieving conservation goals (McDonald-Madden et al. 2010; Martin et al. 2015).

Here, we review the literature to identify: (1) potential CAEs that may impact small or sparse bird populations (Table 1); and (2) life-history, ecological and demographic traits (hereafter ‘traits’) that may increase a species’ susceptibility to each CAE (Table 1). To demonstrate this, we concurrently evaluate the potential for undetected CAEs, and hence a DAE, to exist in 14 of the 16 species or subspecies (hereafter ‘taxa’ where both are considered) currently listed federally as critically endangered in Australia (Commonwealth of Australia 2017, Table 2). We omit the Mount Lofty quail-thrush *Cinlosoma punctatum anachoreta*, which is presumed extinct (Garnett et al. 2011) and jointly evaluate herald and Round Island petrels *Pterodroma arminjoniana/heraldica* given the similarity of their traits in the context of Allee effects. The 14 case taxa, spanning 7 families and 12 genera, represent a non-arbitrary sample with which to evaluate the potential impact of undetected Allee effects on Australia’s threatened birds (Table 3). Many, though not all, have suffered a rapid decline (Garnett et al. 2011), but as for most threatened bird populations, a lack of detailed monitoring data for these taxa makes confirming the presence of a DAE extremely challenging

(Gilroy et al. 2012). We then discuss the feasibility of accounting for undetected Allee effects via precautionary conservation management of the data-deficient case taxa (Table 4).

REVIEW OF COMPONENT ALLEE EFFECTS

Habitat selection

Selection of quality breeding habitat is critical to breeding success (Fletcher 2006; Gunnarsson et al. 2005). Adaptations to select high quality habitat include philopatry (Part 1991; Gunnarsson et al. 2005), imprinting (Teuschl et al. 1998) and conspecific attraction (Fletcher 2006; Schmidt et al. 2015). However, conspecific attraction can result in a DAE because socially-acquired information on habitat quality can be unreliable at low population density (Schmidt et al. 2015), particularly in highly variable environments (Stodala & Ward 2017). Individuals use the presence of small numbers of conspecifics as an inaccurate cue that occupied habitat is of high quality (Schmidt et al. 2015). Cues that historically provided reliable information on habitat quality can also become unreliable following habitat modification (Kokko & Sutherland 2001). At low density, competitive exclusion from poor quality, modified habitat by conspecifics is reduced and a larger proportion of individuals settle there, setting an ‘ecological trap’ for the population (Kokko & Sutherland 2001). Despite theoretical support, Allee effects pertaining to habitat selection are challenging to detect empirically and the relative influence of social information in determining an individual’s assessment of habitat quality is poorly understood (Fletcher 2006; Schmidt et al. 2015).

Species that exhibit high conspecific attraction, have low philopatry in highly variable environments and small populations that are sparsely distributed across wide areas, should be at risk from a habitat selection CAE. Given their semi-nomadic movement patterns and tendency to aggregate when breeding (Ford et al. 1993; Webb et al. 2014), swift parrots and regent honeyeaters are the most susceptible of the case taxa to a habitat selection CAE. The very small breeding ranges of the King Island scrubtit, helmeted honeyeater and the herald / Round Island petrels makes these taxa least susceptible to a habitat selection CAE.

Table 1 (overleaf): Component Allee effects in birds and ecological, demographic or life-history traits that increase susceptibility to each at small population size or density.

Component Allee Effect	Rationale for susceptibility to component Allee effect	Susceptible life-history/ demographic/ecological traits	Examples
Habitat selection	Lack of exclusion from poor quality habitat leads to ecological trap. Small nesting aggregations provide unreliable social information on habitat quality, leading to suboptimal habitat selection	Low philopatry/high mobility High conspecific attraction High environmental variability	Models (Kokko and Sutherland 2001) Models (Schmidt <i>et al.</i> 2015) Least flycatcher (Fletcher 2009) Models (Stodala and Ward 2017)
Mate-finding	Reduced capacity to find mates at low densities High susceptibility to male-biased operational sex ratio	High or female-biased dispersal Large range Aggregative nesting Historically common Socially monogamous High female mortality	Models (Gilroy and Lockwood 2012) Willow warbler (Morrison <i>et al.</i> 2016) Theory (Gascoigne <i>et al.</i> 2009) Models (Berec <i>et al.</i> 2017) Models (Shaw <i>et al.</i> 2017) Glossy Black Cockatoo (Lee <i>et al.</i> 2015) Black-Eared Miner (Ewen <i>et al.</i> 2001) Swift Parrot (Stojanovic <i>et al.</i> 2014)
Mate choice/ facilitation	Lack of mate choice leads to less, or less successful, breeding activity Lack of nesting conspecifics reduces female impetus to nest.	All species Aggregative or colonial nesting	Models (Møller and Legendre 2001) Puerto Rican Parrot (Brock and White 1992) Passenger Pigeon (Halliday 1980)
Nest success	Lower nest survival High susceptibility to stochastic events e.g. storms or heatwaves	Aggregative or colonial nesting Open nesting Aggregative or colonial nesting	Fieldfare (Andersson and Wiklund 1978) Lesser Kestrel (Serrano <i>et al.</i> 2005) Passenger Pigeon (Halliday 1980) Little Tern (Medeiros <i>et al.</i> 2007) Theory (Gascoigne <i>et al.</i> 2009)
Dispersal	Reduced efficiency of dispersal movements Reduced capacity for optimal location of food resources Reduced capacity to overcome a DAE once established	High mobility Movement in flocks High variability of food resources Low population structure	Whooping Crane (Mueller <i>et al.</i> 2013) Domestic Pigeon (Biro <i>et al.</i> 2006; Pettit <i>et al.</i> 2015) Black-Browed Albatross (Grünbaum and Veit 2003) Models (Boukal and Berec 2002)
Foraging and anti-predation	Reduced foraging efficiency Increased predation risk	Group feeding Obligate intraspecific flocking	Speckled Warbler (Gardner 2004) Redshank (Cresswell and Quinn 2011)
Interspecific competition	Reduced competitiveness for access to common resources	Relatively small body size in guild	Honeyeaters (Ford 1979; Ford <i>et al.</i> 1993)
Genetics	Reduced hatching success Reduced disease resistance Reduced survival	Small effective population size Low dispersal	Meta-analysis (Heber and Briskie 2010) Galapagos Hawk (Whiteman <i>et al.</i> 2006) Darwin's Finches (Keller <i>et al.</i> 2002)
Cultural adaptation, social learning and song learning	Reduced capacity to learn, or slower spread, of socially-acquired adaptations Song learning errors / small vocal repertoire hinder mate/ territory acquisition	High sociality Long generation time Close-ended song learning.	Great Tit (Morrand-Ferron and Quinn 2011; Aplin <i>et al.</i> 2015) Models (Kokko and Sutherland 2001) Florida Grasshopper Sparrow (Hewett <i>et al.</i> 2015) Dupont's Lark (Laiolo and Tella 2008)
Anthropogenic	Disturbance by eco-tourists reduces nesting success Illegal harvesting for food or animal products	High species profile Value or demand increase with rarity	Humboldt Penguin (Ellenberg <i>et al.</i> 2006) Helmeted Hornbill (Beastall <i>et al.</i> 2016) Indonesian songbirds (Harris <i>et al.</i> 2017)

Table 2: The 14 Australian case species or subspecies listed federally as critically endangered, their estimated population size, cause of population decline (declining population paradigm, Caughley 1994) and quality of available monitoring data in the context of detecting Allee effects (Gilroy et al. 2012).

Species	Estimated population size	Declining population paradigm	Quality of available monitoring data
Round Island / herald petrel <i>Pterodroma arminjoniana/heraldica</i>	25-50	N/A (range-restricted)	Poor
plains-wanderer <i>Pedionomus torquatus</i>	250-1000	Habitat loss + degradation	Poor
eastern curlew <i>Numenius madagascariensis</i>	<20,000	Habitat loss + degradation	Poor
bar-tailed godwit <i>Limosa lapponica menzbieri</i>	150,000 – 170,000	Habitat loss + degradation	Poor
great knot <i>Calidris tenuirostris</i>	<250,000	Habitat loss + degradation	Poor
curlew sandpiper <i>Calidris ferruginea</i>	50,000-100,000	Habitat loss + degradation	Poor
swift parrot <i>Lathamus discolor</i>	2,000	Habitat loss + introduced predator	Poor
western ground parrot <i>Pezoporus flaviventris</i>	<100	Habitat loss + degradation	Poor
orange-bellied parrot <i>Neophema chrysogaster</i>	20-40	Habitat loss + introduced predator	Poor
Grey Range thick-billed grasswren <i>Amytornis modestus obscurior</i>	10	Habitat loss + degradation	Poor
regent honeyeater <i>Anthochaera phrygia</i>	300-350	Habitat loss + degradation	Poor
helmeted honeyeater <i>Lichenostomus melanops cassidix</i>	<100	Habitat loss	Moderate
Capricorn yellow chat <i>Epthianura crocea macgregori</i>	200-300	Habitat loss + degradation	Poor
King Island scrubtit <i>Acanthornis magnus greenianus</i>	<50	Habitat loss + degradation	Poor

Mate-finding, mate choice and facilitation

Selection of a high quality mate is central to maximising individual fitness (Andersson 1994), but finding or choosing between potential mates can be limited in species that have become anthropogenically rare, sparsely-distributed (Veit & Lewis 1996; Gascoigne et al. 2009; Berec et al. 2017), or in which the operational sex ratio (OSR; the local ratio of fertilizable females to sexually active males) has become biased (Clout et al. 2002; Donald 2007). Dispersal in birds is typically female-biased (Dale 2001), which at low densities or in small populations may cause females to become lost to the effective population, if potential mates are distributed sparsely across large areas. High female mortality can also lead to an OSR bias (Ewen et al. 2001). A mate-finding CAE can be exacerbated if unpaired males disturb breeding pairs by attempting to steal mates (Goodburn 1984), harassing females (Ewen et al. 2011) or increasing nest exposure to predators (Taylor et al. 2001).

Species can avoid a mate-finding CAE by evolving strategies to enable the location of mates at low densities (Berec et al. 2017), or by avoiding low population density during breeding via aggregative nesting (Gascoigne et al. 2009). If species that adopt avoidance strategies *do* find themselves at low densities, however, their ability to find mates and recover from low densities can be severely limited (Gascoigne et al. 2009; Berec et al. 2017). CAEs can also occur if individuals do not nest or have low reproductive success through the poor quality of available mates in small populations (Møller & Legendre 2001). Many social species also require the presence of conspecifics to initiate breeding, which limits reproduction at low density via a ‘facilitation’ CAE (Stephens & Sutherland 1999).

Species that have low breeding philopatry within a large breeding range, nest in aggregations and have high dispersal should be susceptible to a mate-finding CAE. Regent honeyeaters should therefore be most at risk from a mate-finding CAE. Species with monogamous breeding strategies that have a male-biased sex ratio should also be susceptible to a mate-finding CAE (Shaw et al. 2017). Evidence from orange-bellied parrots (4 males per female, Stojanovic et al. 2018, swift parrots (estimated 2 males per female, D. Stojanovic, pers. obs.), regent honeyeaters (1.2 males per female, R. Crates, in press), curlew sandpipers and bar-tailed godwits (Nebel 2007) suggests that these species should all be susceptible to a mate-finding CAE through a male biased OSR. Low breeding participation in orange-bellied parrots may be due to the poor quality of potential mates (Holdsworth, Dettmann, and Baker 2011).

Nesting success

Nesting success can be positively influenced by the size or density of nesting aggregations (Halliday 1980; Redondo 1989). Small or sparse nesting aggregations are less able to defend against predators or competitors through mobbing (Andersson & Wiklund 1978; Oro et al. 2006). A nest success CAE may be compounded if predators preferentially target small aggregations (Cuthbert 2002).

Species that build open nests in dense aggregations should therefore be susceptible to a nesting success CAE. Regent honeyeater nest survival has declined by 30-40% since the 1990s (Crates et al. in press), at which time pairs nested in aggregations of 2 to 11 nests spaced just 40 – 80 m apart (Geering & French 1998; Oliver et al. 1998). In contrast, no monitored regent honeyeater nests had > 1 pair nesting within 100m of a focal nest in 2015-2017 (Crates et al. in press). Eastern curlews can also nest in aggregations of 2-3 pairs (Del Hoyo et al. 1992), which may assist nest defence against Corvids (Gerasimov et al. 1997). Although swift and orange-bellied parrots also nest in aggregations (Holdsworth et al. 2011; Webb et al. 2014) both species nest in hollows, do not defend nests by mobbing predators and are therefore unlikely to be at risk from a nest success CAE.

Dispersal

Dispersal encompasses a range of movements as a three-part process of departure, transience and settlement (Clobert et al. 2009). Dispersal decisions are influenced by multiple social, environmental and genetic factors (Pasinelli et al. 2004). A dispersal CAE may occur if dispersal is influenced by a collective decision making (i.e. social) process, with dispersal efficiency (departure time, direction / duration of transience, settlement location) a function of group size (Couzin et al. 2005). The ‘many wrongs hypothesis’ proposes that navigation accuracy increases with group size (Simons 2004), as a smaller proportion of informed individuals is required for accurate navigation as group size increases (Couzin et al. 2005; Biro et al. 2006). Experienced individuals reduce the distance and duration of migratory flights (Mueller et al. 2013), so the loss of experienced individuals from flocks should disproportionately affect dispersal efficiency. In addition, highly mobile species typically have less population structure (few or no sub-populations) than more sedentary species (Newton 2006), which means single, panmictic populations of mobile species have no buffer against a DAE once established (Boukal & Berec 2002; Gilroy et al. 2012).

Highly mobile species that disperse in flocks should therefore be susceptible to a dispersal CAE. Nomadic species may be particularly susceptible to a dispersal CAE in small flocks, because the location of food resources they depend upon are highly variable in space and time (Grünbaum & Veit 2003). Of the case taxa, all four shorebirds and both parrots may be susceptible to a dispersal CEA as they migrate in flocks between their breeding and wintering grounds (Del Hoyo et al. 1992). Given their semi-nomadic dispersal patterns, swift parrots and regent honeyeaters may be particularly susceptible to a dispersal CAE with decreasing flock size. Although herald and Round Island petrels are also highly mobile, both species typically undertake solitary movements (Commonwealth of Australia 2015^a) and are unlikely to suffer from a dispersal CAE.

Foraging and anti-predation

Flocking or group living are adaptations to increase foraging efficiency and decrease predation risk (Krause & Ruxton 2002). Cooperative species benefit from obligate group living, because helpers increase reproductive output or survival via augmentation (Kokko & Johnstone 2001). Similarly, interspecific flocking facilitates efficient resource location, higher foraging rates and lower rates of predator vigilance (Sridhar et al. 2009). Although species that form obligate cooperative groups or join interspecific flocks are susceptible to foraging or anti-predator CAEs from a reduction in group size (Courchamp & Macdonald 2001), both formations may serve to prevent the emergence of such CAEs. A reduction in group or flock size can be compensated for by immigration or group fusion, thus maintaining high local density despite a reduction in overall population size (Angulo et al. 2013).

Consequently, birds that form obligate *intraspecific* flocks may be among the most susceptible to a foraging or anti-predation CAE (Gardner 2004; Cresswell & Quinn 2011).

All four shorebird species, but particularly the great knot and curlew sandpiper, form large flocks during the non-breeding season (Del Hoyo et al. 1992) and should therefore be at risk from a foraging/anti-predation CAE. So too should orange-bellied parrots, swift parrots and regent honeyeaters, which all form non-breeding, single-species flocks (Franklin et al. 1989; Saunders & Heinsohn 2008). Regent honeyeater flocks historically numbered in the thousands (Geering & French 1998), but contemporary flocks containing more than 10 individuals are extremely rare (BirdLife Australia, unpubl.). In contrast, the plains-wanderer, Grey-Range thick-billed grasswren and Capricorn yellow chat have not evolved to form obligate large flocks (Baker-Gabb et al. 1990; Houston et al. 2013), resulting in a low risk from a foraging / antipredation CAE at low population size or density for both.

Interspecific competition

Species that compete for access to common resources are susceptible to a CAE if access to resources depends upon local conspecific density (Connell 1983). For many species that compete for patchy resources (Ford 1979; Ford et al. 1993), access is positively correlated with body size (Ford & Paton 1982). Thus, high local conspecific density of smaller species can facilitate access to rich patches by overcoming the territorial defences of larger competitors (Foster 1985; Dubois et al. 2003).

Species that have a relatively small body size within their feeding guild and compete for food resources that are spatially aggregated may therefore be at risk from an interspecific competition CAE with decreasing group size. Regent honeyeaters should be most susceptible to an interspecific competition CAE, as they compete with multiple larger-bodied species for access to rich nectar patches (Ford 1979; Franklin et al. 1989). Historically, regent honeyeaters likely overcame their body size disadvantage by ‘swamping’ larger competitors with many individuals occurring at high density (Ford et al. 1993). Assuming heterospecific competition exceeds conspecific competition, the co-occurrence of many regent honeyeaters at rich nectar patches should make displacement by larger competitors uneconomical (Dubois et al. 2003), diluting individual displacement and increasing the efficiency of foraging bouts (Ford et al. 1993). As the local density of regent honeyeaters declines, so too could their foraging efficiency (Kvistad et al. 2015).

Genetics

Negative genetic effects of small effective population size on fitness have been widely documented (Allendorf et al. 2012). Loss of genetic diversity through inbreeding or genetic drift (Lande 1976) in

small effective populations can reduce hatching success (Heber & Briskie 2010), survival (Keller et al. 2002) and increase susceptibility to disease (Whiteman et al. 2006). For a detailed review of the negative genetic impacts of small effective population size on population growth rates, see Frankham (2005).

Whilst by definition all small effective populations may be susceptible to a genetic CAE, Grey Range thick-billed grasswrens, King Island scrubtits, helmeted honeyeaters, orange-bellied and western ground parrots may be most at risk from a genetic CAE given their very small effective populations (Table 2) or limited dispersal capabilities. High incidence of infertility and disease outbreaks in recent years (Peters et al. 2014; Stojanovic et al. 2018) suggests that the orange-bellied parrot in particular may suffer from a genetic CAE.

Cultural adaptation, social learning and song-learning

Behavioural plasticity provides an important mechanism for adapting to environmental change, particularly in long-lived species (Kokko & Sutherland 2001). While many adaptations are acquired through individual experience (Badyaev 2005), the importance of cultural factors in shaping changes to individual behaviour are increasingly apparent (Firth & Sheldon 2016; Firth et al. 2016). For example, novel behaviours can be learned through the cultural transmission of information (Aplin et al. 2015), which is spread more efficiently in larger groups (Morand-Ferron & Quinn 2011). Birds song is also learned socially in many species (Thorpe 1958; Beecher 2017), and song anomalies can arise in small or sparse populations where isolated individuals have few opportunities to learn accurately songs from conspecifics in early life (Kelley et al. 2008). A song-learning CAE may therefore exist in populations of sparsely-distributed species, if song anomalies or small vocal repertoires natively influence mate acquisition or metapopulation dynamics (Laiolo & Tella 2008; Hewett et al. 2015).

Long-lived species that are highly social should be most susceptible to a cultural adaptation CAE at low population size or density. The four shorebirds, both parrots and the regent honeyeater should be the case taxa most at risk from a cultural adaptation CAE. Three of the four songbirds (Capricorn yellow chat, King Island scrubtit and helmeted honeyeater) have very small ranges, meaning juveniles of these species are unlikely to be isolated whilst learning songs. In contrast, regent honeyeaters are sparsely distributed throughout their vast range and have highly variable vocal repertoires (Veerman, 1992; Powys 2010). In some individuals, interspecific song appears to have completely replaced the species' typical song (R. Crates, unpubl.), which may be caused by a lack of conspecific demonstrators during song-learning (Thorpe 1958). Consequently, the regent honeyeater is the sole case species judged to be at risk of a song-learning CAE.

Anthropogenic

An anthropogenic CAE can occur in species of high socio-economic importance if negative human impacts on fitness increase with decreasing population size (Courchamp et al. 2006). For species of economic importance (e.g. for illegal pet trade), negative population growth rates occur as the value (i.e. demand) of species increases as their populations (i.e. supply) decline. (Beastall et al. 2016; Harris et al. 2017). An anthropogenic CAE may also arise through disturbance, as observer pressure increases with species' rarity (Sekercioglu 2002; Ellenberg et al. 2006). Indeed, conservation interventions may themselves inadvertently lead to negative population growth, for example through disease spill-over events from captive to wild subpopulations, Stojanovic et al. 2018).

Species that have a high profile and are particularly rare or sensitive to disturbance (Blumstein 2006) are at risk from an anthropogenic CAE. In this respect, orange-bellied parrots, eastern curlews and regent honeyeaters should be susceptible to an anthropogenic CAE. Hundreds of observers visit the last remaining breeding site of the orange-bellied parrot each year (Commonwealth of Australia 2014). Eastern curlews are thought to be particularly susceptible to anthropogenic disturbance (Reid & Park 2003; Commonwealth of Australia 2015^b), and as one of the most elusive passerines in Australia, there is high demand among eco-tourists to observe regent honeyeaters. Between 2015 and 2016, 78% of regent honeyeater nesting attempts have been in publicly accessible areas (R. Crates, unpubl.), knowledge of which can potentially spread rapidly through observer networks (Kronenberg 2014). Whilst there is also high demand to observe plains-wanderers, the small proportion of the population at risk from anthropogenic disturbance makes an anthropogenic CAE in the plains-wanderer unlikely.

Likelihood of multiple CAEs

Although populations become susceptible to an increasing number of CAEs, and hence a DAE, as their size or density decrease (Berec et al. 2007, Table 3), there remains relatively sparse empirical evidence of multiple CAEs affecting the population growth rates of threatened species (but see Serrano et al. 2005; Berec et al. 2007): a likely consequence of the challenge of detecting multiple Allee effects in such populations (Gilroy et al. 2012). Nonetheless, many potential CAEs could operate together in a complex and unpredictable fashion, necessitating further management actions to address effectively an Allee-mediated population decline (Berec et al. 2007).

THE EVIDENCE FOR UNDETECTED ALLEE EFFECTS IN AUSTRALIA'S CRITICALLY ENDANGERED BIRDS

Our review suggests that the susceptibility of the case taxa to CAEs (and hence a DAE), is highly variable (Table 3). The Round Island / herald petrels, Grey Range thick-billed grasswren, helmeted honeyeater, Capricorn yellow chat and King Island scrubtit, and have few traits that make them susceptible to CAEs (Table 3). These taxa are unlikely to suffer from a DAE, supported by the fact that each has persisted at a small population size for a relatively long period. In contrast, the four shorebird and both parrot species exhibit a suite of traits that make them susceptible to a number of CAEs (Table 3). These species have suffered rapid population declines, which may be underpinned by an undetected DAE. However, our review highlights the regent honeyeater as the case species most at risk from the greatest number of CAEs, strongly suggesting that this species' decline may be driven by an undetected DAE (Table 3). Regent honeyeaters have declined at a faster rate than sympatric honeyeater species (Lindenmayer & Cunningham 2011; Commonwealth of Australia 2016). Despite extensive loss, available habitat suggests that regent honeyeaters should occur across large areas of extant woodland (Commonwealth of Australia 2016), implying the species' decline is exacerbated by factors beyond the direct impact of habitat loss (Reed & Dobson 1993).

Table 3. Estimated susceptibility of Australia's critically endangered bird taxa to undetected component (and hence demographic) Allee effects based on their traits and current population sizes (Table 2). Ticks denote relevance of each trait to the species in the context of each CAE. Shading for each CAE denotes overall estimated susceptibility of each taxon to each CAE (white, not susceptible (0); light grey, moderate susceptibility (1); dark grey, high susceptibility (2)). Each taxon must exhibit all or most traits (ticks present for each) to be considered at risk from each CAE. Overall susceptibility of each taxon to an undetected DAE (bottom row) estimated by summing the risk of each CAE occurring.

CAE	Susceptible traits	Round Island/ Herald Petrel	Plains-Wanderer	Eastern Curlew	Bar-tailed Godwit	Great Knot	Curlew Sandpiper	Swift Parrot	Western Ground Parrot	Orange-bellied Parrot	Grey Range Thick-billed Grasswren	Regent Honeyeater	Helmeted Honeyeater	Capricorn Yellow Chat	King Island Scrubtit
Habitat selection	Low philopatry	✓	✓	✓	✓	✓	✓	✓		✓		✓			
	Large range	✓	✓	✓	✓	✓	✓	✓		✓		✓			
Mate-finding	High conspecific attraction			✓	✓	✓	✓	✓		✓		✓			
	High/female-biased dispersal		✓	✓	✓	✓	✓	✓		✓		✓			
	Large breeding range		✓	✓	✓	✓	✓	✓		✓		✓			
	Sparse distribution		✓	✓	✓	✓	✓	✓		✓	✓	✓			
	Historically common		✓	✓	✓	✓	✓	✓		✓		✓			
	Aggregative nesting		✓	✓	✓	✓	✓	✓		✓		✓			
	Socially monogamous	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Male-biased operational sex ratio			✓	✓	✓	✓	✓		✓		✓			
Mate choice	Aggregative nesting							✓				✓			
Facilitation	Breeding influenced by conspecifics									✓					
Nest success	Aggregative/colonial nesting			✓				✓		✓		✓			
	Open nesting		✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓
Dispersal	High mobility	✓		✓	✓	✓	✓	✓		✓		✓			
	Dispersal in flocks			✓	✓	✓	✓	✓		✓		✓			
	High variability in food resources	✓		✓	✓	✓	✓	✓		✓		✓			
	Low population structure		✓	✓	✓	✓	✓	✓	✓	✓		✓		✓	✓
	Large range/sparse distribution	✓	✓	✓	✓	✓	✓	✓		✓		✓			
Foraging/anti-predation	Group feeding			✓	✓	✓	✓	✓		✓		✓			
	Obligate intraspecific flocking			✓	✓	✓	✓	✓		✓		✓			
Interspecific competition	Relatively small body size in guild					✓	✓			✓	✓	✓	✓		✓
	Spatial aggregation of food resources							✓				✓			
Genetics	Small population size	✓	✓					✓	✓	✓	✓	✓	✓	✓	✓
	Small geographic range								✓	✓	✓	✓	✓	✓	✓
	Low dispersal	✓	✓						✓	✓	✓	✓	✓	✓	✓
Cultural adaptation/social learning	High sociality			✓	✓	✓	✓	✓		✓		✓			
	Long generation time	✓	✓	✓	✓	✓	✓	✓	✓			✓	✓		
Song-learning	High juvenile dispersal											✓			
	Close-ended song-learning										✓	✓	✓	✓	✓
	Sparse distribution											✓			
Anthropogenic	High species profile (demand)		✓	✓	✓	✓	✓	✓	✓	✓		✓	✓		
	High 'observability' when nesting									✓		✓			
	High susceptibility to disturbance			✓						✓		✓			
Estimated overall susceptibility to a DAE		1	2	8	6	7	8	11	2	12	2	18	2	2	2

IMPLICATIONS FOR CONSERVATION

The presence of undetected Allee effects in threatened species should lead to management actions that (1) address the initial cause of the population decline (Caughley 1994); (2) increase population density; and (3) decrease the negative fitness effects of low population density (Stephens & Sutherland 1999; Deredec & Courchamp 2007). We discuss ways in which potential CAEs, where relevant, could be accounted for in management actions for the case taxa. Given the high susceptibility of the regent honeyeater to multiple CAEs (Table 3), we focus in particular on ways in which precautionary conservation actions for the Regent honeyeater could account for likely undetected Allee effects (Table 4).

Address the declining population paradigm

The declining population paradigm for the case taxa undergoing rapid declines is extensive habitat loss and degradation (Ford et al. 2001; Cresswell & Murphy 2017, Table 1). Without habitat restoration, these species will have little chance of long-term population recovery.

Increase local population density

The wild populations of four case taxa (Helmeted and regent honeyeater, plains-wanderer and orange-bellied parrot) are or will soon be supplemented by the introduction of captive-bred birds. To mitigate a DAE, the primary goal of introductions should be to maximise local population density (Stephens & Sutherland 1999), which can be achieved by introducing captive-bred birds in the core range, where the majority of wild birds persist. Maximising local population density using introductions should assist wild birds in overcoming potential CAEs relating to interspecific competition and foraging / anti-predation (Table 2). Captive-bred birds often lack socially-acquired knowledge (Caro 1999; 2005) which, in combination with small group size, can inhibit the success of introductions (Fischer & Lindenmayer 2000). Maximising population density can facilitate interactions between wild and captive-bred birds to aid the cultural transmission of information from wild to captive-bred conspecifics (cultural adaptation CAE). The social acquisition of ‘wild knowledge’ may be critical for the survival and successful breeding of naïve, captive-bred birds.

The likelihood of a DAE should also influence the size and procedure of introductions (Deredec & Courchamp 2007; Armstrong & Wittmer 2011). In highly dispersive species such as the Orange-Bellied Parrot and Regent honeyeater, the ‘minimum founding population’ may be substantially larger than the size of introduced groups (Goodsman & Lewis 2016), reducing the benefits of releasing large groups. To maximise breeding density in dispersive species, captive releases should therefore occur

either as close to the start of the breeding season as possible, where and when wild birds are present (Table 4).

For species with high conspecific attraction, actions that improve the reliability of habitat cues and encourage settlement in optimal habitat may also be possible (Reed & Dobson 1993; Stodala & Ward 2017). Decoys and acoustic lures can attract birds to settle in optimal habitat where risks can be most effectively managed (Jeffries & Brunton 2001). The targeted release of captive regent honeyeaters and orange-Bellied Parrots in optimal breeding habitat (where the abundance of natural food sources is highest) could also attract wild birds and assist them in overcoming habitat selection or interspecific competition CAEs. A mate-finding CAE due to a male-biased sex ratio could be accounted for by introducing proportionally more females, or by temporally segregating captive releases by sex (Wedekind 2002). Recently, the release of female orange-bellied parrots has allowed previously unpaired wild males to breed (Stojanovic et al. 2018). A similar strategy could prove equally effective for regent honeyeaters (Table 4).

Decrease the fitness costs of low population density

For taxa at risk of a genetic CAE (e.g. King Island scrubtit, Capricorn yellow chat), the translocation of individuals amongst isolated subpopulations could facilitate gene flow and reduce the negative genetic effects of small effective population size (Webb et al. 2016). For small effective populations lacking population structure (e.g. helmeted honeyeater), the introduction of genes from sister taxa could aid genetic rescue (Harrisson et al. 2016). For species that may suffer from a nesting success CAE, the implementation of nest protection measures and predator suppression could increase nesting success in small or sparse aggregations (Fulton & Ford 2001; Major et al. 2014, Table 4). Careful management of tourists at breeding or wintering sites should overcome potential anthropogenic CAEs.

Table 4: Potential undetected component Allee effects in the regent honeyeater and management options for accounting for their potential presence based on the precautionary principle.

Component Allee effect	Actions to reduce negative fitness effects of low population size or density
Interspecific competition	<p>Reduce local density of competitors via targeted control.</p> <p>Release captive bred-birds in core range when and where wild birds are present.</p> <p>Increase availability of feeding habitat via habitat restoration.</p> <p>Exclude larger competitors by selective caging of critical food resources / supplementary food.</p> <p>Increase competitiveness of captive birds by exposing them to competition in captivity.</p>
Habitat selection	<p>Release captive birds in high quality breeding habitat shortly before breeding commences.</p> <p>Increase availability of breeding habitat via habitat restoration.</p> <p>Use decoys / song broadcast to attract conspecifics to high quality habitat.</p>
Mate-finding	<p>Targeted release of captive-bred females at breeding aggregations with a male-biased sex ratio.</p> <p>Release captive-bred birds in core range during early breeding season where wild birds are present.</p> <p>Facilitate mate-finding using artificial social cues such as song broadcast.</p>
Mate choice / facilitation	<p>Release captive bred-birds in core range when and where wild birds are present.</p> <p>Ensure high phenotypic quality of captive breeding stock.</p>
Nesting success	<p>Employ nest protection measures such as tree guards, branch collars and nest cages.</p> <p>Targeted control or translocation of known nest predators at breeding sites.</p> <p>Increase size of breeding habitat patches via habitat restoration.</p>
Dispersal	<p>Ensure captive-bred birds interact with wild conspecifics to maximise flock size/group knowledge of future resource location.</p> <p>Increase size and abundance of suitable habitat patches via habitat restoration.</p>
Foraging / anti-predation	<p>Maximise flock size by releasing captive-bred birds in core range when wild birds are present.</p> <p>Targeted control of predators.</p>
Genetics	<p>Ensure genetic integrity of captive stock.</p> <p>Maximise mate choice by releasing captive-bred birds in the core range.</p> <p>Facilitate gene flow by translocating individuals between regional subpopulations.</p>
Cultural adaptation, social learning and song learning	<p>Release captive bred-birds in core range when and where wild birds are present</p> <p>Ensure interaction of captive bred birds with wild birds during song-learning.</p> <p>Use playback or demonstrator individuals to ensure captive birds have full vocal repertoire</p>
Anthropogenic	<p>Limit human disturbance during breeding by restricting access to breeding areas.</p>

CONCLUSION

Allee effects present a major challenge for conservation, because their probability of occurrence and impact on population growth rates may be greatest in the rare and declining species in which they are hardest to detect and overcome. This challenge could explain the lack of empirical evidence for DAEs in threatened species (Gregory et al. 2010; Kramer et al. 2009). An inability to test for the existence of a DAE in the majority of threatened species is an unavoidable consequence of a lack of comprehensive contemporary and historical population data (Franklin et al. 1989, Table 2).

Nonetheless, a growing literature can be used to critically evaluate the range of potential CAEs that could lead to a DAE, as well as traits that make species susceptible to each (Tables 1 and 3). Given monitoring constraints (Crates et al. 2017) and a race against time (Gilroy et al. 2012), adopting a precautionary approach currently offers the most effective means of attempting to eliminate or reduce the strength of Allee effects in threatened bird populations (Cooney 2004; Courchamp et al. 2008). Although general, our approach could be used to assess the relative susceptibility of any data-deficient taxa to undetected Allee effects, prioritising early intervention for those deemed most at risk (Drake & Griffen 2010).

Managers must ask three questions to address a potential undetected DAE:

- 1) By which means and over what time scale could a DAE be proven to exist?
- 2) How would the presence of an undetected DAE influence management actions?
- 3) Should a DAE not exist, would accounting for it compromise viability of the target population or success of current management actions?

For example, despite being most at risk from a DAE, we argue that it would not be possible to detect a DAE in the regent honeyeater population in a timescale that would not inhibit the success of recovery actions, should a DAE indeed exist. The presence of an undetected DAE should lead to alterations to current management, as multiple, potentially interacting CAEs could be accounted for by adapting existing actions and employing a small number of new management strategies (Table 4). Given an ongoing population decline despite two decades of recovery effort (Oliver & Lollback 2010), adopting a precautionary approach to account for an undetected DAE in the regent honeyeater is unlikely to impede the success of current management actions. Indeed, the regent honeyeater population may have already passed the Allee threshold, in which case urgent and intensive population management is required to prevent extinction (Courchamp et al. 2008).

We urge conservationists to explicitly consider the potential for undetected Allee effects to influence negatively the population growth rates of threatened species, and to critically assess how management

actions could be targeted accordingly. By adopting a precautionary approach, an inability to detect Allee effects in threatened species need not preclude efforts to account for their potential presence in management. Such actions are urgently needed if regent honeyeaters, and many species alike, are not to follow the course of the passenger pigeon and paradise parrot into oblivion.

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CHAPTER 4: Contemporary breeding biology of critically endangered regent honeyeaters: implications for conservation.

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ABSTRACT

Identifying factors influencing the demographics of threatened species is essential for conservation, but a lack of comprehensive demographic data often impedes the effective conservation of rare and mobile species. We monitored breeding of critically endangered and semi-nomadic regent honeyeaters *Anthochaera phrygia* (global population = c100 pairs) over three years throughout their range. Overall nest success probability (0.317) was highly spatially variable and considerably lower than previous estimates for this (and many other honeyeater) species, as was productivity of successful nests (mean 1.58 juveniles fledged). Nest surveillance revealed high predation rates by a range of birds and arboreal mammals as the primary cause of nest failure. An estimated 12% of pairs failed to establish a territory or their nests did not reach the egg stage. We also found a male bias to the adult sex ratio, with an estimated 1.18 males per female. Juvenile survival for the first two weeks after fledging was high (86 %). Management interventions that aim to increase nest success in areas of low nest survival must be investigated to address an apparent decline in reproductive output and avoid extinction of the regent honeyeater. We show that temporal and spatial variation in the breeding success of rare and highly mobile species can be quantified with robust population monitoring using sampling regimes that account for their life-histories. Understanding the causes of spatio-temporal variation in breeding success can enhance conservation outcomes for such species through spatially and temporally targeted recovery actions.

KEY WORDS

Allee effect, *Anthochaera phrygia*, juvenile survival, nest predators, nest survival, sex ratio.

INTRODUCTION

Robust modelling of population trajectories requires accurate and precise estimates of demographic parameters (Brook et al. 2000) and identification of factors influencing these parameters is vital for effective conservation (Caughley 1994). For rare and highly mobile species, collecting demographic data can be challenging (Runge et al. 2014; Cottee-Jones et al. 2015). Consequently, identifying the drivers of population decline in such species is often based on limited data (Rayner et al. 2014), reducing the accuracy of estimated demographic parameters. Sampling populations of widespread species in a subset of their range or over short time periods could lead to biased estimates of breeding success if reproductive parameters vary in space and time (Paradis et al. 2000, Stojanovic et al. 2014), or are a function of population size or density (Stephens & Sutherland 1999). Thus, data limitations can inhibit detection of limits to population recovery, potentially leading to misallocation of conservation resources (McDonald-Madden et al. 2010).

Population growth rates can also be compromised by biased adult sex ratios (the ratio of sexually active males to fertilizable females, Donald 2007), because members of the more abundant sex may be unable to find mates (Gascoigne et al. 2009). Biased adult sex ratios are relevant to threatened species recovery efforts, via conservation measures to increase numbers of the rarer sex (Ewen et al. 2001). Sex ratios are also often spatially variable (Steifetten & Dale 2006) with implications for determining where recovery effort should be invested (Wedekind et al. 2002). However, robust sex ratio estimates are seldom available for rare and mobile species due to perceived challenges of collecting necessary data (Donald 2007).

We monitored reproduction in the wild population of the critically endangered regent honeyeater. The species' estimated population of 350-400 individuals (Kvistad et al. 2015) and patchy occurrence across up to 600,000 km² of south-eastern Australia (Commonwealth of Australia 2016), poses challenges for conservation research (Clarke et al. 2003). Historically, regent honeyeaters were considered abundant until a rapid population decline since the 1960's (Franklin et al. 1989). Population decline is primarily attributed to extensive clearing of preferred box-gum-ironbark woodland habitats (Ford et al. 1993; Ford et al. 2001), but the demographic factors underlying contemporary population trends are poorly understood (Clarke et al. 2003; Crates et al. 2017a).

Regent honeyeaters build open cup nests, typically in the outer branches of large trees. They form socially monogamous pairs and nest in loose aggregations in association with the flowering of a select number of *Eucalyptus* tree species (Franklin et al. 1989). Parents provision juveniles in separate family groups for 2 - 3 weeks post fledging, almost exclusively within 200 m of the nest site (R. Crates, pers. obs). Post-breeding, regent honeyeaters form flocks consisting of adults and independent

juveniles (Geering & French 1998). Estimates of nesting success (probability of fledging ≥ 1 juvenile) in the 1990's were comparable to other Australian honeyeaters (38 - 47 %), leading to the conclusion that low nesting success was not driving population decline (Geering & French 1998; Oliver et al. 1998). Current recovery actions focus on biannual releases of captive-bred individuals to the southern edge of the species' contemporary range and small-scale protection and restoration of habitat (Commonwealth of Australia 2016). No standardised nest monitoring has been undertaken for over 20 years, but given the regent honeyeater's conservation status, reassessment of key breeding parameters is a conservation priority (Commonwealth of Australia 2016).

To inform conservation management of the wild regent honeyeater population, we aimed to develop a range-wide monitoring program to locate breeding regent honeyeaters. Then, we aimed to estimate the adult sex ratio and contemporary nesting success and to identify factors affecting these parameters. We also aimed to estimate short-term post-fledging juvenile survival. Lastly, we compare our estimates with historical studies for the regent honeyeater and other Australian honeyeaters.

METHODS

Locating regent honeyeaters

We searched for regent honeyeaters over three breeding seasons. In 2015 we surveyed 321 sites over 880 km² of the Capertee Valley, New South Wales, a known core breeding region for the remaining population (Crates et al. 2017b). We then expanded our sampling across the entire contemporary range, surveying 777 and 896 sites in 2016 and 2017 respectively (Supporting Information Table S1, Figure S1). Sites were spaced ≥ 150 m apart in Regent honeyeater breeding habitat (Commonwealth of Australia 2016) and were selected based on a combination of: 1) habitat identified as high priority by a species distribution model constructed in 'MaxEnt' (Rayner et al. in prep); 2) expert advice and 3) extensive field searches for potential breeding habitat.

We visited all sites in the Capertee Valley three times in 2015. In 2016 we visited 777 sites twice, with 371 of those visited a third time. In 2017 we increased the number of survey sites to 896, of which 610 sites were visited twice (supporting information Table S1). In total, 5949 site visits were made over the course of study. Site visits comprised a 5-minute point count within a 50 m radius, with regent honeyeater song broadcast (Pizzey & Knight 2014) from portable speakers in the first survey minute (Crates et al. 2017b). We also recorded and monitored all regent honeyeaters detected (visually or audibly) > 50 m from survey sites or outside the 5-minute survey window.

Estimating sex ratios

Where regent honeyeaters were detected, we undertook repeated adaptive sampling to search for additional birds nearby (Smith et al. 2004). Follow up searches commenced as soon as possible to, and not more than 4 days after the first regent honeyeater detection at a site. We marked a sample of individuals (20 females, 48 males) with coloured leg bands using mist-nets and assigned sex based on wing length, plumage and mass (Geering 2010). We then determined the number of individuals present in each aggregation (defined as a group of birds loosely distributed over an area of 2 - 20 ha), their sex and nesting status. The maximum number of individuals present in each aggregation was estimated during adaptive sampling through combined monitoring of nests, colour banded individuals and unpaired males. Because aggregations were largely monitored simultaneously within years, movement of individuals between aggregations was minimised. Only 2 colour banded individuals were detected in > 1 aggregation in the same year, these individuals and their unmarked partners were accounted for in counts. We sexed unmarked birds in the field based on plumage, size and song characteristics, as only males vocalise a full song (R. Crates, pers. obs). We estimated the sex ratio of nesting aggregations by collating data from all Regent honeyeaters observed at sites (including all colour-marked birds, unmarked pairs and individuals). Unpaired males at nesting aggregations were readily identified by their persistent singing, lack of an accompanying female and the small number of birds in each aggregation. Because nests were invariably inaccessible, we were unable to estimate nestling sex ratios.

Locating and monitoring nests

We located nests by observing bird behaviour (i.e. nest construction, incubating females or parents provisioning young). For nests where egg laying dates were not known, we estimated initiation date based on a 34 day nesting period comprising 2 eggs laid on consecutive days, 14 days incubation and a 19-day nestling period. The nestling period was calculated based on our own observations of 13 successful nests monitored from the date the first egg was laid, which was 2-3 days longer than Geering and French (1998) and Oliver et al. (1998). Initiation date could not be estimated to within ± 2 days for 2 nests, which were excluded from analyses. Because of the small number of pairs at each nesting aggregation ($n = 22$ aggregations, mean n pairs = 6, $SD = 4.7$, range = 1 – 14), we were confident that effectively all nesting attempts were located and monitored. We observed nests (mean height = 13 m, $SD = 4.69$, range = 3 – 25 m) from the ground every 2 to 7 days using the presence and behaviour of adults to determine whether nests were active. We recorded the nectar sources associated with each nest by observing the tree and mistletoe species the adults were foraging in.

To identify the causes of nest failure, we deployed wireless infra-red video cameras between 2 and 8 metres from 14 nests accessible by tree climbing. Where we could not confirm the cause of nest

failure, we assumed the cause of failure where possible. For instance, if failed nests were damaged or empty, we assumed that predation had occurred. If intact nests were found on the ground following a period of high winds, we assumed that wind had dislodged the nest.

Post-fledging juvenile survival

At successful nests and where logistically possible, we monitored juvenile survival every 2-4 days for the first two weeks after fledging. Juveniles were readily detected by their persistent begging calls (R.Crates, pers. obs). We identified juveniles via the colour bands on the parents provisioning them, or because there were no other recently-fledged juveniles present concurrently within 200m of them.

Data analysis

We modelled the daily survival rate (DSR) of regent honeyeater nests in R v3.2.3 (R core development team 2017) using package *RMark* v2.2.2 (Laake et al. 2016), an R-interface for the nest survival model (Dinsmore et al. 2002) in the program *MARK* (White & Burnham 1999, Cooch & White 2005). By calculating daily survival probabilities, these models account for undetected nests in survival estimates (Dinsmore & Dinsmore 2007). We obtained the best-supported nest survival models based on lowest *AICc* values (Burnham & Anderson 2002). Because it is difficult to assess reliably the goodness of fit of nest survival models (Sturdivant et al. 2007), we avoided overfitting by including a maximum of 3 covariates per model (Table 1).

Table 1: Description of covariates included in regent honeyeater nest survival models. Further details of covariates are provided in supporting information Table S2.

Covariate	Description
Tree cover	Estimated % tree cover within 50 m of nest, to nearest 10%.
Edge	Log-transformed distance to (positive) or from (negative) edge of continuous woodland.
Concealment	4 - level factor- concealment of nest by surrounding 2 m ³ vegetation: <i>Low</i> < 25 %; <i>Moderate</i> < 50 %; <i>High</i> < 75 %; <i>Very High</i> > 75 %.
Position	3 - level factor- position of nest within tree crown: <i>Outer</i> = outer 10 % of crown; <i>Mid</i> = 11 – 25 % of crown; <i>Inner</i> > inner 75 % of crown.
Height	Height of nest in metres above ground.
Camera	Presence / absence of nest monitoring camera.
Flower	5 - level factor- relative flower (a proxy for nectar) abundance within 100 m of nest: 0 = none; 1 = light; 2 = moderate; 3 = high; 4 = very high.
Noisy miner	Detection / non-detection of noisy miners within 50 m of each active nest during nest monitoring.
Temperature	Days during nesting period where maximum temperature exceeded 35°C.
Conspecifics	Number of Regent honeyeater nests, active synchronously for ≥ 50 % of focal nest duration, within 100 m of focal nest.
Region	2 - level factor: 1 = greater Blue Mountains, 2 = Northern Tablelands.
Site	9 - level factor indicating nest location within regions: 1 = Capertee north; 2 = Capertee north-west; 3 = Capertee west; 4 = Capertee central; 5 = Capertee south; 6 = Goulburn River; 7 = Burragorang 8 = Barraba; 9 = Severn River.
Location	Spatial location (Lat/Long) of nest.
Habitat type	3 - level factor: 1 = box-ironbark woodland; 2 = box-gum woodland; 3 = riparian.
Nest Age	Age of nest in days since first egg.
Time	Continuous timing of nest (Julian date) within overall breeding season.
Year	3 - level factor: 1 = 2015 2 = 2016 3 = 2017.

We assessed spatial autocorrelation in range-wide nesting success (binomial response: fail or fledge ≥ 1 juvenile, sampling distance 50 km) and within the core range of the Capertee Valley (sampling distance 500 m) using correlograms (based on Moran’s I, Tiefelsdorf 2000) using the R-package *ncf* (Bjornstad 2015). To explicitly account for spatial dependence in nest success (which is not possible with nest survival models using RMark), we modelled range-wide nest success including a spatial term. The response metric was daily nest failure probability, calculated using the ‘successes per failure’ syntax (Aebischer 1999, Shaffer & Thompson 2007). Where the exact day of nest failure was unknown, we assumed nest failure occurred on the median day between visits. We evaluated three spatial modeling approaches: spatial autoregressive lag models, generalized linear models with a spatial autocovariate, and generalized additive models (GAMs) with a spatial covariate. Model diagnostics (analysis of residuals and adequacy of basis dimensions of smoothed spatial terms, sensu Wood 2017) indicated that GAMs were the most appropriate choice given the distribution of nests

throughout the range. We fitted GAMs using package *mgcv* (Wood 2018), with a binomial link function and nest location (latitude/longitude) as a smoothed spatial covariate. Unlike nest survival models, GAMs also allow the inclusion of random terms. We therefore included Pair ID as a random term because 15% of nests were known to be second attempts by the same pair in the same season. We used *MuMin* v1.40.4 (Bartoń 2018) to find the most parsimonious models from the global model, based on lowest AICc (Burnham & Anderson 2002).

To estimate juvenile survival for the fortnight post fledging, we fit constant and age-trend nest survival models using *RMark* for 56 juveniles from 42 nests in the greater Blue Mountains. Constant nest survival models assume daily survival does not change during the post-fledging period, whereas age-trend models account for potential temporal changes in daily juvenile survival (Dinsmore & Dinsmore 2007). Thus, each juvenile was ‘found’ on the first day it fledged, and ‘succeeded’ if it survived for 14 days post-fledging, regardless of the fate of any siblings. To account for potential non-independence of the fate of siblings (n=13 nests), we ran the models twice more, randomly sampling just one juvenile from each nest.

To compare our regent honeyeater nest success and productivity estimates with other studies of Australian honeyeaters, we conducted a literature search in GoogleScholar of the terms ‘nest survival’, ‘nest success’, ‘honeyeater’ and ‘Australia.’ We included all independent studies of honeyeater species returned by the literature search to accumulate a database of nest success estimates and associated spatio-temporal metadata.

RESULTS

We detected regent honeyeaters at 27 monitoring sites in 2015, at 39 sites in 2016 and at 53 sites in 2017 (Table S1). We detected a minimum of 70 adult birds (age ≥ 1 year) in the Capertee Valley in 2015, 73 adult birds range-wide in 2016, and 145 adult birds range-wide in 2017. This represented 30 – 65% of the estimated effective population each year (Kvistad et al. 2015; Commonwealth of Australia 2016). We colour-marked individuals in the Severn River (1 f, 7 m), Barraba (2 m), Goulburn River (1 f, 3 m) and Capertee Valley (18 f, 38 m), 94% of which were >1 year old. The range-wide adult sex ratio was 1.18, but small breeding aggregations in the Northern Tablelands (Barraba and Severn River) were more male-biased (Figure 1).

An estimated 12% of potential breeding pairs (exhibiting territorial aggression and male singing) either failed to gain a territory or lay eggs. In the Capertee Valley, 17 pairs fitted these criteria over 3 years, and in the broader range, 6 pairs failed to breed in this way.

We found 119 regent honeyeater nests reaching the egg stage in five regions of New South Wales: The Capertee and Burragorang Valleys, Goulburn River, Barraba and Severn River (Figure 1). In total, 51 successful nests produced 82 fledglings. Although nests were associated with a variety of nectar resources, yellow box *Eucalyptus melliodora* was disproportionately the most common (Table S3). Thirty four percent of nests were found on or before the day the first egg was laid. Median nest age when found was 4 days, mean = 7.5.

Figure 1: Regional variation in the population size, adult sex ratio and nest success probability of wild regent honeyeaters in 2015 (orange), 2016 (yellow) and 2017 (blue). Northern Tablelands: Severn River (SR) and Barraba (BA). Greater Blue Mountains: Goulburn River (GR), Munghorn Gap (MG), Capertee Valley (CV), lower Hunter Valley (LH), Burraborang Valley (BU). Figures in parentheses denote, overall years: (nests, juveniles, nest success probability). Overlapping population symbols (circles) denote same regional sites occupied in > 1 year. Inset: Regent honeyeater range based on 2000 - 2010 sightings data. * Sex ratio data not available for lower Hunter Valley. Unknown fate of 4 nests in the Burraborang Valley not included in DSR models.

The best-supported range-wide nest survival model (Table 2) provided a daily nest survival estimate of 0.967 ± 0.004 (95% $CI = 0.959 - 0.975$, effective sample size (N_e) = 1895), giving a nest survival probability over the 34 day nesting period of 0.317 (95% $CI = 0.24 - 0.42$). DSR models showed high regional variation in nest survival. In the Northern Tablelands (Barraba and Severn River), nest survival probability was substantially lower (0.093, 95% $CI = 0.014 - 0.27$, $n = 11$) than in Central NSW (Blue Mountains, 0.337, 95% $CI = 0.245 - 0.431$, $n = 108$, Figures 1 and 2), with no juveniles fledged from 11 nests found in the Northern Tablelands. Nest survival also differed markedly at the site level between nesting aggregations within the Capertee Valley, ranging from 0.14 in the centre, to 0.74 in the north-east (Figure 2, Tables S4 and 5).

Figure 2: Variation in regent honeyeater daily nest survival rate (DSR) $\pm se$ by factors included in top ranked nest survival models (Table 2), plus year: A, breeding site; B, nest position within tree crown; C, presence/absence of nesting conspecifics within 100 m; D, year. Estimates derived from separate models of each factor. See Tables S4, S5 and Figure S3 for additional information.

Correlograms of nest success indicated that nests located close to each other tended to share a similar fate (Figure S2). Nest success was spatially autocorrelated at distances separating discrete breeding sites within the Capertee Valley, and also between regional sub-populations range-wide (Figure S2).

DSR models also identified nest position and the presence of conspecifics as additional factors influencing nest survival. Nests built in the outer canopy and in close proximity to other nesting pairs had the greatest survival probability (Figure 2, Table S5). Nest survival decreased slightly as nest age increased, but the age-trend model fitted the data no better than constant survival ($\Delta AIC_c = 1.59$). In the Capertee Valley, nest survival was more than twice as high in 2017 than in other years (Figure 2, Table S4).

The top-ranked GAMs confirmed that nests positioned in the outer crown of trees had highest nest success (Tables 2 and S5). Noisy miner presence was included in 2 of the 4 top ranked models (Table 2) and had a negative though non-significant effect on the probability of regent honeyeater nest success (Table S5). The smoothed spatial term of nest location improved the fit of the model (Table 2).

Table 2: Top-ranked ($\Delta AIC_c < 2$) nest survival (S) and daily failure probability (F) generalised additive models (GAMs) for 119 regent honeyeater nests ($N_e = 1895$) from 2015 - 2017.

Model type	Model	AIC_c	ΔAIC_c	W_i
Nest survival	$S(\text{Site})$	438.88	-	0.40
	$S(\text{Site} + \text{Conspecifics} + \text{Position})$	440.13	1.26	0.21
GAM	$F(\text{Position} + s(\text{Lat}, \text{Long}) + 1 \text{pair ID})$	269.86	0	0.19
	$F(\text{Position} + s(\text{Lat}, \text{Long}) + \text{Noisy miner} + 1 \text{pair ID})$	270.19	0.33	0.16

Predation was the main known cause of regent honeyeater nest failure, accounting for 89% of nests where the cause of failure was confirmed. Avian predators were pied currawongs *Strepera graculina* ($n = 3$), noisy miners *Manorina melanocephala* ($n = 2$), a pied butcherbird *Cracticus nigrogularis* and a laughing kookaburra *Dacelo novaeguineae*. Mammalian predators included a brush-tailed possum *Trichosurus vulpecula* and sugar glider *Petaurus breviceps*. Inclement weather (high wind, hail, extreme heat) was inferred as the cause of seven additional failures. A second nesting attempt was initiated by 8 pairs whose first attempt failed, and by 10 pairs following a successful first attempt.

Mean number of young fledged per nesting attempt was 0.78 ± 0.1 CI. Mean number of juveniles fledged from 59 successful nests was 1.58 ± 0.53 . The age-trend juvenile survival model ($AIC_c = 92.65$, $W_i = 0.86$) fitted the data better than the constant model ($\Delta AIC_c = 3.67$, $W_i = 0.14$), with daily juvenile survival increasing with time since fledging (Figure 3). Estimated DSR of juveniles ($n = 56$ juveniles, $N_e = 649$) averaged across the fortnight post-fledging was 0.989 ± 0.009 , giving a survival probability of 0.859 (95% CI = 0.46 – 0.97). Models containing only 1 juvenile from each nest (i.e. excluding random nest effect) did not substantially alter juvenile survival estimates (Figure S4).

Figure 3: Effect of days since fledging on short-term post-fledging survival of juvenile regent honeyeaters from 2015-2017 ($n = 56$).

Table 3: Published estimates of nest survival probabilities and mean fledglings per successful nest for Australian honeyeaters (Meliphagidae). Estimates are ranked by % nest success. Historical and contemporary estimates for Regent honeyeaters highlighted in grey. Unavailable data denoted by ‘-’.

Species	Location	Year	% Nest success (nests)	Method ¹	Fledglings / nest (nests) ²	Citation
white-cheeked honeyeater	C. NSW	1987-88	68.3 (43)	Binary	-	Armstrong & Pyke 1991
bell miner	S. VIC	1988-1990	57.4 (122)	Binary	-	Poiani 1993
regent honeyeater	C. NSW	1996	46.9 (42)	Mayfield	1.88 (18)	Geering & French 1998
brown honeyeater	NT	1985-1999	42 (75)	Binary	1.74 (19)	Franklin & Noske 2000
new holland honeyeater	C. NSW	1987-88	41	Binary	-	Armstrong & Pyke 1991
regent honeyeater	C. NSW	1995	38.7 (73)	Mayfield	1.78 (50)	Geering & French 1998
noisy friarbird	N. NSW	1990 and 1997	38.4 (224)	Binary	2.07 (85)	Ford 1999
regent honeyeater	N. NSW	1993-1996	38.3 (41)	Mayfield	2.1 (21)	Oliver et al. 1998
yellow-faced honeyeater	C. VIC	1997-2000	37 (69)	DSR	1.73 (50)	Clarke et al. 2003
new holland honeyeater	W. VIC	-	37 (-)	-	-	Paton 1985
regent honeyeater	C. NSW	2015 - 2017	33.7 (108)	DSR	1.58 (59)	This study
red wattlebird	N. NSW	1990 and 1997	33.3 (90)	Binary	1.6 (50)	Ford 1999
helmeted honeyeater	C. VIC	1984-1992	32.9 (257)	Mayfield	1.63 (40)	Franklin et al. 1995
new holland honeyeater	SA	2004	32.2 (-)	Binary	-	Lambert & Kleindorfer 2006
fuscous honeyeater	N. NSW	1984-1988	28.2 (137)	Binary	2 (33)	Dunkerley 1989
crescent honeyeater	S. VIC	1996-1997	20.1 (32)	Mayfield	2.7 (9)	Clarke & Clarke 2000
helmeted honeyeater	C. VIC	1984-1996	17.5 (526)	DSR	-	Smales et al. 2009
regent honeyeater	N.NSW	2016 and 2017	9.3 (11)	DSR	- (0)	This study

¹ ‘Binary:’ percentage of nests that produced one or more fledgling; ‘Mayfield:’ Mayfield (1975) method. ‘DSR:’ method used in this study (see methods). Parameters presented are from first egg to fledging. We do not provide a comparable Mayfield estimate for our data because Mayfield estimates are prone to inconsistent bias and DSR estimates between the two methods are invariably very similar (Shaffer and Thompson 2007). ²data presented for successful nests only.

DISCUSSION

By developing a targeted and spatially stratified sampling design, we consistently located between 30 - 65% of the estimated global population of regent honeyeaters. Poor breeding success limits recruitment to the population. High rates of nest predation drives poor reproduction, which is exacerbated by the failure of some pairs to participate in breeding, a decrease in the number of juveniles fledged from successful nests, and a lack of females. Contemporary nesting success (9 - 34 %) is considerably lower than estimates from previous studies (Table 3). Nest success is also highly variable at multiple spatial scales, suggesting complex factors are suppressing population growth in regent honeyeaters.

Approximately 1 in 6 males was unable to find a mate. Although our sex ratio estimate is based primarily on the sexing of individuals in the field, we are confident that our estimate is accurate. Unpaired males sang prolifically, which confirmed they were not nesting as singing ceases prior to egg laying (R. Crates, pers. obs). Singing males may be more detectable than females, but these males would have facilitated detection of any associated females during follow-up searches (e.g. through courting behaviour), had females been present. Given the very sparse distribution of nesting aggregations throughout the range (Figure 1), dispersing females may fail to locate other flocks, causing them to become lost from the breeding population (Dale 2001; Gilroy & Lockwood 2012). Alternatively, a male-biased sex ratio could be due to predation of some females during nesting (Grüebler et al. 2008; Stojanovic et al. 2014). Male-biased sex ratios may be common in bird species (Donald 2007), but a lack of females is a clear limit to regent honeyeater population recovery, considering the species' estimated effective population of just 100 pairs (Kvistad et al. 2015). This effect appears to be greatest at the range edge in northern NSW, indicating the sex ratio bias may increase with population decline (Stojanovic et al. 2018). Unpaired males could also reduce breeding success of pairs by attempting to steal mates or increasing nest exposure to predators (Dale 2001).

The failure of 12% of pairs during territory acquisition or nest construction may be a consequence of high attraction to nesting conspecifics in fragmented habitat patches that are too small to support the number of pairs attempting to settle there (Kokko & Sutherland 2001; Schmidt et al. 2015). Although some species regularly abandon nests during construction if concealment is considered to be insufficient (Beckmann & Martin 2016), our observations suggest that the failure of regent honeyeater nests during construction was more likely through competitive displacement by larger honeyeater species. These results emphasise an urgent need for targeted restoration of yellow box - mugga ironbark *Eucalyptus sideroxylon* habitats (Tulloch et al. 2016), to increase the size and availability of breeding habitat and reduce competition for nest sites.

The best nest survival models included breeding site and nest position as factors influencing nest survival. Given high levels of observed nest predation by an assemblage of species, local predator presence/abundance at breeding sites may explain spatial structure in regent honeyeater nest success. Variation in nest position could be explained by the age of nesting pairs, with more experienced pairs selecting less accessible nest positions within trees (Eggers et al. 2006). Although the exact age of birds was unknown, 94% of colour-marked individuals were adults, suggesting the results were not driven solely by young, first-time breeders. The positive effect of the presence of conspecifics may be due to greater probability of attraction to nesting conspecifics at sites with high nest survival (Schmidt et al. 2015). Alternatively, nearby nesting conspecifics could facilitate nest survival through antipredator defences including alarm calling and predator mobbing (Serrano et al. 2005). Declining nest success over time may therefore reflect a concurrent reduction in the size and density of breeding aggregations (Crates et al. 2017a).

Although only 22% of identified nest predations were attributable to noisy miners, their negative impact on regent honeyeater nest success, supported by the GAMs, may primarily occur at the settlement phase by excluding regent honeyeaters from preferred breeding habitat (Piper & Catterall 2003). Noisy miners were removed from the north-eastern breeding site in the Capertee Valley in winter 2017 (BirdLife Australia unpubl. data), where 14 regent honeyeater pairs subsequently nested. Nest success at this site was the highest observed during the study, which is reflected by the importance of ‘site’ (and in association ‘year’) in nest survival models. Whilst negative effects of noisy miners are understood to be a function of their local abundance (Piper & Catterall 2003), our observations indicate the presence of a single pair of noisy miners poses a risk to regent honeyeater nest survival. Since some pairs fledged young despite the presence of noisy miners, however, the nature of interspecific interactions between the two species appears to be highly context-specific, representing an area for future study.

Our contemporary estimates of regent honeyeater nest success and productivity were lower than historical estimates for the regent honeyeater and many other honeyeater species (Table 3). Declining nest success, particularly in the Northern Tablelands, may be explained by a concurrent increase in the abundance of nest predators (Bayly & Blumstein 2001; Remes et al. 2012). Reporting rates for pied currawongs and noisy miners have increased substantially since the 1980s (Barrett et al. 2003). Unlike some species (Schmidt et al. 2006), regent honeyeaters rarely avoid nesting at sites with high perceived predator abundance, likely because a severe lack of breeding habitat makes settlement at high predation risk sites unavoidable (Gilroy & Sutherland 2007). Compounding the effects of decreased nest success, productivity of successful nests (1.58 ± 0.58 juveniles) was also lower than historical estimates (1.78 - 2.1, Table 3). Reduced nest productivity could be explained by a reduction

in nectar abundance due to senescence of food trees or mistletoe or increased interference competition from competitors such as the noisy miner (Ford et al. 1993). The decrease in the size and density of nesting aggregations could also reduce nest productivity, forcing pairs to invest more time defending nests and less time provisioning young (Ford et al. 1993).

Recent work by Taylor et al. (2018) shows that captive-bred regent honeyeaters have a 48 % lower rate of nest success than our estimate for wild conspecifics. Given substantial funds invested in the breeding and release of captive birds (Canessa et al. 2014), spatial variation in nest success and adult sex ratios have implications for the management of reintroduction efforts (Armstrong & Wittmer 2011). For instance, captive-bred females could be released strategically to stabilize sex ratios at nesting aggregations (Wedekind et al. 2002; Deredec & Courchamp 2007). Captive birds could also be released early in the breeding season at sites known to have high nest success that are occupied by wild birds. Intensive management intervention aimed at reducing nest predation at high-risk breeding sites and stabilising sex ratios may be more effective at facilitating population recovery than by releasing captive-bred birds alone.

Detailed demographic data are seldom available for rare and mobile species, yet potentially vital for their conservation (Heinsohn et al. 2015). We show that it is possible to consistently locate and monitor a substantial proportion of breeding regent honeyeaters from the early stages of nesting. Consequently, implementation of rapid conservation actions including nest protection (Major et al. 2014), competitor suppression (Debus et al. 2006; Fletcher et al. 2010) and complementary release of captive-bred birds are more achievable and necessary than previously thought (Clarke et al. 2003). Our study highlights the need for vigilance against deteriorating demographic parameters in declining populations. Comprehensive, spatially-explicit demographic data facilitates more effective investment of conservation resources, allowing high-risk areas to be identified, thus maximising the likelihood of population recovery.

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CHAPTER 5: Spatially and temporally targeted suppression of despotic noisy miners has conservation benefits for highly mobile and threatened woodland birds.

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ABSTRACT

Interactive effects of habitat loss and interspecific competition are major threats to global biodiversity. Managing disruptive competitors in modified landscapes is a conservation priority, but implementing actions to benefit rare and highly mobile species is challenging. In Australia, overabundance of hyperaggressive noisy miners following woodland fragmentation and degradation is a threatening process given their impact on songbirds including the nomadic, critically endangered regent honeyeater. Recent studies have found rapid noisy miner recolonization following their experimental removal, questioning the efficacy of miner removal as a conservation measure. We estimated the relative habitat saturation of noisy miners at a hotspot of threatened bird diversity. We then experimentally removed 350 noisy miners and assessed the effect of this removal on subsequent noisy miner abundance, relative to a control area. We monitored the occurrence of noisy miners near regent honeyeater nests and modelled the effect of noisy miner removal on songbird populations. Noisy miner removal significantly decreased noisy miner abundance throughout the breeding season, when 15-18 regent honeyeaters nested in the miner removal area. Songbird abundance and species richness increased significantly in the treatment area, relative to the control area. We provide a rare example of how spatially and temporally targeted preventative action can reduce threats for nomadic and highly threatened species during breeding and prevent ongoing avian diversity loss more broadly.

KEY WORDS

Precautionary Principle; Adaptive Management; Threatened Species; Population monitoring; Invasive species; Australia.

INTRODUCTION

Interactive effects of habitat loss and interspecific competition are major and ongoing threats to global biodiversity (Byers et al. 2002; Didham et al. 2007). Habitat loss increases niche overlap and subsequent interspecific competition for remaining resources (Scheele et al. 2017). Increases in the abundance of territorial and disruptive generalists or edge specialists (hereafter ‘despotic generalists’) following habitat loss and fragmentation can cause biotic homogenisation through competitive exclusion of smaller, rare or mobile species from habitat in which they may otherwise persist (Ford et al. 2001; Robertson et al. 2013).

Following habitat modification, the length of time that interspecific competition can affect population trends of co-occurring species is unclear (Didham et al. 2007). This uncertainty is likely because population trends of competing species can change for decades following habitat modification (Didham et al. 2007). In many modified environments, population changes due to interspecific competition are therefore likely to be ongoing (Sanderson et al. 2006). Even less clear are the circumstances under which interventions to suppress populations of despotic generalists can be successful and cost-effective (Grey et al. 1998; Davitt et al. 2018).

Highly mobile (i.e. nomadic, semi-nomadic or migratory) species pose unique challenges for conservation because predicting where and when to implement applied conservation action is difficult (Runge et al. 2014). Competitor suppression may represent wasted investment if mobile species do not subsequently occupy that location, or if competitors recolonise shortly afterwards (Stojanovic et al. 2014). Meanwhile, at locations mobile species do occupy, threats from despotic competitors continue unabated. Difficulties predicting when and where mobile species will settle, and associated risk of wasting conservation resources means these species are under-conserved and disproportionately threatened globally (Webb et al. 2014; Cottee-Jones et al. 2015). Nonetheless, competitor suppression is most likely to benefit threatened, mobile species when preventative action is taken at times and locations when both species are present, but before the negative impacts of despotic generalists have fully manifested (Cooney, 2004; Crates et al. 2017a; Leung et al. 2002; Pluess et al. 2012).

In southeast Australia, widespread and ongoing vegetation clearance has led to extreme fragmentation of lowland woodland communities (Bradshaw, 2012; Tulloch et al. 2016). The noisy miner *Manorina melanocephala*, a medium sized (~ 63 g), native generalist honeyeater occupies sparsely-vegetated habitats and has benefitted greatly from habitat fragmentation and degradation (Maron, 2007; Piper and Catterall, 2003). Noisy miners are sedentary cooperative breeders and establish colonies that aggressively exclude smaller-bodied songbirds (passerines, order Passeriformes) from potential

breeding habitat (Piper and Catterall, 2003). The presence of even small numbers of noisy miners during breeding risks decreasing reproduction of co-occurring species through nest destruction or disturbance (Thomson et al. 2015; Crates et al. in press). Severe woodland clearance and noisy miner invasion interact to homogenise bird communities via population declines of threatened woodland specialists (Ford et al. 2001; Mac Nally et al. 2012). The noisy miner is therefore listed as a key threatening process under biodiversity legislation and development of methods to reduce their impact on avian diversity is an urgent conservation priority (Threatened species scientific committee, 2014).

Recent studies have experimentally removed noisy miners to assess the viability of culling as an avian conservation measure (Davitt et al. 2018; Beggs et al. in review). A common result of these studies is rapid noisy miner recolonization, often within days, with minimal decrease in miner abundance or increase in songbird abundance (Davitt et al. 2018; Beggs et al. in review). Since earlier studies found songbird populations increased following experimental miner removal (Grey et al. 1998), the factors determining the success of noisy miner removal for avian conservation remain unclear. Here we build on recent work by experimentally removing noisy miners from a known breeding site of the critically endangered and nomadic regent honeyeater *Anthochaera phrygia*. Regent honeyeaters (contemporary population 350-500, Kvistad et al. 2015) are disproportionately impacted by the ongoing spread of noisy miners because lowland woodland clearance has led to extensive overlap between the two species' remaining breeding habitat throughout their 600,000km² range (Commonwealth of Australia, 2016; Ford et al. 2001; Ford, 2011). Where they co-occur, regent honeyeaters compete with noisy miners and other large honeyeater species for nectar and invertebrates (Ford, 1979). Increases in noisy miner abundance over the past two decades may have contributed to a decrease in regent honeyeater nesting success over this period (Crates et al. in press). Challenges associated with the regent honeyeater's small population size, vast range and irregular breeding locations have constrained attempts to implement targeted actions such as competitor suppression to aid population recovery.

We aimed to assess the effectiveness of noisy miner suppression as a means of; 1) reducing noisy miner abundance; 2) preventing and reducing competition from co-occurrence of noisy miners and regent honeyeaters during nesting; and 3) increasing songbird abundance and species richness. Based on the absence of potential source habitats for noisy miners nearby, we predicted that noisy miner removal would lead to a sustained reduction in their abundance, which would prevent their co-occurrence with any breeding regent honeyeaters. We also predicted that songbird diversity and species richness would increase following miner removal, relative to the control area.

METHODS:

Study location

The study was conducted in woodland surrounding a 7.75 km stretch of the Goulburn River in the Greater Blue Mountains, New South Wales, Australia (Figure 1). The location is typical of remaining regent honeyeater breeding habitat, with largely cleared agricultural river flats varying in width from 5 to 400 m. Regent honeyeaters breed on lower slopes and valley floors with remnant patches of box-gum *Eucalyptus* spp. woodland and riparian gallery forest (Crates et al. 2017b). We considered that all potential regent honeyeater breeding habitat (box-gum Eucalypt and riparian vegetation communities, Crates et al. in press) was also potential habitat for noisy miners, as these vegetation communities were never > 200 m from a habitat edge (Piper and Catterall, 2003). Surrounding the cleared river flats is largely continuous dry shrubby woodland. In contrast to many areas within the regent honeyeater's range, including the study areas of Davitt et al. (2018) and Beggs et al. (in review), the heavily-forested matrix surrounding the study location is unsuitable for noisy miners, which are rare in the surrounding area (Maron, 2007, Figure 1). In November 2016, a range-wide regent honeyeater monitoring program detected 4 regent honeyeater pairs breeding within the study location, all of which were frequently observed aggressively defending nests from co-occurring noisy miners (Crates et al. in press).

Pre-removal bird surveys

During the week commencing 1st August 2017, 189 monitoring sites were established within the treatment and control areas (145 treatment sites and 44 sites control sites, Figure 1). Although multiple treatment and control areas would have been desirable, the experimental design was determined by external factors including cost, the number of miners that could be removed under licence and the known distribution of breeding regent honeyeaters. Each monitoring site was a point count of the surrounding 50 m radius centred on a fixed location. Monitoring sites were spaced at least 140 m apart, firstly to account for fine-scale variation in noisy miner occupancy, habitat characteristics and associated effects on songbirds (Piper and Catterall, 2003) and second to maximise detection of regent honeyeaters given their small breeding territories (Crates et al. 2017b). During each site visit, maximum counts of noisy miners and other songbirds within each site during a 5-minute survey period were recorded. Adaptive sampling was used to add sites adjacent to those occupied by noisy miners, oriented towards the woodland interior until miners were no longer detected (Smith et al. 2004; Maron, 2007). Each site was visited twice during a 5-day period from 3-7th August 2017, prior to the removal of noisy miners. Detection probability of noisy miners ($p = 0.82$)

and other songbirds including the regent honeyeater ($p = 0.59$) using this survey design have been shown previously to be high (Crates et al. 2017b).

Noisy miner removal

Noisy miners were removed from 430 hectares of woodland within the treatment area by two licenced marksmen over a 5-day period commencing 8th August 2017. This date was specifically chosen to be as close as possible to, but before the potential arrival of any regent honeyeaters to the area (Ford et al. 1993; Crates et al. 2017b). Noisy miner calls were broadcast (Pizzey and Knight, 2014) from portable speakers to attract miners, which were subsequently removed from the treatment area using 2 x 12-gauge shotguns and size 8 shot. The treatment area was divided into 4 sections of approximately equal size and miners were removed via a daily unstructured search of each section. On the fifth day, a follow-up sweep of the entire treatment area was conducted until dusk to remove as many remaining miners as possible.

Post-removal bird surveys

Repeat site visits were made to all monitoring sites over 3 sets of 6 day periods, commencing 2 days, 1 month and 3 months after miner removal. As per pre-removal surveys, maximum counts of all songbirds detected during each repeat 5-minute site visit were recorded.

Regent honeyeater monitoring

Nesting activity of all regent honeyeaters detected (visibly or audibly) at the study location during bird surveys or opportunistically was monitored every 2-7 days by a single observer (RC). Each active regent honeyeater nest was observed for ten minutes during each visit from a distance of > 50m, to determine whether noisy miners and regent honeyeaters co-occurred during nesting (i.e. if miners were observed within 50m of an active regent honeyeater nest) and any aggressive interspecific interactions were recorded.

Statistical analysis

To estimate the extent to which the study location was saturated with noisy miners prior to their removal, a centred and scaled principal component analysis (PCA) was implemented using ‘factoextra’ v1.0.5 (Kassambara and Mundt 2017). The PCA included all habitat and vegetation covariates that were predicted to potentially influence noisy miner presence or abundance (Table 1). The first two principal components (cumulatively explaining 26.1 % of total variation) were plotted and grouped by pre-removal noisy miner presence/absence at each site using ‘ggfortify’ v0.4.3 (Tang, 2018). The 95% ellipsis was fitted to quantify the ‘effective niche space’ of noisy miners within the

study site. The relative saturation of the study site with noisy miners was estimated by calculating the proportion of monitoring sites within the 95% niche space (i.e. potential noisy miner habitat) where noisy miners were detected during pre-removal surveys.

Habitat covariates were checked for multicollinearity, but no pairs had a Pearson's $r > 0.6$. To account for variation in tree species composition, a second centred and scaled principal component analysis was implemented including the percentage cover of each tree species present within each monitoring site. The first component, explaining 11.1 % of total variation, was included in bird models as 'vegetation composition' (Figure S1).

Table 1: Description of site-level covariates tested in models of noisy miner abundance and the abundance and diversity of other songbirds before and after experimental noisy miner removal.

Covariate	Description	Justifying citation
Tree cover	Estimated % canopy cover > 4 m to nearest 5 %.	Maron et al. 2007
Shrub cover	Estimated % cover of vegetation of height 30 cm - 1.5 m to nearest 5 %.	Val et al. 2018
Grass cover	Estimated % ground cover comprised of grass to nearest 5 %.	Val et al. 2018
Mid-storey	Estimated % cover of vegetation height 1.5 - 4 m to nearest 5 %.	Maron et al. 2013
Mistletoe	Number of clumps of live mistletoe grouped into none (0), 1 - 2 plants (1), 3 - 5 plants (2), 6 - 10 plants (3), 10 - 15 plants (4), >15 plants (5).	Watson & Herring 2012
Woody debris	Amount of coarse woody debris present in survey area, grouped into none (0), light (1), moderate (2), extensive (3), very extensive (4).	Mac Nally et al. 2001
Large old trees	Number of trees present with a diameter at breast height > 80 cm.	Crates et al. 2017b
Stand age	Estimated mean age of trees to nearest 5 years.	Law et al. 2014
Flower	Eucalypt and mistletoe flower score of none (0), low (1), moderate (2), high (3). Included in saturated models as an interaction with period to account for temporal changes in flower location and abundance. Flower considered a proxy for nectar abundance.	Mac Nally & McGoldrick 1996 Crates et al. 2017b
Vegetation composition	Principal components based on proportion of all tree species present within each monitoring site, estimated to nearest 5%.	Debus 2008 Maron et al. 2011
Noisy miner	Maximum count of noisy miners across repeat site visits at each time period. Implemented in models of songbird diversity.	Maron et al. 2013 Mac Nally et al. 2012 Piper & Catterall 2003
Treatment	2 - level factor- noisy miner removal site or control site.	Davitt et al. 2018
Period	4 - level factor- pre noisy miner removal, 2 days, 1 month and 3 months post noisy miner removal.	Grey et al. 1998 Beggs et al. in revision
Location	WGS 84 decimal latitude/longitude, modelled as a smoothed bivariate term.	Webb et al. 2014

Noisy miner models

To assess the relative impact of noisy miner removal on their abundance, noisy miner abundance was modelled as a function of habitat covariates, treatment area (hereafter ‘treatment,’ and time period (hereafter ‘period’, Table 1). Modelling frameworks included general linear models (GLMs, using ‘MASS’ v7.3-50, Ripley, 2018) and generalised additive models (GAMs, using ‘mgcv’ v1.8-23, Wood, 2018). Given the clear spatial structure in the noisy miner data, spatial location was included as a smoothed bivariate term $s(Lat/Long)$ to account for the spatial autocorrelation (sensu Webb et al. 2014). The appropriate level of smoothing was selected using the *mgcv* default settings and cross validation. GLMs and GAMs were fitted with negative binomial or Poisson distributions, both with a log-link. Analysis of residuals and comparison of AICc values for the 4 model types indicated that GAMs with a Poisson distribution and including the bivariate spatial term were best suited to noisy miner data (Wood, 2017). Overall goodness of fit was assessed using % of deviance explained and R^2 . A global model was first fitted including all habitat covariates, an interaction term ‘TREATMENT x PERIOD’ and $s(Lat/Long)$. Package ‘MuMIn’ v1.40.4 (Bartoń, 2018) was used to rank all models derived from the global model by AICc. Model averaging was implemented on all models with Akaike weight (W_i) > 0.1 to obtain averaged beta coefficients for each covariate and relevant interaction terms (Burnham and Anderson, 2002). Goodness of fit of the most parsimonious GAM was assessed using function *gam.check* and correlograms of Moran’s I test for spatial autocorrelation of residuals.

Songbird models

To assess songbird responses to noisy miner removal, the same modelling procedure was repeated using songbird abundance (maximum count of each species) and species richness (sum of species detected) as response variables. Site-level abundance metrics were calculated as the maximum count of each species across repeat site-visits at each time period, summed across all relevant species. Site-level species richness indices were the same as those for abundance, replacing count data with binary presence-absence. Analysis of residuals and AICc values indicated GAMs with a negative binomial distribution, and a smoothed bivariate spatial term, were the best fit to the passerine data. Songbird abundance and species richness GAMs were first fitted for all species and subsequently for a set of functional groups therein (Table S1). Functional groups were defined based on factors known or predicted to affect bird abundance at monitoring sites in response to changes in noisy miner abundance, such as body size (Mac Nally et al. 2012) and residency status.

The effect of noisy miner removal on songbird abundance and species richness was examined in two ways: First, GAMs were fitted to all data with a TREATMENT x PERIOD interaction term, as per

noisy miner models described above. The effect size and significance estimate of this interaction term were assessed to examine the effect of noisy miner removal on songbird abundance and species richness, relative to the control area. Second, GAMs were fitted with a NOISY MINER ABUNDANCE x PERIOD interaction term on only the monitoring data from treatment area (i.e. excluding data from control area). This was designed to assess changes in the direct effect of noisy miner abundance on songbird abundance and species richness at each time period, avoiding a 3-way interaction term of NOISY MINER ABUNDANCE x PERIOD x TREATMENT. All songbird abundance and richness metrics were calculated excluding noisy miner data. All statistical analyses were implemented in R v3.4.3 (R core team, 2017).

RESULTS

Statistical analysis

Model diagnostics of the most parsimonious GAMs assessed using *gam.check* confirmed that they were an appropriate model choice in each case. Summary statistics and tests for spatial autocorrelation indicated that the spatial term in the GAMs had successfully accounted for almost all of the spatial structure in the data (Figure S1).

Noisy miner abundance

Noisy miners were initially detected at 93/187 monitoring sites. Pre-removal mean noisy miner density was 1.85 ($\pm$ sd = 0 – 2.3) / ha at occupied sites. Principal component analysis suggested noisy miners had not saturated suitable habitat within the study location prior to their removal, as miners were not detected at 40% of monitoring sites falling within their niche space (Figure 2).

Figure 1: Spatial distribution of monitoring sites at the Goulburn River study site, New South Wales, Australia. Colour shading represents the maximum count of noisy miners detected across repeat site visits at each time period, as defined in legend to right. Dashed polygon denotes control sites. Removal data shows locations within treatment area from where noisy miners were removed (not constrained to within monitoring sites).

Figure 2: Ordination scatter plot of principal component analysis of site-level habitat covariates at monitoring sites within the Goulburn River study site. Points denote the habitat composition as defined by the PCA of each monitoring site. Blue ellipsis effectively denotes 95% noisy miner ‘niche space’ within the study location.

Noisy miner abundance was significantly and negatively associated with shrub cover, mistletoe abundance, average tree (stand) age and the vegetation index, and positively associated with grass cover (Tables 2 and 3, Figure S2). They tended to occupy sites dominated by tree species including rough-barked apple *Angophora florubunda*, river she-oak *Casuarina cunninghamii*, yellow box *Eucalyptus melliodora* and grey gum *E. punctata*. (Table 3, Figure S2).

A total of 350 noisy miners were removed from the treatment area (Figure 1). Noisy miner removal led to a 40 – 45 % reduction in their presence and a 21% decrease in their mean abundance at occupied sites within the treatment area (Figures 1 and 3), which lasted the duration of the study (Figures 2 and 3, Table 3). By contrast, noisy miner abundance increased by 16% at the control area over the season (Figures 1 and 3). Relative to the control area and their pre-removal abundance, there was a significant negative effect of TREATMENT x PERIOD interaction on noisy miner abundance over all time periods (Figures 2 and 3, Table 3). This effect was strongest at 1 month, and weakest at 3 months post-noisy miner removal (Table 3).

Table 2: Best (lowest AICc, Akaike weight > 0.1) generalised additive models of noisy miner abundance before and after their experimental removal from the Goulburn River study site, New South Wales, Australia. Goodness of fit metrics for top model: R² = 0.471, deviance explained = 49.7%.

Model	DF	AIC	ΔAIC	w _i
Grass + Mid storey + Period + s(Lat,Long) + Shrub + Stand age + Treatment + Tree + Period*Treatment	40.18	1766.47	-	0.45
Grass + Mid storey + Mistletoe + Period + s(Lat,Long) + Shrub + Stand age + Treatment + Tree + Period*Treatment	41.39	1767.63	1.16	0.25
Grass + Mid storey + Period + Vegetation composition + s(Lat,Long) + Shrub + Stand age + Treatment + Tree + Period*Treatment	41.18	1768.14	1.67	0.19
Grass + Mid storey + Mistletoe + Shrub + Stand age + Period + Treatment + Tree + Vegetation composition + Period*Treatment	42.36	1679.22	2.75	0.11

Table 3: Conditional model-averaged beta coefficients of covariates included in top ranked (Akaike weight > 0.1) generalised additive models of noisy miner abundance over the course of a breeding season at the Goulburn River study site, New South Wales. Significant effects defined as p < .05 highlighted in bold.

Covariate	Factor level	B	se	Z	P
(Intercept)		1.890	0.42	4.52	<0.01
MISTLETOE		-0.142	0.03	5.04	<0.01
SHRUB		-0.034	0.01	6.13	<0.01
STAND AGE		-0.017	0.00	4.40	<0.01
PERIOD ¹	Post 2 day	-0.096	0.16	0.62	0.54
PERIOD	Post 1 month	0.056	0.15	0.37	0.71
PERIOD	Post 3 month	0.077	0.15	0.52	0.60
TREATMENT ²		-0.768	0.26	2.93	<0.01
TREE COVER		-0.010	0.00	3.31	<0.01
VEGETATION					
SPECIES		-0.341	0.04	8.36	<0.01
PERIOD *					
TREATMENT ³	Post 2 day	-0.820	0.21	3.94	<0.01
PERIOD *					
TREATMENT	Post 1 month	-0.870	0.20	4.34	<0.01
PERIOD *					
TREATMENT	Post 3 month	-0.744	0.20	3.81	<0.01
GRASS		0.007	0.01	2.69	0.01
MID-STOREY		-0.014	0.01	2.40	0.02
			edf	χ ²	P ⁴
S(Lat,Long)			27.36	242.3	<.01

¹ relative to pre-noisy miner removal. ² relative to control site. ³ relative to pre-noisy miner removal and control site. ⁴ Approximate significance of smoothed spatial term.

Figure 3: Relative changes in noisy miner abundance (mean $\pm$ 95% CI) at treatment and control areas over the study period. Estimates derived from conditional model-average of generalised additive models with Akaike weight > 0.1 . Points denote partial residuals.

Regent honeyeaters

The first regent honeyeaters were detected at the study site 2 days after miner removal ceased. Between 15 - 18 regent honeyeaters were detected during the study, potentially representing 8 – 10% of the global effective population (Kvistad et al. 2015). Whilst one colour marked individual observed in 2016 returned to breed at the treatment site in 2017, at least three others were not detected in 2016, having been colour marked in previous years >100 km away (BirdLife Australia, unpublished data). All regent honeyeaters were located within the treatment area, as were 6 associated nesting attempts. Nests were located in two aggregations, one of 4 nests in the centre of the treatment area where no co-occurrence with noisy miners was observed, and a second of two nests near the northern boundary of the treatment area. Although noisy miners co-occurred with regent honeyeaters at this second aggregation, competitive interactions between noisy miners and these two breeding pairs were only observed during 2 / 7 nest observation bouts. Three nests (including one co-occurring with noisy miners) were successful, together fledging 5 juveniles.

Songbird response

Sixty-six songbird species were detected at monitoring sites, including 8 species with a population status of ‘vulnerable’ or higher (Table S1). Whilst songbird abundance increased following the removal of noisy miners in the treatment and control areas (Table 4, Figure 4), the increase was significantly greater in the treatment area than the control area at 1 month after miner removal (Table 4). Increases in abundance were similar across all functional groups (Figure 4), suggesting that the positive effect of miner removal on songbird abundance was not solely driven by increases in small-bodied species or the subsequent return of summer migrants in spring. Effects of miner removal on songbird species richness were broadly similar to those for passerine abundance, suggesting that increases in species richness were not due to increases in the abundance of a small number of resident species (Table S2, Figure S2). Overall, the effect of miner removal on songbird abundance and species richness was significant but relatively small (Figure 4). Habitat covariates, including mistletoe and flower abundance, shrub and mid-storey cover and stand age also positively influenced songbird abundance and diversity (Tables 4 and S2). As with the previous models, the smoothed spatial location term that was used to account for spatial autocorrelation was also significant.

Table 4: Best generalised additive models (GAMs) of the effect of noisy miner removal on temporal changes in total songbird abundance and species richness at the Goulburn River study site, New South Wales. Associated beta coefficients for TREATMENT x PERIOD derived from conditional average of models with Akaike weight > 0.1. Significant effects defined as $p < .05$ highlighted in bold. Best models for functional groups are shown in Table S2 and beta coefficients for other covariates in best models are presented in Table S3. Goodness of fit metrics for abundance and species richness models, respectively: $R^2 = 0.45$ and 0.49 , deviance explained = 50.6 % and 50.9 %.

Response metric	Best model	$\Delta AICc^1$	w_i	TREATMENT x PERIOD term				
				Level	β	SE	Z	P
Abundance	Period + Flower + Shrub + Stand age + Treatment + Mistletoe + s(Lat/Long) + Period*Treatment	-0.1	0.3	Post 2 days	0.12	0.18	0.66	0.51
				Post 1 month	0.42	0.18	2.36	0.02
				Post 3 month	0.22	0.17	1.29	0.20
Species richness	Period + Flower + Shrub + Stand age + Treatment + Mistletoe + s(Lat/Long) + Period*Treatment	-0.15	0.28	Post 2 days	0.13	0.16	0.8	0.42
				Post 1 month	0.40	0.16	2.5	0.01
				Post 3 month	0.22	0.15	1.4	0.15

¹ $\Delta AICc$ presented as difference between best and second best models, hence negative values.

Figure 4: Relative temporal changes in songbird abundance at noisy miner treatment and control area on the Goulburn River, New South Wales. Estimates derived from conditional model average of models with Akaike weight > 0.1 . Points denote partial residuals. Further results shown in Table S2.

Within the treatment area, noisy miner removal led to a significant reduction in the negative effect of noisy miner abundance on overall songbird abundance. Although the effect was significant just 2 days post- miner removal, it was greatest at 1 month and 3 months post miner removal (Figure 5).

Figure 5: Effect of noisy miner abundance on overall songbird abundance before, 2 days, 1 month and 3 months following noisy miner removal within the treatment (removal) area at the Goulburn River, New South Wales.

DISCUSSION

Effective management of despotic generalists is critical to minimising biodiversity losses following habitat loss and fragmentation (Sakai et al. 2001). For the conservation of threatened and highly mobile species, robust evidence is required to ensure that competitor suppression is implemented using methods that obtain the greatest biodiversity benefits for the smallest financial and ethical costs (Beggs et al. in review). Here we report a rare contemporary example of the successful reduction in abundance of the despotic noisy miner. Preventative noisy miner suppression provided relief from a known source of nesting failure for the critically endangered and nomadic regent honeyeater at an ecologically relevant time and location. Noisy miner suppression also increased abundance and species richness of the broader songbird community.

Consistent with previous studies, noisy miners occupied sites with minimal shrub cover and young trees (Piper and Catterall 2003; Mac Nally et al. 2012). Noisy miners were positively associated with a particular tree species assemblage (Table 3, Figure S2), potentially due to the high abundance of lerp (*Psyllidae* sp.) associated with these species (Woinarski and Cullen, 1984). Principal component analysis suggested the noisy miner population had not yet saturated the habitat available to them within the study site, prior to their removal (Figure 1). Pre-removal noisy miner density was 25 - 110

% lower than at the locations of other recent studies (Davitt et al. 2018; Beggs et al. in review) and noisy miner abundance increased by 16% over the breeding season within the control area. Further, 8 threatened species were detected, which have already vanished from many parts of their former range where noisy miners are now ubiquitous (Ford et al. 2001; Ford, 2011).

In contrast to recent studies and despite lower removal effort (Davitt et al. 2018; Beggs et al. in review), removal of noisy miners here led to a significant and sustained decrease in their abundance within the treatment area. Three factors may have inhibited the success of other recent miner removal experiments. First, removal efforts in these studies were focused in highly fragmented agricultural landscapes where noisy miner abundance has increased in recent decades, facilitating rapid recolonization (Grey et al. 1998; Davitt et al. 2018; Beggs et al. in review). Second, degraded woodland remnants may no longer represent suitable habitat for specialist songbird species such as the regent honeyeater, and it is possible that no nearby source populations of other threatened species persisted to allow immigration following miner removal (Davitt et al. 2018; Beggs et al. in review). Due to the forested matrix surrounding our study site, noisy miners were uncommon in the wider landscape, minimising the opportunity for removal to be negated by immigration (Figure 2, Hanski, 1998). A third potentially critical factor is the timing of removal actions (Zavaleta et al. 2001). We specifically removed noisy miners during the early breeding season, to pre-empt the likely return date of any regent honeyeaters (Crates et al. 2017b). During this period, most noisy miners are settled into breeding territories (Dow, 1978), and opportunities to suppress subsequent population growth by minimising breeding activity is greatest.

Noisy miner suppression reduced, but did not eliminate, co-occurrence with regent honeyeaters during nesting. The presence of miners in the vicinity of two regent honeyeater nests is likely explained by the location of these nests. Pre-removal abundance of noisy miners here was very high, and although > 30 individuals were removed from this location, it is likely some likely persisted or immigrated from the control site. Given their pre-removal abundance at this location, it is likely that noisy miners would otherwise have excluded regent honeyeaters from settling in this area altogether, or disturbed their reproductive attempt (Mac Nally et al. 2012). The low level of aggressive interaction observed between noisy miners and nesting regent honeyeaters could be explained by removal efforts selectively targeting individuals with the most aggressive behavioural syndrome via their territorial response to call playback (Sih et al. 2004). Alternatively, fragmentation of the social structure of noisy miner colonies could have reduced cooperative aggression in remaining individuals (Davitt et al. 2018). Follow-up removal efforts are required to minimise noisy miner abundance and associated risk to breeding regent honeyeaters and other threatened species.

The effect of noisy miner removal on songbird abundance and richness was significant, positive but relatively small. Noisy miner density at the Goulburn River exceeded a threshold of 0.6 ha^{-1} , beyond which miners can diminish songbird abundance and species richness (Thomson et al. 2015). Yet, noisy miners did not occupy all suitable habitat (Figure 2) and their abundance here was still very low relative to other areas (Thomson et al. 2015; Davitt et al. 2018; Beggs et al. in review). This suggests that miners had not yet, or only recently, reached densities sufficient to substantially diminish the broader songbird community at the Goulburn River (Piper and Catterall, 2003; Beggs et al. In review).

In line with previous studies (Debus, 2008; Watson and Herring, 2012), songbird abundance and species richness were also positively associated with habitat complexity (shrub, mid-storey and mistletoe) as well as stand age and flower abundance (Bennett et al. 2014; Mac Nally and McGoldrick, 1996). Noisy miners were negatively associated with these habitat features, emphasising the potential double benefit of targeted habitat restoration to increase songbird abundance and decrease noisy miner abundance (Debus, 2008; Law et al. 2014).

By identifying critical locations in time and space, our study takes steps towards maximising the cost-effectiveness of targeted noisy miner suppression for conservation of threatened and highly mobile species. Although our study lacked replication at the treatment level, applied management involving lethal actions have an ethical obligation to maximise broader inferences, especially when despotic species are native (Soulé et al. 2005). Future landscape-scale removal experiments could confirm the role of the wider habitat matrix (i.e. forest extent and noisy miner abundance) and removal timing on the success of noisy miner removal actions.

Long-term, repeated removal experiments could also help quantify the importance of preventative action for conserving those species most at risk from ongoing noisy miner invasion (Cooney, 2004; Crates et al. 2017a). Experimental quantification of the importance of early intervention is extremely rare in conservation (see Thompson et al. 2000 for theoretical example). However, such evidence may be critical for justifying and funding actions to conserve the most at-risk species, where causal evidence is not only lacking, but also challenging and time-consuming to obtain (Crates et al. 2017a). For species such as the regent honeyeater, population recovery is unlikely unless the initial cause of population decline, severe habitat loss, is addressed (Caughley, 1994; Crates et al. 2017a). Short term interventions such as noisy miner removal are therefore likely to be essential for minimising interacting effects on small populations until the time restored and regenerating habitat becomes functional breeding habitat and unsuitable for despotic generalists.

Although effective in the past (Grey et al. 1998; Debus, 2008), recent research suggests high financial costs for low conservation returns makes noisy miner removal uneconomical in agri-environments

where habitats are highly degraded, miners are now widespread and threatened species have already disappeared (Beggs et al. in review). Informed by a spatially-extensive monitoring programme to locate regent honeyeaters (Crates et al. in press), we were able to identify a critical breeding area where noisy miners were at relatively low abundance, uncommon in the surrounding matrix and threatened songbird populations persist. Thus, we provide evidence that spatially and temporally targeted competitor suppression can be a viable short term preventative measure to reduce threats for rare and highly mobile species during the critical breeding period. To overcome interacting effects of habitat loss and noisy miner expansion on songbird populations, at-risk diversity hotspots should also be the focus for urgent and large-scale habitat restoration (Didham, 2007; Mortelliti et al. 2016). Together, targeted preventative competitor suppression and habitat restoration offers a promising approach to minimise Australia's avian extinction debt.

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CHAPTER 6: Impact of severe population decline on the population genomics of a critically endangered, nomadic songbird.

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Citation:

ABSTRACT

Uncovering the population genetic histories of non-model organisms is increasingly possible through advances in next generation sequencing and DNA sampling of museum specimens. This new information can inform conservation of threatened species, particularly those for which historical and contemporary population data are unavailable or challenging to obtain. The critically endangered, nomadic regent honeyeater *Anthochaera phrygia* was abundant and widespread throughout south-eastern Australia prior to a rapid population decline and range contraction since the 1970s. A current estimated population of 300–500 individuals is distributed sparsely across 600,000 km² from northern Victoria to southern Queensland. Using hybridisation RAD (hyRAD) techniques, we obtained 3,524 Single Nucleotide Polymorphisms from 64 museum specimens (date 1880–1960), 100 ‘recent’ (1989–2012) and 50 ‘current’ (2015–2016) wild birds sampled throughout the historical and contemporary range. We aimed to estimate population genetic structure, genetic diversity and population size of the regent honeyeater prior to its rapid decline. We then assessed the impact of the decline on recent and current population size, structure and genetic diversity. We found minimal population structure in the remaining regent honeyeater population, which appears to be a consequence of the species’ life-history, rather than population decline and range contraction. Population decline has led to detectable loss of genetic diversity since the 1980’s. However, capacity to quantify the extent of genetic diversity loss and population decline in a historical context was inhibited by the quality of genomic data derived from museum specimens. We discuss our results in the context of the genetic management of mobile species and enhancing the value of museum specimens in population genetic studies.

KEY WORDS

Australia; Conservation genetics; hyRAD; museum DNA.

INTRODUCTION

Large-scale habitat loss and fragmentation is a widespread and ongoing global process (Tilman et al. 2001), affecting the demographics and population genetics of species through altered patterns of dispersal, reproduction and selection (Martinez-Cruz et al. 2007). Demographic and genetic effects of habitat loss differ substantially between species, depending upon life-history traits (Romiguier et al. 2014). Species with limited dispersal capacity and short generation times are particularly susceptible to rapid loss of genetic variation within populations, increases in genetic structure among populations and reduced fitness, following anthropogenic habitat fragmentation (Frankham 2005). In contrast, highly mobile species are typically, but not universally (Barr et al. 2008), less susceptible to negative effects of habitat fragmentation on genetic diversity. This is because they often have the capacity to maintain gene flow over larger distances among patches of suitable habitat (Mason and Taylor 2015; Stojanovic et al. 2018). However, while mobile species may tolerate certain levels of structural fragmentation of habitat before population processes are impacted, they may be particularly susceptible to other environmental changes. Mobile species often depend upon a spatial network of habitats over time. Therefore, loss or degradation of critical habitat elements for mobile habitat specialists is a major risk (Runge et al. 2014). Likewise, alternative population pressures such as harvesting can have major negative impacts (Hung et al. 2014; Kamp et al. 2015). In the case that functional population connectivity is reduced, mobile species may be particularly susceptible to negative genetic effects (e.g. inbreeding depression, accumulation of deleterious mutations) once their populations become small or fragmented (Frankham 2005), as rapid decline limits opportunities to purge deleterious alleles from the population (Crnokrak and Barrett 2002).

For rare and highly mobile species, quantifying the impacts of habitat loss on demographics and population genetics is challenging (Bi et al. 2013; Stojanovic et al. 2018). Limited spatio-temporal sampling can hinder detection of the genetic effects of population decline, which may manifest before or after sampling windows (Kvistad et al. 2015; Diez del Molino et al. 2018). Similarly, obtaining sufficient contemporary samples to detect recent changes to the size and genetic composition of small and sparsely-distributed populations is challenging (Palstra & Ruzzante 2008; Athrey et al. 2012). When possible, however, understanding historical demographics can quantify anthropogenic impacts on population size (Hung et al. 2014). Because contemporary population genetics are the result of past demographic processes, combined population genetic information from historical and contemporary samples can also assist conservation efforts (Spurgin et al. 2014; Harrison et al. 2014). Such information can delineate conservation units (Mikheyev et al. 2017; Stojanovic et al. 2018), aid genetic management of captive populations (Kvistad et al. 2015), identify priority areas for habitat

restoration or translocation (Ralls et al. 2017), and accurately quantify genetic threats in high risk species (Díez-del-Molino et al. 2018).

Continuing developments in high-throughput sequencing offers increasingly greater genetic resolution to uncover genetic effects of population decline, despite small sample sizes (Ellgren 2014). Capacity to obtain SNP data from ancient DNA sources such as museum specimens offers exciting opportunities to infer the historical demographics and population genetics of threatened species, prior to their decline (Bi et al. 2013; Suchan et al. 2016; Ewart et al. 2018). However, for non-model organisms, the information inferred about past population processes using museum SNP data, along with the most robust techniques for doing so, is subject to ongoing research (Díez-del-Molino et al. 2018; Stronen et al. 2018).

The regent honeyeater *Anthochaera phrygia* is a critically endangered Australian songbird, for which contemporary population data is severely limited. Regent honeyeaters were common and widespread throughout their vast range extending from Adelaide to southern Queensland (Franklin et al. 1989, Figure 1). Historical ecological data is lacking, but records document ‘immense flocks,’ occasionally containing thousands of individuals, which undertook semi-nomadic wanderings to track seasonal shifts in the location of flowering *Eucalyptus* species (Franklin et al. 1989). Breeding regent honeyeaters specialise on woodland *Eucalyptus* species that grow on fertile soils (Franklin et al. 1989). Since the 1940s, these tree species have been extensively cleared for agriculture, with less than 5% of pre-European vegetation remaining (Bradshaw 2012). Concurrently, the regent honeyeater has undergone rapid population decline and range contraction (Peters 1979). Regent honeyeaters were extinct in South Australia by the 1980’s (Franklin and Menkhorst 1988, Figure 1). Since then, nest records have waned from traditional breeding sites in Victoria, the Australian Capital Territory, and western-central New South Wales (Commonwealth of Australia 2016). Although occasional sightings of non-breeding birds are distributed throughout their 600,000 km² range, current breeding activity is almost exclusively restricted to two regions of NSW: the greater Blue Mountains (BMTN), and the Northern Tablelands (N. NSW, Crates et al. in press, Figure 1).

Using 10 microsatellite markers and 108 samples collected throughout the species’ recent range between 1989-2012 (Figures 1 and 2), Kvistad et al (2015) found no evidence that population decline and fragmentation had led to recent population structure, estimating the effective population at 2012 to be 90-150 pairs. Whilst these results likely reflect biological reality, it is possible that loss of genetic diversity and changes in population structure were not detected because they occurred before or after the sampling period, or through a lack of genetic resolution in microsatellite markers or sample size (Kvistad et al. 2015). We build on the work of Kvistad et al (2015) to place the extant

patterns of genetic diversity and structure in the context of historical patterns in this species by: (1) incorporating 64 museum specimens sampled prior to 1960 from throughout the species' former range; (2) collecting 50 'current' samples from the remaining wild population; and (3) using next generation sequencing technology to construct and analyse a SNP database of the species. Our sampling therefore covers three distinct time periods: 'historic' (pre 1960), when the species was abundant and widespread (no robust population estimate), 'recent' (1989-2012) when the estimated population declined from 1500 to 400, and 'current' (2015-2016) with an estimated 350 individuals largely restricted to two remaining breeding regions separated by > 300km (Figures 1 and 2). Using hyRAD techniques (Suchan et al. 2016), we aimed to quantify the impact of widespread habitat loss on the population genetic structure, effective population size, and genetic diversity of the wild regent honeyeater populations over these time periods.

METHODS

Figure 1: Location of regent honeyeater DNA samples by *a-priori* population (denoted by ellipses) and sampling date. Inset: location of recent and current samples within Capertee Valley, a core remaining breeding site for the species. N.B due to map scale and spatial clustering of samples, not all individuals are visible on the map. See Table S1 and Figure S1 for further information.

Sample collection and DNA extraction

We included 230 samples in this study from 5 geographic regions (Figure 1). The collection date of 74 museum specimens from which toe-pads were sampled ranged from 1879 to 1960 (Table 1). Toe pad fragments were approximately 1.5 x 1.5 x 2 mm in size, obtained using a scalpel and stored dry in sterile sample tubes. Recent samples were sourced from Kvistad et al. (2015) in the form of extracted DNA from 104 wild birds captured from 1989-2012. We obtained current samples from 52 individuals captured in 2015 and 2016 for this study. We located regent honeyeaters by developing a range-wide survey protocol, detailed in Crates et al. (2018). We captured 48 adults and one juvenile with a single 9 m mist net. We used standard brachial venepuncture to sample blood. For 10 birds, we collected body feathers shed during the capture and handling process and from one dead nestling we obtained a tissue sample. We stored all current samples in 70% ethanol. Metadata on all samples are provided in Table S1 and Figure S1.

We conducted DNA extraction at the EcoGenomics and BioInformatics Laboratory at the Australian National University (ANU), where avian DNA extraction had not been implemented before. For aDNA extraction from museum samples and for DNA extraction from contemporary feather samples, we used Qiagen DNeasy Blood and Tissue kits (QIAGEN, California), with modifications to the manufacturer's instructions per Olah et al. (2016). DNA extraction from blood followed standard salting out protocol (Miller et al. 1988).

Probe preparation using ddRAD

Laboratory procedures followed the hybridization RAD protocol of Suchan et al. (2016) with some modifications. In order to reach the necessary amount of SNP loci, we used the 'SimRAD' package (Lepais and Weir 2014), applying in-silico digest on a simulated genome of the target species, and estimated the target size for 155-235 base pairs (bp) without adapter regions. For the ddRAD library preparation, we selected 5 samples with high quality genomic DNA (1,500 ng each), representing the species recent/current distribution, digested individually with 15 U SphI (NEB) and 15 U MluCI (NEB). After confirming digestion profiles using the LabChip GXII (Caliper Life Sciences, USA), we purified DNA using Sera-Mag SpeedBeads (GE Healthcare) in bead:sample ratio of 2:1. Following ligation of short adapters containing only primer binding sites for amplification (see online protocol), we pooled the 5 samples and bead purified as above. We used a LabChip XT fractionation system (Caliper Life Sciences, USA) to size-select the ligation product with a DNA 300 Chip to isolate fragments between 210-290 bp (including adapter regions). We PCR amplified the size-selected DNA in 20 replicates using MyTaq HS Mix (Bioline) for 38 cycles. We then pooled amplicons, and purified them with a bead: sample ratio of 1:1, quantitated with a Qubit 3.0 fluorometer (Life Technologies).

In order to yield sufficient amplified product (500-1,000 ng/capture), we re-amplified some of the PCR products, achieving a total of 30,000 ng DNA after purification. We converted the whole ddRAD library into probes by removing the short adapter sequences using the same restriction enzymes, purified with a bead:sample ratio of 1.5:1. Finally, we biotinylated the DNA fragments with DecaLabel DNA Labeling Kit (Thermo Fisher Scientific), followed by a final purification using bead:sample ratio of 1.5:1. We used these DNA probes as baits in the subsequent hybridization sequence capture.

Genomic library preparation

We prepared the whole genome library using all available samples, including those used for the ddRAD probes. We first quantitated all samples by fluorometry using the Quant-iT dsDNA Assay kit (Invitrogen). We sheared the 156 contemporary samples using a Bioruptor sonicator (Diagenode) with variable number of cycles depending on the sample age and level of degradation. We sheared samples to a mode of 300 bp and visualised by GXII chip electrophoresis. Where possible, we used the same quantity of DNA samples while preparing the whole genome library. We followed the methods of Suchan et al (2016) with some modifications to prepare the libraries. Full protocol detail can be found online (<https://protocols.io/view/hyrad-for-regent-honeyeater-mt2c6qe>).

We then hybridized the DNA probes with the whole genome library template. For hybridisation, we pooled the 230 samples in 10 groups, each containing samples of similar age. Each hybridization mix contained 500-1,000 ng of each DNA library group and 500-1,000 ng of the biotinylated probes. The hybridization buffer contained 1 mg/ml of Chicken Hybloc Cot-1 DNA (Applied Genetics Laboratories, USA), 2 μ M of each blocking oligo, 6x SSC, 50 mM EDTA, 1 % SDS, and 2x Denhardt's solution. We denatured each mix at 95 °C for 5 min and incubated at 65 °C for 48-72 hours. We captured the probes hybridized with the targeted fragments on streptavidin Dynabeads M-280 (Life Technologies) and removed target DNA from the probes and beads, purified with SSC/SDS buffer. We enriched the resultant library via 15 cycles of PCR amplification using NEBNext High-Fidelity PCR Master Mix (NEB), and performed a final purification with bead:sample ratio of 1:1 on each capture. We verified the profile and concentration of each captured library with GXII assays, and pooled the 10 hybridisations in equimolar ratios.

Scaffold sequence

We used a single contemporary sample of high molecular weight to make a 550 bp average insert library using TruSeq Nano DNA LT Kit (Illumina). We spiked this sample into the hyRAD library at approximately 40 % for the purposes of increasing the complexity of template sequence in the subsequent HiSeq lane and providing a scaffold low coverage whole genome sequence for a single

individual. We sequenced the library using 100 bp paired-end option on a single lane of the Illumina HiSeq 2500 sequencing machine at the Australian National University's Biomolecular Resources Facility.

Bioinformatics pipeline

We processed the sequenced data using the Computational Biology and Bioinformatics Unit (CBBU) Linux-based computational cluster at the Research School of Biology, ANU. A total of 504,269,284 raw sequencing reads were received. We demultiplexed barcodes using the 'fastq-multx' function of the 'ea-utils' package (Aronesty 2011), resulting in 399,241,340 unique sequences from 230 individuals, and 98,426,238 reads from the single sample whole genome. We checked raw sequencing reads for quality using FastQC (Babraham Bioinformatics 2011) and performed read trimming with Trimmomatic (Bolger et al. 2014).

We used SOAPdenovo2 (Luo et al. 2012) with kmer size of 23 to construct a whole genome scaffold for the single individual library. We polished the genome scaffold and closed gaps following the HaploMerger2 pipeline (Huang et al. 2017) using default parameters. We blasted the final scaffold against the NCBI 'nt' database and the external contamination was negligible (1.4% of reads with at least one hit).

We used the assembled contigs for reference mapping of the hyRAD unique sequences with the BWA-MEM aligner (Li 2013). We used the MarkDuplicates tool from the Picard toolkit (<http://broadinstitute.github.io/picard>) to remove PCR duplicates, and mapDamage 2.0 (Jónsson et al. 2013) to rescale base quality scores of putatively post-mortem damaged bases in the museum samples. Finally, we used Freebayes (Garrison and Marth 2012) to produce an initial set of 3,667,699 raw SNP sites. We used VCFtools (Danecek et al. 2011) to filter SNPs, retaining variants that were biallelic, present in at least 50% of individuals, had a sequencing depth between 5 and 1,000, a minimum quality score of 30, and a minimum minor allele frequency of 5%. Further, we removed indels, along with 16 samples that contained more than 90% missing SNP data. The structure of the sequenced genomic library is shown in Figure 2.

Figure 2. Structure of the sequenced genomic library.

Data analysis

Additional sample and SNP filtering

We used R v3.4.3 (R core team, 2017) for all analyses unless otherwise stated. To remove loci that were (in sequence) monomorphic, missing in > 70% of samples, under possible selection, showing linkage disequilibrium and deviating significantly from Hardy-Weinberg expectations ($p < .05$) we used package *dartR* v1.0 (Gruber and Georges 2017). Filtering steps and descriptive statistics of the filtered datasets for each analysis are provided in Table S2.

Population genetic structure

For the analyses on genetic structure, we grouped samples into two time periods: contemporary (N = 156, containing recent and current samples between 1989–2016) and historic (N = 74, containing museum samples between 1879–1960). Within these time periods, we assigned samples to five *a priori* defined populations (Figure 1), based on known breeding movements of colour-marked individuals within populations and the rarity of known movements between populations (Australian Bird and Bat Banding Service, unpublished data). A lack of a current N.VIC population reflects a failure of current monitoring to detect regent honeyeaters in this region (Crates et al. In press). We ran all analyses described below separately for the contemporary and historic datasets, unless otherwise stated.

Using poppr v2.8 (Kamvar 2018), we ran an AMOVA to determine the proportion of total molecular variance explained by differentiation between the five *a priori* populations (Figure 1), testing a null hypothesis of no genetic differentiation between them. We replaced remaining missing data with mean allele values for the whole dataset and estimated significance of genetic differentiation at the population level via 999 permutations and P-values corrected for false discovery rate (FDR). We used stAMPP v1.5.1 (Pembleton et al. 2013), to calculate Weir and Cockerham pairwise F_{ST} estimates between *a priori* populations with significance estimated via 10,000 permutations and FDR correction. We fitted an unrooted, bootstrapped ($n = 10,000$) dendrogram based on Prevosti's genetic distance (which can handle missing data) in poppr to determine whether *a priori* populations clustered by geographic distance.

We inferred population structure using fastSTRUCTURE (Raj et al. 2014). In order to choose the appropriate number of model components that explain structure in the dataset, we first ran the algorithm for multiple choices of genetic clusters (K) ranging between 1–10. Then, we used a built-in utility tool to parse through the outputs and determine the cluster size that best explained structure in the data. We visualised the expected admixture proportions inferred by fastSTRUCTURE using

Distruct plots (Rosenberg 2004). Given the sensitivity of this analysis to missing data, it was only run on the contemporary dataset.

Using adegenet v 2.1.1 (Jombart 2018), we implemented discriminant analysis of principal components (DAPC). We first allowed the package to infer the number of clusters in the data, but for subsequent analysis we based the number of clusters on the number of *a priori* populations ($n = 5$). We used the optimal *a*-score and cross-validation per Jombart (2018) to determine the number of principal components to retain, and tested the capacity of the DAPC to correctly assign individuals to populations with supplementary individuals excluded from the initial model.

A spatial principal component analysis (sPCA), mantel test, and spatial autocorrelation test were also run (see Supplementary file 1 for associated methods and results). The number of private alleles within each *a priori* historical population was counted using poppr.

Genetic diversity and effective population size

To calculate observed (H_O) and expected (H_E) individual heterozygosity and rarefied allelic richness (A_R) at each time period, we used Hierfstat v0.04-22 (Goudet and Jombart 2015). We used Mann-Whitney U-tests to compare levels of historic, recent and current observed heterozygosity between individuals, and tested pairwise differences in H_O , H_E , and A_R between the three time periods using 10,000 permutations, with P-values corrected for FDR. We used poppr to count the number of private alleles within each time period.

To estimate effective population sizes with the linkage disequilibrium (LD) method (Waples 2006), we used NeEstimator v2.1 (Do et al. 2014) with a threshold frequency of 0.02 for screening out rare alleles, assumed random mating, and 95% CIs using jackknifing among pairs of loci. We used BEAST v2.4.8 (Drummond and Rambaut, 2007) for additional, coalescent-based demographic analysis with only SNPs present in at least 70% of the samples included. We performed 10 Markov chain Monte Carlo (MCMC) runs to ensure convergence of the chains, using known sampling dates and the Jukes-Cantor substitution model. We ran each chain for 1 billion samples. After examining the likelihood traces of each chain in Tracer v1.6.0 (Rambaut et al., 2018), we chose appropriate burn-in percentages, ranging from 30–90 %, to construct extended Bayesian skyline plots.

RESULTS

The size of the whole genome scaffold of regent honeyeater was 815,195,808 base pairs organized in 265,122 contigs (N50 = 4,482 bp). After aligning the hyRAD sequences to this genome scaffold and initial filtering, the final dataset contained 3,524 SNPs for 214 individuals. Summary plots of read depth and missingness are shown in supplementary file S2.

Population structure

The contemporary dataset (recent and current samples, 1989-2016) comprised 2580 SNPs and 150 individuals, while the historic dataset (museum samples) comprised 931 SNPs and 61 individuals after filtering (Table S2).

AMOVA revealed weak but significant genetic differentiation between both contemporary and historic a priori populations (Contemporary Obs = 2.77, Simulated Obs = 6.40, $P = 0.003$, Historic Obs = 1.87, Simulated Obs = 5.71, $P = 0.003$, Figure S2). The proportion of total molecular variance attributable to variation between populations was very small (Contemporary = 0.33 %, Historic = 0.27 %). Pairwise F_{ST} statistics revealed weak but significant population differentiation between all recent and current populations, apart from between the recent and current N.NSW populations, and the recent populations of N.NSW and N.VIC (Table 1). Pairwise F_{ST} estimates were small and non-significant between all historical a priori populations, apart from between ADL / N.NSW and S.VIC / BMTN (Table 1).

Table 1. Pairwise population genetic differentiations of regent honeyeater between (A) contemporary and (B) historic *a priori* populations (Fig. 1). F_{ST} values are below horizontal and P values (FDR corrected via 10,000 permutations) are above horizontal. Sample sizes for each population are shown in parentheses. *Significant ($P < 0.05$) population genetic differentiations.

A	Recent BMTN	Recent N.NSW	Recent N.VIC	Current BMTN	Current N.NSW
Recent BMTN (56)	-	0.0160*	0.0263*	0.0000*	0.0070*
Recent N.NSW (22)	0.0009	-	0.8593	0.0000*	0.30120
Recent N.VIC (23)	0.0008	-0.0006	-	0.0000*	0.0160*
Current BMTN (37)	0.0029	0.0028	0.0026	-	0.0070*
Current N.NSW (12)	0.0019	0.0005	0.0021	0.0019	-

B	ADL	S.VIC	N.VIC	BMTN	N.NSW
ADL (13)	-	0.3226	0.3226	0.5035	0.0390*
S.VIC (22)	0.0007	-	0.2785	0.0000*	0.2785
N.VIC (7)	0.0013	0.0022	-	0.2785	0.3226
BMTN (16)	0.0000	0.0043	0.0028	-	0.3226
N.NSW (3)	0.0023	0.0065	0.0047	0.0053	-

Dendrograms revealed both historic and contemporary *a-priori* populations largely clustered by geographic distance with high bootstrap support, but divergence estimates between populations were low (Figures 1 and 3).

Figure 3: Bootstrapped dendrogram of historic (left) and contemporary (right) samples by *a-priori* population based on Prevosti's genetic distance.

When the contemporary samples were analysed in fastSTRUCTURE (i.e. only three recent and two current populations), the model complexity that maximised marginal likelihood was one ($K = 1$), and the number of components used to explain structure in the data was three ($K = 3$), indicating that true number of K can be between those values. When the results were plotted, only $K = 3$ showed detectable structure, but this was not explained by population or time groupings (Figure S3).

DAPC found weak clustering of individuals by populations in both the historic and contemporary samples (Figures 4 and 5). Discriminant analysis using ‘find.clusters’ with all principal components initially retained also supported the existence of just 1 cluster within the contemporary data (delta Bayesian Information Criterion, ΔBIC , was 2.63 for 1–2 clusters and 12.71 for 1–5 clusters). The number of retained principal components (PCs) that maximised the α -score was 15–18 and the number that minimised root mean squared error (RMSe) via cross-validation was 20. Of 50 supplementary individuals, 40% were correctly assigned by the model to their a priori populations (Figures 4 and 5). ‘Find.clusters’ also supported the existence of just one cluster in the historic data (ΔBIC was 2.01 for 1–2 clusters, and 9.83 for 1–5 clusters). The number of retained PCs that maximised the α -score and minimised RMSe was 7 and 10, respectively. Of 15 supplementary individuals, 47 % were correctly assigned to their historic a-priori population.

Figure 4: Discriminant analysis of principal component (DAPC) plots for historic (45 % cumulative variance explained) and contemporary (21 % cumulative variance explained) samples by *a-priori* population.

Figure 5: DAPC compoplots showing the probability of assignment to *a-priori* populations for historic (top) and contemporary (bottom) regent honeyeater samples.

Supplementary spatial genetic analyses yielded congruent results to those described above (Supplementary file S1).

Within the five contemporary populations, there were two private alleles each present in a single individual: in the current BMTN and current N.NSW populations, respectively. Within the historic samples, private alleles were detected in ADL (2 loci, 4 individuals), N.VIC (3 loci, 3 individuals) and S.VIC (4 loci, 8 individuals).

Genetic diversity

Post filtering, the dataset contained 1,529 SNPs and 198 individuals (Table S2). Overall missing data was 10.36% (22% missing in museum, 7% missing in contemporary samples).

Within the contemporary population, there were 2 private alleles (5 individuals) in the current and 6 private alleles (66 individuals) in the recent populations. When comparing all three time periods, there

were 2 private alleles present in 27 individuals in the contemporary population. No private alleles were found in historic or current samples.

Expected heterozygosity and allelic richness were significantly lower in the historic population than in the recent (Mann-Whitney U test: $W_{HE} = 1073900$, $P_{HE} < 0.001$; $W_{AR} = 1095600$, $P_{AR} = 0.003$) and current ($W_{HE} = 107900$, $P_{HE} < 0.001$; $W_{AR} = 10965600$, $P_{AR} = 0.003$) populations. Allelic richness was lower in the current than in recent samples, though neither AR nor HE differed significantly between the recent and current populations ($W_{HE} = 1171100$, $P_{HE} = 0.9$; $W_{AR} = 1152000$, $P_{AR} = 0.507$; Figure 6). Within the contemporary populations, AR was significantly lower in the current BMTN population than the recent BMTN population ($W = 5898000$, $P = 0.00028$), and in the current N.NSW population than the recent N.NSW population ($W = 6386000$, $P = 0.038$).

Figure 6: Spatio-temporal variation in expected heterozygosity (A and C) and allelic richness (B and D) for regent honeyeaters by time period and *a-priori* population. No current data available for N.VIC.

Effective population size

All model runs in NeEstimator resulted in infinite population size and CI. Similarly, convergence was difficult to achieve in some chains in BEAST. At all ranges of burn-in, skyline plots depicted similar trends in population size (Figure 7). All skyline plots suggested an increase in population size around 200–250 years ago. Although confidence intervals showed some evidence of a decrease in population size commencing around 35 years ago, a decrease in effective population size was not detectable.

Figure 7: Bayesian skyline plot of estimated regent honeyeater effective population size.

DISCUSSION

Using a large, spatially-stratified dataset spanning over 130 years, we conducted a comprehensive population genomic analysis of the impact of severe population decline and range contraction in the regent honeyeater. We obtained hundreds of SNPs from museum samples from as early as 1879. Despite evidence of weak population differentiation throughout their vast former range, the regent honeyeater population appears to have comprised a single, intermixing population prior to their rapid decline. Thus, we provide genetic evidence that regent honeyeaters have historically been a highly

mobile species, and that nomadic movements observed within the contemporary population are unlikely to be an obligate response to severe habitat loss. We confirm that the remaining wild population still represents a single conservation unit (Moritz 1994), despite severe population decline and range fragmentation. Population decline has also led to the erosion of genetic diversity from the contemporary population since 1989.

Despite evidence that the regent honeyeater population did, and still does, represent a single genetic unit, this does not imply that the populations were, and still are panmictic (Mills and Allendorf 1996). Complementary spatial analyses indicated a degree of weak genetic differentiation within the historic and contemporary populations. Given a minimum distance of 2,000 km between the species' historic north-south range boundary, and an historic range of over 600,000 km², a degree of historic genetic differentiation is perhaps unsurprising. Within the current samples, there was still no detectable differentiation between the Blue Mountains and northern New South Wales populations. This is despite these small breeding populations (potentially as few as 50 remaining individuals in N.NSW) being separated by over 300 km and substantial song differences between them (Powys 2012, R. Crates pers. obs). Given the rapid rate of population decline, our genetic data lend support to an unusual scenario whereby local extinction and associated range contraction may be outpacing the establishment of spatial genetic differentiation in the regent honeyeater.

Spatio-temporal patterns of genetic diversity

We found no evidence that population decline has led to loss of heterozygosity within the wild birds. However, allelic richness, a metric more sensitive to population decline (Cornuet and Luikart 1966), did show some temporal decrease. Similar to recent findings in a study of endangered grasshoppers (Schmid et al. 2018), allelic richness was lower in the current (2015-2016) regent honeyeater samples relative to the recent (1989-2012) samples. Within the recent samples, AR was also lower in the smaller a priori populations of northern Victoria and northern New South Wales than in the core Blue Mountains population. In addition, most private alleles within the contemporary populations were present in individuals sampled prior to 2012 (i.e. they were not detected in current samples). Due to the poorer quality of data derived from the historic samples, we were unable to quantify the overall magnitude of genetic diversity loss as a consequence of population decline. Measures of HO and AR were both significantly lower in the museum samples. Rather than reflecting a true biological pattern, apparent homozygote excess is likely due to allelic dropout, whereby one allele fails to amplify in degraded heterozygote samples, resulting in a bias in favour of homozygotes (Taberlet et al. 1996; Wandeler 2007).

Changes in effective population size

Despite evidently rapid and severe population decline, we were unable to derive reliable estimates of historic and current effective population sizes. Population decline may have occurred too rapidly for genetic evidence of a decline to be detectable (Wang et al. 2016). However, Kivstad et al. (2015) were able to derive plausible population estimates from the contemporary data with a limited microsatellite dataset. Simulations show that NeEstimator can derive robust population estimates using 200 SNPs (Do et al. 2014). Thus, it should have been possible to derive effective population size estimates with our dataset. Sampling error or biases associated with large SNP datasets could have led to an infinite population estimate (Do et al. 2014). Coalescent-based population estimates derived from BEAST support this supposition. There was more missing data in the historic sample, which could explain an apparent increase in population size over the past 500 years depicted by the skyline plot. Although skyline plots can be confounded by the presence of population structure (Heller et al. 2013), our spatial results suggest that this should not have biased regent honeyeater population estimates.

Implications for regent honeyeater conservation

Given the current wild regent honeyeater population represents a single conservation unit, capacity for active genetic management of the remaining population through translocation or supplementation is limited (Ralls et al. 2017). High mobility of regent honeyeaters means that artificial gene flow that could be initiated via translocation is likely to occur naturally. Yet, similar to other nomadic resource specialists like the swift parrot (Stojanovic et al. 2018), lack of population structure means the global regent honeyeater population is subject to the same threats. Regent honeyeaters appear to have no spatial buffer to prevent ongoing population decline (Crates et al. 2017). Introduction of genes from sister taxa could aid genetic rescue of another critically endangered honeyeater (Harrison et al. 2016), but this does not appear to be a feasible option for genetic management of regent honeyeaters. Preservation of adaptive potential within the remaining population would appear to be best achieved at present through indirect conservation actions, including urgent attempts to increase breeding success, breeding participation and large-scale restoration of breeding habitat (Crates et al. 2017, in press).

Implications for spatial population genetic studies using hyRAD

Genomic analysis of museum specimens is a rapidly expanding area of research (Bi et al. 2013; Suchan et al. 2016). A specific aim of our study was therefore to determine what spatio-temporal population genetic questions could be answered with a relatively large, long-term SNP dataset. We therefore present the results of all research questions that we attempted to answer, even if dataset limitations prohibited us from doing so. With 230 individuals stratified in space and time, we assumed the sample should have been sufficient to answer the questions we were unable to answer, had data quality permitted (Ryman et al. 2006).

Due to a higher proportion of missing data in museum samples, and despite rigorous filtering (O’Leary et al. 2018), the historic and contemporary datasets were not directly comparable (Supplementary file S1). Temporal sampling of individuals was non-random throughout the range, as only museum samples were available from Adelaide and southern Victoria. Separating samples by time (museum vs. contemporary) did not hinder our capacity to test for spatial population structure. At worst, the smaller number of markers in the historic dataset may have reduced the power of the historic analysis. With more than 900 SNPs, similar number of markers used in other recent studies (Schmid et al. 2018), we were still able to derive reliable spatial inferences from the museum data. However, potential allelic dropout and non-random fragmentation of aDNA likely hindered the capacity of the dataset to answer questions relating to temporal changes in genetic diversity and population size (Wang et al. 2016; Taberlet et al. 1996). Quantifying genetic diversity loss using museum specimens, though highly desirable in terms of deriving more accurate estimates of genetic threats in endangered species (Diez-del-Molino et al. 2018), appears to be an ongoing challenge (Stronen et al. 2018). Recently proposed delta methods, which account for possible spatial or temporal biases in the degree of missingness or sequence depth in SNP datasets, such as the number of heterozygous sites per genotyped sites could help derive more robust estimates of temporal genetic diversity loss in the near future (Diez-del-Molino et al. 2018).

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CHAPTER 7: CONCLUSION

The aims of this thesis were to enhance understanding of the factors driving severe and disproportionate population decline in the regent honeyeater, and to use this information to develop novel ways to prevent the species' extinction in the wild. Before this research programme commenced, standardised, spatially-extensive monitoring data on the wild regent honeyeater population was acutely lacking. This is a result of conceptions that the rarity, mobility and vast range of the regent honeyeater would make useful monitoring extremely challenging or prohibitively expensive to implement (Clarke et al. 2003). A lack of contemporary population data means the factors driving an ongoing population decline are poorly understood. Without this information, it is impossible to effectively address the suite of threats facing the species. Without real-time monitoring data, it is also impossible to address these threats at ecologically relevant times and locations (i.e. during nesting).

Based on a similar monitoring programme for swift parrots (Webb et al. 2014), we were able to rapidly and successfully implement a novel occupancy survey design that accounts for the regent honeyeater's rarity and unusual life history attributes (Crates et al. 2017a). By timing the survey period in early spring, locating small monitoring sites in known breeding habitat, using call playback and adaptive sampling, we were able to locate a large proportion of regent honeyeaters from the early stages of breeding. In addition, we were able to quantify the detectability of regent honeyeaters, giving us confidence that a spatially-extensive, rapid survey design would allow us to detect regent honeyeaters present at monitoring sites throughout their range. In combination, standardised collection of habitat covariates such as blossom and local competitor abundance and modelling approaches that account for spatial autocorrelation allowed us to infer statistically the factors driving regent honeyeater habitat selection, despite their rarity. In the long term, these data and modelling approach can help explain dynamic distributions of breeding regent honeyeaters (Webb et al. 2017), which will directly benefit conservation efforts by (1) enhancing our understanding of the fine-scale habitat features that regent honeyeaters select for breeding; (2) inform more effective habitat restoration efforts and (3) predict where regent honeyeaters may breed in future to facilitate pre-emptive conservation action. Nomadism is far from a unique avian life-history strategy, especially in Australia but also globally (Cottee-Jones et al. 2015). Our study thus provides an example of how monitoring programs could be developed to aid the conservation of other such species.

Why has the regent honeyeater suffered disproportionate population decline?

Given the regent honeyeaters' rarity, it is extremely challenging to test statistically many hypotheses which may explain why the species has suffered a disproportionate population decline (Gilroy et al.

2012). In chapter 3, I attempted to overcome some of these challenges by conducting a literature review to identify components of fitness that may be impacted negatively by a decrease in population size or density, as well as life-history traits that may make species susceptible to each component (Crates et al. 2017b). I found that, of all Australia's critically endangered birds, the regent honeyeater possesses the most life-history traits that should make the species particularly susceptible to population decline driven by Allee effects. Here I combine the findings of this review with insights from my extensive field observations of regent honeyeaters during breeding, in an attempt to explain why the regent honeyeater has suffered a disproportionate population decline. Much of the following three paragraphs is unavoidably speculative, which has prevented my capacity to discuss these ideas in previous chapters. Nonetheless, I have spent much time thinking about how the regent honeyeater's traits may interact, which I believe is worth discussing briefly here. It is my belief that interacting effects of the regent honeyeaters' body size relative to competitors and the species' specific breeding habitat requirements can to a large extent explain its imperilled population status.

Body size

Regent honeyeaters are the smallest of the 'rich patch specialists' (Ford 1979) and have evolved to live in flocks to overcome their size disadvantage (Franklin et al. 1989). When flock sizes decrease, the regent honeyeater's capacity to forage efficiently at the richest nectar sources also decreases, which is likely to impact individual survival and breeding success (Ford et al. 1993). Similarly, regent honeyeaters have evolved to nest in aggregations (Geering & French 1998; Oliver et al. 1998), which may facilitate nest defence through alarm calling and mobbing of larger predators (Serrano et al. 2005). Nesting at low density due to reduced population size and interspecific exclusion from the richest patches likely reduces regent honeyeater nest success and productivity. I have observed many regent honeyeater pairs nest in close proximity to larger species, particularly Australian magpies and noisy friarbirds. Similar to breeding behaviour of hummingbirds (Greeney et al. 2015), I consider this to be an attempt by pairs nesting at low density to indirectly obtain nest protection from these larger species defending their own nests.

Because they breed in aggregations, regent honeyeaters exhibit attraction to nesting conspecifics. However, most remaining breeding habitat patches are too small to support the number of pairs that may attempt to settle there (Fletcher 2006; Schmidt et al. 2015). The result, as shown in chapter 4, is that some pairs fail to secure a breeding territory, or intense competition between pairs may delay nesting, leading to asynchrony between nest timing and peak nectar abundance. A likely reliance on group predator mobbing during aggregative nesting has also likely minimised the strength of selection on regent honeyeaters to choose strategic (i.e. concealed and inaccessible) nest positions.

Some of the nests I have monitored have been glaringly obvious, for example in the crowns of senescing, isolated paddock trees. Given the high provisioning rates of adults (Ford et al. 1993), likely a consequence of their use of nectar as a nestling food source, it is unsurprising that such nests are readily predated. It is intriguing to speculate whether the regent honeyeater would be in such a perilous situation if, instead of weighing c40 g, mean body mass was 70g- larger than noisy miners.

Habitat specialism

Regent honeyeaters are habitat specialists during nesting (Ford et al. 2001), which means they have disproportionately suffered from the clearing of lowland woodland habitats. From observations, I believe nectar forms a particularly high proportion of the diet of nestling regent honeyeaters. This constrains breeding birds to nest sites that provide consistent nectar supplies for the duration of the nesting period at relatively small spatial scales. Regent honeyeaters rarely forage further than 200 m from their nest which, I suspect, is a much shorter distance than other breeding nectarivores have the capacity to forage at. Consistent nectar supplies may come from large, old growth box-ironbark trees with long flowering durations, or clusters of smaller trees that are asynchronous in their flowering phenology. Given extreme clearing of these habitats, intra and interspecific competition for such nest sites is very high. As a consequence, some breeding pairs fail to settle at their desired location. I have frequently observed regent honeyeaters unsuccessfully attempt to obtain nest sites in large old yellow box trees in heavy bud. By breeding in other, less productive sites, nest productivity and offspring fitness may decrease (leading to potential carry-over effects on survival, Harrison et al. 2011). In such scenarios, breeding adults frequently have to forage further from the nest, which reduces both provisioning rates and the capacity for adults to defend nests against predators. If, as is frequently the case (Crates et al. 2018), nests do fail, severe habitat loss and spatial autocorrelation in flowering phenology mean many pairs may fail to find alternative breeding sites nearby, potentially reducing the number of broods in a given season. Although not presented in this thesis, I have been collecting detailed data on fine-scale flowering phenology of regent honeyeater nests sites and the associated timing of nesting and foraging behaviour of adults. With more data from forthcoming breeding seasons, I hope to be able to test some of the hypotheses I have described above.

Despite the advances we have made in obtaining robust estimates of key population parameters, capacity to model accurately regent honeyeater population trajectory remains limited. Adult survival rates are unknown, yet could contribute significantly to population decline. Nonetheless, we have shown that low nest success and nest productivity is a clear limit to contemporary population recovery, which could be addressed. If regent honeyeater nest success rates can be artificially increased through the implementation of nest protection measures (the aim should be to ensure every

nestling hatched successfully fledges), it may be possible to sustain a small wild population until the declining population paradigm of severe breeding habitat shortage has been addressed. Consequently, the following actions are required to prevent imminent extinction of the regent honeyeater:

- Large scale, targeted habitat restoration of box-gum-ironbark woodlands and riparian zones, particularly at known breeding areas. Focus on the greater Blue Mountains area where monitoring suggests the majority of the regent honeyeater population persists.
- Legal protection of all known remaining breeding habitat, specifically including protection of the Burratorang Valley from inundation under a major infrastructure development proposal (WaterNSW 2018).
- Targeted efforts to eliminate threats from noisy miners at critical breeding sites via spatially and temporally targeted noisy miner culls.
- Targeted efforts to reduce nest predation risk from known threats via selective culling of pied currawongs, physical exclusion of brush-tailed possums from nest trees.
- Continued range-wide population monitoring to identify unknown breeding sites, implement, evaluate and refine intervention measures via adaptive management.
- More effective captive breeding strategies- releasing captive-bred individuals in the remaining core area of the Blue Mountains when and where wild birds are present.
- Trialling and development of supplementary nectar resources to facilitate nesting during drought periods when natural nectar resources are rare or absent.

Figure 1: Achieving key conservation aims for the regent honeyeater through monitoring, actions and refinement.

The regent honeyeater represents the tip of an extinction iceberg for Australia's woodland bird community (Ford et al. 2001). Many threatened species are severely under-recorded in long-term monitoring programs (Rayner et al. 2014; Lindenmayer et al. 2018). Perversely then, monitoring data is most lacking for those species for which it is most needed to inform conservation. Through targeted monitoring of the most vulnerable species, we have laid the foundations to address this data shortfall for an entire community of threatened woodland birds. In the long term, these data can be used to assess population trends of species which, like the regent honeyeater, are likely to be in decline, but for which statistical evidence of the magnitude and drivers of population change is currently lacking. Even if the regent honeyeater cannot be saved, by obtaining complementary, value-added monitoring data on other declining woodland birds, our monitoring efforts will not have been in vain.

By gathering standardised data on flowering (i.e. nectar) abundance, regent honeyeater monitoring will also facilitate research into *Eucalypt* flowering phenology at an unprecedented spatial and temporal scale. In comparison to other continents (Burgess et al. 2018), detailed phenological knowledge of ecological phenomena such as flowering, and its impact at higher trophic levels, is severely lacking in Australia. Yet, spatio-temporal variation in flowering phenology underpins the abundance, distribution and breeding activity of large numbers of woodland bird species, including the regent honeyeater (Mac Nally & McGoldrick 1996; Woinarski et al. 2000; Crates et al. 2017). Long-term, spatially-extensive monitoring of breeding swift parrots exemplifies how the delivery of effective conservation depends crucially on a thorough understanding of spatio-temporal variation in the flowering phenology of their food trees (Stojanovic et al. 2014, 2018; Webb et al. 2014, 2017, 2019).

Our spatially-extensive monitoring programme is also of great importance for negating the impacts of a major and growing threat to Australian woodland birds- the noisy miner (Mac Nally et al. 2012; Bennett et al. 2014). In chapter 5 we were able to identify a critical site where, in contrast to recent studies (Davitt et al. 2018; Beggs et al. In press), we successfully reduced the abundance of noisy miners for the duration of a regent honeyeater breeding season. This exemplifies how the success of conservation actions hinge on where and when they are implemented. Successful noisy miner suppression for the benefit of threatened woodland birds also highlights the importance of precautionary conservation action. Although a lack of historical monitoring means direct evidence is lacking, there is little doubt that spread of noisy miners through Australia's fragmented woodlands has contributed to regent honeyeater population decline (Commonwealth of Australia 2016). Indeed, a possible reason why regent honeyeaters still persist at our Goulburn River study site is that noisy miners had not yet reached a population density to exclude regent honeyeaters from breeding there. Future reactionary management following noisy miner population expansion at this location, may not only lead to the

failure of population suppression attempts, their despotic effects may have already manifested, meaning management occurs too late to benefit regent honeyeaters and other threatened woodland birds. The imperilled population status of the regent honeyeater and other threatened taxa such as the orange-bellied parrot exemplifies why action paralysis is unacceptable (Crates et al. 2017; Stojanovic et al. 2018). The development of informed, precautionary management action thus represents a critical area of future conservation research (Stojanovic et al. in review).

Concluding remarks

Our work on one of the most challenging species in Australia here demonstrates that it is entirely possible to obtain robust ecological data to inform conservation of any threatened mobile species. It has been a heartbreaking privilege to study regent honeyeaters. It is my grave fear that the species has passed the Allee threshold and that its extinction may be unavoidable. Regent honeyeaters are a flagship species for avian conservation in Australia and, unlike many other threatened fauna (Woinarski et al. 2017), have had a long-term recovery team dedicated to preventing the species' extinction. It is therefore deeply frustrating that this research and associated conservation action did not commence many years ago. Nonetheless, the next five years represent a critical period for the species' survival. We have shown that regent honeyeaters still persist, sometimes in moderate numbers, in fragments of their vast range (Crates et al. In press). It has also provided new information on how, when and where urgent conservation efforts can be implemented, and will continue to do so in future. With political will, effective legislation, sufficient funding and strong collaboration, there is still slim hope. But, the time is now.

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“One of the penalties of an ecological education is that one lives alone in a world of wounds. Much of the damage inflicted on land is quite invisible to laymen. An ecologist must either harden his shell and make believe that the consequences of science are none of his business, or he must be the doctor who sees the marks of death in a community that believes itself well and does not want to be told otherwise.”

Aldo Leopold

APPENDIX

CHAPTER 2 SUPPLEMENTARY INFORMATION

Figure S1. Detectability curve ($\pm 95\%$ CI) of regent honeyeaters in the Capertee Valley, New South Wales, Australia, spring 2015 by number of site visits, using method outlined in (Garrard et al. 2008). Constructed based on the formula $D = 1 - (1 - d)^v$, where D is the probability of detection after v visits and d is the average single-visit detectability (i.e. 0.59).

Figure S2. Frequency distribution of estimates of regent honeyeater site occupancy probability in the Capertee Valley, New South Wales, Australia, spring 2015, from models implemented in PRESENCE and SAR models implemented using the ‘spdep’ package in R.

Figure S3. Close up of north western section of study area (inset map) in the Capertee Valley, New South Wales, Australia, spring 2015, showing the distribution of occupied and unoccupied sites and nests subsequently located.

Table S1. Vegetation species associated with potentially suitable regent honeyeater habitat and their estimated nectar productivity scores (adapted from Oliver, 2000).

Species	Nectar productivity score
Mugga ironbark <i>Eucalyptus sideroxylon</i>	1.0
Yellow box <i>Eucalyptus melliodora</i>	0.9
Blakely's red gum <i>Eucalyptus blakelyi</i>	0.3
Rough barked apple <i>Angophora floribunda</i> *	N/A
River she-oak <i>Casuarina cunninghamii</i>	0.0
Needle-leaf mistletoe <i>Amyaema cambageii</i>	0.8
Slaty box <i>Eucalyptus dawsonii</i>	0.4
Brittle gum <i>Eucalyptus mannifera</i> *	N/A
White box <i>Eucalyptus albens</i>	0.9
Narrow-leaved ironbark <i>Eucalyptus crebra</i>	0.05
Box mistletoe <i>Amyema miquelii</i>	0.8

* Not recorded flowering during surveys

CHAPTER 4 SUPPLEMENTARY INFORMATION

Table S1: Breakdown of range-wide regent honeyeater monitoring sites by region and number of sites regent honeyeaters detected in 2016 and 17. Location of regions shown on Figure S1 via map codes.

State	Map code	Region	2016		2017	
			N(sites)	N(sites regent honeyeaters detected)	N(sites)	N(sites regent honeyeaters detected)
VIC	1	Chiltern-Killawarra-Benalla	79	0	79	0
QLD	2	Karara-Pikedale	12	0	16	0
NSW	3	Ashford-Emmaville	79	4	79	2
	4	Bundarra-Barraba	188	5	188	0
	5	Capertee Valley	355	20	355	34
	6	Goulburn River	61	10	60	5
	7	Limbri	18	0	9	0
	8	Lower Hunter Valley	83	0	83	1
	9	Mudgee-Wollar	17	0	17	1
	10	Pilliga-Warrumbungles	26	0	35	0
	11	Tenterfield-Torrington	18	0	18	0
	12	Widden-Baerami Valley	65	0	60	0
	13	Burraborang Valley	0	0	121	10
Total			777	39	896	53

Table S2: Covariates included in models of regent honeyeater nest survival.

Covariate	Description	Descriptive statistics	Citations*
Tree cover	Estimated % tree cover within 50 m of nest, to nearest 10%, estimated using vegetation cover with a 50m radius circle centred on the nest on GoogleEarth.	Mean = 0.48, SD = 0.23, range = 0.1-0.9.	Stojanovic et al. 2014; Major et al. 2014
Edge	Log-transformed distance to edge of continuous woodland, estimated using the ‘measure’ tool in GoogleEarth. Values are positive when nests within continuous woodland and negative when nests are within a semi-cleared agricultural matrix.	Mean = -1.04, SD = 4.30, range = -8.29 – 5.63	Newmark and Stanley 2011
Concealment	4-level factor indicating concealment of nest by surrounding 2m ³ vegetation, estimated visually from the ground: <i>Low</i> < 25% ; <i>Moderate</i> < 50% ; <i>High</i> < 75% ; <i>Very High</i> > 75%.	Median = 2, range = 1-4.	Oliver et al. 1998; Coates and Delehanty 2010
Position	3-level factor indicating location of nest within tree crown: <i>Outer</i> = outer 10% of crown; <i>Mid</i> = 11-25% of crown; <i>Inner</i> > inner 75% of crown.	Median = 2, range = 1-3.	Dinsmore and Dinsmore 2007
Height	Height of nest in metres above ground.	Mean = 13, SD = 4.69, range = 3-25.	Newmark and Stanley 2011
Camera	Presence / absence of nest monitoring camera.	Present on 14 nests.	Richardson et al. 2009
Flower	5-level factor indicating relative flower (a proxy for nectar) abundance within 100 m of nest location: 0 = none; 1 = light; 2 = moderate; 3 = high; 4 = very high.	Median = 3, range = 0-4.	Stojanovic et al. 2015; Crates et al. 2017b.
Noisy miner	Detection / non-detection of noisy miners; a hyper-aggressive and despotic competitor species, within 50 m of each active nest at any point during nest monitoring.	Present near 44/119 nests.	Piper and Catterall 2003
Temperature	Number of days during nesting period where maximum temperature exceeded 35°C.	Mean = 1, SD = 2.3, range = 0-14.	Jiguet et al. 2006; McKechnie and Wolf 2010
Conspecifics	Number of regent honeyeater nests, active synchronously for ≥ 50% of focal nest duration, within 100 m of focal nest.	Median = 0, range = 0-2.	Serrano et al. 2005
Region	2-level factor: 1 = greater Blue Mountains, 2 =	See Figure 1.	Paradis et al. 2000; Stojanovic et al. 2014
Site	9-level factor indicating nest location	See Table 5.	Geering and French 1998.
Location	Spatial location (Lat/Long) of nest.	Smoothed bivariate term- see reference.	Webb et al. 2014
Habitat type	3-level factor: 1 = box-ironbark woodland; 2 = box-gum woodland; 3 = riparian.	Box-ironbark = 22 nests, box-gum = 46 nests, riparian = 51 nests.	Geering and French 1998, Oliver et al. 1998
Nest Age	Age of nest in days since first egg.		Dinsmore and Dinsmore 2007
Time	Continuous timing of nest (Julian date) within overall breeding season.	Mean = 1 st Oct, range = 1 st Sep – 16 th Dec.	Dinsmore et al. 2002
Year	Three-level factor: 1 = 2015 2 = 2016 3 = 2017	2015 = 33 nests, 2016 = 34 nests, 2017 = 52 nests	Oliver et al. 1998

*Additional citations not in citation list:

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Table S3: Nectar sources with which regent honeyeater nesting attempts were associated throughout the species' range from 2015 to 2017.

Primary nectar resource	Frequency
Yellow box <i>Eucalyptus melliodora</i>	42
Mugga ironbark <i>E. sideroxylon</i>	13
Needle-leaf mistletoe <i>Amyema cambagei</i>	4
Yellow box + Needle-leaf mistletoe	30
Yellow box + Mugga ironbark	19
No nectar (invertebrates only)	4
Forest red gum <i>E. tereticornis</i>	3
Grey box <i>E. molluccana</i>	1
Mugga ironbark + Blakely's red gum <i>E. blakelyi</i>	1
Red stringybark <i>E. macrorhyncha</i>	1

Table S4: Site-level and annual variation in regent honeyeater nest survival within the Capertee Valley, New South Wales between 2015 and 2017, $N_e = 1562$. Beta estimates for year relative to 2015. Spatial distribution of nests shown in supporting information Figure S3.

Factor	Level (nests)	DSR	SE	$P(succeed)$	95% CI
Site	North west (9)	0.99	0.01	0.64	0.17 , 0.89
	West (47)	0.96	0.01	0.25	0.14 , 0.37
	Central (12)	0.94	0.02	0.14	0.02 , 0.36
	South (12)	0.97	0.01	0.33	0.07 , 0.63
	North (14)	0.99	0.01	0.74	0.40 , 0.91
Year	2015 (33)	0.961	0.01	0.257	0.11 , 0.42
	2016 (20)	0.955	0.01	0.212	0.08 , 0.39
	2017 (42)	0.977	0.01	0.461	0.29 , 0.61

Table S5: Beta coefficients of covariates included in top-ranked regent honeyeater nest survival models and generalised additive model (GAM), using data from 119 nests ($N_e = 1895$) monitored throughout the species' range from 2015 - 2017.

Model	Covariate	Factor level (nests)	β	SE	Lower CI	Upper CI
Nest survival	Site ^a	Goulburn River (10)	1.37	0.65	0.10	2.63*
		Barraba (4)	0.12	0.66	-1.17	1.41
		Burraborang Valley (3)	-1.44	0.94	-3.29	0.41
		Capertee: south (12)	0.82	0.61	-0.37	2.01
		Capertee: north-west (9)	1.70	0.82	0.09	3.30*
		Capertee: north (14)	2.16	0.71	0.77	3.54*
		Capertee: west (47)	0.59	0.44	-0.28	1.45
		Capertee: central (12)	0.23	0.53	-0.80	1.27
	Position ^b	mid crown (48)	0.56	0.35	-0.13	1.25
		outer crown (58)	0.95	0.48	-0.01	1.90
	Conspecific ^c	present (54)	0.64	0.25	0.15	1.14*
			β	SE	Z	P
GAM	Intercept		- 4.13	0.27	15.17	0.00*
	Position ^b	mid crown (48)	0.53	0.29	1.84	0.07
		outer crown (58)	1.22	0.39	3.10	0.00*
	Noisy miner ^e	present (44)	0.41	0.28	1.42	0.15
	$S(Lat, Long)^f$	-	edf	ref.df	χ^2	
	Pair ID	-	7.32	8.73	21.5	0.01*
			0.00	1	0	0.68

Relative to: ^aSevern River (8); ^binner crown (13); ^cno nesting conspecifics (65); ^dlow nest concealment (14); ^eNoisy miners absent (75); ^fapproximate significance of smoothed spatial term and pair ID.

*confidence intervals do not overlap 0 (nest survival models) or $p < .05$ (GAMs).

Figure S1: Location of range-wide regent honeyeater monitoring sites. Ellipses and numbers delineate survey regions as described in Table S1. Inset, study area in a national context.

Figure S2: Spatial autocorrelation in regent honeyeater nest success between a) regional aggregations range-wide (sampling distance = 50 km, n = 119 nests, see Figure 1) and b) breeding sites within the Capertee Valley (sampling distance = 0.5 km, n = 95 nests). Shaded area denotes $p > 0.05$, black points denote $p < 0.05$, grey points outside the shaded area are due lack of data at those distance classes.

Figure S3: Spatial distribution of regent honeyeater nests within the Capertee Valley in 2015-2017. Ellipses denote sites as defined in Tables 1, S4 S5 and Figure 2. Inset: location of Capertee Valley on a national scale.

Figure S4: Post-fledging survival ($\pm$ 95% CI) of all monitored juvenile regent honeyeaters (black, $n = 56$) and excluding random duplicate juveniles from the same nests (red and blue, $n = 42$).

CHAPTER 5 SUPPLEMENTARY INFORMATION:

Table S1: Species groups classifications for models assessing the effect of noisy miner removal on the abundance and distribution of songbirds at the Goulburn River, New South Wales, Australia. Body size defined as relative to body size of noisy miners. Species with state or national level population threat status of ‘vulnerable’ or greater highlighted in bold.

Resident		Migrant	
Small-bodied	Large-bodied	Small-bodied	Large-bodied*
Spotted pardalote	Spotted quail-thrush	Grey fantail	Olive-backed
Striated pardalote	Grey shrike-thrush	Silvereye	oriole
Weebill	Magpie lark	Dusky woodswallow	Cicadabird
Golden whistler	White-browed babbler	Restless flycatcher	Noisy friarbird
Buff-rumped thornbill	Eastern whipbird	Rufous whistler	Blue-faced
Yellow thornbill	Satin bowerbird	White-throated	honeyeater
Brown thornbill	Grey-crowned	gerygone	
Mistletoebird	babbler	White-winged triller	
Striated thornbill	Pied butcherbird	Leaden flycatcher	
Willie wagtail	Grey butcherbird	Yellow-faced	
Crested shrike-tit	White-bellied	honeyeater	
White-throated	cuckooshrike	White-naped	
treecreeper	Black-faced	honeyeater	
Brown-headed	cuckooshrike	Striped honeyeater	
honeyeater		Regent honeyeater	
White-eared honeyeater		Spiny-cheeked	
White-plumed		honeyeater	
honeyeater		Scarlet honeyeater	
Eastern spinebill		Little friarbird	
Fuscous honeyeater		Painted honeyeater	
Black-chinned		Fan-tailed cuckoo	
honeyeater		Bronze cuckoo sp.	
Brown treecreeper		Black-eared cuckoo	
Speckled warbler		Australian reed	
Varied sittella		warbler	
Superb fairy-wren		Rose robin	
Rockwarbler		Red-capped robin	
Yellow-rumped thornbill			
Jacky winter			
Eastern yellow robin			
Brown treecreeper			
White-browed scrubwren			
Red-browed finch			
Double-barred finch			

* Due to insufficient data, these species were grouped as ‘migrants.’

Table S2: Best generalised additive models (GAMs) of the effect of noisy miner removal on temporal changes in songbird abundance and species richness at the Goulburn River study site, New South Wales. Associated beta coefficients for TREATMENT x PERIOD derived from conditional average of models with Akaike weight > 0.1. Significant effects defined as $p < .05$ highlighted in bold.

Functional group	Response metric	Best model	$\Delta AICc_1$	W_i	TREATMENT x PERIOD term				
					Level	β	SE	Z	P
Small	Abundance	Mid-storey + Flower + Mistletoe + Period + s(Lat/Long) + Stand age + Shrub + Treatment + Tree + Period*Treatment	-0.02	0.27	Post 2 days	0.06	0.20	0.27	0.79
					Post 1 month	0.48	0.20	2.39	0.02
					Post 3 month	0.11	0.19	0.55	0.58
	Species richness	Mid-storey + Mistletoe + Flower + Period + s(Lat/Long) + Shrub + Stand age	-0.08	0.30	Post 2 days	0.07	0.18	0.36	0.72
					Post 1 month	0.42	0.18	2.35	0.02
					Post 3 month	0.15	0.17	0.87	0.39
Resident	Abundance	Mid-storey + Mistletoe + Flower + Period + s(Lat/Long) + Shrub + Stand age + Treatment + Tree + Period*Treatment	-0.78	0.37	Post 2 days	0.36	0.18	1.97	0.05
					Post 1 month	0.61	0.18	3.36	<.01
					Post 3 month	0.39	0.17	2.29	0.02
	Species richness ¹	Mid-storey + Mistletoe + Flower + Period + s(Lat/Long) + Shrub + Stand age + Treatment	-0.63	0.33	Post 2 days	0.13	0.16	0.79	0.43
					Post 1 month	0.40	0.16	2.52	0.01
					Post 3 month	0.22	0.15	1.44	0.15
Migrant	Abundance	Mid storey + Mistletoe + Period + Flower + s(Long/Lat) + Treatment + Tree + Period*Treatment	-0.43	0.38	Post 2 days	0.38	0.27	1.41	0.16
					Post 1 month	0.80	0.25	3.21	<.01
					Post 3 month	0.56	0.23	2.38	0.02
	Species richness	Mid storey + Mistletoe + Flower + Period + s(Lat/Long) + Stand age + Treatment + Period*Treatment	-0.65	0.29	Post 2 days	0.45	0.29	1.56	0.12
					Post 1 month	0.82	0.26	3.12	<.01
					Post 3 month	0.51	0.25	2.07	0.04

¹Treatment*Period not included in best models- beta coefficient therefore estimated by including Treatment*Period in the best model ($\Delta AICc = 0.17$).

Table S3: Conditional model-averaged beta coefficients of habitat covariates included in best GAMs of songbird diversity and species richness for resident, migrant and all songbird species.

Songbird group	Response metric	Covariate	β	SE	Z	P
ALL	Abundance	Mistletoe	0.171	0.018	9.32	<.01
		Shrub	0.009	0.001	5.64	<.01
		Stand age	0.008	0.002	4.33	<.01
		Flower	0.196	0.037	5.31	<.01
	Richness	Mistletoe	0.137	0.015	9.08	<.01
		Flower	0.118	0.030	3.94	<.01
		Shrub	0.007	0.001	5.31	<.01
		Stand age	0.007	0.002	4.45	<.01
SMALL	Abundance	Mid storey	0.004	0.003	1.52	0.13
		Flower	0.162	0.044	3.72	<.01
		Mistletoe	0.174	0.021	8.28	<.01
		Shrub	0.011	0.002	5.52	<.01
		Stand age	0.008	0.002	3.73	<.01
		Tree	0.008	0.002	3.89	<.01
	Richness	Mid storey	0.004	0.002	1.75	0.08
		Mistletoe	0.145	0.017	8.63	<.01
		Flower	0.106	0.034	3.09	<.01
		Shrub	0.010	0.002	6.33	<.01
		Stand age	0.008	0.002	4.32	<.01
RESIDENT	Abundance	Mistletoe	0.154	0.020	7.78	<.01
		Mid-storey	0.004	0.002	1.57	0.12
		Flower	0.113	0.041	2.76	0.01
		Shrub	0.009	0.002	4.63	<.01
		Stand age	0.006	0.002	2.75	0.01
		Tree	0.006	0.002	2.98	<.01
	Richness	Mid storey	0.003	0.002	1.63	0.10
		Mistletoe	0.143	0.018	8.15	<.01
		Flower	0.100	0.036	2.69	0.01
		Shrub	0.011	0.002	6.26	<.01
		Stand age	0.008	0.002	3.90	<.01

Figure S1: Figure S1: Correlograms of spatial autocorrelation (SAC) in: (left column): noisy miner abundance, overall songbird abundance and overall songbird species richness; (right column): residuals in the associated best GAM for noisy miner abundance, overall songbird abundance and overall songbird species richness. Significant SAC (Moran's $I > 0.05$) at each distance class depicted by red points. Non-significant SAC denoted by grey points. Also shown are adjusted R^2 and % deviance explained statistics in the title of each GAM.

Figure S2: Eigenvector loadings of tree species including in centred and scaled principal component analysis of habitat composition at the Goulburn River study site, New South Wales.

Figure S3: Relative temporal changes in songbird species richness at noisy miner treatment and control sites on the Goulburn River, New South Wales. Estimates derived from conditional model averages (models with Akaike weight > 0.1). Points denote partial residuals.

CHAPTER 6 SUPPLEMENTARY INFORMATION

Figure S1: Temporal distribution of regent honeyeater DNA samples: historic museum samples (green), recent (yellow) and current (blue).

Figure S2A: Plots of observed (black points) and simulated (grey histograms) variation within and between samples and between contemporary (recent and current) a-priori regent honeyeater populations, with FDR correction.

Figure S2B: Plots of observed (black points) and simulated (grey histograms) variation within and between samples and between historic a-priori regent honeyeater populations, with FDR correction.

Figure S3A: Complot of assignment of individuals to populations as identified by *fastSTRUCTURE* when $k = 3$.

Figure S3B: Assignment of contemporary regent honeyeater samples to time-structured population when $K = 3$ in *fastSTRUCTURE*.

Figure S3C: Assignment of contemporary regent honeyeater samples to space-structured population when $K = 3$ in *fastSTRUCTURE*.

Table S1: Regent honeyeater sample metadata.

SAMPLE ID	YEAR	POP	AGE	LOCATION	LOCATION NAME	SEX	SAMPLE TYPE	ABBBS BAND No.	COLLECTION
N001	2015	BMTN	current	Available on request	Capertee	M	BLOOD	053-11089	Australian National University
N002	2015	BMTN	current	Available on request	Capertee	M	BLOOD	053-12303	Australian National University
N003	2015	NNSW	current	Available on request	Gwydir River	M	BLOOD	053-11086	Australian National University
N004	2015	BMTN	current	Available on request	Capertee	F	BLOOD	053-12301	Australian National University
N005	2015	BMTN	current	Available on request	Capertee	M	BLOOD	053-12302	Australian National University
N006	2015	BMTN	current	Available on request	Capertee	M	BLOOD	053-11094	Australian National University
N007	2015	BMTN	current	Available on request	Capertee	F	BLOOD	053-11096	Australian National University
N008	2015	BMTN	current	Available on request	Capertee	M	BLOOD	053-11098	Australian National University
N010	2015	BMTN	current	Available on request	Capertee	M	BLOOD	053-11088	Australian National University
N011	2015	BMTN	current	Available on request	Capertee	M	BLOOD	053-11097	Australian National University
N012*	2015	BMTN	current	Available on request	Capertee	F	BLOOD	053-12315	Australian National University
N013	2015	BMTN	current	Available on request	Capertee	M	BLOOD	053-12307	Australian National University
N014	2015	BMTN	current	Available on request	Capertee	M	BLOOD	053-11095	Australian National University
N015*	2015	NNSW	current	Available on request	Lake Cathie	M	BLOOD	053-11087	Australian National University
N016	2015	BMTN	current	Available on request	Capertee	M	BLOOD	053-11093	Australian National University
N017	2015	BMTN	current	Available on request	Capertee	M	BLOOD	053-11091	Australian National University
N018	2015	BMTN	current	Available on request	Capertee	F	BLOOD	053-11090	Australian National University
N019	2015	BMTN	current	Available on request	Capertee	M	BLOOD	053-12312	Australian National University
N020	2015	BMTN	current	Available on request	Capertee	F	BLOOD	053-12310	Australian National University
N021	2015	BMTN	current	Available on request	Capertee	M	BLOOD	053-12313	Australian National University
N022	2015	BMTN	current	Available on request	Capertee	M	BLOOD	053-12308	Australian National University
N023	2015	BMTN	current	Available on request	Capertee	F	BLOOD	053-12311	Australian National University
N024	2015	BMTN	current	Available on request	Capertee	F	BLOOD	053-12314	Australian National University
N025	2015	BMTN	current	Available on request	Capertee	M	BLOOD	053-12309	Australian National University
N026	2015	BMTN	current	Available on request	Capertee	F	BLOOD	053-12304	Australian National University
N027	2015	BMTN	current	Available on request	Capertee	M	BLOOD	053-12306	Australian National University
N028	2015	BMTN	current	Available on request	Capertee	M	BLOOD	053-12305	Australian National University

N029	2015	BMTN	current	Available on request	Capertee	M	FEATHER	053-11100	Australian National University
N030	2016	BMTN	current	Available on request	Capertee	F	BLOOD	053-12316	Australian National University
N031	2016	BMTN	current	Available on request	Capertee	F	FEATHER	053-12317	Australian National University
N033	2016	BMTN	current	Available on request	Capertee	M	BLOOD	053-12319	Australian National University
N034	2016	BMTN	current	Available on request	Capertee	M	BLOOD	053-12320	Australian National University
N035	2016	BMTN	current	Available on request	Capertee	M	BLOOD	053-12321	Australian National University
N036	2016	BMTN	current	Available on request	Capertee	M	BLOOD	053-12322	Australian National University
N037	2016	BMTN	current	Available on request	Capertee	F	FEATHER	053-12323	Australian National University
N038	2016	NNSW	current	Available on request	Severn River	F	FEATHER	053-12324	Australian National University
N039*	2016	NNSW	current	Available on request	Severn River	M	BLOOD	053-12325	Australian National University
N040	2016	NNSW	current	Available on request	Severn River	F	BLOOD	053-12326	Australian National University
N041	2016	NNSW	current	Available on request	Severn River	M	FEATHER	053-12327	Australian National University
N042	2016	NNSW	current	Available on request	Severn River	M	FEATHER	053-12328	Australian National University
N043	2016	BMTN	current	Available on request	Capertee	F	BLOOD	053-12329	Australian National University
N044	2016	NNSW	current	Available on request	Severn River	M	BLOOD	053-12330	Australian National University
N045	2016	NNSW	current	Available on request	Severn River	M	FEATHER	053-12331	Australian National University
N046	2016	BMTN	current	Available on request	Capertee	F	BLOOD	053-12332	Australian National University
N047	2016	BMTN	current	Available on request	Capertee	M	BLOOD	053-12333	Australian National University
N048	2016	NNSW	current	Available on request	Barraba	M	BLOOD	053-12334	Australian National University
N049	2016	NNSW	current	Available on request	Barraba	M	FEATHER	053-12335	Australian National University
N050	2016	NNSW	current	Available on request	Severn River	U	TOE PAD	N/A	Australian National University
N051	2016	BMTN	current	Available on request	Goulburn River	M	FEATHER	053-12336	Australian National University
N052	2016	BMTN	current	Available on request	Goulburn River	M	FEATHER	053-12337	Australian National University
M001	1880	ACT	historic	-N/A	N/A	M	TOE PAD	N/A	American Museum of Natural History
M002	N/A	ADL	historic	-35.245,138.885	Strathbalbyn	U	TOE PAD	N/A	American Museum of Natural History
M003	1900	ADL	historic	-34.845,138.725	Tree Gully	M	TOE PAD	N/A	American Museum of Natural History
M004	1904	NVIC	historic	-36.061, 146.8	N/A	F	TOE PAD	N/A	American Museum of Natural History
M006	1885	BMTN	historic	-33.415, 151.379	Gosford	F	TOE PAD	N/A	American Museum of Natural History
M007	1897	BMTN	historic	-33.415, 151.379	Gosford	F	TOE PAD	N/A	American Museum of Natural History
M008	1879	BMTN	historic	-33.935, 151.129	Cooks River	F	TOE PAD	N/A	American Museum of Natural History

M009	1879	BMTN	historic	-33.935, 151.129	Cooks River	F	TOE PAD	N/A	American Museum of Natural History
M010	1898	BMTN	historic	-33.935, 151.129	Cooks River	F	TOE PAD	N/A	American Museum of Natural History
M011	1893	NVIC	historic	-35.671, 147.05	N/A	M	TOE PAD	N/A	American Museum of Natural History
M012	1916	BMTN	historic	-33.98, 151.1208	Hogarah, Sydney	M	TOE PAD	N/A	American Museum of Natural History
M013	1916	SVIC	historic	-38.071, 145.35	N/A	U	TOE PAD	N/A	American Museum of Natural History
M014	1916	SVIC	historic	-38.071, 145.35	Bimberry, Manilara	U	TOE PAD	N/A	American Museum of Natural History
M015	NA	BMTN	historic	-33.760, 150.780	St. Marys	M	TOE PAD	N/A	American Museum of Natural History
M016	NA	BMTN	historic	-33.760, 150.781	St. Marys	M	TOE PAD	N/A	American Museum of Natural History
M017	NA	BMTN	historic	-33.760, 150.782	St. Marys	M	TOE PAD	N/A	American Museum of Natural History
M018	NA	BMTN	historic	-33.760, 150.783	St. Marys	F	TOE PAD	N/A	American Museum of Natural History
M019	NA	BMTN	historic	-33.760, 150.783	St. Marys	F	TOE PAD	N/A	American Museum of Natural History
M020	NA	NNSW	historic	-30.755631,152.9759	N/A	F	TOE PAD	N/A	American Museum of Natural History
M021	NA	ACT	historic	N/A	N/A	U	TOE PAD	N/A	American Museum of Natural History
M022	1909	ADL	historic	-35.0290, 138.6	Blackwood	M	TOE PAD	N/A	American Museum of Natural History
M024	1901	SVIC	historic	-37.986, 145.215	Dandenong	M	TOE PAD	N/A	American Museum of Natural History
M025	1901	SVIC	historic	-37.986, 145.215	Dandenong	F	TOE PAD	N/A	American Museum of Natural History
M026	1908	SVIC	historic	-37.856, 145.275	Bayswater	F	TOE PAD	N/A	American Museum of Natural History
M028	1905	SVIC	historic	-37.6866, 144.575	Melton	M	TOE PAD	N/A	American Museum of Natural History
M029	1897	SVIC	historic	-37.726, 144.575	Castlemaine	U	TOE PAD	N/A	American Museum of Natural History
M030	1897	SVIC	historic	-37.726, 144.575	Castlemaine	U	TOE PAD	N/A	American Museum of Natural History
M031	1897	SVIC	historic	-37.726, 144.575	Castlemaine	U	TOE PAD	N/A	American Museum of Natural History
M032	1914	SVIC	historic	-37.876, 145.095	Ashburton	M	TOE PAD	N/A	American Museum of Natural History
M033	1914	SVIC	historic	-37.876, 145.095	Ashburton	M	TOE PAD	N/A	American Museum of Natural History
M034	1914	SVIC	historic	-37.876, 145.095	Ashburton	M	TOE PAD	N/A	American Museum of Natural History
M035	1914	SVIC	historic	-37.876, 145.095	Ashburton	F	TOE PAD	N/A	American Museum of Natural History
M036	1914	SVIC	historic	-37.876, 145.095	Ashburton	F	TOE PAD	N/A	American Museum of Natural History
M037	1914	SVIC	historic	-37.876, 145.095	Ashburton	F	TOE PAD	N/A	American Museum of Natural History
M038	1898	SVIC	historic	-38.276, 145.595	Lang Lang	M	TOE PAD	N/A	American Museum of Natural History
M039	1895	SVIC	historic	-38.076, 144.295	Moorabool	U	TOE PAD	N/A	American Museum of Natural History
M040	1908	SVIC	historic	-37.916, 145.155	Mulgrave	M	TOE PAD	N/A	American Museum of Natural History

M041	1898	ACT	historic	-N/A	N/A	F	TOE PAD	N/A	American Museum of Natural History
M042	1966	QLD	historic	-28.23, 151.75	Greymare	M	TOE PAD	N/A	Queensland Museum
M045	N/A	QLD	historic	-26.74, 150.65	Chinchilla	M	TOE PAD	N/A	Queensland Museum
M047	1919	ADL	historic	-35.050, 138.7	Clarendon,	M	TOE PAD	N/A	South Australia Museum
M048	1919	ADL	historic	-35.150, 138.6	Happy Valley	F	TOE PAD	N/A	South Australia Museum
M049	1917	ADL	historic	-35.0290, 138.6	Blackwood	M	TOE PAD	N/A	South Australia Museum
M050	1926	ADL	historic	-35.69, 137.59	Kangaroo Island	M	TOE PAD	N/A	South Australia Museum
M051	1918	ADL	historic	-35.0290, 138.6	Blackwood	M	TOE PAD	N/A	South Australia Museum
M052	1918	ADL	historic	-35.0290, 138.6	Blackwood	F	TOE PAD	N/A	South Australia Museum
M053	NA	ADL	historic	-34.60, 138.75	Gawler	U	TOE PAD	N/A	South Australia Museum
M054	1914	ADL	historic	-35.0605, 138.68	Cherry Gardens	M	TOE PAD	N/A	South Australia Museum
M055	NA	NVIC	historic	-35.75, 147.29	Holbrook	U	TOE PAD	N/A	South Australia Museum
M056	1936	ADL	historic	-35.0605, 138.59	Coromandel Valley	F	TOE PAD	N/A	South Australia Museum
M057	NA	ADL	historic	-35.380, 138.63	Square waterhole	M	TOE PAD	N/A	South Australia Museum
M058	1894	BMTN	historic	-33.790, 150.941	Toongabbie	F	TOE PAD	N/A	South Australia Museum
M059	1900	BMTN	historic	-33.85, 151.13	Abbotsford	M	TOE PAD	N/A	Museum Victoria
M060	1906	BMTN	historic	-33.92, 151.03	Bankstown	M	TOE PAD	N/A	Museum Victoria
M061	1909	SVIC	historic	-37.65, 145.52	Healesville	M	TOE PAD	N/A	Museum Victoria
M062	1903	SVIC	historic	-37.72, 145.15	Eltham	M	TOE PAD	N/A	Museum Victoria
M063	1907	SVIC	historic	-37.05, 144.8	Tooborac	F	TOE PAD	N/A	Museum Victoria
M064	1905	SVIC	historic	-37.52, 145.13	Whittlesea	M	TOE PAD	N/A	Museum Victoria
M065	1931	NVIC	historic	-36.47, 147.25	Eskdale district	U	TOE PAD	N/A	Museum Victoria
M067	1917	BMTN	historic	-34.05, 151.15	Sutherland shire	F	TOE PAD	N/A	Australian Museum
M072	1912	BMTN	historic	-33.783, 150.95	Parramatta	M	TOE PAD	N/A	Australian Museum
R041	1996	BMTN	recent	-33.08327, 150.18502	Capertee**	M	BLOOD	7-rhe orange	Museum Victoria
R050	1996	BMTN	recent	-32.4, 149.87	Cumbo Road	F	BLOOD	041-57207	Museum Victoria
R073	1997	NNSW	recent	-30.3, 150.79	Armidale	F	BLOOD	042-03907	Museum Victoria
N009	2015	BMTN	new	-33.01383, 150.03304	Capertee	M	BLOOD	053-11099	Australian National University
R001	1995	NNSW	recent	-30.435, 151.225	Armidale	U	BLOOD	041-48942	Museum Victoria
R002	1995	NNSW	recent	-30.43, 151.22	Armidale	U	BLOOD	041-48943	Museum Victoria

R003	1996	NNSW	recent	-30.43, 151.22	Armidale	M	BLOOD	041-48945	Museum Victoria
R004	1996	NNSW	recent	-30.43, 151.22	Armidale	M	BLOOD	041-48946	Museum Victoria
R005	1995	NNSW	recent	-30.436, 151.217	Armidale	U	BLOOD	041-48951	Museum Victoria
R006	1995	NNSW	recent	-30.43, 151.22	Armidale	F	BLOOD	041-48952	Museum Victoria
R007	1995	NNSW	recent	-30.43, 151.22	Armidale	M	BLOOD	041-48953	Museum Victoria
R008	1996	NNSW	recent	-30.43, 151.22	Armidale	M	BLOOD	041-48985	Museum Victoria
R009	1995	BMTN	recent	-33.04627, 150.16257	Capertee	M	BLOOD	041-57159	Museum Victoria
R010	1995	BMTN	recent	-33.04627, 150.16257	Capertee	M	BLOOD	041-57161	Museum Victoria
R011	1995	BMTN	recent	-33.04627, 150.16257	Capertee	M	BLOOD	041-57162	Museum Victoria
R012	1995	BMTN	recent	-33.04627, 150.16257	Capertee	M	BLOOD	041-57163	Museum Victoria
R013	1995	BMTN	recent	-33.04627, 150.16257	Capertee	F	BLOOD	041-57164	Museum Victoria
R014	1995	BMTN	recent	-33.04627, 150.16257	Capertee	M	BLOOD	041-57165	Museum Victoria
R015	1995	BMTN	recent	-33.04627, 150.16257	Capertee	M	BLOOD	041-57166	Museum Victoria
R016	1995	BMTN	recent	-33.04627, 150.16257	Capertee	M	BLOOD	041-57167	Museum Victoria
R018	1995	BMTN	recent	-33.04627, 150.16257	Capertee	M	BLOOD	041-57169	Museum Victoria
R019	1995	BMTN	recent	-32.95627, 150.10257	Capertee	M	BLOOD	041-57170	Museum Victoria
R020	1995	BMTN	recent	-32.95627, 150.10257	Capertee	F	BLOOD	041-57171	Museum Victoria
R021	1995	BMTN	recent	-32.95627, 150.10257	Capertee	M	BLOOD	041-57173	Museum Victoria
R022	1995	BMTN	recent	-33.01756, 150.03326	Capertee	F	BLOOD	041-57176	Museum Victoria
R023	1995	BMTN	recent	-33.01756, 150.03326	Capertee	M	BLOOD	041-57177	Museum Victoria
R024	1995	BMTN	recent	-33.01756, 150.03326	Capertee	F	BLOOD	041-57178	Museum Victoria
R025	1995	BMTN	recent	-33.01756, 150.03326	Capertee	M	BLOOD	041-57179	Museum Victoria
R026	1995	BMTN	recent	-33.01756, 150.03326	Capertee	M	BLOOD	041-57180	Museum Victoria
R027	1995	BMTN	recent	-33.01756, 150.03326	Capertee	M	BLOOD	041-57181	Museum Victoria
R028	1995	NVIC	recent	-36.15, 146.65	Chiltern	U	BLOOD	041-87302	Museum Victoria
R029	1995	NVIC	recent	-36.154, 146.645	Chiltern	U	BLOOD	041-87303	Museum Victoria
R030	1995	NVIC	recent	-36.15, 146.65	Chiltern	U	BLOOD	041-87305	Museum Victoria
R031*	1995	NVIC	recent	-36.158, 146.605	Chiltern	U	BLOOD	041-87306	Museum Victoria
R032	1995	ACT	recent	-35.265, 149.175	Canberra	F	BLOOD	041-87307	Museum Victoria
R033	1995	ACT	recent	-35.265, 149.175	Canberra	M	BLOOD	041-87308	Museum Victoria

R034	1995	ACT	recent	-35.265, 149.175	Canberra	M	BLOOD	041-87309	Museum Victoria
R035	1995	ACT	recent	-35.265, 149.175	Canberra	F	BLOOD	041-87310	Museum Victoria
R036	1995	ACT	recent	-35.265, 149.175	Canberra	M	BLOOD	041-87311	Museum Victoria
R037	1995	ACT	recent	-35.265, 149.175	Canberra	F	BLOOD	041-87312	Museum Victoria
R038*	1995	ACT	recent	-35.265, 149.175	Canberra	M	BLOOD	041-87313	Museum Victoria
R039	1996	BMTN	recent	-33.08327, 150.185025	Capertee**	F	BLOOD	2-rhe yellow	Museum Victoria
R040	1996	BMTN	recent	-33.08327, 150.185025	Capertee**	F	BLOOD	4-rhe white	Museum Victoria
R042	1996	BMTN	recent	-33.08327, 150.185025	Capertee**	M	BLOOD	8-rhe yellow/orange	Museum Victoria
R043	1996	BMTN	recent	-33.08327, 150.185025	Capertee**	F	BLOOD	9-rhe black	Museum Victoria
R044	1996	BMTN	recent	-36.2, 146.7	Chiltern**	M	BLOOD	1-rhe red	Museum Victoria
R045	1996	BMTN	recent	-36.2, 146.7	Chiltern**	F	BLOOD	3-rhe blue	Museum Victoria
R046	1996	BMTN	recent	-36.2, 146.7	Chiltern**	F	BLOOD	5-rhe purple	Museum Victoria
R047	1996	BMTN	recent	-36.2, 146.7	Chiltern**	M	BLOOD	6-rhe light green	Museum Victoria
R048	1996	BMTN	recent	-32.4, 149.87	Cumby Rd, NSW	F	BLOOD	041-57204	Museum Victoria
R049	1996	BMTN	recent	-32.4, 149.87	Cumby Rd, NSW	M	BLOOD	041-57206	Museum Victoria
R051	1996	BMTN	recent	-32.25, 150.05	Goulburn River NP	M	BLOOD	041-57208	Museum Victoria
R052	1996	BMTN	recent	-32.25, 150.05	Goulburn River NP	M	BLOOD	041-57209	Museum Victoria
R053	1996	BMTN	recent	-32.389, 149.835	Munghorn East	M	BLOOD	041-57205	Museum Victoria
R054	1996	NNSW	recent	-30.43, 151.22	Armidale	M	BLOOD	041-48948	Museum Victoria
R055	1995	BMTN	recent	-33.08327, 150.185025	Capertee	M	BLOOD	041-57172	Museum Victoria
R056	1995	BMTN	recent	-33.08327, 150.185025	Capertee	U	BLOOD	041-57183	Museum Victoria
R057	1997	NVIC	recent	-36.15, 146.65	Chiltern	U	BLOOD	041-87401	Museum Victoria
R058	1997	NVIC	recent	-36.15, 146.65	Chiltern	M	BLOOD	041-87402	Museum Victoria
R059	1997	NVIC	recent	-36.15, 146.65	Chiltern	M	BLOOD	041-87403	Museum Victoria
R060	1997	NVIC	recent	-36.15, 146.65	Chiltern	U	BLOOD	041-87404	Museum Victoria
R061	1997	NVIC	recent	-36.15, 146.65	Chiltern	M	BLOOD	041-87405	Museum Victoria
R062	1997	NVIC	recent	-36.15, 146.65	Chiltern	U	BLOOD	041-87406	Museum Victoria
R063	1997	NVIC	recent	-36.15, 146.65	Chiltern	F	BLOOD	041-87407	Museum Victoria
R064	1997	NNSW	recent	-30.43, 151.22	Armidale	M	BLOOD	042-03910	Museum Victoria
R065	1995	NNSW	recent	-30.43, 151.22	Armidale	U	BLOOD	041-48912	Museum Victoria

R066	1996	NNSW	recent	-30.43, 151.22	Armidale	M	BLOOD	041-48983	Museum Victoria
R067	1997	NNSW	recent	-30.415, 151.165	Armidale	F	BLOOD	041-48986	Museum Victoria
R068	1997	NNSW	recent	-30.43, 151.22	Armidale	F	BLOOD	041-48987	Museum Victoria
R069	1995	NVIC	recent	-36.15, 146.65	Chiltern	U	BLOOD	041-87301	Museum Victoria
R070	1995	NVIC	recent	-36.15, 146.65	Chiltern	U	BLOOD	041-87304	Museum Victoria
R071	1997	NNSW	recent	-30.3, 150.79	Armidale	M	BLOOD	042-03901	Museum Victoria
R072	1997	NNSW	recent	-30.3, 150.79	Armidale	U	BLOOD	042-03902	Museum Victoria
R074	1997	NNSW	recent	-30.3, 150.79	Armidale	M	BLOOD	042-03908	Museum Victoria
R076	1997	NNSW	recent	-30.3, 150.79	Armidale	M	BLOOD	042-03911	Museum Victoria
R077	1997	NNSW	recent	-30.3, 150.79	Armidale	U	BLOOD	042-03912	Museum Victoria
R078	1997	NNSW	recent	-30.3, 150.79	Armidale	F	BLOOD	042-03914	Museum Victoria
R079	1997	NNSW	recent	-30.3, 150.79	Armidale	M	BLOOD	042-03915	Museum Victoria
R080	1989	ACT	recent	-35.185, 149.25	Sutton	F	BLOOD	f724	Museum Victoria
R081	1989	ACT	recent	-35.185, 149.25	Sutton	M	BLOOD	f725	Museum Victoria
R082	2011	BMTN	recent	-33.15327, 150.135025	Capertee	F	BLOOD	042-99755	Museum Victoria
R083	2011	BMTN	recent	-33.15327, 150.135025	Capertee	M	BLOOD	042-99757	Museum Victoria
R084	2011	BMTN	recent	-33.15327, 150.135025	Capertee	M	BLOOD	042-99703	Museum Victoria
R085	2011	BMTN	recent	-33.15327, 150.135025	Capertee	M	BLOOD	042-99704	Museum Victoria
R086	2011	BMTN	recent	-33.15327, 150.135025	Capertee	F	BLOOD	042-99753	Museum Victoria
R087	2011	NVIC	recent	-36.18, 146.775	Indigo Valley	M	BLOOD	042-99754	Museum Victoria
R088	2011	NVIC	recent	-36.6, 146.1	Lurg	M	BLOOD	042-99705	Museum Victoria
R089	2012	BMTN	recent	-33.10327, 150.205025	Capertee	M	BLOOD	042-99767	Museum Victoria
R090	2012	BMTN	recent	-33.10327, 150.205025	Capertee	M	BLOOD	042-99768	Museum Victoria
R091	2012	BMTN	recent	-33.11127, 150.265025	Capertee	M	BLOOD	042-99769	Museum Victoria
R092	2012	BMTN	recent	-33.11127, 150.265025	Capertee	M	BLOOD	042-99773	Museum Victoria
R093	2012	BMTN	recent	-33.11127, 150.265025	Capertee	M	BLOOD	042-99774	Museum Victoria
R094	2012	BMTN	recent	-33.11127, 150.265025	Capertee	M	BLOOD	042-99775	Museum Victoria
R095	2012	BMTN	recent	-33.11127, 150.265025	Capertee	F	BLOOD	042-99776	Museum Victoria
R096	2012	BMTN	recent	-33.11127, 150.265025	Capertee	M	BLOOD	042-99777	Museum Victoria
R097	2012	BMTN	recent	-32.915, 151.25	Quorrobolong	M	BLOOD	042-99758	Museum Victoria

R098	2012	BMTN	recent	-32.915, 151.25	Quorrobolong	M	BLOOD	042-99759	Museum Victoria
R099	2012	BMTN	recent	-32.915, 151.25	Quorrobolong	M	BLOOD	042-99761	Museum Victoria
R100	2012	BMTN	recent	-32.915, 151.25	Quorrobolong	M	BLOOD	042-99762	Museum Victoria
R101	2012	BMTN	recent	-32.915, 151.25	Quorrobolong	F	BLOOD	042-99763	Museum Victoria
R102	2012	BMTN	recent	-32.915, 151.25	Quorrobolong	M	BLOOD	042-99764	Museum Victoria
R103	2012	BMTN	recent	-32.915, 151.25	Quorrobolong	F	BLOOD	042-99765	Museum Victoria
R104	2012	BMTN	recent	-32.915, 151.25	Quorrobolong	M	BLOOD	042-99766	Museum Victoria
* Denotes sample included in ddRAD									
** Denotes wild bird captured for captive breeding									

Table S2: Overview of filtering steps and summary data used for each regent honeyeater population genomic analysis.

				SNPs		SAMPLES		
Analysis	Time period	Function	threshold	removed	remaining	removed	remaining	% missing
AMOVA, Pairwise F _{st} , Private alleles, DAPC, sPCA, Correlograms, Mantel tests, Ne Estimates	Recent and current	gl.filter.monomorphs	n/a	0	3524	0	153	18.95
		gl.filter.callrate (loc)	0.7	823	2701	0	153	13.74
		gl.filter.callrate (ind)	0.3			3	150	12.31
		gl.outflank	0.05	12	2689			
		gl.filter.hwe	0.05	0	2689			
	Historic	gl.filter.monomorphs	n/a	473	3051	0	61	52.15
		gl.filter.callrate (loc)	0.65	2120	931	0	61	13.31
		gl.filter.callrate (ind)	0.4	0	931	0	61	13.31
		gl.outflank	0.05	16	915	0	61	13.31
		gl.filter.hwe	0.05	0	915	0	61	13.31
H _{obs} , H _{exp} , A _r , Private alleles	All	gl.filter.monomorphs	n/a	0	3524	0	214	29.93
		gl.filter.callrate (loc)	0.7	1980	1544	0	214	14.27
		gl.filter.callrate (ind)	0.5	0	1544	16	198	10.36
		gl.outflank	0.05	15	1529	0	198	10.36
		gl.filter.hwe	0.05	0	1529	0	198	10.36

SUPPLEMENTARY FILE S1: Additional regent honeyeater spatial population genomic analysis.

METHODS: A spatial principal component analysis was implemented using package *adegenet* v2.1.1 (Jombart 2018). A neighbourhood by distance connection network (Type = 5) was used, given the spatial distribution of samples throughout the range. Isolation by distance was tested based on Euclidean geographic distance and Prevosti's genetic distance matrices calculated in *poppr*. Mantel tests of these data were used to test significance of isolation by distance, first in the historic (museum) data and then in the contemporary (recent and current) data with 1000 permutations using *ade4* v1.7-10 (Chessel et al. YEAR). Spatial autocorrelation in genetic distance was tested using *GENALEX* v6.501 (Peakall and Smouse 2006, 2012), with confidence intervals based on 1000 bootstraps.

RESULTS: Spatial PCA found no evidence of any historical or contemporary spatial genetic clines within the species' range (Figure 1). Mantel tests revealed weak but significant negative genetic differentiation by distance in both the historic and contemporary populations (historic, observation = -0.1399071, simulated P = 0.034, contemporary observation = -0.111, simulated P = 0.055, Figure 2). There was no strong pattern of spatial autocorrelation in genetic distance at any time period (Figure 3).

Figure 1: Spatial PCA with interpolated map of regent honeyeater genetic differentiation across the species' range by time period. Note variable scales of axes due to concurrent regent honeyeater range contraction over time.

Figure 2: Isolation by distance plots (left column) for contemporary (recent and current) and museum reagent honeyeater samples. Also shown are histograms of mantel test. Observed statistic denoted by black diamond and line.

Figure 3: Spatial autocorrelation in regent honeyeater population genetic structure through time. Note variable scale of x-axis, representing concurrent range contraction. Blue line denotes r -values at each distance class, error bars denote 95% confidence intervals. Dashed red lines denote bootstrapped upper and lower 95% confidence intervals.

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SUPPLEMENTARY FILE S2: SNP dataset summary statistics.

Figure 1: Histogram of % missingness per sample_UNF

Figure 1: Histogram of % missingness of the regent honeyeater hyRAD dataset.

Figures 2 and 3: % Data missingness and read depth by sample. Sample IDs on X-axes starting with 'm' denote museum samples

ADDITIONAL WORK CONDUCTED DURING PhD ENROLMENT

Publications

Crates, R. A., Garroway, C. J., and Sheldon, B. C. (2015). Causes and consequences of individual variation in the extent of post-juvenile moult in the blue tit *Cyanistes caeruleus* (Passeriformes: Paridae). *Biological Journal of the Linnean Society* 116: 341-351.

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Austral Ecology: 1

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Frontiers in Ecology and Evolution: 1

Oikos: 1

Emu: 2

Ringling and Migration: 1

Conferences

Conservation in action, Bathurst, 2018

Talks

Conservation in Action conference, May 2018

Canberra Ornithologists Group, February 2018

Hunter Bird Observers Club, March 2018

Birding New South Wales, March 2017

BirdLife Australia environmental policy forum, May 2018

Cover images for our articles in The Journal of Wildlife Management 2017, © Dean Ingwersen and EMU 2017, © Murray Chambers.

Regent honeyeater feeding 2 juveniles, Bogee, Capertee Valley 2017. ©Mick Roderick

Incubating female, Capertee National Park 2015. © Nathan Sherwood.

A selection of the regent honeyeaters that contributed to this thesis. I am extremely grateful to each and every one of them for still being alive and allowing me to find them.