Adaptive Management of the Regent Honeyeater: Song Culture and Conservation

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CANDIDATES DECLERATION

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 my knowledge, it contains no material previously published or written by another person, except where due reference is made in the text. This thesis is primarily my own research and for all chapters I was the first author and principal contributor, but the research was part of a collaborative project. I use “we” whenever I am referring to a collaborative piece of work. Due acknowledgement is made to co-authors at the beginning of each chapter.

Daniel Appleby

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Abstract

Captive breeding for release is an increasingly important tool in the conservation of species. Despite ever more rigorous management, the success of translocations, population supplementations and re-introductions remains variable. A suite of factors may negatively impact the success of these programs, amongst these are a divergence between captive and wild animal cultures. Animal cultures are behaviours which are acquired from conspecifics through some form of social learning and maintained at a population or community level. In recent years the importance of animal cultures has been increasingly recognised in conservation.

A key example of animal culture is provided in the context of birdsong where some 40% of bird species learn their songs. Birdsong has important functions in territory establishment, pair formation and group cohesion, and as such impacts individual fitness. A divergence between captive and wild song cultures, therefore, may hinder the efforts of reintroductions. In such cases, adaptive management of captive breeding programs is necessary to identify and reduce the divide between captive and wild song cultures. The critically endangered regent honeyeater (*Anthochaera phrygia*) provides a clear example where captive breeding and release is critical to the recovery of the species, but song culture differs between captive and wild populations. This thesis explores the song culture of the regent honeyeater, both in the wild and captivity. It assesses the fitness implications to individuals and the risks to the success of the reintroduction program and provides a rare demonstration of cultural restoration within an applied conservation breeding setting.

In chapter 2, I review the literature surrounding animal cultures, and the implications for the preservation and restoration of animal cultures. In chapter 3 I (with my colleagues) tested the song preferences of zoo-bred females. Females were presented with bouts of familiar zoo-bred songs, unfamiliar wild-type songs, and the songs of another species. We found that female zoo-bred birds

showed a significant preference for the songs of zoo-bred males over the songs of wild males, across all measures tested.

We show for the first time that there is a risk of assortative mating based upon divergent wild and captive song cultures. In chapter 4, we attempted to reduce the divide between wild and captive regent honeyeaters by implementing a song tutoring program. Over three breeding seasons within the applied breeding system, we undertook adaptive song tutoring experiments using combinations of song broadcast and live tutoring from two wild-origin males to teach zoo-bred juveniles the wild song. Using just two wild founders, we show how animal cultures can be restored in *ex-situ* populations with simple modifications to husbandry protocols. In chapter 5, we monitor ongoing changes in wild regent honeyeater song culture and report a dramatic shift in the dominant song type. Between 2015 and 2019, most males in the Blue Mountains sang a typical regent honeyeater song ('Typical Blue Mountains song'), but 5-10% sang an abbreviated version of the song with half the number of syllables (the 'Clipped Blue Mountains song'), which was associated with lower pairing success. Since 2020, the proportion of males singing the Clipped Blue Mountains song has increased to 50-75%. The likelihood of successful pairing in these males showed a significant concomitant increase, suggesting that the fitness costs associated with singing the abbreviated song decreased as it became the dominant song type. In chapter 6, we draw upon 3 years of study of regent honeyeater song culture research to provide insights into the species' song learning process. This chapter provides guidelines for animal managers in the breeding program for critical learning periods and provides evidence of the long-term retention of songs in individuals. Finally, I conclude by synthesizing the results of three years of experiments and monitoring regent honeyeater song culture, demonstrating the critical gains in understanding song learning, cultural persistence, and the implications for adaptive management both within this species and as a model for broader applications in conservation programs.

"Birds were flying from continent to continent long before we were. They reached the coldest place on Earth, Antarctica, long before we did. They can survive in the hottest of deserts. Some can remain on the wing for years at a time. They can girdle the globe. Now, we have taken over the earth and the sea and the sky, but with skill and care and knowledge, we can ensure that there is still a place on Earth for birds in all their beauty and variety – if we want to... And surely, we should."

Sir David Attenborough

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Figure 1. Logistic regression plots showing the relationship between age and (a) the presence of the adult Typical Blue Mountains song; and (b) the presence of any elements of the adult Typical Blue Mountains song in successfully song-tutored juvenile zoo-bred regent honeyeaters. (c) shows the inverse predictions for the age at which the probability of juveniles singing the adult Typical Blue Mountains song reached 0.5, 0.75 and 0.95. The colour of the line in (c) denotes the tutoring method.

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Zoo-bred female birds prefer songs of zoo-bred males: Implications for adaptive management of reintroduction programs.

Daniel L. Appleby, Joy S. Tripovich, Naomi E. Langmore, Robert Heinsohn, Benjamin J. Pitcher, Ross Crates

Author contributions were as follows:

D.A. conceptualized the study, lead the experimental design, experiments, data curation and manuscript writing. R.C. supported the experimental design, experiments, data curation formal analysis and manuscript. N.L., J.T. and B.P. supported the experimental design, and manuscript. R.H. supported the manuscript and provided supervision.

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Chapter 4

Reintroduction of wild song culture to a critically endangered songbird

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Chapter 5

Frequency-dependence may moderate fitness costs linked to reduced bird song complexity.

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Chapter 6

Insights into the timing of song learning in Regent Honeyeaters: implications for adaptive management and animal husbandry.

Daniel L. Appleby, Joy S. Tripovich, Naomi E. Langmore, Robert Heinsohn, Benjamin J. Pitcher, Ross Crates

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Chapter 1: Introduction

The rapid decline in global biodiversity, often dubbed the 'biodiversity crisis' or the 'sixth mass extinction' (Barnosky *et al.,* 2011) stands as one of the defining environmental challenges of the 21st century (Ceballos *et al.,* 2015; IPBES 2019). Species extinctions have risen to between 100 and 1000 times the pre-human rate (Pimm *et al.,* 1995; Barnosky *et al.,* 2011), and animal population trends suggest that as many as 48% of species are currently in decline (Finn, Grattarola & Picheira-Donoso, 2023). Driven primarily by anthropogenic habitat destruction and fragmentation (Pimm et al., 2014; Diaz et al., 2019; Chase et al., 2020), climate change (Bellard et al., 2012; Ceballos et al., 2015; Martay et al., 2017), and competition with invasive species (Blackburn et al., 2004; Burchart et al., 2010; Hejda, Pyšek & Jarošík, 2009), biodiversity loss threatens the stability of ecosystems worldwide (Loreau & de Mazancourt 2013) and the vital ecosystem services and processes that facilitate human existence (Cardinale et al., 2012). This crisis has spurred an urgent call for action (Grubb *et al.,* 1993; Brooks *et al.,* 2006; Pattberg, Widerberg & Kok, 2019) compelling conservation practitioners to implement a range of strategies aimed at preserving biodiversity (Van Jaarsveld *et al.,* 1998; Hannah, Midgely & Millar 2002).

Among these approaches, *in-situ* conservation—efforts that aim to protect species in the ecosystem or community of which it forms a part (Rotach, 2005) — are considered by many researchers to be the preferred approached for preserving biodiversity (Whitmore, 1990; Rahbek, 1993, Synder *et al.,* 1996), for two primary reasons. First, it is within these environments that species maintain their ecological roles, relationships, and evolutionary potential (Zegeye, 2017). Secondly, by conserving crucial habitat and as such, more complex ecosystems, the conservation actions may thereby confer the benefits and protections to a broader range of co-occurring species (Bifolchi & Lode, 2005; Branton & Richardson, 2011). Despite this, there are many species, under many scenarios, in which effective *in-situ* methods may prove challenging (Pritchard *et al.,* 2012). Exacerbated by climate change (Gregory *et al.,* 2009), the shifting of species' ranges, both in terms of distribution and extent, interacts with increased habitat

fragmentation (Thomas *et al.,* 2006), meaning that *in-situ* conservation action may become redundant where target species may no longer be able to survive within historic ranges and have limited dispersal potential (Odpan & Wascher 2004). Even without the consideration of future climactic conditions, the life histories and current population paradigms of many species make *in-situ* conservation particularly challenging in the short term. Nomadic species, for example, may require protection and restoration of habitats at very large spatial scales (Nandintsetseg *et al.,* 2019). While every attempt should be made to preserve and restore as much habitat as necessary to preserve a species, the temporal and spatial scale required to address threats sufficiently may render efforts intractable, particularly where urgent intervention is necessary to prevent imminent extinction (Acosta & Perry 2002). Furthermore, where extreme population decline has already occurred in a species, conservation actions taken *in-situ* may not be sufficient to overcome the effects of small populations (Schrott, With & King, 2005). As a result, in cases where species are on the brink of extinction, *in-situ* measures alone may not suffice (Franzreb, 1997).

Ex-situ conservation, such as captive breeding, has emerged as a critical tool in the conservation of threatened species (Conde *et al.,* 2011). This is particularly true for species on the brink of extinction (Rahbek, 1993; Pritchard *et al.,* 2012), or for those species for which *in-situ* conservation is particularly complex (Pritchard *et al.,* 2012). Captive breeding has two primary aims and applications. The first is to establish insurance populations of species that may otherwise become extinct (Bowkett, 2009). Secondly, populations reared in the relative haven of captivity, may serve as a platform for reintroduction or population supplementation (Conde *et al.,* 2011). Captive breeding has proven effective in some high-profile conservation efforts, including the recovery of the California condor (*Gymnogyps californianus*) and the Arabian oryx (*Oryx leucoryx*) (Walters *et al.,* 2010; Spalton, Lawerence & Brend, 1999). Yet, despite these notable successes, the overall outcomes of captive-breeding for release programs are mixed (Fisher & Lindenmayer, 2000). While some programs

successfully reintroduce individuals into the wild, others struggle with low survival rates and poor integration of captive-bred individuals into natural ecosystems.

One of the central challenges in the assessment of *ex-situ* conservation is the lack of standardized criteria by which to evaluate success (Fisher & Lindenmayer, 2000; Seddon & Armstrong, 2007; Taylor *et al.,* 2017). Success is often self-reported and the metrics by which they measure success vary, with programs, particularly those in pilot stages, emphasizing different outcomes such as genetic diversity, population size, or survival rates post-release (Fisher & Lindemayer, 2000; Taylor *et al.,* 2017). Despite the variable metrics by which the success of captive breeding programs are measured and the poor documentation of their outcomes (Fisher & Lindemayer, 2000), it is generally agreed that the ultimate goal of captive breeding programs is to use the captive population to establish viable, self-sustaining populations in the wild (Griffith, 1989; Fisher & Lindenmayer,2000; Robert, 2009). The variable success of captive breeding programs can be attributed to multiple factors. To establish viable captive populations, species managers must address a range of considerations, including diet (Cooper *et al.,* 2023), health (Kołodziej-Sobocińska *et al.,* 2018), and environmental conditions (i.e., Enclosure size; Ali, 2016). Furthermore, post-release success may be affected by genetic factors, such as inbreeding depression, or phenotypic factors. Phenotypic limitations may arise from the inability to replicate natural conditions in captivity and the neglect of critical behavioural and ecological needs essential for survival in the wild. These divergences may be evident in morphological (Stojanovic *et al.,* 2021), physiological (Davis *et al.,* 2020), health-related (e.g., Love, Lovern & DuRant, 2017), and behavioural differences (e.g., Larocque, Johnson & Fisk, 2020). With conservation resources finite, it is essential that captive breeding programs be well-justified and grounded in sound scientific and evidence-based practices (Fisher & Lindenmayer, 2000; Taylor *et al.,* 2017).

As more rigorous and sophisticated approaches have been implemented in the management of captive breeding programs (Harding, Griffiths & Pavajeu 2009; Berger-tal *et al.,* 2020; Tripovich *et al.,* 2021), adaptive management – a dynamic process that 'combines the need for practitioners to take immediate

action with a plan for learning' has emerged as a key principle of effective *ex-situ* conservation (Westgate, Likens & Lindenmayer, 2013). Initially, captive breeding programs focused largely on maintaining genetic diversity and minimizing inbreeding (Weeks *et al.,* 2015). Over time, the scope of adaptive management has expanded to include behavioural interventions aimed at enhancing the fitness and survival of captive-reared individuals so that they thrive when released (Berger-Tal *et al.,* 2016 Greggor *et al.,* 2016; Greggor *et al.,* 2020)

Increasingly, the focus of adaptive management of captive-bred animals has shifted towards understanding and manipulating animal behaviour (Shier, 2016). These manipulations primarily focus on efforts to train naïve captive individuals through conditioning, to recognise predators (Griffin, Blumstein & Evans 2001; Moseby, Cameron & Crisp, 2012), toxic prey (O'Donnell Webb & Shine 2010) or to hunt prey (Houser *et al.,* 2011) and enhance natural foraging behaviours (Hocking, Salverson & Evans 2015). Amongst these behaviours, culturally acquired traits—behaviours learned from social interaction and maintained across generations—play a critical role in the survival and reproductive success of many species. However, the manipulation of animal culture for conservation purposes remains a largely underexplored area (Witten, 2021). While efforts to influence behaviour through environmental enrichment or training are common (Shier 2016), less attention has been paid to the transmission and maintenance of culture in captive populations. This gap in research is particularly relevant to species where behaviours with cultural aspects, such as vocalizations (Lewis, Williams & Gilman 2020) or migratory patterns (Mueller *et al.,* 2013), are vital to their survival and reproductive success in the wild.

Birdsong provides one of the most ubiquitous and pervasive examples of animal culture. Unlike innate calls, the songs of the Oscine birds are learned (Beecher & Brenowitz, 2005). Vocal learning is also observed in other bird groups, such as the calls of parrots (Bradbury & Balsby, 2016), hummingbirds (Araya-Salas & Wright, 2013), New Zealand wrens (Moran *et al.,* 2024), and even in one suboscine species, the three-wattled bellbird (*Procnias tricarunculatus*) (Saranathan *et al.,* 2007). In many bird

species, song learning is a complex process that is critical for communication, it has functions in mate attraction, social cohesion, and territory defence (Catchpole & Slater, 2008). The ability to learn and replicate species-specific songs is often vital to reproductive success and integration into wild populations. Song learning is widespread across avian taxa, with the Oscines (comprising more than 4,000 species across 115 families) and parrots (Psittacines, 387 species across 4 families), together accounting for over 40% of global bird diversity. Its loss in captive populations can have significant implications for conservation (Lewis, Williams & Gilman, 2020). The failure of captive-bred individuals to learn appropriate songs may reduce the fitness of reintroduced individuals if they struggle to communicate or establish territories in the wild (i.e. Crates et al. 2021).

The study of song learning in birds offers valuable insights into the broader concept of cultural transmission and its importance in conservation. While much attention has been given to the genetic and ecological aspects of species recovery, the role of culture—particularly in captive breeding and reintroduction programs—has received comparatively little focus. As conservation science continues to evolve, a more comprehensive approach that integrates the management of both genetic and cultural diversity is likely to enhance the success of future conservation efforts (Brakes *et al.,* 2021).

Study Species

The regent honeyeater (*Anthochaera phrygia*) is a medium-sized songbird weighing between 35 – 46g which was historically widespread across much of south-eastern Australia (Franklin *et al.,* 1989). Its range once extended from the Adelaide Hills in South Australia to southern Queensland, but they are now restricted to northern Victoria, New South Wales and rarely, southern Queensland. Regent honeyeaters are known to inhabit a variety of habitats, including swamp mahogany and spotted gum-ironbark forests, coastal heath, and riparian gallery forests. However, regent honeyeaters show a strong preference for temperate box-gum woodlands, especially areas dominated by yellow box (*Eucalyptus melliodora*) and mugga ironbark (*Eucalyptus sideroxylon*), which overlap extensively with their historic

range (Geering & French 1998). Their diet consists mainly of nectar from these trees, but they also consume insects, lerp, and manna (Franklin *et al.,* 1989).

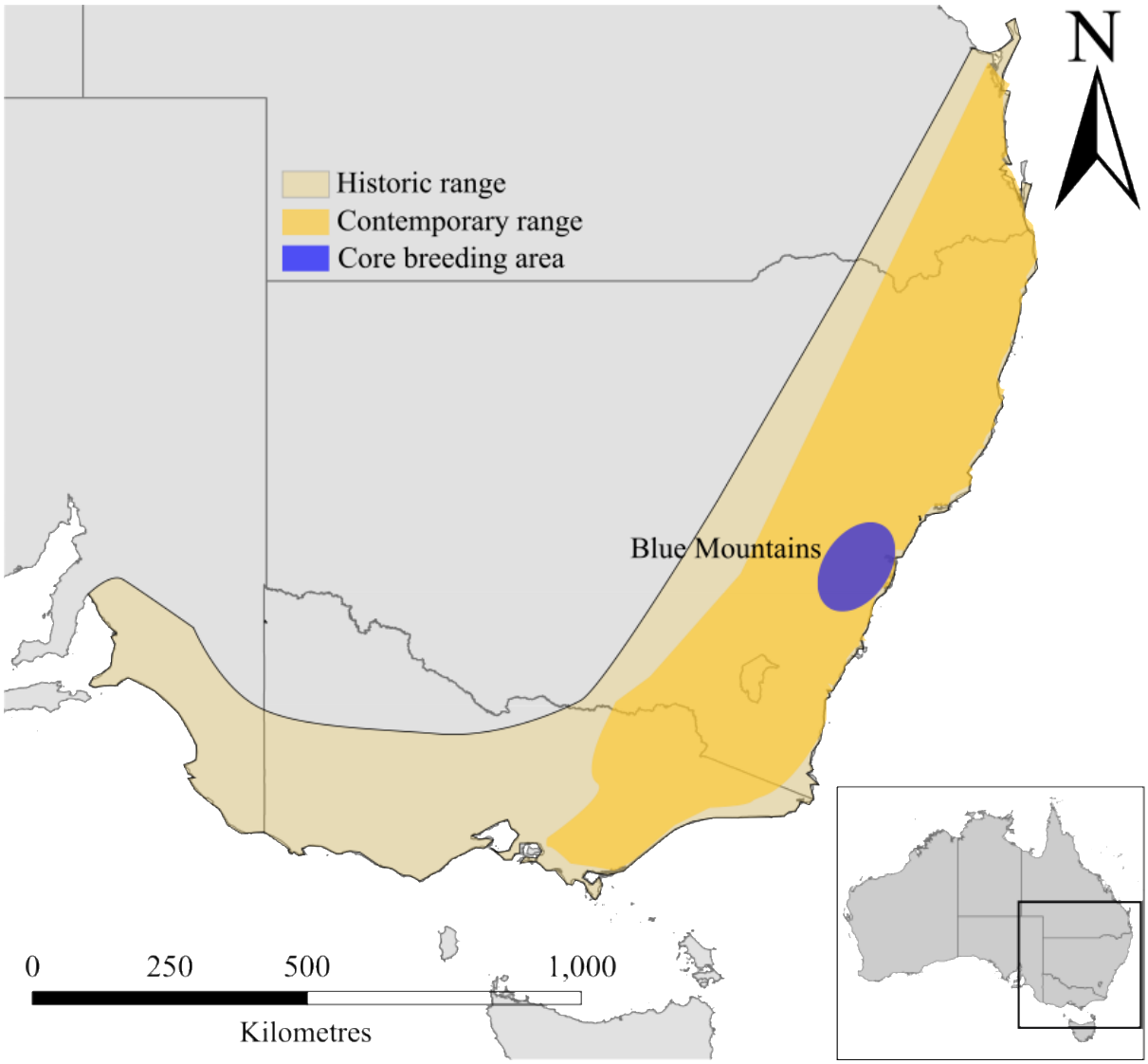

Figure 1 – Historic and contemporary range of the regent honeyeater including core breeding area. Inset map shows the range on a national scale.

These birds have evolved a highly mobile, nomadic, or semi-nomadic lifestyle, enabling them to track nectar resources over large areas (Franklin *et al.,* 1989). Nesting tends to coincide with flowering events, and though they often nest in loose aggregations, regent honeyeaters do not breed cooperatively (Geering & French 1998). Males defend small territories around the immediate nest site but do not maintain exclusive foraging territories, resulting in colonial breeding aggregations where multiple pairs may nest in close proximity (Franklin et al., 1989; Geering & French, 1998). The female

incubates a clutch of 2-3 eggs in an open cup nest built from bark, grass, and spider web, usually located in the outer forks of large trees. After hatching, both parents provision the chicks, which fledge after about 16-18 days. Juveniles become independent roughly three weeks after fledging. Historically, non-breeding season flocks could exceed 100 individuals, but such gatherings are now rare due to small population size (Franklin *et al.,* 1989). Regent honeyeaters typically begin breeding at one year of age, with a known maximum lifespan of just over 11 years, and an average lifespan of 5 –6 years (Geering & French 1998; Commonwealth of Australia, 2016).

The species' preferred habitat, particularly the fertile river flats where their nesting trees grow, has been disproportionately cleared since European settlement, primarily for agriculture. Over 90% of their historic breeding habitat has been lost, and what remains is highly fragmented. (For, 2011; Commonwealth of Australia 2016). This habitat destruction is the primary driver of the species' dramatic decline. Since major population declines were noted in 1970s, the regent honeyeater population fell to an estimated 1000 individuals by the 1990's (Garnett 1993; Liu *et al.,* 2013) and dropped further to 350 –500 birds by 2015 (Kvistad *et al.,* 2015). Monitoring the wild population is challenging, and estimates are uncertain, but there has been a clear reduction in both the range and frequency of sightings. Since 2010, the species has largely disappeared from Victoria, southern New South Wales, Bundarra-Barraba-Severn River district of northern New South Wales, the Australian Capital Territory, and is increasingly rare in Queensland.

Today, the remaining wild population is primarily confined to one region, the greater Blue Mountains area. This region includes the Capertee, Wolgan, Lower Hunter, and Burragorang Valleys, as well as parts of the Upper Hunter catchment (Crates *et al.,* 2019). Due to its sharp population decline, the regent honeyeater was listed as critically endangered in 2015 (Department of Environment 2015).

Owing to major population declines (Franklin *et al.,* 1988, Menkhorst, 1993; Garnett 1992), a captive breeding program was established in 1995 (Liu *et al.,* 2013). The first trial released 10 birds into the

Capertee Valley in 2000, followed by the release of 285 birds in subsequent years across northern Victoria (Tripovich *et al.,* 2021). Since then, releases have been relocated to the remaining core breeding range (Crates *et al.,* 2021). Despite breeding readily in captivity (Liu *et al.,* 2013) and adhering to rigorous breeding protocols (Tripovich *et al.,* 2021), the survival and reproductive success of released birds remain lower than that of their wild counterparts with some evidence of captive-bred birds breeding post release but virtually no records of breeding between wild and captive birds(Taylor *et al.,* 2018; Heinsohn *et al.,* 2022). Recent analyses of the population's viability indicate that captive breeding and subsequent releases are essential to any scenario that avoids extinction for this species in the wild (Heinsohn *et al.,* 2022). Previous studies have identified significant song differences between captive and wild birds (Liu *et al.,* 2013; Crates *et al.,* 2021), with captive birds typically producing simpler, more rudimentary songs resembling those of juvenile birds. These differences may impede their ability to attract mates, as song complexity and cultural conformity play a critical role in mate selection (Baker, 1983) and reproductive success (Crates *et al.,* 2021) in many species. Additionally, the inability of captive birds to produce more sophisticated wild-type songs may affect their integration into wild flocks, limiting opportunities for social learning and the transmission of song culture from experienced wild individuals. This cultural gap between captive and wild populations could further exacerbate challenges in post-release survival and long-term population viability.

Thesis Structure and Rationale

Regent honeyeaters present an unusual opportunity for studying the importance of culturally acquired behaviours in captive breeding settings. The aim of this thesis is to improve the understanding of regent honeyeater song culture and its applications to the adaptive management of this species to improve conservation outcomes. To achieve this, I first review the emerging role of animal cultures in conservation with a detailed literature search. Secondly, using novel song preferences within the active breeding program, we (my collaborators and I) quantify the song preferences of female zoo-bred regent honeyeaters. Third, we implement a three-year song tutoring program with the aim of reducing a

cultural divide between wild and captive birds and to test the efficacy of a range of song tutoring protocols in achieving this reduction. We next extend the long-term monitoring of wild male regent honeyeater songs, measuring the proportion of males singing various song types and quantify temporal changes in the fitness correlates of singing different song types. Finally, we use song data collected in the previous chapters to provide critical insights into the song learning process in regent honeyeaters to provide best practice advice for the maintenance of culturally conforming songs in captivity and potential for wild song restoration.

Chapters 3-6 are written in collaboration with colleagues as stand-alone scientific papers. Chapters three and five are published in *Biological Conservation* and *Royal Society Open Science*, respectively. Chapter four has been submitted to the *Proceedings of the Royal Society Series B*. Chapter 6 is in preparation for submission to *Emu, Austral Ornithology*.

Context Statement

Chapter 1: The first chapter introduces the challenges and frontiers of *ex-situ* conservation and discusses factors influencing post release success. It introduces the regent honeyeater as a novel study system whereby a critically endangered species with a managed *ex-situ* population has culturally acquired behaviours that differ between wild and captive populations.

Chapter 2: In the second chapter, I discuss in detail the advances in animal culture research and their application to conservation. This chapter translates examples of cultural learning to applied conservation in both wild and captive settings.

Chapter 3: A central tenet of adaptive management is to increase knowledge and reduce uncertainty while conducting management practices (Westgate, Likens & Lindenmayer, 2013). Given the observed divergence in song culture between zoo-bred and wild regent honeyeaters described by Liu (2014) and Crates *et al*. (2021) and the well-known function of bird song in mate attraction, it is reasonable to assume that this divergence may have fitness consequences that hinder post-release success. However, this is unconfirmed for this species, and it is difficult to measure explicitly the role of song in mate attraction in the wild, post-release. In chapter three, we conducted song preference experiments to test this hypothesis empirically. The results of this study underpin the work conducted in chapter four and are useful in interpreting the results of chapter five. Critically, research contained in chapter three adds to an extremely limited body of work that empirically tests fitness implications based on divergent culturally acquired behaviours between wild and captive populations.

Chapter 4: Based on an extensive history of laboratory experiments that successfully tutor captive birds with novel songs, we aimed to reduce the divide in song culture between wild and captive birds by testing the efficacy of several tutoring methods on juvenile birds reared in captivity. The methods

explored, while well established in behavioural studies of birds, had until this point never been implemented in an active conservation program. The challenges associated with the limited space and resources in a zoo setting meant that it was prudent to explore multiple tutoring options. Playback tutoring was more flexible and scalable but was associated with lower success in previous research, while live tutoring was severely limited by suitable adult tutors and hence scalability.

Chapter 5: Effective adaptive management requires continuous monitoring to inform the decision-making process (Westgate, Likens & Lindenmayer, 2013). In tandem with our song tutoring experiments, wild-song culture was monitored over the same period. This provided evidence of ongoing population decline, a rapid shift in the dominant song, and evidence that frequency dependence may ameliorate the fitness impacts associated with reduced song complexity. With the dramatic shift in the dominant song type this meant that the zoo-population tutored in chapter four is now the primary source of the traditional song culture in this species. This presents the exciting possibility of wild cultural restoration from captive stock but highlights the dynamic nature of the *in-situ* environment and the need for constant monitoring to best inform adaptive management.

Chapter 6: To manage the ongoing song tutoring program, and to understand the ongoing erosion of song culture in the wild it is critical to understand the timing and process of song learning in regent honeyeaters. In chapter six we use song data collected in chapters four and five to estimate the timing of critical song learning periods. This in turn contributes vital information to refine the husbandry of this species and provides insights into the processes leading to the erosion of song culture in the wild. Finally, it provides a window of time in which species managers may target the transfer of cultural information from captive to wild individuals.

Chapter 7: In chapter seven I synthesise the results from the previous chapters to summarise the gains in knowledge and the implications for the management of this species and for adaptive management practices more broadly.

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Chapter 2: Culture Crash: Mitigating animal cultural loss for conservation.

Interest in animal culture has seen a steady increase over the past two decades, enabled by major advances in methodological approaches (Whiten 2021). An accumulation of evidence of socially transmitted behaviours over the past seventy years has turned a once highly controversial topic (i.e., Galef 1992) into a burgeoning field (Whiten 2021). There is still debate around the exact definition of animal culture, with behavioural ecologists, evolutionary psychologists, anthropologists, and sociologists varying in how strictly the definition is applied (Whitehead et al. 2004). Some psychologists strictly adhere culture to social transmissions that involve objective teaching and learning, that distinction is unnecessary to highlight the importance of animal cultures for conservation. The definition used throughout this review refers to behaviours that are acquired through some form of social learning between individuals and are shared at a population or sub-population level (Rendell and Whitehead 2001). This definition is intrinsically linked to conservation as (1) behaviours affect phenotypes, (2) culture is a group phenomenon, and (3) it is an inheritance system, like genes (Boyd and Richardson 1985). While the principal interest in animal culture has traditionally been as a tool to understanding how socially transmitted behaviours evolve in humans, there has been a significant shift toward their applications in the conservation of biodiversity (Ryan 2006, Brakes et al. 2019).

Why Culture Matters

A greater understanding of how common and widespread socially transmitted behaviours are in the animal kingdom has presented a great deal of opportunity for conservation (Whiten, 2021). The ability for animals to learn and spread behaviour may allow wildlife managers to introduce novel behaviours that mitigate anthropogenic threats (Greggor *et al.,* 201). This is particularly important where conflict exists between humans and animals (Brakes *et al.,* 2019).

Although the advances in understanding animal cognition and social learning have presented many novel methods for adapting animal cultures for conservation, they represent expedited or artificial behaviours that deal with novel, anthropogenic threats. Preserving naturally acquired animal behaviours is an equally important endeavour. Animal cultures can be seemingly arbitrary, like the various grooming handclasps observed in different groups of chimpanzees (*Pan troglodytes*) (McGrew *et al.,* 2001), or even maladaptive in the case of the plains bison (*Bison bison bison*) that experienced a population-wide shift from avoidance to selection of an agricultural area where mortality was 12% higher (Sigaud *et al.,* 2016). However, Animal cultural behaviours may confer a local adaptive advantage (Whiten, 2021). In the famous case of tool use in chimpanzees, as first described by Goodall (1970), some sub-populations display tool use for extractive foraging that others seemingly do not (McGrew & Rodgers, 1983). Variation also exists between populations that do exhibit tool use when targeting food, the materials from which tools are made, and the way in which tools are made. As Goodall (1970) observed, young chimpanzees learn the cultural norms of tool use during infancy, learning from older individuals through social facilitation, observation, imitation and trial and error. This is certainly an important process, as Teleki (1974) points out, fishing for termites is a difficult task. Having tried himself after months of observing chimpanzees in West Africa, he was unable to consistently locate tunnel entrances or select appropriate probes with the same ease as adult chimpanzees. Without appropriate social learning facilitation, it is possible for the chimps to lose key behaviours that aid in the foraging of a key diet component within a single generation (Teleki, 1974).

As social learning can lead to animal cultures with local adaptive advantage, it can also mediate population structure, giving rise to distinct sub-populations with unique behavioural profiles, which erect social barriers (Brakes *et al.,* 2019). Culturally mediated population structure can influence phenotypic diversity at a species-wide level, and this has important implications for conservation efforts. If culture can shape species-wide phenotypic diversity, then like genetic diversity, it can increase the resilience and adaptability of a species to changing environmental conditions (Garland *et al.,* 2015).

Where preserving genetic diversity in unique population segments below the species level has been a major focus for conservation biologists through the designation of evolutionarily significant units (ESUs) (Waples, 1991; Moritz 1994), several authors have proposed terms such as culturally significant units (CSUs) (Whitehead *et al.,* 2004; Ryan, 2006). CSUs operate in much the same way as ESUs where segments of a population that are not differentiated as separate species or sub-species but exhibit significant cultural differences from other segments of the population. Within these segments another factor must be considered, that is, the role of culturally significant individuals. As McComb and colleagues (2001) suggest, individuals may possess cultural knowledge, which makes their significance in the population much greater than only their reproductive capacities The loss of such individuals can result in the extinction of unique cultural variants within a population, complicating conservation efforts. As a result, CSUs present the problem of maximizing cultural diversity while protecting unique cultures (Ryan, 2006), requiring managers to consider both the preservation of culturally distinct population segments and the key individuals who maintain those traditions."

ESUs, CSUs and considerations for animal cultures

ESUs and CSUs have many similarities; both focus on diversity at scales below the wider population, and both recognise that variations within a population can lead to phenotypic variation and, as such adaptability to a changing environment or distinct evolutionary trajectories (Whiten, 2021; Moritz, 1994). As a result, many generic conservation strategies apply to both, for example, maintaining larger populations appears to increase genetic diversity (i.e., Hague & Routman, 2016). Larger population sizes have also been linked to greater cultural diversity and innovation (I.e., Ashton *et al.,* 2019). On the other hand, population decline, and fragmentation have been shown to reduce diversity of traits and song complexity in bird populations (Williams *et al.,* 2013; Paxton *et al.,* 2019). The same can be said for the protection of critical habitat, it is equally important to identify and preserve specific habitat sites for separate ESUs as it for CSUs rather than focusing on sites that may, for instance contain a larger population.

Two distinctions must be drawn between these concepts. Firstly, culture may be significantly more plastic than genetics, with cultural norms able to change in very few generations with environmental or demographic shifts (Boyd & Richerson, 1985). This is demonstrated where sub-populations are behaviourally distinct but not genetically distinct. An Amazonian parrot, the yellow-naped amazon (*Amazona auropalliata*), for example displays discrete vocal dialects amongst sub-populations but also exhibits high gene flow across dialectal boundaries and shows no greater genetic distance across groups than within groups (Wright & Wilkinson, 2001). A similar phenomenon exists within orcas (*Orcinus orca*) along the US-Canada border where feeding behaviour stratifies the species into two distinct populations (Barrett-Lennard, 2000).

Secondly, while ESUs predominately assess diversity at a group level, CSUs recognise the cultural importance of certain individuals within a population (Ryan, 2006; McComb, 2001). ESUs may be able to identify significant individuals based on their reproductive capacity, for example prioritising younger individuals for conservation (Brakes *et al.,* 2019), whereas CSUs recognise experienced adults as culturally significant as repositories of cultural information and potential for tutoring (McComb, 2001). A simple way to distinguish the difference is to consider the role of an isolated group of juvenile animals taken into captivity as an insurance stock. If these juveniles are of genetic significance and can be reared successfully in isolation, their reproductive capacity is high and serves the express purpose of maintaining population of that ESU. Conversely, if the group of juveniles is from a CSU and have no social facilitation of important cultural behaviours, their cultural uniqueness will not be expressed or maintained and will become insignificant for conservation. This is demonstrated frequently in the literature, where, in absence of proper access to social learning, captively reared individuals lack important cultural knowledge and, in some cases, are unable to rear offspring of their own (i.e., Ryan, 2002; Shier, 2016).

Regarding conservation, the applications of ESUs and CSUs are very similar once units are identified. Compared with ESUs, CSUs are a relatively new concept (Ryan, 2004) and have not been as readily integrated into conservation management strategies. With comprehensive reviews of the wide reach of animal culture (Whiten, 2021; Brakes *et al.,* 2019), this is beginning to change, with many influential conservation bodies and international legal instruments, such as the Convention on the Conservation of Migratory Species of Wild Animals formally recognising the role of culture in conservation.

The remainder of this review will focus on the strategies available to mitigate the loss of cultural diversity for *in-situ* conservation with a particular focus on *ex-situ* contexts, as well as remedial solutions for populations in *ex-situ* breeding programs that have lost or are unable to maintain advantageous behavioural traits.

Preserving animal cultures in the wild

As with any designatable conservation unit, to preserve it, it must first be identified. Indeed, for culturally significant units, this can present a challenge. Populations can often be sympatric and show little genetic difference, researchers must have significant knowledge of behaviour of the populations of interest. The famous example of bottlenose dolphins (*Tursiops aduncus*) near shark bay off the western coast of Australia demonstrates this paradigm where the population is organised into subgroups based on foraging behaviour (Krützen *et al*., 2005). Populations that form based on foraging and habitat use, may benefit by increasing their adaptability to changing environments. The loss of cultural behaviours that are adapted to a specific habitat may have irreversible effects. The decline of commercial whaling in most waters saw a recovery of many whale populations around the world (Whitehead *et al.,* 2004), but many important habitats in the North Atlantic remain deserted. The Southern rightt whale (*Eubalena australis)* was hunted to near extinction in the region, and while populations in parts recovered, right whales remain completely absent some traditional calving grounds

(Caroll *et al.,* 2011),. The absence of whales in a vacant niche is probably a result of the loss of culturally transmitted local adaptation (Whitehead *et al.,* 2004; Caroll *et al.,* 2011).

By identifying culturally significant units, researchers can focus conservation efforts towards vulnerable or environmentally important sub-populations (Brakes *et al.,* 2019). While habitat protection is a fundamental of conservation, understanding cultural stratification and the movement between groups can guide conservationists toward the most appropriate habitat to protect. With the identification of culturally significant units, international conservation organisations may be able to increase international cooperation and the identification of sub-populations that may be considered vulnerable outside of conserved habitat (Brakes *et al.,* 2019).

Preserving culture in captivity

Captive-breeding and re-introductions are increasingly common conservation tools (Ewen & Canesta, 2014). Degraded habitat and anthropogenic threats make many geographically defined populations unviable (Fischer & Lindenmayer, 2000). In such cases conservationists may seek to create insurance populations in captivity (Farhadinia *et al.,* 2020) or reinforce wild population numbers through increased breeding effort in captivity. The role of genetic diversity and suitable diet in captive breeding planning is well documented in the literature, however consideration of culture is strikingly rare (Ryan, 2004; Berger-Tal et al. 2015). As mentioned earlier, preserving culture for conservation has the conundrum of two opposing goals, preserving the uniqueness of individual cultural units, and maximising cultural variation at the species level. In captivity the focus is rather more straightforward.

Captive breeding for reintroduction can have two aims, bolstering wild populations, or re-establishing extirpated species (Ewen & Canesta 2014). Firstly, the aim of bolstering wild populations with planned reintroductions can target specific release sites. In such cases it is critical to identify significant cultural

behaviours of the target release site. For example, many species of bird exhibit distinct regional dialects (Marler & Tamura, 1962; Catchpole & Slater, 2008). Song in birds facilitates communication and information signalling (Marler & Sherman, 1985). Like language in humans, it is important to have cultural norms in communications. Stability in acoustic properties allows for group cohesion and the transmission of social knowledge among group members (Catchpole & Slater, 2008). As song plays a vital role in both mate acquisition and breeding territory establishment, individuals varying significantly from a cultural norm may have lower chances of survival and reproduction (Catchpole & Slater, 2008). This is clearly demonstrated in the literature, with many species exhibiting assortative mating based on regional dialects (Baker, 1983; Chilteon *et al.,* 1990; O'Loughlen & Rothstein, 1995), in these cases, it is essential that the captive breeding program first identifies the correct dialect for the planned release site and maintains suitable tutors within the population.

Secondly, where a species is planned to be re-established where it has been extirpated, the widest possible range of CSUs should be represented within the captive cohort. Chimpanzees for example may display several different culturally inherited grooming behaviours amongst subgroups of a single population (Holbaiter *et al.,* 2014, Wrangham *et al.,* 2016). Similarly, tool use in chimpanzees is known to vary between populations in geographically distinct regions, where researchers have hypothesised that ecological differences are the primary driver of differences in tool use (Laland and Jannick, 2006; van Schaik, 2006). Recent studies, however, show that chimpanzees from neighbouring populations also exhibit differences in the manufacture and use of tools in foraging where no ecological difference is observed (Pascual-Garrido, 2019). Considering this, where multiple socially learned behavioural adaptations are present, they may each vary in how advantageous they are to an environment and as such, wildlife managers should aim to represent as much cultural diversity within a captive breeding or translocation program design. The capture of a wide culturally significant behaviour may be possible where other wild populations still exist but of course, where wild populations are completely extinct, preserving what cultural behaviours remain will become the primary focus.

The impact of socially learned behaviours on captive breeding success are vast, even the most basic aim of captive breeding – that of reproduction – can be jeopardised by the lack of social transmission between individuals. As Ryan (2002) noted, female gorillas (*Gorilla gorilla gorilla)* that were hand reared without their mothers and did not join a social group were not able to of raise their own offspring. Once important socially learned behaviours have been identified, captive breeding programs should then consider the most appropriate ways to facilitate their transmission utilising three important principles.

Firstly, population size should be adequate to support cultural transmission. This serves the dual purpose of preserving the complexity of socially learned signals, such as song in birds, where population has been shown to be correlated with signal complexity (Paxton *et al.,* 2019) and capturing the broadest range of behaviours, even where some behavioural variations may not have been identified initially (Ryan, 2006). Secondly, as significant behaviours may be evolved as a form of local adaptation or niche modification, the habitat in the captive environment must be sufficiently like the proposed release site to facilitate area-specific socially learned behaviours (Laland & Janik, 2006). Insect fishing techniques in chimpanzees, certainly cannot be taught between individuals without the ability to interact with the environment in that way. Finally, access to cultural significant individuals as tutors is paramount. Without behavioural exemplars, maintenance of significant socially learned behaviours is highly improbably (Ryan, 2002). Many bird species, such as the zebra finch (*Taeniopygia guttata*), produce abnormal song when raised in isolation, and will readily learn the songs of other isolate birds (Fehér *et al.,* 2009). Further to this, culturally significant individuals may be behavioural information repositories and offer population-level fitness advantages. The presence of an experienced matriarch in elephants has been shown to increase the breeding success of younger elephants (McComb *et al.,* 2001).

Subsequent releases should consider the cultural behaviours exhibited in the captive population when assessing the appropriateness of individuals for release. Individuals who do not display culturally

appropriate behaviours are less likely to both survive and reproduce post-release. Where species are to be reintroduced to areas that they have previously been extirpated from, captive bred individuals who display cultural similarities and will readily co-exist and interbreed should be considered for release together (Ryan, 2006).

Restoring Lost Cultures

In some cases, establishing captive breeding programs that cater to the maintenance of cultural behaviours may not be practicable due to habitat requirements (Edwards, 2014) or may have been established prior to the detection of significant cultural behaviours. Where a culturally self-maintaining captive population cannot be established, significant cultural behaviours may be rapidly lost and may significantly reduce reintroduction success. In such cases it may be possible re-seed culturally appropriate behaviours (Brakes *et al.,* 2019).

Desirable behaviours may be 'seeded' into populations to avoid certain foods or sites (Greggor, 2017) reducing conflict with agricultural areas or roads (Proppe *et al.,* 2017). Introducing novel behaviours to naïve animals also has great utility in reducing population decline from toxic introduced species, where social learning is inhibited by lethal first encounters (Brown, 2013). A key example is the northern quoll (*Dasyurus hallucatus*). Where other Australian carnivores such as the common planigale (*Planigale maculata*) naturally learned aversion to eating toxic cane toads (*Rhinella marina*) through non-lethal encounters (Webb et al. 2008), the northern quoll failed to learn the dangers of cane toads due to the bold hunting strategies they employ, targeting large toads that cause deadly encounters (O'Donnell *et al.,* 2010). Researchers successfully trained northern quolls to avoid toad encounters through aversive conditioning (O'Donnell *et al.,* 2010). Although this training is not explicitly culture it provides clear evidence of the ability of species managers to introduce novel behaviours. Speices trained with novel behaviours may socially transmit this information to future generations aiding the protection of a globally endangered species.

The need to re-seed important cultural behaviours into the population is particularly evident in migratory species where captive rearing prevents social learning of this behaviour. For over 15 years, researchers studying migratory birds in North America (Canada geese (*Branta canadensis*), Lishman 1996; Trumpeter Swans (*Cygnus braccinator*), Sladen *et al.,* 2001; Whooping cranes (*Grus americana*), Urbanek *et al.,* 2005) have devised novel strategies to retrain captively reared or isolated birds on appropriate migration routes, though with mixed success.

Whooping cranes had been virtually extirpated from the wild, by the 1930's only two small populations remained, one sedentary and a single migratory population of 15 individuals (Allen, 1952). Some *in-situ* conservation and habitat protection saw this number grow slightly, however the populations were still considered extremely vulnerable. Researchers attempted several reintroductions aimed at re-establishing a viable migratory population (Texas Parks and Wildlife, 2007). Sandhill cranes (*Antigone canadensis*) were used as surrogates to imprint migratory patterns. However, the whooping cranes failed to pair or mate due to imprinting on Sandhill cranes. The attempts to reintroduce a migratory population were abandoned in 1989. In 2001 a new migratory reintroduction program was launched (Urbanek, 2005). This time researchers attempted to seed artificially migratory behaviour by imprinting costumed humans on the captively reared birds, then guiding the birds along a migratory path using an ultralight aircraft (Urbanek, 2005). While the overall success of this reintroduction has faced criticism and was eventually ended after 15 years (US Fish and Wildlife service ,2016), the success in seeding the migratory behaviour is striking. The return rate for birds trained with ultralight aircraft was 90.5% compared with 69.2% for birds released directly into their autumn grounds (Urbanek, 2016), and survival at one-year post-release was 85.1% compared with 65.7% for direct release (Urbanek, 2016).

The overall success of the breeding program was largely assessed on the poor reproduction of released birds (Urbanek, 2016), with future strategies aiming for less human intervention. This certainly should

not dissuade conservationists from attempting to re-seed behaviours in other future projects. The conservation goal in this case was to re-establish a migratory colony, which, without the appropriate behaviours is unlikely to succeed. Indeed, even with a larger population of tutors, direct autumn release birds showed lower return rates than the birds lead by ultralight aircraft (Urbanek, 2016). In this case, many of the original issues leading to population decline, such as predation, illegal hunting, and harassment of incubating birds by black flies (*simulium spp.*) were still leading causes of mortality and breeding failures (Urbanek, 2016). Reintroductions are complex endeavours with multiple factors affecting the success (Fischer & Lindemayer, 2000) including habitat quality, competition, and genetics. The ability to improve one component of a species' fitness may still contribute to greater reintroduction outcomes and as such, behavioural manipulation attempts should be judged on the success of seeding the desired behaviour, not the overall success of the reintroduction.

Conversely, behavioural modification may show signs of improving a conservation goal before that behaviour becomes expressly evident. The Regent Honeyeater (*Anthochaera phrygia)* is a critically endangered songbird that has suffered a major reduction in habitat (Crates *et al.,* 2021). Crates *et al.* (2021) have observed a reduction in song complexity amongst the wild population and, along with others (Powys, 2010) who noted radical abnormalities within the captive breeding program. A recent study by Tripovich *et al.* (2021) has shown that birds exposed to wild type song before release had greater survival post-release but lower reproduction rates. Interestingly, the birds exposed to wild type song still sang significantly different songs to the wild birds (Pers comm.). In this case it is possible that the attempts to modify the behaviour may have transferred a partial advantage. All birds may have become familiar with wild type song, facilitating group integration, and perhaps even mate recognition for females, while males were unable to emulate the wild song and as such, were less likely to attract wild mates. While it is unclear why this attempt at seeding wild type song failed, a greater understanding of the species-specific song learning process may assist in optimising the protocol.

As Greggor and colleagues (2017) point out, "Conservationists often lack sufficient knowledge of cognitive theory to implement successful behavioural manipulations". Many behavioural interventions achieve sub-optimal outcomes where researchers overlook key cognitive mechanisms that influence learning and the specific applications to a target species. Some conservation issues need only address learned behaviours at the perceptual stage, but many learned behaviours require multiple learning phases. While some bird species may learn song effectively in a critical learning period in their natal area (Catchpole & Slater, 2008), others require changes at multiple stages of the learning process. Where non-seasonal breeding birds like Zebra finches show a critical learning period with the final song evident within 90 days (Williams, 2004), canaries have been shown to crystalise their songs in the spring following their breeding (Nottenbohm *et al.,* 1986). Understanding when and how to target specific cognitive processes involves an integrated approach to utilising cognitive mechanisms.

To reseed adaptive behaviours effectively into captive populations, wildlife managers must first identify the key cultural behaviours, understands the important learning processes, and implement them in a way that minimises negative side effects.

Summary

A growing body of evidence has shifted the discussion from debating the existence of animal cultures to applying our understanding of culture toward practical and well-informed conservation practices. Sensible decision making requires practitioners to plan for conservation tasks and implement actions that take into consideration the role of social learning and animal culture in successful conservation attempts. A summary table of strategies in different conservation settings is listed below.

Table 1: Conservation tasks with corresponding planning and action.

CONSERVATION TASK	Planning	Action
IN-SITU CONSERVATION	• Identify culturally significant units • Increase international cooperation (i.e., migratory birds/cetaceans)	• Protect critical habitat • Minimise human interaction • Prioritise culturally significant individuals
REINTRODUCTION	• Identify important cultural behaviours specific to target release site • Explore possibilities of earlier release where juveniles can learn from wild conspecifics	• Create captive environment similar enough to natural habitat to facilitate learning of release site-specific behaviours • Maintain sufficient population and culturally significant individuals to facilitate social learning • Avoid unnecessary human or foreign interspecific contact
RE-SEEDING CULTURAL BEHAVIOUR	• Identify maladaptive behaviour • Identify key cognitive processes, timing of learning and best learning cues • Consider conflicting/complementary behaviours	• Facilitate artificial/natural learning of cultural behaviour. • • Minimise human contact during intervention

While addressing animal culture is unlikely to solve most of the conservation issues facing the rapid loss of biodiversity, the integration of these considerations can be a useful and complimentary tool for decision makers to maximise success across a range of conservation goals.

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Chapter 3: Zoo-bred female birds prefer songs of zoo-bred males: implications for adaptive management of reintroduction programs.

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Abstract

Understanding the factors affecting the success of animal reintroductions is crucial for facilitating endangered species recovery. Cultural differences between individuals from different animal populations can lead to assortative mating, whereby individuals prefer to breed within their own familiar cultural cohort. Assortative mating can hinder the assimilation of zoo-bred individuals into wild populations, so quantifying this risk becomes important for informing adaptive management strategies in conservation. We conducted mate choice experiments on zoo-bred female regent honeyeaters (*Anthochaera phrygia)* to assess whether they showed stronger behavioural responses to the familiar but abnormal songs of zoo-bred male regent honeyeaters than those of their wild counterparts or an interspecific control song. Female regent honeyeaters were no more likely to enter the playback aviary in response to the songs of zoo-bred males than those of wild males or a different species. However, females that did enter the playback aviary were significantly more likely to respond quickly, approach the speaker closely and remain in the playback aviary for longer in response to the songs of zoo-bred males than wild songs or control songs. Our study is the first to show experimentally that zoo-bred females prefer familiar, but abnormal, zoo-bred songs over unfamiliar wild-type songs. We suggest that assortative mating among released birds is a risk in reintroduced regent honeyeaters, given recent evidence that the breeding success of reintroduced pairs is low. Our study demonstrates how small-scale experiments conducted within applied zoo-breeding settings can yield useful

information to refine husbandry techniques and reduce cultural divides between wild and reintroduced populations.

Introduction

Zoo breeding programs are a widely used conservation strategy to bolster populations of endangered species and to supplement declining wild populations undergoing concurrent *in-situ* conservation (Rahbek, 1993; Seddon *et al.,* 2014). Although many species readily breed in captivity, the success of reintroduction programs, whereby zoo-bred individuals are released to the wild, is often low or variable (Crates *et al.,* 2022; Fischer & Lindenmayer, 2000; Sheean *et al.,* 2012). Identifying the factors that determine the success or failure of reintroductions is complex, but is a priority to maximise returns on conservation investment (Fischer & Lindenmayer, 2000; Robinson *et al.,* 2020; Sheean *et al.,* 2012). Many studies have assessed common issues, ranging from poor planning (Ewen & Armstrong, 2007) and a failure to address the initial causes of population decline (Kingsford *et al.,* 2009; Sheean *et al.,* 2012) to the effects of the captive environment, including inbreeding (Araki *et al.,* 2009; Frankham, 2008; Fraser, 2008) and adaptations to captivity (Frankham, 2008; McPhee, 2004). More recently, erosion of animal cultures – a lack of socially-transmitted behaviours in captivity – is being recognised as a potential barrier to successful re-introduction to the wild (Brakes *et al.,* 2019; Rutz *et al.,* 2016). Although zoo breeding programs are increasingly being run with more rigorous protocols (Ewen & Armstrong, 2007; Frankham, 2010; Greggor *et al.,* 2018), animal cultures are rarely considered in their planning and implementation (Brakes *et al.,* 2019).

Cultures in animals are behaviours that are acquired through social learning between individuals and are shared at a population or sub-population level and maintained through generations (Rendell & Whitehead, 2001; Rutz *et al.,* 2016; Whiten, 2021). Culture is key to kin recognition, social cohesion, and mate attraction, amongst other social factors (Rutz *et al.,* 2016; Whiten, 2021). However, culturally-

acquired behaviours such as birdsong can be lost or altered in zoo environments through changes to the social composition and dynamics of zoo-bred populations relative to their wild counterparts (Crates et al. 2022). Devising innovative ways to maintain wild animal cultures in zoo environments through adaptive management is increasingly being recognised as an important conservation consideration (Brakes et al. 2021).

Songbirds learn songs and song preferences from older conspecific tutors, including parents or associated adults (Beecher *et al.,* 2007; Catchpole & Slater, 2008). While copying errors and cultural drift can lead to changes in song culture over time, rapid and drastic changes to population-level song cultures are likely to be an outcome of population decline or fragmentation (Paxton *et al.,* 2019). Songs in birds are used in mate attraction and territory defence (Catchpole & Slater, 2008; Laiolo & Tella, 2007), and assortative mating between individuals that belong to the same song dialect (where either both the male and female sing or only the male sings songs that non-singing females were exposed to during development) is widespread (Wang et al. 2022, Planque et al. 2014). Therefore, changes to song structure may isolate social groups even if they co-occur (Crates *et al.,* 2021). Assortative mating, whereby individuals preferentially pair within the same cultural cohort based on sexual or social preferences formed through developmental auditory experience (Jiang *et al.,* 2013, Chen *et al.,* 2017), can hinder the assimilation of zoo-bred individuals into wild societies (Slade *et al.,* 2014). Although successful pairing between captive-reared and wild individuals is essential to the success of captive-rearing programmes, no studies have previously investigated whether dialect-based assortative mating occurs in this context (Lewis *et al.,* 2020). As songs are learnt from adults during early development, and typically become fixed before their release, zoo-bred birds may fail to integrate and interbreed with wild populations. This potentially limits the ability of naïve, zoo-bred birds to acquire behaviours socially from wild conspecifics that may be crucial for their long-term survival.

The regent honeyeater (*Anthochaera phrygia*) is a critically endangered Australian songbird with an estimated population of fewer than 300 individuals remaining in the wild (Commonwealth of Australia, 2016, Garnett & Baker, 2021). To mediate the risk of imminent extinction in the wild, zoo breeding to bolster the wild population through reintroductions and provide an insurance against further population decline is a high priority strategy in the species' national recovery plan (Commonwealth of Australia, 2016). However, the ongoing supplementation of the wild population with zoo-bred regent honeyeaters has had limited success in arresting the population decline (Heinsohn *et al.,* 2022; Tripovich *et al.,* 2021), with the majority of zoo-bred birds failing to establish a breeding territory or pair with wild mates (Kvistad *et al.,* 2015; Tripovich *et al.,* 2021). In the past, a failure to pair with wild conspecifics could be explained by the small number of wild birds persisting at the traditional reintroduction site in northern Victoria (Kvistad *et al.,* 2015). However, the reintroduction strategy shifted in 2021 such that zoo-bred birds are now reintroduced into the greater Blue Mountains in New South Wales (NSW), where the vast majority of wild regent Honeyeaters persist (Heinsohn *et al.,* 2022). Despite this shift, zoo-bred birds still show poor breeding success (unpublished release data).

Regent honeyeaters sing a soft, metallic-sounding song that is locally variable and forms loose geographic dialects (Crates *et al.,* 2021). Over the past 25 years their songs have gradually changed within all populations (Powys 2009, Crates *et al.,* 2021). Now, the vast majority of all males recorded birds sing the dialect, referred to in Crates *et al.,* (2021) as the "Blue Mountains typical" dialect. Zoo-bred male regent honeyeaters sing an abnormal song that is distinct from any wild population (Crates *et al.,* 2021). Their songs are simpler, shorter and contain fewer distinct syllables, none of which overlap with the wild population (Crates *et al.,*2021). The founding population of zoo-bred regent honeyeaters were hand-reared from wild nestlings that never had the opportunity to learn the wild song. This simplified song has persisted for nine generations (assuming three years per generations per Heinsohn *et al.,* 2022) in the zoo population. Post-release, interbreeding between zoo-bred and wild regent honeyeaters of either sex has rarely been observed (Ingwersen, 2014; Kvistad *et al.,* 2015). This may be

because females of many bird species exhibit preferences for familiar song dialects when selecting a mate (Beecher *et al.,* 2007). This assortative mating strategy may occur if wild females show lower levels of attraction to the simplified, unusual songs of zoo-bred males. Conversely, most zoo-bred females are typically only exposed to zoo-bred song dialects prior to release, meaning they may show a preference for the familiar songs of zoo-bred males over wild conspecifics based on their developmental auditory experience (Chen *et al.,* 2017). The observation that zoo-bred regent honeyeaters of either sex have lower breeding success than their wild counterparts (Taylor et al. 2018) means that quantifying the risk of assortative mating based on differences in song dialects is a conservation priority.

We adopted an experimental approach within the regent honeyeater zoo-breeding facilities to test the hypothesis that zoo-bred female regent honeyeaters show a behavioural preference for the song dialect of zoo-bred male regent honeyeaters, with which they are familiar, over the culturally dominant song dialect of wild male regent honeyeaters occupying future release sites within the greater Blue Mountains.

Methods

Study populations

Female regent honeyeaters (n=18) were raised at Taronga Zoo, Sydney, and/or Taronga Western Plains Zoo, Dubbo in NSW, Australia. All birds spent a minimum of one year in the Taronga Western Plains Zoo population, which was founded from part of the Taronga Zoo population in 2019.

We conducted our experiments within the breeding season, when we expect females would be naturally most receptive to males' songs. So as not to interfere with the breeding effort, we did not implement subcutaneous oestradiol implants, as has been conducted in similar experiments in other species (Dunning *et al.,* 2014). We implemented two of the three trials at the beginning of the breeding

season, within one week of the regular pairing of birds for the breeding program in June 2021 (n = 6 females) and June 2022 (n = 6). The third trial took place later in the breeding season in January 2022 (n = 6), but still within the known regent honeyeater breeding period (Geering & French, 1998).

Selection of playback songs

We selected zoo-bred regent honeyeater songs from recordings of contemporary males recorded at Taronga Zoo between 2018 and 2021. Each female received unique wild and zoo-bred songs. Male song at the Taronga Western Plains Zoo closely resembles these recordings, as the population was largely founded from Taronga Zoo. To ensure previous experience of a male's song did not bias female responses, we vetted the identity of the recorded males to ensure that females were not presented with songs of previous mates, or of their own fathers. The zoo breeding aviaries are in multiple blocks of four individual aviaries, so while it is possible to isolate juvenile females from the songs of males in other blocks, it is not possible to totally isolate them from the songs of males in neighbouring aviaries within their own block**.** As future releases are planned for areas within the greater Blue Mountains, NSW, we selected wild songs from recordings categorised as 'Typical Blue Mountains' in Crates et al. (2021), which is the cultural norm within this part of the species' range. We selected songs with the highest fidelity and signal to noise ratios from Crates et al. (2021) field recordings, which we assigned to females randomly. We used an interspecific song as a neutral control to test whether females respond to a vocal stimulus simply out of curiosity. For the interspecific song we selected superb fairy-wren (*Malurus cyaneus*) song as this species is abundant around the zoo-breeding facilities and are presumed not to threaten regent honeyeaters in any way. We acquired the superb fairy-wren songs from xeno-canto (https://xeno-canto.org/) recorded within NSW to maximise familiarity. We selected the highest possible fidelity recordings of similar lengths to those of the regent honeyeater songs (range = 1.0 - 1.3 seconds), which we presented at the natural superb fairy wren frequency range. Example songs of wild and zoo-bred regent honeyeaters and the control songs of superb fairy-wrens are shown in Figure 1. A list of songs is provided in the Supplementary Information Table S2.

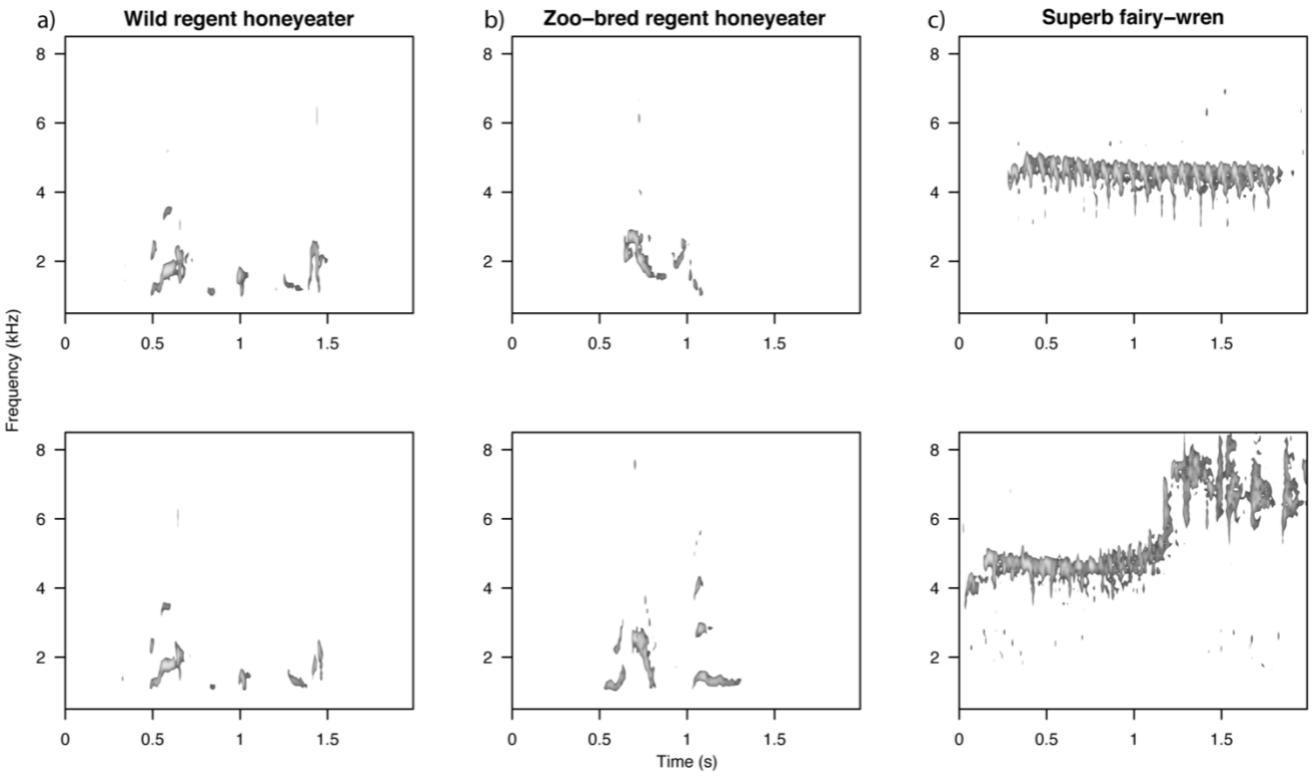

Figure 1: Example spectrograms of: column a) typical Blue Mountains wild regent honeyeater; column b) superb fairy-wren; and column c) zoo-bred regent honeyeater songs used in female regent honeyeater song preference experiments.

Song Preference Testing

We implemented song preference experiments in a bank of aviaries ordinarily used for breeding. The experimental area consisted of three 4 x 4 x 6 m aviaries with opaque Perspex dividers between them and a metre wide, floor to ceiling door linking each aviary (Fig.2). The aviaries are vegetated with a combination of live trees and shrubs, and trimmed browse affixed to the walls. We provided food, water, and flowers in the central aviary and installed four speakers on the wall 1.7m from the ground at the front and rear corners of the outer two aviaries (i.e. aviaries b and d in Fig.2).

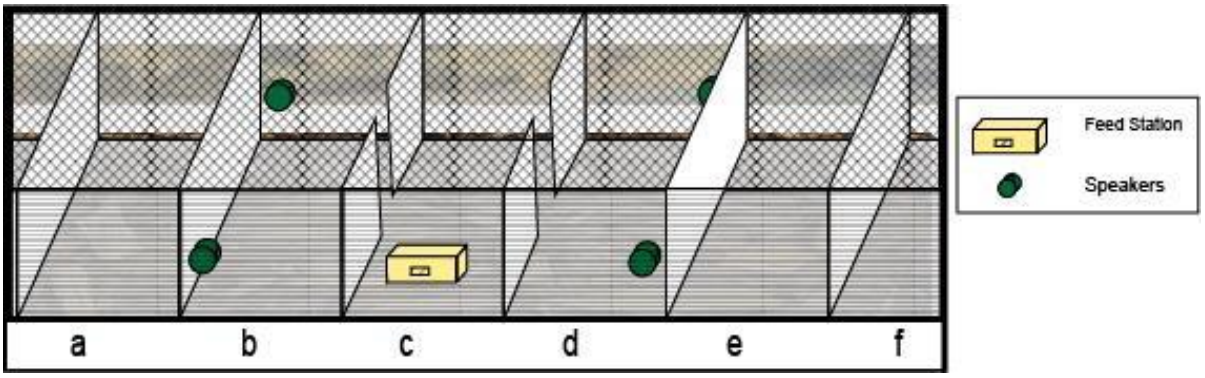

Figure 2: Floor plan of aviaries used to explore song preference in zoo-bred female regent honeyeaters. Aviaries were in a bank of 10. We selected 3 aviaries to use for the experiments at least 2 aviaries away from the edge to reduce bias toward one side. Aviaries 'a', 'e' and' 'f' were not used in the experiment.

For the description of the experimental design, we use the following definitions:

- Song bout: One song (as shown in a panel in Fig 1) from each of three different males singing the same song type, played successively in a random order, with randomized intervals of between three and fifteen seconds between the three individual songs. We chose this interval range to represent the males' natural singing frequency during the breeding season.
- Presentation: the three-minute period during which we played a female a single song bout and measured her responses to it.
- Treatment: Five song bouts of each of the three different song types: zoo-bred regent honeyeater, wild regent honeyeater and interspecific superb fairy-wren control.

To minimise stress, we undertook the experiment when females were due to be moved between free-flight and breeding aviaries. We introduced 18 females, one at a time, from the free-flight aviary to the experimental area (aviary block b, c & d in Fig 2) and gave each a minimum of two hours to acclimatise. After experimentation on each female was complete, they were moved from the experimental area to neighbouring individual breeding aviaries (e.g. aviaries e and f in Fig 2).

For the experiments, aviary 'c' was always the neutral aviary and contained the feed station. We used aviaries 'b' and 'd' for playback and they were deemed 'occupied by a male' when we played song bouts

from speakers in that aviary or 'unoccupied' when we played songs from the opposite treatment aviary. We alternated the 'occupied' and 'unoccupied' aviaries within treatments, ensuring song bouts in each treatment were played from both outer aviaries b and d. We also randomized (i) whether song bouts were played from the front or back speakers in the occupied aviary; and (ii) the order in which we presented the three treatments to females.

We tested female responses to three 15-minute treatments; unfamiliar wild-type regent honeyeater song, familiar zoo-type regent honeyeater song, and familiar but heterospecific superb fairy-wren song. During each presentation, we measured female responses to songs for three minutes, from the moment the first song within a bout was broadcast. Treatments were separated by 20 minutes of silence.

We began each song bout only when the female was settled in the central (neutral) aviary. For each song bout, we measured four female response metrics on separate stopwatches:

- Association time: The total amount of time during each presentation the female spent within the playback aviary.
- Close association time: The total amount of time during each presentation the female spent within 1m of the playback speaker.
- Latency: The time interval between the commencement of the first song in a song bout and the female entering the playback aviary.
- Negative association time: The total amount of time spent during each presentation in the outer aviary not broadcasting song.

A list of the randomised experimental protocol is provided in Supplementary Table S1.

Sample Sizes

We considered each presentation as a separate response measure as females had to return and settle in the central aviary prior to commencement of the next song bout. As each treatment had five presentations (i.e. five three-minute periods containing one song bout each), there are 20 data points for each of the 18 females, equating to 360 data points in total (90 each for zoo-bred and wild type regent honeyeater songs and 180 for control superb fairy-wren songs).

Statistical analysis

We used RStudio version 4.0.3 for all data analysis (R Core Team, 2020). We first checked the distribution of each of the four response measures. Because association, close association, and negative association times had zero-inflated distributions, we implemented two complementary hierarchical approaches for the response measures.

Binomial association and close association responses

First, we reclassified the association time within each presentation into a binomial response; 0 = no time spent in the playback aviary, 1 = time spent in the playback aviary. We then fitted a logistic regression with the binomial response of association or no association, with song type as a fixed effect and individual identity, song presentation order and aviary side (left or right) as random terms. We also included time within breeding season as an interaction term with song type:

Binomial association model <- glmer (Association or not ~ Song type + Song type:Time within breeding season + (1|Female ID) + (1|Presentation order) + (1|side of aviary), family = 'binomial', data = all data)

In cases where the female entered the playback aviary (154 times, 44/90 in response to zoo-bred, 73/180 in response to control, 37/90 in response to wild), we also reclassified close association time

into a binomial response; 0 = no time spent within one metre of the playback speaker, 1 = time spent in one metre of the playback speaker. We then fitted the same logistic regression described for the binomial association, but with the binomial response of close association or no close association using only the positive response data:

Binomial close association model <- glmer (Close Association or not ~ Song type + Song type: Time within breeding season + (1|Female ID) + (1|Presentation order) + (1|side of aviary), family = 'binomial', data =positive response data)

Association time

We then explored two approaches to analysing the full dataset, including null responses: these were a zero-inflated Poisson general linear mixed effects model (ZIP) using function *glmmTMB* via package 'glmmTMB' v1.14 (Brooks *et al.,* 2017) and a negative binomial generalized linear mixed model in 'lme4' 1.1-27.1 (Bates *et al.,* 2015). Goodness of fit measures including conditional R^2 values, normality of residuals and Akaike Information Criterion (AIC) values indicated the ZIP models were best suited to the data (see results and Supplementary Table 4). We therefore modelled association time as a function of song type, again including an interaction term for time within the breeding season (early versus late), as well as random terms for song presentation order and the side of aviary from which we broadcast the song during each bout of playback. We also included female identity within the zero-inflated component of the model (ziformula):

Association time model <- glmmTMB (Association time ~ Song type + Song type: Time within breeding season + (1|Presentation order) + (1|side of aviary), ziformula = ~1 + (1|Female identity), family=Poisson, data= all data).

Latency, close association time and negative association time

For these three measures, we focused only on the response data (i.e., positive when the female entered the playback aviary for latency and close association [154 responses] or negative when the female entered the non-playback aviary for negative association [132 responses]). For the close association and negative association models, the data were still zero-inflated, and we maintained the default zero-inflation formula. Latency response data was not zero-inflated, so we removed the zero-inflation term from the latency model formula but kept the female identity random term in the model:

Close association time model <- GlmmTMB (Close association time ~ Song type + Song type:Time within breeding season + (1|side of aviary) + (1|Presentation order), family=poisson, ziformula=~1 + (1|Female identity), data = positive response data).

Latency model <- GlmmTMB (Latency ~ Song type + Song type:Time within breeding season + (1|Female identity) + (1|Presentation order) + (1|side of aviary), family=poisson, data = positive response data).

Negative association model <- GlmmTMB (Negative association time ~ Song type + Song type:Time within breeding season + (1|Female identity) + (1|Presentation order) + (1|side of aviary), family=poisson, ziformula=~1 (1|Female identity), data = negative response data).

Results

Binomial response for association and close associations

The probability of a female entering the playback aviary in response to zoo-bred song was not significantly higher than in response to either the control playback (relative to zoo-bred songs, control β = -0.40, se = 028, p = 0.159) or to wild playback (wild β = -0.3, se = 0.32, p = 0.256, Figure 3a, Table S3). During many trial presentations females preferred to stay in the central aviary where food and water were provided. However, of the birds that did enter the playback aviary, the probability of

females subsequently approaching within 1m of the playback speaker was significantly higher in response to zoo-bred song playback than wild songs or control songs (relative to zoo-bred songs, wild song β = -3.10, se = 0.66, p = <.001, control songs β = -3.48, se = 0.63, p = <.001, Figure 3b, Table S3).

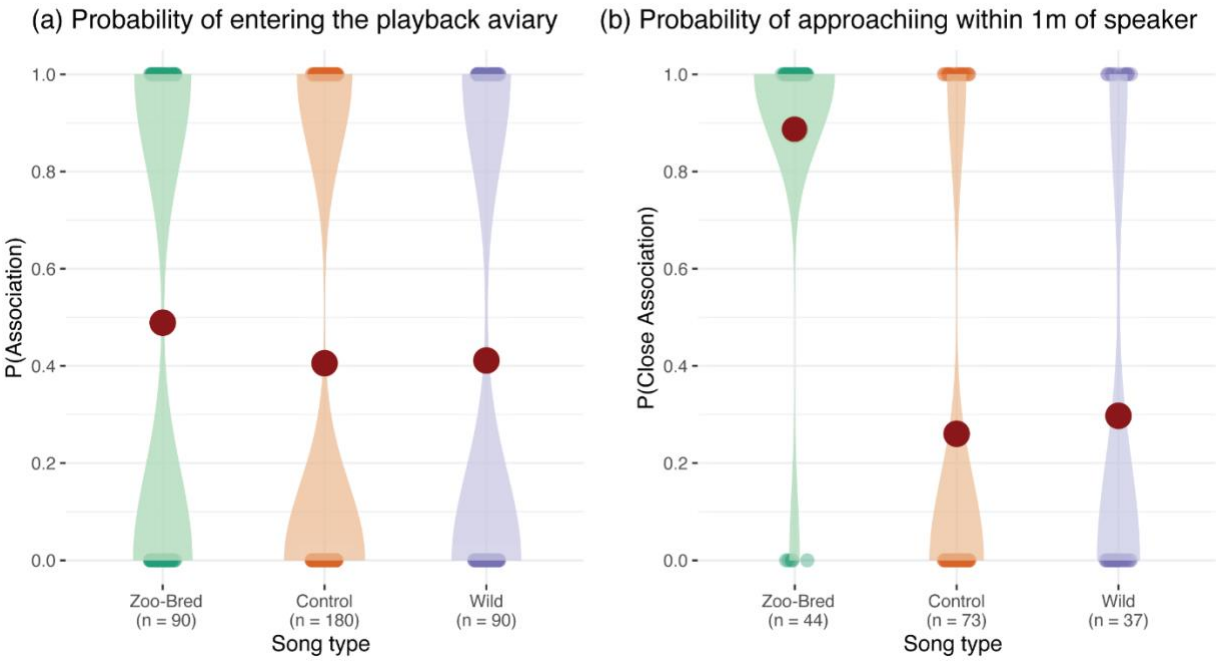

Figure 3: Logistic regression plots showing the probability of (a) positive association of zoo-bred female regent honeyeaters to playback song type; and (b) of associated birds making a close association within 1m of the speaker. The red circle represents the estimated probability, the shaded areas represent the kernel density and numbers in parentheses denote the sample sizes (i.e. the number of song bout presentations) included in each analysis.

Association time

Females spent significantly more association time in the aviaries that broadcast zoo-bred songs than in the aviaries that broadcast either the control or the wild song types in both the early breeding season (relative to zoo-bred songs, control songs β = -0.46, se = 0.04, p <.001, wild songs β = -0.48, se = 0.03, p = <.001), and the late breeding season (relative to zoo-bred songs, control songs β = -0.25, se = 0.05, p <.001, wild songs β = -0.17, se = 0.06, p = 0.03). Association time was greater in the late season for all song types (Figure 4a). The interaction between 'time in season' and 'song type' was significant (χ^2=509.77, df = 3 p = < .001, Table S6). The conditional R^2 value of the model was 0.30.

Close association time

The ZIP model for close association time revealed that the females that responded to playback spent significantly more time within one metre of the speaker (i.e. close association) in response to zoo-bred song than either wild songs or control songs in the early breeding season (relative to zoo-bred songs, wild songs β = -0.81, se = 0.10, p = <.001, control songs β = -0.43, se = 0.15, p = <.001). In the late breeding season, females associated closely with zoo-bred song playback more than either wild or control songs, but this was only significant for control songs (relative to zoo-bred songs, wild songs β = -0.97 se= 0.47, p = .29, control songs β = -1.28, se = 0.22, p = <0.001). A significant interaction between 'time in the season' and 'song type' was revealed by this model (χ^2 = 12.09, df = 3, p = 0.007, Table S6).

Latency

Of the females that responded to playback, the latency to approach the speaker was significantly shorter in response to zoo-bred song playback than control and wild song playbacks in the early breeding season (relative to zoo-bred song type, control songs β = 0.64 se = 0.07, p = <.001, wild songs β = 0.72, se = 0.04, p = <.001). In the late breeding season females' latency to approach the speaker was shorter in response to zoo-bred songs than both control and wild songs, however, this was only significant compared with control songs (relative to zoo-bred song type, control songs β = 0.36, se = 0.10, p = <.001, wild songs β = 0.003, se = 0.10, p = 0.10). Latency did not differ significantly within groups for any song type between early season and late season (Figure 4d, Table S7). The interaction between 'time in season' and 'song type' was significant (χ^2 = 52.96, df = 3, p = <.001, Table S6).

Negative association

Negative time indicates the time spent in the aviary opposite the playback aviary. We include it to show that the bird may move from its initial position in the central aviary to another aviary without being directly influenced by playback. It sets a benchmark to compare females' response to playback with.

For females that entered the non-playback outer aviary, song type was a significant predictor of the amount of time spent in the non-playback outer aviary (χ^2= 159.74, df =2, p = <.001). Females that entered the non-playback outer aivary spent significantly less time during the zoo-type playbacks than during the control and wild playbacks in the early season (relative to zoo-bred songs, control songs β = 0.47, se = 0.06, p = <.001, wild songs β = 0.51, se = 0.04, p = <.001, Figure 4c). In the late season, there were no significant differences in the amount of time spent in the non-playback outer aviary (relative to zoo-bred songs, control songs β = -0.30, se = 0.11, p = 0.11, wild songs β = -0.55 se = 0.12, p = <.001). The interaction of time in season and treatment was significant (χ^2 = 50.12, df = 3, p = <.001).

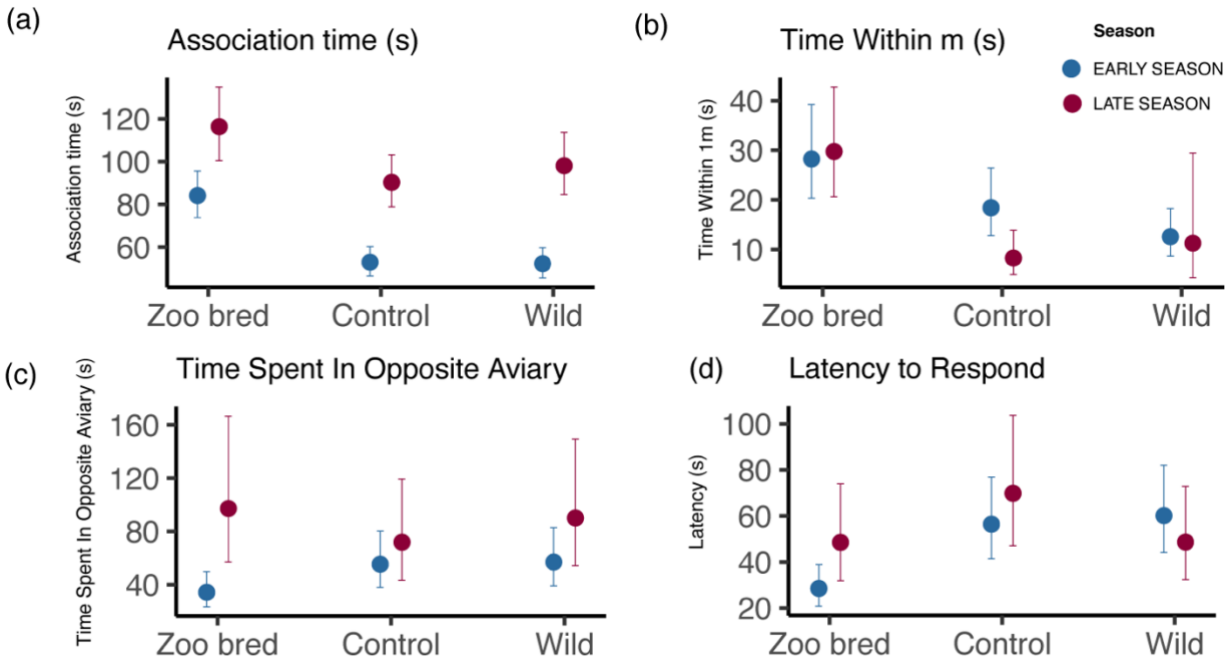

Figure 4: Prediction plots for linear mixed models showing mean +/- SE amount of time spent by zoo-bred female regent honeyeaters in (a) the playback aviary; (b) within 1m of the playback speaker; and (c) in the non-playback outer aviary, and (d) the latency to enter the playback aviary, by song playback type and time within breeding season.

Discussion

Understanding the fitness consequences of captivity on individual animals bred for release into the wild is critical to the success of *ex-situ* conservation efforts (Baskett & Waples, 2013; Crates *et al.,* 2022). Using a rigorous experimental protocol within an applied zoo breeding environment, we demonstrated that female regent honeyeaters were no more likely to enter an aviary containing playback of the familiar songs of zoo-bred males than the unfamiliar songs of wild regent honeyeaters or an interspecific control. However, of those individuals that did enter the playback aviary, females spent a longer time in the aviary and were more likely to approach to within 1m of the playback speaker in response to familiar, zoo-bred males' songs than unfamiliar wild males' songs or the songs of an interspecific control. This is the first demonstration that female zoo-bred birds prefer familiar but abnormal songs from the captive environment to those of a wild counterpart. We conclude that assortative mating based on a preference for familiar song dialects is a risk for the regent honeyeater zoo-breeding program, with potentially negative consequences for reintroduction efforts. However further work is required post-release to better quantify these risks. Our study adds to the growing body of literature exploring potential fitness consequences of divergent cultural norms between wild and zoo-bred populations in conservation programs (Lewis *et al.,* 2020, Crates *et al.,* 2022). Here we discuss the implications of our findings for adaptive management of zoo-bred populations.

Playback studies have the difficult task of interpreting the meaning of the female's behaviour (Fujii *et al.,* 2022). Our results showed a significant difference in the probability of females entering the playback aviary in response to the familiar, zoo-bred songs compared with the control but not to the unfamiliar, wild song type. While the individual responses were inconsistent, 16/18 females responded to zoo-bred playback in at least one of the presentations of playback. There are at least two possible explanations for this. First, a female could enter the playback aviary at any time during the trial, not just when the playback was being broadcast. As such, an approach could be misinterpreted as interest in the song

when the motivation for entering the aviary could be related to other factors. The probability of entering the non-playback outer aviary was lower but comparable across song types suggesting that bird movements can be independent of broadcasted playback. Second, some females may have had a greater interest in visiting the feeder in the neutral aviary than in responding to playback, thereby weakening any effect of preferences for song types.

Arguably, more meaningful measures of preference than entry to the playback aviary are the amount of time spent in the playback aviary and the probability of approaching the speaker closely. The amount of time spent associating with a potential mate has been shown to be a reliable indicator of mate preference in birds (Witte, 2006). We found that females spent a significantly greater amount of time in the playback aviary, and were more likely to approach the speaker closely, in response to familiar, zoo-bred songs than either wild songs, or those of another species, regardless of the time within the season. We included close association time (time spent within one metre of the speaker) as a clearer indication of female response due to the separation of the speakers from the vegetated areas elsewhere in the aviary and the direct investigation of the source of sound. Close association time was also significantly greater in response to familiar songs than unfamiliar ones. Latency to respond is also a consistent indicator of behavioural response in passerine birds (Freeman & Montgomery, 2017; Vargas-Puentes *et al.,* 2021). Our results showed that the latency to approach the playback aviary was significantly shorter in response to familiar, zoo-bred songs than unfamiliar ones. We included negative association time, not as a direct measure of interest but to demonstrate the nature of movement in the experimental aviary and the amount of time spent in an area with no playback. Our results demonstrated that birds spent a similar overall amount of time in the non-playback outer aviary as in the playback aviary in response to wild and control type songs. In response to familiar songs, birds spent less time in the non-playback outer aviary than when they were exposed to the unfamiliar song types.

The two clearest indicators of response, close association time and latency, were the same or stronger in the early breeding season, while association time was greater in the late breeding season (Fig. 4). The early breeding season represented experiments undertaken in late June. At this time zoo-bred birds are observed attempting to form pairs and collecting nesting material (pers comm.). The late season represented the experiments undertaken in January, a time when birds may be underway with a second or third clutch, potentially with a new partner (Geering & French, 1998). At this time wild regent honeyeaters may also have finished breeding and be gathering in post breeding flocks. Thus, some females may have been less motivated to seek out mates at this time, either because of the limited time remaining for breeding and/or because of hormonal changes as breeding winds down (Maney *et al.,* 2008). Further, although females spent longer in the playback aviary than the non-playback outer aviary during the late breeding season, they did not necessarily investigate the speaker. This may have been a function of the increased temperature at this time of year, leading to a trade-off between activity and temperature regulation (Pattinson *et al.,* 2020). Although familiar songs resulted in significantly less negative association time than unfamiliar songs in the early season, this was the not the case in the late season. Combined with the fact that negative time was lower in the early breeding season for all song types suggests if interest in the playback stimulus is lower in the late breeding season, then bird movements may be independent of the playback stimulus. Alternatively, this may reflect the smaller sample size of females assayed later in the breeding season.

In tandem with the wider regent honeyeater literature, our study suggests that variations in song dialects and associated differences in female familiarity with these dialects could contribute to assortative mating based on song type in this species. The results from our study are consistent with Crates et al. (2021) who demonstrated that wild males that sang songs different to the regional cultural norm were less likely to be paired with a female, and those that did pair were significantly less likely to build nests that reached the egg stage. Further, both sexes showed a higher propensity to survive post-release when exposed to song-tutoring (Tripovich *et al.,* 2021). Our results may also help

to explain the rarity of pairings between released zoo-bred birds and wild birds, despite releases taking place within known breeding areas (Ingwersen, 2014; Kvistad *et al.,* 2015). None of these observations show that song differences alone are responsible for the lack of breeding success. Birds with non-locally conforming songs in Crates' study were often isolated from other birds, and as such may have had limited opportunities to attract females. Likewise, the lack of zoo-bred male – wild female pairs after release may be due to a paucity of females at release sites or be influenced by differences between zoo-bred and wild males in other sexual signals such as plumage, ornamentation or physical condition (Burley, 1981; Candolin, 2003; Wang *et al.,* 2022). However, even amongst paired males, those with an abnormal song were significantly less likely to initiate nesting, suggesting that song abnormalities could carry a fitness cost (Crates et al. 2021).

Similar to other studies (Fujii *et al.,* 2022; O'Loghlen & Beecher, 1997; Vargas-Puentes *et al.,* 2021), our results show that female responses to male vocal stimuli were not limited exclusively to familiar songs but were significantly stronger than to unfamiliar ones. The evidence of wild females pairing with males who sing non-local songs, and zoo-bred birds pairing with wild birds suggests that abnormal song is not an absolute barrier to pairing. Despite this, aberrant song may still have fitness consequences for released birds. Females constrained to non-preferred partners by a lack of choice due to small population size or low population density may experience deleterious effects to both their physical condition and to the success of the breeding effort (Griffith *et al.,* 2011). This appears to be consistent with Crates et al. (2021) who showed that aberrant song was not an absolute barrier to pairing, but it did result in significantly lower likelihood of producing a nest that reached the egg stage.

Our study provides evidence that females prefer to associate with males that sing familiar songs, suggesting that they may be more likely to pair with these males than those that sing unfamiliar songs. Assortative mating is likely to inhibit gene flow that is pivotal in the conservation of critically endangered species (Slade *et al.,* 2014) and has the potential to reduce the overall number of birds forming pairs

and hence the number of offspring produced. If dialect differences inhibit released birds from integrating into established flocks, the experience of wild birds is unlikely to be conferred to re-introduced birds (Kleiman, 1989), which models show will compromise the capacity of zoo-bred birds to population recovery post-release (Heinsohn et al. 2022).

Considering the song differences between wild and zoo-bred birds, we have now implemented a song tutoring program at Taronga Zoo that exposes young birds to the songs of wild males with the aim of improving post-release outcomes. Our study has important implications for adaptive management of the zoo-breeding program, highlighting the importance of exposing not only males, but also females, to wild-type songs given the evidence that courtship preferences in birds can be shaped by juvenile auditory experiences (Chen *et al.,* 2017). Secondly, it reinforces the need to release females in places and at times they can gain rapid exposure to wild males and their songs prior to the breeding season. More broadly, our study shows the importance of the potential, but often-overlooked fitness consequences of cultural isolation in zoo-breeding settings (Brakes *et al.,* 2019). The initial implementation of the regent honeyeater breeding program aimed to understand husbandry requirements and consequently began with a small number of nestling wild founders and implemented hand-rearing (Liu *et al.,* 2014). This prevented crucial social learning of wild song dialects from the commencement of the breeding program, which has influenced the song culture of all successive generations. The selection of founders for *ex-situ* conservation programs must consider not only their life-stage but also their behavioural competency/diversity. Programs must also be designed to maintain these traits across generations and facilities. Conservation breeding programs must strive to consider socially learned behaviours in their management of species.

Small scale behavioural experiments are rarely a priority in zoo-breeding programs (Kemp *et al.,* 2015) as limited resources are often directed towards husbandry and breeding outcomes. However, it is increasingly acknowledged that the phenotypic quality of zoo-bred animals is as important as their

quantity in determining the success of reintroductions (Crates et al. 2022). Despite the limitations of working on critically endangered species amongst a raft of research and conservation priorities, our study demonstrates that carefully planned experiments can be incorporated into husbandry practices with minimal inconvenience. We suggest such experiments should be considered an essential part of reintroduction programs, as they can inform the adaptive management of husbandry and release protocols to increase the fitness of zoo-bred animals and thereby the success of *ex-situ* conservation more broadly.

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Ethics

Research was conducted under New South Wales scientific licences #SL101580 and #SL101603. All experiments were conducted in accordance with Taronga Zoo Ethics approval #4a/02/20 and Australian National University Animal Ethics permit #A2015/28.

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Chapter 4: Rescue of the traditional song culture of a critically endangered songbird

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ABSTRACT:

Animal cultures are learned behaviours that are maintained in populations through social learning and conformity. Global reductions in species' populations can lead to the loss of animal cultures, which may further reduce individual fitness. The Critically Endangered regent honeyeater (*Anthochaera phrygia)* is an Australian songbird whose population is declining to the extent that individuals have insufficient contact with conspecifics to maintain their song culture. Zoo-bred males that supplement the wild population sing songs that differ from all wild birds, representing a cultural barrier that may impact their fitness post-release. Over three breeding seasons within the captive breeding system, we undertook adaptive song tutoring experiments using song playback and live tutoring to teach zoo-bred juveniles the wild song. The proportion of juveniles that learned the wild song increased from zero to 42% within three years. The full version of the wild song taught to zoo-bred males disappeared from the wild during the study, making the zoo population the only remaining source of traditional song culture. Using just two wild founders, we show how animal cultures can be restored in ex-situ populations with simple modifications to husbandry protocols. Ex-situ populations could then help maintain and restore wild animal cultures through reintroductions.

Introduction

To address the global biodiversity crisis, ex-situ breeding of animals for release into the wild is now an integral conservation tool[1]. The success of animal reintroductions has been the subject of much scrutiny however[2-4]. Whilst practitioners increasingly apply more rigorous approaches to the management of ex-situ populations and the decision-making process around release planning[5-7], the success of ex-situ reintroduction programs in terms of their contribution to recovery of wild populations remains questionable[8] and their success may depend on practitioners' consideration of natural behavioural repertoires in captive animals.

The suite of factors known to affect reintroduction success is large and growing[2,3,9]. Whilst these factors are often species-specific and linked to whether the initial drivers of population decline have been mitigated[10], there is increasing evidence that the phenotypes and associated post-release fitness of zoo-bred animals can be impacted negatively by life in captivity[11]. This in turn can hinder the contribution of zoo-bred animals to the recovery of wild populations[12-14].

The predominant focus of ex-situ breeding programs has traditionally been on genetic diversity and disease prevention[15,16]. Whilst both factors are fundamental for maintaining viable ex-situ populations, behavioural differences between zoo-bred and wild animals rank among the top reported issues hindering the success of reintroduction programs[9]. Manipulating behaviours to improve translocation success is an emerging tool for adaptive management of translocation programs[17]. Such manipulations have largely focused on pre-release exposure to novel-stimuli such as antipredator training, or soft-release strategies where individuals can learn and adapt behaviours to novel-environments before being released fully[18,19]. Culturally acquired behaviours that may impact translocation success have received relatively little focus in translocation planning,[17] but are increasingly being recognised as a critical component of effective conservation[20,21].

Animal culture refers to the transmission and maintenance of learned behaviours, traditions, and knowledge within animal populations through social learning processes such as observation, imitation, and teaching[20,22]. Similar to human culture, animal cultures encompass a wide range of behaviours, including feeding techniques, communication signals, mating rituals, and tool use, which are transmitted within and across generations and may vary between different social groups or populations[20,23]. These cultural behaviours often contribute to the adaptation and survival of individuals within their respective environments and can shape social dynamics and ecological interactions within animal communities. In captivity, culture may be degraded or lost by preventing necessary social interactions and exposure to suitable adults from which juveniles learn key behaviours[11]. This is problematic where ex-situ populations contain candidate individuals for release into the wild, as culturally-acquired behaviours may be critical for post-release fitness[20]. Birdsong is one of the most well-known examples of animal culture[20,22]. Learned vocalizations in songbirds serve critical functions in mate attraction, territory establishment and defence, species recognition, and social cohesion[21]. The loss or degradation of song culture in ex-situ and/or wild populations can therefore have direct fitness consequences by reducing breeding success and limiting social integration.

The regent honeyeater (*Anthochaera phrygia*) is a Critically Endangered Australian songbird[24] whose population has declined primarily due to extensive loss and degradation of woodland habitats through agricultural clearing, dieback, and forestry practices, while remaining habitat is further threatened by climate-change driven drought and bushfire[27]. With an estimated wild population of fewer than 250 individuals[25], zoo breeding to bolster the wild population through reintroductions and provide an insurance against further population decline is a high priority recovery strategy[26]. Supplementation of the wild population with zoo-bred birds has had limited success in arresting the population decline however[27,28], with the majority of zoo-bred birds failing to establish a breeding territory or pair with wild mates[28-29].

Wild male regent honeyeaters sing only one of several song types with loose geographic dialects[30]. Females of this species do not produce the full song that the males sing, and instead produce subtle calls, warbles and 'mews' which may be innate . The dominant wild song type within the core range of the remaining wild population in New South Wales is defined as the 'Typical Blue Mountains' song[30]. A severe decline in the size and density of the remaining wild regent honeyeater population is leading to loss of the species' song culture – many young males either learn the songs of other species or sing an abbreviated version of the 'typical Blue Mountains' song with half the number of syllables[30,31]. The nomadic ecology of regent honeyeaters means young males encounter different heterospecific models at dispersed breeding sites, precluding convergence on a shared, species-specific song culture[30]. As population density declines and social learning opportunities diminish, song degradation likely impairs mate attraction and territorial function, potentially contributing to Allee effects[45,46.]

Zoo-bred males sing a distinct abnormal song that is different from all wild song types[30]. As passerines, young male regent honeyeaters learn their songs via exposure to and imitation of conspecific tutors[32]. The zoo population was founded through the collection of nestlings in 1995 and since then juvenile males have been 'crèched' together during their critical song-learning period in early life[30]. Zoo-bred regent honeyeaters have therefore never had the opportunity to learn wild regent honeyeater song culture from older conspecifics. Instead, they crystallise an adult song that resembles the warbling calls of juveniles, suggesting zoo-bred juveniles learn songs from each other in the crèche. The distinct differences between the songs of wild and zoo-bred birds may represent a significant cultural barrier to the assimilation of zoo-bred birds into wild flocks due to assortative mating[33]. Zoo-bred females prefer the familiar but aberrant songs of zoo-bred males over the unfamiliar songs of wild males[14].

We aimed to eliminate an important cultural divide between a zoo-bred and wild animal population within an applied zoo-breeding environment by teaching zoo-bred regent honeyeaters the dominant

wild song culture in the area they will be reintroduced to through song tutoring experiments and adaptive management. Song tutoring experiments occurred over three years within six different treatment groups that varied principally in tutoring method and cohort size (Table 1). At the start of the study, only two wild origin male regent honeyeaters that were recruited to the zoo population sang the Typical Blue Mountains song and were available as live tutors. The alternative tutoring approach was to use song playback, based on recordings of other wild males' songs from the Blue Mountains population. We adaptively modified treatments in later years of the study based on the results of the previous years' tutoring approaches. Our overall goal was to seed the dominant wild song type into the zoo-bred population culturally, such that the wild song culture can be sustained in captivity as part of routine management practice. Our secondary goal was to gain insights into the key factors determining song learning in regent honeyeaters.

Results

Before the first year of the experiment, only the two wild-origin males sang the typical Blue Mountains song within the zoo population. By the end of the experiment, 32 males representing 42% of the male zoo population, which fluctuates annually depending on the number bred and the number reintroduced to the wild, sang songs that were statistically indistinguishable from the typical Blue Mountains song based on discriminant function analysis (DFA) and general linear models (GLMs, Fig. 1). We successfully developed a sustainable song tutoring protocol based on simple modifications to existing husbandry practices that will give future generations of regent honeyeaters born in captivity the opportunity to learn culturally conforming songs from older conspecifics.

During the first year of the experiment, no juveniles successfully learned the typical Blue Mountains song (Treatments one – three: control group, playback only large cohort and tutor only large cohort, Table 1, Fig. 1a). Model predictions based on Mahalanobis distances showed the average songs of males in all three groups were significantly different from the wild reference group (Fig. 1b, Table 2).

Table 1: Summary of the zoo-bred regent honeyeater song tutoring experimental design under an adaptive management framework showing cohorts of juveniles, their tutoring treatment, site, aviary type and cohort size. Breeding site TWPZ refers to Taronga Western Plains Zoo, Dubbo; TZ refers to Taronga Zoo, Sydney. Zoo-bred birds in the Tutor origin column refer to zoo-bred males that successfully learned the typical Blue Mountains song in previous years of the study. See Fig. S1 for description of aviary types.

Treatment group	Breeding season	Breeding site	Aviary type	No. males in cohort	No. live tutors	Tutor origin
1. Control	2020/21	TWPZ	2	9	N/A	N/A
2. Playback only, large cohort	2020/21	TWPZ	2	9	N/A	N/A
3. Live tutoring only, large cohort	2020/21	TZ	2	13	1	Wild
4. Playback only, small cohort	2021/22	TWPZ	2	4	N/A	N/A
	2021/22	TWPZ	2	5	N/A	N/A
5. Live tutoring + playback, small cohort	2021/22	TZ	1	4	1	Wild
	2021/22	TZ	1	4	1	Wild
	2022/23	TZ	1	4	1	Wild
	2022/23	TZ	1	5	1	Zoo-bred
6. Live tutoring only, small cohort	2022/23	TZ	2	5	2	Zoo-bred
		TZ	2	5	2	Zoo-bred
		TWPZ	3	4	2	Zoo-bred

By the end of the second breeding season, no juveniles in Treatment four (playback only, small group) produced the typical Blue Mountains song, however eight juvenile males in Treatment five (live tutor + playback) learned to sing songs that closely-resembled the typical Blue Mountains song type. DFA was unable to distinguish the songs of these birds from the wild reference songs (Fig. 1a), with the GLM confirming the songs of birds in this group were not significantly different from the wild reference songs (Fig. 1b, Table 2).

Four males from Treatment group five were employed as live tutors in the third year of the experiment. Together with the two wild origin birds, these four males successfully tutored an additional 19 juveniles in year three; all within Treatment group six (live tutor only, small cohort). The songs of these birds could also not be distinguished from the typical Blue Mountains reference songs by DFA (Fig. 1a). Again, there were no significant differences between the songs of birds in Treatment six and those of the wild reference group (Fig. 1b-c). Despite having a shorter tutoring period, songs of younger juvenile males

hatched later in the season were no less similar to the reference songs than those of older males from first clutches (Table S1).

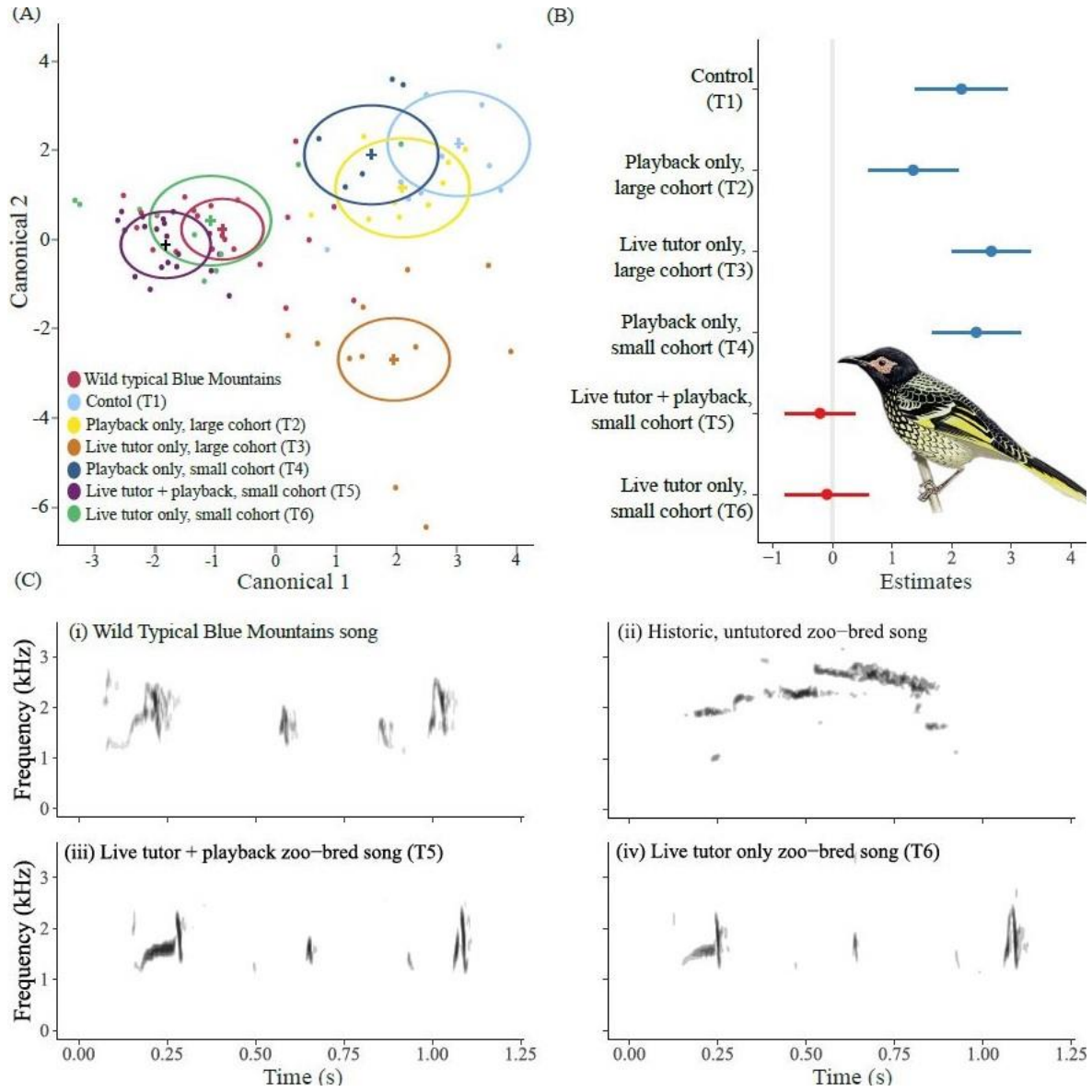

Fig 1: (a) Discriminant function analysis (DFA) of regent honeyeater songs by treatment group. DFA labels each treatment group or reference group multivariate mean with a circle corresponding to a 95% confidence limit for the mean. Groups whose songs are significantly different have nonintersecting circles. The first two discriminant functions explained over 84% of the total variance in the song samples. The model's overall significance was supported by multiple test statistics (wilks-lambda= 0.062, f = 20.10, p = <0.001; Pillai's Trace = 1.83, f= 13.90 p= <0.001); (b) Model estimates showing the effect of regent honeyeater song tutoring experimental treatment on the similarity of songs to the culturally dominant 'typical Blue Mountains' wild regent honeyeater songs based on Mahalanobis distances. Points show predictions, lines show 95% confidence intervals. Estimates in red show the two treatment groups (5 & 6) whose songs show no statistically significant difference to the typical Blue Mountains reference songs as per Table 2. (c) Spectrograms showing example songs of: (i) wild Typical Blue Mountains; (ii) Historic zoo-bred song; (iii) tutored zoo-bred male with live tutor and playback

(Treatment 5); (iv) tutored zoo-bred male with live tutor only (small cohort- Treatment 6). See Fig. S2 for indicative spectrograms of juveniles in treatment groups 1 – 4.

Table 2: Model summary derived from a general linear model showing the effect sizes of treatment group in explaining the differences between the songs of zoo-bred regent honeyeaters. Models are based on the Mahalanobis distance between the mean acoustic attributes of males in each group relative to the average culturally-dominant typical Blue Mountains wild song. P-values in bold indicate significant differences at the $p < 0.05$ level. See Fig. S3 for model diagnostics.

Coefficient	*Estimate*	*SE*	*t*	*p*
Control (T1)	2.16	0.40	11.39	**<0.001**
Playback Only (Large, T2)	1.35	0.30	-0.72	**<0.001**
Live Tutor (Large, T3)	2.66	0.34	7.91	**<0.001**
Playback Only (Small, T4)	2.41	0.38	6.33	**<0.001**
Live Tutoring & Playback (T5)	-0.21	0.30	-0.72	0.48
Live Tutoring (Small, T6)	-0.10	0.36	-0.27	0.79

Discussion

The importance of considering animal cultures in conservation programs is increasingly being acknowledged[20]. We show that it is possible to reintroduce lost wild culture into an ex-situ animal population by providing the first example of successful song tutoring in an active conservation program. Both live tutoring (small cohort) and live tutoring with playback supplementation were successful in training birds to sing songs that did not differ significantly from the reference wild song. We were able to utilise birds from previous year's successful experiments as additional tutors to increase the rate at which the wild song culture was culturally seeded in the zoo population. Playback only tutoring in both large and small tutor groups was unsuccessful and did not differ significantly from the control. Live tutoring (large cohort) alone did differ from the control but was also significantly different from the reference wild song. The results of our study, whilst limited in design by the unavoidable constraints of operating within an applied zoo-breeding facility, suggest that being able to interact with a live tutor in a social group with a high ratio of adult tutors to juveniles are the key factors influencing whether birds are able to successfully acquire the wild song culture.

In numerous bird species, tutoring via playback is observed to be less effective than instruction from a live tutor[34,35]. The understanding of avian learning processes predominantly stems from a limited selection of taxonomic groups. In this study, birds subjected solely to playback tutoring exhibited an expansion in song complexity relative to untutored, zoo-bred birds but failed to replicate accurately the target wild-type song. It has been suggested that birds exclusively exposed to playback tutoring may exhibit lower success rates in learning the reference song[35,36]. As the birds progress into the sensorimotor stage of learning, the songs of associates may provide a more enriched form of learning than the playback[36], which could facilitate horizontal transmission of song among juveniles within the aviary. This horizonal transmission would then be compounded by the addition of younger birds throughout the experiment who may learn predominantly from older juveniles rather than the audio playback. This hypothesis aligns with the findings of Derégnaucourt and Gahr[37], who show that within generation song learning can occur in zebra finches *Taeniopygia castanotis* where a young bird exposed to songs of a tutor can teach another bird not exposed to the tutor some elements of reference song. In that study, juvenile songs converged more closely with each other than with the initial reference tutor.

The live-tutoring only (large cohort) also failed to facilitate juveniles to accurately produce wild-type regent honeyeater songs, although the songs of juvenile males within this group were closer to the wild reference songs than those of the playback treatments. We propose a similar mechanism for this result; in this instance juvenile birds did not receive playback or live tutoring during the earliest stages of independence, but rather had no tutoring at all until the adult tutor was moved into the experimental aviary. While the exact timing of song learning in this species is unknown, we propose that the introduction of the live tutor fell between the sensory and sensorimotor stages and as such limited the amount of sensory learning opportunity for the juvenile males in Treatment three. Experiments depriving young birds of tutors have demonstrated that the absence of a tutor in critical learning phases reduces the likelihood that young birds will learn new syllables after the introduction of a tutor[38]. It is

also possible that as the oldest birds were deprived of a live tutor for 2.5 months, their maladapted songs may have then been horizontally transmitted to other birds in the cohort.

The two successful treatments shared two key features: opportunities for multimodal learning from live tutors and small cohorts. There is probably a relationship between smaller cohorts and truly multimodal learning; where the live tutoring only (large cohort) presented the *opportunity* for multimodal learning, it was likely not realised due to the large ratio of students to tutors. The small cohort size may have allowed for frequent and prolonged interaction with the tutor, and this is consistent with previous research that links the number of tutors with song learning fidelity[39]. Anecdotally, we observed this occurring in real time; in the large cohort group the tutor was overwhelmed by practicing juveniles and frequent song practicing was seen between juveniles. In the small cohorts, the adult male dominated high value areas like the feeders more easily. During these interactions we observed head-bobbing and bill-snapping typically associated with the crescendo of the typical Blue Mountains song.

While both successful cohorts were not statistically distinguishable from the reference wild song, birds that only received live tutoring resembled their specific tutor more closely than the wild reference. This is perhaps due to the increased variation in reference songs available to these birds but critically, unlike the playback only cohort, the live tutoring + playback cohort had exposure to a wider range of songs, that may impact the reference template and birds own song experience consistent with the eavesdropping hypothesis[40].

While it is possible that natural cultural drift caused the divergence over time between wild and zoo-bred regent honeyeaters, it is highly likely that hand rearing of founder juveniles caused significant cultural divergence and drift within the zoo population from the very start[14,30]. Our results show that although the control group remained the least similar of all treatments to the typical Blue Mountains song, those birds' songs were considerably more similar to the typical Blue Mountains wild song than

historical ex-situ songs. This may be reflective of the increased socialisation that a larger ex-situ population affords or may be due to changes to housing arrangements that crèche juvenile birds closer to adult, albeit ex-situ tutors.

The results of our study may have significant implications for conservation efforts. Regent honeyeaters bred in captivity have generally shown a low propensity to integrate with wild birds after being released and an even lower propensity to pair and breed[30]. Assortative mating based on differences in song culture is common amongst birds[41,42] and previous research suggests that zoo-bred female regent honeyeaters prefer the familiar songs of zoo-bred males to the unfamiliar typical Blue Mountains song produced by most wild males[14]. Our experimental design exposed female juveniles to wild song tutoring alongside males in all treatment groups, potentially influencing their subsequent mate preferences. While we did not measure female song preferences in this study, exposure to wild-type songs during development could shift female preferences toward males singing wild-type songs, potentially reinforcing rather than undermining the cultural restoration we achieved in tutored males. Future research should examine whether early exposure to wild song culture affects female mate choice in this species. Reintroducing wild songs to the ex-situ population may increase the likelihood of breeding events between reintroduced and wild regent honeyeaters[14] and increase social cohesion at the flock level[43,44].

At the inception of this project the typical Blue Mountains song was the most common song sang in the target release site[31]. Since 2020, monitoring has shown that the typical Blue Mountains song has effectively disappeared from the wild population and has been replaced by the more simplified 'clipped Blue Mountains' song type that contains half the number of syllables[31]. This raises an important consideration: if current wild birds predominantly sing the simplified song, teaching zoo-bred birds the traditional complex song could theoretically reduce initial pairing success due to assortative mating preferences. However, restoring traditional song culture aligns with the broader hierarchy of

conservation objectives for this species. While maximizing short-term pairing success between reintroduced and wild birds is important, the primary goals remain long-term population recovery and maintenance of functional breeding systems. The simplified song may itself reflect ongoing population decline and could perpetuate Allee effects that undermine population viability[45,46]. Moreover, as released zoo-bred birds now represent an increasingly large proportion of the wild population and are the sole repository of traditional song, reintroductions offer an opportunity to reverse cultural erosion rather than simply accommodate it. From a practical standpoint, continuously adapting tutoring protocols to match rapidly eroding wild song culture is unlikely to be feasible given the multi-year timescales of captive breeding programs and any associated cultural tutoring that may be required. Providing a stable cultural reference through traditional song tutoring creates beneficial inertia against further simplification, and our results demonstrate that even imperfect song learning produces substantially more complex and functional songs than those of untutored zoo-bred birds.

Whilst cultural drift and revolution are common features of animal populations, it is more likely that simplification of the song culture is reflective of an ongoing decline in the size and density of the wild regent honeyeater population[27], making it increasingly challenging for cultural diversity to be maintained at the population level through social interactions[30,31]. This decline in song complexity may have downstream Allee effects that negatively impact the viability of the wild population[45,46]. The results of our study mean that zoo-bred birds are now the sole source of traditional song cultural knowledge in regent honeyeaters. As it has been shown previously that songbirds can learn songs experimentally in the wild[47], the results of our study open up the exciting opportunity to use the reintroduction of zoo-bred birds to re-establish or reinforce the species' traditional song culture in the wild population.

Our results highlight several questions for future research. First, determining whether song learning can begin during the nestling period, and whether early playback exposure would enhance or interfere

with subsequent learning, remains unclear for this species. Second, investigating whether developmental exposure to wild songs shifts female mate preferences would clarify the long-term viability of cultural restoration in reintroduced populations. Finally, systematically varying tutor introduction timing could reveal the critical windows for sensory and sensorimotor learning in regent honeyeaters, improving our ability to optimize tutoring protocols.

As global biodiversity declines, it is likely that the erosion or loss of animal cultures will become more apparent over coming years[30]. Whilst bird song provides arguably the most prominent examples across a range of species whereby declines in population size or density can impact the persistence of culture[48], maintaining a sufficient number and frequency of social interactions is important for conserving socially-learned behaviours across multiple domains[49]. Our study highlights the important role of experienced adults as repositories of cultural knowledge in conservation programs. It also demonstrates the importance of facilitating social interactions at all stages of the husbandry process in ex-situ breeding, to help maintain socially acquired behaviours that may have a significant bearing on post-release fitness and the overall effectiveness of reintroduction programs[11]. While the song learning timeline and processes we describe are shared across many songbird species, the specific details vary between taxa, and we encourage researchers to adapt this protocol based on the developmental trajectories and learning mechanisms of their target species. For species where wild populations are or too small or sparsely distributed to practically provide live tutors, we recommend proactive recording of vocal cultures before populations decline to critical levels; however, our results suggest that recordings alone are insufficient and conservation practitioners should prioritize maintaining access to live adult tutors even in small captive populations. Given the increasing recognition of the need to consider animal cultures in conservation[22], we provide clear evidence that this can be achieved rapidly and at little cost within applied conservation settings through adaptive management. There is great scope for similar studies on other endangered populations s (e.g. Cirl Buntings[63]) to help reduce to the phenotypic divide between wild and zoo-bred populations[11]. This will require improved monitoring of

wild animal populations to better understand the dynamics of culturally-acquired behaviours[50], as well as increased collaboration between academics and applied conservation practitioners to facilitate research, reporting and implement evidence-based changes to zoo-husbandry and reintroduction strategy[11]. Such actions could make a significant contribution to minimising the loss of not only global biodiversity over coming decades, but also its emergent properties such as birdsong that form an intrinsic part of human culture and wellbeing.

Methods

Study population and tutoring protocols

Birds were born and housed in Taronga Zoo (TZ), Sydney and/or Taronga Western Plains Zoo (TWPZ), Dubbo. All birds included in this study were part of the active zoo-breeding program and therefore subject to routine husbandry protocols. Experiments needed to be incorporated in the least disruptive manner to ensure they achieved the overall aim of establishing wild song culture in the zoo population without impacting negatively the breeding capacity of the program.

During the experiments birds were housed in one of three aviary types (Fig. S3). We conducted tutoring experiments over three Austral post-breeding seasons (September - March) commencing in 2020. We implemented five treatments and one control treatement across the two breeding institutions in the first season and adaptively refined the treatment groups before each subsequent season based on the results of previous seasons' experiments. See Supplementary text S1 for further information on the study population and treatment groups.

We considered two common experimental song tutoring techniques as options for teaching juvenile birds to sing the 'typical Blue Mountains' wild song.; live tutors and audio playback. Live tutoring has shown more success than audio-only tutoring[34,51], however due to the rarity of this species in the wild, acquiring wild birds is a logistical and ethical challenge that is compounded by regional song differences

and the presence of wild birds with abnormal songs[30]. Only two wild males recruited to the TZ breeding population in 2019 sang the 'typical Blue-Mountains' song and were available as live tutors in the first year of the experiment. Regent honeyeaters are probably 'closed-ended' song learners, with males' songs becoming largely fixed after a period of learning and crystallisation in the first year of life[30].

Playback tutoring has proven successful in many species[52] and is logistically feasible in the zoo-breeding setting. Wild regent honeyeater song recordings were available from previous research[30] and could be implemented with minimal disruption to the zoo's routine husbandry activities (Supplementary text S2). The remaining years of the study were adapted based on preliminary results of the previous years, the rationale for which can be found in Supplementary text Text S3.

Standard timeline

All treatments shared the same timeline, with juvenile males transferred from their natal aviaries to their respective experimental crèche aviaries (Fig. S1) approximately two to three weeks post-fledging (~30 days old). Juveniles are typically moved out of their natal aviaries at this point as fathers become territorial and aggressive towards juveniles prior to re-nesting with second or third clutches per season[53]. Juvenile males were exposed to song tutoring from the time they entered the experimental crèche until the conclusion of the breeding season when they are transferred to a larger, multi-species flight aviary,[28]. See Table S2 for details of experimental timelines. Some juveniles from second or third broods received a shorter duration of song tutoring (Range = 128 – 208 days, mean = 162), but all were moved into the experimental aviaries at approximately 30 days old. This reflects the typical wild song-learning environment, where juveniles from different broods join larger, mixed-age post-breeding flocks towards the end of the breeding season[54]. Table 1 summarises the five treatments and control. In summary, the experiment involved 68 birds: two wild origin males (tutors) and 66 juvenile zoo-bred males, of which four were used as live adult tutors in subsequent years (Table 1). See Supplementary materials for full details of each treatment group.

Female juveniles were housed alongside males in all treatment aviaries at approximately equal sex ratios and were exposed to the same tutoring protocols. However, as female regent honeyeaters do not sing, this study focused exclusively on measuring song learning outcomes in males.

Acoustic environment of natal enclosures

Breeding pairs were housed in adjacent aviaries at both TZ and TWPZ. Male regent honeyeaters do not sing during active breeding or while tending to chicks[30], but non-breeding males housed in nearby single-sex groups were occasionally audible from natal enclosures, providing incidental exposure to adult vocalizations prior to fledging. We did not implement playback protocols in natal enclosures for two reasons: first, to avoid inducing territorial stress in breeding males from perceived rival songs within their territory, and second, because males cease vocal activity during the nestling period to reduce nest predation risk. Experimental tutoring protocols therefore commenced when juveniles were transferred to crèche aviaries post-fledging at approximately 30 days old.

Playback parameters

Playback tracks consisted of songs from 25 different wild male regent honeyeaters singing the typical Blue Mountains song type (each song ~2 seconds duration). To approximate natural variation in songbird vocal activity, songs were broadcast at higher rates during peak periods (~one song per 25 seconds; first four hours and last two hours of daylight) than during off-peak midday periods (~one song per 93 seconds). Playback rate was doubled in the 2021-2022 season based on observations that juveniles vocalized more frequently than the initial playback rate. Tracks were broadcast from sunrise to sunset daily via Bose FreeSpace outdoor speakers mounted within aviaries. Playback was broadcast at amplitudes approximating natural song volume (55-75 dB SPL measured at 3 meters from the speaker), with variation reflecting the natural range of vocal amplitudes across the 25 recorded

individuals. Complete details of track composition and playback systems are provided in Supplementary Text S2.

Song recording and editing

Juveniles' songs were recorded in their tutoring aviaries at the end of each tutoring period (October-March; Age range: 2.5 months – seven months) before they were moved into a multi-species flight aviary in preparation for potential release to the wild. Songs of each juvenile male were recorded over two days at a typical distance of one to four metres using a Sennheiser ME66 microphone and a Zoom H4n Pro recording device (48KHz sampling rate, 16 bit). For comparison with the songs of zoo-bred juveniles included in the tutoring experiments, we also obtained recordings of (i) 'typical Blue Mountains' wild birds that were made in the wild between 2015 and 2019 (which formed the playback tracks used for audio tutoring); and (ii) zoo-bred birds that had not been exposed to song tutoring either one week after release into the wild in 2017 (n = 12) or within captivity in 2019 (n = nine). These additional recordings used the methods detailed in Crates et al. (2021)[30]. We first edited individual songs (342 total, min four max 13 per individual) in Apple Garageband (v10.3.3) to trim them to include only the target individual's song and no other background noise.

Spectral and statistical analysis

To assess the similarity between the songs of tutored juveniles and the reference wild-type typical Blue Mountains males, we first performed semi-autonomous syllable segmentation using the open-source Python software 'Chipper v1.0'[55]. Chipper allows users to set default parameters for automated segmentation of syllables within each bout of song. We used only the top two percent on signal and used the default settings of 10 ms minimum silence duration and 30 ms minimum syllable duration. Occasionally we adjusted the syllable onsets and offsets (syllable selections) to remove environmental noises or other artefacts from recordings. We subsequently performed Chipper's song analysis to obtain spectral, song and syllable measurements, from which we selected nine acoustic features of the

songs for subsequent analysis (Table S3). These 9 acoustic features were selected through stepwise discriminant function analysis using forward selection, whereby acoustic features were sequentially added to the model based on their contribution to discrimination between groups. Only features that significantly improved model discrimination ($p < 0.05$) were retained in the final model.

After ensuring none of the acoustic attributes showed strong positive or negative correlation with any others using the 'ggcorrplot' function in the R-package 'GGally'[56] (Fig. S4), we averaged each spectral measure across multiple recordings of each individual's song. We then performed discriminant function analysis (DFA) on the average spectral measures using JMP v17.2.0[57]. The DFA contained seven pre-defined groups: the five experimental groups and the control group described above, plus the songs of untutored zoo-bred males from previous years and the typical Blue Mountains wild male reference songs, which included songs of the two wild-origin live tutors.

We then calculated the Mahalanobis distance between each individual's average song attributes and the centroid of the culturally-dominant typical Blue Mountains reference songs recorded in the wild between 2015 and 2017 and used in the playback tutoring experiments. The Mahalanobis distance represents a measure of acoustic (dis)similarity between the average song of each individual regent honeyeater and the target song of the tutoring experiment[58]. Increasing Mahalanobis distances indicate increasing dissimilarity between an individual's learned song and the mean of the culturally dominant wild song type.

To assess whether the songs of birds in each treatment group differed from the typical Blue Mountains reference songs (and from those in other treatment groups), we used the Mahalanobis distances described above as the response variable in a generalised linear model (GLM) using the package 'stats' in R 4.0.3[59]. The model contained treatment group as a factorial fixed effect and individual age in days

at song recording as a continuous fixed effect. We conducted backwards model selection using the 'stepAIC' function from the package 'MASS'[60], which revealed that removing age as a non-significant predictor improved model fit (AIC= 1.97, Table S4). While this difference is marginal, age showed no significant effect (p = 0.854), and we retained the simpler model based on parsimony principles. We selected a generalised linear model containing only treatment as a factorial fixed effect. We could not include breeding facility or aviary type as random terms due to unavoidable restrictions on the space available within the breeding facilities for experimentation. We assessed the quality of the model by using the 'simulateResiduals' function in the 'DHARMa' package[61] (Fig. S3) and conducted post-hoc tests of pairwise significance between treatment groups using the package 'multcomp' v1.14-17[62].

Ethics

Research was conducted under New South Wales scientific licences #SL101580 and #SL101603. All experiments were conducted in accordance with Taronga Zoo Ethics approval #4a/02/20 and Australian National University Animal Ethics permit #A2015/28.

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Chapter 5: Frequency-dependent shifts in song-preference may decrease fitness costs associated with reduced bird song complexity

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Abstract

Animal cultures can undergo rapid changes associated with innovations, revolutions, or population decline. Where a rapid shift results in reduced complexity of cultural behaviours, it may have fitness consequences for individuals. Here we report a dramatic shift in the dominant song type of critically endangered wild regent honeyeaters *Anthochaera phrygia*. Between 2015 and 2019, most males in the Blue Mountains sang a typical regent honeyeater song ('Typical Blue Mountains song'), but 5-10% sang an abbreviated version of the song with half the number of syllables (the 'Clipped Blue Mountains song'), which was associated with lower pairing success. Since 2020, the proportion of males singing the Clipped Blue Mountains song has increased to 50-75% each year. The likelihood of successful pairing in these males showed a significant concomitant increase, suggesting that the fitness costs associated with the abbreviated song decreased as it became the dominant song type. Our results suggest that the fitness consequences of loss of song complexity in declining and fragmented populations may be ameliorated by frequency-dependent shifts in song type preference.

Introduction

Animal cultures are learned behaviours, traditions or knowledge that are transmitted within a group of animals through social learning or imitation, and maintained across generations (Fragaszy & Perry, 2003; Brakes *et al.,* 2021). They encompass the shared behaviours, skills, or customs that are maintained between generations, shaping the behaviour and practices of societies or populations. Cultures may aid or hinder the rate of survival (Chiyo *et al.,* 2012; Wild *et al.,* 2019), reproduction (McComb *et al.,* 2001) or dispersal (Sapage, Varela & Kokko, 2021) for individuals within a species and as such, understanding these processes is vital for the conservation and management of threatened species.

Recent studies have shed light on instances wherein animal cultures undergo transformative changes. Research conducted by Aplin *et al.* (2015) and Klump *et al.* (2021) has elucidated cases of animal cultures having been subject to the influence of innovation, manifesting in the adoption of novel feeding techniques, tool use, or other behavioural innovations that subsequently permeate through a population. Animal cultures may also undergo revolutionary changes (Noad *et al.,* 2000). For example, humpback whales *Megaptera noevaeagliae* in the South Pacific, underwent a cultural revolution, in which the population's entire song repertoire was replaced in a period of two years following the introduction of novel songs sung by individuals originating from a geographically distinct population (Noad et al. 2000). The novelty of introduced behaviours may indicate some adaptive advantage such as increased genetic diversity or other fitness traits which can rapidly be selected for (Sergeant & Mann, 2009). Such cultural modifications could hold profound implications for the dynamics and ecological interactions within a species and its respective ecosystem.

Population decline (Laiolo & Tella 2006) and the fragmentation of habitats (Laiolo & Tella 2005) can also significantly alter animal cultures. Investigations conducted by Paxton et al. (2019), Crates et al. (2021), and Backhouse et al. (2023) have demonstrated that dwindling animal populations may result in the loss of cultural knowledge and associated behaviours within remaining individuals. Revolutionary changes due to environmental or anthropogenic change have been observed in recent years. In these

cases, cultural traditions may shift to facilitate exploitation of a new resource, deal with a novel threat or simply decline, where a niche is no longer suitable (Pressuto *et al.,* 2020, Roncero *et al.,* 2023). This loss of culture can impair the adaptability and resilience of the population, potentially exacerbating the rate of population decline through Allee effects (Stephens *et al.,* 1999).

In songbirds, song repertoires and dominant song types change over time (Garland & McGregor 2020). This is a normal outcome of song learning and is generally not costly to individuals. However, several recent studies have revealed that when population size declines, there is often a concomitant loss of complexity in cultural traits such as birdsong (Laiolo *et al.,* 2008; Paxton et al 2019; Crates et al. 2021, Backhouse et al. 2023). Moreover, many studies have shown that female songbirds prefer males that sing complex songs and have complex repertoires (Catchpole & Slater 2008), so it is predicted that males with reduced song complexity will be less attractive to females, reducing reproductive success and potentially impeding conservation efforts in threatened species (Valderrama *et al.,* 2013; Lewis *et al.,* 2021).

Regent honeyeaters (*Anthochaera phrygia*) are a critically endangered nectarivorous songbird whose males sing a single song that is learned early in life and maintained throughout adulthood. Within the population there are a range of song types (Crates *et al.,* 2021) that form loose geographic dialects. It has been demonstrated previously that males that sing the song type that is the cultural norm have greater reproductive success than males that sing an abbreviated version of that song type (Crates *et al.,*2021). However, it is unclear whether males with the simpler song type were less successful because their songs were a) not the cultural norm, or b) less complex in terms of its duration, number of syllables and number of unique syllables. A recent, rapid change in song culture provides a natural experiment that facilitates comparison between complex sexual signals (complex songs) and cultural conformity. Specifically, we aim to test whether males that sing the simpler song type continue to suffer fitness costs when the simpler song type becomes the cultural norm.

Methods

Study species and previous song culture research

Regent honeyeaters are a medium-sized (~40g) songbird endemic to south-eastern Australia with a generation time of 3.4 years, a mean lifespan of 5-6 years and a monogamous breeding strategy (Heinsohn et al. 2022). The population has been in significant decline from ~1500 (Geering and French, 1998) to <300 over three decades (Crates *et al.,* 2021a) and is headed for extinction within a decade without enhanced conservation action (Heinsohn et al. 2022). Severe habitat loss is the primary driver of population decline (Franklin et al. 1989).

Crates et al. (2021) and Powys (2010) identified that male regent honeyeaters' songs have geographic song types within the two remaining New South Wales breeding areas – the greater Blue Mountains and the Northern Tablelands. The greater Blue Mountains contains the largest remaining population of regent honeyeaters and males originating from this area that sing species-specific songs produce one of two song types – the "Typical Blue Mountains" or the "Clipped Blue Mountains". The Clipped Blue Mountains song is a simplified version of the Typical Blue Mountains song with half the number of syllables (Crates et al. 2021). Males in the Northern Tablelands that sing species-specific songs produce one distinct song referred to as the "Northern Tablelands" song type. Moreover, 12 % of wild males across both locations fail to sing any version of the species-specific song and instead sing songs resembling those of other bird species- the "Interspecific" song type. Interspecific songs were more common in males occurring in areas of low population density. Aberrant songs (including the Clipped Blue Mountains song) were associated with fitness costs in regent honeyeaters: males who sang songs that differed from the regional cultural norm were less likely to be paired to a female, and those that were paired to a female were less likely to initiate a nesting attempt (Crates et al. 2021). Aberrant songs also occurred in zoo-bred males contributing to a reintroduction program, which sang rudimentary songs differing from all wild song types (Crates *et al.,* 2021).

Data collection

We compiled a database of all wild-born male regent honeyeaters located between 2015 and 2022. The database did not contain zoo-bred males released to the wild because of (i) the relatively small number of males resighted at least 6 months post-release; and (ii) the fact that the release location of zoo-bred regent honeyeaters shifted from northern Victoria to the greater Blue Mountains in the middle of our study period. We obtained data on wild males from two sources: the National Regent Honeyeater Monitoring Program- a standardised survey protocol surveying over 1200 sites throughout the species' range each spring (Stojanovic et al. 2021); and a public sightings database managed by BirdLife Australia. We were able to sex birds in the field based on a combination of colour bands, size, plumage traits, singing and/or nesting behaviour. We were able to identify males individually through a combination of: colour bands on a proportion of wild males (n = 57) and/or their partner females (n = 21), the unique song attributes of some males, the location of nests, and/or a lack of other birds nearby at the time they were sighted. Whilst it is possible that occasionally an unbanded male was included as separate data points in different years, colour mark resighting data suggest the overall resighting rate is <20 % and the annual resighting rate is much lower than this. This relatively low resighting rate is likely to be explained by a combination of the highly nomadic life-history of the regent honeyeater, generational turnover and population decline. Therefore, we are confident that the majority of unbanded males included in the dataset represent different individuals. Nine colour-banded males were resighted in more than one breeding season. We accounted for possible non independence by including 'Male ID' as a random term in the models. For each male, we calculated an index of population density by counting the number of other males sighted within 1km and 50km of each male in the same year. The full methodology used in this study is described in Crates et al. (2021), incorporating an additional 3 years' data on the wild population.

Song classification

Juvenile regent honeyeaters learn songs by associating with older conspecific tutors (Crates *et al.,* 2021). Only males produce a full song, and recent evidence from zoo-breeding experiments shows male regent honeyeaters are close-ended song learners that crystallise their adult-type song within the first nine months of life (Appleby *et al.,* in prep). There is no evidence in the wild or zoo-bred population that individual males sing more than one song type.

We assigned wild males' songs to one of five song types following Crates et al. (2021, Figures 1, 2 & S1):

- Typical Blue Mountains - the 'regional cultural norm' song type of wild males occurring in the greater Blue Mountains - the largest remaining wild population- during the period 2015-2019.
- Clipped Blue Mountains - similar to the Typical Blue Mountains song, but males singing this song type omit the final three syllables of the Typical Blue Mountains song.
- Typical Northern Tablelands - birds predominantly occurring in the northern NSW breeding population with a song type distinct from both Blue Mountains song types.
- Interspecific singer - males that do not sing any of the above three song types and have instead learned to sing the song of one of a number of different bird species including little wattlebird *Anthochaera chrysoptera*, noisy friarbird *Philemon corniculatus*, little friarbird *Philemon citreogularis* and spiny-cheeked honeyeater *Acanthagenys rufogularis* (Crates et al. 2021).
- Unknown - males that were located in the wild but either did not sing, their song was not recorded, or was not heard by a species' expert.

Crates et al. (2021) used discriminant function analysis of the spectral attributes of high-quality recordings of 47 males' songs to demonstrate the similarities within and the differences between the song types. They then used blind classification tests of song recordings by recognised species' experts to further demonstrate the repeatability of the song classification approach. For the additional 3 years' data utilised in this study, we utilised the same species' experts for classification of song types that

contributed to Crates et al. (2021) and obtained field recordings from a further 14 males (Supplementary File T1).

Statistical analysis

Changes in population density and dominant song type

We first used Mann-Whitney U tests to assess how the density of the wild male population differed between the 2015-2019 and 2020-2022 study periods and between the 1km and 50km scales. We then calculated the proportion of males singing each song type for each year. To assess the changes in the proportion of males singing each song type, we calculated the mean proportion for each song type within the periods 2015-2019 and 2020-2022. This provided values for the proportions of individuals associated with each song type in each year and within each period. To determine if there were significant differences between the proportions of males singing each song type in each period, we conducted Mann-Whitney *U* tests using the base 'stats' package in R (R core team, 2020).

Fitness correlates of male song type

To assess fitness associations with song type, we examined the likelihood that birds detected during the breeding season (July to January) were a) paired or unpaired; and b) in paired birds, whether or not they successfully initiated a nesting attempt in which their partner female laid at least one egg. Details on nest monitoring methodology are provided in Crates et al. (2019). We fitted mixed-effects logistic regressions with the binomial response variables of 'paired or unpaired', and then, for paired birds 'nested or failed to nest', including song type as a fixed effect (formula: Paired or not ~ Song Type + (1|Male Id), Family= "binomial"; Nested or Not ~ Songtype + (1|Male Id), Family "Binomial"). We tested this on both the 2015-2019 and the 2020-2022 datasets, as this was the period during which we noted a rapid shift in the dominant wild song type (see results). In Crates et al. (2021) a fitness cost was reported for birds that sang songs outside the regional cultural norm. To assess changes to this fitness

cost we repeated the logistic regressions on the 2020-2022 dataset keeping 'Typical Blue Mountains' and 'Northern Tablelands' song types as the cultural norm for their respective geographic regions. We then conducted the same 'paired' and 'nested' logistic regressions on this dataset. Due to the small sample size of birds singing 'Typical Blue Mountains' songs in the 2020-2022 period, we conducted an additional analysis of the temporal changes in fitness correlates for birds singing the 'Clipped Blue Mountains' song. We fitted mixed-effects logistic regressions using time period as the predictor and binomial response variables of 'paired or unpaired', and then, for paired birds 'nested or failed to nest', including period as a fixed effect (formula: Paired or not ~ Time Period + (1|Male Id), Family= "binomial"; Nested or Not ~ Time Period + (1|Male Id), Family "Binomial"). We also fitted the same models with a subset of the data including only birds whose songs were classified as the predominant song type detected during each study period (2015-2019 – 'Typical Blue Mountains', 2020-2022 – 'Clipped Blue Mountains').

Results

The sightings database contained 324 individual males located during the periods 2015-2019 (n=241) and 2020-2022(n= 75) (Figure 1).

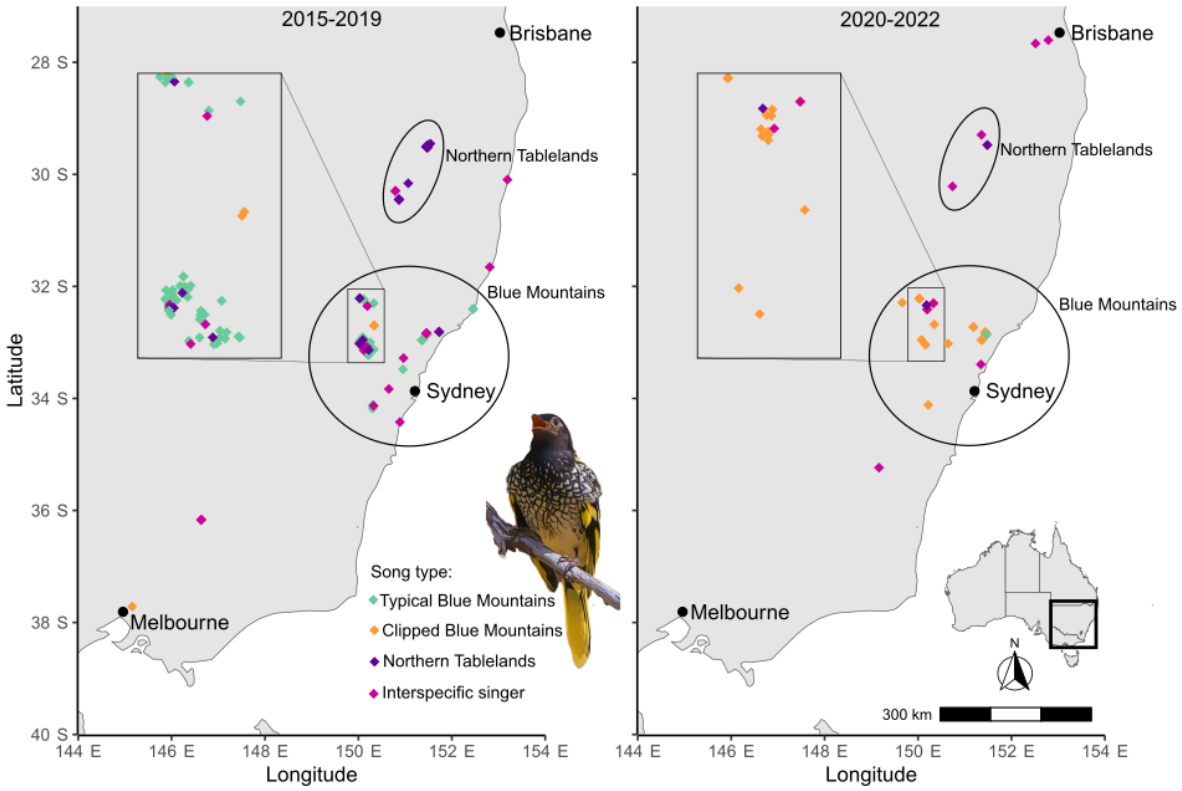

Figure 1: The location of wild male regent honeyeaters with colour labels indicating their song type, from 2015 to 2019 (left) and from 2020 to 2022 (right). Ellipses identify the location of the Blue Mountains and Northern Tablelands breeding areas, with the Blue Mountains key breeding areas shown at finer scale in insets. Due to map scale, not all individuals' records in areas of high population density are visible. Inset on right: location of study area on a national scale.

The total number of males located annually varied considerably but showed an overall downward trend (Figure 2). The density of the population also decreased significantly at both the 1 km range (Mann-Whitney U-tests W =13465, p = <0.001) and at the 50 km range (W =14174, p = <0.001, Figure 3). Thus, there was a significant decrease in the number of potential song tutors for juvenile males over time (Figure 3).

The songs of 218 males were classified as belonging to one of four song types, whereas those of 106 males were either not recorded or not heard in the field by a species' expert and were therefore classified as unknown.

Song Types

Major changes were detected in the proportion of males singing the Typical Blue Mountains and Clipped Blue Mountains song types over time (Figure 2). Among males with known song types, males singing the Clipped Blue Mountains song type represented only 5% of the population in 2015, and this proportion remained relatively consistent until 2019 (11% of birds with identified song type). However, the proportion of males singing the Clipped Blue Mountains song type had increased to 91% of birds with an identified song type by 2022. Conversely, in 2015, 73% of males with a known song type sang the Typical Blue Mountains song, which also remained relatively consistent until 2019 (52% of birds with an identified song type). After 2020, the proportion of males singing the Typical Blue Mountains song type had fallen to 5%. No birds were identified to be singing this song type by 2022.

The average proportion of males singing the Typical Blue Mountains song type was significantly lower (U = 15, p = 0.03) in the 2020-2022 period (2.8 % of birds) than the 2015-2019 period (34.7 %). The average proportion of males singing the Clipped Blue Mountains song type increased significantly (U = 0, p = 0.03) in the 2020-2022 period (58 %) compared with the 2015-2018 (5 %). There was no significant difference in the proportion of males singing other song types between the two time periods (Northern Tablelands, U = 12, p = 0.25; Interspecific, U = 6, p = 0.79; Unknown, U = 10, p = 0.57, Figure 2).

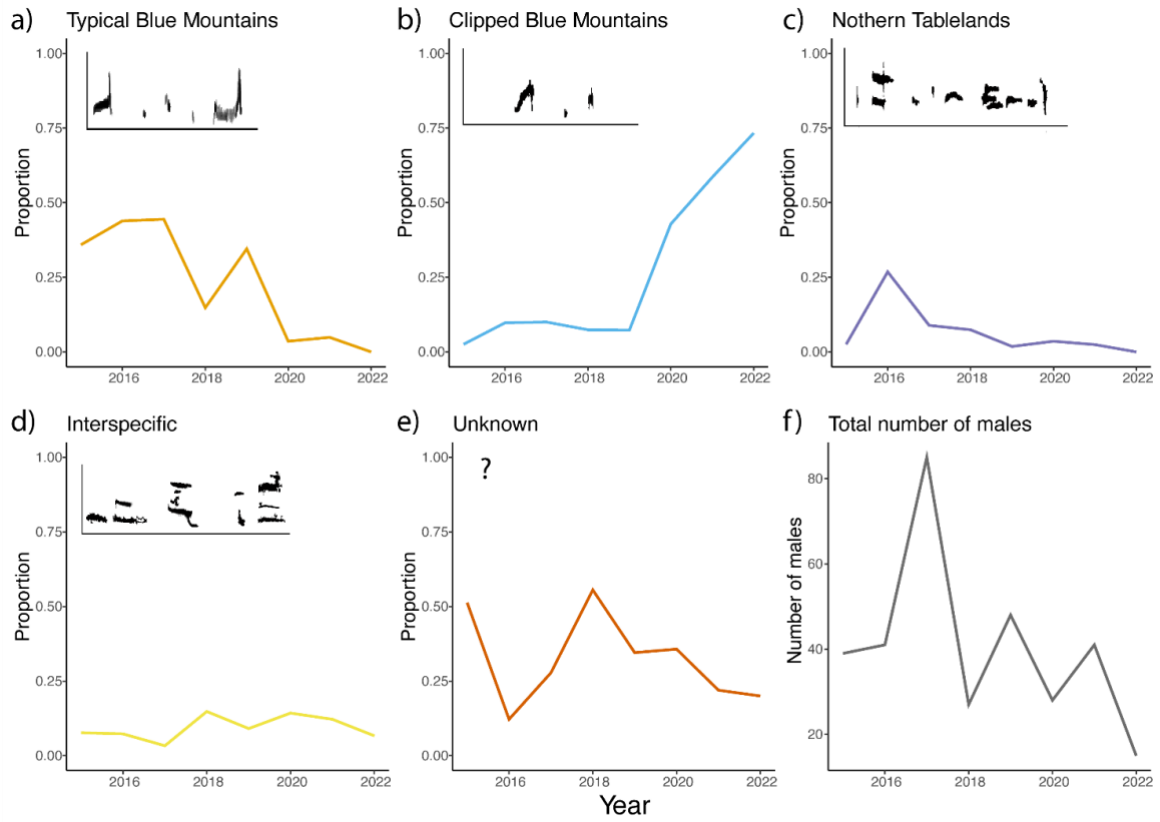

Figure 2: The annual proportion of wild male regent honeyeaters singing each song type by year (a-e); and the total number of males detected per year (f). Insets in (a-d) show representative spectrograms of each song type. The frequency on the Y-axis is between 0 – 10 kHz the time on the X-axis is variable. See Supplementary Material for more detailed spectrograms.

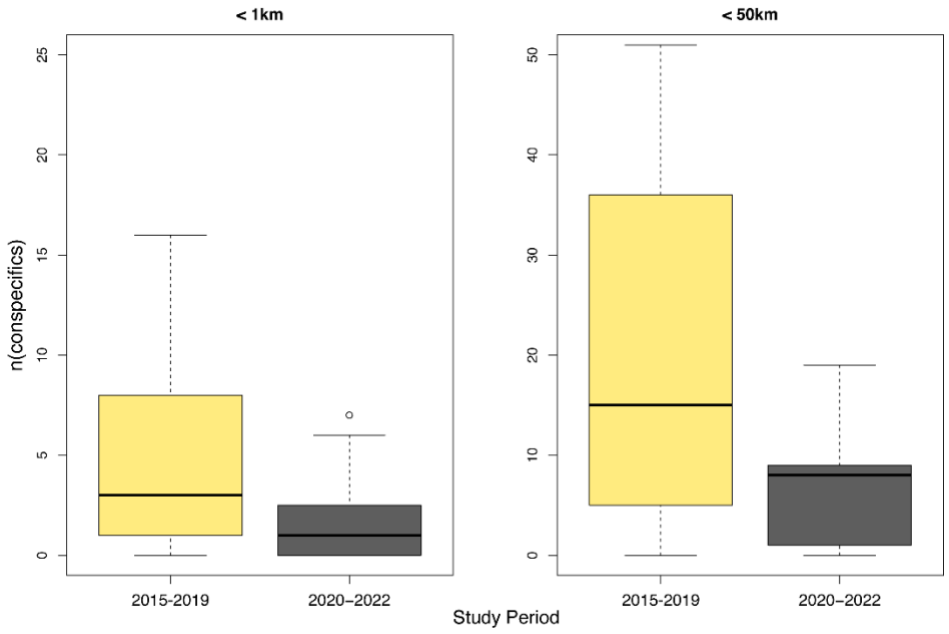

Figure 3: Boxplots showing the number of male regent honeyeaters detected within a) 1km; and b) 50km of each focal male in each of the two study periods (2015-2019: n= 252, 2020-2022: n= 84).

Fitness associations with song type

Pairing and Nesting by song type

During the 2015-2019 period, the probability of pairing in males singing songs other than the 'Typical Blue Mountains' song type was significantly lower than for males that did sing this song type (Table 1). However, during the 2020-2022 period, the probability of a male being paired to a female did not differ by song type. During the 2015-2019 period, the probability of pairs successfully initiating a nest was lower when the male sang the "Clipped Blue Mountains" song type than when the male sang the Typical Blue Mountains song type. However, this difference was also not present during the 2020-2022 period (Table S1, Figure 4b).

Overall, during the 2015-2019 period, there was a significantly lower probability of both nesting and pairing for males whose songs did not conform culturally (Table 1). During the 2020-2022 period there was no significant difference in the probability of males singing songs outside the cultural norm (as defined by the 2015-2019 standard) being paired or of paired males nesting (Table S1, Figure 5). Despite comprehensive census of this species, its nomadic life-history, cultural song shifts and continued population decline result in unavoidable small samples of males singing the Typical Blue Mountains and Northern Tablelands songs the 2020-2022 period, and should therefore be interpreted with caution. However, relative to the 2015-2019 period, the pairing and nesting success of birds singing the 'Clipped Blue Mountains' song type increased significantly in the 2020-2022 period (Table 1, Fig. 4). Comparison between birds singing the most common song type in each time-period (2015-2019: 'Typical Blue Mountains', 2020-2022: 'Clipped Blue Mountains') revealed that the probability of successfully pairing was not significantly different, however the probability of paired birds successfully nesting was significantly lower for Clipped Blue Mountains singers between 2020-2022 than for Typical Blue Mountains singers between 2015-2019 (Table 1).

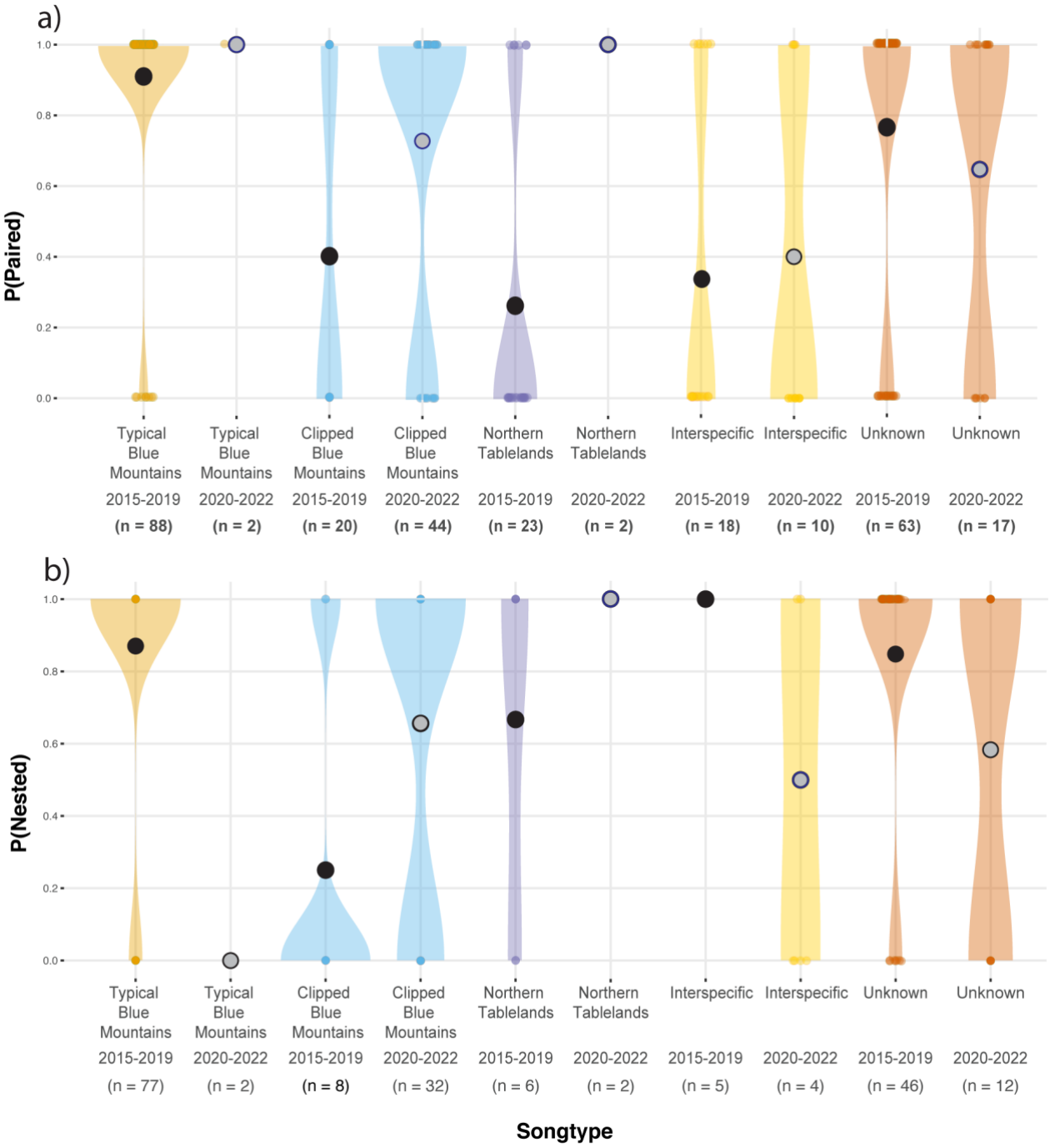

Figure 4: Violin plots showing the probability of wild male regent honeyeaters detected during the breeding season being a) paired; or b) paired males successfully initiating a nesting attempt according to song type in the periods 2015-2019 and 2020-2022. The Y-axis shows the probability, the X-axis shows song type and year. The colour of the violins is according to song type, while the colour of the estimates (i.e., circles) denotes time period (black for 2015-2019, grey for 2020-2022).

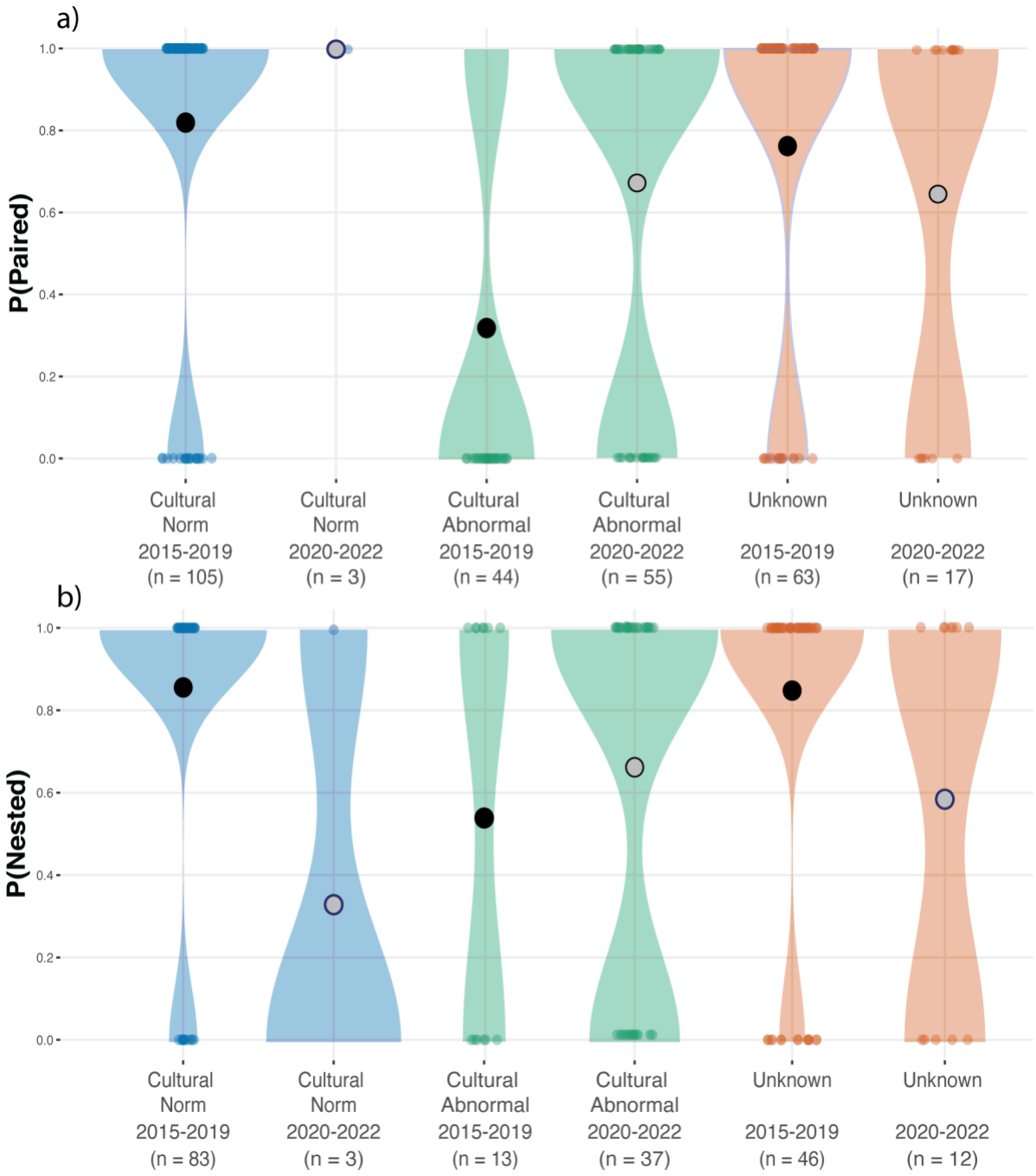

Figure 5. Violin plots displaying the probability of wild male regent honeyeaters detected during the breeding season being a) paired; or b) paired males successfully initiating a nesting attempt according to whether males sang a song-type belonging to the prevailing regional cultural norm in the periods of 2015-2019 and 2020-2022. We consider the Typical Blue Mountains and the Northern Tablelands song types as the cultural norm in their respective regions across both time periods. The Y-axis shows the probability, the X-axis shows whether the birds song belonged to the regional cultural norm or not and the time period. The colour of the violins is according to cultural norm, while the colour of the predictions (i.e., circles) denotes time period (black for 2015-2019, grey for 2020-2022).

Table 1: Results of generalised linear mixed-effects models showing the relationship between the probability of being paired to a partner female and of paired males to successfully initiate a nesting attempt and the time period. β is the coefficient which describes the change in probability relative to the reference time period of 2015-2019.

Response	Test	Reference Group (n)	Level	n	β	se	Z	p
Paired	Time Period (Clipped Blue Mountains only)	2015-2019 (20)	2020-2022	44	1.39	0.57	2.44	**0.02**
Nested		2015-2019 (8)	2022-2022	32	1.75	0.90	1.95	0.05
Paired	Predominant Song Type	Typical Blue Mountains and Northern Tablelands 2015-2019 (105)	2022-2022	46	-0.47	0.42	-1.11	0.266
Nested		Clipped Blue Mountains and Northern Tablelands 2015-2019 83	2022-2022	32	-1.13	0.49	-2.33	**0.02**

Discussion

Between 2015 and 2022, there was a substantial decrease in both the annual number of wild male regent honeyeaters located and the density of the remaining population. Over the same period, the average proportion of males singing the Typical Blue Mountains song decreased from 35 % to 0 %, whilst the proportion singing the Clipped Blue Mountains song increased from 2.5 % to 73.3 %. The fitness costs associated with singing songs that differed from the cultural norm during the 2015-2019 period significantly decreased in the 2020 to 2022 period, as the previously culturally dominant Typical Blue Mountains song disappeared from the population. While unavoidably small sample sizes prevented robust comparison of the fitness correlates of contemporary birds singing 'Typical Blue Mountains ' songs with the now dominant 'Clipped Blue Mountains' songs, comparison of contemporary birds singing 'Clipped Blue Mountains' songs with their 2015-2019 counterparts show that the fitness cost associated with singing the abbreviated song type has decreased as that song type has become more dominant, suggesting that females become more accepting of males singing less complex songs as they become more common in the population.

Cultural song shifts linked to generational turnover

Understanding the underlying mechanisms of rapid, population level changes toward a simpler song in nomadic species such as the regent honeyeater is a difficult task. We suggest that rather than a cultural revolution of song in regent honeyeaters, the observed rapid change may be caused by a combination of generational turnover and decreased population density, which in turn may influence the number and type of song tutors available to juvenile males. Although many species undergo rapid changes through innovation or cultural revolution (Aplin *et al.,* 2015), it is unlikely that these processes are responsible in the case of the observed changes to regent honeyeater song. Whereas most cultural innovations are adaptive (Sasaki & Biro, 2017; Wild *et al.,* 2019; Pravosudov, 2021), loss of song complexity and learning the songs of a different species were associated with fitness costs in regent honeyeaters (Crates *et al.,* 2021). The more simplistic Clipped Blue Mountains song may instead indicate a landmark in generational turnover, whereby the remaining adult birds singing the Typical Blue Mountains song declined in numbers, and the emerging generation were limited in their exposure to tutors capable of singing the typical Blue Mountains song. The Clipped Blue Mountains song is an abbreviated version of the Typical Blue Mountains song with no new elements, so it is unlikely that innovation or immigration is driving a revolution as is often the case in cultural revolutions (Noad et al. 2000). Although the specific details of regent honeyeater song learning are unknown in the wild, evidence from the zoo's song tutoring program suggests that core learning occurs within 100 days of fledging (Appleby, *in prep*). Therefore, it is unlikely that a cultural revolution, whereby adult birds switch song types, is occurring within regent honeyeaters. All available evidence combining colour-marked wild birds and wild birds recruited to the zoo-breeding program indicate adult regent honeyeaters remain faithful to the single song type they learn as juveniles (Crates et al, 2021).

Population size, and in particular, population density are likely to be additional key factors contributing to the rapid change in the dominant regent honeyeater song type. This is because population size and density indirectly influence the structure and diversity of songs that juveniles are exposed to during crucial phases of song learning. Song complexity and diversity may emerge from the introduction of

new song elements through cultural mutations, such as copying errors and innovation, as well as the transmission of new songs among dispersing individuals (Paxton *et al.,* 2019). With fewer overall tutors, convergence on simpler songs may occur (Laiolo & Tella, 2005), especially as the proportion of adult birds (i.e. tutors for future generations) conforming to the simpler Clipped Blue Mountains song increases. Regent honeyeaters breed in loose aggregations and the breeding season may represent the time at which there is the highest density of birds in a single location. Juvenile birds are likely to have the most social interactions during this period. However regent honeyeaters are unlikely to learn from their fathers (Crates *et al.,* 2021), as males do not sing whilst raising their young and actively chase juveniles from the nesting area. As such it is likely that juveniles learn from other suitable neighbours. As co-occurring Clipped Blue Mountains singing male birds were significantly more likely to be single, and as such more vocal during the 2015-2019 period, it also is possible that a demonstrator bias toward the most vocal birds (Lachlan *et al.,* 2018) influences the early song learning during birds' sensory learning phase. Given that the Clipped Blue Mountains song is an abbreviated version of the Typical Blue Mountains song, a conformist bias (Lachlan *et al.,* 2018) toward the most common syllables may reinforce song learning of the Clipped Blue Mountains song through selective attrition (Nelson & Marler 1994).

Crates et al. (2021) showed that a decline in the regent honeyeater population was correlated with an increase in the proportion of interspecific singers, however, further decline appears not to have increased the proportion of detected interspecific singers. In this case the number of interspecific singers may be a function of the number of isolated birds in the population, which may have remained stable or declined. In 2015-18, the Typical Blue Mountains song type represented the dominant cultural norm and was most prevalent in birds that occurred in higher densities. In the 2020-2022 period the Clipped Blue Mountains song type was prevalent in birds that occurred in higher densities, suggesting that oblique transmission occurs between other suitable adult tutors and juvenile birds.

Temporal fitness associations with song type

The fitness costs associated with singing the simpler, but now culturally dominant Clipped Blue Mountains song type decreased during the 2020-2022 period. Given that female regent honeyeaters have been shown to prefer the songs of birds they are exposed to and familiar with as juveniles (Appleby *et al.,* 2023), this decrease may primarily reflect a change in female preference for the familiar songs of present-day males, demonstrating a concomitant frequency-dependent shift in the preferences for song types. This could also explain the prevalence of males singing both Typical Blue Mountains and Northern Tablelands song types being paired. It is possible that males singing Typical Blue Mountains and Northern Tablelands songs may be paired with older females with whom they have previous breeding experience or that those females experienced these now uncommon song types as a juvenile. Alternatively, this may reflect a relaxation of selective pressures on the quality of song in mate choice. As Crates et al. (2021) posited, where mate choice is severely limited either through competition for optimal males, or as likely the case here the cost of female search time (Duval & Kapoor, 2015) the fitness costs associated with female choosiness may force females to select males with songs that do not indicate the optimum level of fitness (Penterniani, 2009). It is evident that song type is not an absolute barrier to reproduction in regent honeyeaters (Crates et al. 2021), as some birds with even the most aberrant songs were able to successfully find a mate. In these cases however pairing with a non-preferred mate may carry a fitness cost in its own right. The most direct mate competition between dialects occurred in the 2015-2019 period where a choice between males singing Typical Blue Mountains and Clipped Blue Mountains songs was most available. During this period, paired Clipped Blue Mountains birds only proceeded to build a nest 25% of the time, this may be due to females being paired with 'unattractive' mates. This is consistent with findings from Griffith et al. (2011); where mate choice was constrained to non-preferred mates, female Gouldian finches (*Erythrura gouldiae*) had higher levels of corticosterone and delayed egg -laying relative to females that were paired with preferred mates. During the 2020-2022 period paired Clipped Blue Mountains singers proceeded to nesting 62.5% of the time. This may indicate that Clipped Blue Mountains songs are at least neutral in

terms of preference. However, the probability of birds singing the now dominant Clipped Blue Mountains song type being paired is lower than that of birds singing the Typical Blue Mountains song type for the 2015-2019 period. This could be due to an increasingly male-biased wild population (Crates et al. 2019), as a lack of adult females is a pervasive problem in many endangered populations (Donald 2007).

The presence of Allee effects can impose fitness costs on the population (Crates *et al.,* 2017), and may arise in regent honeyeaters from reduced communication and the loss of critical cultural knowledge within their unnaturally small and sparsely distributed population. These effects may decrease cooperation and diminish the transmission of important behaviours. Therefore, while the fitness costs for birds singing the now dominant, but simpler Clipped Blue Mountains song may have diminished – potentially due to females selecting mates that conform to the new cultural norm – the decrease in population at large might still cause further loss of critical cultural information and impose potential fitness costs.

Previous studies on song complexity in birds show that song complexity may predict the viability of small, fragmented populations (Laiolo *et al.,* 2008). On the contrary, our results suggest that loss of song complexity entailed an initial decrease in reproductive success, but as the simpler songs became the cultural norm there was some increase in reproductive success in males singing the simpler song, suggesting that populations may adjust to loss of some forms of cultural complexity.

Ethics

Research was conducted under New South Wales scientific licences #SL101580 and #SL101603. All experiments were conducted in accordance with Taronga Zoo Ethics approval #4a/02/20 and Australian National University Animal Ethics permit #A2015/28.

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Chapter 6: Insights into the timing of song learning in Regent Honeyeaters: implications for adaptive management and animal husbandry.

Citation: Appleby DL, Langmore N, Pitcher B, Tripovich K, Heinsohn R, Crates R. Insights into the timing of song learning in Regent Honeyeaters: implications for adaptive management and animal husbandry. *In Prep*

Abstract

Captive environments impose different selective pressures on individuals than those in the wild. This can lead to emergence of phenotypic differences between wild and captive populations that may hinder conservation efforts involving reintroductions. To combat maladaptation due to captive environments and maximise the success of zoo-breeding for release programs, practitioners must manage populations informed by monitoring and research. The regent honeyeater (*Anthochaera phrygia*) is a critically endangered Australian songbird which relies on captive breeding to supplement the wild population through reintroduction but does not produce the wild-type song in captivity. We undertook three years of experimental song tutoring of zoo-bred males to improve understanding of the process of song learning in regent honeyeaters. Of the birds that successfully learned the wild-type song, the first features of the wild adult 'Typical Blue Mountains' song were detectable at around 50 days of age approximately 20 days after their first exposure to tutoring. The probability of juveniles singing the wild song was 50 % and 95 % at 110 and 150 days (80 and 120 days from exposure to tutoring), respectively. Two males that were introduced into the tutoring program at 108 days post-hatching failed to learn the wild song. We provide the first estimate of the regent honeyeater song learning timeline and discuss the implications this has for adaptive management of zoo population.

Introduction

Captive breeding and the subsequent release of individuals into the wild have become increasingly critical tools in the conservation of endangered species and prevention of biodiversity loss (Matthews *et al.,* 2005; Conde *et al.,* 2011). However, these programs do not always achieve their desired outcomes (Fischer and Lindemayer 2000). Many factors may hinder captive breeding for release efforts (Snyder *et al.,* 1996). These include the genetic and demographic challenges of small population sizes (Caughley, 1994; Bouzat, 2010), persistence of the original causes of decline (Dolman *et al.,* 2015), and the phenotypic costs associated with captivity (Crates *et al.,* 2023). The captive environment differs significantly from natural habitats, imposing distinct developmental pressures that lead to phenotypic divergences between wild and captive populations. Often lacking the complexity and selective pressures present in the wild (Frankham, 2002; Williams and Hoffman, 2009), differences in diet (Curtis et al., 2018; Cooper et al., 2023), space (Cannington et al., 2018, Latorre et al., 2020), social structure (Saraiva & Pompeu, 2016), and exposure to natural predators (Henyen, Bunnefeld and Borcherding, 2016; Terry & Duda, 2021) result in morphological changes such as body size (Turner et al., 2016), limb form (Stojanovic, 2021) or limb size (Pulcini et al., 2013; Cooper et al., 2023). Physiological changes may include alterations in long-term stress responses (Dickins, Delehanty & Romero, 2009), immune function (Kuhlman & Martin, 2010), and metabolic rates (McKechnie, Freckleton & Jetz, 2006), which may reduce the ability of captive-bred individuals to survive and reproduce after release. Health-related issues such as increased exposure (Sheelings Lightfood & Holz, 2011; Kołodziej-Sobocińska et al., 2018) or susceptibility to novel disease (Das et al., 2020) are also commonly observed. These divergences can impose substantial fitness costs, ultimately hindering the success of reintroduction, translocation, or population supplementation efforts (Berger-Tal, Blumstein & Swaisgood 2020, Crates et al., 2023).

Behavioural differences between wild and captive animals are amongst the most critical. These can include alterations in foraging behaviour (Rodewald, Hyvarinen & Hirvonen, 2011) predator avoidance (El Balaa & Blouin-Demers, 2011; Greggor *et al.,* 2021), social interactions (Kelley, Magurran & Garcia, 2006; Cenni, Parisi & Gehradi, 2010), and vocal communication (Crates *et al.,* 2021; Appleby *et al.,* 2023). Such behavioural changes can severely impact the ability of released individuals to integrate into wild populations, establish territories, attract mates, and ultimately contribute to the establishment of self-sustaining populations (Shier, 2016).

To mitigate the phenotypic differences between wild and captive populations and enhance the success of zoo-breeding programs, adaptive management is essential (Westgate, Likens & Lindenmayer, 2013; Canessa *et al.,* 2016). Adaptive management is a dynamic process that "combines the need for practitioners to take immediate action with a plan for learning" (Westgate, Likens and Lindemayer 2013). To facilitate the plan for learning, it is critical to continue testing hypotheses, monitoring outcomes (Nichols & Williams 2006), and adjusting management strategies based on the results (Crates *et al.,* 2022). This approach is particularly important in conservation, where the goal is to maximize the fitness of released individuals and ensure their successful integration into wild populations. Effective adaptive management requires a deep understanding of the factors that may predict the success or failure of animal reintroductions (Runge, Converse & Lyons, 2011). This means identifying traits that are most critical to survival and reproduction in the wild, as well as the factors that influence these traits in captivity (Crates *et al.,* 2022).

The regent honeyeater (*Anthochaera phrygia*) is a critically endangered songbird endemic to south-eastern Australia (Garnett & Baker, 2021). In response to severe population decline, a captive breeding program was initiated in 1995 (Commonwealth of Australia, 2016), resulting in the release of over 400 birds into the wild. While these birds have adapted well to the captive environment and exhibit high breeding success in captivity (Liu *et al.,* 2014), their post-release survival and reproductive success are significantly lower than those of wild birds (Taylor et al. 2018; Heinsohn *et al.,* 2022). One key factor

that may contribute to this discrepancy is the divergence in song culture between captive and wild populations (Crates *et al.,* 2021; Tripovich *et al.,* 2021; Appleby *et al.,* 2023). Regent honeyeaters in captivity exhibit simple and abnormal songs that do not resemble those of birds in the wild. They are shorter in duration and contain fewer syllables. Previous research has described them as being like those of juvenile males (Crates *et al.,* 2021).

Birdsong provides one of the best examples of how cultural traits can differ between populations (Aplin *et al.,* 2019; Crates et al. 2021). Amongst the ~9000 species of birds, approximately 40% learn their songs from appropriate models. These include the Oscines (~4,000 species), Parrots (Psittacines, ~387 species) as well as many hummingbirds (Trochillidae). While the patterns of learning are highly variable in the duration of the sensitive periods in which songs can be learned, ranging from short to indefinite, from whom individuals learn and the degree of fidelity with which songs are copied, certain general learning phases are ubiquitous (Marler, 1991; Kroodsma, 1996; Beecher & Brenowitz, 2005). Songbirds undergo three main learning phases: First birds undergo a 'sensory' phase in which they listen and begin to learn songs from their tutors. Second is a 'sensorimotor' phase in which birds practise vocalisations in an attempt to match their vocal output to the learning template they had previously memorised (Beecher & Brenowitz, 2005). The sensorimotor phase is often split in sub-phases which may vary across species. Generally, the sensorimotor phase is split between at least two stages: subsong, in which syllable morphology of adult song elements are not present and vocal bouts are highly variable duration (Marler, 1991) and the plastic song stage, where song syllable morphology of adult songs is present, but song duration and the order of presentation may remain variable. Finally, the crystallisation phase, in which song syllables and song morphology both become stereotyped and stable (Marler, 1991). For songbirds, song plays a vital role in mate attraction, territory establishment, defence, and group cohesion (Catchpole & Slater, 2008).

For regent honeyeaters, the observed divergence in song type between zoo-bred and wild birds may inhibit mating and thus critical gene flow between wild and reintroduced zoo-bred birds (Appleby *et*

al., 2023 Chapter 3). Since 2020, the zoo-breeding program has implemented a song tutoring initiative to train zoo-bred birds to sing wild-type songs, with the aim of improving post-release outcomes (Appleby *et al., 2024b, Chapter 4*). However, the success of this program hinges on a detailed understanding of the timing and mechanisms of song learning in regent honeyeaters that is currently lacking.

The zoo-breeding program for regent honeyeaters involves regular movements of birds for genetic management, health treatment, and space optimization (Taronga Zoo, personal communication). These movements can coincide with the critical periods of song learning described above (Marler, 1989; Beecher & Brenowitz, 2005). To ensure effective song tutoring, it is essential to understand when these sensitive song learning periods occur. This knowledge would allow managers to optimise timing of the song tutoring program, ensuring that only those juveniles still within the critical learning window are trained, thereby maximizing the effectiveness of the program.

Song learning has been extensively studied in model species such as zebra finches and white-crowned sparrows, establishing well-characterized sensitive periods and learning mechanisms (Marler, 1970; Immelmann, 1969; Beecher & Brenowitz, 2005). However, far less is known about song learning timelines in threatened species, where conservation imperatives limit the scope for controlled experimentation. Understanding these timelines remains critical for adaptive management of captive breeding programs, even when experimental constraints preclude the conditions typical of laboratory studies. Our study focuses on informing captive management protocols for regent honeyeaters through opportunistic experiments conducted within the constraints of the zoo-breeding program, while contributing data on song learning variation in a critically endangered oscine species.

In this study, we draw upon three years of song tutoring experiments and wild population monitoring to investigate the song learning process in regent honeyeaters. Our objectives are to determine the critical periods for song learning, assess the effectiveness of current song tutoring methods, and

provide recommendations for optimizing the song training program. This research aims to contribute to the broader field of conservation biology by offering insights into the management of behavioural traits in captive breeding programs and improving the success of reintroduction efforts for endangered species.

Materials and Methods

Song tutoring and recording.

We used recordings of the songs of regent honeyeaters tutored between 2021 and 2023 according to the protocols detailed in Appleby *et al.* (2024b, *in review*). All birds (except for 3 males, detailed below) entered experimental aviaries at the age they reached independence (~30 days post hatch), at which point they were transferred from their natal aviaries where no tutoring had been provided. Male regent honeyeaters do not sing while tending nests or nestlings (Crates et al., 2021), meaning juveniles received minimal song exposure during this initial period. This allowed us to investigate song learning from a well-defined starting point with tutoring representing the primary source of targeted song exposure. They received song tutoring until the conclusion of the breeding season when they are transferred to a larger, multi-species flight aviary, which has been linked to small increase in post-release survival (Tripovich *et al.,* 2021). While five different methods of song tutoring were used in Appleby et al. (2024b, *in review*) only birds that received tutoring from two of those were included in this study. Prior to the commencement of the song tutoring program, un-tutored zoo-bred regent honeyeaters exhibited a highly variable song type that does not occur in wild birds. Consequently, birds from 'unsuccessful' tutoring cohorts were excluded from this study. The 'successful' cohorts were exposed to either a live tutor or both a live tutor and playback of wild-type songs (Appleby 2024b, *in review*), together totalling 27 individuals.

In the 'Live Tutor + Playback' group, birds were transferred from their natal aviary to a crèche aviary adjacent to a wild-origin that sang the traditional blue mountains typical song adult male upon reaching independence. The median age of juveniles entering the crèche aviaries was 32 days. During the initial

2.5 months of song tutoring, the juveniles could see and hear the adult male but could not physically interact with him. During this period, playback of wild regent honeyeaters singing the 'Typical Blue Mountains' song from a loudspeaker was used to supplement the tutoring. Following the integration of the juveniles with the adult male in the same aviary, playback continued until the juveniles were transferred either into mixed species free-flight aviaries, or other single species aviaries at ~10 months of age.

In the 'Live Tutor Only' group, fourteen birds were housed in three separate aviaries, each containing five juveniles. These juveniles were moved from their natal aviary at independence (~32 days post hatching) to a crèche aviary with a zoo-bred adult male tutor aged approximately 18 months, who had successfully learned the typical Blue Mountains song in the previous season. The juveniles had the opportunity to see, hear, and physically interact with the live tutor during the tutoring period.

Songs of each juvenile male were recorded monthly over a two-day period (~0700-1300) at a typical distance of 1 – 4m using a Sennheiser ME66 microphone and a Zoom H4n Pro recording device (48KHz sampling rate, 16 bit).

During the 2021 breeding season, three birds were opportunistically added to the 'Live Tutor + Playback' group due to routine husbandry activities and space requirements. One of the birds was added to the tutoring program at 62 days post hatch (dph) and two birds were added at 108dph. These birds were recorded using the same methods described above.

Statistical Analysis

We conducted subjective auditory assessments and visual assessments of spectrograms of monthly recordings of each individual (n=27) made during the tutoring experiments (n = 1222, recordings/individual range = 2 – 9). While quantitative acoustic similarity measures (as used for the late-tutored birds, see below) would have provided more objective assessments, the large number of

recordings and high variability in juvenile vocalizations during subsong and plastic song phases made subjective classification more practical for determining the binary presence/absence of crystallized adult song. We recorded during each month whether each juvenile (i) produced a song that was recognisable as the target Typical Blue Mountains song; (ii) if any elements of the Typical Blue Mountains song were present; and (iii) the age of the individual in days post hatching (dph). We performed semi-autonomous syllable segmentation using the open-source Python software 'Chipper v1.0' (Searfoss *et al.,* 2020). Chipper allows users to automate segmentation of syllables within each bout of song. We used the default settings of 10 ms minimum silence duration and 30 ms minimum syllable duration and used on average the top two percent on signal (Range = 1 – 2.6%). Where necessary we adjusted the syllable onsets and offsets (syllable selections) to remove environmental noises or other artefacts from recordings. We used Chipper's song analysis function to obtain the bout duration, number of syllables per bout and the overall frequency range. We chose these three acoustic indices because of their broad relationship to the song learning phases described above. We subsequently took an average of each of these song features for each individual.

To evaluate the timing of song learning, we first conducted logistic regression using JMP statistical software (https://www.jmp.com) to analyse the presence/absence of the final song or any of its syllables. We used inverse predictions to estimate the age at which the probability of juveniles singing the adult Typical Blue Mountains song was 0.5, 0.75 and 0.95, respectively. Finally, we conducted linear regression in JMP to assess the relationship between juvenile age and the average number of syllables per bout duration, average bout duration and average frequency range.

We then used recordings of the five zoo-bred birds that had been successfully tutored in 2021 (the only birds available for longitudinal assessment at these ages) at 12, 18 and 24 months old to assess the similarity of songs across years once the song learning phase was over. To assess within-individual song stability, we conducted cross correlations using Raven Pro: Interactive Sound Analysis Software (https://ravensoundsoftware.com/). Cross-correlation measures the degree of acoustic similarity

between two recordings by comparing their spectrograms, producing a correlation coefficient where values closer to 1 indicate greater similarity. This method is well-suited for comparing recordings of the same individual across time.

Additionally, to assess the upper limit of the sensitive period, we assessed the song types produced at the end of the tutoring period of three birds that were opportunistically added to a tutoring cohort in 2021. To compare similarity with birds who had successfully learned the wild adult song, we first conducted discriminant function analysis in the same manner as Appleby (2024b) using the same parameters (supplementary table 1). Discriminant function analysis is a multivariate classification technique that creates a statistical model to distinguish between predefined groups. We measured the mean Mahalanobis distance of each of the two birds and classified them as one group. The Mahalanobis distance represents a measure of (dis)similarity in the acoustic properties between the average song of each individual regent honeyeater and the target song of the tutoring experiment (De Maesschalck *et al.,* 2000). We tested the mean Mahalanobis difference between groups using a Wilcoxon sum rank test. We also conducted spectrogram cross-correlations in Raven Pro using the batch correlator function with normalized spectrograms. We then conducted Wilcoxon rank sum tests to test for significant differences in correlation between groups.

Results

The logistic regression models for both adult song crystallization and production of adult song elements revealed that age was a highly significant predictor (both $P < 0.0001$, Figure 1). The probability of juveniles producing the crystallized song increasing substantially between 110 (0.50) and 150 days post-hatching (0.95). Birds in the 'Live Tutor Only' group demonstrated slightly faster song crystallization, with a 50% probability of crystallization by 98 days, compared to 118 days for juveniles in the 'Live Tutor plus Playback' group. While this suggests a marginal difference in timing, it did not reach statistical significance ($P = 0.1690$). In the model assessing the emergence of adult song elements, age was again

a significant factor (P < 0.0001), with the probability of adult Typical Blue Mountains song elements being present in juveniles' songs increasing steeply between 63 days (0.5) and 80 days (0.95), signalling the beginning of the plastic song phase that precedes full song crystallization.

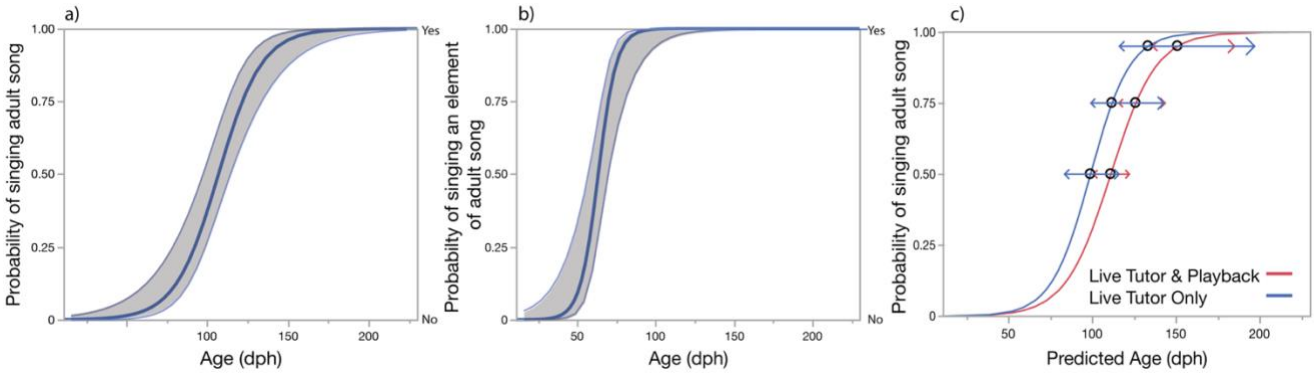

Figure 1. Logistic regression plots showing the relationship between age and (a) the presence of the adult Typical Blue Mountains song; and (b) the presence of any elements of the adult Typical Blue Mountains song in successfully song-tutored juvenile zoo-bred regent honeyeaters. (c) shows the inverse predictions for the age at which the probability of juveniles singing the adult Typical Blue Mountains song reached 0.5, 0.75 and 0.95. The colour of the line in (c) denotes the tutoring method.

For the analyses exploring song duration, syllable count, and frequency range, age was again revealed as a significant predictor across all three traits (Figure 2). Song duration decreased significantly with age (Estimate = -10.41, SE = 1.68, p < 0.0001). Similarly, the number of syllables decreased with age (Estimate = -0.037, SE = 0.0055, p < 0.0001). Frequency range also showed a significant decline (Estimate = -23.08, SE = 4.53, p < 0.0001).

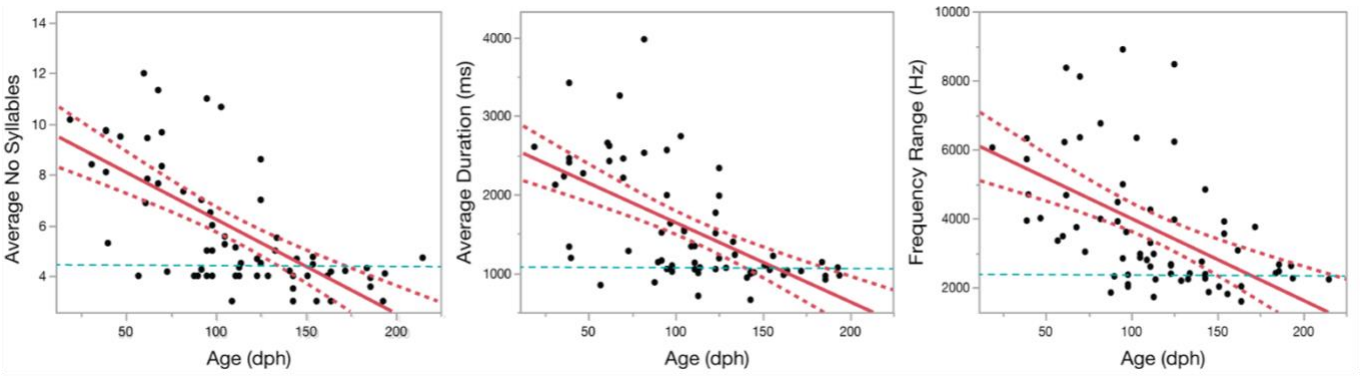

Figure 2. Linear regression plots showing the relationship between age and (a) the average number of syllables sung, (b) the average duration(ms) of a song bout and (c) the average overall frequency range (Hz) of a song bout. Dashed blue line represents the mean of each acoustic feature for adult birds.

The bird introduced to the tutoring program at 62dph successfully learned the adult Typical Blue Mountains song (Figure 3b). The two birds introduced to the song tutoring program at the later than usual age of 108 dph failed to produce a faithful copy of the adult Typical Blue Mountains song (Figure. 3c). Discriminant function analysis revealed that neither of the birds were classified as belonging to any of the successful tutoring groups. The mean Mahalanobis distance from the multivariate mean of the reference song for birds introduced at 108dph was 12.89 compared with 3.30 for birds in the 'Live Tutoring + Playback' cohort, and 5.18 for birds in the 'Live Tutor Only (small)' cohort. This difference was statistically significant (Chi-square = 12.90, p = 0.005). Spectrogram cross-correlation revealed significant differences between the mean correlation scores of birds introduced to the song tutoring program at 62 dph, 108 dph and the standard age of ~31 dph. The mean correlation of birds introduced at the standard age of 31 dph was 0.392 compared with 0.429 for the bird introduced at 62 dph and 0.222 for the birds introduced at 108 dph. The Wilcoxon sum test showed a significant difference in song learning success across the three groups (Chi-square = 809.9047, p < .0001). The two birds added to the tutoring program at 108 dph group had the weakest cross-correlations, indicating poor song learning. Pairwise comparisons reveal significant differences between all groups, with the largest disparity between the 31 dph group and the two birds added 108 dph (Z = -26.75, p < 0.0001). Cross correlations, however, did reveal that elements of the typical adult song were present in the

vocalisations of the birds added at 108 dph indicating some degree of learning the 'Typical Blue Mountains' song.

The five males that were tutored in 2021 and recorded at 12, 18 and 24 months retained the same song type at each interval (Figure 3d-f). The mean Mahalanobis distances were 3.1, 3.7 and 2.99 respectively. None of these were statistically significant in non-parametric comparisons of each pair using Wilcoxon method (p = 0.40, 0.59, 0.63).

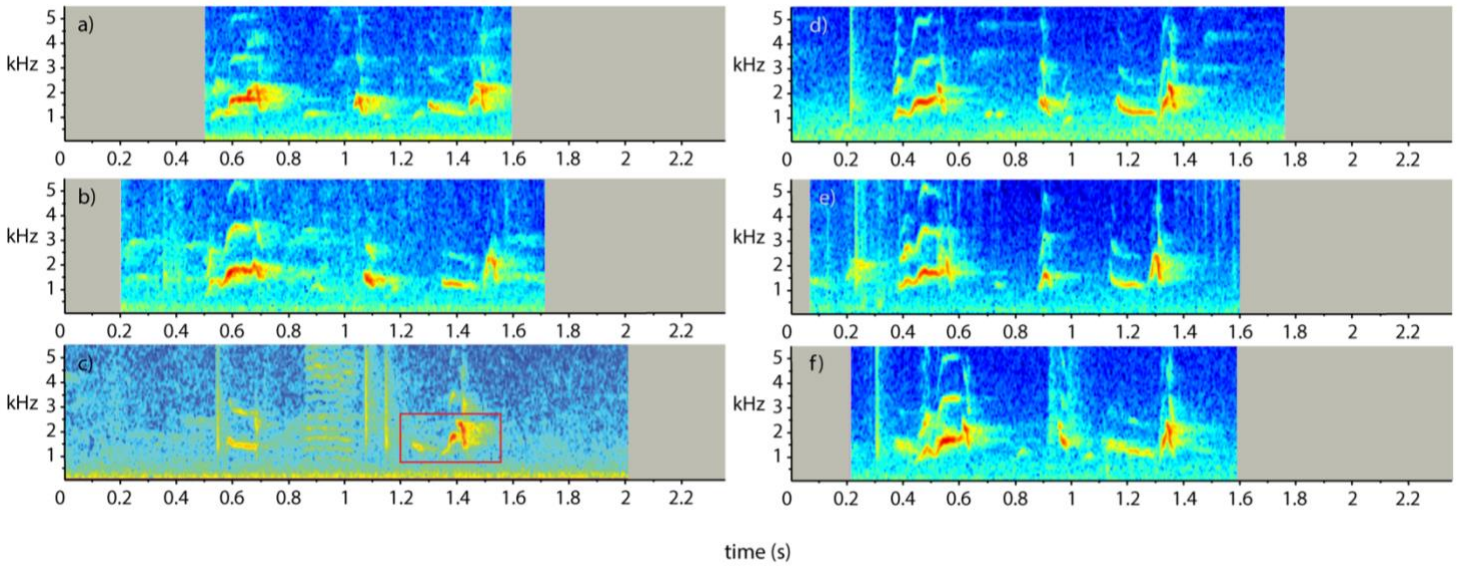

Figure 3. Sample spectrograms of birds recorded during three years of song tutoring experiments. a) shows the 'Typical Blue Mountains' song of an adult male bird collected from the wild but recorded in captivity. b) Shows the song of a male introduced to the tutoring program at 62dph. c) Shows the song of male introduced to the tutoring program at 108dph. d-f) show the songs of a male added at 31 dph at three different ages d) 186 dph, e) ~12 months and f) ~18 months old. The Y axis shows the frequency in hertz and the x axis shows time in seconds. The red rectangle in c) highlights an element of the adult song.

Discussion

Adaptive management actions, such as behavioural manipulations based on improved understanding of behavioural pathways, may be necessary to minimize negative impacts of divergent wild and zoo-bred phenotypes. Our results offer valuable insights into the timing and nature of song learning in

regent honeyeaters; information that is critical for the management of song within the captive environment, as well as understanding the ongoing erosion of vocal culture in wild regent honeyeaters.

These results suggest that regent honeyeaters are closed or only semi-open-ended learners. The timing of song learning in regent honeyeaters aligns with patterns observed in many other well-studied species (Catchpole & Slater 2008). During the initial 40 days post-hatching (dph), very few vocalizations were produced (tutoring commenced ~32 dph), indicating that the sensory phase extends beyond the age of independence. Between 40 and 100 dph, songs exhibited high variability in both bout duration and average number of syllables (Figure 2), likely reflecting the sensorimotor phase, which incorporates aspects of the subsong phase and the plastic song phase (Doupe 1999). Before 60 dph, there is less than a 60% probability of birds being observed to sing any elements of the adult song. The vocalisations produced during this period resembled the babbling or subsong phase, typified by long, highly variable song bouts (Figure 2). By 80 dph, there was a 95% probability that a bird would sing elements of the adult song but in variable ways representing the plastic song phase. These songs, while not matching adult songs, usually contained multiple elements of the adult song but were either arranged in variable ways or only contained part of the adult Typical Blue Mountains song. By 150 dph all birds had produced their final song, suggesting that song crystallization occurs between 130 and 150 dph. From these findings we propose a likely timeline of song learning in regent honeyeaters (Fig. 4).

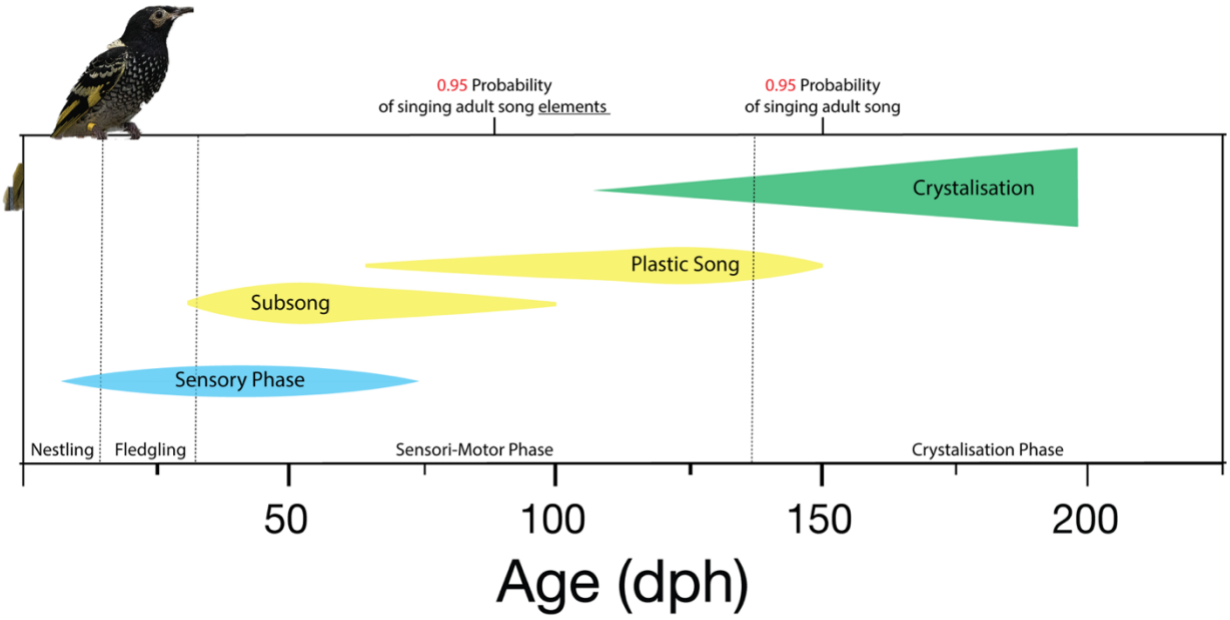

Figure 4. A conceptual song learning timeline for regent honeyeaters. Shapes denote sections of song learning while colours denote broader song learning phases as defined by Marler (1991). The two notches at the top side of the figure denote the age post hatching at which logistic regression estimated that the probability of zoo-bred juvenile males singing either the adult Typical Blue Mountains song or elements thereof was 0.95.

The absence of male song during the nestling period (Crates et al., 2021), combined with our experimental design in which no tutoring was provided in natal enclosures, meant juveniles had minimal deliberate song exposure before entering the tutoring program at 30 days. While we cannot rule out the possibility of some early auditory learning during the nestling period or even in Ovo, as has been documented in other species (Colombelli-Négrel et al., 2012), the consistent success of our tutoring protocol suggests that any such early exposure had limited influence on final song acquisition. Our results demonstrate that targeted tutoring from 30 days onward was sufficient to establish wild-type song templates, suggesting that the sensory phase either begins at or after fledging, or that later social learning from live tutors may override earlier song learning.

Our findings also indicate that birds exposed to a live tutor from the beginning of their independence produced a crystallized song more quickly than those that only experienced playback during their early

development (Figure 1c), however this was not statistically significant. As playback alone has proven ineffective for tutoring regent honeyeaters (Appleby et al. 2024b), juveniles may delay their sensitive period during times without access to suitable tutor males, a behaviour observed in other species, for example zebra finches *Taeniopygia guttata* (Eales 1987, Gobes, Jennings & Maeda, 2019). The ability of the male added at 62 dph to produce a Typical Blue Mountains adult song, despite the absence of suitable tutors before that point in time, may be a consequence of the same process.

The inability of the two males introduced at 108 dph to learn the Typical Blue Mountains song, however, suggests a limit to the sensitive period. Both males failed to learn the final song despite several months of access to appropriate tutors and all other males introduced at 32 dph in the same treatment group successfully learning the song. This is further supported by evidence that all repeated sightings of colour banded males recording in Crates et al. (2021), and Appleby et al. (2024a) whose song type was known retained the same song type across years. Zoo-bred males also retained song-types across years which indicates a level of closed-ended song learning. For the latter, it is possible that the lack of change in song-type could be attributed to the stable zoo environment in which novel songs are not introduced by immigration and birds exist in a relatively high density compared to the remaining wild population. This cannot be true of the wild birds, however. Wild birds travel great distances through their nomadic movements (Crates *et al.,* 2021) and form loose breeding aggregations where interactions with novel birds, and possibly novel song types, would be facilitated.

The seemingly closed-ended nature of regent honeyeater song learning is complicated by their ability to learn the songs of other species. This phenomenon is often described in the literature as either mimicry (Veerman, 1992; Ley, 2022) or maladaptive learning (Crates, 2019). This singing has been proposed as a form of Batesian mimicry, given the tendency of regent honeyeaters to sing the songs of larger species (Veerman, 1992; Veerman, 1994), which may offer an advantage by making the bird appear larger. For this mimicry to occur, regent honeyeaters would need to exhibit a high level of vocal

learning plasticity, a notion for which our results offer limited support. The literature primarily documents regent honeyeaters singing only the songs of one other species, with few records of 'bilingual' males singing both regent honeyeater songs and those of their presumed models (Higgins *et al.,* 2001; Ley 2022). However, these records are often based on the subjective assessment of regent honeyeater song without substantiating audio recordings and may not detail the specific elements of regent honeyeater songs incorporated or provide recordings of these songs. Regent honeyeaters produce a range of vocalisations beyond those described in recent research (Crates *et al.,* 2021, Appleby *et al.,* 2024b) and include innate calls and less directed song elements. It is possible that other vocalisations are being accounted for in mimicry reports, and these may constitute innate vocalizations such as calls. On the other hand, it is possible that regent honeyeaters demonstrate additive learning, where the core component of their song is fixed during the critical learning period, but additional elements are incorporated into their repertoire seasonally or throughout their lives (Slater, Jones & ten Cate, 1993; Cornez *et al.,* 2020). This is somewhat supported by the result that birds added at 108 dph did successfully learn at least some element of the 'Typical Blue Mountains' song. Our results provide stronger evidence for this theory than for continuous learning. Males tutored in 2021 retained the same song type in 2022, 2023, and 2024. In captivity, the lack of necessity or opportunity to acquire new or attractive songs contrasts with the inconsistency of male song types observed in the wild, where novel songs or those from other species are more common. Regent honeyeaters are nomadic and extremely rare, and observations of males singing offer only a limited view of their full singing behaviour. This leaves open the possibility of unobserved behaviours not accounted for in this study and highlights the need for further research on the full vernacular of regent honeyeater song.

Our study contributes to the limited body of knowledge on song learning in threatened species, where experiments must be conducted within the practical and ethical constraints of conservation programs. While our opportunistic experimental design and sample sizes do not permit the level of experimental control typical of laboratory studies with model species, our findings provide information essential for

evidence-based management decisions. The developmental timeline we document places regent honeyeaters within the range observed for closed-ended learners (Catchpole & Slater, 2008), though more controlled comparative studies would be needed to fully resolve questions about the flexibility of sensitive periods or the mechanisms underlying song template formation in this species.

Implications for Adaptive Management and Husbandry

Our results have important implications for adaptive management and husbandry practices. They indicate that in instances where regent honeyeaters need to be moved between aviaries or facilities they can be successfully tutored if they are returned to the tutoring aviary by 60 days old, but not beyond 100 days old. These estimates are based on the current data and may be subject to revision as additional information is obtained. This flexibility allows for the transfer of juvenile birds between breeding institutions, facilitating cross-institutional tutoring arrangements when certain facilities lack the ideal conditions for tutoring, or the removal from and subsequent reintroduction to the tutoring program where injury or illness may necessitate veterinary care.

For wild adaptive management, our findings provide insights into the timing of song learning and the potential for restoring wild song traits in zoo-tutored birds, provided that wild juveniles are within the specified age range. With the evidence that other bird species have successfully been tutored in the wild (Mennill *et al.,* 2018), understanding critical song learning windows may allow species managers to time releases to best prevent the ongoing loss of song in the wild population (Crates *et al.,* 2021; Appleby *et al.,* 2024a).The study highlights an effective framework for adaptive management, demonstrating the process of identifying maladaptive traits in captive environments, addressing them, and improving management practices accordingly. More broadly, this research demonstrates how experiments within the zoo-based component of a captive breeding program can yield valuable insights for adaptive management actions. In this case study, continued monitoring, and application of the

results to management actions may greatly improve outcomes for the conservation of this and many other species.

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Animal Ethics

Research was conducted under New South Wales scientific licences #SL101580 and #SL101603. All experiments were conducted in accordance with Taronga Zoo Ethics approval #4a/02/20 and Australian National University Animal Ethics permit #A2015/28.

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Chapter 7 Conclusion

The aim of this thesis was to improve the understanding of regent honeyeater song culture and its applications to adaptive management and improved conservation outcomes. Specifically, I set out to quantify the risk of assortative mating in this species based on song type; reduce the division of song culture between wild and captive populations; better understand the timing of song learning; and to monitor the ongoing changes to wild song culture. Before this research, the relative contribution of song culture to the success of regent honeyeaters after release was inferred from studies on wild birds (Liu *et al.,* 2015; Crates *et al.,* 2021). However, the lack of focused study of the consequences of divergent song culture between wild and captive birds made it impossible to assess accurately the risk when releasing zoo-bred birds. Without understanding the song learning pathways for this species it is difficult to understand properly the ongoing erosion of wild song culture, or how to manage it properly in captivity. Finally, with a general lack of captive examples of manipulating culturally acquired behaviour, it is unclear how practical and effective these actions are for the adaptive management of threatened species.

Song culture had previously been shown to be linked to lower reproductive success in regent honeyeaters (Crates *et al.,* 2021). However, it was not possible to disentangle completely whether birds who sang songs different from the regional norm were also impacted by their higher propensity to be isolated from conspecifics. It was also unclear whether females preferred males who sang songs that conformed to cultural norms (Fuji, Ikebuchi & Okanoya 2021), that is, the most common song-type sung in a particular area, or whether they preferred males who sang songs that were more complex (i.e., Mountjoy & Lemon, 1995). Given that the dominant wild song at the beginning of this research, the 'Typical Blue Mountains' dialect was also the more complex dialect (Crates *et al.,* 2021), either hypothesis was conceivable, and as such the impact on conservation actions was unclear. Song preference based on either familiarity or complexity, posed a risk of assortative mating that may inhibit the success of reintroduction attempts. Prior to this research, zoo-bred males sang simpler songs than

wild-born males, and female zoo-bred birds may have preferred the songs of wild males. This could lead to zoo-bred females pairing with wild-male pairs and decrease the chances of zoo-bred males of finding mates. Conversely, the preference for familiar songs could inhibit pairing between both zoo-bred males and females with wild birds. In chapter three we showed that female birds preferred the familiar songs of zoo-bred males despite their reduced complexity. In terms of fitness consequences and implications for the zoo-breeding program in regent honeyeaters, this discovery provided strong evidence that assortative mating based on song type was a serious risk, and the preference for familiar songs may inhibit both males and females from finding wild mates. While offspring of zoo-bred pairs may eventually learn wild songs and integrate, assortative mating prevents immediate gene flow into the wild population and risks multi-generational cultural isolation if offspring lack access to wild tutors in the critically fragmented populationThe research contained in chapter three represents some of the first empirical evidence that divergent, culturally acquired behaviours between captive and wild populations poses a threat to the successful reintroduction and establishment of captive stock to the wild population. These findings also support the long-held suspicion (Liu *et al.,* 2015; Crates *et al.,* 2021) that divisions in song culture may hinder the efforts of zoo-breeding programs for release to the wild.

Now, with the knowledge that assortative mating is a quantifiable risk, the possibility of reducing the division between wild and captive birds' song culture allows improvement of the fitness of birds prior to release. In chapter four, we demonstrated the potential for previously lost culturally learned behaviours to be reintroduced into captive populations through a structured song tutoring program. Over a three-year period, our program increased the proportion of juvenile birds that successfully learned the wild-type song from 0 to 42%. This finding holds significant promise, as it suggests not only that vital cultural traits can be reinstated within captively bred populations but also that captive birds may serve as a reservoir for such traits in the event of their extirpation from the wild.

The playback experiments in chapter four revealed that playback tutoring alone was not sufficient for juvenile birds to learn and produce wild-type adult song. This indicates that regent honeyeaters require

multimodality to learn their songs successfully (Varkevisser, 2022). The multimodal interaction that facilitates song learning between adult male tutors and their juvenile 'students' appears to be aggressive or dominant social interactions (personal observations). Adult, and even older juveniles, tended to chase other younger or smaller males from resources or prominent perching positions within the aviary. This observation suggests that juveniles may preferentially attend to and learn from socially dominant individuals. This type of learning has been observed in indigo buntings (*Passerina cyanea*) and zebra finches (*Taeniopygia guttata*), where juveniles show stronger learning from aggressive or dominant tutors (Payne 1981; Clayton, 1987). If regent honeyeaters similarly use social dominance as a cue for selecting song models, this may explain how regent honeyeaters come to learn the songs of other species in the wild: in the absence of conspecific tutors, isolated juveniles may learn from heterospecific individuals who display aggression toward them during competitive interactions at nectar resources. This behaviour is unusual within the taxonomic family and poorly understood (Powys 2010; Crates 2019a).

The singing of other species' songs in regent honeyeaters has been referred to as a form of Batesian mimicry by Veerman (1992; 1994), due to the propensity for regent honeyeaters to copy the songs of larger species almost exclusively. By doing so they may reduce aggression from other species thereby conferring an advantage to the 'mimic'. Crates (2019a; *et al.,*2021) cast doubt on this idea by showing that no fitness benefits were conferred to birds that sang abnormal 'interspecific' songs because those birds had lower likelihood of both pairing and nesting than those who sang species typical songs (Crates 2021). Despite the strong evidence that 'true' mimicry is unlikely in regent honeyeaters, it failed to explain why larger species were almost exclusively the song 'model'. Without a conferred advantage from singing the songs of larger species, it should be equally likely that regent honeyeaters would mimic the songs of smaller species. Our research in chapter four indicated that direct interactions are necessary to facilitate song learning. This makes the simple eavesdropping hypothesis (Beecher & Akcay, 2020), whereby birds learn simply from hearing a song, unlikely. If regent honeyeaters

preferentially learn from more aggressive tutors, it may be that isolated birds, without access to suitable tutors are learning the songs of birds who display aggression to them, probably while defending nectar resources. Aggressive interactions with the species that regent honeyeaters are known to learn the songs of are likely. It is surprising then, that the abundant and despotic noisy miner (*Manorina melanocephala*) is not a species that regent honeyeaters have been known to learn the songs of. As Ley (2022) suggests, in cases where no suitable tutor is available to a juvenile, there may still be some advantage relative to learning no song at all. The role of social interaction in regent honeyeater song learning could be explored further through detailed social network analysis (Ogino *et al.,* 2023; Aplin *et al.,* 2015).

As Crates (2019a) suggests, interspecific singing and indeed the ongoing erosion of regent honeyeater song culture (Crates *et al.,* 2021) is probably due to a lack of suitable adult tutors. The research presented in chapters four, five and six all support this hypothesis. Chapter four showed the direct learning relationship between adult tutors and juvenile males and chapter five revealed an ongoing erosion of song culture with continued population declines. In chapter six, we showed that regent honeyeater song learning probably occurs between two and six months of age. Although our methods to estimate this differed in a number of ways, the results were consistent with the only other study of song learning available (Vecsei, 2015) in terms of the song learning phase. Critically, Vescei (2015) did not provide evidence of the upper limit of the critical period, which the results of chapter six suggest is around 100 days of age. As Crates (2019a) has pointed out, young regent honeyeaters disperse away from the loose breeding aggregations before the song learning phase begins, and do not return to breeding areas until after the critical-period closes as suggested by our estimates.

Contribution to regent honeyeater Conservation

Although the success of the song tutoring program in restoring the species' typical wild song is promising, the ultimate benefit to the fitness of zoo-bred birds released to the wild is yet unknown. Without monitoring birds that have been successfully tutored after release, it is unclear whether

reducing the division in song culture between wild and captive birds translates to improved reproduction or survival. The first successfully tutored males were of high value to the song tutoring program to propagate further the wild song through the population. As such, no males that successfully learned the wild song were released during that period. In August 2024, the first males known to sing the wild-type dialect were released and monitoring such individuals is an essential area for future research. Already data are being collected to assess the post-release success of song-tutored birds in terms of survival, reproduction, and social integration. Previous evidence suggests that exposure to wild songs through playback, regardless of whether the adult song was successfully produced, had a positive impact on survival when released to the wild (Triopvich *et al.,* 2021). It therefore seems likely that recently released males who now sing songs that are statistically indistinguishable from wild type songs, will have a greater chance of attracting a wild mate who is familiar with the 'Typical Blue Mountains' song type. Female birds exposed to song tutoring may now also prefer the songs of any remaining wild birds that sing the 'Typical Blue Mountains' song type, which will have major implications important as the wild population becomes increasingly male-biased. This point underscores the need to tutor not only juvenile males, but also juvenile females.

The findings from chapter five raise questions regarding the long-term feasibility and efficacy of the song tutoring program for improving conservation outcomes. The rapid shift in the dominant song observed in the wild population meant that the song type learned by juvenile zoo-bred males was virtually extinct in the wild by the end of this study. This leads to the question of whether the song being taught in the zoo-breeding program can still improve post-release outcomes. This is an important consideration, and the arguments made elsewhere in this thesis suggest that female birds prefer familiar songs. However, there are a number of arguments in favour of continuing the song tutoring program in its current form. Firstly, the observed rate of change in the wild suggests it is not feasible to update the song being taught in captivity to match wild populations. This leaves managers with only two choices, persist with the current tutoring program or provide no tutoring at all. Secondly, the songs

of adult zoo-bred birds prior to the commencement of this program were extremely simple and had been likened to juvenile subsongs. The link between song complexity and the viability of populations (Laiolo *et al.,* 2008, Crates *et al.,* 2021) suggests that more complex songs would be preferable within the zoo population. Moreover, as the number and proportion of zoo-bred birds in the wild increases, it is increasingly likely that previously released and newly released individuals will sing, and be familiar with the songs originating from the tutoring program. Ensuring consistency in the song repertoire is therefore crucial for maintaining cultural cohesion among released birds and promoting gene flow. Finally, it is important to acknowledge that songs change naturally over time through 'cultural drift' (Williams, 2021). It may not be possible, or even necessary to preserve song culture in a permanent static state. It may be equally important to preserve the *potential* for cultural maintenance and evolution. Without suitable tutors, zoo-bred birds maintained simple rudimentary songs, despite more than 20 years of captive breeding. By continuing the song tutoring program birds will have access to tutors from whom they can learn songs, facilitating cultural maintenance and the potential for cultural evolution. While this still poses potential risks to the fitness of zoo-bred birds post-release, it appears favourable to the alternative of continued simplification and erosion of song culture.

As song complexity in the wild population continues to decline (chapter five), it becomes more likely that the zoo-bred population is the sole reservoir of the previously dominant 'Typical Blue Mountains' song type. This suggests the exciting prospect for future research of exploring the restoration of song culture from a captive origin. However, reintroducing these traits in a meaningful way requires careful implementation and detailed knowledge of the song learning pathway. Our research presented in chapter six suggests that managers may be able to time releases to coincide with the period when juvenile birds in their critical song learning period are present. Research on other species (Mennill *et al.,* 2018) has demonstrated the success of tutoring programs in wild settings, providing additional evidence that timing releases of zoo-tutored birds to align with key learning periods could prevent further loss of cultural traits.

Zoos engaged in conservation often prioritize public education (Roe, McConney, & Mansfield, 2014). It is important that *ex-situ* individuals be representative of their wild counterparts both behaviourally and morphologically. While it is not possible to account for all geographic song dialects in a population, the singing behaviour of regent honeyeaters in the zoo-breeding program did not display songs that were typical of any wild adult. There is the real possibility that regent honeyeaters may soon be extinct in the wild (Heinsohn *et al.,* 2023). It will be critical that song culture is well established and consistent before re-introduction are attempted. As we have demonstrated, for the song tutoring program to be successful, live tutors are necessary. If the wild population becomes extinct, there is a real risk of losing typical adult singing behaviour in captivity. It is fortunate that we have been able to establish a representative wild song culture within the zoo population in a timely manner, based first on the present of just two wild origin males.

Broader implications for adaptive management of captive breeding programs

The research contained in this thesis showed the feasibility of implementing effective and minimally disruptive experimentation to inform the active management of *ex-situ* conservation programs. Evidence-based adaptive management can ten improve the success of such programs. Animal culture and its implications for conservation is an emerging field (Brakes *et al.,* 2019; Witten, 2021). Culturally acquired behaviours are widespread, and probably understudied (Witten, 2021). For species involved in captive breeding programs these behaviours may have significant implications for their fitness, either within captivity or after release into the wild. We show for the first time that it is possible to re-seed cultural behaviours in avian captive breeding programs, and this can spread quickly through the population with subsequent generations if husbandry practices are appropriate to facilitate such spread. The utility of this may have far reaching implications for other species. While birds predominantly learn songs in specific sensitive periods, and this means that the spread of culturally acquired behaviours may be limited to the rate at which individuals breed and are born, other cultural

behaviours, for example novel foraging techniques, may spread much faster amongst the population and may not be limited to juveniles (Aplin *et al.,* 2015).

The challenges and approaches documented here have relevance to other contexts where culturally transmitted behaviours are threatened. Cultural behaviours are widespread across taxa (Whiten, 2021), and understanding how to maintain or restore these behaviours in captive settings becomes increasingly important as more species decline.

The sensitive period constraints we documented raise questions about interventions in species with different learning windows. Species with open-ended learning may offer more flexibility for cultural interventions across life stages, while those with extremely narrow sensitive periods may require even more precise timing. For behaviours acquired across the lifespan—such as socially learned foraging techniques (Aplin et al., 2015)—opportunities for intervention may be broader, though transmission mechanisms within captive populations may differ substantially from song learning.

Behaviours requiring large social groups, complex environments, or live prey interactions may be difficult or impossible to replicate in captivity. In such cases, alternative strategies such as carefully timed releases into populations with intact cultural traditions may be necessary.

More broadly, cultural traits should be considered alongside genetic and demographic factors in conservation planning for any species where social learning occurs. The methods we developed—identifying sensitive periods, testing transmission mechanisms, monitoring cultural change—provide a template adaptable to other species and, though each will present unique challenges dependent on species-specific ecology and the practical constraints of captive management.

Concluding Remarks

Many species face the paradigm of "death by a thousand cuts" when it comes to extinction, with a variety of compounding threats ultimately undermining their survival. It is my belief that in threatened species recovery, particularly in captive breeding programs, the inverse is likely to be true. It is clear that a number of incremental improvements through adaptive management strategies, such as song tutoring, have the potential to accumulate and contribute to more significant conservation outcomes over time. However, it is not likely that song culture alone will determine the success or failure of the regent honeyeater, though it may play a crucial role in shaping reproductive behaviours and long-term population viability.

Five years ago, Crates (2019b) expressed concern that regent honeyeaters may have passed the Allee threshold, and that extinction may be inevitable, noting the importance of the next five years for the species survival. Since then, releases of captive-bred birds from the zoo-breeding program have been moved to the remaining core habitat and reintroduced more than 200 birds to the wild. While limited data are available, reports suggest that individuals may be persisting longer than in previous years post release, and I have received a number of anecdotal reports of various levels of integration and even pairing between wild and captive birds over the past year. Despite the continued debate about the appropriateness of *ex-situ* conservation, the rigor with which captive-breeding is conducted for regent honeyeaters is heartening. While I echo Crates' 2019 fears, I'm confident that the breeding program will make an in increasingly positive contribution to the conservation of this species, and with this the slim hope of the species' recovery remains.

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Appendix

Chapter 3 Supplementary Material

Supplementary Table 1 – List of recordists of superb fairy-wren song with URLs to Files on Xeno- Canto

File Name	Recordist	URL
XC576508	Marc Anderson	https://xeno-canto.org/576508
XC576507	Marc Anderson	https://xeno-canto.org/576507
XC576506	Marc Anderson	https://xeno-canto.org/576506
XC194815	Diego Caiafa	https://xeno-canto.org/194815
XC389376	Marc Anderson	https://xeno-canto.org/389376
XC278419	Marc Anderson	https://xeno-canto.org/278419
XC340988	Marc Anderson	https://xeno-canto.org/340988
XC233828	Marc Anderson	https://xeno-canto.org/233828
XC340989	Marc Anderson	https://xeno-canto.org/340989
XC340990	Marc Anderson	https://xeno-canto.org/340990
XC609326	Marc Anderson	https://xeno-canto.org/609326
XC609335	Marc Anderson	https://xeno-canto.org/609335
XC201471	Marc Anderson	https://xeno-canto.org/201471
XC460624A	Marc Anderson	https://xeno-canto.org/460624
XC460624B	Marc Anderson	https://xeno-canto.org/460624
XC172263	Marc Anderson	https://xeno-canto.org/172263
XC30651	Vicki Powys	https://xeno-canto.org/30651
XC146635	Marc Anderson	https://xeno-canto.org/146635

Supplementary Table 2 – Model summaries for the logistic regressions of Association and Close association times

	Association			
Fixed Effects	*Estimate*	*std. Error*	*Z value*	*P*
Captive	-0.045	0.30	-0.16	0.871
Control	-0.40	0.28	-1.41	0.159
Wild	-0.37	0.32	-1.14	0.256
Random Effects	Group	Variance	Std. Dev.	
	Id	7.446e-01	8.629e-01	
	Side	1.374e-10	1.172e-05	
	Close Association			
Fixed Effects	*Estimate*	*std. Error*	*Z value*	*p*
Captive	2.2236	0.54	4.104	<0.001
Control	-3.48	0.63	-5.56	<0.001
Wild	-3.10	.66	-4.681	<0.001
Random Effects	Group	Variance	Std. Dev.	
	Id	5.196e-01	0.7208170	
	Side	7.003e-08	0.0002646	

Supplementary Table 3 – Model Summaries for Linear mixed models for Association time, Close Association Time, Latency and Negative Time

	Association			
Predictors	*Log-Mean*	*std. Error*	*Statistic*	*p*
Captive	4.42	0.07	66.96	<0.001
Control	-0.43	0.03	-17	<0.001
Wild	-0.57	0.03	-17.65	<0.001
Captive*LATE SEASON	0.48	0.12	4.01	<0.001
Control* LATE SEASON	0.61	0.16	5.33	<0.001
Wild * LATE SEASON	0.46	0.12	3.76	<0.001
Zero-Inflated Model				
(Intercept)	0.29	0.11	2.73	0.006
Random Effects				
σ^2	0.27	ICC	0.15	
τ_{00}	0.05 $_{Id}$	N	18 $_{Id}$	
Observations	360	Mar. R^2 / Cond. R^2	0.256 / 0.369	
	Close Association			
Predictors	Log-Mean	std. Error	Statistic	p
Captive	3.28	0.18	18.51	<0.001
Control	-0.29	0.07	-4.36	<0.001
Wild	-0.83	0.1	-8.46	<0.001
WILD*LATE SEASON	-0.44	0.37	-1.18	0.237
Control* LATE SEASON	-0.29	0.45	-0.65	0.519
Wild * LATE SEASON	-1.29	0.62	-2.07	0.038
Zero-Inflated Model	0.17	0.16	1.01	0.312
(Intercept)				
Random Effects				
σ^2	0.34	ICC	0.52	
τ_{00}	0.36 $_{Id}$	N	18 $_{Id}$	
Observations	154	Mar. R^2 / Cond. R^2	0.252 / 0.638	
	Latency			
Predictors	Log-Mean	std. Error	Statistic	p
Captive	3.37	0.11	31	<0.001
Control	0.67	0.03	19.5	<0.001
Wild	0.85	0.04	21.73	<0.001
WILD*LATE SEASON	0.22	0.2	1.11	0.266
Control* LATE SEASON	0.13	0.19	0.71	0.481
Wild * LATE SEASON	-0.32	0.19	-1.652	0.099
Random Effects				
σ^2	0.02	ICC	0.87	
τ_{00}	0.13 $_{Id}$	N	18 $_{Id}$	
Observations	154	Mar. R^2 / Cond. R^2	0.421 / 0.926	
	Negative			
Predictors	Log-Mean	std. Error	Statistic	p
Captive	3.53	0.35	9.96	<0.001
Control	0.5	0.04	13.7	<0.001
Wild	0.51	0.04	12.7	<0.001
WILD*LATE SEASON	0.59	0.66	0.9	0.369
Control* LATE SEASON	-0.61	0.65	-0.94	0.346
Wild * LATE SEASON	-0.34	0.65	-0.52	0.601
Zero-Inflated Model				
(Intercept)	0.49	0.12	4.19	<0.001
Random Effects				
σ^2	0.51	ICC	0.75	
τ_{00}	1.50 $_{Id}$	N	18 $_{Id}$	
Observations	360			
Mar. R^2 / Cond. R^2	0.035 / 0.754			

Supplementary Table 4 – Post-hoc ANOVAs for Association time, Close Association time, Latency and Negative time

	Association			*Close Association*			*Latency*			*Negative Time*		
	Sum Sq	Df	Pval.	Sum Sq	Df	Pval.	Sum Sq	Df	Pval	Sum Sq	Df	Pval.
Treatment	521.17	2	<0.001	88.13	2	<0.001	534.23	2	<0.001	117.63	2	<0.001
Treatment: Season	33.48	3	<0.001	4.33	3	0.24	47.98	3	<0.001	183.54	3	<0.001

Supplementary Table 5 – Planned Comparisons for linear mixed models; Association time, Close association time, Latency and Negative time using function 'glht' in package 'multcomp' adjusted p- values using Holm-Bonferroni corrections.

Association Time	**Estimate**	**Std. Error**	**t value**	**P**
EARLY SEASON Captive - LATE SEASON Captive	-0.48035	-0.11979	4.01	0.000371 ***
EARLY SEASON Captive - EARLY SEASON Control	0.42756	0.02515	17.003	< 2e-16 ***
EARLY SEASON Captive - LATE SEASON Control	-0.18591	0.11494	-1.617	0.213341
EARLY SEASON Captive - EARLY SEASON Wild	0.56594	0.03207	17.646	< 2e-16 ***
EARLY SEASON Captive - LATE SEASON Wild	0.10818	0.1201	0.901	0.368348
LATE SEASON Captive - EARLY SEASON Control	0.90791	0.11997	7.568	3.69e-12 ***
LATE SEASON Captive - LATE SEASON Control	0.29444	0.04895	6.015	3.61e-08 ***
LATE SEASON Captive - EARLY SEASON Wild	1.04629	0.12155	8.608	2.66e-15 ***
LATE SEASON Captive - LATE SEASON Wild	0.58853	0.05751	10.234	< 2e-16 ***
EARLY SEASON Control - LATE SEASON Control	-0.61347	0.11513	-5.329	1.24e-06 ***
EARLY SEASON Control - EARLY SEASON Wild	0.13838	0.03222	4.294	0.000136 ***
EARLY SEASON Control - LATE SEASON Wild	-0.31938	0.12028	-2.655	0.024857 *
LATE SEASON Control - EARLY SEASON Wild	0.75185	0.11677	6.439	3.95e-09 ***
LATE SEASON Control - LATE SEASON Wild	0.29409	0.04831	6.088	2.69e-08 ***
EARLY SEASON Wild - LATE SEASON Wild	-0.45776	0.12185	-3.757	0.000806 ***
Close Association	**Estimate**	**Std. Error**	**t value**	**P**
Captive EARLY SEASON - Control EARLY SEASON	0.28927	0.06629	4.364	0.000313 ***
Captive EARLY SEASON - Wild EARLY SEASON	0.83164	0.09835	8.456	3.93e-13 ***
Captive EARLY SEASON - Captive LATE SEASON	0.43729	0.36989	1.182	1
Captive EARLY SEASON - Control LATE SEASON	0.57812	0.4458	1.297	1
Captive EARLY SEASON - Wild LATE SEASON	2.11909	0.61527	3.444	0.008975 **
Control EARLY SEASON - Wild EARLY SEASON	0.54237	0.10799	5.023	2.05e-05 ***
Control EARLY SEASON - Captive LATE SEASON	0.14802	0.37335	0.396	1
Control EARLY SEASON - Control LATE SEASON	0.28885	0.44878	0.644	1
Control EARLY SEASON - Wild LATE SEASON	1.82982	0.61735	2.964	0.035473 *
Wild EARLY SEASON - Captive LATE SEASON	-0.39436	0.38004	-1.038	1
Wild EARLY SEASON - Control LATE SEASON	-0.25352	0.45439	-0.558	1
Wild EARLY SEASON - Wild LATE SEASON	1.28745	0.62141	2.072	0.320334
Captive LATE SEASON - Control LATE SEASON	0.14083	0.4335	0.325	1
Captive LATE SEASON - Wild LATE SEASON	1.68181	0.50258	3.346	0.011455 *
Control LATE SEASON - Wild LATE SEASON	1.54097	0.67015	2.299	0.206083
Latency	**Estimate**	**Std. Error**	**t value**	**P**
EARLY SEASON Captive - LATE SEASON Captive	-0.22193	0.19953	-1.112	1
EARLY SEASON Captive - EARLY SEASON Control	-0.67206	0.03447	19.499	< 2e-16 ***
EARLY SEASON Captive - LATE SEASON Control	-0.80258	0.18647	-4.304	0.000305 ***
EARLY SEASON Captive - EARLY SEASON Wild	-0.84793	0.03903	21.726	< 2e-16 ***
EARLY SEASON Captive - LATE SEASON Wild	-0.53213	0.19179	-2.775	0.043724 *
LATE SEASON Captive - EARLY SEASON Control	-0.45013	0.19826	-2.27	0.147804
LATE SEASON Captive - LATE SEASON Control	-0.58064	0.084	-6.912	1.76e-09 ***
LATE SEASON Captive - EARLY SEASON Wild	-0.62599	0.19891	-3.147	0.015973 *
LATE SEASON Captive - LATE SEASON Wild	-0.3102	0.09254	-3.352	0.009184 **
EARLY SEASON Control - LATE SEASON Control	-0.13051	0.18511	-0.705	1
EARLY SEASON Control - EARLY SEASON Wild	-0.17586	0.03156	-5.572	1.40e-06 ***
EARLY SEASON Control - LATE SEASON Wild	0.13993	0.19047	0.735	1
LATE SEASON Control - EARLY SEASON Wild	-0.04535	0.18581	-0.244	1
LATE SEASON Control - LATE SEASON Wild	0.27044	0.06202	4.361	0.000267 ***
EARLY SEASON Wild - LATE SEASON Wild	0.31579	0.19114	1.652	0.503215
Negative Time	**Estimate**	**Std. Error**	**t value**	**P**
EARLY SEASON Captive - EARLY SEASON Control	-0.499045	0.036427	-13.7	< 2e-16 ***
EARLY SEASON Captive - EARLY SEASON Wild	-0.507334	0.039952	-12.699	< 2e-16 ***
EARLY SEASON Captive - LATE SEASON Captive	-0.589437	0.65611	-0.898	1
EARLY SEASON Captive - LATE SEASON Control	0.114744	0.652417	0.176	1
EARLY SEASON Captive - LATE SEASON Wild	-0.166392	0.652487	-0.255	1
EARLY SEASON Control - EARLY SEASON Wild	-0.008289	0.029747	-0.279	1
LATE SEASON Captive - EARLY SEASON Control	-0.090392	0.655617	-0.138	1
EARLY SEASON Control - LATE SEASON Control	0.61379	0.651921	0.942	1
EARLY SEASON Control - LATE SEASON Wild	0.332653	0.651991	0.51	1
LATE SEASON Captive - EARLY SEASON Wild	-0.082103	0.65578	-0.125	1
LATE SEASON Control - EARLY SEASON Wild	0.622078	0.652085	0.954	1
EARLY SEASON Wild - LATE SEASON Wild	0.340942	0.652155	0.523	1
LATE SEASON Captive - LATE SEASON Control	0.704181	0.082595	8.526	5.77e-15 ***
LATE SEASON Captive - LATE SEASON Wild	0.423045	0.087084	4.858	1.97e-05 ***
LATE SEASON Control - LATE SEASON Wild	-0.281136	0.046091	-6.1	3.36e-08 ***

Chapter 4 Supplementary Material

Reintroduction of wild song culture to a critically endangered songbird

Supplementary Materials

Supplementary Text

Study population

The regent honeyeater zoo-breeding program was established in 1995 in response to a dramatic decline in the wild population. Nine nestlings collected from the wild formed the original zoo population, with birds subsequently recruited in 1997 (n=2), 2012 (n = 4), 2019 (n = 3) and 2023 (n = 2). To date, ~400 zoo-bred regent honeyeaters have been released into the wild.

Birds in this study were born and housed in Taronga Zoo (TZ), Sydney and/or Taronga Western Plains Zoo (TWPZ), Dubbo. All birds included in this study were part of the active zoo-breeding program and therefore subject to routine husbandry protocols. As is often the case in conservation management, resources for time and space within the breeding facilities were limited. As such, experiments needed to be incorporated in the least disruptive manner to ensure they achieved the overall aim of establishing wild song culture in the zoo population without impacting negatively the breeding capacity of the program.

Treatment groups

Treatment 1 - Control: The control tutoring was conducted at TWPZ in the 2020-2021 breeding season. Nine juvenile males were crèched together according to traditional husbandry protocols on the standard timeline described above and in Table S1. Birds were housed in aviary type 2 (Fig. 1a) at the end of a breeding block of aviaries and were unable to see birds in other treatments. A dummy speaker (Bose FreeSpace 360p Series II) was mounted within the aviary but did not emit sound. The only sounds juvenile males in the control group were exposed to were from each other, and from the surrounding environment such as nearby zoo and wild animals and suburban noises such as air and road traffic.

Treatment 2 – Playback only (large cohort): The playback only (large cohort) treatment was conducted at TWPZ in the 2020-2021 breeding season. Nine birds were housed together in an aviary type 2 (Fig. S1a) according to the standard timeline. A composite track consisting of the songs of 25 different wild male regent honeyeaters singing the typical Blue Mountains song type (each with a duration of ~2 seconds) was broadcast to the juvenile birds from sunrise to sunset every day of the experiment from a broadcast speaker mounted within the aviary. Given the regent honeyeater evolved to be a flocking species[51], we considered 25 individuals as a reasonable estimate of the number of adult tutors a juvenile could have expected to interact with in a post-breeding flock prior to severe population decline. To approximate the natural variation in songbird singing activity[52,53], songs were emitted at a more frequent rate (~four songs per minute) for the first four hours and last two hours of the day, than during the middle of the day (~one song per minute). The length of the playback track was increased two months into the experiment to reflect the lengthening of the daylight hours during the Austral summer. A description of the process of composing the playback track, playback system parameters and playback track itself can be found in the supplemental text (Text S2 & Text S3).

Treatment 3 – Live tutoring only (large cohort): The live tutoring only (large cohort) was conducted at TZ in the 2020-2021 breeding season. Thirteen juvenile males were housed together in an aviary type 1 (Fig. S1b) according to the standard timeline. Juveniles were moved into an aviary adjacent to one of the two wild-origin adult males that sang the typical Blue Mountains wild song. Juveniles could both see and hear the adult male but could not physically interact with him for the first 2.5 months, during which period the adult male was breeding and would otherwise have shown aggression to juveniles in the same aviary (Regent honeyeater husbandry guidelines, Taronga Zoo, 2013). After 2.5 months, the two aviaries were merged, and the juveniles could directly interact with the adult male.

Treatment 4 – Playback only (small cohort): The playback only (small cohort) was conducted at TWPZ in the 2021-2022 breeding season. Four juvenile males were housed in an aviary type 2 (Fig. S1a) according to the

standard timeline and were unable to see birds in adjacent aviaries. All other experimental parameters were as described above for Treatment 2- Playback only (large cohort).

Treatment 5: Live tutoring + playback: The live tutoring + playback treatment was conducted at TZ in the 2021-22 and 2022-2023 breeding seasons. Eighteen juvenile males were housed in two separate aviaries: eight (2021-2022) in a type 1 aviary (Fig. S1d) and 10 (2022-2023) also in a type 1 aviary (Fig. S1d). Birds were moved from their natal aviary into a crèche aviary adjacent to a wild-origin adult male according to the standard timeline. As in treatment 3, juvenile birds were able to both see and hear the adult male but could not physically interact with him for the first 2.5 months until the aviaries were merged. Unlike treatment 3, during the period that juveniles could not interact with the wild-origin adult male, they were exposed to playback in the same way as treatment 4. After the juveniles and adult male were united, the broadcast playback continued until the end of the breeding season.

Treatment 6: Live tutoring only (small cohort): The live tutoring only (small cohort) experiment was conducted at both TZ and TWPZ in the 2022-2023 breeding season. Fourteen birds were housed in three separate aviaries, with five juveniles each in two type 2 aviaries (Fig. S1c) at TZ and four juvenile males in one type 3 aviary at TWPZ (Fig. S1c). In the live tutoring only (small cohort) experiments, juvenile males were transferred from their natal aviary according to the standard timeline to a crèche aviary with a zoo-bred adult male tutor (~18 months old) that had successfully learned the typical Blue Mountains song in the previous season's Treatment 5 experiment. During the tutoring period they could see, hear and physically interact with the live tutor. In summary, the experiment involved 68 birds: two wild origin males (tutors) and 66 juvenile zoo-bred males, of which four were used as live adult tutors in subsequent years (Table 2).

Supplementary Text S2 – Composing the Playback Track and Playback systems

Composing the playback tracks

We first gathered 25 recordings of wild-origin males who sang the Typical Blue Mountains song-type. These were sourced largely from the wild using recordings obtained in Crates et. al (2021, n = 23), as well as one recording made by D. A of a wild-origin bird at TZ who sang Blue Mountains typical song-type and one recording from Xeno-Canto.

We initially composed 14 playback tracks. Each day of the week had a unique playback track, and two time periods had a set of seven tracks. The first seven of these time periods were 12 hours in length to fill the average Austral daylight hours between September and November. The second seven were 14 hours in duration, designed to fill the average Austral daylight hours between December and March. We divided tracks between peak and off-peak periods. The peak period was designed with the intention of mimicking the increased call activity of songbirds early in the morning and late evening periods (Catchpole and Slater, 2008). During the peak period, songs were broadcast at a more frequent rate than the off-peak period during the middle of the day. On our tracks the peak period ran for the first four, and the last two hours of the day, regardless of day length. The off-peak period ran in the warmer, middle part of the day in between peak periods. The off-peak period of the playback track broadcast songs at a less frequent rate than the peak period. Between September and November, the off-peak period was six hours in length. Between December and March, the off-peak period was eight hours in length.

To compose the tracks, we developed two randomisation matrices each for the peak and off-peak periods. The first randomisation matrix was designed to set the order of the song presentation and then the timing of the song presentations. For the peak period, songs were emitted at an average rate of one song per 25 seconds (randomised silence between 12- 75 seconds). For the off-peak period, songs

were emitted at an average rate of one song per 93 seconds (randomised silence between 60-180 seconds).

We first post-processed recordings in audacity to remove any unwanted sounds. We then ran a 300Hz High Pass Filter to all songs to reduce low end noise. We then created 10, one-hour long blocks of both peak and off-peak playback, by arranging songs in Apple's 'garageband' according to the randomisation matrix. We created another randomisation matrix to decide the presentation of one-hour blocks to make the peak and off peak tracks for each day. These one-hour blocks were arranged in apple's 'garageband' according to the randomisation matrix to make seven peak periods of four hours (morning) and seven peak periods of two hours (evening). We repeated this to make seven off peak periods of six hours of length, and seven off peak periods of eight hours in length. To compose the final 12- and 14-hour playback tracks we randomly assigned one morning peak period, one off peak period and one evening off peak period to create one track. This was done in Audacity software owing to the extremely large file size that was inappropriate for garage band. Files were exported as high-quality '.wav' files and assigned one day each per week for broadcast.

In 2021/2022 season we increased the song playback rate. To do this we followed all of the above steps this time decreasing the randomised silence period by half (effectively doubling the playback rate).

Playback systems

We employed two playback systems throughout the three years. At both zoos the broadcast speakers were Bose 'Freespace' outdoor speakers. In TZ we used a XX amplifier connected to a windows computer. Playback was initiated using the 'Windows Task Scheduler' software. At TWPZ, where a permanent indoor space was not available, we used a portable set up housed in a waterproof box. We used a Swamp Industries line amplifier connected to an iPod touch. We used the default automation app to schedule playback.

Text S3 – Rationale for adaptive decisions for song tutoring protocols.

In the 2020/21 season (year one), we trained one cohort with playback at Taronga Western Plains Zoo (TWPZ), one cohort with live tutors at Taronga Zoo (TZ) and one cohort remained un-tutored as a control group at TWPZ. It was clear that live tutoring resulted in better song outcomes than playback, while playback alone was only marginally better the control group. Songs from the live tutoring only (Large cohort) did appear to be more complex than the playback and control groups, however, the final songs of all groups did not approach the wild reference song from a listener's perspective and subsequent statistical analysis of acoustic features confirmed this (as reported in the main text). This evidence, combined with observations from researchers and husbandry staff showed that these methods had potential if some minor changes were made to the protocols.

During the first season, we observed that in the playback group, the vocalisations of the juveniles were far more frequent than the playback itself, and that juvenile birds tended to interact and vocalise more often in response to each other than in response to playback emitted from the loudspeakers. We believed that interactions with real birds are more enriching than playback and as such juveniles would preferentially learn from a live model, even if they were also juvenile (i.e., Varkevisser 2022; Derregnaucourt 2013). A similar pattern of behaviour was observed in the live tutor group, whereby juveniles did interact with the tutor, but the volume of juveniles appeared to result in most interactions being with other juveniles instead of the adult tutor. We also noted that for the earliest period of their life, young birds didn't hear wild song as the tutor was not vocal during his own breeding period. This resulted in the following changes for season 2021/22:

(1) Rate of song playback was doubled in the both the playback only and live tutoring cohorts.
(2) Adult tutor to juvenile ratio was limited to a maximum of1:5.
(3) Playback was introduced during the early phases of the live tutoring cohort. The previous live tutoring cohort in 2021 was not exposed to additional playback.

Given the severity of ongoing population decline in regent honeyeaters and the importance of the zoo-breeding program to their recovery efforts (Heinsohn *et al.,* 2022; Appleby *et al.,* 2024), we prioritised implementing all the above changes at once to maximise the chances of successful song learning over a more rigorous step-by-step experimental approach. This was also in-part driven by a lack of space in the zoo setting, where implementing each experimental change in separate cohorts would have taken several years to deploy.

In 2021/22 the playback only (small) cohort showed little improvement compared with the playback only (large) cohort. This indicated that song learning is likely multimodal (Varkevisser et al. 20220) in regent honeyeaters; that is, interaction with a tutor is critical and as such, we decided to cease playback only cohorts in future years in favour of expanding live tutoring. In the live tutoring + playback cohort, we frequently detected wild-type songs by ear during the song tutoring which was later confirmed statistically and are displayed below in fig. S1.

While the 2021/22 season produced juvenile birds whose songs resembled the reference wild song type, the decision to implement multiple changes to the protocols in one year created ambiguity around whether the smaller cohort or the reinforcement of the tutor with playback was the critical factor determining successful song learning. To disambiguate this, we introduced the live tutoring only (small) cohort.

Supplementary Tables

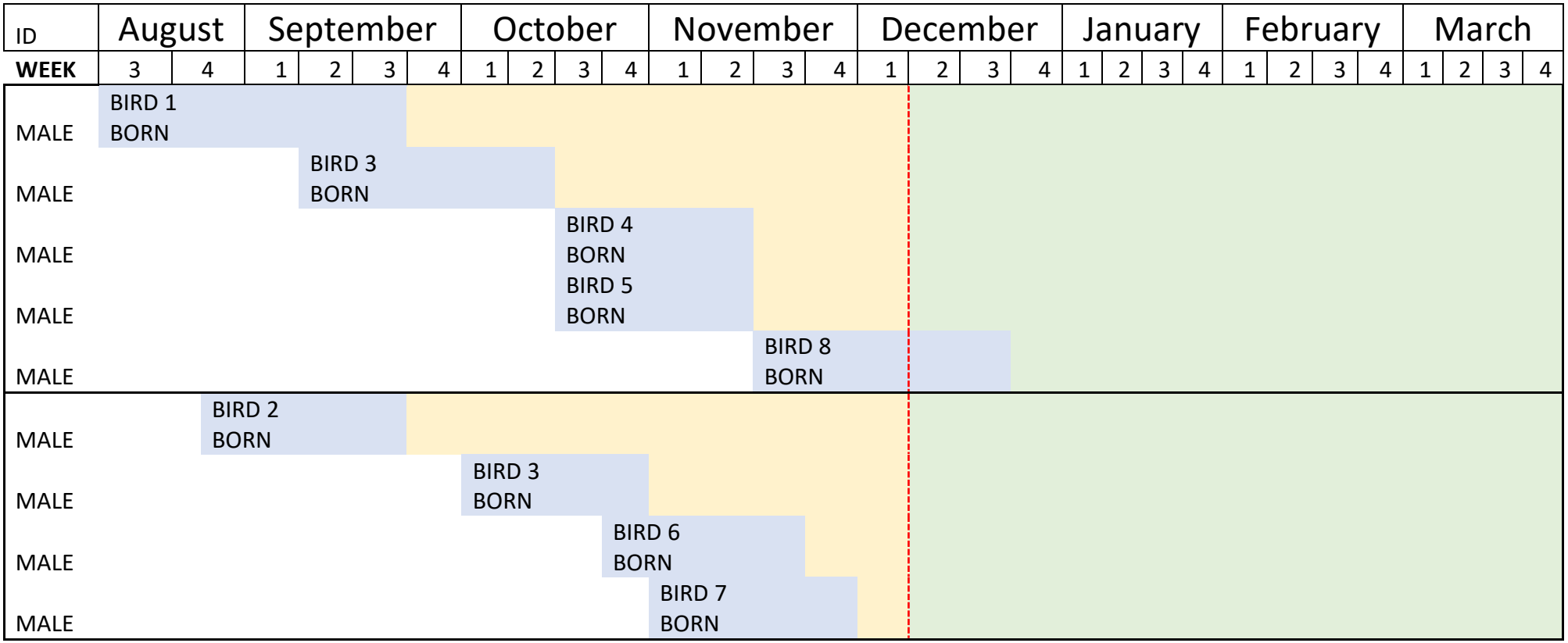
Supplementary Table 1

Table showing the model estimates of generalised linear model regressions using to formula Mahalanobis distance ~ Treatment + Age

	Mahalanobis Distance		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	2.56	0.13 – 4.99	**0.039**
Control (T1)	2.03	0.40 – 3.65	**0.014**
Playback (Large, T2)	1.22	-0.32 – 2.77	0.121
Live Tutoring Only (Large, T3)	2.55	1.16 – 3.94	**<0.001**
Playback (Small, T4)	2.30	0.90 – 3.70	**0.001**
Live Tutoring + Playback (T5)	-0.33	-1.74 – 1.08	0.643
Live Tutor (Small, T6)	-0.22	-1.75 – 1.30	0.773
Age	-0.00	-0.01 – 0.01	0.854

Supplementary Table 2 **–**

Timeline Example 2022/23 season (Live Tutor + Playback). Table shows the timeline for two live tutoring + playback cohorts in the 2022/23 season. Light-blue shading represents the period where the juvenile bird is in the natal aviary with its parents. Pale-yellow represents the period when the bird is in a an experimental creche exposed to playback while next to a wild male with who they can't physically interact but can see and hear. The pale-green shading represents the period when birds are in the creche aviary with the live tutor as well as exposed to song playback. The dashed red line denotes the addition of the live to tutor to the creche aviary.



Supplementary Table 3: Acoustic features measured in 'Chipper' and whether they were included in the discriminant function analysis.

Feature	Included in DFA?
Number of Unique Syllables	**Yes**
Smallest Syllable Frequency Range (Hz)	**Yes**
Average Syllable Lower Frequency (Hz)	**Yes**
Largest Syllable Frequency Range (Hz)	**Yes**
Number Syllables per Bout Duration (1/ms)	**Yes**
Number of Syllables	**Yes**
Overall Syllable Frequency Range (Hz)	**Yes**
Std. Deviation Syllable Frequency Range (Hz)	**Yes**
Smallest Syllable Duration (ms)	**Yes**
Average Silence Duration (ms)	**No**
Average Syllable Duration (ms)	**No**
Average Syllable Duration (ms)	**No**
Average Syllables Upper Frequency (Hz)	**No**
Bout Duration(ms)	**No**
Largest Silence Duration (ms)	**No**
Largest Syllable Duration (ms)	**No**
Largest Syllable Freq Range (Hz)	**No**
Max Syllables Frequency (Hz)	**No**
Min Syllables Frequency (Hz)	**No**
Smallest Silence Duration (ms)	**No**
Std. Dev. Silence Duration (ms)	**No**

Supplementary Table 4

Table showing results of backwards model selection. Each step removes a predictor and shows the resulting AIC. Text in bold shows the strongest performing model.			
Model Formula	AIC	Predictor to Remove	New AIC
Mahalanobis Distance~ Treatment + Age	264.71	Age	262.74
		Treatment	334.25
Mahalanobis + Distance~ Treatment	**262.7**	Treatment	340.3

Supplementary Figures

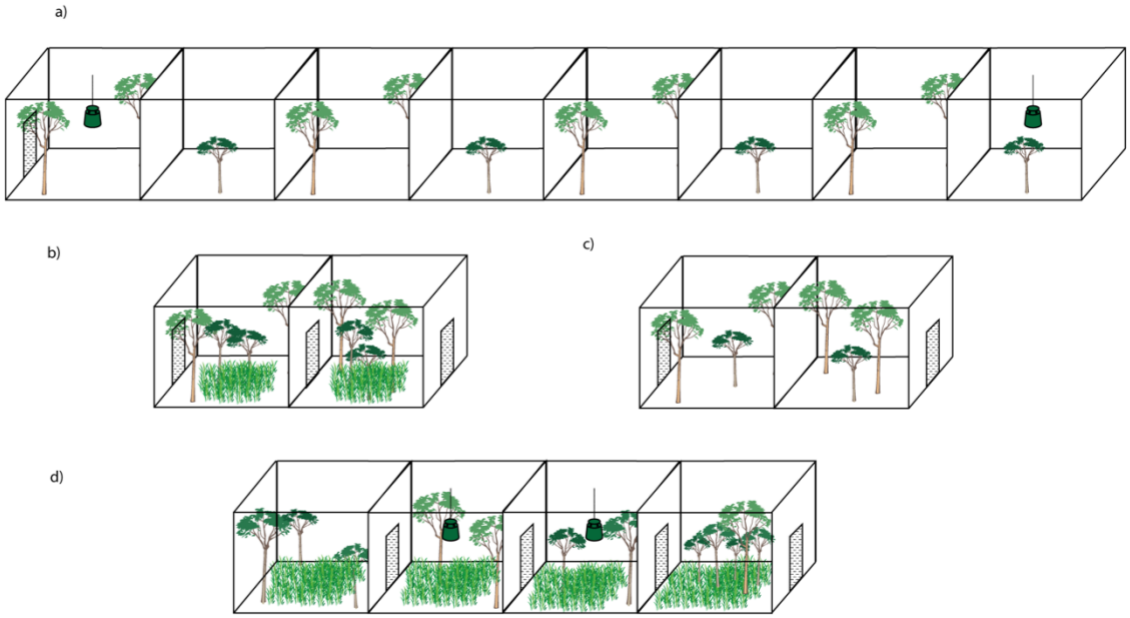


Fig. S1: Schematic of regent honeyeater song tutoring aviaries for the different song tutoring protocols. The first, aviary type 1 (a) at Taronga Zoo, (6m x 3.3m x 3m), the second two (b & c) at Taronga Western Plains Zoo, aviary type 2 (4m x 4m x 6m) and type 3 (d) (6m x 4m x 6m). Aviaries were typical of those used as crèche aviaries during normal breeding and post-breeding procedures. A) shows playback only tutoring aviary configuration, where juvenile pupil groups were placed in outer aviaries of an eight-aviary block with broadcast speakers. Breeding pairs housed in the aviaries between. Aviaries were separated by solid dividers preventing visual contact between aviaries. B) shows the configuration of the live tutor only cohort (large) where adult tutor was initially housed in the left aviary while juvenile pupils were housed in the right aviary but could both see and hear the tutor. The tutor was subsequently introduced to the juveniles at the conclusion of the breeding period by opening the adjoining door. C) shows the configuration of the live tutor only (small) cohorts where both the adult tutor and juvenile pupils were housed together in one aviary. Two cohorts were housed side by side with a solid divider between preventing visual contact. D) shows the live tutor + playback configuration where the adult tutor was initially housed in the left aviary while juvenile pupils were housed in the right aviary, but could both see and hear the tutor as well as exposed to broadcast playback. The tutor was subsequently introduced to the juveniles at the conclusion of the breeding period by opening the adjoining door.

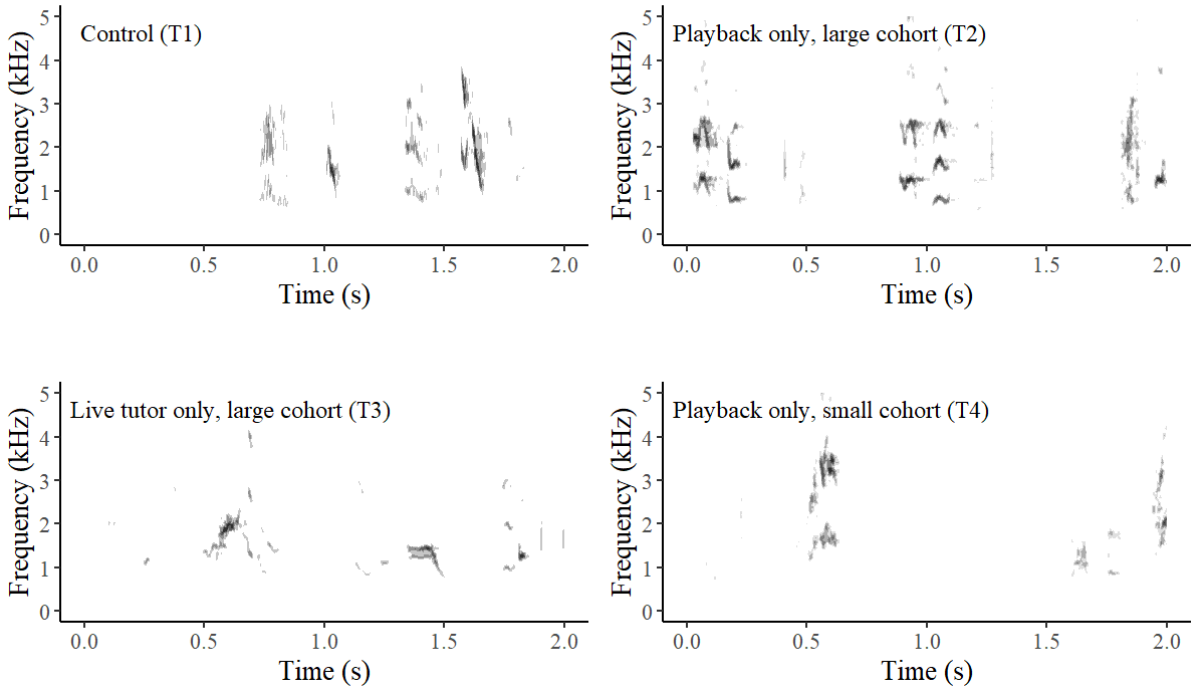


Fig. S2: Indicative spectrograms of the songs of juvenile regent honeyeaters included in song tutoring treatment groups 1 – 4.

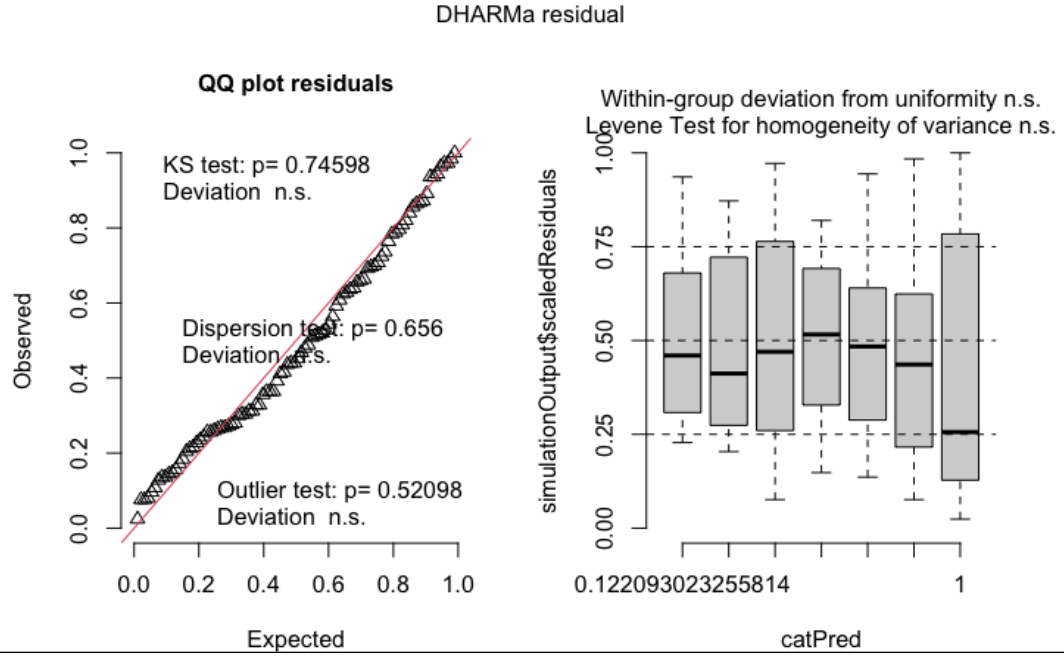


Fig. S3. Simulated Residuals of Generalised Linear Regression (Mahalanobis Distance ~ Treatment)

Chapter 5 Supplementary Material

Title: Frequency-dependence may moderate fitness costs linked to reduced bird song complexity

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Supplementary Material

Table S1: Results of generalised linear mixed-effects models showing the relationship between male regent honeyeater song type and the probability of being paired to a partner female and of paired males to successfully initiate a nesting attempt. **β** is the coefficient which describes the change in probability relative to males singing the Typical Blue Mountains song. Cultural norm effects are relative to males singing the regional cultural norm (i.e. Typical Blue Mountains in the Blue Mountains, Northern Tablelands in the Northern Tablelands.

Response	Test	Study Period	Reference Group (n)	Level	n	β	se	*Z*	*p*
Paired	Song type	2015-2019	Typical Blue Mountains (88)	Clipped Blue Mountains	20	-23.13	3.36	-5.92	**<0.001**
				Northern Tablelands	23	-23.71	3.43	-6.91	**<0.001**
				Interspecific	18	-23.312	3.46	-6.74	**<0.001**
				Unknown	63	-1.21	2.41	-0.50	**0.016**
		2020-2022	Typical Blue Mountains (2)	Clipped Blue Mountains	44	-1.66	2.79	-0.01	0.99
				Northern Tablelands	2	-3.785	3.96	0	0.99
				Interspecific	10	-1.80	2.78	-0.01	0.99
				Unknown	17	-1.67	2.78	-0.01	0.99
	Cultural Norm	2015-2019	Cultural norm (105)	Non-cultural norm	44	-22.65	2.58	-8.77	**<0.001**
				Unknown	63	-0.37	1.92	-0.20	0.85
		2020-2022	Cultural norm (from 2015-19) (3)	Non-cultural norm	55	-15.85	1385.38	-0.01	0.99
				Unknown	17	-15.96	1385.38	-0.01	0.99
Nested	Song type	2015-2019	Typical Blue Mountains (77)	Clipped Blue Mountains	8	-20.60	4.97	-4.15	**<0.001**
				Northern Tablelands	6	-2.23	5.02	-0.45	0.66
				Interspecific	5	2.08	28.59	0.07	0.94
				Unknown	46	-0.19	3.39	-0.56	0.58
		2020-2022	Typical Blue Mountains (2)	Clipped Blue Mountains	32	18.21	2797.44	0.01	0.99
				Northern Tablelands	2	35.13	3956.18	0.01	0.99
				Interspecific	4	17.57	2797.44	0.01	0.99
				Unknown	12	18.13	2797.44	0.006	0.99
	Cultural Norm	2015-2019	Cultural Norm (83)	Non-cultural norm	13	-20.23	3.68	-5.50	**<0.01**
				Unknown	46	-0.12	2.45	-0.05	0.96
		2020-2022	Cultural Norm (from 2015-19) (3)	Non-cultural norm	37	1.31	0.344	1.78	0.07
				Unknown	12	1.25	1.38	0.91	0.36

Additional relevant work undertaken during PhD enrolment.

Publications:

Heinsohn, R., Zdenek, C.N., **Appleby, D.**, and Endler, J.A. (2023) *Individual preferences for sound tool design in a parrot.* Proceedings of the Royal Society of London Series B, Biological Sciences, 290 (2006). 20231271.

Heinsohn, R., **Appleby, D.**, Wilson, D., & Legge, S. (2024). Large, overlapping home ranges reveal male mating strategies in polygynandrous *Eclectus* parrots. *Emu - Austral Ornithology*, 1–7. https://doi.org/10.1080/01584197.2024.2368006

Crates, R.A., **Appleby, D.L.**, Bray, W., Langmore, N.E., Heinsohn, R. (2024). Conserving avian vocal culture, *Philosophical Transactions (in review)*

Nature Research Highlight: This parrot taps out beats — and it custom-builds its instruments *Nature* 621, 447 (2023) *doi: https://doi.org/10.1038/d41586-023-02829-6*

Interviews & Media:

BBC Wildlife Magazine: *The bird that lost its song*: https://www.pressreader.com/uk/bbc-wildlife-magazine/20210701/281599538467178

BBC Earth Podcast:

https://podcasts.apple.com/au/podcast/whose-story/id1444357023?i=1000584585026

Wilderness Society: *Wilderness Journal #022; Songs of Hope.*

https://www.wilderness.org.au/journal/issue-022

The Times: *Singing lessons give threatened birds a chance of survival*.

https://www.thetimes.com/world/article/singing-lessons-give-threatened-birds-a-chance-of-survival-xm7pvmdwp

The Mirror : *Half of world's glaciers 'will be gone by 2100' - but singing lessons for birds could help*

https://www.mirror.co.uk/news/world-news/half-worlds-glaciers-will-gone-28903387

Newcastle Herald: *It's a race against time - and industry - to save the regent honeyeaters of the Tomalpin Woodlands in the Lower Hunter.*

https://www.newcastleherald.com.au/story/7730793/saving-the-regent-honeyeaters-of-the-tomalpin-woodlands/

CBOC Newsletter: October 2022. *Singing for survival*.

Presentations:

Australasian Ornithological Conference, Brisbane, November 2023: *Lost in translocation: the impacts and restoration of lost song culture in an Critically Endangered song bird (Regent honeyeater Anthochaera phrygia)*

ZAA Bird TAG workshop: *Regent Honeyeater Song Tutoring Program*

Regent Honeyeater Panel Discussion: Blue Mountains Cultural Centre. *'Lost Song' – Expert Panel Discussion*