

1 Video monitoring reveals novel threat to Critically Endangered captive bred and released  
2 Regent Honeyeaters

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13 **1. Abstract**

14 Nest predation is a primary cause of nest failure in open cup nesting woodland birds and low  
15 reproductive success is a common reason that reintroduced species fail to establish in the wild. We  
16 used video monitoring to record the breeding outcomes and identify the causes of nest failure in a  
17 reintroduced population of the Critically Endangered Regent Honeyeater. We intensively monitored  
18 28 nesting attempts of 13 pairs during the 2015 breeding season, and found that the probability of  
19 individual nest success was 0.21 (from egg laying to fledging). We report for the first time Sugar and  
20 Squirrel Gliders depredating Regent Honeyeater nests. In addition to losses attributed to predation,  
21 a high proportion of chicks died in the nest from unknown causes. Our results show that rates of  
22 nest initiation and success are low in reintroduced Regent Honeyeaters, and future reintroductions

23 should attempt to mitigate the threat of nest predation. Other sources of nest failure and barriers  
24 to nest initiation and egg laying are priority areas for future research.

25 Key words: *Anthochaera phrygia*; predation; threatened species; breeding success; nest survival

## 26 **2. Introduction**

27 Reproduction is a key vital rate determining the demographics of populations. There are numerous  
28 external influences that can reduce nest success in birds (here defined as the proportion of nests  
29 that fledge at least one young), including extreme weather (Jovani & Tella 2016), limited resources  
30 (Sherley et al. 2014), competition (Frei *et al.* 2015), brood parasitism (Wei et al. 2015), parasites  
31 (Scott-Baumann and Morgan 2015) and anthropogenic habitat disturbance (Ibáñez-Álamo et al.  
32 2015). However, perhaps the most important driver of nest failure is predation (Ricklefs,1969;  
33 Major et al. 2014). Predation risk to eggs, nestlings and attending adults, impact a variety of  
34 behaviours (e.g. nest construction and position)(Lee & Lima, 2016) and influences the evolution of  
35 life history traits (Martin, 1995) to ultimately shape population dynamics and densities (Lahti, 2001).  
36 When predation is the main driver of decline for a threatened species, management actions are  
37 often focused on reducing predation risks. For example, predator removal (Armstrong *et al.* 2002)  
38 and predator exclusion methods (Major et al. 2014) have been employed to reduce predation and  
39 therefore increase adult survival and reproductive output. Seeking to maximise reproductive  
40 success is particularly important when attempting to establish or reinforce a population through the  
41 release of breeding adults. Predation of nests is known to be a major limiting factor for  
42 establishment success in reintroduced populations (Moseby et al. 2015; Ashbrook et al. 2015) and  
43 this risk may be further elevated when releasing captive bred individuals due to their naivety to  
44 predation pressures in the wild (Moseby *et al.* 2015).

45

46 Here, we report findings from nest monitoring of captive bred and released Regent Honeyeaters  
47 (*Anthochaera phrygia*) during an initial post-release breeding event in 2015 at Chiltern-Mt Pilot  
48 National Park in northeast Victoria. This species has been subject to intensive recovery actions over  
49 the last two decades in response to a precipitous decline in population size. Although once  
50 widespread across the woodland belt of south eastern Australia, Regent Honeyeaters have lost  
51 >85% of their primary habitat through land clearing (Mac Nally *et al.* 2000), and sightings are now  
52 largely restricted to regions in New South Wales (Bundarra-Barraba, the Hunter Valley and the  
53 Capertee Valley) and north-east Victoria (centred around the Chiltern-Mt Pilot National Park). The  
54 total wild population was estimated to number 350-400 individuals in 2010 (Garnett *et al.* 2010),  
55 with further subsequent declines likely. Previous releases at Chiltern-Mt Pilot in 2008, 2010 and  
56 2013, indicated that nest success was low (D. Ingwersen unpubl. data). As the drivers of this low  
57 reproductive success have remained largely unknown, here we sought to explicitly identify the  
58 factors limiting nest success.

### 59 **3. Methods**

#### 60 ***Study species and site***

61 The Regent Honeyeater is a Critically Endangered, nectarivorous passerine endemic to south  
62 eastern Australia. The breeding season typically occurs from August to January (the Austral spring  
63 and summer). Regent Honeyeaters are open cup nesters, with the nest built solely by the female.  
64 Regent Honeyeaters lay 2-3 eggs per clutch and have an incubation period of 14 days and a nestling  
65 period of ~16 days (Oliver *et al.*, 1998). Both parents feed the chicks. Although nests are often  
66 spatially aggregated, Regent Honeyeaters do not appear to be synchronised breeders (Oliver *et al.*  
67 1998). Regent Honeyeaters are often associated with riparian habitat during the breeding season  
68 (Geering and French 1998; Crates *et al.* 2017).

69 Chiltern-Mt Pilot National Park (36°7'59.00"S 146°36'4.00"E), was the chosen release site for all  
70 releases (2008, 2010, 2013, 2015 and 2017) because wild Regent Honeyeaters have historically used  
71 this area for breeding, and wild individuals are occasionally still observed here. It covers 21,600 ha  
72 of primarily regrowth open box-ironbark forest of which roughly 4,600 ha in the northern section  
73 comprises the core habitat, predominantly Mugga Ironbark (*Eucalyptus sideroxylon*), for Regent  
74 Honeyeaters. The northern section of the park also supports Red Stringybark (*E. macrorhyncha*) and  
75 box eucalypts (*E. albens*, *E. macrocarpa* and *E. polyanthemos*). It is located on the traditional lands  
76 of the Dhudhuroa-Waywurru and Pangerang people (Blake and Reid 2002). Seventy-seven Regent  
77 Honeyeaters (36 female and 41 male) of mixed ages (39 were < 1 yr, 31 were between 1 and 2 yrs  
78 and 7 were between 2 and 3 yrs) were selected for release from birds bred at Taronga Zoo and  
79 affiliate zoos. None of the birds had prior breeding experience in captivity.

#### 80 ***Nest location and monitoring***

81 All released birds were fitted with unique combinations of colour bands. Forty-two of these birds  
82 (19 female, 23 male) were also fitted with Holohil systems BD-2 radio transmitters using a backpack  
83 style harness incorporating a weak point designed to break when exposed to resistance or wear.  
84 The transmitters weighed no more than 5% of the bird's body weight. The release occurred in April  
85 2015, three months prior to any anticipated breeding events, and timed to coincide with the  
86 commencement of seasonal flowering of key eucalypt food plants species. The average battery life  
87 of functioning radio transmitters was 10-12 weeks, so transmitters were redeployed at intervals  
88 during the release such that at any point in time a selection of birds could be tracked. Over the  
89 course of the release 59 of the released birds wore a functioning transmitter at least once, with  
90 eight of those refitted with transmitters two or three times.

91 Established pairs were identified on the basis of intense calling and territory defence by the male,  
92 both birds displaying courtship positions (lowered straightened body with wings slightly opened),

93 and subsequent close association when foraging, inspecting potential nest sites, and nest building.  
94 Once pairs were identified they were monitored daily and their nest attempts followed. Nest  
95 building was confirmed when the birds regularly took material to the same place and a clear base of  
96 a nest was seen (they often took one or two sticks to a site before ceasing activities at that site). A  
97 complete nest was defined as a nest where adult attendance at that nest continued beyond the  
98 nest building stage. All nests were discovered during the early nest building stage providing  
99 confidence that, amongst monitored pairs, few if any nests were overlooked. For each nesting  
100 attempt we recorded the pair ID, the tree species in which the nest was built, height of nest and  
101 nest tree height, distance to water, degree of visual concealment, clutch size and nest outcome  
102 (Table 1). The degree of visual concealment was assessed by one observer; from each cardinal  
103 direction at a distance of 2m from the base of the nest tree, acknowledging that nest height may  
104 impact the accuracy of our concealment estimate. We estimated the percentage, to the nearest 5%,  
105 of the nest that was concealed by foliage with the mean of these four values providing a relative  
106 measure of nest concealment.

107 *\*Table 1 near here*

108 Modified video surveillance cameras (Network 4 Channel AHD DVR Kit with 4 x 720p Cameras) with  
109 DVR monitors were used to monitor ten nests (eight that received eggs and two that didn't). Each  
110 camera was connected to an 18m cable and fixed to a 6m extendable pole. The batteries and DVR  
111 were housed in a 780 x 380 x 380mm cargo box at the base of the tree, minimising the climbing  
112 required and therefore disturbance. Cameras were only deployed on completed nests and then only  
113 if they were in a position that allowed easy and safe access to the tree with minimal disturbance to  
114 the breeding pair. Cameras were always positioned 3-4 metres from the nest, which still enabled  
115 quality footage to identify predators. No vegetation or other potential forms of concealment were  
116 modified. After a camera had been installed, nests were observed from a distance of 10+ m once

117 per day. If it appeared the nest had been abandoned (e.g. absence of the parents or reduced  
118 visitation rates), we scrutinised the video footage to identify the time and cause of predation.  
119 Nestlings that were found dead in the nest were stored at  $\sim 4^{\circ}\text{C}$  and air-freighted to Taronga Zoo for  
120 post-mortem (n=3 chicks from two broods). In one instance, footage showed the adults removing  
121 dead chicks and this allowed us to recover those bodies.

## 122 ***Statistical analysis***

123 An initial basic model for constant daily survival rate (DSR) from laying to fledgling or failure of  
124 Regent Honeyeater nests (based on a 30 day nesting period) was estimated using the R-package  
125 'RMark' v2.2.2 (Laake et al. 2016), an R- interface for the nest survival model (Dinsmore et al. 2002)  
126 in the software program 'MARK' (Cooch and White, 2005). We then included concealment and  
127 height in a second and third model respectively to calculate if DSR varies with these covariates.  
128 Only those nests that reached the egg stage were included in analyses. As two pairs reached the egg  
129 stage twice, we first ran all models with the complete data set and then re-ran the models after  
130 excluding the second of each of these nests to assess the influence of repeated measures. Akaike's  
131 information criterion corrected for small sample sizes (AICc) was used for model selection (Shaffer,  
132 2004). Means  $\pm$  one standard deviation are presented throughout this paper.

## 133 **4. Results**

134 Twenty-eight nesting attempts by 13 pairs (26 individuals as all pairs remained unchanged through  
135 the study) were recorded during the 2015 breeding season (Table 1). There was a mean of  $2.2 \pm 1$   
136 nests per pair (range 1-4). Ten of these nest attempts, from seven different pairs, were  
137 subsequently filmed. Two nests that were filmed never received eggs, and two nests that reached  
138 the egg stage were not filmed. In total 10 nests reached at least the egg stage and were used in our  
139 DSR analysis.

140 ***Timing of breeding***

141 The first pairing was confirmed on 1<sup>st</sup> July 2015, 78 days after the birds were released. At this date  
142 78% of the released individuals (60 of 77 birds), and 86% of those known to be alive (60 of 69 birds)  
143 were being regularly sighted. Most pair bonds were confirmed during August (54%, 7/13). By the  
144 end of August almost half of all released birds (45%, 35/77) were no longer being detected in the  
145 area, most likely due to a combination of mortality, dispersal and transmitter loss. For example by  
146 31<sup>st</sup> August 2015 10 transmitters had been recovered in settings that indicated the focal bird had  
147 died (e.g. a mass of feathers and/or bones). Once a pair had secured a breeding territory, the male  
148 typically ceased to call and the pair became increasingly difficult to detect. We therefore assume  
149 breeding attempts from additional unmonitored pairs occurred. The first nest to reach the egg stage  
150 was recorded on 23<sup>rd</sup> August 2015, 131 days after birds were released.

151 ***Characteristics of nesting sites***

152 Nest building typically took 4-5 days, followed by a day with little activity before egg laying took  
153 place. The mean height of nests was  $7.6 \pm 4.8$  m (range: 0.7 m in a dead stump to 16 m in a Mugga  
154 Ironbark). A total of 13 different tree and shrub species were used as nest sites (Table 1). The most  
155 commonly used tree species for nesting were Mugga Ironbark (7 of 28 nests) and Red Stringybark (5  
156 of 28 nests). The mean distance of Regent Honeyeater nests from surface water was  $61 \pm 76$  m.

157 ***Causes of nest failure***

158 Of the 28 nest attempts monitored (both with and without video surveillance), 18 (64%) were  
159 abandoned before eggs were laid, four (14%) failed at the egg stage, four (14%) failed at the  
160 nestling stage, and two fledged young. For the 10 active nests (those that reached egg stage) the  
161 best supported DSR model was our null model, although a second model with concealment was also  
162 equally plausible ( $\Delta AIC < 2$ ; but less than half as well supported based on model weights; see  
163 Supplementary Information, Table 1 & 2). Given a lack of influence from our predictor variables we

164 calculated DSR from the null model as  $0.95 \pm 0.002$ , giving a nest survival probability over a 30 day  
165 nesting period of 0.21 ( $0.95^{30} = 0.21$ ).

166 Five of the pairs abandoned all nest attempts and were never observed to reach the egg stage,  
167 whereas eight pairs reached at least the egg stage before failure. Causes of failure or abandonment  
168 before an egg was laid could not be ascertained. Three categories of failure at the egg or nestling  
169 stage were identified; these were mammalian predation (3/10; Sugar Glider (*Petaurus breviceps*)  
170 and Squirrel Glider (*P. norfolcensis*)), avian predation (2/10<sup>1</sup>; Australian Magpie (*Cracticus tibicen*)  
171 and House Sparrow (*Passer domesticus*)) (Figures 1a-d) and unexplained nestling mortality (i.e.  
172 chicks found dead in the nest or ground: 4/10). (Note that one nest had one chick predated and one  
173 chick that escaped and fledged, hence nine nests were subject to predation and two nests that  
174 successfully fledged one or more young). The results of post mortems were inconclusive for chicks  
175 found dead in nests (Taronga Zoo, unpublished data).

176 Video monitoring captured important information that would likely have been otherwise missed  
177 and/or misinterpreted. Both females whose eggs were predated by nocturnal marsupials returned  
178 to the nest the following morning and continued to briefly adopt a sitting position within the empty  
179 nest that resembled incubation. Without the camera this behaviour would have inferred that nest  
180 failure occurred during subsequent daylight hours. A female Regent Honeyeater was also shown to  
181 defend her nest by continuing to incubate despite attempts by a Sugar Glider to get underneath  
182 her. The glider eventually abandoned its attempt, but the eggs were predated the following night by  
183 Squirrel Gliders.

184 *\*Figures 1 near here*



185       **5. Discussion**

186 This study is the first to provide detail on the breeding outcomes of captive bred and released  
187 Regent Honeyeaters. Furthermore, we report the first records of native Sugar and Squirrel Gliders  
188 as nest predators of this species. Nest survival was worryingly low at 0.21 and 64% of nest attempts  
189 never reached the egg stage. Video footage has provided important information on the impact of  
190 predation to the nest success of released bird as well as documenting adult behaviour that would  
191 have otherwise been misinterpreted.

192 Nest abandonment prior to egg laying is not unique to Regent Honeyeaters, yet remains poorly  
193 understood (Flegeltaub et al, 2017; Beckman & Martin 2016). This may reflect a mix of  
194 inexperienced breeders in the captive-released cohort and the greater intensity of tracking  
195 individual captive-released pairs (aided by transmitters) through an entire breeding season.  
196 Furthermore, we know from observations in captivity that breeding pairs will frequently initiate  
197 multiple nests before settling and completing one (Taronga Zoo pers. comm.). Given that we did not  
198 film nests during nest building we are unable to offer additional insight into the drivers of this  
199 abandonment. Further monitoring to establish the causes of failure during the nest building phase  
200 may have merit.

201 Predation by native species was the principal cause of nest failure where eggs or nestlings were  
202 present. This is consistent with many previous studies that identify nest predation as a substantial  
203 threat to open-cup nesting bird species (Beckmann & McDonald 2016; Stojanovic et al. 2014). A  
204 single nest failure was attributed to the activities of an introduced avian species when a House  
205 Sparrow was filmed destroying eggs. Sugar Gliders have previously been identified as a significant,  
206 novel threat to Swift Parrots (*Lathamus discolor*) in Tasmania, where the glider is an introduced  
207 species (Stojanovic *et al.* 2014). We are not aware of any observations where Sugar Gliders or  
208 Squirrel Gliders have previously been recorded depredating the eggs of bird species within their

209 native range. Stojanovic et al. (2014) also reported that Sugar Gliders killed and ate incubating  
210 female Swift Parrots. Whilst no predation of adult Regent Honeyeaters by gliders was recorded in  
211 our study, video footage does document two instances where gliders lunge at an incubating female  
212 Regent Honeyeater, indicative of a possible predation attempt.

213 We also recorded nestlings found dead in four nests with no obvious sign of predation or predator-  
214 caused nest abandonment. This is common in birds and can be attributed to various factors such as  
215 exposure to extreme weather, disease and parasites (Smith et al. 1998) and limited food availability  
216 (Jovani & Tella 2016). In order to investigate if nestling mortality could be due to extreme weather  
217 conditions we retrieved the daily temperatures for the week prior to chicks dying in the nest. The  
218 mean maximum temperature in the week prior to chick death for the first two nests was  $26.6^{\circ}\text{C} \pm 3.7^{\circ}\text{C}$ ,  
219 (max  $32.4^{\circ}\text{C}$ ), for the third nest it was  $27^{\circ}\text{C} \pm 2.9^{\circ}\text{C}$  (max  $32.4^{\circ}\text{C}$ ) and for the fourth nest it  
220 was  $32.1^{\circ}\text{C} \pm 3.3^{\circ}\text{C}$  (Max  $36.6^{\circ}\text{C}$ ) (BOM, 2017). None of these mean maximum temperatures were  
221 substantially higher than the means for their respective months (Oct  $26.9^{\circ}\text{C}$  and December  $31.7^{\circ}\text{C}$ ).  
222 In addition, whilst post mortems were inconclusive, no apparent sign of disease was detected  
223 (Taronga Zoo unpublished data). On this basis limited food availability was considered a more  
224 plausible driver of nestling mortality than either extreme temperatures or disease. Assessment of  
225 this food limitation hypothesis is the focus of on-going research.

226 Whilst we acknowledge the role that human disturbance may play in nest failure, we are confident  
227 that the presence of researchers and the placement of cameras was not a significant driver of nest  
228 abandonment or failure. Cameras were only placed near nests when nest building had been  
229 completed. The birds were of captive origin and habituated to the presence of humans since  
230 hatching, however we ensured that nest-building attempts were observed from a distance. In  
231 addition, there was no evidence from the video footage that visiting predators were aware of, or  
232 attracted specifically to, the cameras.

233 The seemingly poor nest success we have recorded in reintroduced Regent Honeyeaters is  
234 concerning. There are no known records of nest success rates prior to significant population  
235 declines, however previous studies on the breeding biology of wild Regent Honeyeaters have  
236 reported much higher rates than found in this study: 46% (Geering and French 1998) and 38.3%  
237 (Oliver *et al.* 1998). It may be that captive bred birds, with no prior breeding experience, are  
238 particularly naïve to nest building, nest defence, and feeding of young in wild settings. Surviving  
239 birds might therefore be expected to improve in future breeding attempts. However recent  
240 observations of wild breeding birds have also reported high rates of failure, seemingly due to  
241 predation (R. Crates pers. comm.). Taken together these observations suggest poor reproduction is  
242 a proximate limiting factor for the Regent Honeyeater population and not solely related to birds in  
243 our study being captive-bred and reproductively naïve.

244 Knowing that low rates of reproduction may be an important limiting factor in the recovery of  
245 Regent Honeyeaters means it can become the focus of management. Our study offers critical insight  
246 into the causes of nest failure and suggests targets for possible intervention. We have highlighted  
247 two separate areas of concern. Firstly we provide direct evidence for predation by mammals and  
248 birds. Secondly, we document nestling mortality that did not appear to be related to disease or  
249 temperature extremes and may be due to starvation. Developing interventions and testing their  
250 utility requires care and needs to consider the objectives of management (Canessa *et al.* 2016).  
251 Furthermore, appropriate interventions need to consider other affected groups and species. For  
252 example, control of predators through culling or translocation is unlikely to be acceptable because  
253 most identified predators were native species, and some such as the Squirrel Glider are considered  
254 regionally threatened. Alternatively, management actions may consider strategies such as predator  
255 surveys in the planned release area to assess predation risk (Chalfoun & Martin, 2009) or barriers at  
256 nests that prevent or reduce predator access (Homeberger *et al.*, 2017).

257 Either way, managers of the Critically Endangered Regent Honeyeater are now better informed  
258 regarding the causes of poor reproductive success and are thus better positioned to develop,  
259 deploy and monitor an appropriate management strategy.

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366 Figure1 . Selected video frames showing (a&b) a Sugar Glider (*P. breviceps*) climbing on the branch  
367 where a Regent Honeyeater is incubating (circled), before flushing her off the nest and consuming  
368 the egg. (c&d) showing an adult male Australian Magpie attacking two Regent Honeyeater nestlings.  
369 In the second frame a Regent Honeyeater can be seen defending the nestlings (circled).

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