Native fly parasites are the principal cause of nestling mortality in endangered Tasmanian pardalotes

A. B. Edworthy[1] (iD), N. E. Langmore[1] & R. Heinsohn[2]

1 Research School of Biology, Australian National University, Canberra, ACT, Australia
2 Fenner School of Environment and Society, Australian National University, Canberra, ACT, Australia

Abstract

Established host–parasite interactions at an evolutionary equilibrium are not predicted to result in host population decline. However, parasites may become a major threat to host species weakened by other factors such as habitat degradation or loss of genetic diversity in small populations. We investigate an unusually virulent Tasmanian ectoparasite, *Passeromyia longicornis*, in its long-term hosts, the endangered forty-spotted pardalote (*Pardalotus quadragintus*) and striated pardalote (*Pardalotus striatus*) in southeastern Tasmania, Australia. We conducted a parasite elimination experiment to determine the net effect of parasites on forty-spotted pardalote nestling mortality, and monitored nestling parasite load and mortality in forty-spotted and striated pardalote nestlings during two breeding seasons (Aug–Jan, 2013–2015). *Passeromyia longicornis* larvae killed 81% of all forty-spotted pardalotes nestlings. Across 2 years, forty-spotted pardalotes fledged fewer nestlings (18%) than sympatric striated pardalotes (26%), and this difference was generated by a combination of higher parasite load and virulence in forty-spotted pardalote nests. These results show that native ectoparasites can cause substantial and potentially unsustainable mortality in their hosts.

Introduction

Established host–parasite interactions at an evolutionary equilibrium are not predicted to result in host population decline (Anderson & May, 1982). However, parasites may become a major threat to host species weakened by other factors such as habitat degradation (Lafferty & Kuris, 1999; Kutz *et al.*, 2005; Hudson *et al.*, 2006) or loss of genetic diversity in small populations (Coltman *et al.*, 1999; Cassinello, Gomendio & Roldan, 2001; Whitehorn *et al.*, 2011). Environmental change can favor parasites or stress hosts disproportionately (Lafferty & Kuris, 1999; Hudson *et al.*, 2006). For example warming temperatures can accelerate parasite life cycles relative to those of their host, resulting in pulses in parasite abundance (Kutz *et al.*, 2005). In addition, hosts with declining population abundance, loss of genetic diversity or increased stress can have reduced immune response to parasites (Coltman *et al.*, 1999; Cassinello *et al.*, 2001; Whitehorn *et al.*, 2011).

In Tasmania, the endemic fly *Passeromyia longicornis* (Diptera: Muscidae) parasitizes two native pardalotes: the forty-spotted pardalote (*Pardalotus quadragintus*), an endangered habitat specialist, and the striated pardalote (*Pardalotus striatus*), a common generalist (BirdLife International 2016; Edworthy, 2016a) (Fig. 1). Forty-spotted pardalotes currently occupy an area <50 km² on islands and isolated mainland Tasmania

forests in the southeast, and Flinders Island in the northeast of the state (Woinarski & Rounsevell, 1983; BirdLife International 2016). They are diet and habitat specialists, largely dependent on 'manna', a sugary exudate of *Eucaylptus viminalis* trees (Case & Edworthy, 2016). Between 1997 and 2010 the population of forty-spotted pardalotes declined by 60% to 1000–1500 individuals (Bryant, 2010). Although causes of this decline are uncertain, *P. longicornis* larvae are highly prevalent ectoparasites in forty-spotted pardalote nests, are often lethal (Edworthy, 2016a), and thus are potentially a significant threat to their hosts. Although there is little information about effects of *Passeromyia* spp. or other ectoparasites in Australian nestling birds, sympatric striated pardalotes (*Pardalotus striatus*) allow us to explore whether parasite load (number of parasites per nestling) and virulence (mortality associated with parasites) may be unusually high in forty-spotted pardalotes.

Invasive fly parasites of nestling birds have become a threat to rare and endangered bird species, particularly in the Galapagos Islands, where an introduced botfly, *Philornis downsi*, has caused extirpation in several local finch populations (O'Connor *et al.*, 2010; Kleindorfer *et al.*, 2014a,b; Kleindorfer & Dudaniec, 2016). In natural systems, however, studies of the impact of ectoparasites on their avian hosts have thus far found marginal or no effects of environmental disruption, reduced genetic diversity, or insular populations on ectoparasite virulence (Møller
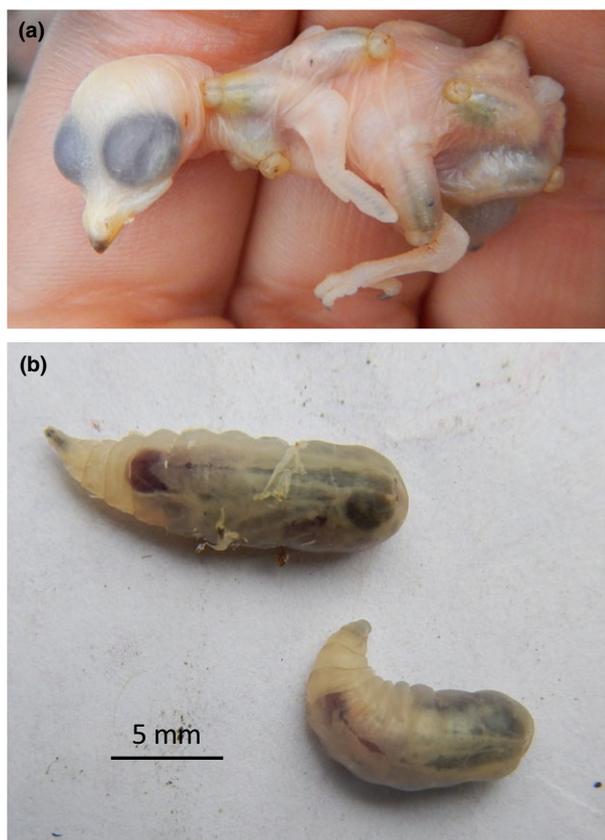
Figure 1 (a) Forty-spotted Pardalote nestling parasitized by *Passeromyia longicornis* larvae. (b) Third instar *P. longicornis* larvae recently emerged from their host. [Colour figure can be viewed at zslpublications.onlinelibrary.wiley.com.]

et al., 2009, 2013; Antoniazzi *et al.*, 2011). Here, we investigate whether a native parasite is a significant cause of mortality in its long-term host, the forty-spotted pardalote. Specifically, we (1) quantify nest success and sources of failure in forty-spotted and sympatric striated pardalotes, (2) use a parasite elimination experiment to determine the net effect of *P. longicornis* parasites on forty-spotted pardalote nestling survival and (3) compare *P. longicornis* load and associated mortality in forty-spotted and striated pardalote nests.

Materials and methods

Study species and sites

Both forty-spotted pardalotes and *P. longicornis* are endemic to Tasmania, likely the result of speciation generated by at least 12 000 years of geographical separation from mainland Australia (Rldpath & Moreau, 1966; Pont, 1974). The closest relative of *P. longicornis,* is *Passeromyia indecora*, and is endemic to mainland Australia; nucleotide divergence between these species is 5% (Nelson & Grzywacz, 2017), and they are considered distinct species (Hajibabaei *et al.*, 2007). Striated pardalotes are distributed throughout much of Tasmania and

continental Australia; however, the Tasmanian form is a morphologically distinct subspecies whose non-breeding range overlaps with mainland subspecies (Higgins & Peter, 2002).

Study sites were located on North Bruny Island, Tasmania, Australia, at Waterview Hill (43.10°S, 147.36°E) and Murrayfield Farm (43.12°S 147.38°E), comprising three patches of habitat: South Waterview (15 ha), Dennes (12 ha), and Murrayfield patch #16 (5 ha; see Brown, 1986 for patch numbering scheme). All sites were within 3 km of each other, and were a patchwork of dry woodlands containing *Eucalyptus viminalis* and grazed pasture. South Waterview and Dennes sites were dominated by *E. viminalis*, but also contained *Eucalyptus globulus*, *Eucalyptus pulchella* and *Eucalyptus obliqua*. Murrayfield patch #16 had similar species composition, but no single dominant species. In 2008, the Tasmania Department of Primary Industries, Parks, Wildlife and Environment installed 100 boxes intended for use by forty-spotted pardalotes, including 50 boxes at South Waterview and 50 boxes at Dennes (32 of which were within suitable habitat). In 2013, we installed 20 additional boxes at Murrayfield patch #16. Boxes were spaced 15–50 m apart to achieve a density of ~3.4 per ha across all sites. They were constructed of plywood with dimensions of 12 cm x 12 cm x 30 cm, oriented horizontally, with a 28–30 mm entrance diameter (La Trobe University, Melbourne, VIC, Australia).

Forty-spotted and striated pardalotes were common at the study sites. Forty-spotted pardalotes lay 3–5 eggs (typically 4–5), striated pardalotes lay 2–4 eggs (typically 3–4) and both produce one to two broods per year (A. Edworthy, unpublished data). Both species nest in natural cavities and nest boxes, but we were unable to access nestlings in natural cavities and this study relied on birds nesting in nest boxes. However, timing and frequency of nest failure does not differ significantly between nest boxes and natural hollows (Edworthy, 2016c). Clutch size of forty-spotted pardalotes was somewhat higher in nest boxes (4.3 ± 0.13 eggs) than in natural cavities (3.68 ± 0.18) (Edworthy, 2016c).

Passeromyia longicornis was present in 87% of forty-spotted pardalotes nests and 88% of striated pardalote nests at the study sites (Edworthy, 2016a). *Passeromyia longicornis* larvae are obligate parasites of nestling birds, often causing mortality in their hosts (Pont, 1974; Edworthy, 2016a). First instar larvae burrow under the skin of nestlings where they remain for 4–7 days feeding on blood and dead tissue until emerging from the host as 3rd instar larvae that develop a cocoon in the nest material (Edworthy, 2016a).

Nest monitoring

We monitored reproductive success of forty-spotted and striated pardalotes and their ectoparasite loads during two breeding seasons, Aug–Jan, 2013–2015. We located nests by checking all nest boxes at least every 4 days throughout Aug–Oct, and watching for re-nesting activity or the presence of new pairs at boxes Nov–Dec. Nests were accessed using ladders to determine timing of breeding, number of eggs laid (clutch size), number of nestlings hatched and number of nestlings fledged. At each check, nestlings were

removed from the nest and examined for parasitic fly larvae, which were counted. To identify individual nestlings, we marked one or both legs with permanent marker (Sharpie, Oak Brook, IL, USA) until they were banded at ~20 days of age. Nestlings were aged by mass and size at the first check after hatching. We developed growth curves for forty-spotted pardalotes based on 30 nestlings which we found on hatch day. Nests were monitored until nestlings fledged or died.

Parasite elimination experiment in forty-spotted pardalote nests

During the 2014/15 (hereafter, '2014') field season, we conducted a parasite elimination experiment using an eco-friendly insecticide (Avian Insect Liquidator; Vetapharm, Wagga Wagga, New South Wales, Australia) to fumigate nests. The active ingredients of Avian Insect Liquidator (hereafter, 'insecticide') are permethrin, piperonyl butoxide and methoprene. We sprayed nest material with insecticide within the week prior to hatching. The treatment lasts for 6 weeks, and was only reapplied if parasites appeared on the nestlings, in which case they were individually dabbed with insecticide, eliminating almost all remaining parasites. Control nests were untreated but were visited with the same frequency as treated nests. Treatment and control nests were assigned randomly in pairs (blocked randomization).

This design included 30 nest attempts in boxes, by 25 forty-spotted pardalote pairs, five of which re-nested in the same box after an initial failure. We kept these second attempts in the control group, and included both nest attempts in the study. Three nests were found within a day of hatching and were included in the study as additional controls. Most nests were at the Dennes and Waterview sites, with an additional two nests at Murrayfield patch #16. Although forest composition was slightly different at these sites, we did not include site in any models given the small sample size at Murrayfield ($n = 2$). To assess the effects of insecticide treatment on parasite load (maximum number of parasites observed on a nestling), we constructed a GLMM with a Poisson error distribution, parasite load (a count variable) as the response variable, insecticide treatment (fumigated versus untreated) as a fixed effect, and pair ID as a random effect to account for inclusion of second nesting attempts by five pairs in the control group. There was no evidence of overdispersion in this model.

To determine the effect of parasite reduction on fledging success, we constructed a GLMM with a binomial error distribution. Because insecticide treatment was applied at the nest level, counts of number of nestlings fledged vs. died at the nest level was used as the response variable. Treatment (fumigated vs. untreated) was a fixed effect, and pair ID was a random effect to account for inclusion of second nesting attempts. We also tested for effects of nest attempt number and initial brood size, and found none ($z = 0.21$, $P = 0.834$ and $z = 0.99$, $P = 0.323$, respectively), so we dropped these variables from the final model to avoid overfitting.

Relationship of parasite load and probability of fledging across host species

We used data from the 2013 and 2014 breeding seasons to examine the relationship between *P. longicornis* load and nestling mortality in nests of both forty-spotted and striated pardalotes. There were 32 nests by 20 forty-spotted pairs and 44 nests by 24 striated pairs, all of which nested in boxes at the Dennes (23 nests) and Waterview (51 nests) and Murrayfield (2 nests) sites. Fumigated nests were excluded from this analysis because the goal was to compare parasite effects between forty-spotted and striated pardalotes, and we did not fumigate striated pardalote nests. To determine the effect of parasite load on probability of fledging, we constructed a GLMM with a binomial distribution. In this analysis, we tracked the fates of individual nestlings, and whether a nestling fledged or died was the binary response variable. Parasite load (maximum number of parasites observed on a nestling), host species (forty-spotted vs. striated pardalote), the interaction of parasite load and host species were fixed effects. Nest ID was included as a random effect to account for sampling of multiple nestlings within broods. We also tested for an effect of brood size, but found none ($z = 0.04$, $P = 0.968$) and dropped this variable from the model to avoid over-fitting.

To estimate differences in parasite load between species and years, we constructed a GLMM with a Poisson distribution (no evidence of overdispersion), in which parasite load was the response variable and host species was a fixed effect. Nest ID and year were included as random effects to account for sampling multiple nestlings within broods, and seasonal variation in parasite variation, respectively.

All statistical analyses were conducted using the statistical program R (R Core Team 2015). We constructed linear mixed effects models (LMEs) and generalized linear models (GLMs) using the package 'nlme' (Pinheiro *et al.*, 2016), and constructed generalized linear mixed effects models (GLMMs) using the package 'lme4' (Bates *et al.*, 2015). We used Wald's test to assess the significance of dichotomous and continuous variables, and likelihood ratio tests to assess the significance of variables with more than two levels. All reports of uncertainty are ± SE unless indicated otherwise.

Results

Parasite elimination experiment in forty-spotted pardalote nests

We treated 11 nest attempts by 11 pairs with insecticide, and 19 nest attempts by 14 pairs (five pairs re-nested following failure) were untreated controls. Treatment with insecticide resulted in a substantial reduction of parasite intensity, with just 0.3 ± 0.2 SE parasites per nestling in treated nests, compared to 4.6 ± 1.5 parasites per nestling in untreated nests (difference = 4.3 parasites, $t = 5.02$, $P < 0.001$) (Fig. 2a, see Table S1). The few parasites that remained were killed within their first 4 days of life by direct application of insecticide.
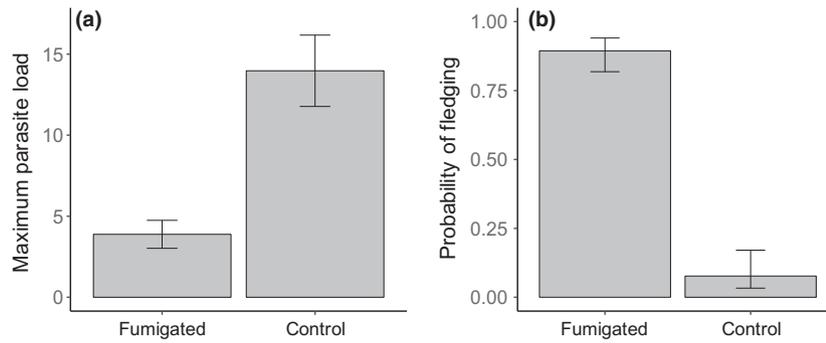
Figure 2 Results of a parasite-reduction experiment to assess the impacts of *Passeromyia longicornis* parasitism on forty-spotted pardalote nestlings in southeastern Tasmania, Australia, during the 2014 breeding season (Aug 2014 – Jan 2015). (a) Effect of insecticide treatment on nestling-level parasite load (maximum number of parasites observed on a nestling) (n treated = 42 nestlings in 11 broods, n control = 69 nestlings in 19 broods). (b) Effect of parasite elimination on probability of fledging (no. nestlings in brood that fledged vs. died). Values are back-transformed from GLMM estimates, and error bars show standard error.

Treatment and control nests had similar initial brood sizes (4.27 ± 0.47 and 3.73 ± 1.31 nestlings, respectively). However, percent of nestlings fledged was just 8% in control nests compared with 89% in treated nests ($z = -5.05$, $P < 0.001$) (Fig. 2b, see Table S2). From this we infer that 81% of nestling mortality in untreated nests can be explained by the presence of parasitic fly larvae. Eleven percent of nestlings died in fumigated nests, possibly from a combination of hypothermia, starvation and disease.

Relationship between parasite load and probability of fledging across host species and year

We monitored nestling survival to fledging and parasite load in 156 forty-spotted and 171 striated pardalote nestlings. Probability of fledging declined with increasing parasite load in both pardalote species ($z = -2.68$, $P = 0.007$). While the effects of species and the interaction of species and parasite load were both non-significant (see Table S3), forty-spotted

pardalotes had greater mortality in response to parasitism than striated pardalotes, especially at high parasite loads (Fig. 3a,b).

Parasite load was also higher in forty-spotted pardalote nestlings (14.8 ± 1.8 larvae; range = 0–28) than in striated pardalote nestlings (10.8 ± 1.1; range = 0–29) ($z = -2.01$, $P = 0.045$; see Table S4). Parasite load was higher in 2013 than 2014 (Fig. 4), but when tested as a fixed effect this difference was not statistically significant ($z = -0.505$, $P = 0.614$).

Discussion

Nestling mortality was 81% in forty-spotted pardalote nests parasitised by *P. longicornis*. This is a native host–parasite relationship, and such high virulence is considered unusual in established host–parasite systems. We show that ectoparasites can become a significant and likely unsustainable source of mortality in their long-term hosts. In addition, the presence of a relatively tolerant host (striated pardalote)
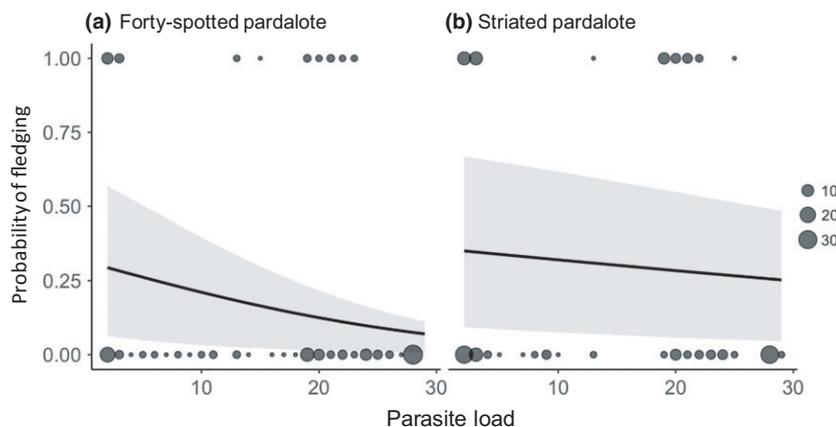

Figure 3 Effect of ectoparasite load on probability of fledging in untreated nests for (a) forty-spotted pardalotes and (b) striated pardalotes nesting in boxes at Bruny Island, Tasmania, during two breeding seasons (Aug–Jan, 2013–2015). Circle area represents sample size, and shaded regions are 1st and 3rd quartiles.
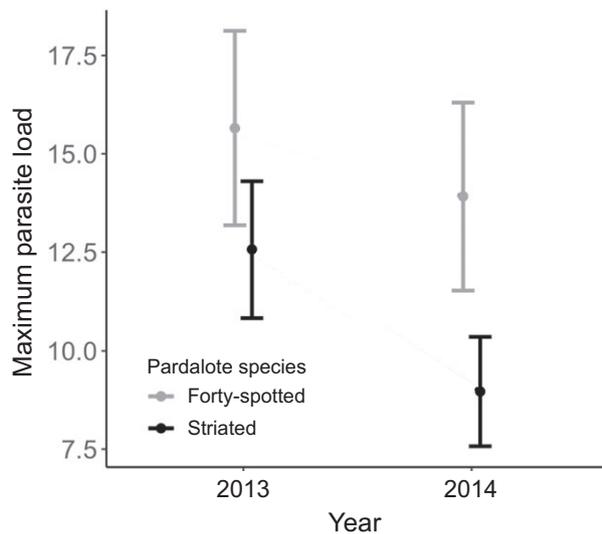
Figure 4 Comparison of parasite load on forty-spotted (grey) and striated pardalote (black) nestlings at Bruny Island, Tasmania, during two breeding seasons (Aug–Jan, 2013–2015). Error bars represent standard error.

potentially increases risk to forty-spotted pardalotes; if striated pardalotes maintain their densities while forty-spotteds decline, then the effect of density dependence is lost, and the continued transmission of parasites from striated to forty-spotted pardalotes may allow parasites to drive forty-spotted pardalotes to extinction (Tompkins *et al.*, 2000; Hudson *et al.*, 2006). Although all nests monitored in this study were in nest boxes, patterns of mortality were similar between these two nest types. Nest success (probability of fledging at least one nestling) did not differ between natural cavities and nest boxes, and mean nestling age at death was <7 days in both nest types, consistent with parasite-induced mortality (Edworthy, 2016*c*).

Nest failure is particularly costly for forty-spotted pardalotes given their heavy investment into individual nesting attempts. They lay clutches of 4–5 eggs and their incubation and nestling periods last up to 55 days in total. The fecundity data available for other cavity- and burrow-nesting Australian passerines suggest that nestling mortality was unusually low in both pardalote species. Treecreeper nest success (percent of nests that fledged at least one young) ranges from 48% in to 78% with a mean of 67% across four species (rufous treecreeper [*Climacteris rufus*], brown treecreeper [*Climacteris picumnus*], white-throated treecreeper [*Cormobates leucophaea*] and red-browed treecreeper [*Climacteris erythrops*]) (Noske, 1991; Luck, 2001; Doerr & Doerr, 2006). Nest success in Gouldian finches (*Erythrura gouldiae*), which are classified as endangered under the EPBC act, was 56% over three years (*n* = 157 nests; Tidemann *et al.*, 1999; Department of the Environment 2018). Forty-spotted and striated pardalotes had nest success of 35% and 38%, respectively, both substantially lower than estimates for other cavity-nesting species. Demographic modeling in combination with long-term population monitoring

throughout their island and mainland ranges is needed to determine whether *P. longicornis* is a main driver of population decline in forty-spotted pardalotes.

Passeromyia longicornis load was higher in forty-spotted pardalotes than striated pardalotes, and there was a non-significant effect of higher parasite-associated mortality in forty-spotted pardalotes, especially at high parasite loads. Together these effects likely account for the lower nest success in forty-spotted pardalotes. Physiological and immune differences between pardalote species may influence parasite load. Whereas, striated pardalotes are common generalists, Forty-spotted pardalotes have a history of range contraction and population decline, which can result in inbreeding, reduced immune diversity (*e.g.* MHC complex), and poor capacity to resist parasites (Radwan, Biedrzycka & Babik, 2010; Owen, Nelson & Clayton, 2010). In addition, capacity to mount an immune response is closely tied to body condition (Møller *et al.*, 1998), and the forty-spotted pardalote's specialized diet may make it vulnerable to food shortages (Owens & Bennett, 2000; Higgins & Peter, 2002; Case & Edworthy, 2016). All remaining forty-spotted pardalote habitat is secondary growth, and much of their habitat is fragmented by agricultural or residential development (Fensham, 1989; Bradshaw, 2012). Grazing, altered fire regime and a recent decade of drought have changed the forest structure and composition, all of which may affect food supply and body condition of forty-spotted pardalotes (Bryant, 2010; Bureau of Meteorology 2015). Finally, warming temperatures can increase rates of reproduction in invertebrate parasites, whereas their vertebrate hosts are more constrained (Kutz *et al.*, 2005). The life cycle of *Passeromyia longicornis* is accelerated by warm temperatures (Edworthy, 2016*a*), and warm years in southeastern Tasmania may promote increased parasite abundance. Further research into environmental factors influencing parasite abundance, as well as factors related to declining host populations (*e.g.* inbreeding, poor body condition), is needed to determine whether high parasite-induced mortality is a cause or consequence of population decline.

Our results show that parasitism by *P. longicornis* is a major conservation concern for protection of forty-spotted pardalotes. *Passeromyia longicornis* kills most (81%) nestlings, and methods of fly control or management need to be considered. Causton, Cunningham & Tapia (2013) outline possible management strategies for controlling flies that parasitize birds. These include trapping adult flies with pheromones or other attractants to reduce their population size. This would require identifying species-specific attractants for *P. longicornis*, and regular trap maintenance, but may be feasible as forty-spotted pardalotes are confined to a small range area. Second, release pheromones can be used to disrupt mating among adult flies. This would require identification and synthesis of species-specific pheromones, but can be highly effective in causing population collapse in the target species. Third, classical or augmentative biological control using parasitoid wasps can reduce the impact of the target species. Because *P. longicornis* is a native species, augmentative control that supplements populations of native

parasitoid wasps is a better option than introduction of non-native biocontrol agents. However, no parasitoid wasps have yet been detected in *P. longicornis* larvae.

Chemical control through spraying nests with eco-friendly insecticides is among the most effective of control measures (Koop *et al.*, 2011; Causton *et al.*, 2013), and is likely the most immediately viable option for forty-spotted pardalotes. Application of Avian Insect Liquadator to nest material eliminated most parasites from nests and increased fledging success from 8 to 89%. In the presence of a nest-monitoring program, this is a highly effective method for boosting reproduction output. However, at a broader scale, manually fumigating large numbers of nests is challenging, and research into alternate methods of fumigating nests is needed. Knutie *et al.* (2014) developed a method of placing cotton balls treated with insecticide, which the birds then use to line their nests, effectively self-fumigating their nests and eliminating the need for managers to know the location and stage of nests. Forty-spotted pardalotes line their nests with soft materials including sheep wool, which makes them a promising candidate for this method. In addition, knowledge of fly abundance and distribution across forest types and within forest structure (e.g. vertical layers), may allow us to manage habitat for low fly abundance, or place nest boxes in locations with low fly density.

Overall, our results show that native ectoparasites can become a major conservation concern for their long-term avian hosts. Forty-spotted pardalotes are an endangered and declining species, and parasitism by *P. longicornis* larvae is the principle cause of nestling mortality. This is a critical threat, and ectoparasite management should be a conservation priority for the species.

Acknowledgements

Data accessibility

Data presented in this study will be archived in Dryad.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Effects of nest fumigation on *P. longicornis* load in forty-spotted pardalote nestlings. Parameter estimates of fixed effects for GLMM (Poisson distribution) predicting parasite load as a function of insecticide treatment (fumigated vs. untreated), with nest ID as a random effect (corresponds to results in Figure 2A).

Table S2. Effects of nest fumigation on forty-spotted pardalote nestling survival to fledging. Parameter estimates of fixed effects for GLMM (binomial distribution) predicting probability of nestling fledging as a function of insecticide treatment (fumigated vs. untreated), with pair ID as a random effect (corresponds to results in Figure 2B).

Table S3. Relationship of *P. longicornis* load with nestling mortality between forty-spotted (n = 156 nestlings from 39 broods) and striated pardalotes (171 nestlings from 51 broods).

Table S4. Difference in *P. longicornis* load between forty-spotted spotted (n = 156 nestlings from 39 broods) and striated pardalotes (171 nestlings from 51 broods).