Avian hosts, prevalence and larval life history of the ectoparasitic fly *Passeromyia longicornis* (Diptera : Muscidae) in south-eastern Tasmania

Amanda B. Edworthy

Research School of Biology, Australian National University, Building 116, Daley Road, Acton, ACT 2601, Australia.
Email: amanda.edworthy@anu.edu.au

Abstract. Blood-sucking fly larvae are widespread parasites of nestling birds, but in many systems we lack knowledge of their basic biology. This study reports the first observation of an endemic Tasmanian fly species, *Passeromyia longicornis* (Diptera : Muscidae), parasitising the forty-spotted pardalote (*Pardalotus quadragintus*), another Tasmanian endemic. Because the forty-spotted pardalote is an endangered and declining songbird, *P. longicornis* is a species of interest to conservation biologists. Its larval form is an obligate, subcutaneous parasite of nestling birds, but before this study, there were just two published records of the species infesting avian hosts, and little known about its ecology or life cycle. This study documented hosts, prevalence, and larval life history of *P. longicornis* by locating and monitoring nests and ectoparasites of the forest bird community in south-eastern Tasmania. I also reared *P. longicornis* larvae in captivity to determine the length of the pupal stage in relationship to ambient temperature. Hosts of *P. longicornis* included forty-spotted pardalotes (87% prevalence across nests), striated pardalotes (*Pardalotus striatus*) (88% prevalence), and New Holland honeyeaters (*Phylidonyris novaehollandiae*) (11% prevalence). Both pardalote species were new host records. *P. longicornis* larvae burrowed under the skin of nestlings where they developed for 4–7 days, feeding on nestling blood. When fully grown, larvae dropped into the surrounding nest material and formed pupae. Length of the pupal stage was 14–21 days, and declined with increasing ambient temperature. Median parasite abundance was 15 larvae in infested forty-spotted pardalote nests and 11 larvae in infested striated pardalote nests. Nestling mortality was frequently associated with ectoparasite presence. This study provides the first survey of *P. longicornis* hosts, prevalenve and life cycle, and shows that this species is likely a major player in the ecology of pardalotes, and possibly other forest bird species in Tasmania.

Additional keywords: ectoparasite, endangered species, hematophagous parasite, host–parasite relationship, myiasis, parasitic fly larvae, subcutaneous parasite, virulence.

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Introduction

Nestling birds are a vulnerable target for ectoparasitic fly larvae (Clayton *et al*. 2010). The nest environment provides a warm, humid microclimate that is ideal for many ectoparasites (Heeb *et al*. 2000), and nestling birds are captive hosts until they leave the nest (Loye and Carroll 1995). Within the widespread family Muscidae (houseflies), the genera *Passeromyia* and *Philornis* include species whose larvae parasitise nestling birds by sucking nestling blood externally, or by burrowing under skin or into the body cavity to feed on blood and tissues (Couri and Carvalho 2003). Their impacts on nestling birds include blood loss, infection, slowed development, deformities lasting into adulthood, and death (Whitworth and Bennett 1992; Dudaniec and Kleindorfer 2006; O'Brien and Dawson 2008; Galligan and Kleindorfer 2009). Despite their potentially strong impacts on nestling birds, we know very little about the ecology and life history of many dipteran bird-nest parasites.

In 2012, larvae of *Passeromyia longicornis* Macquart (Diptera : Muscidae) were found parasitising nestlings of endangered forty-spotted pardalotes (*Pardalotus quadragintus*) in Tasmania, Australia (A. Edworthy, pers. obs.; BirdLife International 2012). As a result, *Passeromyia longicornis* has become a species of interest to wildlife managers. *Passeromyia* is an Old World genus, and the only group of dipteran bird-nest parasites found in Australia. Within this genus, only *P. longicornis* is found in (and endemic to) Tasmania (Pont 1974; Couri and Carvalho 2003). *P. longicornis* was originally discovered and described in its adult form (Macquart 1851), which is a free-living fly. Its food sources as an adult are uncertain, but may include rotting fruit and resin (Pont 1974). In its larval form, *P. longicornis* is a subcutaneous parasite of nestling birds (Green and Munday 1971; Pont 1974). Despite its endemic status, the hosts and prevalence of *P. longicornis* among Tasmanian birds are unknown. Two previous records of *P. longicornis*

parasitising nestling birds are from nests of an introduced house sparrow (*Passer domesticus*) and a New Holland honeyeater (*Phylidonyris novaehollandiae*) (Green and Munday 1971; Green 1988). The Australian National Entomological Collection holds 25 adult specimens and pupal casings collected from Sidmouth, Tasmania, in the north of the state near Launceston (collected by R. Mahon). There are also several accounts of unidentified subcutaneous fly larvae parasitising nestling birds, including European goldfinches (*Carduelis carduelis*) in the Tasmanian Midlands at Antill Ponds (Green 1988), noisy miners (*Manorina melanocephala*) in Bellerive near Hobart (Sharland 1923), and brown thornbills (*Acanthiza pusilla*) in Trevallyn Nature Recreation Area near Launceston (C. Young, pers. comm.). On the basis of photographs, written descriptions, and the lack of other candidate species in Tasmania, all of these were likely *P. longicornis*. These records span much of the north–south range of eastern Tasmania, suggesting that *P. longicornis* may be widespread throughout the dry sclerophyll forests and woodlands in this region.

Green (1988) documented *P. longicornis* as a subcutaneous parasite of nestling New Holland honeyeaters, from a single observation, and was unable to determine the behaviour of larvae across all three instar stages (e.g. subcutaneous versus external feeding), their primary food source (e.g. tissues versus fluids), and their impacts on host nestlings. This study aims to provide a basic understanding of its role in the ecology of Tasmanian forest bird communities, as well as its larval and pupal life history. I focused on forty-spotted pardalotes as hosts, primarily because of their status as an endangered and declining species, but the study also included striated pardalotes (*Pardalotus striatus*) and 10 other forest bird species as potential hosts. My specific objectives were to (1) identify hosts of *P. longicornis* in south-eastern Tasmania, (2) quantify the prevalence of *P. longicornis* in identified hosts, (3) describe the behaviour and life-history timing of the larval and pupal stages of *P. longicornis*, and (4) report the effects of *P. longicornis* on their hosts.

Materials and methods

Study sites

I conducted fieldwork in south-eastern Tasmania at Maria Island (five study plots, 2–19 ha; 42.65°S, 148.05°E), Bruny Island (eight plots, 4–15 ha; 43.10°S, 147.36°E), and mainland Tasmania at Tinderbox Peninsula (four plots, 5–20 ha; 43.04°S, 147.32°E), during three breeding seasons (August to January, 2012–15). Bruny Island and Tinderbox Peninsula are separated by a 1.4-km channel, and Maria Island is 65 km north-east of Bruny Island, separated from mainland Tasmania by a 4-km channel. Sites were dry coastal forests and woodlands, which were either dominated by *Eucalyptus viminalis* or included a mix of *E. viminalis*, *Eucalyptus pulchella*, *Eucalyptus globulus*, *Eucalyptus obliqua*, *Eucalyptus ovata*, and *Eucalyptus amygdalina* trees. Maria Island sites were embedded in continuous native forest, Bruny Island sites were patches of forest surrounded by grazed pasture, and mainland sites were a mix of continuous forest and patches surrounded by residential or industrial development. All sites were previously logged, regenerating forest. At two Bruny Island patches there were 50

pardalote nest boxes placed two per tree, and spaced at intervals of 15–50 m. At one mainland Tasmania patch there were two nest boxes installed by local residents. Another 153 nest boxes were installed throughout the study regions, but few of these were occupied. See Edworthy (2016) for further description of study sites.

Identification of parasites and potential hosts

In November 2012, I collected three subcutaneous parasites from two forty-spotted pardalote nestlings. These fly larvae were reared to adulthood and identified by Guy Westmore (entomologist, Biosecurity Tasmania) using adult characteristics following the key in Pont (1974). After the initial discovery of *P. longicornis* infesting pardalotes during the 2012/13 breeding season, I monitored nests for the presence of parasites during the next two breeding seasons (August to January, 2013–15). Study sites were systematically searched for nests of forty-spotted and striated pardalotes by checking nest boxes and previously occupied tree hollows, and by following birds to their nest sites. Nests of all other forest bird species were located by flushing adults off their nest, or by observing nest-building or nestling-provisioning behaviours. Nests of forty-spotted and striated pardalotes were located in both nest boxes and natural hollows, and all nest sites of other forest bird species were natural (i.e. open cup, dome, or tree hollow). In addition to forty-spotted and striated pardalotes, nesting bird species detected at the study sites included New Holland honeyeater, brown thornbill, superb fairy-wren (*Malurus cyaneus*), Tasmanian scrubwren (*Sericornis humilis*), grey fantail (*Rhipidura albiscapa*), yellow-throated honeyeater (*Lichenostomus flavicollis*), dusky woodswallow (*Artamus cyanopterus*), grey shrike thrush (*Colluricincla harmonica*), green rosella (*Platycercus caledonicus*), and brush bronzewing (*Phaps elegans*).

Host nest monitoring

I accessed nests in tree hollows or nest boxes with climbing ropes or ladders. Open cup and dome nests were accessible from the ground. For nests in boxes or open cups, I removed nestlings at each check to inspect them for signs of parasites, which were clearly visible under the skin (Fig. 1*a*). For nests in front-opening nest boxes, I removed nestlings and felt inside the domed nest for the presence of free-living larvae in the nest material. Both pardalote species build domed nests at the back of their boxes with narrow 3–4-cm tunnel entrances, making it difficult to directly observe activity in the nest cup. In 2013 I installed new nest boxes that opened on top, enabling me to inspect the nest material for the presence of parasite larvae and pupae without destroying the nest; however, only three forty-spotted pardalotes and five striated pardalote pairs used these boxes. For nests in natural hollows, I was unable to remove nestlings, and these nests were inspecting using a video camera on a flexible stalk (Burrowscope, Faunatech Australia, Mount Taylor, Victoria, Australia).

I monitored nests every four days until nestlings failed or fledged. In Tasmania, the length of the nestling period was 26–32 days (median = 30 days) for forty-spotted pardalotes and 23–27 days (median = 25 days) for striated pardalotes. I assumed that nestlings fledged if they survived to within four
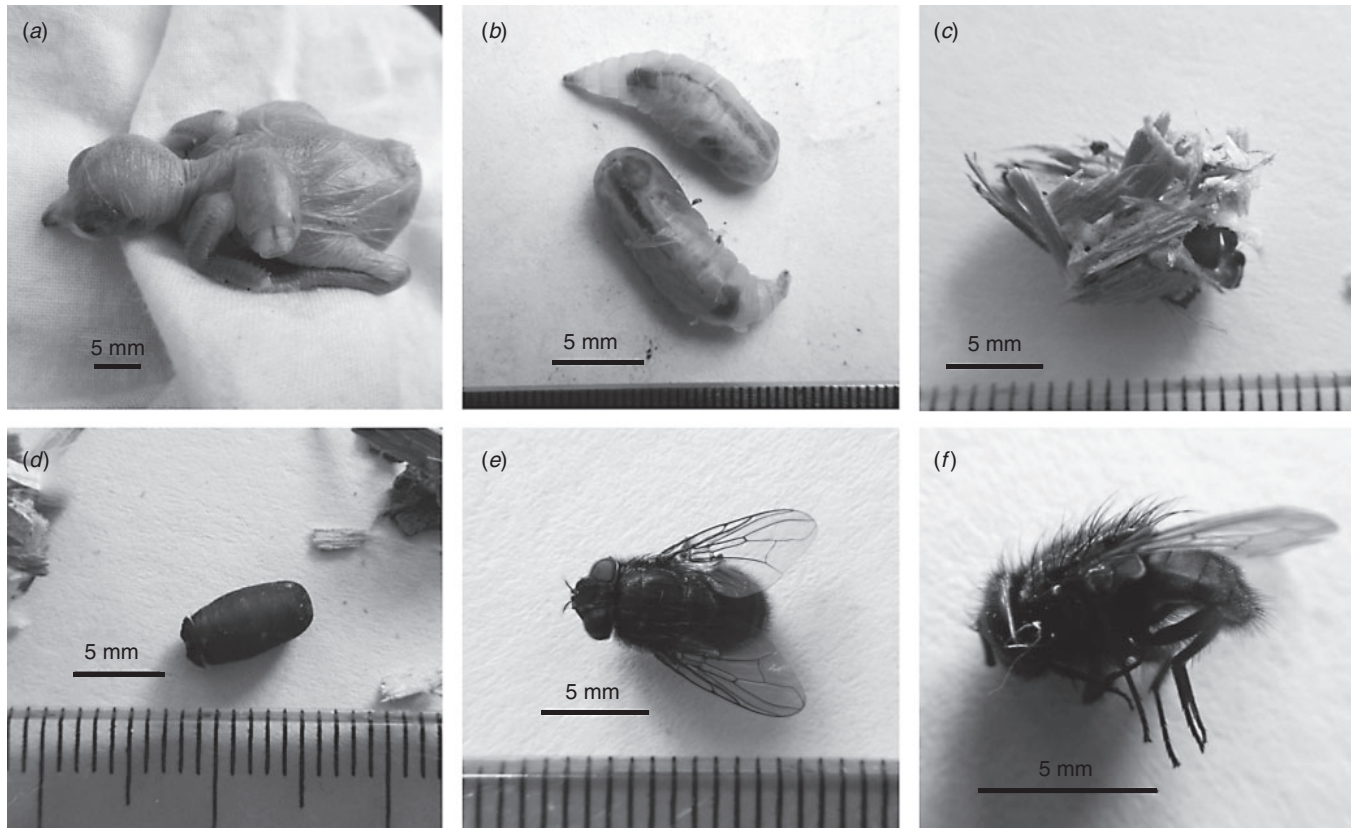
Fig. 1.   Developmental stages of *Passeromyia longicornis*. (*a*) A six-day-old forty-spotted pardalote nestling infested with a single larva, from a nest on north Bruny Island, Tasmania The larva spends 4–7 days in its nestling host. (*b*) Two third-instar larvae recently emerged from their host. These larvae drop into the nest material and form pupae within 3–4 days. (*c*) A foamy white cocoon anchors the pupa into surrounding nest material, which is wood shavings in this photograph. (*d*) An empty puparium separated from nest material. The pupal stage lasts 14–21 days, depending on temperature. (*e*, *f*) The adult form of *P. longicornis*, a free-living fly.

days of their minimum nestling period, and there was no evidence of mortality (e.g., dead nestlings in the nest or box, destroyed nest material, crushed eggs). Nestlings that died in the nest were often left in the nest cup or near the front of the nest box, where ants removed all tissue within a few days, leaving skeletons behind. Thus, I was usually able to distinguish between fledging and failure. However, my results may underestimate mortality if nestlings died and were removed from the nest by adult birds during the last four days of the nestling period.

Collection and rearing of fly larvae

In 2014 I collected 18 *P. longicornis* larvae from three forty-spotted pardalote nestlings (in two nests), and seven striated pardalote nestlings (in seven nests). Larvae were subcutaneous but were relatively easy to extract while they were in the process of emerging (a multihour process). When I found a larva emerging during routine nest checks, I pulled it out of its host, which allowed me to collect larvae while minimising my effect on the system. Collected larvae were reared in plastic containers with a bed of wood shavings, and kept at ambient temperature at Dennes Point, Bruny Island, within 10 km of the study sites. I checked captive fly larvae daily and recorded dates of their pupation and emergence as adult flies.

Statistical analysis

I used Pearson's Chi-square test to assess differences in parasite prevalence among forty-spotted pardalotes, striated pardalotes, and the grouping of all other forest bird species (pooled because of low sample sizes). This analysis excluded nests that were found later than two days after hatching, in order to eliminate nests for which parasites might been present but undetected early in the nestling period.

I used a simple linear regression to estimate the effect of temperature on the duration of the pupal stage. The temperature metric used was mean daily maximum temperature during the pupal stage. Temperature data were obtained from the Dennes Point weather station archives (Bureau of Meteorology 2015). Larvae collected from the same nest were possibly siblings, and to avoid pseudoreplication I grouped larvae collected from a single nest, and took the mean duration of the pupal stage within each group.

Finally, to examine the impact of *Passeromyia longicornis* larvae on their hosts, I report the abundance of parasites found in forty-spotted and striated pardalote nests as a box-and-whisker plot, and test for a difference in parasite abundance across these species using a generalised linear mixed-effects model (GLMM), with parasite abundance as the response variable, species as

a fixed effect, pair ID as a random effect, and a Poisson error distribution for count data (Bates *et al.* 2015). As an exploratory analysis of the potential for *Passeromyia longicornis* to cause mortality in pardalotes, I examined mortality in nests infested by these parasites. I calculated mortality as the number of nestlings that failed to fledge divided by the number of nestlings that originally hatched. None of these nests were depredated, but factors such as cold temperatures or limited food supply may have contributed to mortality in parasitised nests (A. Edworthy, pers. obs.). The sample size of nests where parasites were undetected was low ($n = 3$–6), and the absence of parasites was uncertain in cases where all nestlings died before the first nest check, so I was unable to assess mortality in unparasitised nests. Margins of error reported in the Results section are standard errors unless otherwise indicated. All analyses were done using the statistical software R 2.15.0 (R Development Core Team 2013).

Results

Hosts and prevalence of Passeromyia longicornis

Hosts of *Passeromyia longicornis* included forty-spotted pardalotes, striated pardalotes, and New Holland honeyeaters. Both forty-spotted and striated pardalotes were new host records for the fly parasite. I located a total of 11 nests of nine other forest bird species and there were no parasites in any of these nests; however, sample sizes were low ($n = 1$–3 nests per species) (Table 1). Prevalence of *P. longicornis* was 87% in forty-spotted pardalote nests ($n = 45$), 88% in striated pardalote nests ($n = 49$), and 11% in New Holland honeyeater nests ($n = 9$) (Table 1).There was no significant difference in parasite

prevalence between forty-spotted and striated pardalotes ($\chi^2 = 0.03$, $P = 0.87$). Both pardalote species had higher parasite prevalence than the grouping of other forest bird species; prevalence was 16.6 times higher in striated pardalotes and 16.4 times higher in forty-spotted pardalotes than in other forest birds ($\chi^2 = 43.37$, $P < 0.001$, and $\chi^2 = 40.23$, $P < 0.001$, respectively).

All striated pardalote nests were in nest boxes and 37 of 45 forty-spotted pardalote nests that allowed a clear view of nestlings were in nest boxes (Table 1). There were eight forty-spotted pardalote nests in natural hollows that were both accessible and allowed a clear view of nestlings. These nests were located at Tinderbox Peninsula and Maria Island. At Tinderbox Peninsula, three of four nests were parasitised by *P. longicornis* (subcutaneous larvae were observed by means of a video burrowscope), and at Maria Island two of four nests were parasitised (Table 1).

Life cycle of Passeromyia longicornis

Adult females of genus *Passeromyia* typically deposit their offspring as eggs rather than larvae, but this has not been confirmed in *P. longicornis* (Couri and Carvalho 2003). *Passeromyia* species have three larval instar stages, and their general morphology is described in Skidmore (1985). The youngest *P. longicornis* individuals that I detected were first-instar larvae (~1–2 mm long), which spread across the body of nestling birds, and burrowed under the skin of their hosts. These observations of first-instar larvae were often made on the day that host nestlings hatched, suggesting that the larvae burrowed into their hosts within one day (and likely within hours) of hatching or being deposited by an adult female. The fly larvae did not need

Table 1. Summary of nest sample sizes and parasite prevalence of *Passeromyia longicornis* across potential host species, sites, and nest types
Nests were located in south-eastern Tasmania, at three study areas (Maria Island, Bruny Island and Tinderbox Peninsula), in both nest boxes and natural nest sites (tree hollows, dome nests, open cup or stick platform nests). Prevalence (proportion of nest attempts with parasitised nestlings) is bolded for species totals across sites, and nest types

Species	Site	Nest type	No. of nest attempts monitored	No. of pairs	No. of nests with parasites	Prevalence
Forty-spotted pardalote	Bruny	Box	35	21	33	0.94
	Maria	Natural hollow	4	3	2	0.5
	Tinderbox	Box	2	2	1	0.5
	Tinderbox	Natural hollow	4	4	3	0.75
Total forty-spotted pardalote			45	30	39	**0.87**
Striated pardalote	Bruny	Box	49	39	43	**0.88**
New Holland honeyeater	Maria	Open cup	4	4	1	0.25
	Tinderbox	Open cup	5	5	0	0
Total New Holland honeyeater			9	9	1	**0.11**
Brown thornbill	Tinderbox	Open cup	1	1	0	0
Superb fairy-wren	Tinderbox	Dome	3	3	0	0
Tasmanian scrubwren	Maria	Dome	1	1	0	0
Grey fantail	Bruny	Open cup	1	1	0	0
Yellow throated honeyeater	Bruny	Open cup	1	1	0	0
Dusky woodswallow	Tinderbox	Open cup	1	1	0	0
Grey shrike thrush	Tinderbox	Natural hollow[A]	1	1	0	0
Green rosella	Bruny	Natural hollow[B]	1	1	0	0
Brush bronzewing	Tinderbox	Stick platform	1	1	0	0
Total forest birds excluding pardalotes			20	20	1	**0.05**
Grand total			114	89	83	**0.72**

[A]The grey shrike thrush built an open cup nest inside a large tree hollow.
[B]Green rosellas differ from the other hollow-nesting birds listed in that they do not add nest material (other than a bed of wood chips) to their hollows.

an existing lesion or opening, and were found in a variety of locations on their hosts, including the head, body, wings, and legs. Once embedded under the skin of their host, larvae did not move from their initial site of entry, where they developed through second and third instars, reaching a mean length of 13.6 ± 1.3 mm (maximum = 15 mm, $n = 18$) (Fig. 1*a*). Larvae kept their posterior segment level with the skin of their host, or slightly protruding (Fig. 1*a*). After 4–7 days larvae emerged from their hosts and burrowed into the surrounding nest material, where they pupated. The larvae that I collected as they were emerging from their hosts started to pupate within 3–6 days of collection ($n = 18$ larvae). As is characteristic of the genus, *P. longicornis* larvae produced a spongy white cocoon surrounding the puparium. The cocoon anchored the pupal case (Fig. 1*c*) to the nest material (Fig. 1*d*), and may have provided protection from adult birds or hyperparasites (though none were observed). Median duration of the pupal stage was 17 days, but was negatively related to the mean of daily temperature maxima during the pupal stage (Fig. 2). Length of the pupal stage decreased by 2.00 days (s.e. = 0.24) with each 1°C increase in average daily temperature maximum ($t = -8.291$, $P < 0.001$, adjusted $r^2 = 0.89$) (Fig. 2). After emerging, adults left the nest box as free-living flies (Fig. 1*e, f*). The food sources, habitat requirements, and overwintering strategies of adult *P. longicornis* flies are still unknown.

Host–parasite interactions

P. longicornis larvae were often found on nestlings within the first day after hatching, but new infestations also occurred as nestlings aged (parasite infections appeared in previously healthy nestlings). Abundance of these parasites in host nests ranged from 1 to 46 larvae (median = 15) in forty-spotted pardalote nests and 1 to 61 larvae (median = 10) in striated pardalote nests (Fig. 3),
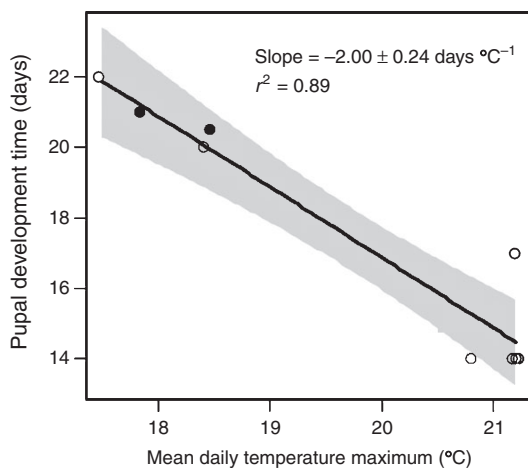
but host species was not a significant predictor of parasite abundance (likelihood ratio test: $\chi^2 = 2.08$, $P = 0.150$). All larvae observed in nests were subcutaneous parasites of nestling birds (Fig. 1*a*), unless the nestlings had died recently, in which case fly larvae were found feeding in the body cavity or nearby in the nest material. These included third-instar larvae feeding on nestlings that had died within the previous 24 h, and were likely *P. longicornis* larvae; however, I did not identify them, and they may have been a different species of fly. In living hosts, larvae stayed just under the skin, and did not appear to cause significant tissue damage. Blood was visible in the gut of third-instar larvae (Fig. 1*b*) and, thus, *P. longicornis* appeared to be mainly haematophagous. Emerging larvae left behind a wound in the nestling that scabbed over and healed after several days. Other effects of *P. longicornis* on nestling birds included blood loss, infection, swelling in the surrounding tissue, and death. In nests harbouring *P. longicornis* larvae, mortality was 85 ± 5% (s.e.) of forty-spotted pardalote nestlings ($n = 33$ nests), and 65 ± 6% of striated pardalote nestlings ($n = 43$ nests). There was no evidence of predation in any of these nests (e.g. ripped open nest boxes, destroyed nest material, crushed eggs); however, extended periods of cold weather or starvation may have contributed to mortality.

Discussion

These results show that *Passeromyia longicornis* is a common parasite of nestling forty-spotted and striated pardalotes in south-eastern Tasmania, and provide the first information about its hosts, prevalence, and larval life history. Three of 12 forest bird species found nesting at the study sites hosted *P. longicornis*, and in all hosts, the larval form of *P. longicornis* was a subcutaneous, blood-feeding parasite of nestling birds. Although *P. longicornis* is endemic to Tasmania, the only previous records of it parasitising nestling birds were in house sparrows and New Holland honeyeaters (Green and Munday 1971; Green 1988).
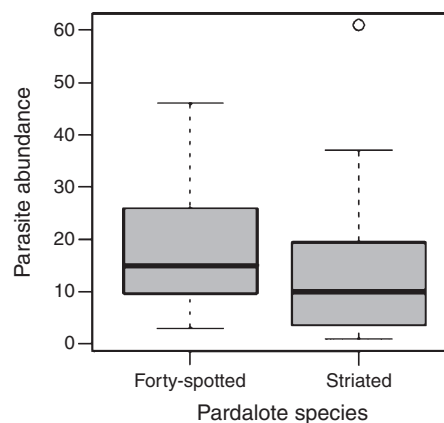

Fig. 2. Duration of the pupal stage for *P. longicornis* as a function of mean daily temperature maxima throughout the pupal development period. Larvae were collected from nine nests of pardalotes on North Bruny Island, Tasmania, during October–December 2014. One data point was used per nest, and development time was averaged across larvae in the single case where it differed among larvae from the same nest. Filled circles denote larvae collected from forty-spotted pardalote nestlings and open circles denote larvae collected from striated pardalote nestlings. The shaded area represents the 95% confidence band, and points were jittered to avoid overlap.

Fig. 3. Box-and-whisker plot showing abundance of the ectoparasitic fly *Passeromyia longicornis* in host nests (forty-spotted pardalotes versus striated pardalotes at sites) on north Bruny Island, Tasmania, during October–December 2014. Parasite abundance is the maximum number of parasites observed in a nest on any one check. The thick black line represents the median abundance value, boxes contain lower and upper quartiles of the data, and whiskers contain the least and greatest values excluding outliers (values that fall outside 1.5 box heights).

There has been a general lack of studies of nesting birds in Tasmania (especially for the small hollow- and burrow-nesting species), so previous interactions have likely gone undocumented. Parasite prevalence and nestling mortality were high in striated and forty-spotted pardalote broods, but the causal relationship between parasites and mortality is uncertain. A parasite elimination experiment is needed in order to determine the effect of *P. longicornis* abundance on nestling mortality. Nonetheless, parasitism by *P. longicornis* did appear to be a major source of nest failure; there was no predation detected in nest boxes, and other sources of mortality were minor, including hypothermia during extended periods of cold weather, possibly combined with starvation (A. Edworthy, unpubl. data).

Most pardalote nests with observable nestlings and parasites were in nest boxes. Although I was able to climb to about half of the natural nests detected, I was unable to get a clear view of nestlings in most natural tree hollows because pardalotes build domed nests at the base of their hollow. However, I did detect parasites in three of four nests with observable nestlings at Tinderbox Peninsula, and two of four such nests at Maria Island. These 50–75% prevalence levels likely underestimate actual prevalence, as I was much more likely to access nests with healthy nestlings due to their longer survival times (e.g. 4 weeks versus <1 week). Despite limited sample sizes, these observations of nests' natural hollows are valuable as they show that parasites were present throughout the main range of forty-spotted pardalotes (Maria Island, Bruny Island, and Tinderbox Peninsula), and in both nest boxes and natural hollows.

Confirmed hosts of *P. longicornis* now include forty-spotted pardalotes, striated pardalotes, New Holland honeyeaters, house sparrows, and European goldfinches (Green and Munday 1971; Green 1988). Early reports of *P. longicornis* parasitising honeyeaters and other bird species on mainland Australia (e.g. Gilbert 1919; Gilbert 1923; Hindwood 1930) were later shown to refer to *P. indecora* or *P. steini* (Pont 1974). Of the currently known hosts, most records come from pardalotes. Prevalence was high in both forty-spotted and striated pardalotes nests at 87–88% of nests. In a recent meta-analysis of all studies reporting prevalence and virulence of avian ectoparasites up to the year 2008, 48 bird species were infected by dipteran ectoparasites, with an average prevalence of 63% across nests of each species (s.d. = 34%, range = 85–100%) (Møller *et al*. 2009). Prevalence of *P. longicornis* in forty-spotted and striated pardalotes falls into the 66th percentile of this dataset, well above the median. Unfortunately, the lack of historical data limits our ability to interpret current prevalence in this system. High parasite prevalence can occur in host–parasite relationships that share a long evolutionary history (Møller *et al*. 2009), as well as in novel relationships (e.g. Fessl and Tebbich 2002).

Although nest sample sizes were low for most bird species aside from pardalotes, the high prevalence in pardalotes compared with that in other forest birds (just one of 20 nests parasitised) indicates that pardalotes are important hosts for *P. longicornis*. Selection of pardalote nests may be related to their location in nest boxes and natural tree hollows. These nest sites are often reused within a breeding season or across years, providing a predictable source of hosts, as well as a humid microclimate, well suited to ectoparasites (Heeb *et al*. 2000). Additionally, *Passeromyia* species tend to parasitise species with densely packed nest material (Pont 1974). Both pardalote species pack their nest boxes or hollows full of bark strips and grass, which was used by *P. longicornis* during its pupal stage. Parrots and cockatoos are the most abundant hollow-nesters in Australia but, in contrast to pardalotes, they don't add nest material to their hollows (aside from wood shavings or stick platforms). Thus, pardalotes may be an ideal target for dipteran ectoparasites among the Australian hollow-nesting birds, as their hollows provide both a warm, humid microclimate, and a substrate of grass or bark nest material for safe development of fly pupae.

Of the *Passeromyia* species, the life history of *P. longicornis* is most similar to that of *P. indecora*, which is also an obligate, subcutaneous, and hematophagous parasite of nestling birds (Couri and Carvalho 2003). Similar to other fly species, duration of the pupal stage in *P. longicornis* was strongly temperature-dependent (e.g. Anderson 2000). The relationship of temperature with rate of pupal development (as well as parasite abundance in nests) is often parabolic, reaching an optimum at moderate temperatures (Anderson 2000; Dawson *et al*. 2005). However, the breeding season in Tasmania is mild compared with the rest of Australia, and pupal development rate increased throughout the spring and summer, from 21 days in October to 14 days in late December and early January (Fig. 2). This pattern may allow fly populations to increase more rapidly later in the breeding season, as well as in warmer years. In other systems, the abundance and virulence of ectoparasitic fly larvae has increased in response to warm, wet conditions, as well as to changes in forest structure (Dudaniec *et al*. 2007; Antoniazzi *et al*. 2011). Thus, the response of *P. longicornis* to environmental change is an important area for future research.

This study is the first to examine the hosts, prevalence, and larval life cycle of *P. longicornis*; however, there is still much to learn about the basic ecology of the adult fly, including its food supply and habitat factors influencing abundance and distribution. *P. longicornis* is of particular interest because of its high prevalence in endangered and declining forty-spotted pardalotes. Fly parasites are emerging as a threat to nestling birds in several systems (e.g. O'Connor *et al*. 2010; Antoniazzi *et al*. 2011; Møller *et al.* 2013; Koop *et al*. 2016), and there is potential for the same to occur in Tasmania. Whether increasing in abundance or maintaining stable populations, *P. longicornis* was widespread in Tasmanian pardalotes, and is likely a major player in the ecology of this system.

Acknowledgements

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