

## BOTFLY PARASITISM EFFECTS ON NESTLING GROWTH AND MORTALITY OF RED-CRESTED CARDINALS

LUCIANO N. SEGURA<sup>1,3</sup> AND JUAN C. REBOREDA<sup>2</sup>

**ABSTRACT.**—We collected observational data in three consecutive breeding seasons to study interactions between the botfly *Philornis seguyi* and Red-crested Cardinals (*Paroaria coronata*) in a temperate zone near the southern limit of *Philornis* distribution. We analyzed: (1) seasonal trends in prevalence of parasitism, (2) influence of botfly parasitism on nestling growth rate and survival, and (3) the association between nest site vegetation at different scales (i.e., nest tree, vegetation surrounding the nest tree, and landscape) and probability of botfly parasitism. Prevalence of parasitism was 28% and was higher later in the breeding season. Botfly parasitism produced sub-lethal (lower growth rate of nestlings that survive) and lethal (lower nestling survival) effects. The lethal effect was negatively associated with age at the time nestlings were parasitized. Botfly parasitism was not associated with vegetation characteristics at the level of nesting tree or vegetation surrounding the nesting tree, but was associated with landscape features. Parasite prevalence was higher in large continuous woodland patches than in small isolated patches. However, we did not observe increased use of isolated patches of forest by Red-crested Cardinals, suggesting that use of nest sites with high botfly parasite intensity could be the consequence of high host density. Received 6 April 2010. Accepted 19 October 2010.

Nestling birds are hosts to a wide range of ectoparasites that capitalize on the brief period of rapid host development and resource availability (Loye and Carroll 1995). Three dipteran families (Calliphoridae, Muscidae, and Piophilidae) represent most of the hematophagous parasites of birds (Uhazy and Arendt 1986, Ferrar 1987). Many species of the genus *Philornis* (botflies) within the Muscidae parasitize nestlings and adults of cavity and open-nesting birds in the Neotropics (Arendt 1985a). Studies of the interactions between the genus *Philornis* and their hosts have been limited to a few species (i.e., *P. downsi* and Darwin's finches in the Galápagos Islands) (Fessl et al. 2001; Fessl and Tebbich 2002; Fessl et al. 2006a, b; Dudaniec et al. 2006; Dudaniec et al. 2007; Huber 2008; Kleindorfer and Dudaniec 2009; O'Connor et al. 2010c), or to species distributed in tropical and subtropical regions (Dudaniec and Kleindorfer 2006).

The genus *Philornis* includes ~50 species of flies, all ectoparasites of birds (Couri and Carvalho 2003, Dudaniec and Kleindorfer 2006). The life cycle of most of these species as well as relationships with their hosts is frequently unknown (Couri 1999, Teixeira 1999, Dudaniec and Kleindorfer 2006). Flies of this genus are distributed from central Argentina to the southern

United States (Couri 1999, Fessl et al. 2001). Botflies have been reported to parasitize at least 127 species of birds without marked host specificity (Couri 1991, Teixeira 1999). Most botfly species have subcutaneous larvae (Couri et al. 2005) and nestlings can be parasitized as soon as they hatch (Arendt 1985b; Delannoy and Cruz 1988, 1991; Spalding et al. 2002; Rabuffetti and Reboresda 2007). Botfly larvae feed on red blood cells (Uhazy and Arendt 1986) and remain in nestlings for 5–8 days (Arendt 1985b, Young 1993, Rabuffetti and Reboresda 2007, Quiroga 2009) when they leave the nestling as third instars and pupate in nest material (Uhazy and Arendt 1986). Adult flies emerge after a pupation period of 1–3 weeks (Oniki 1983, Young 1993, Rabuffetti and Reboresda 2007, Quiroga 2009).

Most studies indicate botfly parasitism produces sublethal (i.e., lower growth rates) or lethal effects on their hosts (Arendt 1985a, b; Delannoy and Cruz 1991, Young 1993, Fessl and Tebbich 2002, Rabuffetti and Reboresda 2007). One of the predictor variables for nestling survival is parasite intensity (number of larvae/nestling) (Dudaniec and Kleindorfer 2006). Some studies have reported only 5–6 larvae caused nestling death (Arendt 1985b, Delannoy and Cruz 1991), but others report similar intensities were not lethal (Nores 1995) and were only associated with lower growth rates (Young 1993). The other variable that influences nestling survival is age at the time they are parasitized (Arendt 1985a, 2000; Rabuffetti and Reboresda 2007) although this association has been less studied. Parasite prevalence (the percentage of nests with larvae) increases as the

<sup>1</sup>Laboratorio de Investigaciones en Sistemas Ecológicos y Ambientales, Universidad Nacional de La Plata, Diagonal 113 # 469, B1904CCA, La Plata, Argentina.

<sup>2</sup>Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, C1428EGA Buenos Aires, Argentina.

<sup>3</sup>Corresponding author; e-mail: lsegura79@yahoo.com.ar

host breeding season advances (Arendt 1985a, b; Young 1993; Rabuffetti and Reboreda 2007), although some studies did not find a trend (Nores 1995, Fessl and Tebbich 2002, Quiroga 2009).

The role of nest-site vegetation on prevalence and intensity of botfly parasitism has received little research attention, except for the work of O'Connor et al. (2010a) who reported higher prevalence and intensity of botfly (*P. downsi*) parasitism in moist forest highlands than in arid lowlands of the Galapagos Islands and suggested that size and continuity of forest patches could influence botfly dispersal ability. Understanding the role of nest-site vegetation on parasite infestation may help predict the likelihood of parasitism for a given host in a given environment (Loye and Carroll 1998).

We used a large set of observational data collected during three consecutive breeding seasons to study the interactions between *Philornis seguyi* and Red-crested Cardinals (*Paroaria coronata*), a species that has been previously reported as a host of botflies in central Argentina (De la Peña et al. 2003). We examined: (1) seasonal trends in parasite prevalence and intensity, (2) the influence of botfly parasitism on nestling growth and survival, and (3) the association between nest-site vegetation at different scales and probability of botfly parasitism. We hypothesized that survival of Red-crested Cardinal nestlings may be negatively associated with parasite intensity and positively associated with age at time of parasitism. We expected nests in small isolated patches of forest would have lower parasite prevalence than those in large continuous patches, because grassland areas that separate isolated from continuous patches may act as barriers for dispersal.

#### METHODS

*Study Site.*—The study was conducted near the town of Punta Indio, Buenos Aires Province, Argentina (35° 20' S, 57° 11' W). The vegetation at the study site consists of woodlands arranged in several strips (50–100 m in width and up to several km in length) parallel to the edge of the “de la Plata” river surrounded by small areas of grassland. In addition, there are also small patches of forests (between 10 and 70 m in diameter) more distant from the edge of the river surrounded by large areas of grassland. These small patches are separated from woodland strips by 300–1,200 m. The woodlands are dominated by *Celtis tala*

(Tala) and *Scutia buxifolia* (Coronillo). The annual rainfall for the study site is 891 mm and rainfall during the study period varied between 772 (2005) and 845 mm (2007). We collected data during Red-crested Cardinal breeding seasons (early Oct–mid Feb) 2005–2006 to 2007–2008. Average annual rainfall during the breeding season is typically 441 mm and, during the breeding seasons of 2005–2006, 2006–2007, and 2007–2008, it was 431, 439, and 487 mm, respectively. Ranges of mean monthly ambient temperatures during the study period were 14.8 (Oct) to 21.9° C (Jan) in 2005–2006, 17.0 (Oct) to 23.2° C (Feb) in 2005–2006, and 17.1 (Oct) to 24.6° C (Jan) in 2007–2008.

*Study Species.*—Red-crested Cardinals inhabit semi-open areas with scattered trees and shrubs from east central Argentina to southern Brazil, Paraguay, eastern Bolivia, and Uruguay (Ridgely and Tudor 1994). Their nests at our study site were at a height of 2–6 m, primarily in Talas and secondarily in Coronillos and Molles (*Schinus longifolius*) (Segura and Arturi 2009). Nests are open-cups with external and internal diameters of 13 and 6.5 cm, respectively, a depth of 4.5 cm and lateral translucent walls of 2 cm in width (LNS, unpubl. data). The wall of the nest is built with thin dry branches of Tala and small stems of grass while the chamber is lined with thin rootlets, vegetation fibers, and cattle hair. Clutch size varies between two and four eggs, nestlings hatch after 12 days of incubation and fledge ~14 days after hatching (LNS, unpubl. data). Average mass of nestlings at hatching is 3–3.5 g and 30–31 g at time of fledging (LNS, unpubl. data).

The species of botfly recorded previously in our study area is *P. seguyi* (Couri et al. 2005, Rabuffetti and Reboreda 2007). We collected botfly larvae from Chalk-browed Mockingbird (*Mimus saturninus*,  $n = 21$ ), House Wren (*Troglodytes aedon*,  $n = 9$ ) and Baywing (*Agelaioides badius*,  $n = 3$ ) nestlings, all identified (Martin Quiroga, INALI-CONICET, Argentina) as *P. seguyi* (Garcia 1952, Couri et al. 2009). Quiroga (2009) described the life cycle of this species. *P. seguyi* larvae feed and develop subcutaneously in the host for 5–6 days reaching a length of ~8–9 mm and a mass of 0.11–0.13 g. Larvae drop from the host to undergo pupation, emerging as adult flies after 9–10 days. Host larvae do not pupate at the bottom of the nest due to the scarce material that forms the cardinal nest, but drop to the ground where they undergo pupation (LNS, unpubl. data).

*Data Collection.*—Nests were found by searching systematically in potential nest sites and by observing nesting behavior of territorial pairs (Martin and Geupel 1993) of Red-crested Cardinals. We found 367 nests ( $n = 108, 120,$  and  $139$  for the breeding seasons of 2005–2006, 2006–2007, and 2007–2008, respectively). Nearly 50% of the nests ( $n = 177$ ) were found during construction and laying with the remainder found during incubation ( $n = 152$ ) and after hatching ( $n = 38$ ). We used 131 nests that survived at least 6 days after the first nestling hatched ( $n = 36, 45,$  and  $50$  for 2005–2006, 2006–2007, and 2007–2008, respectively). We used this criterion in our study because botfly parasitism occurred while nestlings were between 1 and 6 days of age. Inclusion of nests depredated before nestlings were 6 days of age would result in underestimation of parasite prevalence.

Nests were checked daily until all eggs hatched and then every 2 days until the nestlings fledged or the nest failed. Nestlings were marked after hatching on the tarsus with black ink for individual identification and color banded after day 6. We recorded: (1) day of hatching for each nestling, (2) number of nestlings hatched, (3) day we found the first larvae in the nestling, (4) day the nest failed or fledged young, and (5) number of young fledged for each nest. We recorded: (1) body mass, (2) lengths of the beak, right tarsus, and wing, and (3) parasite intensity (number of botfly larvae/nestling) for each nestling at each nest visit.

We measured body mass with 30 and 50 g Pesola spring scales (accuracy  $\pm 0.2$  and  $\pm 0.5$  g, respectively), length of the tarsus and beak with a dial caliper (accuracy  $\pm 0.1$  mm), and length of the wing with a ruler (accuracy  $\pm 0.1$  mm). We minimized the effect of daily variation in body mass and size by collecting these data between 1600 and 1900 hrs.

We analyzed the structure of the vegetation surrounding the nest by measuring vegetation characteristics at three different scales: (1) nest tree, (2) vegetation surrounding the nest tree, and (3) landscape. We measured (1) tree species, (2) nest height, (3) distance from the nest to the edge of the canopy, and (4) cover of the canopy at the nest tree scale. We measured the cover of tree canopy within a 15-m radius of the nest at the surrounding nest tree vegetation scale, and whether the nest tree was in the continuous strips of forest parallel to the river or in small isolated

forest patches more distant from the river at the landscape scale. We used images QuickBird (5 m) extracted from Google Earth (Digital Global Coverage, 6 October 2008) to calculate the cover of individual nest tree and proportion of canopies in the nest surrounding area using Program IDRISI Kilimanjaro 14.01 (Clark Labs 2003).

*Data Analysis.*—We assumed a nest was successful if it fledged at least one young and depredated if all nestlings disappeared between two consecutive visits. We did not observe abandonment of nests with nestlings in circumstances other than botfly parasitism. We assumed that a nestling died as a result of botfly parasitism if it was previously parasitized and found dead or disappeared between visits with no evidence of attack by predators (i.e., feathers or blood in the nest).

We estimated the lethal effect of botfly parasitism by comparing nestling survival (proportion of nestlings that fledged) between non- and parasitized nests excluding nests that were depredated. We estimated the sub-lethal effects of botfly parasitism by comparing growth rates of: (1) body mass, (2) tarsus length, (3) beak length, and (4) wing length between non- and parasitized chicks that survived. We used brood means to avoid pseudoreplication. We calculated growth rates as the slope of a linear regression of the values of each variable versus age of nestlings between 2 and 8 days of age (hatching day = age 0). Growth rates of all the variables were almost linear for nestlings 2–8 days of age (body mass:  $y = 3.0x + 1.6, r = 0.99, P < 0.001$ ; tarsus length:  $y = 2.1x + 5.9, r = 0.99, P < 0.001$ ; beak length:  $y = 0.56x + 4.9, r = 0.99, P < 0.001$ ; and wing length:  $y = 4.7x - 0.79, r = 0.99, P < 0.001$ ;  $n = 222$  data points from 66 non-parasitized nests). We only considered nests in which we had three or more measurements in that period (16 parasitized and 66 unparasitized nests). We used nests with nestlings during January and February only for analysis of the association between vegetation characteristics and botfly parasitism, as the occurrence of parasitism in nests with nestlings during the previous months was practically zero.

We used parametric tests for normally distributed data only, and nonparametric tests with corrections for ties. We used Mann-Whitney  $U$  or Kruskal-Wallis tests for independent comparisons. We used logistic regressions to analyze the association between botfly parasitism (binary dependent variable) and one or more independent

TABLE 1. Parasite prevalence (percentage of nests parasitized), parasite intensity (mean number of larvae/nestling and per nest), latency of parasitism (time elapsed since hatching of the first nestling and nest parasitism), and date first brood of Red-crested Cardinals was parasitized for three breeding seasons (2005–2008) in central Argentina woodlands.

	2005–2006	2006–2007	2007–2008
Parasite prevalence	8/36 (22.2%)	12/45 (26.7%)	17/50 (34%)
Parasite intensity	14.4 ± 1.6	10.8 ± 1.8	13.4 ± 1.7
( $\bar{x}$ ± SE of larvae/nest)	(range: 9–22)	(range: 3–26)	(range: 3–29)
Parasite intensity	6.7 ± 1.3	6.5 ± 1.4	6.4 ± 0.9
( $\bar{x}$ ± SE of larvae/nestling)	(range: 4.5–16)	(range: 2.5–19)	(range: 1.7–16)
Latency of parasitism	3.0 ± 0.38	3.3 ± 0.35	3.2 ± 0.39
( $\bar{x}$ ± SE of age of nestlings)	(range: 2–5)	(range: 2–6)	(range: 1–6)
Date first brood parasitized	10 Jan	31 Dec	11 Jan

variables. We used Fisher's exact or Chi-square tests for the analysis of contingency tables. Reported values are means ± SE. All tests were two-tailed and differences were considered significant at  $P < 0.05$ . Statistical tests were completed using STATISTICA 7.0 (StatSoft Inc. 2004).

## RESULTS

*Prevalence and Intensity of Parasitism during the Breeding Season.*—The prevalence of botfly parasitism was 28.2% (37/131 nests) and did not differ between years ( $X^2_2 = 0.85$ ,  $P = 0.65$ ; Table 1). There was a positive association between occurrence of botfly parasitism and time of breeding for the three breeding seasons (logistic regressions: 2005–2006,  $X^2_1 = 14.2$ ,  $P < 0.001$ ; 2006–2007,  $X^2_1 = 17.1$ ,  $P < 0.001$  and 2007–2008,  $X^2_1 = 36.1$ ,  $P < 0.001$ ) with most parasitized nests (36/37) occurring in January and February (Fig. 1). We divided the breeding season into 15-day intervals and calculated the proportion of nests that were parasitized with botflies for each interval to examine if the seasonal increase in parasite prevalence during January and February was associated with a decrease in availability of nests. We combined the data for the 3 years because of the small number of periods per year. There was no significant association between number of nests with nestlings and botfly prevalence (Spearman's rank correlation:  $\rho = -0.20$ ,  $P = 0.52$ ,  $n = 12$ ; Fig. 1). All nestlings were parasitized in 35 of 37 nests. Botfly intensity was  $6.5 \pm 0.66$  larvae/nestling (range: 1.6–19,  $n = 37$  nests; Fig. 2A) and was not statistically different between years (Kruskal-Wallis test:  $H_2 = 0.12$ ,  $P = 0.93$ ; Table 1). Mean parasite intensity per nestling did not differ between nests with one, two or three

nestlings (Kruskal-Wallis test:  $H_2 = 3.4$ ,  $P = 0.18$ ,  $n = 37$ ) and was not associated with date of hatching (Spearman's rank correlation:  $\rho = 0.1$ ,  $P = 0.55$ ,  $n = 37$ ). Latency of parasitism (time elapsed since hatching of the first nestling and nest parasitism) was  $4.2 \pm 0.2$  days (range 2–6 days,  $n = 35$  nests; Fig. 2B) and did not differ across years (Kruskal-Wallis test;  $H_2 = 0.6$ ,  $P = 0.74$ ; Table 1).

*Lethal and Sub-lethal Effects of Botfly Parasitism.*—Thirteen of 37 nests parasitized by botflies were depredated and excluded from analysis of nestling survival. No nestlings fledged in 4/24 nests (17%), there was partial fledging (some nestlings fledged, some died) in 7/24 nests (29%), and all nestlings fledged in 13/24 nests (54%). Nestling survival was lower in parasitized than in non-parasitized nests (parasitized:  $0.6 \pm 0.07$ ,  $n =$

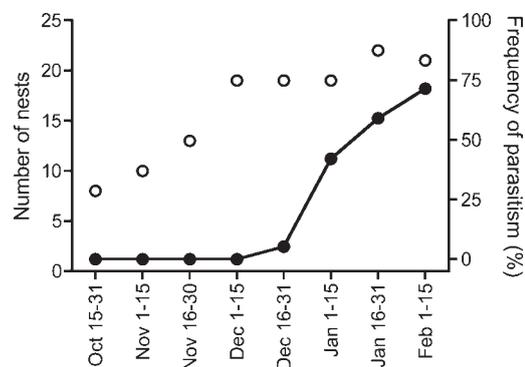


FIG. 1. Botfly parasitism of Red-crested Cardinals at different times of the breeding season in Buenos Aires Province, Argentina. White circles show the number of nests that produced nestlings during the 15-day interval and black circles = the percentage of those nests parasitized by botflies. Data correspond to the breeding seasons of 2005–2006, 2006–2007, and 2007–2008 combined ( $n = 131$  nests).

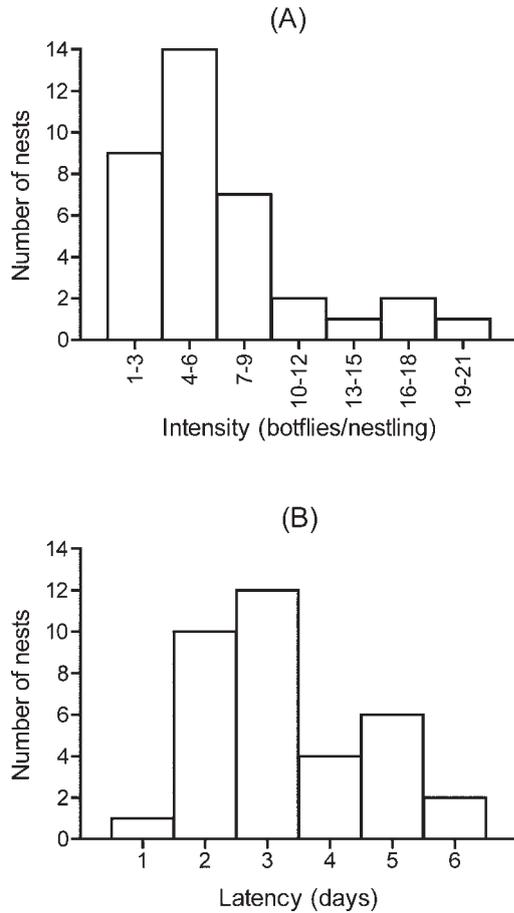


FIG. 2. Frequency distributions of the number of botfly larvae per nestling of Red-crested Cardinals; (A) = parasite intensity and (B) = time elapsed since hatching of the first nestling and nest parasitism (latency).

24; non-parasitized:  $0.86 \pm 0.02$ ,  $n = 74$ ; Mann-Whitney  $U$ -test:  $Z = 3.35$ ,  $P = 0.0008$ ). We did not detect an association between nestling survival and parasite intensity/nestling (Spearman's rank correlation:  $\rho = -0.19$ ,  $P = 0.37$ ,  $n = 24$ ; Fig. 3A), but nestling survival was positively associated with latency of parasitism (Spearman's rank correlation:  $\rho = 0.59$ ,  $P = 0.002$ ,  $n = 23$ ; Fig. 3B). Intensity and latency of parasitism were negatively associated (Spearman's rank correlation:  $\rho = -0.48$ ,  $P = 0.02$ ,  $n = 23$ ).

Predation outcome did not differ between parasitized and non-parasitized nests ( $X^2_1 = 0.33$ ,  $df = 1$ ,  $P = 0.56$ ) for the three breeding seasons combined. Young that fledged from parasitized nests had lower growth rates for body

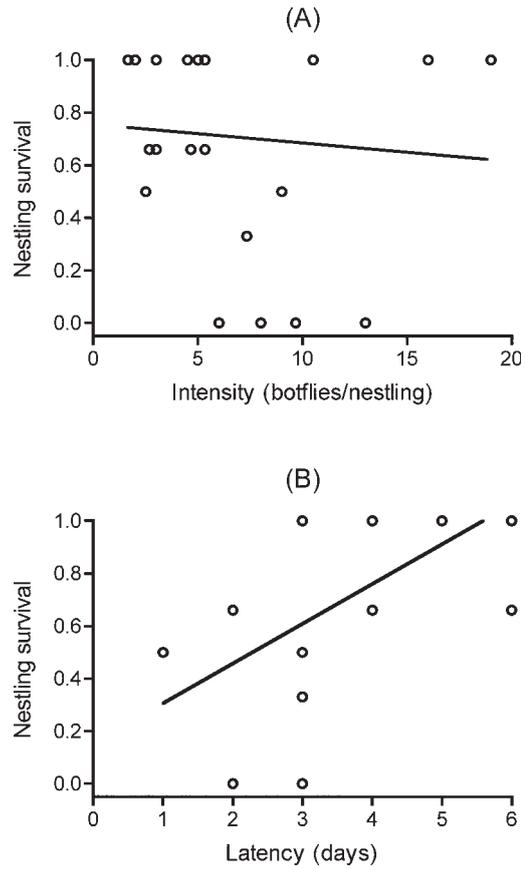


FIG. 3. Red-crested Cardinal nestling survival as a function of the number of botfly larvae/nestling; (A) = parasite intensity and (B) = time elapsed since hatching of the first nestling and nest infestation (latency).

mass (Fig. 4A), tarsus length (Fig. 4B), beak length (Fig. 4C), and wing length (Fig. 4D) than young that fledged from non-parasitized nests (Mann-Whitney  $U$ -test; body mass:  $Z = -2.94$ ,  $P = 0.003$ ; tarsus length:  $Z = -3.02$ ,  $P = 0.002$ ; beak length:  $Z = -2.54$ ,  $P = 0.01$ ; wing length:  $Z = -2.13$ ,  $P = 0.03$ ).

*Characteristics of the Vegetation and Botfly Parasitism.*—We did not detect a significant association between occurrence of botfly parasitism and species of nest trees (Talas: 22/44; Coronillo: 15/23;  $X^2 = 0.04$ ,  $P = 0.52$ ). We also did not observe a significant association between botfly parasitism and other characteristics of the vegetation at the nest-tree level (logistic regressions for nest height:  $X^2_1 = 1.24$ ,  $P = 0.26$ ; distance from the nest to the edge of the canopy:  $X^2_1 = 0.04$ ,  $P = 0.83$ ; and cover of the canopy:

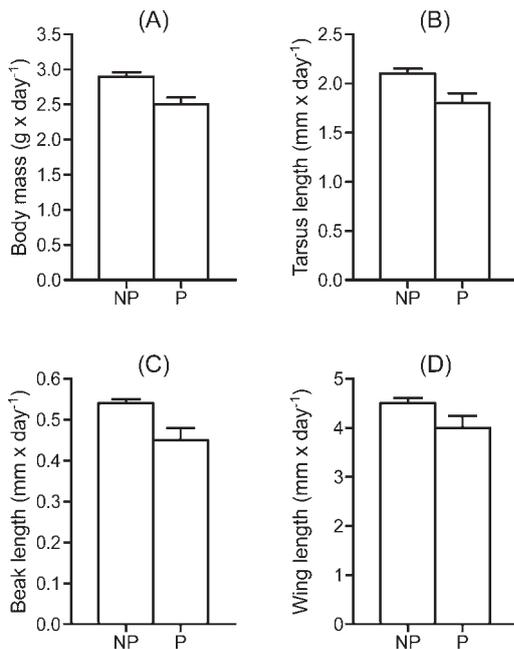


FIG. 4. Growth rates of (A) body mass ( $\text{g} \times \text{day}^{-1}$ ), (B) tarsus length ( $\text{mm} \times \text{day}^{-1}$ ), (C) beak length ( $\text{mm} \times \text{day}^{-1}$ ), and (D) wing length ( $\text{mm} \times \text{day}^{-1}$ ) for Red-crested Cardinal nestlings that fledged from unparasitized nests (NP,  $n = 66$ ) and from nests parasitized by *P. seguyi* botflies (P,  $n = 16$ ). Growth rates were estimated as the slope of the linear regression between the values of each variable and the age of nestlings between 2 to 8 days of age (hatching day = age 0).

$X^2_1 = 0.86$ ,  $P = 0.35$ ;  $n = 67$ ), or at the level of vegetation surrounding the nest tree (logistic regression:  $X^2_1 = 2.67$ ,  $P = 0.1$ ,  $n = 67$ ). There was a significant effect of landscape features and botfly parasite prevalence: nests in connected riverine woodlands had higher parasite prevalence than nests in small woodland patches more distant from the river (large riverine woodlands patch: 32/46 nests; small woodlands patch: 5/21 nests;  $X^2_1 = 4.0$ ,  $P = 0.04$ ).

#### DISCUSSION

Botfly parasitism of Red-crested Cardinals increased during the breeding season, but was not significantly different across years. Parasite prevalence and intensity had lethal (lower nestling survival) and sub-lethal (lower growth rate of nestlings that survive) effects. Nestling survival in parasitized nests was positively associated with age at which nestlings were parasitized. In addition, botfly parasitism was more prevalent in nests in large continuous patches of riverine

woodland than in small isolated patches further from the river.

Red-crested Cardinals start breeding in early October, but first records of botfly parasitism occurred in late December-early January. Similarly, Rabuffetti and Reborada (2007), in a study conducted only 35 km north of our study site, observed low prevalence of botfly parasitism at the beginning of the Chalk-browed Mockingbird breeding season (mid Oct-early Dec), but high prevalence towards the end of the season (late Dec-Jan). Quiroga (2009) studied House Wrens 500 km north our study area and reported botfly parasitism in late October-November and did not detect any seasonal trend in parasite prevalence. It is likely our study site is close to the southern limit of *Philornis* distribution, as studies of House Wrens (a regular host of botflies; Young 1993, Quiroga 2009) 100 km south of our study site did not detect parasitism (Llambías and Fernández 2009; Paulo Llambías, pers. comm.). The increasing absence of botfly parasitism approaching the southern limit of the parasite's distribution could be the result of lower ambient temperatures that delay emergence of new adults early in the season. Bennett and Whitworth (1991a, b) reported population size of flies of the genus *Protophila* increased over the breeding season because new adults emerge while older flies persist. Thus, as the season advances, the number of adult flies increases which would explain the finding of higher parasite prevalence.

Many studies report parasitic botflies (*Philornis* spp.) have detrimental effects on nestling survival (Arendt 1985b, Delannoy and Cruz 1991, Fessl and Tebbich 2002, O'Connor et al. 2010c), growth (Young 1993), and malformation that may persist in adults (Galligan and Kleindorfer 2009). Our results conducted in a temperate area close to the southern limit of the distribution of botflies indicate that botfly parasitism can lower host's reproductive success. Many nests had total and partial fledging success (83%), but we found lethal (lower nestling survival) and sub-lethal (lower growth rate of nestlings that survive) effects of parasitism. Our study also showed that low parasite intensity (6-7 larvae/nestling) could produce lethal effects. Botfly parasitism in our study decreased nestling survival from 0.86 to 0.6. This effect was less than reported by Rabuffetti and Reborada (2007) for Chalk-browed Mockingbirds, where botfly parasitism decreased nestling survival from 0.78 to 0.3. Differences in the

impact of botfly parasitism on host nestling survival between studies may be due to differences in parasite intensity, which was three-fold higher in mockingbirds than in cardinals.

Latency of parasitism, as in other studies (Arendt 1985a, 2000; Rabuffetti and Reboresda 2007), was an important factor that influenced nestling survival. We found a positive relationship between nestling survival and age at time of parasitism. We also observed a negative association between latency and parasite intensity. The latter association possibly indicates that changes in the skin of nestlings as they grow (likely the presence of feathers after day 5–6) may prevent larvae from penetrating the skin. We did not find an association between nestling survival and parasite intensity, probably due to low intensity in this study.

Nests in large continuous forest along river edges were more parasitized than those in small isolated patches, even when the distance between both types of patches was a few hundred meters. Bennett and Whitworth (1991b), in an experimental study with adult flies of the genus *Protophthora*, found that adult flies do not move large distances and that new infestations occur <50 m from where adults emerged. Dudaniec et al. (2010) also found lower levels of genetic relatedness in *P. downsi* when nests were located in more arid environments and host nesting density was lower. Open areas of grasslands that separate patches of forest may act as barriers for botfly dispersal. Alternatively, isolated patches of forest could have microclimate conditions (i.e., lower humidity and higher temperatures) that may reduce their suitability for botflies. O'Connor et al. (2010a) found habitat differences in *Philornis* parasite prevalence and intensity with higher levels of parasitism in moist forest highlands than arid lowlands on Floreana Island, in the Galapagos.

Some authors have proposed birds should avoid selecting nesting sites where they are more exposed to parasites, and that selection for good sites would be the first line of defense against parasitism (Loye and Carroll 1991, 1998; O'Connor et al. 2010b). We did not observe behavior by Red-crested Cardinals that suggested increased use of isolated patches of forest and earlier onset of breeding to avoid the higher parasite prevalence typical of riverine forest patches. Use of nest sites with high botfly parasite intensity could be the consequence of high host density; we have

observations of high nesting density of Red-crested Cardinals in riverine forest study sites (LNS, unpubl. data). Kleindorfer and Dudaniec (2009) and Kleindorfer et al. (2009) found an effect of high host nesting density on high *P. downsi* parasite intensity in the Galapagos Islands. Little is known about the role of parasite infestation for host nest site selection behavior: more studies on these interactions are needed.

#### ACKNOWLEDGMENTS

We are grateful to Luis del Sotro and Emiliano F. Torres for allowing us to conduct this study at Estancia 'La Matilde'. We thank Rachel E. McNutt, Danielle Castle, Sharon A. Fee, Kathleen Masterson, Anahí E. Formoso, Diego A. Masson, Diego I. Isaldo, Roberto F. Jensen, Tobias Mika, Ross H. Crandall, Marie M. Kalamaras, Rachel Buxton, Amy Nixon, Leigh Marshall, Yamila S. Obed, and Miguel A. Diferdinando for help in data collection and nest monitoring. We also thank Sarah A. Knutie and one anonymous reviewer for helpful comments to a previous version of this manuscript. We are grateful to 'Base Aeronaval de Punta Indio' for rainfall and temperatures data in the study area. LNS was supported by a fellowship from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). JCR is a Research Fellow of CONICET.

#### LITERATURE CITED

- ARENDR, W. J. 1985a. *Philornis* ectoparasitism of Pearly-eyed Thrashers. I. Impact on growth and development of nestlings. *Auk* 102:270–280.
- ARENDR, W. J. 1985b. *Philornis* ectoparasitism of Pearly-eyed Thrashers. II. Effects on adults and reproduction. *Auk* 102:281–292.
- ARENDR, W. J. 2000. Impact of nest predators, competitors and ectoparasites on Pearly-eyed Thrashers, with comments on the potential implications for Puerto Rican Parrot recovery. *Ornitología Neotropical* 11:13–63.
- BENNETT, G. F. AND T. WHITWORTH. 1991a. Host, nest and ecological relationships of species of *Protophthora*. *Canadian Journal of Zoology* 70:51–61.
- BENNETT, G. F. AND T. WHITWORTH. 1991b. Studies on the life history of some species of *Protophthora* (Diptera: Calliphoridae). *Canadian Journal of Zoology* 69:2048–2058.
- CLARK LABS. 2003. IDRISI Kilimanjaro. GIS software package. Clark Labs, Worcester, Massachusetts, USA.
- COURI, M. S. 1991. *Philornis carinatus* Dodge, 1968 (Diptera, Muscidae) data on morphology, biology and taxonomy. *Revista Brasileira de Entomologia* 35:109–112.
- COURI, M. S. 1999. Myiasis caused by obligatory parasites. Ia. *Philornis* Meinert (Muscidae). Pages 51–70 in *Myiasis in man and animals in the neotropical region; bibliographic database* (J. H. Guimarães and N.

- Papavero, Editors). Editora Plêiade/FAPESP, São Paulo, Brazil.
- COURI, M. S. AND C. J. B. CARVALHO. 2003. Systematic relations among *Philornis* Meinert, *Passeromyia* Rodhain and Villeneuve and allied genera (Diptera, Muscidae). *Brazilian Journal of Biology* 63:223–232.
- COURI, M. S., F. L. RABUFFETTI, AND J. C. REBORDA. 2005. New data on *Philornis seguyi* García, 1952 (Diptera, Muscidae). *Brazilian Journal of Biology* 65:29–41.
- COURI, M. S., L. R. ANTONIAZZI, P. BELDOMENICO, AND M. QUIROGA. 2009. Argentine *Philornis* Meinert species (Diptera: Muscidae) with synonymic notes. *Zootaxa* 2261:52–62.
- DELANNOY, C. A. AND A. CRUZ. 1988. Breeding biology of the Puerto Rican Sharp-Shinned Hawk (*Accipiter striatus venator*). *Auk* 105:649–662.
- DELANNOY, C. A. AND A. CRUZ. 1991. *Philornis* parasitism and nestling survival of the Puerto Rican Sharp-shinned Hawk. Pages 93–103 in *Bird-parasite interactions: ecology, evolution, and behaviour* (J. E. Loye and M. Zuk, Editors). Oxford University Press, Oxford, United Kingdom.
- DE LA PEÑA, M. R., P. M. BELDOMENICO, AND L. R. ANTONIAZZI. 2003. Pichones de aves parasitados por larvas de *Philornis* sp. (Diptera: Muscidae) en un sector de la provincia biogeográfica del Espinal de Santa Fe, Argentina. *Revista FAVE – Sección Veterinaria* 2:141–146.
- DUDANIEC, R. AND S. KLEINDORFER. 2006. Effects of the parasitic flies of the genus *Philornis* (Diptera: Muscidae) on birds. *Emu* 106:13–20.
- DUDANIEC, R. Y., S. KLEINDORFER, AND B. FESSL. 2006. Effects of the introduced ectoparasite *Philornis downsi* on haemoglobin level and nestling survival in Darwin's Small Ground Finch (*Geospiza fuliginosa*). *Austral Ecology* 31:88–94.
- DUDANIEC, R. Y., S. KLEINDORFER, AND B. FESSL. 2007. Interannual and interspecific variation in intensity of the parasitic fly, *Philornis downsi*, in Darwin's finches. *Biological Conservation* 139:325–332.
- DUDANIEC, R. Y., M. G. GARDNER, S. DONELLAN, AND S. KLEINDORFER. 2010. Offspring genetic structure reveals mating and nest infestation behaviour of an invasive parasitic fly (*Philornis downsi*) of Galápagos birds. *Biological Invasions* 12:581–592.
- FERRAR, P. 1987. A guide to the breeding habits and immature stages of Diptera Cyclorrhapha. Scandinavian Science Press Ltd., Copenhagen, Denmark.
- FESSL, B. AND S. TEBBICH. 2002. *Philornis downsi*. A recently discovered parasite on the Galápagos archipelago. A threat for Darwin's finches? *Ibis* 144:445–451.
- FESSL, B., M. S. COURI, AND S. TEBBICH. 2001. *Philornis downsi* Dodge and Aitken, new to the Galapagos Islands (Diptera, Muscidae). *Studia Dipterologica* 8:317–322.
- FESSL, B., S. KLEINDORFER, AND S. TEBBICH. 2006a. An experimental study of the fitness costs of *Philornis downsi* in Darwin's Ground Finches. *Biological Conservation* 127:55–61.
- FESSL, B., B. J. SINCLAIR, AND S. KLEINDORFER. 2006b. The life cycle of *Philornis downsi* (Diptera: Muscidae) parasitizing Darwin's finches and its impacts on nestling survival. *Parasitology* 133:739–747.
- GALLIGAN, T. H. AND S. KLEINDORFER. 2009. Naris and beak malformation caused by the parasitic fly, *Philornis downsi* (Diptera: Muscidae), in Darwin's Small Ground Finch, *Geospiza fuliginosa* (Passeriformes: Emberizidae). *Biological Journal of the Linnean Society* 98:577–585.
- GARCÍA, P. C. 1952. Las especies argentinas de género *Philornis* Mein., con descripción de especies nuevas (Diptera, Anthom.). *Revista de la Sociedad Entomológica Argentina* 15:277–293.
- HUBER, K. S. 2008. Effects of the introduced parasite *Philornis downsi* on nestling growth and mortality in the Medium Ground-Finch (*Geospiza fortis*). *Biological Conservation* 141:601–609.
- KLEINDORFER, S. AND R. Y. DUDANIEC. 2009. Love thy neighbour? Social nesting pattern, host mass and nest size affect ectoparasite intensity in Darwin's Tree Finches. *Behavioral Ecology and Sociobiology* 63:731–739.
- KLEINDORFER, S., F. J. SULLOWAY, AND J. A. O'CONNOR. 2009. Mixed species nesting associations in Darwin's Tree Finches: nesting pattern predicts predation outcome. *Biological Journal of the Linnean Society* 98:313–324.
- LLAMBÍAS, P. E. AND G. J. FERNÁNDEZ. 2009. Effects of nestboxes on the breeding biology of Southern House Wrens *Troglodytes aedon bonariae* in the Southern Temperate Zone. *Ibis* 151:113–121.
- LOYE, J. E. AND S. P. CARROLL. 1991. The effect of nest ectoparasite abundance on Cliff Swallow colony site selection, nestling development, and departure time. Pages 222–241 in *Ecology, behavior and evolution of bird-parasite interactions* (J. E. Loye and M. Zuk, Editors). Oxford University Press, Oxford, United Kingdom.
- LOYE, J. AND S. CARROLL. 1995. Birds, bugs and blood: avian parasitism and conservation. *Trends in Ecology and Evolution* 10:232–235.
- LOYE, J. E. AND S. P. CARROLL. 1998. Ectoparasite behavior and its effects on avian nest site selection. *Annals of the Entomological Society of America* 91:159–183.
- MARTIN, T. E. AND G. R. GEUPEL. 1993. Nest-monitoring plots: methods for locating nests and monitoring success. *Journal of Field Ornithology* 64:507–519.
- NORES, A. I. 1995. Botfly ectoparasitism of the Brown Cacholote and the Firewood-gatherer. *Wilson Bulletin* 107:734–738.
- O'CONNOR, J. A., R. Y. DUDANIEC, AND S. KLEINDORFER. 2010a. Parasite infestation and predation in Darwin's Small Ground Finch: contrasting two elevational habitats between islands. *Journal of Tropical Ecology* 26:285–292.
- O'CONNOR, J. A., F. J. SULLOWAY, AND S. KLEINDORFER. 2010b. Avian population survey in the Floreana highlands: is Darwin's Medium Tree Finch declining in remnant patches of Scalesia forest? *Bird Conservation International* 20: In press.

- O'CONNOR, J. A., F. J. SULLOWAY, J. ROBERTSON, AND S. KLEINDORFER. 2010c. *Philornis downsi* parasitism is the primary cause of nestling mortality in the critically endangered Darwin's Medium Tree Finch (*Camarhynchus pauper*). *Biodiversity and Conservation* 19:853–866.
- ONIKI, Y. 1983. Notes on fly (Muscidae) parasitism of nestlings of South American birds. *Gerfaut* 73:281–286.
- QUIROGA, M. A. 2009. Interacciones entre moscas parásitas del género *Philornis* (Diptera: Muscidae) y su hospedador *Troglodytes aedon* (Aves: Trogloditidae): ciclo de vida del parásito e impacto sobre el éxito reproductivo del hospedador. Dissertation. Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina.
- RABUFFETTI, F. L. AND J. C. REBORESDA. 2007. Early infestations by botflies (*Philornis seguyi*) decreases chick survival and nesting success in Chalk-browed Mockingbirds (*Mimus saturninus*). *Auk* 124:898–906.
- RIDGELY, R. S. AND G. TUDOR. 1994. The birds of South America. II. University of Texas Press, Austin, USA.
- SEGURA, L. N. AND M. F. ARTURI. 2009. Selección de sitios de nidificación del Cardenal Común (*Paroaria coronata*) en bosques naturales de Argentina. *Ornitología Neotropical* 20:203–213.
- SPALDING, M. G., J. W. MERTINS, P. B. WALSH, AND K. C. MORIN. 2002. Burrowing fly larvae (*Philornis porteri*) associated with mortality of Eastern Bluebirds in Florida. *Journal of Wildlife Diseases* 38:776–783.
- STATSOFT INC. 2004. STATISTICA (data analysis software system), Version 7. StatSoft Inc., Tulsa, Oklahoma, USA.
- TEXEIRA, D. M. 1999. Myiasis caused by obligatory parasites. Ib. General observations on the biology of species of genus *Philornis meinert*. Pages 51–70 in *Myiasis in man and animals in the neotropical region; bibliographic database* (J. H. Guimarães and N. Papavero, Editors). Editora Plêiade/FAPESP, São Paulo, Brazil.
- UHAZY, L. S. AND W. J. ARENDT. 1986. Pathogenesis associated with *Philornis* myiasis (Diptera: Muscidae) on nestling Pearly-eyed Thrashers (Aves: Mimidae) in the Luquillo rain forest, Puerto Rico. *Journal of Wildlife Diseases* 22:224–237.
- YOUNG, B. E. 1993. Effects of the parasitic botfly *Philornis carinatus* on nestling House Wrens, *Troglodytes aedon*, in Costa Rica. *Oecologia* 93:256–262.