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"The effect of *Philornis downsi* in the reproductive success of the Little Vermilion Flycatcher on the Isabela Island – Galápagos"

verfasst von / submitted by

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1. Introduction

Nearly all oceanic archipelagos have lost part of their original avifauna following human colonisation (Steadman 1995, 2006 & Blackburn *et al.* 2004). The Galápagos archipelago is a notable exception. The archipelago is known as one of the most intact refuges for native avifauna in the world**.** This is about to change: decreases in bird populations have been recorded in several bird species including the endemic Darwin's finches (Dvorak, *et al.* 2012, 2017; Grant *et al.* 2005; Fessl *et al.* 2017). Also the populations of the Vermilion Flycatchers *Pyrocephalus spp.* have declined during the last decades on three inhabited islands (Merlen G. 2013). Previously considered as two subspecies of the continental Vermilion Flycatcher *Pyrocephalus rubinus*, the two populations have now been recognized as two different endemic species (Carmi *et al.* 2016). The Least Vermilion Flycatcher *Pyrocephalus dubius* was restricted to the San Cristóbal Island and was not found in most recent searches (Fessl *et al.* 2017a). If this bird species cannot be rediscovered in the next years, it would represent the first Galápagos bird extinction in modern history. The Little Vermilion Flycatcher *Pyrocephalus nanus is* described for the rest of the mayor islands except Genovesa. Two phenotypically distinct clades have been described for this species: one occurring on the northern central islands, Pinta, Marchena, Santiago, Rábida, Pinzón, and Santa Cruz, the other occurring on the south western islands, Fernandina, Isabela, and Floreana (Carmi *et al.* 2016). *P. nanus* populations are threatend: on Floreana Island it has already disappeared and in Santa Cruz Island its population is down to 30 to 40 breeding pairs (Fessl *et al.* 2017a). It was thus classified as "vulnerable" by IUCN in 2016 (BirdLife International 2017).

The reasons for the decline of the Little Vermilion Flycatcher are unknown, but studies on other bird species in Galápagos point towards several possible explanations, namely predation by rodents (Fessl et al, 2010 – Mangrove Finch), brood loss due to parasitism by the introduced fly *Philornis downsi* (Fessl *et al.* 2006b; Huber 2008; O'Connor *et al.* 2010), habitat change and extreme climatic events (Dvorak *et al. 2012;* Cimadom *et al.* 2014).

There is a clear relation between the population declines of Darwin's finch species and the presence of the invasive fly *Philornis* (Fessl *et al.* 2017b). This fly is an obligatory bird parasite in its larval stage (Causton *et al.* 2013) and was first described for the Galápagos in 1997 (Fessl & Tebbich 2002) and entomological records show that it has been probably introduced during the sixties of the last century (Causton *et al.* 2006). The adult flies lay their eggs in bird nests during incubation or chicks feeding. Hatched fly larvae develop their first stage mostly in the chick's nares. They cause beak malformations, which in case of survival could persist until adulthood (Galligan & Kleindorfer 2009). Second instar larvae then move to the bottom of the nest from where they suck blood from nestlings during the night, which produces a reduction in haemoglobin concentration with blood losses from 18 to 55% of nestlings total blood volume, (Fessl *et al.* 2006). This leads to anaemia, reduction of mass gain and therefore a reduced fledgling success (Fessl *et al.* 2006). It especially affects bird species with small body size (Dudaniec *et al.* 2007), and low clutch numbers (Fessl *et al.* 2017b; Kleindorfer *et al.* 2016).

Experiments demonstrated that small Ground Finch *Geospiza fulliginosa* and Medium Ground Finch *Geospiza fortis* had significantly higher reproductive success in parasite-reduced nests than in parasitized nests (Fessl *et al.* 2006; Koop *et al.* 2013; O'Connor *et al.* 2014).

In addition to parasitism by *Philornis*, climatic factors and habitat management influences the breeding success in Darwin's tree finches: Wabler Finch *Certhidea olivacea* and Small Tree Finch *Camarhynchus parvulus* nestlings, which were exposed to periods of heavy rain showed reduced fledgling success. Furthermore the habitat management may also have an impact as Warbler Finches had lower breeding success in areas where Galápagos National Park managed introduced plant species by removing the understory (Cimadom *et al.* 2014).

In the present work we studied a population of the Little Vermilion Flycatcher (from here on LVF) on Isabela Island in the west of the Galápagos archipelago during three breeding seasons. From November 2014 to March 2017 we investigated the breeding success and identified reasons for breeding failure. Foremost we investigated the role of *Philornis* and tested its influence by experimentally reducing parasites in the nests. Additionally, we estimated the influence of habitat and climate on breeding success.

The extent and effect of *Philornis* parasitism is rather well known in Darwin's finches on Santa Cruz and Floreana (Dudaniec *et al.* 2006; Fessl *et al. 2006a & 2006b & 2017;* Kleindorfer *et al.* 2014 & 2017; O'Connor *et al.* 2010). To get comparative data on Darwin's finches for our study area we also collected data on the Warbler Finch (from here on WF), which is similar in size and diet to the LVF.

1. Methodology

2.1. *Study zone*

The study was conducted in the humid zone of Isabela Island in the highlands of the Sierra Negra volcano, in the surroundings of "El Cura" (0° 50.208' S, 91° 05.425' W, 550m to 1000m altitude). Nests were searched and monitored on an area of 8km^2 in both farmlands as well as in Galápagos National Park areas (Figure 1). The most common tree species at the study site was the Guayaba tree *Psidium guajava*, an invasive plant threatening several islands ecosystems (Cronk and Fuller 2001). The area was characterized by active and abandoned farmlands. Active farmlands consisted of Guayaba forest mixed with pastures. Farmers keep the pastures open by manual control and fumigating the regrowth of young Guayaba trees. Abandoned farmlands were characterised by more dense Guayaba stands and less pastures. Farmland borders are commonly marked by huge trees, predominantly Nogal *Juglans neotropica*, and other introduced species.

2.2. Monitoring of reproductive activity

Data for LVF were collected during three reproductive seasons: 2014 (between November 10th of 2014 and May 25th of 2015), 2015 (between December 4th of 2015 and April 4th of 2016), and 2016 (between November 9th of 2016 and March 25th of 2017). For WF data were collected in two reproductive seasons: 2014 and 2015 (see above). Subsequently we will refer to the three breeding seasons with the year they started in.

During 2014 season, LVFs were captured using a temporary fixed mist-net (6x4m, 2,5cm mesh size). Birds were measured (wing length, bill length, bill depth, tarsus length, primary feathers length, primaries feathers length), weighted and banded with a unique combination of three coloured rings and one numbered metal ring. Re-sightings of marked individuals were recorded during all subsequent field seasons.

At the beginning of each field season, intensive searches for LVFs individuals were conducted. Potential reproductive territories of LVF were determined by the presence of an aggressive male, aggressive female, displaying male, male and female interacting, male and female copulating or male or female feeding the chicks. All the territories and any nest found were marked by GPS waypoints.

LVFs are able to make more than one breeding attempt if their previous nests fails. Therefore, active nests found in the proximity of a monitored failed nest (within the boundaries of a single territory, defined by intensive observation), were assumed to be a re-nesting attempt of the territorial breeding pair. In order to avoid pseudo-replication, we randomly chose only one nest per breeding pair for the analysis and excluded 39 nests from analysis. The remaining sample consisted of 118 monitored nests. In 2014 we monitored 50 nests, in 2015 30 nests and, in 2016 38 nests. For WF we monitored 6 nests during 2014 and 21 nests during 2015.

Depending on the current breeding status the nest status was checked at different intervals to determine onset of breeding, number of eggs, hatching day, number of nestlings and date of failure or fledgling: During nests building a five day interval, during incubating a three day interval, during feeding a two day interval, and close to fledging daily (see Cimadom *et al.* 2014).

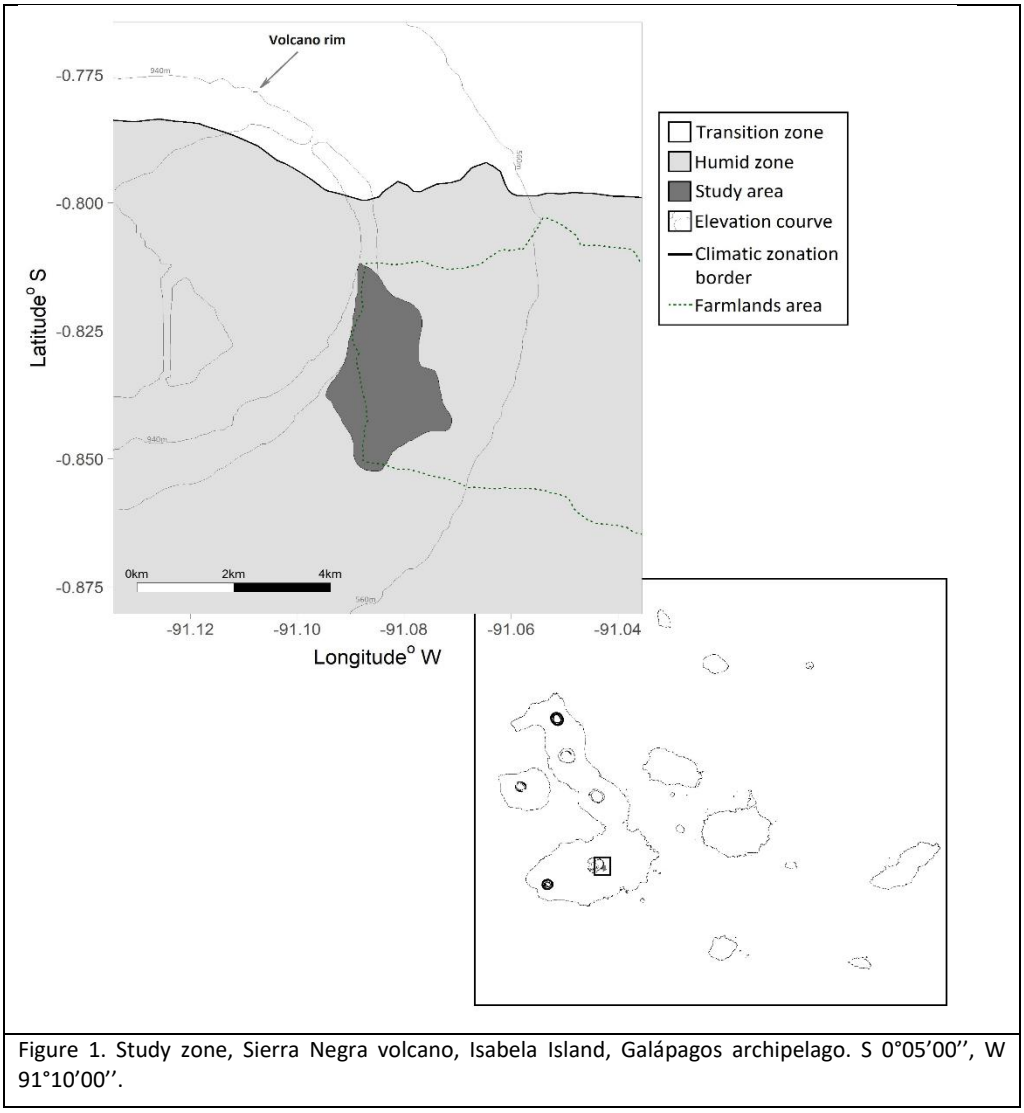

Figure 1. Study zone, Sierra Negra volcano, Isabela Island, Galápagos archipelago. S 0°05'00'', W 91°10'00''.

The nest status and number of eggs or chicks were determined either by observing adult behaviour or by inspecting the nest with an endoscopic camera (dnt, *Findoo 3.6*). After each visit, the observer waited until one of the adults returned to the nest. Successful nests (fledged nest) were defined as nests that produced at least one fledgling. Failed nests were assigned to the following categories: 1. Abandoned (only eggs in the nest), 2. Empty during incubation (previously incubated nest with no content), 3. Empty during chick feeding (a nest previously with young found with no content, chicks ≤10 days), 4. Dead chicks (chicks dead inside the nest), 5. Predated (destroyed or partially destroyed nests, or empty nests during feeding when chicks older than ≥11 days), 6. Other (e.g. nesting tree fallen down, nest fallen down due to unknown reasons).

Incubating period for LVF was calculated counting the number of days between the first incubation day and the first hatching day. The mean incubation period was 15.5±1.4 SD (n=29, median 16). Chick feeding period was calculated counting the number of days between the first hatching day and the last chick feeding observation in successful nests; the mean chick feeding

period was 16.8±1.8 SD (n=21, median 16). Thus, an incubation period of 15 days and a chick feeding period of 17 days were assumed. Incubation period, feeding period and observations on the date of first egg laying were used to estimate the "date of incubation start". To standardise the date of incubation start between years, we numbered the days beginning with the first breeding attempt of each breeding season (e.g. day one was the 23 October 2014 and day 177 to 17 April 2015). For nests abandoned during incubation, the date of the first observation on incubation was assumed as the date of incubation start. In two nests nestlings hatched before the first observation, here pictures and videos were used to define chick age and expected hatching date. For WF we assumed a mean incubation period of 13.7±2.0 SD (Cimadom *et al. 2014*. methods see above).

2.2.1. *Philornis downsi* survey

Once nest activity had ceased (either successful or failed nests, in both species LVF and WF), nests were collected in individual plastic bags, dismantled and visually inspected for larvae and pupa of *Philornis downsi*. Larvae were classified by size in three groups; small <=0.5cm, medium =>0.5cm<=1cm, large >1cm. Pupae where classified in emerged or not emerged. Parasite prevalence was defined as presence or absence of *Philornis* specimens in nests (presence from 1 specimen onwards). Parasite intensity was defined as the number of *Philornis* specimen per nest (larvae and pupae). *Philornis* variables were used for comparisons between years, species and as variables in models. Both can only be assessed once nest activity ceased. Therefore, parasite intensity and prevalence were assigned to the age of chicks at the moment of nest collection. Like Cimadom *et al* (2014) we found an increase of parasite intensity with chick age due to multiple infestations by *Philornis* (Regression: effect chick age; $F_{37, n = 39} = 4.9$, $p = 0.033$). In infested nests with total brood loss age at which chicks died tended to correlate positively with *Philornis* intensity (N=19, $r_{17} = 0.43$, $p < 0.06$). This leads to the contradictory finding that successful nests may have very high *Philornis* intensities. Because we cannot assess *Philornis* prevalence and intensity in successful nests before they fledge we cannot compare them with failed nests at the same age. Thus, we did not use *P. downsi* prevalence and intensity as predictors for breeding success and excluded them from the respective analysis.

2.2.2. Habitat and climatic data collection

Once nests were collected, several habitat parameters were recorded for LVF. At the nest site we recorded height of nest, height of nesting tree, nesting tree species, and aperture (vegetation density estimation, method see below). Further, we recorded vegetation characteristics at two diameters around the nest. Ten meter diameter: maximum canopy height, vegetation coverage (herb-layer, bush-layer, tree layer), tree height, number of trees, and diameter of five trees. Fifty meter diameter around the nest: tree height average, and vegetation coverage (herb-layer, bush-layer, tree layer. See below).

For measuring aperture (vegetation density around the nest) and vegetation coverage, the observer assigned categories from 1 to 5, 1 being the most open area and 5 the densest vegetation. Height was estimated in meters. Habitat measurements for 2014 and 2016 were collected by Denis Mosquera & Lorena Rojas and by Denis Mosquera & Wislon Iñiguez in 2015.

Climate raw data were provided by Kyoto University by a meteorological station located near the study area (S 0°50'13", W 91°5'27"). The parameters measured were: average temperature (*C°*), average rain (*mm water/m²*). To measure the average temperature, we recorded mean temperature during the days a nest was active. The sum of this daily values was divided by the total active days of each nest. The same procedure was performed to measure rain average. Temperature for 2016 was not available.

2.3. Parasite reduction experiments

In 2016, we treated eight LVF nests with a 0.5% permethrin solution (PermacapCS, Whitmire Micro-Gen Research Laboratories, Inc., St. Louis, MO. Reg. No. 499-528). Permethrin is known

to reduce *Philornis* numbers in other host species (Knutie *et al.* 2014; Koop *et al.* 2014; Fessl *et al.* 2006). The application was performed by injecting the solution directly into the nest base, using a 5ml syringe with a 5cm long metal needle with cut tip. To make treatment comparable between nests each nests used for the experiment fulfilled one of the following conditions: ≥12 days of incubation and feeding chicks ≤6 days of age. The height of treated nests was around 5-6m. For seven of these nests we had information on *Philornis* intensity and nesting success. One treated nest was predated and was not included in the analysis. The treatment group was named "parasite reduced nests". The control group was comprised of ten nests without treatment that were active during the same period; this group was named parasitized nests. We compare breeding success in treated and control nest with a Fisher's exact test.

2.4. Statistical analysis

To test the influence of all habitat variables, and weather conditions on the breeding success of LVFs and WFs we performed Generalized Linear Models (GLMs) with binomial distributed error structure. For LVF the breeding success (yes or no) was the predicted variable; predictors were the date of incubation start, rain average, nest height (log transformed), bush cover, herbs cover and aperture. To account for differences among years we included year (2014, 2015, 2016) as covariate in all LVF models. We excluded nests were neither habitat nor climatic data were available (subset sample n=85). To avoid multicollinearity we assessed correlation between all independent variables. If Pearson correlation coefficient between two variables was higher than 0.45, we chose only one of the two variables depending on their potential ecological importance. Temperature and rain average were highly correlated (2014 and 2015; $r_{45,\ n=47} = 0.54$, $p < 0.001$) and both correlated with date of incubation start (rain: $r_{71,\ n=73}$ 0.69, $p < 0{,}001$, temperature: $r_{45,\ n=47} = 0.92$, $p < 0.001$). As temperature was not available for on 2016 we did not include temperature into the models. The date of incubation start correlated with *Philornis* intensity ($r_{71} = 0.45$, $p = < 0.001$). To test the influence of rain average or date of incubation we performed separated models for each variable. We used the R package "glmulti" to calculate all possible models and select the best model with significant variables (Calcagno & de Mazancourt 2010). Models were compared by calculating the AIC score (Akaike Information Criteria) and we present the model with the lowest AIC. If the AIC score differed less than 2 we always chose the simpler model. To compare the discrimination ability of different binary models we calculated the AUCs, the areas under the receiver operating characteristic (ROC) curves, and tested the difference between them (Delong *et al.* 1988) using the R Package "pRoc"(Robin *et al.* 2011). All statistical analysis were carried out using R 3.4.1 (R Core Team 2017).

For WF we used the same criteria described above (n=21). We only used WF data from 2015 because sample size in 2014 was not representing breeding activity and during 2016 this species was not monitored. Date of incubation start correlated with rain and temperature (Pearson correlation, n=21 r= 0.65, $p < 0.01$, Temperature: n=21, $r = 0.84$, $p < 0.001$) and thus those variables were not used in the same model. Rain average did not correlate with temperature ($n =21 = 0.36$, $p > 0.05$) therefore, temperature and rain were included in each model. Breeding success (yes or no) was the predicted variable, the predictors were the date of incubation start, rain average, temperature (mean temperature during attempt).

To test the influence of all habitat variables, and weather conditions on the prevalence of *Philornis* parasitism in both species we also performed GLMs with binomial distributed error structure. The predicted variables was *Philornis* presence. To predict *Philornis* intensity we performed GLMs with Poisson distributed error structure and corrected for overdispertion. Predictors for *Philornis* (prevalence and intensity) in LVF nests were year, the day of incubation start, nest height, bush cover, herbs cover and aperture. Nest height was log-transformed. Predictors for *Philornis* in WF nests were the date of incubation start, rain average, and temperature average. We excluded nests where *Philornis* information was not available (LVF n=73, WF n=20). Again we performed separated models for rain and date of incubation start and

as above we selected the best models with significant variables. In GLMs with Poisson error structure we used the Kulback Leibler divergence based r^2 for GLMs (Cameron & Windmeijer 1997) using the R Package "rsq". For comparing *Philornis* intensity between LVF and WF we used a Wilcoxon test and for *Philornis* prevalence we performed a Fisher's exact test.

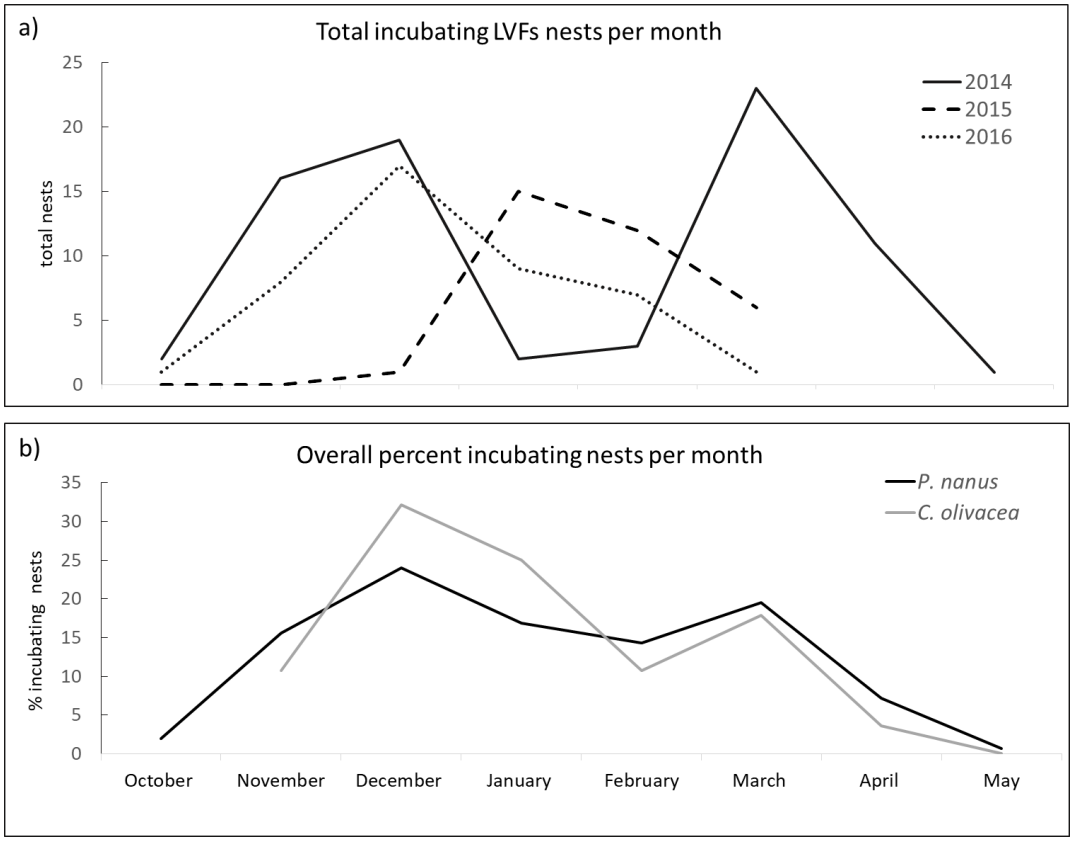

Figure 2. Total monitored nests per month. a) Individual breeding seasons and incubating monitored LVFs nests per month expressed in totals. Three breeding seasons are depicted with different line shape. b) Percentages of monitored incubating nests per month for both species. Species are depicted by the line colour. (Total n=118). Isabela Island, zone "El Cura".

3. Results

3.1. Ringed individuals

In total we colour-banded 49 individuals (12 males, 19 females and 18 juveniles) during the breeding season of 2014. Re-sightings of banded adults showed that all of them occupied the same territory during the whole breeding season (January to April 2015). In 2015 we only re-sighted six banded adults and in 2016 only three with two individuals still in the same territory. None of the banded juveniles were re-sighted in the subsequent breeding season.

3.2. Breeding success of Little Vermilion Flycatcher

Reproductive activity for LVF was registered from the last week of October to May. During 2014, breeding activity showed two peaks: Nests were found between October and end of December and between end of February and May. Breeding activity ceased during January and beginning of February. In 2015, breeding activity showed only one peak between January and February. In 2016 we recorded a single peak in December (Figure 2a).

In total, we monitored 110 nests from incubation onwards (Figure 3). At least one egg hatched in 65.4% of the nests and at least one chick fledged in 28% of the nests.

The type of breeding failure did not differ significantly between years (Chi²-test, effect of year: $x^2_{10,\ n = 79} = 5.613$, p = 0.840). The three most frequent reasons were "Nests with dead chicks "Empty during feeding" and "Abandoned during incubation".

Over the three seasons, a total of 28% nests were abandoned during incubation. Half of the abandoned nest were abandoned before incubation period ends (16 days) and the other half were abandoned later (≥16 days). Nest predation caused 7.5% of the brood failure.

Start of incubation date correlated negatively with the age at which chicks died. Earlier at the seasons chicks got older than later in the season. ($r_{71} = -0.58$, p= < 0.001).

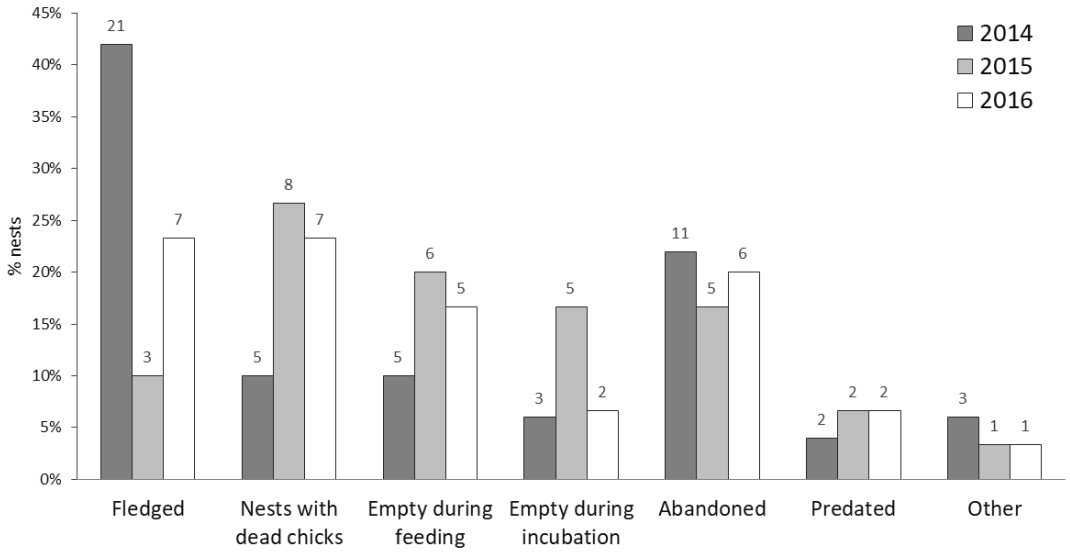

Figure 3. Percentage of fledgling success and types of breeding failure over three years on Isabela Island, zone "El Cura". Sample size is provided above the bars (Total n=110).

Two separated models with either rain or incubation start were calculated. Later in the season nests were less successful (table 1, model 1). Nests which experienced less rain had significantly more success (table 1, model 2). The AUC values showed in both models a strong effect in separating failed and successful nests. The model including date of incubation showed a higher AUC value than the model including rain (0.85 vs. 0.77, z =1.72, p =0.04). None of the habitat parameters had a significant effect.

Table 1. Significant variables to explain variation in breeding success in Little Vermillion Flycatcher (n=85) and Warbler Finch (n=21). Separated binomial GLMs were performed due to the highly correlated variables rain and date of Incubation start. For WF only 2015 data was available. AUC is the area under the ROC to describe the discrimination ability of the models. For each variable estimated value and corresponding p-value is listed. Year was included in all the Little Vermillion Flycatcher models as a covariate variable.

Breeding success (yes/no)	AUC	Date of incubation start	Rain	Temperature
1) Little Vermilion Flycatcher	0.85	-0.05 (0.007)		NA
2) Little Vermilion Flycatcher	0.77		-0.54 (0.004)	NA
3) Warbler Finch	0.81	-0.05 (0.04)		
4) Warbler Finch	0.92			-4.7 (0.03)

3.2.1. *Philornis* parasitism in Little Vermilion Flycatcher (LVF)

We were able to measure *P. downsi* prevalence and intensity for 80 nests. Parasites were present in 64% of all nests. Nests infected with *Philornis* contained a mean number of 12.62±10.31 SD (n=51) parasites (larvae, pupae and puparia combined). In nests abandoned

during incubation the prevalence was 68% with a mean of 6.42±6.27 SD (n=19) parasites. Nests that failed during chick feeding (nests with dead chicks and chicks disappeared during feeding combined) had significantly higher *Philornis* prevalence (65%, n=29); than fledged nests (40%, n=15) (Fisher's exact: n= 44, $p < 0.001$).

Later nests are more likely infested by *Philornis* than earlier ones (table 2, model 1, and 2) and a higher infestation rate was found in rainier conditions. The model including date of incubation had higher AUC value than the model including rain (AUC values: 0.87 vs. 0.80 z = -2.05, p = 0.04). Later and lower nests showed higher *Philornis* infestation rates (table 2, model 5).

Table 2. Significant variables to explain variation in *Philornis* prevalence and intensity in Little Vermillion Flycatcher (n=73) and the Warbler Finch (n=20). Separated GLMs were performed due to the highly correlated variables rain and date of Incubation start. AUC is the area under the ROC to describe the discrimination ability of the binary models. Pseudo R-squared values are listed for the GLMs whit count data. For each variable estimate and corresponding p-value is listed. Year was included in all the LVF models as a covariate variable.

Predicted variable	Model type	Model Quality	Date of incubation start	Rain	Nest height (log)
		AUC			
1) Prevalence in LVF nests (yes/no)	binomial	0.88	0.04 (0.0001)		
2) Prevalence in LVF nests (yes/no)	binomial	0.71		0.4 (0.006)	
3) Prevalence in WF nests (yes/no)	binomial	0.85	0.06 (0.03)		
4) Prevalence in WF nests (yes/no)	binomial	0.71		-0.14 (0.05)	
		Pseudo-r^2			
5) Intensity in LVF nests (continuous)	quasipoisson	0.33	0.01 (0.0005)		-0.63 (0.03)
6) Intensity in WF nests (continuous)	quasipoisson	0.38	0.04 (0.01)		
7) Intensity in WF nests (continuous)	quasipoisson	0.41		-0.2 (0.02)	

3.2.2. Experimentally treating Little Vermilion Flycatcher nests

Using Permacap resulted in a significant reduction of *Philornis* intensity (Wilcoxon-test; w=66.5, n=15, p= 0.002) (Figure 4). The chicks of 3 out of 7 experimentally treated nests fledged, whereas none of the 10 control nests (Fisher's exact: n=17, $p < 0.01$).

3.3. Warbler Finch breeding success and comparisons with Little Vermilion Flycatcher

Overall, onset of breeding is similar to LVF (Figure 2b). Hatching success was 81% (n=27) and breeding success 70% (n=27). Breeding success did not differ between years (Chi²-test, effect of year: $x^2_{1, n = 27} = <0.0001$, p = 1.0; Fisher's exact: $p > 0.05$).

Reasons for breeding failure were abandonment (3 nests), empty during feeding (1 nest), empty during incubation (2 nest), unknown reasons (1 nest), and predated (1 nest).

Later in the season nests were less successful (table 1, model 3). Nests which experienced lower temperature showed more breeding success (table 1 model 4). The model with temperature had a larger AUC value (0.81 vs. 0.92), but the difference to the model including date was not significant (z= -1.41, p = 0.16).

3.3.1. *Philornis* parasitism in Warbler Finch

We found *P. downsi* larvae and pupae in 44% of all monitored nests. Infested nests contained mean number of 26±20.6 SD (n=12) parasites. In nests abandoned during incubation the prevalence was 0% (n=3). *Philornis* was present in 3 of the failed nests (total failed nests 8) and in 9 of the successful nests (total fledged nests 19). In hatched nests we found 50% of prevalence with a mean of 13.45±20.17 SD (n=22) for abandoned during incubation nests 20% a mean of

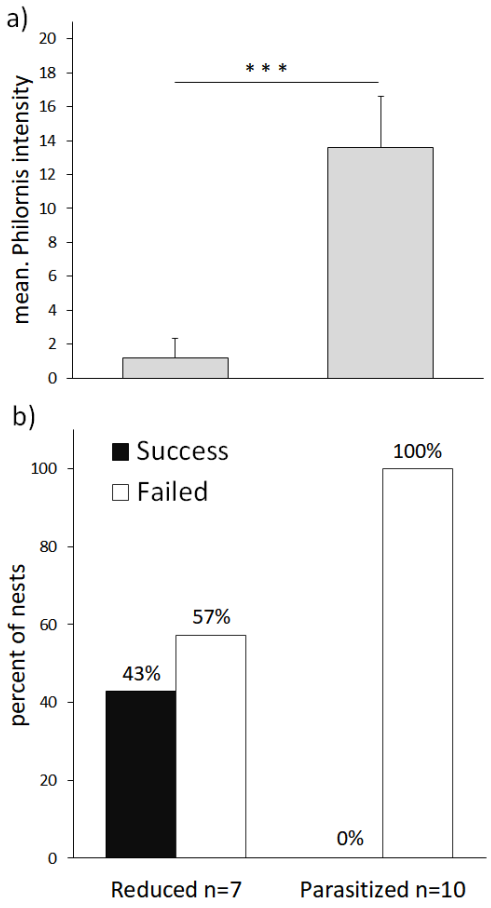

Figure 4. a) Mean number of *Philornis* in parasitized and parasite reduced nests with mean values and standard deviation. b) Reproductive success in parasitized and parasite reduced nests (n=17).

3.2±7.16 SD (n=5). *Philornis* intensity did not differ between fledged nests and failed nests (Fledged nests; mean 14.8±21.3 SD, n=19, *Philornis* in failed nests; mean 3.75±5.9 SD, n=8, Wilcoxon-test: $W_{1, n = 27} = 90$, p = 0.43).

As in the LVFs later nests are more likely infested (table 2, model 3, and 4) and we found more failed nests in rainier conditions. Date showed a larger but not significant effect than rain (AUC values 0.85. vs. 0.71: z = -1.65, p = 0.1). Infestation was higher in later nests and conditions with more rain (table 2, model 7 and 8).

Philornis prevalence was not different between LVFs and WFs (Fisher's exact: n= 107, p < 0.1). *Philornis* intensity of infested nests was significantly lower in the LVF than in the WF (LVF*:* mean 12.49±9.97 SD, n=51, median 11; WF: mean 26±20.6 SD, n=12, median 22; Wilcoxon-test: $W_{1, n = 63} = 105$, p = 0.007) whereas breeding success was higher for WFs compared to LVFs (70% vs. 19%, Fisher's exact: n= 107, p < 0.001).

4. Discussion

Our study revealed that the breeding success of LVF was influenced by the start of incubation date and the mean amount of rain the brood experienced while none of the measured habitat parameters seemed to play an important role. The model comparison indicated that the model with date of incubation start is slightly better to explain breeding success. Nests early in the breeding season had a higher breeding success and all nests after end of January failed. Date of incubation start was highly correlated with rain and also with temperature in 2014 and 2015, in which this environmental parameter was available. Thus, we are unable to disentangle the influence of the climatic parameters and date of incubation start on the breeding success of the LVF. Season is a function of climatic parameters which could affect the breeding success in various ways. Temperature and rain influence food availability but we would expect insect food availability to increase with the start of the wet and warmer climate as insect abundance starts to increase about 10 days after the first rains (Grant & Grant 1989). An alternative hypothesis is that chicks in open cup nests are suffering more from harsh climatic conditions. Cimadom *et al* (2104) showed that nests that experienced days of heavy rain falls have lower breeding success. The authors hypothesised that parents feed chicks less frequently during heavy rain events or that insects are less active and therefore more difficult to find. Cimadom *et al* (2014) also suggested a possible interaction with *Philornis* parasitism, namely that parents cannot compensate for the negative effects parasitism under adverse environmental conditions.

However date of incubation and climatic parameters associated with season also influence *P. downsi* prevalence and intensity. Parasite prevalence and intensity increased as the breeding season progressed, which explains the low breeding success later in the breeding season. This is the first study to find a marked variation of *P. downsi* parasitism within the breeding season. However, all other studies on the interaction of this parasitic fly with the land birds of the Galápagos have been conducted in study areas located from 0-600 masl. Our study site is located higher, at 1050 masl where climatic conditions, especially temperature, differ. Climatic requirements of *P.downsi* are not know but the absence of *P.downsi* larvae early in the breeding season indicates that condition are not favourable for this fly to occur and/or to reproduce. Trapping data would be necessary to assess whether adult flies are only present in low number or do not reproduce. To date, there is no evidence for seasonal migration in *Philornis* but there are observations of single individuals moving across 600m of lava tracts (F. Cunninghame, personal communication). It is possible that adult flies migrate into higher elevation with the onset of the warmer period.

In comparable studies on Darwin's finches *Philornis* intensity was already high at the start of the breeding season and remained more or less stable over the breeding season. The breeding phenology of LVF differed from the breeding phenology reported for Darwin's tree finches in other islands which usually starts in January and lasts until April and coincides with the humid season (Kleindorfer 2007; Cimadom et al 2014). The breeding phenology of the LVF differed between the three monitored breeding seasons. In 2014, breeding activity showed two peaks, one between October and end of December and a second one between end of February and May. In 2015 and 2016, breeding activity showed only one peak in February and December respectively. Although rain correlated positively with day of incubation start the visual inspection of the graph shows that at least in 2014 the early breeding peak was not triggered by rain. However, mist could be sufficient source of humidity to influence the factors that trigger the start of incubation (e.g. insect abundance). More detailed studies are needed to reveal the factors driving breeding activity of the LVF. The early breeding activity coincided with a low number of *P. downsi* prevalence and intensity which point towards a causal role of *P. downsi* parasitism in explaining the low breeding success.

Since *Philornis* intensity can only be measured after breeding activity ceased and *Philornis* intensity increases with chick age, it cannot be included in the model. Thus the effect on breeding success can only be assessed with an experimental approach. We found that nests with experimentally reduced parasite intensity had significantly higher breeding success than parasitized control nests. Other studies, that experimentally reduced numbers *P.downsi* in nests of Small Ground Finches (Fessl et al 2006 & O'Connor et al 2014) and Medium Ground Finches (Fessl et al 2006 & Koop et al 2013) reported similar findings. Although in our study the sample size was small, the results of our experiment point towards a causal role of *P.downsi* in explaining the low breeding success of LVF later in the breeding season.

WFs had higher breeding success than LVFs even later in the breeding season despite experiencing similar *Philornis* intensity. Variation in host susceptibility and host vulnerability has also been found in Darwin's finches (reviewed in: Kleindorfer & Dudaniec 2016; Fessl *et al.* 2017b). On the one hand species sharing the same environmental conditions showed significant differences in *Philornis* prevalence and intensity (Kleindorfer *et al.* 2014; Knutie *et al.* 2016) and on the other hand different host species with similar *Philornis* intensity were affected differently (Cimadom *et al.* 2014; Knutie *et al.* 2016). Variation in susceptibility to infestation may depend on nest size, nest type and nest height whereas host vulnerability may depend on other host traits such as body size, clutch size, immune response or the ability to compensate with increased parental care which in turn may depend on food availability. In Darwin's finches parasite intensity and body size are positively correlated (Dudaniec 2007, but see Kleindorfer & Dudaniec 2009) and species with lower clutch size suffer higher mortality because same number of larvae feeds on lower number of chicks (Fessl *et al.* 2017b; Kleindorfer *et al.* 2016). In our

study none of these two factors is likely to explain the differences in breeding success because the two species are similar in size (WF: mean 9.4g, LVF: mean 9.7g) and clutch size (WF: mean 2.1±0.31 SD, n=28, LVF: mean 2.1±0.41 SD, n=122).

Nest characteristics are another factor influencing parasite intensities. In Darwin's finches bigger nests showed higher *Philornis* intensity. As *WFs* nests are bigger than LVFs nests we expected higher *Philornis* intensity in the WF but found the contrary.

We also found that *Philornis* intensity was negatively influenced by nest height in the LVFs but in the WFs sample size was too small to analyses the effect of this parameter. Evidence of a vertical stratification of *Philornis* infestation has been found in Darwin's finches but in the opposite direction: On Floreana Island higher Darwin's finch nests contained more *Philornis* larvae than lower nests (Kleindorfer *et al.* 2016). The contrasting results between ours and Kleindorefer's study may be an effect of different stratification of the vegetation resulting in different stratification in nest heights: highest nests in Kleindorfer's study were found at 7m while highest nests of LVF were located at 16m. However, in our study nests of the WFs were located significantly lower than LVF and thus this is in an unlikely explanation for the different parasite intensity found in WFs. Other nest characteristics influencing parasite intensity is nest shape. In Darwin's finches lager nests with thicker nest bottoms harbour a higher number of *Philornis* larvae (Kleindorfer & Dudaniec 2009). WF nest are considerably larger with a thicker nest bottom than in LVF which could be responsible for the higher intensities found.

Another factor influencing vulnerability is the age at which nests are infested. Several studies indicated that on Galápagos island *P.downsi* gradually shifting their life cycle forward and attack nests earlier. Previously larvae were only found in nests with chicks but since 2012 larvae are also recorded in nest in the incubation stage where they feed on the incubating females (Cimadom *et al.* 2016). The presence of larvae early in the nesting stage leads to a further reduction in breeding success in Darwin's finches because newly hatched chicks are already attacked by large third instar larvae (Cimadom *et al.* 2014; Kleindorfer *et al.* 2014). We found high proportion of abandoned nests in LVF (28%) which is similar to number reported for the Small Tree Finch (25%) and the WF (28%) on Santa Cruz Island (Cimadom *et a*t. 2014) but higher than for the WF in our study (11%). Seventy one percent of LVF nests abandoned during incubation were infested by *Philornis* with a mean *Philornis* intensity of 9 larvae per nest which is higher prevalence and intensity than in Darwin's tree finches from Santa Cruz Island (Cimadom *in prep*). In our study only three *WFs* nests were abandoned during the incubation stage and none was infested by *Philornis*.

The high number of nest abandonment in LVF could be a direct consequence of *Philornis* infestation. Additionally, it could negatively affect incubation behaviour of the female (Oppliger *et al.* 1994). Reduced incubation time can cause eggs overheating, changes in embryo development, embryo death and changes of incubation duration (Fessl *et al.* 2017b). The effect of infestation during incubation on the breeding success of the WF should be targeted in future studies.

The WF and the LVF could also differ in their ability to compensate for the negative effects of parasitism by increased food provision which is in turn influenced by food availability. Both species are insectivorous (Filek *et al.* 2017; Tebbich *et al.* 2003) but in contrasts to the WF, little is known about the main insect groups this species feeds on.

Nest predation played a minor role in nesting failure for LVF (7.5%) although we observed native birds of prey and introduced mammals which are potential predators in the study area. Predation was less than the reported for several Darwin's finches at the highlands of Santa Cruz Island 9-66% (Cimadom *et al.* 2014; Dudaniec *et al.* 2007). The low nests predation also corresponds to high adult survival during the breeding season. All banded adults during 2014 were re-sighted during the duration of breeding season. On the other hand, during 2015 and

2016 only few re-sightings of birds banded in 2014 were recorded. This could point either towards low year to year survival or low site fidelity between years. Systematic banding and telemetry studies to determine adult and juvenile movements and regular population censuses need to be conducted in future years to get an understanding of the population dynamic of LVF. This is essential to develop population models that should help to predict population trajectories and target protection measures to prevent further islands extinction of the LVF.

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Anhang

Abstract

Several bird species endemic to the Galápagos Islands have declined rapidly during the last decades, among them the Vermilion Flycatchers (*Pyrocephalus dubius and Pyrocephalus nanus*). These two species have been recently elevated to full status based on mitochondrial evidence. *P. dubius,* is possibly extirpated in its natural range and *P. nanus,* has declined on inhabited islands. The reasons for the rapid decline and extinction of these populations are unknown, but identifying them is a precondition for developing efficient conservation strategies. Studies conducted on sympatric Darwin's finches point towards two potential reasons for low breeding success in the Little Vermillion Flycatchers a) parasitism by the invasive parasitic fly *Philornis downsi* and extreme climatic events. In the current study we investigate the role of both on the breeding success of the Little Vermilion Flycatcher (*P. nanus*) during three breeding seasons in the agricultural zone of Isabela Island. We found that, unlike other Galápagos passerines, Little Vermillion Flycatchers have in some years a first breeding peak before the onset of the rainy season. In line with our predictions we found that the Vermilion Flycatcher is strongly affected by the parasitic fly *P. downsi,* depending on the season. Our correlative data show that breeding success was high early in the breeding season (60 % of the observed nests fledged) when infestation with *P. downsi* was very low but was close to zero later in the breeding season when all nests were infested - although seasonal factors like climate might also explain the difference in breeding success. We found that nests which experienced higher rain days during the active period showed lower fledgling success and rain increased later in the season. A parasite removal experiment conducted later in the season confirmed the detrimental effect of the invasive parasite. Nests that were infested with *P. downsi* had significant lower fledging success than nests that were experimentally freed from parasites with an insecticide.

Key words: host parasite interaction, breeding biology, experimental parasite treatment, Warbler Finch, seasonality effect, susceptibility.

Zusammenfassung

Die Bestände vieler endemischer Vogelarten der Galápagos Inseln sind in den letzten Jahren drastisch zurückgegangen, unter ihnen die Rubintyrannen. Auf Grund von mitochondrialen Evidenzen wurden *Pyrocephalus dubius und Pyrocephalus nanus* kürzlich in den Status eigener Arten gehoben. *P. dubius*, ist wahrscheinlich in seiner natürlichen Ausbreitung ausgestorben und die *P. nanus* Bestände sind auf den bewohnten Inseln zurückgegangen. Die Ursachen für den schnellen Rückgang und das Aussterben dieser Populationen sind unklar. Diese zu ergründen ist jedoch grundlegende Voraussetzung für die Entwicklung effizienter Schutzmaßnahmen. Studien an sympatrischen Darwin Finken weisen in Richtung zweier potentieller Gründe für den niedrigen Bruterfolg des Rubintyrannen: Parasitismus durch die invasive parasitische Fliege *Philornis downsi* und extreme klimatische Bedingungen. In dieser Studie haben wir den Einfluss dieser beiden Faktoren auf den Bruterfolg des Kleinen Rubintyrannen (*P. nanus*) über drei Brutsaisonen in der Landwirtschaftszone von Isabela untersucht. Wir haben festgestellt, dass der Rubintyrann, im Gegensatz zu anderen Galápagos Singvögeln, seine erste Brutphase vor dem Beginn der Regenzeit hat. Im Einklang mit unseren Hypothesen fanden wir, dass der Rubintyrann, abhängig von der Saison, stark von der parasitischen Fliege *P. downsi* betroffen ist. Unsere korrelativen Daten zeigen, dass der Bruterfolg in der frühen Brutsaison, wenn der Befall von *P. downsi* sehr gering war, höher war (60% der beobachteten Nester waren erfolgreich), als später in Brutsaison, wenn alle Nester befallen waren. Allerding können auch saisonale Faktoren wie das Klima die Unterschiede im Bruterfolg erklären. Wir stellten fest, dass Nester, die während der aktiven Periode von starkem Regen betroffen waren, einen niedrigeren Bruterfolg hatten, und der Niederschlag nahm später in der Saison zu. Ein Versuch, der später in der Saison durchgeführt wurde und bei dem die Parasiten experimentell entfernt wurden, bestätigte den negativen Effekt des invasiven Parasiten: Mit *P. downsi* befallene Nester hatten einen signifikant niedrigeren Bruterfolg als Nester, die mittels Insektizid von Parasiten befreit wurden.