Wasps, vampires, and carrion flies: addressing the safety of the parasitoid *Conura annulifera* (Hymenoptera: Chalcididae), a promising biological control agent for the Galapagos Islands.

A Thesis
Submitted to the Faculty of
The University of Minnesota
by

Ismael Esai Ramirez

In Partial Fulfillment of the Requirements

for the Degree of

Doctorate in Philosophy

Adviser:

Dr. George E. Heimpel

December 2023

Copyright Permission

Chapter I has previously been published (citation: Ramirez, I. E., Causton, C. E., Gutierrez, G. A., Mosquera, D. A., Piedrahita, P., & Heimpel, G. E. (2022). Specificity within bird–parasite–parasitoid food webs: A novel approach for evaluating potential biological control agents of the avian vampire fly. *Journal of Applied Ecology*, *59*(8), 2189-2198). Permission for use here is granted by the first author, Ismael E. Ramirez, and the Journal of Applied Ecology publisher.

Chapter II has previously been published (citation: Ramirez, I.E., Yar, J., Sinclair, B.J., Torres A.K., Causton, C.E., & Heimpel, G.E. (2024). Competition among invasive and endemic carrion fly species in the Galapagos Islands with implications for biological control risk assessment. *Journal of Insect Conservation and Diversity*, In press. Available from: https://doi.org/10.1111/icad.12706. Permission for use here is granted by the first author, Ismael E. Ramirez, and the publisher of the Journal of Insect Conservation and Diversity.

Acknowledgments

This thesis was completed with the guidance of faculty and staff and the knowledge I have acquired from professors in the Entomology Department and classes along the progress of my degree. My gratitude goes, especially, to my advisor, Dr. George E. Heimpel, for taking me as his graduate student, for believing in me, and for teaching me the valuable skills I need to succeed in a career in academia. I am appreciative of the help and feedback I received on my thesis. I am especially grateful for the help I received from my committee members, Drs. Marlene Zuk, Ralph Holzenthal, and George Weiblen, for their invaluable support and feedback. The generosity has been tremendous.

I want to give recognition to the Charles Darwin Research Station staff, Dr. Charlotte Causton, Joselyn Yar, Andrea Cahuana, Paola Lahuatte, and Magaly Infante, for their support. I want to thank the ESPOL staff and colleagues, Dr. Paolo Piedrahita Piedrahita, Katherine Alban and Saul Aguirre. Thanks also to Dr. Bradley Sinclair. My fellow graduate students in Frenatae, undergraduate students, and my past and present lab-mates, Jonathan Dregni, Hannah Gray, Mary Marek-Spartz, Carl Stenoien, Charles Lehnen, Pamela Rueda, Alyssa Gooding and Sabrina Celis for their support and friendship. To my hosts in mainland Ecuador, Isidora Rosales and her family. To my graduate friends and academics, Carly Crow, Jackie Rodriguez, Isabel Donoso, Christian, Aoifa Galvin, Ilke Geladi, Ana Torres, Grace Vollmers, and Matthew Waller.

More importantly, I want to thank my siblings, Moises Ramirez, and Natalie Ramirez, for all their unconditional support and love. And finally, I want to thank my husband, Andrew Warmuth, for his constant patience, love and for always being there when I needed it the most.

Dedication

To my loving husband, because everything you did mattered.

~

To my mom and dad because I was born to good parents. Thank you for teaching me that hard work requires patience and love. This is for you.

~

To my nieces and nephews, I hope my research can help preserve a part of nature and allow you to experience it as you grow older.

Abstract

The avian vampire fly, *Philornis downsi* (Diptera: Muscidae), is an invasive species in the Galapagos Islands that has caused significant mortality among endemic bird species. This thesis delves into the safety of the neotropical wasp *Conura annulifera* (Hymenoptera: Chalcididae) as a biological control agent against the avian vampire fly in the Galapagos Islands and encompasses four chapters addressing such safety. Chapter One scrutinizes the ecological specificity of pupal parasitoid species found in the native range of the avian vampire fly and other fly species in mainland Ecuador using food web analysis. Additionally, in Chapter Two, I comprehensively characterized the composition of the carrion fly community in the Galapagos Islands, encompassing the abundance and distribution of both endemic and introduced species. Furthermore, I investigated the potential for competitive interactions between introduced and endemic carrion fly species and their implications for biological control. In Chapter Three, I examine the burrowing behavior of endemic and introduced carrion fly species in the Galapagos Islands and evaluate the ability of *C. annulifera* to locate and attack subterranean puparium as the soil is an effective barrier that provides refuge for non-target species that burrow. Finally, in Chapter Four, I assess the physical host preference of *C. annulifera* and its potential to parasitize non-target carrion fly species using no-choice trials in containment facilities of the Charles Darwin Research Station in the Galapagos Islands. The findings of this thesis provide informative insights into the intricate ecological interactions between the avian vampire fly, *C. annulifera,* and carrion fly species in the Galapagos Islands and Mainland Ecuador. Additionally, it sheds light on the safety of *C. annulifera* to serve as a biological control agent for the avian vampire fly. This knowledge is crucial information to decision-making officials regarding the

potential introduction of *C. annulifera* to the Galapagos Islands to mitigate the detrimental effects of *P. downsi* on endemic bird populations and possible extinctions.

Table of Contents

List of Tables

Chapter I.

Chapter II.

Chapter III.

List of Figures

Chapter I.

Chapter II.

Chapter IV.

Introduction

Invasive species threaten the world's ecosystems and biodiversity by outcompeting, preying upon, or parasitizing native species, spreading diseases, and altering critical habitats (Simberloff, 2013). They can also drive native species to extinction and disrupt food webs and ecosystem processes (David et al., 2017). The Galapagos Islands, with their unique and diverse ecosystem, including many endemic species found nowhere else on Earth, are particularly vulnerable to the impacts of invasive species (Reaser et al., 2007; Russell et al., 2017). One is the avian vampire fly, *Philornis downsi* (Diptera: Muscidae), a blood-feeding parasite of birds that has been implicated in the decline of several endemic bird species, including the iconic Darwin's finches (Fessl & Tebbich, 2002; McNew & Clayton, 2018).

To mitigate the negative effects of the avian vampire fly, the Charles Darwin Foundation and the Galapagos National Park have focused on importation biological control (classical biological control), a strategy that involves introducing natural enemies of the invasive species (Heimpel, 2017; Boulton et al., 2019). The neotropical wasp *C. annulifera*, a pupal parasitoid that attacks the pupal stage of flies, has been identified as a potential biological control agent against the avian vampire fly (Bulgarella et al., 2017). Laboratory and field studies have demonstrated that *C. annulifera* is a specialist in the *Philornis* genus (Bulgarella et al., 2017; Ramirez et al., 2022). However, further safety assessments are crucial before a potential release of *C. annulifera* in the Galapagos Islands to ensure non-target effects on endemic species. One primary concern is the potential for *C. annulifera* to parasitize non-target carrion fly species in the Galapagos Islands, as they are closely related taxonomically to the *Philornis* genus and play a vital role in the Galapagos ecosystem, where no large carrion vertebrates exist, acting as essential decomposers.

This thesis aims to investigate the safety of *C. annulifera* as a biological control agent for *P. downsi* in the Galapagos Islands with special consideration for carrion fly species in the families Sarcophagidae, Calliphoridae, and Muscidae. In the Galapagos islands, their role is not well-explored, especially when it comes to understanding the feeding habits of their larvae. While there is limited information on larval feeding behaviors, it is suspected that at least eight species, such as *Lucilia pionia*, *L. setosa* (Diptera: Calliphoridae), *Blaesoxipha insularis*, *B. isla*, *B. violenta*, *B. williamsi*, *Sarothromyiops dasycnemis*, and *Galopagomyia inoa* (Diptera: Sarcophagidae), may function as carrion feeders. Additionally, among the introduced fly species from these families in the Galapagos, at least 12 out of the 21 are either confirmed or suspected to be part of the local necrobiome (Sinclair, 2023).

In this thesis, I introduce an innovative in-field nest-pairing experimental approach to evaluate the level of specificity of *C. annulifera* and other parasitoids associated with *Philornis* spp. in their natural habitat, Mainland Ecuador, which involved incorporating carrion fly pupae into existing food webs that include birds, their *Philornis* parasites, and parasitoids of *Philornis* spp. To estimate specificity, we utilized food-web metrics to quantify *Philornis* flies' targeting birds, parasitoids' targeting of *Philornis* flies, and parasitoid preferences for bird species irrespective of the *Philornis* species affecting them. Additionally, I assessed the relative abundance of various *Philornis* fly species within nests to enhance the understanding of interactions among *Philornis* species, all addressed in Chapter one.

In Chapter two, I report findings on the fly species composition linked to carrion, encompassing introduced, endemic, and native species on Santa Cruz Island in the Galapagos, and I conducted a laboratory experiment to examine the competition dynamics between the dominant introduced carrion fly species, *Peckia chrysostoma*, and other necrophagous fly taxa, including the endemic

S. dasycnemis. I also provide information on the parasitoids in carrion flies' larval and puparial stages. In Chapter three, I attempt to further assess the susceptibility of non-target dipterans in the Galapagos to attacks by *C. annulifera* by carrying out laboratory investigations on carrion flies places of refuge. These studies aimed to learn the frequency of subterranean pupation among native carrion flies in the Galapagos and to assess *C. annulifera* capability to target them. This chapter intends to test the hypothesis that potential non-target hosts of *C. annulifera* in the Galapagos might have diminished ecological vulnerability to parasitism due to a spatial refuge within the soil.

Finally, in Chapter Four, I build upon the study conducted by Bulgarella et al. (2017), by employing no-choice laboratory trials utilizing resident carrion flies as non-target hosts in the Galapagos Islands to assess host preferences further. In my research, I extended this work by subjecting a female *C. annulifera* wasp to an introduced species of carrion flies, namely *Peckia chrysostoma*, *Peckia lambens*, and *Lucilia eximia*.

Chapter I: Specificity within bird-parasite-parasitoid food webs: a novel Approach for evaluating potential biological control agents of the avian vampire fly

Summary

Quantitative food web analyses can provide insights into the specificity of consumers such as herbivores, parasites, and parasitoids. Understanding such patterns can be useful in forecasting the potential benefits and risks of biological control agents being considered for introduction against invasive species. The avian vampire fly, *Philornis downsi* (Diptera: Muscidae), is a neotropical bird parasite that is invasive in the Galapagos Islands, where it is causing substantial mortality of endemic bird species. We used a novel in-field experimental food web approach within the native range of *P. downsi* in Ecuador to test the hypotheses that pupal parasitoids known to attack *P. downsi* specialize on members of the genus *Philornis*, which occur only in bird nests. We deployed pupae of non-*Philornis* fly species adjacent to bird nests to assess the specificity of the parasitoids and used two indices to assess specificity: Resource Range (RR), which evaluates the breadth of host use, and Pair Difference Index (PDI), which evaluates interaction strength. The results revealed very strong compartmentalization within the guild of pupal fly parasitoids, with four species attacking only *Philornis* spp. Both specificity indices indicated significant levels of specificity toward the genus *Philornis* for two of these species: *Conura annulifera* and *Trichopria* sp. *novus.* We also assessed the specificity of the two dominant *Philornis* species attacking 11 bird species and the preference of the two dominant parasitoid species for bird species. Although there was some significant preference for particular bird species by the *Philornis* spp., there was no indication that this drove specificity patterns by the parasitoids. Our results confirm previous laboratory studies indicating specificity by *C. annulifera* and support the hypothesis that this species would produce few, if any, nontarget impacts if released into Galapagos to suppress populations of the *P. downsi*. These results can

inform an environmental risk assessment framework to guide governmental agencies in deliberating potential field releases of parasitoids in the Galapagos Islands.

Introduction

Food webs and ecological networks are commonly used to understand complex relationships in nature and are useful in investigating invasive species (Zavaleta et al., 2001; Frost et al., 2019). Analyses of diet breadth have advanced significantly over the past decade, leading to novel specificity indices (Poisot et al., 2012). These advances offer new opportunities to use food web analyses to investigate interactions between invasive species and their biological control agents. Understanding the nontarget and indirect effects that can occur when biological control agents are introduced into novel geographical areas is an important goal of biological control researchers, and food-web analysis has emerged as a powerful tool that can contribute important insights (Memmott, 2000; Hennemann & Memmott, 2001; Willis & Memmott, 2005; Carvalheiro et al., 2008; Tylianakis & Binzer, 2014; Lopez-Nuñez et al., 2017; Pacheco et al., 2018; Ollivier et al., 2020). Further insights into the safety of biological control introductions can be achieved by studies in the native range of the biological control agent (Veldtman et al., 2011; Todd et al., 2021), particularly when naturally occurring food webs are modified by either adding or removing species (Briese et al., 2002; Frost et al., 2016).

The avian vampire fly, *Philornis downsi*, is a nest parasite that causes high mortality of endemic Galapagos birds, including Darwin's finches, and is the main cause of observed population declines (Kleindorfer & Dudaniec, 2016; Fessl et al., 2018; McNew & Clayton, 2018). The fly is native to mainland South America and was introduced into the Galapagos archipelago before the 1960s, likely as a stowaway on boats or airplanes from mainland Ecuador (Fessl et al., 2018; Koop et al., 2021). Eggs are deposited into bird nests, and the fly larvae feed on the blood of nestlings, causing anemia, blood loss, and death (Dudaniec & Kleindorfer, 2006; Fessl et al.,

2018). Mathematical models suggest that populations of some finch species could go extinct within a century in the Galapagos due to *P. downsi* parasitism (Koop et al., 2016), while critically endangered species are at even greater risk (Bulgarella et al., 2019). Biological control introductions of specialized parasitoids have been deemed to be the most promising long-term solution to control *P. downsi* and protect the Galapagos avifauna by the Charles Darwin Foundation and the Galapagos National Park; however, risks must be fully considered (Boulton et al., 2019). Of particular interest is the parasitoid wasp *Conura annulifera* (Hymenoptera: Chalcididae), which is known as a parasitoid of *Philornis* spp. in Trinidad and Brazil (Burks, 1960; Couri et al., 2006). Bulgarella et al. (2017) also reported this parasitoid attacking two *Philornis* species (including *downsi*) in mainland Ecuador and subjected it to specificity tests under laboratory conditions. *Conura annulifera* is a solitary idiobiont gap-laying parasitoid, meaning that single eggs are laid into the gap between the developing pupa and pupal case and that pupal development ceases upon parasitoid oviposition. Gap-laying parasitoids like *C. annulifera* are restricted to attacking the cyclorrhaphan Diptera because this gap is absent in other insects (Boulton & Heimpel, 2018). Moreover, *C. annulifera* exhibits specificity within the Cyclorrhapha since it did not oviposit in the pupae of five species of non-*Philornis* cyclorrhaphan flies in assays done by Bulgarella et al. (2017). Based on these specificity trials and reports in the literature, *C. annulifera* was categorized as a promising candidate for introduction into the Galapagos Islands to control *P. downsi* (Bulgarella et al., 2017).

We developed a novel in-field nest-pairing experimental technique to assess field-level specificity of *C. annulifera* and other parasitoids associated with *Philornis* spp. in their native range. This was done by adding cyclorrhaphan fly pupae to naturally occurring food webs,

including birds, their *Philornis* parasites, and parasitoids of *Philornis* spp. We then used food-web metrics to characterize the specificity of *Philornis* flies attacking birds and parasitoids attacking *Philornis* flies. Our goal was to better understand the ecological host range of parasitoids and flies found within bird nests, recognizing that patterns of specificity can reflect direct preferences of hosts by parasitoids or indirect pathways of preference mediated through the resources used by those hosts (in this case, bird species) (Singer & Stireman, 2005). We also performed analyses to determine whether any of the parasitoid species preferred either of the two *Philornis* species.

Materials and Methods

Location and Study System

The research was conducted at the Reserva Ecológica Loma Alta (1.85694O S, 80.59938O W) located in Santa Elena province within western mainland Ecuador (Permit: MAE-DNB-CM-2016-0045). The Reserve is composed of a tropical dry forest and a pre-montane cloud forest in the Chongón Colonche Mountain range (Bulgarella et al., 2015), where multiple species of *Philornis* flies have been found, including *P. downsi*, *P. niger* and *P. falsificus* (Bulgarella et al., 2015, 2017, 2019). Along with *Philornis* flies, multiple parasitoid species, including *C. annulifera*, have emerged from *Philornis* puparia at this site. All the *Philornis* spp. that have been found in this location are obligate parasites of altricial birds, feeding on the blood and tissue of their hosts. *Philornis downsi* and *P. falsificus* larvae are free-living ectoparasites that feed on nestling birds at night, while *P. niger* larvae feed subcutaneously on nestlings (Couri, 1999). All three *Philornis* species pupate within bird nests, and egg-adult development takes approximately three weeks, with approximately ten days spent in the pupal stage for *P. downsi* (Lahuatte et al.,

2016). *Conura annulifera* females attack *Philornis* puparia aged between 2 and 7 days and complete their life cycle in approximately 24 days (Bulgarella et al., 2017). We monitored nests throughout the nesting season (January – June) from 2013 to 2018, and our pairing experiment was conducted from March to May 2016 and from February to July 2017. This study did not require ethical animal approval.

Bird nest monitoring

We monitored artificial wooden nest boxes (n=46) and bamboo poles with multiple nesting cavities (n=24) (Bulgarella et al. 2017), as well as naturally occurring bird nests found in trees and other structures between 2013 and 2018 (inclusive). We observed the progress of active nests using an endoscopic fiber-optic camera with a wireless monitor (shaft 17 mm diameter, fiber-optic cable length 91 cm) mounted on a pole (Heimpel et al., 2017). Nest monitoring was carried out biweekly from the incubation phase until nestlings fledged. Bird species, brood size, and nestling mortality were recorded for every nest found.

We continued to monitor nests in the field for three days after fledging to allow for post-fledging parasitoid visitation. Nests were then retrieved, placed into a plastic bag, and taken indoors, where they were dismantled, and *Philornis* puparia were identified to species and counted. All uneclosed puparia in the nests were transferred to individual vials (25x95 mm, diameter x length) at room temperature with ambient humidity and photoperiod to monitor the emergence of either flies or parasitoids. Emerged adult flies, and parasitoids were placed individually into screw-top cryovials (1.2 ml) with 75% ethanol.

Experimental exposure of non-*Philornis* puparia

We reared naturally occurring cyclorrhaphan flies at our field site for the experimental pairing technique using raw chicken meat (0.5 kg, including skin, bone, and fat) as a bait and rearing substrate. The chicken was placed into cylindrical plastic rearing containers (10 cm diameter and 12 cm height) covered by clear plastic lids with 15 holes (diam ca. 8 mm) to allow flies to enter the container. Two additional holes were made at the bottom of the containers for drainage. Two or three baited containers per week were placed outdoors and protected from precipitation and scavengers. Each container yielded approximately 50-60 larvae, and no *Philornis* flies were reared from these containers (see Results). Larvae that had completed feeding and moved away from the meat were transferred to pupation containers, which were identical to the fly attraction containers but had 5 cm of soil at the bottom and non-perforated lids. These puparia were transferred to a third set of containers meant for exposure to parasitoids at the age of one or two days. These parasitoid-exposure containers were identical to the pupation containers but contained 2.5 cm of soil substrate and were covered with lids perforated with holes of the same size as the fly attraction containers to allow the entry of parasitoids.

The parasitoid exposure containers were furnished with 24 +/- 1.75 SEM fly puparia each. Seventeen exposure containers were attached to trees throughout the field site with twine during 2016 and 2017, with no known proximity to bird nests. In addition, 17 exposure containers (5 in 2016 and 12 in 2017) were placed adjacent to active bird nests that were part of the monitoring study described above. These containers were attached singly to trees within 10 cm of active nests during the nesting cycle's last (fourth) week. In 2017, 340 fly puparia reared as described above were placed inside 12 active bird nests in groups of 25-30 within artificial nesting cavities using soft forceps during the fourth week of the nesting cycle. Puparia placed inside or adjacent

to the nests were left for three days after the nest activity ceased to allow for parasitoid visitation. Puparia that were not placed near specific nests were left for four days. All puparia were placed individually into glass vials (25x95 mm) for the emergence of flies or parasitoids.

Insect Identification

Once nests from the monitoring study were retrieved and dismantled, *Philornis* puparia were counted and identified based on characters in Skidmore (1985), Couri (1999), and Bulgarella et al. (2015, 2017) with confirmation of representative adults by Dr. Bradley Sinclair of the Canadian National Collection of Insects (CNCI). Parasitoid adults were identified based on Bulgarella et al. (2017), Nixon (1980), Bouček (1951), with diagnostic photographs of Delvare and Huchet (2017) for *Brachymeria podagrica*. Non-*Philornis* adult flies were identified by Dr. Sinclair as well. Since puparia yielding parasitoids did not provide adult flies for identification, and puparia of the non-*Philornis* flies were not identifiable to species, we categorized the morphology of a subset of non-*Philornis* puparia before allowing them to emerge as flies, which were then identified to species. This provided a basis for determining which fly species had been attacked by parasitoids. Despite these efforts, the puparia of *Peckia* spp. (Diptera: Sarcophagidae) were morphologically indistinguishable, so our specificity statistical analyses were applied at the genus level. Specimens were stored in 90% ethanol and deposited in the University of Minnesota Insect collection.

Food webs and statistical analyses

A food web was constructed for interactions within bird nests, encompassing sampling done from 2013 – 2018. Interactions in bird nests included parasitism of birds by *P. downsi*, *P. niger*,

P. falsificus, and an unidentified species of *Philornis* and parasitism of these *Philornis* puparia by five species of parasitoids. Interactions in the containers included parasitism of seven fly species by two parasitoid species. A null model for the specificity of interactions was generated for this food web using the R Package 'econullnetr' (Vaughan et al., 2018), which takes into account low sampling completeness and allows the detection of resource preferences of consumers. The null model assumes that the interaction frequency is proportional to the combined abundance of the consumer and the resource species. The package estimates food-web metrics for each consumer species to build an interaction matrix with sampling distributions for use in statistical analyses (Vaughan et al., 2018). Once the null models were generated (based on 10,000 iterations), we estimated the specificity metrics of the consumers (*Philornis* spp. attacking birds and parasitoids attacking *Philornis* spp.) using the R Studio Package 'Bipartite.'

Multiple food web metrics have been developed, and Poisot et al. (2012) concluded that the Resource Range (RR) and the Pair Difference Index (PDI) are particularly useful for characterizing specificity due to their robustness and informativity, and we used these in our analyses. RR estimates the proportion of the host species in a web attacked by a given consumer species without considering the strength of interaction, while PDI contrasts the strongest link to a host species that an individual parasite species exhibits to links with the rest of the host species in a web (Poisot et al., 2012). Thus, using both can give a complementary view of specificity. Both indices range from 0 (absolute generalist) to 1 (absolute specialist). The observed indices were then compared to those generated by the null model. We also used econullnetr to generate preference plots, which provide a null expectation for the number of individuals of given host taxa attacked by a given parasite (or parasitoid) species, along with 95% confidence limits used

to establish significant deviations of the observed values from the null model (Vaughan et al., 2018).

We compared the abundances of either *P. downsi* or *P. niger* in nests occupied by a single species or both species of *Philornis* flies using t-tests. We also used a binomial GLM with logit link to determine the effects of the total number of *Philornis* puparia per nest, the bird species (collapsed to the most common host species - the House Wren - and all others combined), and the interaction of these two variables, on the proportion of puparia that were *P. downsi*.

Lastly, we compared parasitism rates on fly pupae occurring within artificial nests versus wild nests, using a GLM with a quasi-binomial error structure with a logit link. We used the same approach to estimate if parasitism rates differed between non-*Philornis* pupae and *Philornis* pupae. All statistical analyses were coded using the program R-Studio (RC Team, 2011).

Results

Parasitism of Birds by *Philornis* Species

We monitored 154 active nests from 2013 to 2018, comprising 34 nests within artificial cavities (nest boxes or bamboo poles) and 120 naturally occurring nests. Fifty nests contained *Philornis*, 52% natural and 48% artificial nests. We found *Philornis* spp. puparia in the nests of 11 bird species (Table S1 in Supporting Information).

The average number of *Philornis* spp. puparia found per parasitized nest was 24.3 +/- 4.65 SEM. This total was composed of three known species, *P. niger* (812 puparia in 37 nests; 21.94 +/- 4.30 per nest), *P. downsi* (388 puparia in 34 nests; 11.41 +/- 2.52 per nest), *P. falsificus* (1 puparium) and one unidentified *Philornis* species (6 puparia in 1 nest). Of the nests containing

any *Philornis*, 46% (23) contained two species, 28% (14) contained only *P. niger*, 24% (12) contained only *P. downsi*, and 2% (1) contained only *P. falsificus*, and the unidentified species of *Philornis*, respectively. In the nests containing both *P. niger* and *P. downsi*, *P. niger* significantly outnumbered *P. downsi* (*P. niger*: 30.86 ± 6.42; *P. downsi*: 14.27 ± 3.62 puparia per nest; t = 2.395, *P*= 0.023, df=28.74), but the numbers of puparia in the nests containing only one of these *Philornis* species were very similar (*P. niger*: 8.86 ± 2.36 SEM; *P. downsi* 7.32 ± 2.06 SEM; t = -0.015, *P*= 0.987, df= 16.39). The proportion of puparia that were *P. downsi* was not significantly affected by the total number of puparia per nest (Z = -1.06; res. df = 45; P = 0.2879) but was significantly lower in House Wren nests than in nests of other host species (Z = -2.82; df = 45; P = 0.0049) (Fig. 1.1). No significant interaction between these two variables was detected (Z = 1.20; df = 45; P = 0.2321).

Bird-*Philornis* food-web metrics.

P. downsi was found attacking nine out of the total 11 bird species sampled, while *P. niger* was found attacking six (Fig.1.1). The Resource Range (RR) indices for both *P. downsi* and *P. niger* were significantly lower than the null expectation, which indicates lower specificity (measured as the number of species attacked) than expected by chance (Table 1.1). The Pair Difference Index (PDI) score was significantly higher than the null expectation for *P. niger* but not significantly different from the null expectation for *P. downsi* (Table 1.1). This reflects the strong association with House Wrens for *P. niger* and a more even distribution of host use for *P. downsi* (Fig. 1.1). The preference plot analyses also showed a significant preference for the House Wren (and the Tropical Parula) for *P. niger*, while *P. downsi* exhibited a significant preference for Tropical Parula only (Fig. 1.2).

Parasitism of *Philornis* and other flies by parasitoids

There was no significant difference in the parasitism rate of *P. downsi* in artificial nests versus wild nests (quasibinomial GLM $F_{1,47} = 1.973$, $P = 0.166$), but parasitoids attacked *P. niger* at a significantly higher rate in artificial nests (wild = 2.5%, artificial = 10.4%; quasibinomial GLM $F_{1,47} = 10.173$, $P = 0.002$). Non-*Philornis* pupae placed within nests exhibited rates of parasitism that were not significantly different from those within exposure containers (n=656) adjacent to the nests (quasibinomial GLM $F_{1,32} = 14.004$, $P = 0.995$; Table 1.2). This latter result suggests that the main parasitoid emerging from non-*Philornis* puparia within nests – *B. podagrica* – mainly attacked flies in the larval stage (see Discussion). The overall rate of parasitism of *Philornis* spp. by all parasitoid species combined was significantly lower (8.5%) than that of the deployed non-*Philornis* flies (27%; quasibinomial GLM $F_{1,126} = 30.852$, $P = 0.0001$; Table 2).

Five parasitoid species emerged from *Philornis* puparia: *Trichopria* sp. (Hymenoptera: Diapriidae, *n* = 40 puparia), *Conura annulifera* (Hymenoptera: Chalcididae, *n* = 20), *Spalangia* sp. (Hymenoptera: Pteromalidae, *n* = 3), *Brachymeria* sp. (Hymenoptera: Chalcididae, *n* = 2), and *Exoristobia* sp. (Hymenoptera: Encyrtidae, *n* = 9), while two parasitoid species were reared from non-*Philornis* puparia: *Brachymeria podagrica* (Hymenoptera: Chalcididae, *n* = 207 puparia) and *Exoristobia* sp. (*n* = 4) (Fig. 1.1). We found 39 *Philornis* spp. puparia with parasitoid emergence holes but were unable to identify which parasitoid emerged; these were thus not included in the analyses but were included in the overall parasitism rate (Table 1.2).

Specificity of parasitoids attacking fly pupae in nests and containers.

The parasitoids *C. annulifera* and *Trichopria* sp. were reared from *P. downsi* and *P. niger,* but none of the non-*Philornis* hosts (Fig. 1.1). Both the RR and PDI specificity indices applied to

hosts at the genus level indicated significant specialization compared to the null models for both parasitoid species (Table 1.1). Two other parasitoid species reared from *Philornis* puparia (*Spalangia* sp. and *Brachymeria* sp.) exhibited numbers too low to assess specificity indices realistically. The preference plot analyses comparing parasitism of puparia at the species level showed a significant preference for *P. niger* by *C. annulifera* and *P. downsi* by *Trichopria* sp. (Fig. 1.3).

Non-*Philornis* fly puparia yielded seven species of cyclorrhaphan flies: *Chrysomya albiceps* (Calliphoridae), *Lucilia eximia* (Calliphoridae), *Hydrotaea* sp. (Muscidae), *Peckia ingens* (Sarcophagidae), *Peckia pexata* (Sarcophagidae), *Peckia pascoensis* (Sarcophagidae), and *Peckia* sp. (Sarcophagidae, Fig. 1.1). Only *Lucilia eximia* and *Chrysomya albiceps* reside in the Galapagos Islands, however all genera are represented in the archipelago (Table S1). Two parasitoid species were reared from one or more of these species: *B. podagrica* and *Exoristobia* sp. *B. podagrica* was reared from three of the four dipteran genera members observed (Fig. 1.1) and exhibited low specificity at the host genus level, with both specificity indices significantly lower than the null expectation (Table 1.1). Specialization of the parasitoid *Exoristobia* sp. was not significantly different from the expected null model, suggesting a more generalized behavior (Table 1.1). Preference plot analyses indicated a preference for members of three of the non-*Philornis* genera for *B. podagrica*, and no significant preference for *Exoristobia* sp. (Fig. 1.3). Lastly, preference plot analyses of interactions between parasitoids and birds indicated that *Trichopria* sp. exhibited a significant preference for House Wren and Smooth-billed Ani nests (Fig. 1.4). However, the preference plot of *C. annulifera* fell within the null model, suggesting no preference for any of the known bird species.

Discussion

In western Ecuador, *Philornis* bird parasites exhibited a broad host range, while at least two parasitoid species attacking *Philornis* puparia specialized on this fly genus. We confirmed this latter result through a novel experimental approach that provided parasitoids a choice between *Philornis*- and non-*Philornis* cyclorrhaphan fly species in a natural field setting. Special interest lies in the parasitoid *C. annulifera* which was previously identified as a promising biological control agent for use against *P. downsi* in the Galapagos Islands (Bulgarella et al., 2017). *Conura annulifera* only attacked *Philornis* puparia in our study despite the presence of other potential hosts, and these results are consistent with laboratory specificity testing (Bulgarella et al. 2017). Another parasitoid, *Trichopria* sp., exhibited a very similar level of host specificity. These results are relevant to biological control risk assessment of *C. annulifera* in Galapagos. They also demonstrate the usefulness of a novel approach to biological control risk assessment: an experimental food-web-based analysis of specificity in a field setting (see Briese et al., 2002 for a similar approach).

A comparison of the parasitoids attacking *Philornis* spp. within bird nests and other cyclorrhaphan fly species showed almost no overlap in parasitoid species, even when the non-*Philornis* puparia were experimentally deployed adjacent to or in bird nests. The main parasitoid attacking non-*Philornis* flies was the known cosmopolitan generalist parasitoid *Brachymeria podagrica* (= *B. fonscolombei,* Delvare and Huchet, 2017) that we reared from multiple species of Calliphoridae and Sarcophagidae. This parasitoid attacks fly larvae (Roberts, 1933), and we reared it from non-*Philornis* puparia placed into bird nests at the same rate as in the containers.

This suggests that female *B. podagrica* entered the chicken-baited containers to attack fly larvae before the puparia were deployed in nests.

It should be noted that naturally occurring non-*Philornis* puparia were only found in 3 of 154 nests monitored between 2013 and 2018 with a cumulative total of 5 puparia. All of these were in the family Sarcophagidae and none were parasitized.

The stark difference between specialist parasitoids attacking *Philornis* spp. and generalists attacking other fly species may reflect the diversification of parasitoid lineages associated with host use (Heimpel et al., 2021). Our results suggest a scenario in which *Philornis* puparia enjoy enemy-free space due to their cryptic location within active bird nests, and specialization of those parasitoid species that can locate the nests is facilitated by low levels of competition. This general scenario was originally posited as a driving force for the specificity of insect herbivores and their enemies (Singer & Stireman, 2005; Singer, 2008) and we hypothesize that enemy-free space has both led to habitat specificity (i.e. attacking altricial birds) of *Philornis* flies as well as specificity of the parasitoids that attack them.

We used two complementary specificity indices to characterize the specificity of *Philornis* flies attacking birds, and parasitoids attacking cyclorrhaphan flies. Resource Range (RR) measures the total number of linkages without considering linkage strength, while the Pair Difference Index (PDI) compares the strength of links between consumers and producers (Poulin et al., 2011; Poisot et al., 2012). These indices showed that both breadth and strength of interactions were consistent with a generalized pattern of host attack for the two species of *Philornis*, and a specialized pattern of attack at the genus level for the *Philornis* parasitoids *C. annulifera* and *Trichopria* sp. Beyond these indices, the null models produced by econullnetr (Vaughan et al.,

2018) allowed me to compare expected (null) and observed levels of attack on a per-species level. These analyses indicated some interactions that were more subtle than could be captured in the web-wide indices. For example, while both *Philornis* species attacked the House Wren, *Troglodytes aedon*, at a relatively high rate, *P. niger* showed a marked preference for this host species, while *P. downsi* attacked it in proportion to its abundance. The House Wren was a particularly abundant bird at our field site, and so this preference likely explains the higher abundance of *P. niger* than *P. downsi*. Also, while both *C. annulifera* and *Trichopria* sp. attacked both *Philornis* species, *Trichopria* sp. exhibited a preference for *P. downsi*, and *C. annulifera* for *P. niger*. Given the disproportionate parasitism of the House Wren by *P. niger*, the preference of *P. niger* by *C. annulifera* could, in principle, have been due to a preference for House Wren nests regardless of the *Philornis* species therein and thus represent an indirect preference through the bird host. We used the econullnetr Preference Plot approach to evaluate this hypothesis by investigating preference of the parasitoid species for bird species. This did not reveal a significant preference for the House Wren by *C. annulifera*, which suggests that the preference for *P. niger* is direct. Instead, *Trichopria* sp. showed a preference for House Wrens despite not exhibiting a preference for *P. niger*. This is also consistent with a direct, rather than an indirect preference for a *Philornis* species (in this case *P. downsi*).

Our study provides guidance to the ongoing considerations of using parasitoids as biological control agents of *P. downsi* in the Galapagos Islands, where it is invasive. First, it confirms reports from previous laboratory and rearing reports that *C. annulifera* is a specialist on *Philornis* species, and second, it provides evidence that other species found in the native range of *P. downsi* are specialists. Principal among these latter species is *Trichopria* sp., which was found in

relatively high abundance. Our studies showed very clearly that the two principal parasitoids at our site are not specific to *P. downsi,* however, and that they attack at least one other species of *Philornis*. Since no native species of *Philornis* occur in Galapagos (Fessl et al., 2018), this is of no concern from a conservation standpoint. Indeed, if established, such parasitoids could potentially provide control of *P. niger* if it were to invade Galapagos. Although our results and methods are promising concerning potential risk, they are limited to species and genera that either do not occur in Galapagos or occur there as introduced species themselves.

Biological control introductions are increasingly being used in natural habitats to protect native biodiversity from invasive species that cannot be effectively or safely controlled using other means (Van Driesche et al., 2010, 2016; Novak et al., 2021). These introductions have been conducted within the context of extensive risk assessment over the past decades (Heimpel & Cock, 2018) and particularly vulnerable habitats such as the Galapagos Islands are subject to added scrutiny (Causton 2009). This study demonstrates the use of experimental food-web-based specificity studies in the native range of prospective biological control agents to supplement laboratory-based studies to obtain a more complete and realistic assessment of nontarget risk. Our results are part of a larger risk assessment process that seeks to provide guidance to governmental agencies deliberating decisions on potential releases of parasitoids in the archipelago.

Table 1.1. Genus-level specialization metric statistics for two *Philornis* and four parasitoid species found at the Loma Alta Ecological Reserve. The tables compare observed values to the 95% confidence limits from the null model including the standardized effect size (SES) for the Resource Range and Pair Difference Index metrices. The 'Test' column indicates if specialization is significantly lower, higher or not significantly different (ns) from the expected value.

Species	Observed	Expected	Lower.CL	Upper.CL	Test	SES
Resource Range (RR)						
Philornis downsi	0.142857	0.602302	0.4981154	0.7098667	Lower	-4.64360
Philornis niger	0.428571	0.598541	0.5370455	0.6604297	Lower	-6.48318
Pair Difference Index (PDI)						
Philornis downsi	0.7946429	0.9180372	0.7780488	0.9500000	ns	-6.68197
Philornis niger	0.7083333	0.9186532	0.8967298	0.9376630	Lower	-20.1427

Species	Observed	Expected	Lower.CL	Upper.CL	Test	SES
Resource Range (RR)						
Conura annulifera	1	0.370808	0	0.75	Higher	3.188526
Trichopria sp.	1	0.237233	0	0.5	Higher	4.358681
Brachymeria podagrica	0.333333	0.040725	0	0.25	Higher	3.159367
Exoristobia sp.	0.666666	0.4640500	0	0.75	ns	1.020421
Pair Difference Index (PDI)						
Conura annulifera	1	0.857772	0.694444	0.955882	Higher	1.997835
Trichopria sp.	1	0.86237	0.75	0.9375	Higher	2.741915
Brachymeria podagrica	0.879386	0.866727	0.806616	0.907285	ns	0.490484
Exoristobia sp.	0.851852	0.854331	0.611111	0.979167	ns	-0.02871

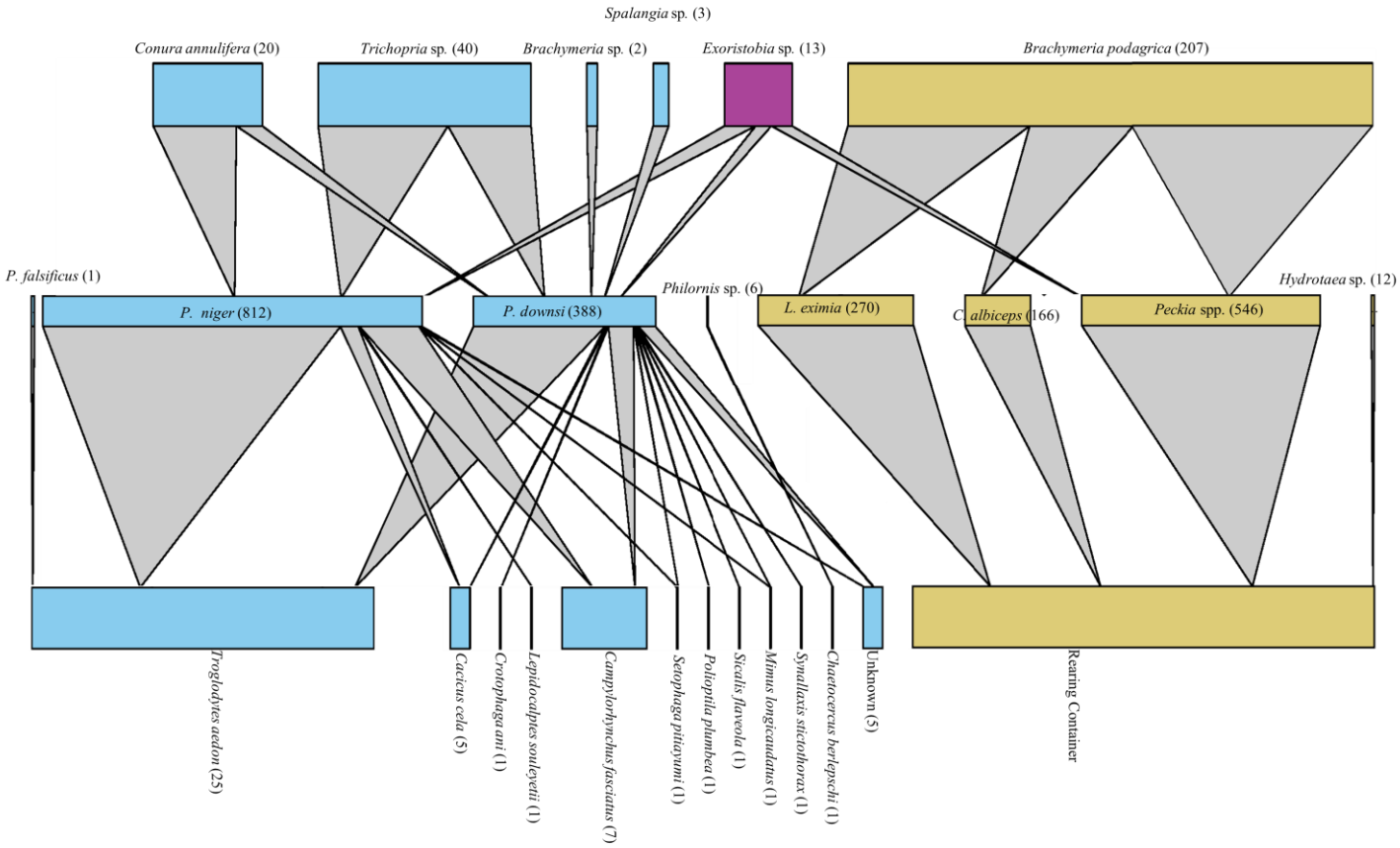

Figure 1.1. Trophic food web of observed parasitoid emergence (top level) from fly puparia (middle level) within bird nests (bottom level). Blue color denotes species that were found emerging from naturally occurring puparia within nests and gold color denotes flies and parasitoids that emerged from the deployed pupae. The numbers in parenthesis after names indicate the observed number of individuals.

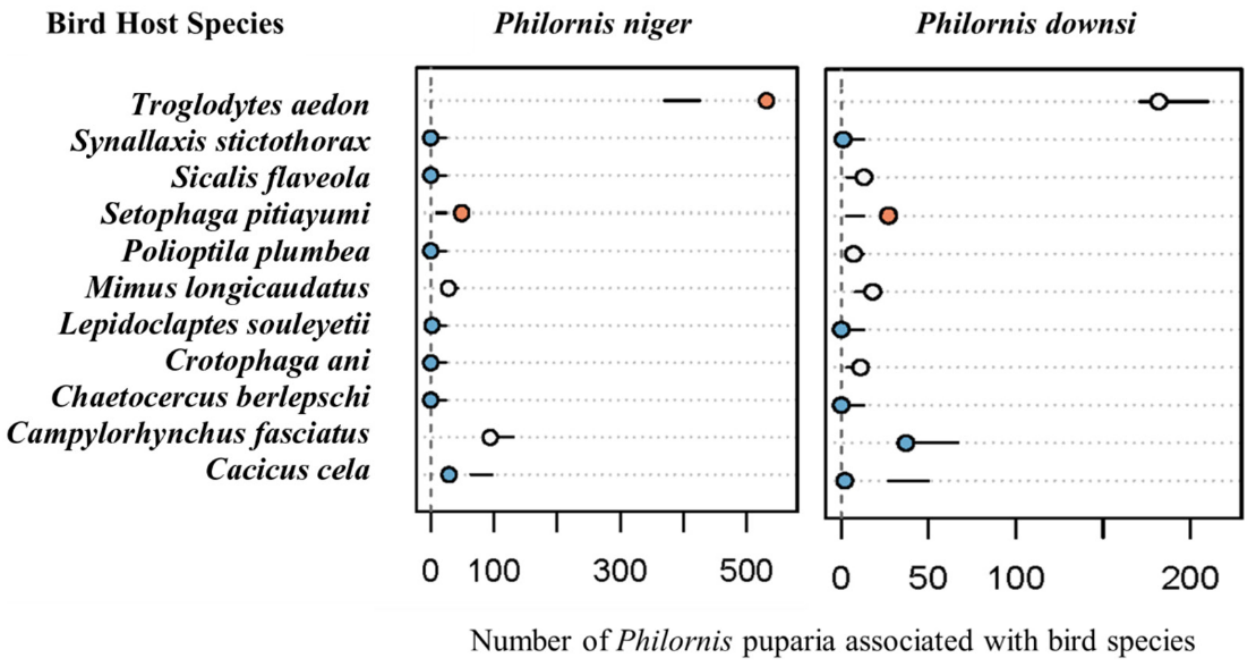

Figure 1.2. Preference plots for two species of *Philornis* parasitizing 11 bird species at the Loma Alta Ecological Reserve. The plots compare the observed interaction frequencies (dots) to the 95% confidence intervals from the null model (bars). Red dots to the right of the bar denote interactions stronger than expected, blue dots to the left of the bar denote interactions weaker than expected, and white dots within the bar denote interactions that are consistent with the null model.

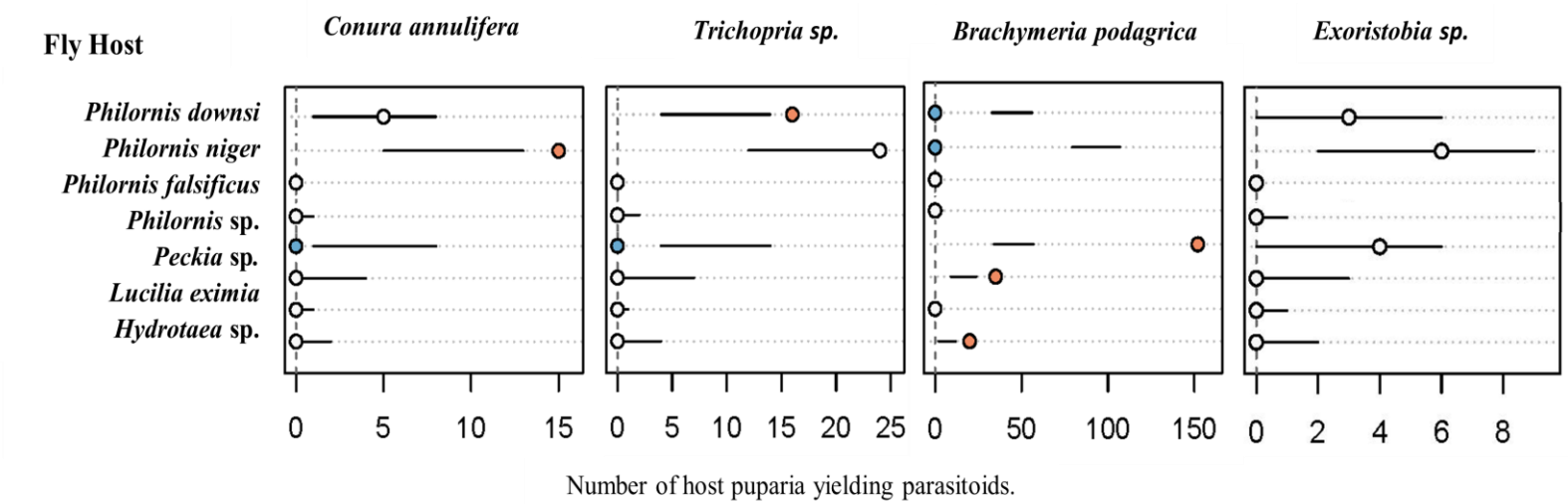

Figure 1.3. Preference plots for four species of parasitoids exposed to eight taxa of fly puparia at the Loma Alta Ecological Reserve. The plots compare the observed interactions frequencies (dots) of interactions to the 95% confidence intervals from the null model (bars). Red dots to the right of the bar denote interactions stronger than expected, blue dots to the left of the bar denote interactions weaker than expected, and white dots within the bar denote interactions that are consistent with the null model.

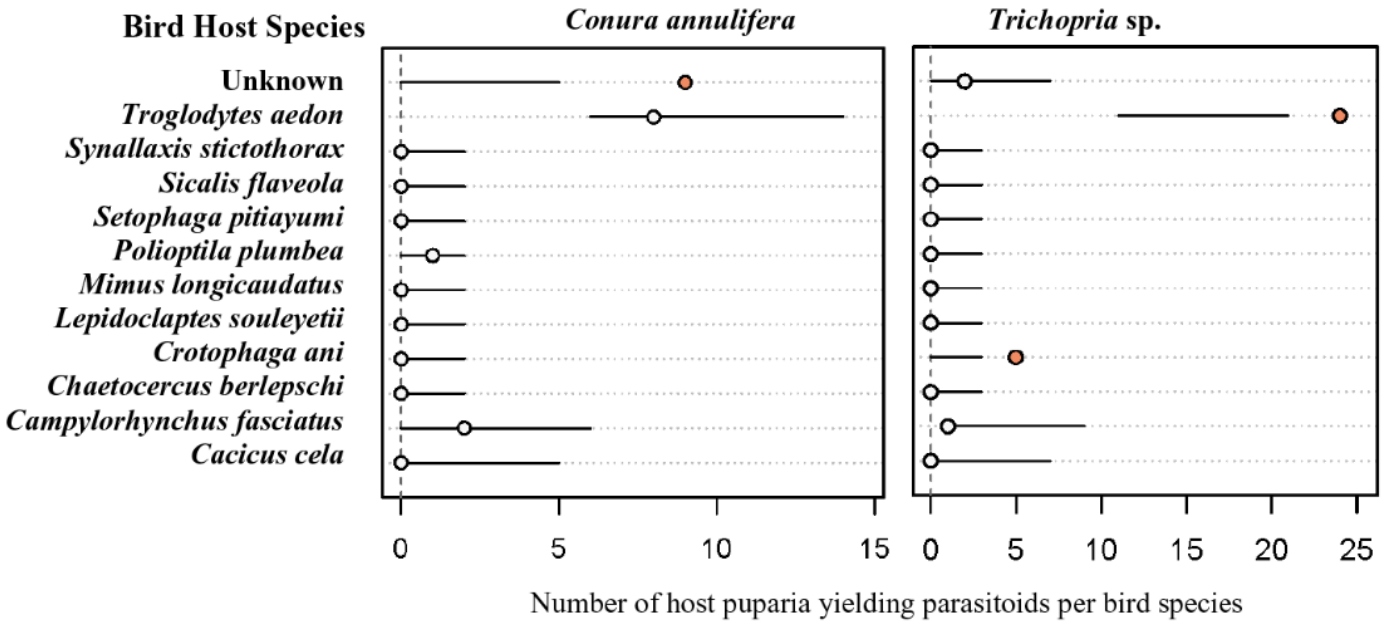

Figure 1.4. Preference plots for two species of parasitoids emerging from *Philornis* spp. puparia found within birds' nests at the Loma Alta Ecological Reserve. The plots compare the observed interactions frequencies (dots) of interactions to the 95% confidence intervals from the null model (bars). Red dots to the right of the bar denote interactions stronger than expected, blue dots to the left of the bar (blue) denote interactions weaker than expected, and white dots within the bar denote interactions that are consistent with the null model.

Chapter II: Competition among invasive and endemic carrion fly species in the Galapagos Islands with implications for biological control risk assessment

Summary

The composition of the necrobiome community in the Galapagos Islands is poorly understood, and nothing is known about the dynamics between endemic species and those introduced through human activity. To determine the composition of the carrion fly community, specifically members of the families Muscidae, Calliphoridae, and Sarcophagidae, We deployed four kinds of carrion bait traps during the cool and hot seasons at two lowland and two highland sites on Santa Cruz Island within the Galapagos archipelago. We also conducted a laboratory experiment to assess resource competition between fly species encountered in the baiting study. Of the eight fly species found in our baited traps, all were introduced except for the endemic sarcophagid, *Sarothromyiops dasycnemis.* Four endemic and one native carrion-feeding species that had been previously recorded on this island were not found. The introduced sarcophagid, *Peckia chrysostoma*, was the most abundant fly species, comprising over half of the collected specimens and it was highly dominant at the lowland sites. The endemic species, *S. dasycnemis*, was only recorded at the lowland sites during the hot season. On the other hand, the calliphorid species were dominant at the highland sites. Experiments demonstrated that *P. chrysostoma* is a strong competitor against other carrion fly species in the Galapagos necrobiome, including the endemic *S. dasycnemis*. A comparison of our data with historical records, combined with the results of our laboratory study, leads to the conclusion that introduced carrion fly species, such as *P. chrysostoma*, represent a threat to endemic carrion fly species, such as *S. dasycnemis*. Three parasitoid species were reared from 19% of the collected fly puparia. Two of these species attacked fly larvae (*Brachymeria podagrica* and *Aphaereta* sp.), while one species attacked fly puparia (*Exoristobia* sp.). We discuss our results in light of the possibility of the purposeful

introduction of a parasitoid as a biological control agent against the avian vampire fly (*Philornis downsi*; Diptera: Muscidae) in Galapagos.

Introduction

Competition among insect species is frequent and can be particularly intense on short-lived, limited and unreliable resources. Decomposing dead animals, or carrion, represent a transient natural resource that is associated with intense competition within and among consumer species (Weatherbee et al., 2017; Carmo et al., 2018). The biota that depends on carrion, or the 'necrobiome' (Benbow et al., 2013), includes a number of invertebrate taxa that can interact as competitors and/or predators during the larval stages (Prinkkilá & Hanski, 1995; Benbow et al., 2019; Komo et al., 2021). Carrion flies are crucial decomposers in the necrobiome, especially flies in the families Calliphoridae, Sarcophagidae and Muscidae as they are consistently the most commonly encountered necrophagous taxa (Kuusela & Hanski, 1982; Merritt et al., 2015; Ren et al., 2018). These flies play a vital role in breaking down and converting dead organic matter into nutrients, which they then disperse for use by other trophic levels (Merritt et al., 2015; Szpila et al., 2015; Benbow et al., 2019). The loss of these services can lead to adverse effects on the environment or human health (Carter, Yellowlees & Tibbett, 2007; Barton et al., 2013).

Necrobiome communities are vulnerable to a variety of processes that affect their component species. In particular, biological invasions can lead to the loss or reduction of populations of native species (Gessner et al., 2010; Brundage et al., 2014; Carmo et al., 2018; Spencer et al., 2020). Effects of biological invasions can be especially strong in island ecosystems where invasive species may outcompete resident species (Causton et al., 2006; Simberloff, 2010; Spatz et al., 2017). The Galapagos Archipelago, located 1,000 km from mainland Ecuador, supports at least nine endemic and two native species belonging to the most common necrophagous Diptera families Sarcophagidae, Calliphoridae and Muscidae, in addition to 21 introduced, two

cryptogenic (i.e., possibly native or introduced) and three taxonomically undetermined species from these same families (Sinclair, 2023). The role of endemic and native fly species as carcass decomposers in Galapagos is understudied, with little information on larval feeding habits, but at least eight species are suspected carrion feeders: *Lucilia pionia* (Walker), *L. setosa* (James) (Diptera: Calliphoridae), *Blaesoxipha insularis* (Townsend), *B. isla* (Curran), *B. violenta* (Walker), *B. williamsi* (Curran), *Sarothromyiops dasycnemis* (Thomson) and *Galopagomyia inoa* (Walker) (Diptera: Sarcophagidae). In addition to these species, at least 12 of the 21 species of flies from these families that have been introduced to Galapagos are known or suspected to be part of the local necrobiome (Sinclair, 2023). Studies are needed to better understand the fly fauna associated with the Galapagos necrobiome and any interspecific relationships that exist between them. Given the rapid pace of species introductions in the Galapagos Islands over the past decades (Causton et al., 2006; Toral-Granda et al., 2017), a particularly important question involves the potential displacement of endemic species by introduced species.

An additional reason for studying the necrobiome fly composition and the families mentioned above is that information on these families (Muscidae, Sarcophagidae and Calliphoridae) is relevant to the management of an introduced fly of particular importance, the avian vampire fly (*Philornis downsi* Dodge & Aitken, Diptera: Muscidae). The avian vampire fly is an invasive bird parasite that causes high nestling mortality in at least 20 endemic landbird species in Galapagos (Kleindorfer & Dudaniec, 2016; Fessl et al., 2018; McNew & Clayton, 2018). Biological control has been deemed the most promising long-term solution for controlling this fly (Fessl et al., 2018) and the parasitoid wasp *Conura annulifera* (Walker) (Hymenoptera: Chalcididae), a purported specialist of flies in the genus *Philornis* Meinert (Bulgarella et al., 2017; Ramirez et al., 2022), is considered a promising agent (Boulton & Heimpel, 2017; Boulton

et al., 2019). Although the avian vampire fly does not play a direct role in the necrobiome, the introduction of *C. annulifera* or other parasitoids could pose a threat to endemic and native carrion flies due to their relatedness to the avian vampire fly and could thus affect the composition of the local necrobiome and critical ecological services such as carcass decomposition.

Here we report on the composition and abundance of fly species associated with carrion, including introduced, endemic and native species in Santa Cruz Island, Galapagos. We also use a laboratory experiment to characterize competition between the most abundant introduced carrion fly species, *Peckia chrysostoma* Wiedemann (Diptera: Sarcophagidae), and other necrophagous fly taxa including the endemic *S. dasycnemis*. Lastly, we report on resident parasitoids of larval and puparial stages of carrion flies.

Materials and Methods

Locations

Sampling and fly collection was done in four distinct areas of Santa Cruz Island in Galapagos from late June to August and from October to early November 2021 (during the cool season), and from February to early May 2022 during the hot season (Trueman & d'Ozouville, 2010). The areas sampled were the littoral (La Playa Ratonera; -0.743717 S, -90.302998 W, elevation 0 m) and arid (El Barranco; -0.737345 S, -90.300233 W, elevation 22 m) zones in the lowlands and the *Scalesia* (Los Gemelos; -0.628276 S, -90.385643 W, elevation 600 m) and *Miconia* (Media Luna; -0.645534 S, -90.33734 W, elevation 655 m) zones in the highlands (Tye et al., 2011). These latter two sampling areas have distinct climatic conditions and plant diversity from the lowland areas; thus, the necrobiome could differ.

Collection and Rearing of Carrion Flies

Four varieties of carrion substrates, raw beef, fish, chicken meat, and broken chicken eggs, were used as bait (~500 g) for the sampling. The meat from all animal sources was not ground and contained fat, skin and bone. To deploy the substrates, we used cardboard cylindrical containers, locally known as 'tarrinas' (10 cm diameter and 12 cm height), with a perforated lid (1 cm holes), enveloped in metal chicken wire to prevent access by vertebrate scavengers and a plastic roof for rain protection. Each of the four substrates was tested once each month at each of the four locations. Only one type of substrate was deployed at a time. The containers were deployed every Monday and left in the field for 72 hours as our goal was to obtain fly larvae weekly for the length of our studies. After the allotted time in the field, the containers were placed on top of rectangular foil pans (22 cm x 30 cm) containing 5 cm of sifted soil, which were placed within mesh cages (30 cm x 30 cm x 30 cm). All mesh cages were kept at ambient temperature, humidity and photoperiod inside a wood-frame structure with mesh and chicken wire walls and a galvanized roof. This building was located at the El Barranco site. Fully developed, post-feeding larval dispersal was observed, and third-instar larvae crawled out of their containers, dug, and pupated within the soil, with pupation occurring 1-2 days later. On the third day, the soil in each tray was sifted, and the puparia were collected and placed in cardboard cylindrical containers (6 cm diameter and 6 cm height) with a mesh lid secured by a rubber band, to wait for insect eclosion. Adult flies and parasitoids emerged from these puparia, and we considered that any emerging parasitoids had attacked the flies during the larval stage. After eclosion, adult flies were identified to species by Ana K. Torres, of the Charles Darwin Research Station and Dr. Bradley J. Sinclair from the Canadian National Collection of Insects and Canadian Food Inspection Agency, and the parasitoids were identified to genus by Dr. John Luhman from the

University of Minnesota Department of Entomology Insect Collection. Exemplars of reared fly and parasitoid specimens are housed in the Charles Darwin Research Station Terrestrial Invertebrates Collection (ICCDRS).

Insect Colonies

Single-species colonies initiated with emerged flies were established with the purpose of controlling the stage and age of flies that were used in our other experiments. Previously identified flies (see above) were placed into individual mesh cages similar to those described above, one species per cage, and the cage was furnished with an aluminum tray with sifted soil and a container similar to the ones used to deploy substrates in the field. Beef meat and fat (~350 g) was placed in the containers and replenished weekly. Adult flies were provided water and granulated sugar *at libitum* within the cages and misted with potable water twice a day. All colonies were kept at ambient temperature, humidity, and photoperiod in the building described above. The soil was sifted every four days to extract puparia and dispersing third-instar larvae. We used the puparia to survey pupal parasitoids (see below) and the third-instar larvae for other experiments and colony growth. Colonies of the following five species were established: *P. chrysostoma*, *Peckia lambens* (Wiedemann) and *S. dasycnemis* (all Sarcophagidae), *Lucilia eximia* (Wiedemann) (Calliphoridae) and *Hydrotaea aenescens* (Wiedemann) (Muscidae).

Survey for pupal parasitoids

Thirty puparia of a mix of different fly species taken from the colonies described above were deployed in the field in the same types of containers used to attract flies. These were set alongside the fly baits for 72 hours. The species of deployed puparia varied depending on availability, but all containers included puparia of *P. chrysostoma*, *P. lambens*, *L. eximia* and *H.*

aenescens (in both seasons) and *S. dasycnemis* puparia during the hot season. After 72 hours, the puparia were recovered and placed into individual emergence vials to allow for fly or parasitoid emergence.

Competition experiment

This experiment assessed whether the larvae of *P. chrysostoma*, the most abundant carrion fly species found in bait containers (see Results), would outcompete larvae of the other fly species in a controlled setting using methods adapted from Ferraz (1993). Females from the following species were taken from our colonies and placed into single-species containers with 1-day-old decomposing meat for oviposition: *P. chrysostoma, P. lambens, S. dasycnemis, L. eximia* and *H. aenescens*. Oviposition occurred within the first 30 hours. Groups of 10 first-instar larvae of a given species were transferred to petri dishes (60 x 15 mm) containing 10 g of raw ground beef along with ten first-instar larvae of *P. chrysostoma*. Petri dishes with 20 first-instar larvae of either *P. lambens, L. eximia, S. dasycnemis* or *H. aenescens* served as controls. The petri dishes with the ground beef and the larvae were placed inside a container as described above ('tarrina'), with a 10-cm layer of sifted soil which served as a pupation medium for post-feeding larval dispersal. The treatments and controls were carried out simultaneously under ambient conditions averaging temperatures of 26.19^{o} C (± 2.822 SD) and humidity of 86.55% (± 7.732 SD). The larvae were left for seven days after which puparia were sifted from the soil and counted. The puparia were then transferred to emergence containers similar to the containers mentioned above.

Statistical analyses

All statistical analyses were performed in R- studio (RStudio Team, 2023) including a one-way ANOVA to determine if there were bait preferences and a post-hoc Tukey test to compare

among them. Species accumulation curves for the different seasons and baits were generated using the R package '*vegan'* with Michaelis-Menten asymptotic curves fitted to the data for each graph. To detect effects of the abundance of introduced flies and parasitoids on the endemic *S. dasycnemis* in the field sampling study we used generalized linear models (GLM) with Quasi-Poisson error structure implemented in the R package '*lme4'*. We only used data gathered at the two lowland sites (littoral and arid) during the hot season, as *S. dasycnemis* was only found at these locations during that period of time. In the first analysis, the number of eclosing *S. dasycnemis* adults emerging per container was the dependent variable and the numbers of eclosing adults of each of five introduced fly species were the independent variables. In the second analysis, the effect of the number of fly puparia from which parasitoids emerged (pooled over fly species) on the number of *S. dasycnemis* adults eclosing per container was estimated for the two parasitoid species that were found in the range of *S. dasycnemis*. For both analyses we used variation inflation factors to detect multicollinearity among the species used as independent variables in the R program '*car'.* For the competition experiment, we compared the abundance of puparia of *P. chrysostoma* vs. the other fly species using t-tests.

Results

Location and seasonality

The bait traps (cool season, n= 44; hot season, n= 50) yielded a total of 3,337 individual fly puparia, 26% (n= 866) of which were found during the cool season and 74% (n= 2,471) of which were found during the hot season. All traps yielded flies and the average number of fly puparia reared per bait trap was 40.80 +/- 4.69 SEM. The number of puparia did not differ between bait types (ANOVA, $F(3, 93) = 1.053$, $p = 0.373$). A total of eight carrion fly species were reared

from the bait traps: *P. chrysostoma* (n = 1,879 adults; 56.3%), *P. lambens* (n = 794; 23.4%), *L. eximia* (n = 293; 8.6%), *Chrysomya albiceps* (Wiedemann) (n = 153; 4.5%), *S. dasycnemis* (n = 131; 3.9%), *Synthesiomyia nudiseta* (Wulp) (Diptera: Muscidae, n = 41; 3%), *H. aenescens* (n = 25; 0.7%) and *Chrysomya megacephala* (Fabricius) (n= 21; 0.6%) (Diptera: Calliphoridae). *Sarothromyiops dasycnemis* was the only endemic species reared (all other species were introduced [Sinclair, 2023]). This species was found in traps baited with eggs, fish and chicken exclusively in the two lowland sites during the hot season.

The greatest number of flies were reared from traps in the littoral (46%) and arid zones (30%) (lowland sites), with fewer reared from traps in the *Miconia* (19%) and *Scalesia* zones (5%) (highland sites (Fig. 2.1)). Seven fly species were recorded in the lowlands, with a greater diversity and abundance of Sarcophagidae than Calliphoridae or Muscidae in the littoral zone (97% of abundance) and the arid zone (92% of abundance). In the highlands, six species were recorded with Calliphoridae being more abundant than Sarcophagidae or Muscidae in the *Miconia* zone (46%) and the *Scalesia* zone (64%) (Fig. 2.1). Some species were found only in the lowland habitats (*S. dasycnemis* and *H. aenescens*) and others were found almost exclusively in the highlands (*C. albiceps* and *C. megacephala*). Only a single species was found in roughly equal proportions at all sites (*P. lambens*). All other species were found in both the lowland and highland habitats but varied in abundance. *Peckia chrysostoma* was the dominant species at both lowland sites but rather rare in the *Scalesia* zone, and *L. eximia* was dominant in both of the highland sites and rarer in the lowlands. *Synthesiomyia nudiseta* was found in the arid and *Miconia* zones, and *C. megacephala* was only found in the *Miconia* zone.

The species accumulation curves suggest that our finding of eight fly species was approximately five below the expected asymptote of 13 ±2.351 (SD) species based on the Michaelis-Menten relationship (Fig. 2.2a). The observed species richness during the hot season reached the expected asymptote of 7 ± 0.938 (SD), while the observed species richness of 5 during the cool season underestimated an expected asymptote of 10 ± 1.484 species (SD; Fig. 2.2 b,c). All bait types attracted between five and eight fly species, with accumulation curves indicating the maximum number of species for specific substrates: chicken (observed = 8, expected = 14 ± 2.088 SD), eggs (observed = 5, expected = 7 ± 1.184 SD), fish (observed = 6, expected = 10 ± 1.760 SD), and beef (observed = 8, expected = 9 ± 1.750 SD) attracted eight species (Fig. 2.2d-g).

The GLM analyses found a significant positive correlation between the abundance of *P. lambens* and the endemic species *S. dasycnemis,* but no other significant correlations were observed (Table 2.2; Fig. 2.3).

Parasitoid emergence

From the 3,337 fly puparia collected from the field, a total of 642 yielded parasitoids, representing three different species (Table 2.1): *Brachymeria podagrica* (Fabricius) (Hymenoptera: Chalcididae, n = 634 puparia containing *B. podagrica*, representing 98.8% of all parasitoids reared), *Exoristobia* sp. (Hymenoptera: Encyrtidae, n = 7 puparia, 1.1%), and *Aphaereta* sp. (Hymenoptera: Braconidae, n = 1 puparium, 0.2%). *Brachymeria podagrica* was found in both seasons and at all locations and baits, emerging exclusively from puparia that had been collected from the field as larvae. Based on puparium morphology, we determined that *B. podagrica* attacked only sarcophagid flies with *P. chrysostoma* being the most common host

(78% of all pupae attacked by this wasp), followed by *P. lambens* (21%), and *S. dasycnemis* (1%). This parasitoid was found parasitizing 26.4% of the *P. chrysostoma* puparia collected (Table 1). The second most abundant parasitoid, *Exoristobia* sp., attacked only *P. chrysostoma* in the *Scalesia* zone (n = 6; representing 86% of all *Exoristobia* sp. reared) and *Peckia lambens* (n = 1; 14%) in the littoral zone. Both of these host species were attacked as puparia during the cool season. Lastly, the parasitoid *Aphaereta* sp. emerged from a single *L. eximia* puparium; the fly was exposed to this parasitoid as a larva in the *Miconia* zone during the cool season. To our knowledge this is the first published report of a species in the genus *Aphaereta* in Galapagos.

The GLM analyses found a significant positive correlation between the endemic fly, *S. dasycnemis* and abundance of the larval parasitoid wasp, *B. podagrica* (Table 2.2; Fig. 2.3).

Experimental competition assays

Larvae of all of the fly species in the interspecific competition experiment experienced significantly greater levels of mortality when paired with *P. chrysostoma* than when paired with equivalent numbers of larvae belonging to their own species (Fig. 2.4): *H. aenescens* ($t = -6.989$, $P = < 0.0001$, $df = 17.015$), *L. eximia* ($t = -9.043$, $P = < 0.0001$, $df = 9.918$), *P. lambens* ($t = -16.2$, $P = < 0.0001$, $df = 14.169$) and *S. dasycnemis* ($t = -9.774$, $P = < 0.0001$, $df = 12.76$).

Discussion

Of the eight fly species reared in our baited traps on Santa Cruz Island, all are listed as having been introduced to Galapagos through human activity except for the endemic sarcophagid *S. dasycnemis*. The sarcophagids *P. chrysostoma* and *P. lambens* dominated the bait traps in the lowlands and the calliphorids were the most abundant in the traps in the highlands. The most abundant species overall was *P. chrysostoma*, which made up over half of the fly individuals

collected and was present in almost all baits during both seasons. Furthermore, we experimentally showed that the presence of this species in carrion induced mortality in the larvae of four other fly species, including the endemic *S. dasycnemis*. Given the information collected in this study, we concluded that *P. chrysostoma* outcompetes other carrion fly species in the Galapagos necrobiome and recommend that *P. chrysostoma* be assigned the status of invasive species in the Galapagos Islands per the definition of the International Union for the Conservation of Nature (IUCN, 2021).

The Michaelis-Menten relationship models suggest that we captured more than half of the available carrion fly species at our study sites. Among the species that were not collected but that were expected at our field sites are four endemic and one native species that are likely associated with carrion and that have been documented on Santa Cruz Island (Sinclair, 2023). These species are the sarcophagids *Blaesoxipha insularis*, *B. violenta* and *Galopagomyia inoa* and the calliphorids *Lucilia pionia* and *L. deceptor*. The absence of these fly species in our traps may be attributed to several factors including temporary absence during our sampling period, the stage of carrion decomposition, bias towards non-natives instead of natives, non-carrion feeding habits, or displacement by introduced species, such as *P. chrysostoma*. It is also possible that these species are specialized on carrion originating in Galapagos, and that they are reluctant to colonize our baits, three of which were sourced from introduced species (beef, chicken, eggs). Such a scenario is possible for *G. inoa*, the larvae of which have been reported feeding on eggs of native sea turtles and eggs and carcasses of endemic land tortoises and sea lions (Sinclair, 2023; S. Aguirre, unpublished; Román et al., 2023). However, it should also be noted that the Galapagos Islands have been subjected to multiple introductions of non-native species, including cattle and chickens, over the past 200 years (Hickman, 1985), so that these bait sources should

not be completely novel to carrion flies. Additionally, a separate study including endemic Galapagos lizards and passerine birds did not yield a higher proportion of endemic vs. introduced carrion flies (C. Lehnen, pers. com.).

Taken together, our results provide support for the displacement hypothesis and suggest a shift in the composition of the dipteran community on Santa Cruz Island from historical records. Lopes (1978) reported that *S. dasycnemis* (as *Sarothromyiops canus* Townsend) was the most common sarcophagid species in samples that he identified from the archipelago. He also reported identifying specimens of *G. inoa*, *B. violenta* and *B. insularis* that were collected on Santa Cruz Island in 1964. To our knowledge, *B. violenta* has not been collected on Santa Cruz Island since this date, and neither have the endemic Calliphoridae in spite of several Diptera surveys (Tantawi & Sinclair, 2013; Sinclair, 2023; C. Lehnen et al., pers. com.; O. Mollá, unpublished; S. Aguirre, pers. com.). The most recent records we found for *B. insularis* are from specimens located at the Charles Darwin Research Station Terrestrial Invertebrates Collection (ICCDRS) that were collected from areas in Santa Cruz not sampled in this study: El Garrapatero and the Northern side of Santa Cruz Island, in 2004. *Galopagomyia inoa*, on the other hand, was collected in 2016 from the arid zone area sampled in this study, and samples are housed at the Canadian National Collection of Insects (B.J.S.; CNCI).

In our studies, *P. chrysostoma* was the dominant sarcophagid species on this island (55% of the total number of flies reared in bait traps), compared to the endemic *S. dasycnemis* at 3.9%, and was especially dominant in the baits set out in the littoral and arid zones, making up 68% and 58% of the carrion flies, respectively. *Peckia chrysostoma* is a forensically important flesh fly native to South America and it is commonly found in decaying human corpses. It was first

recorded in Galapagos in 1935 (Causton et al., 2006) and its prevalence on Santa Cruz Island is consistent with the findings in its native range, especially in Brazil, where *P. chrysostoma* was found to be the most common fly species on carrion (Lopes, 1973; d'Almeida, 1984; Dias et al., 1984; Tavares et al., 1988). Additionally, Ferraz (1993) demonstrated that *P. chrysostoma* is a strong competitor under controlled conditions and suggested that its salivary secretions or metabolic waste could aid in creating a toxic environment for competing species. Another hypothesis for the success of *P. chrysostoma* is that it inhibits oviposition and larviposition by other fly species. (Bradley & Sheppard, 1984).

Sarothromyiops dasycnemis was the only endemic carrion-feeding fly species encountered in our study and it was found in 12% of our baits during the hot season, but not at all during the cool season. Notably, it was only found at the lowland sampling sites, placing it in direct contact with *P. chrysostoma*, which was dominant in the traps at these sites. Both lowland sampling sites were near Puerta Ayora, the most populated town on Santa Cruz Island, with an estimated 12,000 inhabitants (Toral-Granda et al., 2017). The presence of *S. dasycnemis* near human settlements may reflect the adoption of synanthropic (human-associated) behavior in this species as well as a broad feeding range. The presence of *S. dasycnemis* may have been partially enabled by protection through parasitism of its main competitor, *P. chrysostoma*. The parasitoid, *Brachymeria podagrica*, was the most abundant species attacking carrion flies in our study and emerged mostly from *P. chrysostoma*. This parasitoid tends to prefer larger larvae as hosts (Roberts, 1933; Delvare & Huchet, 2017) and *P. chrysostoma* produced the largest larvae of all the carrion flies collected (I.E.R., unpublished).

Our findings show that the necrobiome of the Galapagos Islands is dominated by introduced species has implications for understanding potential interactions between the necrobiome community and any biological control agent that might be released against other fly species, such as the avian vampire fly, *P. downsi*. One proposed biological control agent of *P. downsi*, the wasp *C. annulifera*, is an obligate parasitoid of cyclorrhaphan fly puparia that appears to specialize on *Philornis* species (Bulgarella et al., 2017; Ramirez et al., 2022). All fly species collected in this study were cyclorrhaphans, and thus potential hosts of *C. annulifera* (Boulton & Heimpel, 2018). However, our studies suggest that competition with introduced fly species may be a far greater threat to the survival of endemic or native carrion flies than the release of *C. annulifera* would be. Furthermore, the pupation behavior of many insects including carrion flies (introduced, native and endemic species) would likely protect them from pupal parasitoids, as it usually takes place underground (Frederickx et al., 2014). Indeed, in this study we observed that the endemic *S. dasycnemis* larvae burrow underground to pupate (I.E.R., unpublished).

Carrion-feeding species could be at risk from a parasitoid introduction against *P. downsi* if they are found in bird nests with dead nestlings. In its native range of mainland Ecuador, *C. annulifera* shows a strong association with bird nests, particularly those containing puparia of *Philornis* spp. (Ramirez et al., 2022). Little is known about the prevalence of carrion flies in bird nests in Galapagos Islands and directed surveys are required to determine whether nests are frequented by endemic or native species. To date, the only endemic species found in nests with dead chicks is *B. insularis*, reported in nests of *Geospiza fortis* and *G. fuliginosa* at two locations on Santa Cruz Island in 2004 (B. Fessl, pers. comm). On the other hand, reports of introduced carrion flies are more common. For example, Fessl & Tebbich (2002) found the introduced *P. lambens* (as *Sarcodexia lambens*) in 34 out of 177 wild bird nests surveyed on Santa Cruz Island

and in a subsequent study Fessl et al. (2006) reported two introduced sarcophagids, *P. lambens* (29.6% prevalence) and *Blaesoxipha plinthopyga* (Wiedemann) (14.8% prevalence), in 27 nests.

In summary, this research contributes novel information on the necrobiome community in Galapagos. In particular we highlight the prevalence of introduced carrion flies and the notable paucity of endemic or native carrion fly species within the necrobiome on Santa Cruz Island. We suggest that endemic and native carrion flies have been outcompeted and displaced by introduced species, notably *P. chrysostoma*, which we consider to be invasive in the Galapagos Islands.

Table 2.1. Total number of parasitoids eclosing from the puparia of the eight carrion fly species reared in this study with the proportion of fly puparia parasitized in parentheses.

Fly Species	***Brachymeria podagrica*** **n= 634**	***Exoristobia*** **sp. n= 7**	***Aphaereta*** **sp. n= 1**	**Total parasitism**
Peckia chrysostoma n= 1,879	496 (26.4%)	6 (0.3%)	0	502 (26.7%)
Peckia lambens n= 794	132 (16.6%)	1 (.01%)	0	133 (16.8 %)
Sarothromyiops dasycnemis n= 131	6 (4.6%)	0	0	6 (4.6%)
Synthesiomyia nudiseta n= 41	0	0	0	0
Hydrotaea aenescens n= 25	0	0	0	0
Lucilia eximia n= 293	0	0	1 (0.3%)	1 (0.3%)
Chrysomya albiceps n= 153	0	0	0	0
Chrysomya megacephala n= 21	0	0	0	0

Table 2.2. Results of GLM (Generalized Linear Models) with Quasi-Poisson error structure investigating effect of abundance of fly and parasitoid species reared on abundance of the endemic fly, *Sarothromyiops dascynemis*. Data only gathered from lowland sites during hot season. See Chapter 3.

Fly Species	**Estimate**	**Std. Error**	**Z**	***P***
(Intercept)	1.417	0.559	2.534	0.018 *
Peckia chrysostoma	-0.036	0.021	-1.699	0.102
Chrysomya albiceps	-0.935	295.002	-0.003	0.997
Peckia lambens	0.034	0.010	3.178	0.004 **
Lucilia eximia	0.078	0.113	0.687	0.498
Synthesiomyia nudiseta	0.044	0.116	0.380	0.707
Parasitoid species	**Estimate**	**Std. Error**	**Z**	***P***
(Intercept)	-3.886e-01	2.451e-01	-1.585	0.113
Brachymeria podagrica	3.394e-02	3.312e-03	10.249	<2e-16 ***
Exoristobia sp.	-1.42e+01	1.760e+03	-0.008	0.994

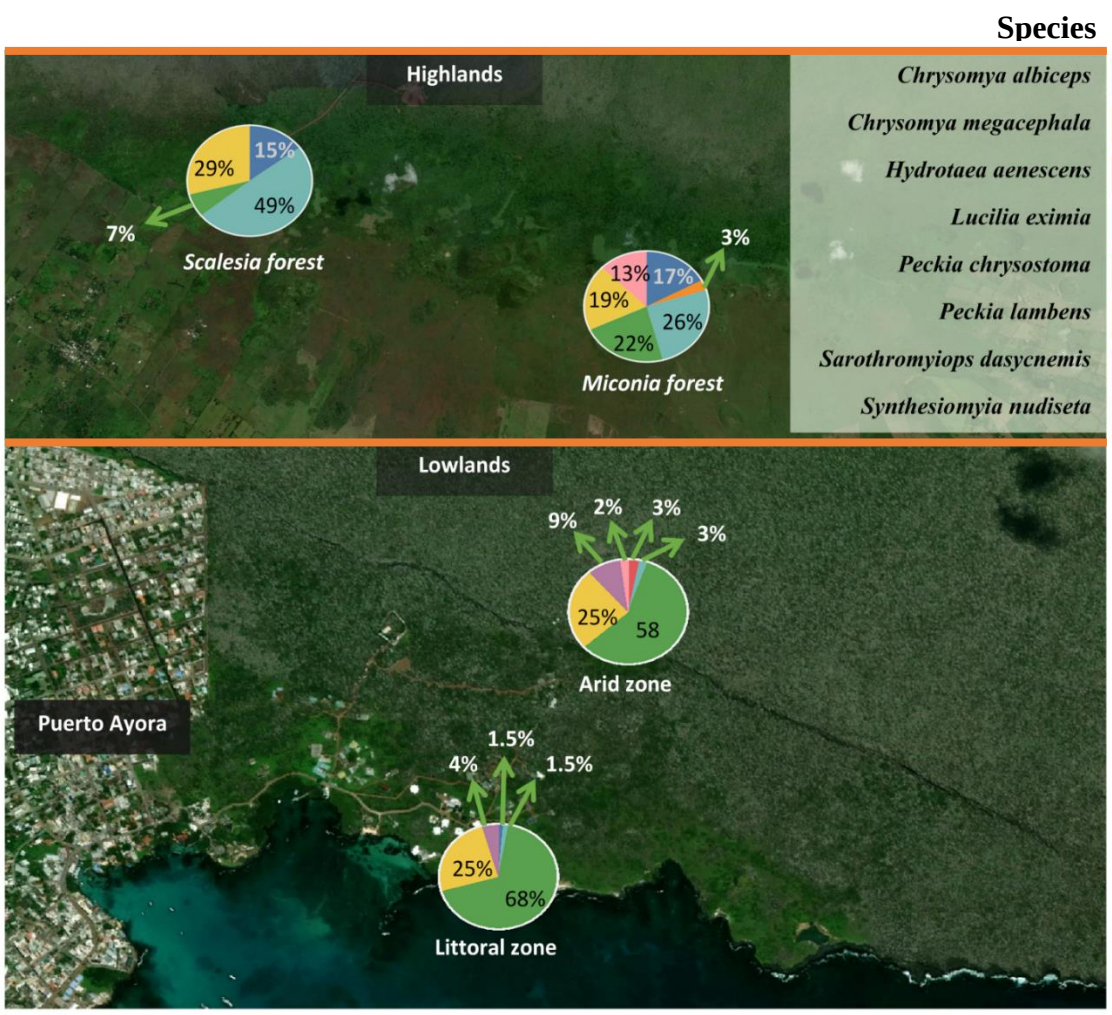

Figure 2.1. Map of the highland and lowland locations where baits were deployed in Santa Cruz Island, Galapagos. Pie charts indicate the proportion of each species reared at each per site. The total numbers of larvae collected per site were: Littoral zone: 1,534, arid zone: 1,006, miconia zone: 619, scalesia zone: 178.

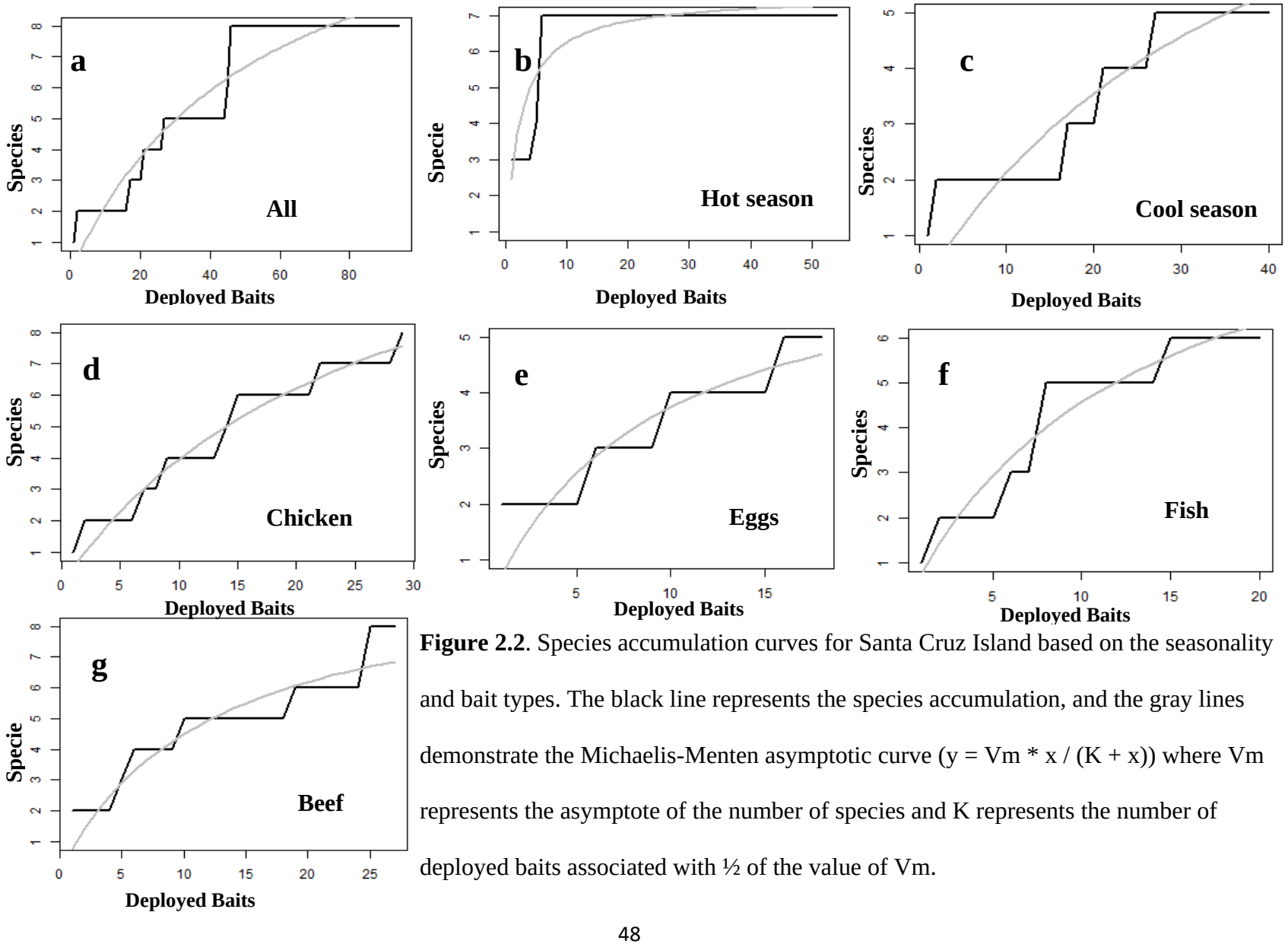

Figure 2.2. Species accumulation curves for Santa Cruz Island based on the seasonality and bait types. The black line represents the species accumulation, and the gray lines demonstrate the Michaelis-Menten asymptotic curve (y = Vm * x / (K + x)) where Vm represents the asymptote of the number of species and K represents the number of deployed baits associated with ½ of the value of Vm.

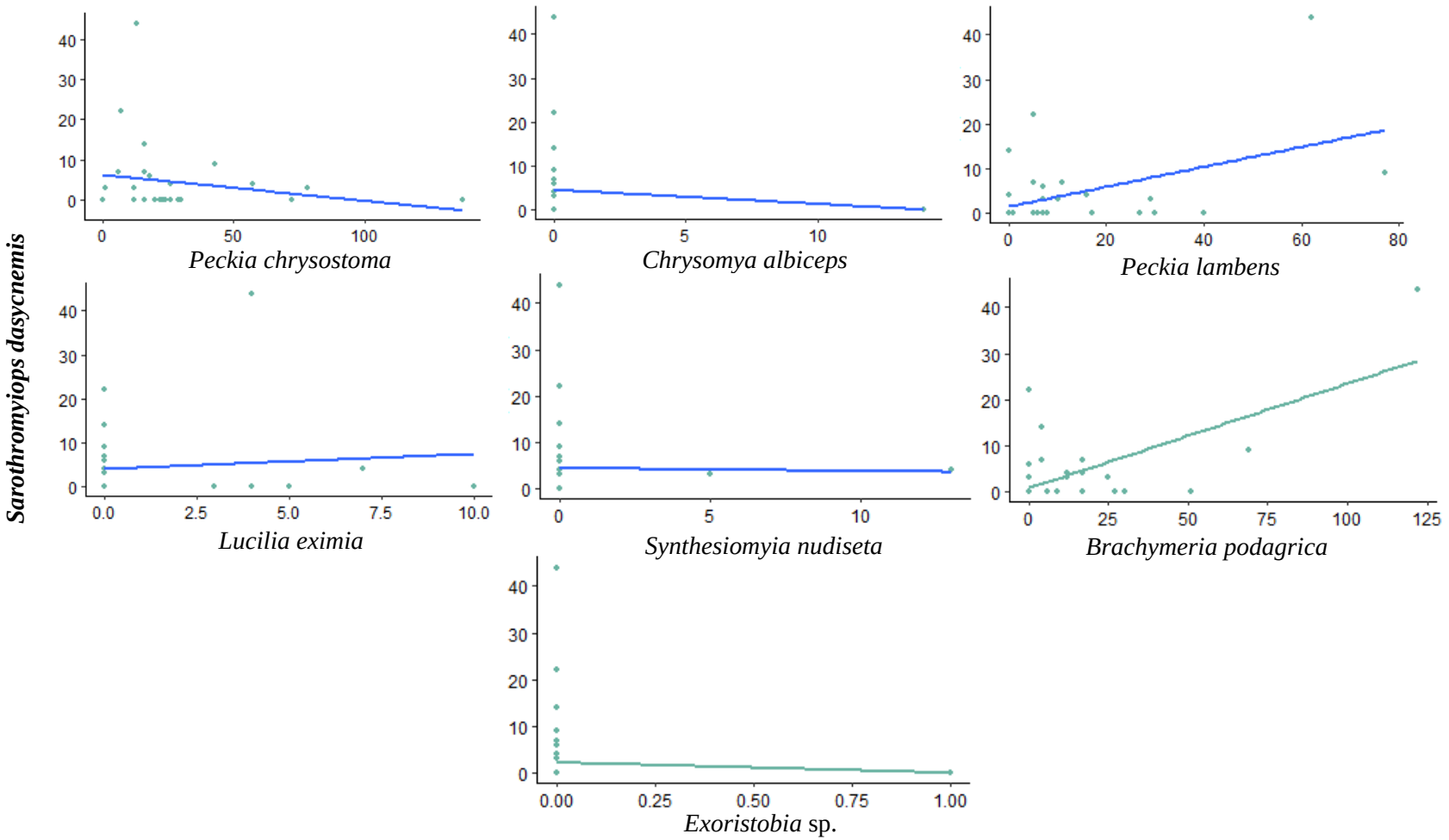

Figure 2.3. Scatterplots of abundance of flies and parasitoids (x-axis) that were found in the habitat of the endemic fly *Sarothromyiops dasycnemis* (y-axis). Linear regression lines were fitted to the graphs for flies (blue) and parasitoids (green).

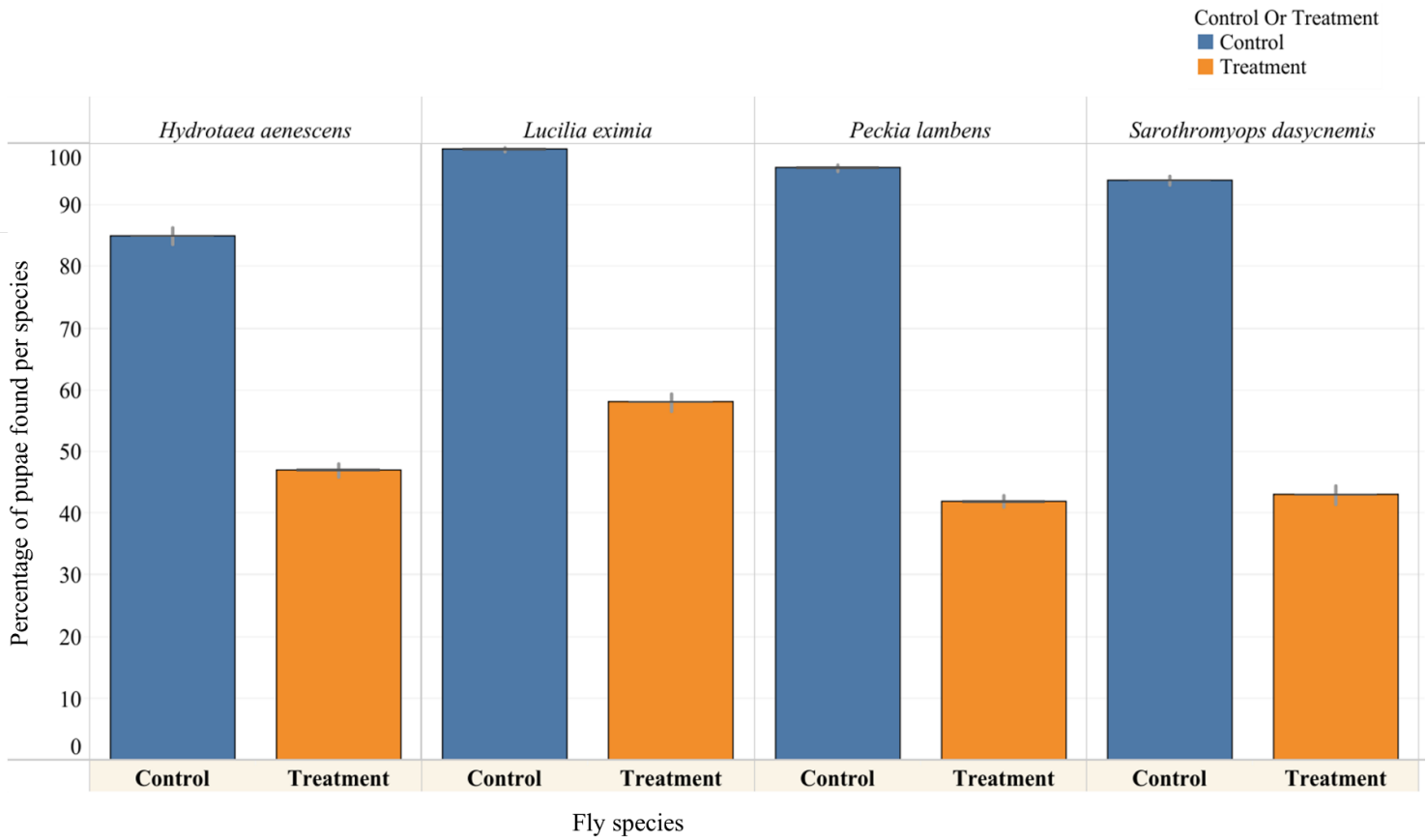

Figure 2.4. Bar plots for the results of the competition experiment in which the percentage of pupae of four fly species is shown for treatment and control exposures. 'Treatment' indicates the presence of *Peckia chrysostoma* larvae while 'Control' indicates the presence of only the indicated species. Gray lines represent the standard error of the mean.

Chapter III: Safety in biological control of the avian vampire fly in the Galapagos Islands: Implications of a refuge from parasitism of non-target hosts.

Summary

The invasive avian vampire fly, *Philornis downsi*, poses a significant threat to endemic bird species in the Galapagos Islands, including Darwin's Finches. The importation of specialized natural enemies is a promising strategy to control *P. downsi* in Galapagos, and the parasitoid *Conura annulifera* has received the most attention as a potential biological control agent thus far, including studies assessing its potential to attack non-target species. The potential native and endemic non-target species that are hypothetically most at risk from a release of *C. annulifera* are those closely related to *P. downsi*, which include carrion flies in the families Muscidae, Sarcophagidae and Calliphoridae. Many of these species pupate in the soil, and since *C. annulifera* attacks the pupal stage of its hosts, I hypothesized that subterranean pupation would constitute a spatial refuge from parasitism, lessening the risk of non-target effects. I thus investigated the burrowing behavior of resident Galapagos carrion flies, as well as the ability of *C. annulifera* to locate and attack underground puparia of these species. Our trials revealed that, of seven species of carrion fly species tested, six exhibited burrowing prior to pupation. This included the Galapagos endemic sarcophagid *Sarothromyiops dasycnemis*. Notably, *C. annulifera* females did not exhibit burrowing behavior and thus were not able to locate or attack subterranean puparia. Our study thus suggests a low level of risk to endemic and native non-target fly species in Galapagos that are most likely to be physiologically suitable hosts since many of these species are known to or expected to pupate within the soil.

Introduction

Predicting the safety of releasing biological control agents often begins with the assessment of acceptance and suitability of potential non-target species under controlled laboratory conditions (Van Driesche & Reardon, 2004; Sheppard et al. 2005; Heimpel & Mills, 2017). A common question, however, is how well such laboratory results translate into host use by biological control agents in the field. And while biological control agents displaying high host specificity in laboratory studies typically display similar patterns in the field (Pemberton, 2000; Kimberling 2004; Ramirez et al. 2022), the situation can be different for agents exhibiting a broader host range in laboratory studies. For some of these cases, the relatively broad host range found in the laboratory overestimates the number of host or prey species actually attacked in the field (Wapshere 1989; Hajek & Butler 2000; Morehead and Feeny 2000; van Driesche et al. 2003; Cock et al. 2021). This is reflective of a commonly seen pattern where the 'physiological' host range is broader than the 'ecological' host range (Heimpel & Mills, 2017), a pattern which is itself an example of the ecological maxim that the 'realized' niche is a subset of the 'fundamental' niche (Futuyma & Moreno, 1988). In the former dichotomy, the 'physiological host range' is a list of host species that a consumer can recognize, attack, and use for development, and the 'ecological host range' is the list of these species attacked in the field. The overestimation of host ranges in laboratory studies can be due to variety of ecological filters that create refuges for non-target species (Abram et al., 2023). A number of such refuges have been identified for non-target species of biological control agents, including phenological mismatch (Hasan & Delfosse 1995; Wyckhuys et al. 2009; Catton et al. 2015), spatial protection from parasitism or predation (Hofkin et al. 1992; Causton et al., 2004; Johnson et al. 2005; Wyckhuys et al. 2007) and mutualistic interactions (Wyckhuys et al. 2007). Non-target species may also be less attractive

than target species to biological control agents (Wyckhuys & Heimpel, 2007; Yong et al. 2007; Malek et al. 2021). All of these factors can lower the ecological availability of suitable non-target species with respect to what may be found in laboratory assays. While lowered ecological availability of non-target species does not necessarily indicate that they are not at risk (Abram et al. 2023), it does reduce the risk that they are attacked, and this lower level of risk should be included in decisions weighing potential risks and benefits of biological control releases (Heimpel et al. in review).

In the Galapagos Islands, an invasive parasitic fly, *Philornis downsi* Dodge & Atkin (Diptera: Muscidae), commonly known as the avian vampire fly, threatens endemic land bird species, in particular the group of bird species known collectively as Darwin's Finches (McNew & Clayton, 2018). *Philornis downsi* females oviposit in bird nests, and the larvae feed on the blood and tissue of nestlings leading to high nestling morality (Kleindorfer & Dudaniec, 2016; Fessl et al., 2018; McNew & Clayton, 2018). Thus, research on the control of *Ph. downsi* in the Galapagos Islands has been prioritized by the Galapagos National Park and the Charles Darwin Foundation (Boulton et al., 2019). Short- and long-term control methods against *Ph. downsi* have been considered (Fessl et al., 2018), and the importation of one or more species of specialized biological control agents has been deemed promising given the potential for long-term sustainable control (Boulton & Heimpel, 2017; Boulton et al., 2019).

Conura annulifera Walker (Hymenoptera: Chalcididae) is a parasitoid of *Ph. downsi* that has been reported emerging from the puparia of various *Philornis* species in Trinidad, Brazil, Argentina and Ecuador (Dodge & Aitken, 1968; Delvare, 1992; Bulgarella et al. 2015, 2017). *Conura annulifera* is not expected to be capable of successfully attacking any insect species

outside of the cyclorrhaphan Diptera, since it oviposits ectoparasitically within the gap between the dipteran pupa and puparial case (Bulgarella et al., 2017), a structure known only in the Cyclorrhapha (Boulton & Heimpel, 2018). But its host range seems to be much narrower than the entirety of the cyclorrhaphan Diptera since both laboratory and field studies have indicated that *Philornis* species were attacked, while other species of cyclorrhaphans were not (Bugarella et al., 2017; Ramirez et al., 2022). The field studies were conducted in mainland Ecuador, where *C. annulifera* was observed attacking two *Philornis* species (*Ph. downsi* and *Ph. niger*) within bird nests, but not other cyclorrhaphan species experimentally placed adjacent to bird nests (Ramirez et al., 2022).

While these studies were promising from the standpoint of the safety of a potential *C. annulifera* release in Galapagos, they did not use non-target species present in the Galapagos Islands. Furthermore, they did not consider the natural pupation behavior of the non-target host species, which could affect the likelihood of attack. This latter consideration is important since many cyclorrhaphan species, particularly carrion flies in the families Calliphoridae, Sarcophagidae, and Muscidae, tend to pupate in the soil (Gomes et al., 2006; Sanford et al., 2015), a behavior that is hypothesized to protect them from parasitism (Geden, 2002; Voss et al., 2009; Frederickx et al., 2014). It is not clear, however, whether resident Galapagos cyclorrhaphans adopt this behavior, and if so, it would provide protection from *C. annulifera* attack. In order to better predict the vulnerability of Galapagos non-target dipterans to attack by *C. annulifera*, I conducted laboratory studies to determine the extent of subterranean pupation by resident Galapagos cyclorrhaphan flies, as well as the ability of *C. annulifera* to attack subterranean puparia. This research therefore evaluated the hypothesis that potential non-target hosts of *C.*

annulifera in Galapagos experience reduced ecological availability for parasitism through a spatial refuge within the soil.

Methods

Location

The fly burrowing trials were conducted in the arid zone of Santa Cruz Island in a wooden structure that had served previously as a bird rehabilitation area (Ramirez et al., in press). The structure has mesh wire as walls and a galvanized tin roof and is located in El Barranco (-0.739376 S, -90.301995 W), adjacent to the Charles Darwin Research Station. The larval burrowing trials were done under environmental conditions of 27ºC ± 1.323 and 88% ± 5.975 RH and ambient photoperiod during March 2022. The *C. annulifera* burrowing trials were done in two different locations, one at the Escuela Superior Politécnica del Litoral University (ESPOL) in Guayaquil during September 2022 and one at the Charles Darwin Research Station (CDRS) at the Santa Cruz Island during December 2022. The conditions were 26ºC ± 1.32 and 69% ± 3.44 R.H. and ambient photoperiod for the ESPOL laboratory and 25ºC ± 0.02 and 70% ± 2.32 R.H. with 12-hour intervals of artificial light and dark for the Charles Darwin Research Station quarantine laboratory.

Insect Colonies

Established colonies of seven species of cyclorrhaphan dipterans were used for the digging trials (see also Ramirez et al., in press) the colonies were in individual per-species mesh cages with 30 cm x 30 cm x 30 cm in dimensions. These consisted of three sarcophagid species: *Peckia chrysostoma*, *Peckia lambens* and the endemic *Sarothromyiops dasycnemis*, two calliphorid

species: *Lucilia eximia* and *Chrysomya albiceps* and two muscid species *Hydrotaea aenescens* and *Synthesiomyia nudiseta*. Other than *S. dasycnemis*, all of these species are considered introduced in Galapagos (Sinclair, 2023). Water was provided to fly colonies through a 90 ml polyethylene container and lid with a hole of approximately 1 cm in diameter. This hole was plugged with a cotton wick extending to the container's bottom, allowing the cotton to absorb and dampen the exposed portion. Additionally, the cages were misted twice daily with potable water. Granulated sugar was provided *ad libitum* within the cages, and all colonies were kept at ambient temperature, humidity, and photoperiod in the building at El Barranco described above.

Conura annulifera were kept inside 30 x 30 cm plastic cages in groups of 20 females per cage, within a larger mesh cage, and were provided with water and honey *at libitum* under the conditions and locations mentioned above.

Burrowing behavior of fly larvae

For each fly species, I identified final-instar larvae from the colonies demonstrating post-feeding dispersal behavior ('wandering'; Denlinger & Zdárek, 1994) and used soft forceps to transfer them individually to soil arenas. The soil arenas were transparent plastic aspirator tubes (6.5 cm height x 2.5 cm diameter) filled to 5 cm with soil covered with mesh lids secured with rubber bands. The soil was a locally collected Alfisol from El Barranco in Santa Cruz (Lasso & Espinosa, 2017) that was dry from which debris were removed. I placed 20 larvae from each of the seven fly species into the arenas individually. In addition, I evaluated 20 control larvae for each species, which were placed individually into identical arenas without soil. The control and treatment trials were run simultaneously, with larvae were left to pupate for three days. The depth at which puparia were found the end of this time period was measured. The pupae were

then placed individually into glass vials (25 x 95mm) for emergence and adult emergence was recorded during the following two weeks.

Conura annulifera burrowing trials in Galapagos and Mainland Ecuador.

Before initiating parasitoid burrowing trials, I exposed *Ph. downsi* puparia to female *C. annulifera* to ensure that the parasitoids were in condition to parasitize. This was done immediately before the initiation of the burrowing treatments, using 14 and 16 *C. annulifera* at ESPOL and CDRS, respectively, exposed to two or three *Ph. downsi* puparia each in identical arenas as described above for the fly larval burrowing trials without sand. I observed parasitoid behaviors for 15 minutes to determine whether puparia were stung.

To determine whether *C. annulifera* females burrow underground and, if so, attack puparia, I placed these parasitoid individuals onto soil arenas that contained a single fly puparium. I used *Pe. chrysostoma* as a model organism for these trials as it was the most abundant carrion fly species in the El Barranco area found by Ramirez et al. (in press) and because larvae of this species were found to burrow in our trials (see Results). The arena for this experiment was identical to the burrowing trials mentioned above using the same type of soil in Galapagos. The same methods were used for ESPOL trials, but I used a Vertisol soil from a dry forest patch at the edge of the Chongón-Colonche Mountain Range adjacent to the ESPOL campus (Moreno et al., 2018). The *Pe. chrysostoma* puparia were placed in the arena individually as third (final) - instar larva, which burrowed into the soil immediately (n = 34 third instar larvae), and all burrowed into the soil, with pupation occurring at an average depth of 4.01 ± 0.160 SE mm. As a second treatment, *Ph. downsi* puparia (n = 20) were manually buried to a depth of two centimeters. Due to the limited number of wasps, most were used for both treatments. I included

this treatment to see whether a known suitable host (*Ph. downsi*) would elicit burrowing behavior despite the fact that larvae of this species do not naturally burrow into the soil. I introduced the parasitoids on the second day after larval burrowing (or placement), and individual parasitoids were left in the arenas for two hours. I observed the parasitoids for the first 15 minutes to document whether behavioral patterns related to burrowing, such as antennation of the soil, attempts at burrowing, or 'stinging' into the soil. Following the two-hour interval, the parasitoids were removed from the arena, and the pupae were placed into individual vials to monitor adult emergence.

Statistical Analyses

I used a linear regression in RStudio (R Core Team, 2023) to examine if the puparial size of the flies would influence the depth at which they were found within the soil. This analysis was done for the six species that exhibited burrowing behavior and thus excluded *C. albiceps* (see Results). I used the estimated volume of a cylinder, $v = \pi(w/2)^2 * l$, where w and l are puparial width and length, respectively, as a measure of pupal size. The per-species average of this size estimate was then used as the independent variable in the regression, and the per-species average of the burrowing depth was used as the dependent variable. Additionally, I employed a Generalized Linear Model (GLM) with a binomial error structure to compare the eclosion outcome between larvae provided with soil and those without soil in the larval digging trials and a GLM with Poisson regression to compare the number of days it took for adults to eclose with and without soil. Finally, I used a GLM with a binomial error distribution to investigate the potential for underground parasitism of *Pe. chrysostoma* and *Ph. downsi* by *C. annulifera*. This analysis included a set of three host-based treatments as the dependent variable: subterranean *Pe.*

chrysostoma and *Ph. downsi*, and *Ph. downsi* without soil, with *C. annulifera* emergence as the dependent variable.

Results

Fly Burrowing trials.

Six of the seven fly species (all except *C. albiceps*) exhibited burrowing behavior. Among these, *Pe. chrysostoma* and *Pe. lambens* puparia were found at the greatest depths, exceeding 4 cm (Table 3.1). However, I found no significant correlation between the estimated puparial volume and burrowing depth among species ($F = 1.104$, $P = 0.320$), and the presence of soil did not significantly influence the timing of adult eclosion ($Z = 0.049$, $P = 0.961$).

Regarding the ability of these fly species to pupate and emerge as adults in the absence of soil, *S. dasycnemis* exhibited a marginally significant improvement in emergence in the presence of soil, while there were no statistically significant trends for any of the other species (Table 3.2).

Conura annulifera burrowing trials.

During our initial 15-minute observation, 24 of the 30 parasitoids attacked and stung at least one *Ph. downsi* puparium. This indicates that most of the parasitoids were capable of stinging suitable hosts at the time of the trials. During the first 15 minutes after placing the female *C. annulifera* wasp onto the soil arena, no antennation, attempted burrowing, or apparent stinging into the soil was observed. Additionally, the emergence of adult parasitoids only occurred for the no-soil *Ph. downsi* control treatment. Fourteen of these hosts yielded parasitoids, while none of the buried puparia (neither *Pe. chrysostoma* nor *Ph. downsi*) yielded adult parasitoids ($\chi^2 =$

20.552, $P < 0.001$). Additionally, I noted that once placed on soil, the parasitoids spent considerable time attempting to dislodge soil particles from their integuments, resulting in lethargy and eventual immobility. These observations were consistent across both study sites. Notably, three parasitoid individuals - one at ESPOL and two in Galapagos - were found dead after two hours of soil exposure.

Discussion

Out of the seven fly species tested, only *C. albiceps* did not dig prior to pupation in our study. Pupation of all of the other species occurred at or below a depth of 1 cm. In addition, I found that *C. annulifera* cannot burrow or reach subterranean fly puparia and thus indicates that soil is an effective barrier to *C. annulifera* attacks, and that insects that exhibit obligate subterranean pupation are at exceedingly low risk of being attacked by this parasitoid species. This applies to the endemic fly species *Sarothromyiops dasycnemis*, which is a Cyclorrhaphan carrion fly species in the family Sarcophagidae that is endemic to the Galapagos Islands. As noted in the Introduction, the potential physiological host range of *C. annulifera* is expected to be limited to Cyclorrhaphan fly species based on the oviposition behavior of females and the development position of larvae (Bulgarella et al., 2017; Boulton & Heimpel, 2018).

The pupation stage in holometabolous insect species is widely acknowledged as a critical phase in their development, marked by increased vulnerability due to limited mobility. During this period, insects are exposed to various biotic and abiotic factors, including the threats of parasitoids and adverse weather conditions (Stenoien, 2017). Carrion fly species, like many other insects, have evolved a defensive strategy involving post-feeding larval dispersal and solitary

underground pupation (Greenberg, 1990; Gomes et al. 2006). Consequently, it is not unexpected to observe such behavior in endemic insect species like *Sarothromyiops dasycnemis* or *Galapagomyia inoa* in the Galapagos Islands. The Galapagos Islands host a total of 13 species of cyclorrhaphan dipterans in the superfamily Oestroidea, comprising both endemic and native species (Sinclair, 2023), and from which pupation behavior is known for two of the 13.

While the pupation behavior of most of these species remains largely unknown, literature on their congeners suggests that many are likely to pupate within soil or other refuge sites, offering protection against predation or parasitism. Table 3.3 presents a comprehensive list of native and endemic Oestroidea in the Galapagos Islands, shedding light on potential refuge sites for these insect species.

Our observation that *Chrysomya albiceps* did not exhibit subterranean pupation was surprising, given that previous studies have reported burrowing behavior in this species (Grassberger et al., 2003; Gomes & Von Zuben, 2005; Gomes et al., 2009). However, the pupation location of this species largely depends on environmental conditions such as temperature, where digging was more frequently observed at 20ºC (Gomes et al., 2009). In our study, the average temperature was above 20º C, which could explain its reluctance to burrow. Thus, the case of *C. albiceps* illustrates the phenomenon of facultative subterranean pupation, which may be present in other cyclorrhaphan species as well.

While our study indicates a spatial refuge from parasitism of some non-target host species in Galapagos, an introduction of *C. annulifera* could still raise concerns for endemic or native carrion-feeding cyclorrhaphan species that pupate above-ground, specifically within bird nests that contain deceased nestlings, as noted by Ramirez et al. (in revision). However, only a single

incident in 2004, on Santa Cruz Island, of an endemic sarcophagid, *Blaesoxipha insularis*, has been documented within a nest with dead nestlings (B. Fessl, pers. comm). On the contrary, introduced carrion flies are more often reported within nests. For example, Fessl & Tebbich (2002) discovered introduced *Peckia lambens* (as *Sarcodexia lambens*) with nests, and in a subsequent study, Fessl et al. (2006) identified two introduced sarcophagids, *Pe. lambens* and *B. plinthopyga* in nests. Thus, the risk that a parasitoid would encounter endemic non-target cyclorrhaphan flies within bird nests seems exceedingly low. In addition, it is conceivable that the release of *C. annulifera* would lead to a substantial in *P. downsi* in Galapagos, and thus improved nestling survival. This would presumably lower the instances of endemic carrion flies within nests and, with it, the risk for attacks of carrion fly pupae within bird nests.

Assessing the safety of releasing biological control agents involves navigating the nuances of host specificity. While laboratory studies typically align with field patterns for agents with high host specificity, more focus is needed for those with a perceived broader range (Heimpel & Mills 2017, and references cited in the Introduction). Ecological filters, including phenological mismatches and spatial and temporal protections, might create refuges for non-target species, influencing their availability in their habitat, as showcased by this study. Acknowledging the potential overestimation of host ranges in laboratories, and the factors mitigating risks to non-target species could prove helpful in decision-making for biological control releases.

Tables

Table 3.1. Means for depth and pupal measurements, including width, length, and volume, for all fly species. It also includes survival rates for species with and without soil treatment, along with the results of Fisher's Exact tests examining the association between soil treatment and the eclosion (emergence) of fly species.

Fly Species	Mean Wid. Pupae ± SE (mm)	Mean Len. Pupae ± SE (mm)	Mean Vol. ± SE (mm^3)	Mean Depth ± SE (cm)	No. of larvae that pupated underground
Chrysomya albiceps	3.845 ± 0.055	7.335 ± 0.088	85.578 ± 2.418	0 ± 0	0/20
Peckia chrysostoma	4.425 ± 0.049	11.940 ± 0.140	183.944 ± 3.975	4.352 ± 0.141	20/20
Hydrotaea aenescens	2.112 ± 0.092	4.625 ± 0.114	14.338 ± 1.798	1.261 ± 0.366	8/20
Peckia lambens	3.501 ± 0.058	8.455 ± 0.063	78.981 ± 4.542	4.645 ± 0.149	20/20
Lucilia eximia	2.645 ± 0.046	6.705 ± 0.038	36.456 ± 2.513	3.352 ± 0.255	20/20
Sarothromyiops dasycnemis	3.000 ± 0.065	6.730 ± 0.121	47.798 ± 2.164	1.511 ± 0.340	11/20
Synthesiomyia nudiseta	3.545 ± 0.076	7.695 ± 0.228	80.533 ± 5.010	1.125 ± 0.278	10/20

Table 3.2. Results of GLM (Generalized Linear Models) with binomial regression investigating the effect of soil in the survival of resident fly species of Galapagos.

Fly Species	Estimate	Std. Error	Z value	P value	Rate of fly survival with soil/without soil
Peckia chrysostoma	18.622	6536.572	0.003	0.998	1.0 / 0.95
Hydrotaea aenescens	-0.606	0.641	-0.946	0.344	1.0 / 0.95
Peckia lambens	0.000	1.126e5	4.095e-11	1.000	0.40 / 0.55
Chrysomya albiceps	18.622	6536.572	0.003	0.998	1.0 / 1.0
Lucilia eximia	18.622	6536.572	0.003	0.998	1.0 / 0.95
Sarothromyiops dasycnemis	1.238	0.663	1.867	0.062	0.65 / 0.35
Synthesiomyia nudistea	19.180	3964.631	0.005	0.996	1.0 / 0.80

Table 3.3. List of Oestroidea species endemic or native to the Galapagos Islands and available information regarding their possible ecological availability during pupation. Entries in the 'Pupation site' and 'References' columns refer to congeners of species listed.

Superfamily	Family	Fly Species	Origin	Pupation site	References
Oestroidea	Calliphoridae	*Lucilia deceptor*	Native	Underground	Gomes et al., 2006, 2009; See results
		L. pionia	Endemic	Underground	Gomes et al., 2006, 2009; See results
		L. setosa	Endemic	Underground	Gomes et al., 2009, 2006; See results
	Sarcophagidae	*Amobia floridensis*	Native	Underground vespid nest	Sinclair, 2023; Verves & Protsenko, 2019
		Blaesoxipha insularis	Endemic	Soil or Host, underground	Pape, 1994; Allen & Pape, 1996
		B. isla	Endemic	Soil or Host, underground	Pape, 1994; Allen & Pape, 1996
		B. violenta	Endemic	Soil or Host, underground	Pape, 1994; Allen & Pape, 1996
		B. williamsi	Endemic	Soil or Host, underground	Pape, 1994; Allen & Pape, 1996
		Galopagomyia inoa	Endemic	Under sand, in turtle nest	Sinclair, 2023
		Sarothromyiops dasycnemis	Endemic	Underground	See results
	Tachinidae	*Chetogena scutellaris*	Native	Unknown	-
		Drino inca	Native	Unknown	-
		Galapagosia minuta	Endemic	Unknown	-

Chapter IV: Host Specificity of *Conura annulifera* (Hymenoptera: Chalcididae) using Galapagos Resident Carrion Diptera in No-choice Trials.

Summary

Human-facilitated biological invasions pose significant threats to ecosystem services and species conservation. In response, classical biological control has emerged as a promising strategy to mitigate the negative effects of invasive species. This chapter focuses on the potential introduction of the neotropical wasp *Conura annulifera* into the Galapagos Islands, targeting the avian vampire fly (*Philornis downsi*), a generalist bird nest parasite that endangers endemic avifauna. Through laboratory experiments, specifically employing two different approaches of no-choice trials, I reveal that *C. annulifera* can successfully attack and parasitize *Peckia chrysostoma,* a carrion fly with forensic importance in the Neotropics. Additionally, my results indicate a strong preference for the avian vampire fly, however such results raise questions about the wasp's behavior and potential implications for non-target species. Because of limitations, such as a restricted sample size and the experienced nature of the wasp, this chapter suggests the need for further research to comprehend *C. annulifera* host-finding behavior and assess its broader impact on the Galapagos carrion fly species. My findings underscore the importance of evaluating safety and ecological factors in biological control interventions, aligning with broader discussions on the safety of releasing biological control agents. The study contributes valuable insights to the ongoing discourse on addressing the avian vampire fly pressure on endemic and native bird species.

Introduction

Invasive species cause substantial damage to ecosystems and their biodiversity by competing, predating or parasitizing naïve endemic and native species (Simberloff, 2010; Bellard et al., 2017; Spatz et al., 2017; Lenzner et al., 2020). Biological invasions, particularly arthropod invasive species, tend to propagate to an easier extent due to human activities (Seebens et al., 2018; Gippet et al., 2019; Meurisse et al., 2019). In response to such effects posed by invasive species, human intervention and a variety of tools for the management have been implemented, and recently, the implementation of biological control for biological conservation has emerged as a promising tool where other methods are not viable (Van Driesche et al., 2010; Heimpel & Cock, 2018; Boulton et al., 2019). Classical or importation biological control is the intentional introduction of natural enemies of invasive species (pest) intended to suppress their pest population and to mitigate their negative effects (Heimpel & Mills, 2017). A relevant example, and the topic of my thesis, is the potential introduction of the neotropical wasp *Conura annulifera* (Hymenoptera: Chalcididae) into the Galapagos Islands.

The wasp *C. annulifera* is an idiobiont pupal-gap parasitoid that has demonstrated a high level of specialization against the genus *Philornis* (Diptera: Muscidae) in field and laboratory experiments (Bulgarella et al., 2017; Ramirez et al., 2022). The use of biological control in the Galapagos has been considered important as the invasive avian vampire fly poses a significant threat to the endemic avifauna in the Galapagos Islands. Originally introduced from South America into the Galapagos (McNew & Clayton, 2018), this fly has become a primary driver for the decline in populations of several native and endemic bird species, including two critically endangered species of Darwin's finches, putting them at risk of extinction (Kleindorfer &

Dudaniec, 2016; Fessl et al., 2018; McNew & Clayton, 2018). While adult flies feed on decaying vegetable matter, the larval stages inflict direct mortality on altricial nestlings by feeding from tissue inside and outside the nostrils, causing beak malformation and enlarged nares, while subsequent instars engage in external feeding on blood and soft tissue, leading to myiasis and eventual death (Dudaniec & Kleindorfer, 2006; Fessl et al., 2006). The larvae then pupate within the nesting material. The success of this free-living ectoparasitic fly has been attributed to the Enemy Release Hypothesis, as no natural enemies of the fly exist in the archipelago, and multiple bird species serve as suitable hosts (Boulton et al., 2019)

Historically prevalent in agricultural settings, the successful implementation of biological control has seen a notable shift towards conservation practices over the past century (Van Driesche et al., 2010; Van Driesche & Reardon, 2017; Abram et al., 2021). This transition is exemplified by its application in natural systems with a higher regard for safety, as highlighted by Heimpel and Cock (2018). A relevant example is the release of the Vedalia beetle (*Novius cardinalis*) as a biological control agent targeting the invasive cottony-cushion scale insect (*Icerya purchasi*) in the Galapagos Islands. This introduction played a pivotal role in protecting numerous species of endemic and native plants from the negative effects of the invasive insect (Hodle et al., 2013). Safety assessments evaluated the beetle's impact on endemic and native-scale insects. Although the Vedalia beetle demonstrated an ability to consume a native species, the Galapagos ground pearl insect (*Margarodes similis*), its underground life history prevented beetle attacks in the field, prompting its prompt release in the Galapagos (Causton et al., 2004).

In the Galapagos Islands, 11 endemic and native fly species belonging to the Sarcophagidae and Calliphoridae families have been identified (Sinclair, 2023). These families, taxonomically

related to the avian vampire fly, represent potential targets for *C. annulifera* (Wapshere, 1974; Kuhlmann et al., 2006; Desneux et al., 2012). Furthermore, the Sarcophagidae and Calliphoridae families play a crucial role in carrion decomposition, providing a valuable ecosystem service in the Galapagos. This service is particularly important to preserve due to the absence of large carrion-feeding fauna. Therefore, understanding the safety of *C. annulifera* concerning these families, particularly resident species of flies in Galapagos, and its potential impact on this vital ecosystem service is considered important, in addition to supplementing existing literature on the safety of *C. annulifera*.

In this chapter, I expand on the research done by Bulgarella et al. (2017) on no-choice laboratory trials using resident carrion flies as non-target hosts in the Galapagos Islands from the families Calliphoridae and Sarcophagidae. I did so by exposing a female *C. annulifera* wasp to introduced species of carrion flies, namely, *Peckia chrysostoma, Peckia lambens* and *Lucilia eximia*.

Materials and Methods

Location, Collection, and Transportation of *C. annulifera* in Mainland Ecuador

Naturally occurring pupae of *Philornis* spp. were collected from recently fledged bird nests at the Reserva Ecologica Loma Alta (1.85694 S, 80.59938 W) located in the Santa Elena province, Ecuador, as in Bulgarella et al. (2017) and Ramirez et al. (2024). The pupae within the nest were extracted by hand and separated based on pupal morphological characteristics to establish the species of *Philornis* (Bulgarella et al. 2015). Then, such pupae were transferred into individual emergence vials from which either adult flies or parasitoids emerged. The emergence vials with fly or parasitoid specimens were transported to the Escuela Superior Politécnica del Litoral

(ESPOL) laboratory in Guayaquil to be reared at 25º C, 12:12 hours D: L, and 85% relative humidity. The emerged *C. annulifera* wasps were kept in a plexiglass container (30 x 35 x 40 cm) with water sprayed twice daily and honey *ad libitum*. To reproduce *C. annulifera*, *P. downsi* pupae reared in the Galapagos (Lahuatte et al., 2016) were transported to the ESPOL laboratory to serve as hosts. The *P. downsi* pupae were exposed to parasitoids for 12 hours. The exposed *P. downsi* pupae to *C. annulifera* at the ESPOL laboratory were then transferred to individual emergence vials and placed inside a containment carrier to be transported to the quarantine facilities at the Charles Darwin Research Station (CDRS) on November 22, 2021, for the no-choice trials.

Non-Target Insect Cultures in Galapagos

Non-target carrion flies were found by deploying bait containers in two locations on The Santa Cruz Island in Galapagos (as in Ramirez et al., in press). The locations were La Ratonera (littoral zone) and El Barranco (arid zone). The bait substrates were chicken and beef meat, all bought locally with skin and fat (~500 g). To deploy the substrates, I used cardboard cylindrical containers, locally known as 'tarrinas' (10 cm diameter and 12 cm height), with a perforated lid (1 cm holes), enveloped in metal chicken wire to prevent access by vertebrate scavengers and added a plastic roof for rain protection as in Ramirez et al. (2024). The bait containers were left for four days to allow naturally occurring flies to oviposit. After the allotted time in the field, the containers were placed on top of rectangular foil pans (22 cm x 30 cm) containing 5 cm of sifted soil placed within mesh cages (30 cm x 30 cm x 30 cm). All mesh cages were kept at ambient temperature, humidity, and photoperiod inside a wood-frame structure with mesh and chicken wire walls and a galvanized roof. Fully developed, post-feeding larval dispersal was observed,

and third-instar larvae crawled out of their corresponding containers, dug, and pupated within the soil, with pupation occurring 1-2 days later. On the third day, the soil in each tray was sifted, and the puparia were collected and placed in cardboard cylindrical containers (6 cm diameter and 6 cm height) with a mesh lid secured by a rubber band to be transported into the quarantine facilities for no-choice testing. I separated and observed every morphologically different pupa yielded by the rearing, which were the identified to species by Ana K. Torres at the Charles Darwin Research Station Invertebrate Collection (CDRSIC) and Dr. Bradley J. Sinclair from the Canadian National Collection of Insects (CNCI). The identifications included two sarcophagid species, *Peckia chrysostoma* and *Peckia lambens,* and one calliphorid species, *Lucilia eximia*. These species are considered to have been introduced to the Galapagos (Sinclair, 2023).

Host Specificity in Galapagos

Using the collected fly pupae, I used no-choice tests to assess the host specificity of *C. annulifera*. More specifically, I approached the no-choice trials in two different ways. The first approach, the 'silver platter' approach, was done by placing a single pupa of a species in a small arena with a single female *C. annulifera* (Fig. 2.1A.). The second approach, the 'mass exposure' approach, consisted of five pupae of a single species instead of one in a larger arena, similar to the methods of Bulgarella et al. (2017). For the 'silver platter' approach, I used a transparent plastic aspirator tube (6.5 cm height x 2.5 cm diameter) placed upside down on top of a petri dish as arena (Fig. 4.1A); for the second approach, the arena was a mesh cage (17.5 x 17.5 x 17.5 cm) were I placed a petri dish with pupae. This cage was then placed inside a larger cage to ensure containment (60 x 60 x 60 cm) (Fig. 4.1B). I interspersed *P. downsi* pupae between trials with the other fly pupae to ensure the wasps could sting before and after the trials. Exposures for the

first approach, ‘silver platter,’ consisted of 20-minute observed exposures annotating observations during that time, and a total of three non-target species (n = 22 total pupae) were exposed to the wasp: *L. eximia* (n =4), *P. lambens* (n = 7), and *P. chrysostoma* (n = 11). *Philornis downsi* served as the positive control (n = 11). The second approach consisted of 24-hour exposure with observations for the first 20 min, and due to the results (see Results) of the previous approach, I decided only to expose *P. downsi* and *P. chrysostoma* to *C. annulifera* where a group of five pupae of a species was exposed. Four repetitions were made of *P. chrysostoma* (four repetitions of groups of five; the total number of pupae exposed is 20) with its control *P. downsi* treated the same way *P. chrysostoma* and interspersed between repetitions. All pupae were between 2 and 6 days old, including the positive controls (*P. downsi*). After every exposure, pupae were placed on individual emergence glass vials (25x95 mm), which were monitored daily for emergence. All trials were done inside the quarantine facility at the Charles Darwin Research Station on Santa Cruz Island at 25º C, 12 hours of light and 12 of dark, and 80% relative humidity. As negative controls, I set aside the same number of pupae of all species, including *P. downsi*, not exposed to *C. annulifera* under the same conditions, to account for natural mortality, as the stinging behavior of parasitoids could lead to death but not parasitism (Bulgarella et al., 2017). Our results are restricted to a single female *C. annulifera* wasp, as all other female wasps died before the beginning of the trials.

Data Analyses

I employed a Generalized Linear Model (GLM) with binomial regression to assess if the quantity of *C. annulifera* wasps emerging from *P. downsi* (target) was significantly different from all other species (non-targets), thus assessing its specificity. I also analyzed the mortality of fly

species exposed to *C. annulifera* that did not result in the emergence of *C. annulifera*, as such mortality could have been caused by the stinging of the host using logistic regressions, where the stinging behavior and presence of *C. annulifera* (predictor variables) would have influenced survivability of fly pupae that did not close as an adult insect (response variable). Both regressions were performed in R-Studio (RStudio Team, 2023).

Results

Silver platter

During the 20-minute observations, I observed antennation by *C. annulifera* of the spiracular slits in all pupae exposed to it. However, *C. annulifera* was observed stinging only two species, *P. chrysostoma* (non-target, n = 6) and *P. downsi* (target, n = 10), right after antennation. Stinging of *P. chrysostoma* occurred within the first 33 ± 4.041 SEM seconds and for *P. downsi* occurred in 74.11± 22.036 SEM seconds after wasp placement, and the handling or manipulation of the pupae, including stinging, lasted 12.644 ± 3.538 SEM minutes for *P. chrysostoma* and 12.429 ± 2.541 SEM minutes for *P. downsi*. *Conura annulifera* stung *P. chrysostoma* individual pupae an average of 1.166 ± 0.166 SEM times and *P. downsi* pupae an average of 1.400 ± 0.221 SEM times. Nevertheless, not all the stinging was productive, as only two wasps emerged from *P. chrysostoma* and seven from *P. downsi* puparia. Higher suitability of *P. downsi* over all other non-targets was observed (binomial GLM, SE = 0.971, Z = 2.948, p = 0.003) with distinct parasitism rates for *P. downsi* of 63% (n= 7 pupae yielded *C. annulifera*) and for *P. chrysostoma* of 18% (n= 2 pupae that yielded *C. annulifera*) and none for the other non-target host species. The stinging behavior and presence of *C. annulifera* suggested a negative impact on the eclosion

of adult insects (logistic regression for stinging: SE = 1.077, Z = -2.005, p = 0.044; and presence: SE = 1.056, Z = -2.835, p = 0.004; Fig. 2.1).

Mass exposure

In this second approach, during the first 20 minutes of observation, no interactions were observed between the female *C. annulifera* and the cluster of pupae in the arena (five pupae of a species). However, *C. annulifera* reproduced and emerged from *P. downsi* (n = 1) pupae and *P. chrysostoma* (n = 2 pupae) with lower rates of parasitism for *P. downsi* than the previous approach as 10% of the exposed pupae were parasitized, however similar rates for *P. chrysostoma* as 20% of pupae were parasitized. No preference for a species was observed in this approach (binomial GLM SE = 1.317, Z = -0.615, p = 0.538), and mortality due to the presence of *C. annulifera* was not significant (SE = 0.742, Z = 0.726, p = 0.467).

Discussion

My results demonstrate that *C. annulifera* can recognize a non-target species, *P. chrysostoma*, as a host and reproduce successfully in it. My results differ from the findings of Bulgarella et al. (2017), where female *C. annulifera* attacked only *Philornis* species under similar conditions (second approach). Important caveats in my results are critical to mention. First, the number of wasps used in the trial is very limited (n = 1). Inferring behavior regarding a species based on an individual does not provide meaningful conclusions about the wasp's reproductive behavior. Second, the *C. annulifera* wasp used was highly experienced, meaning that it was exposed to multiple pupae, and such exposures might have led to higher stinging rates than naïve wasps (Bodino et al., 2016). Despite such caveats, *C. annulifera* is physically able to reproduce in a

non-target species, and more research is needed to understand if such behavior expands to other species in the Galapagos Islands.

Peckia chrysostoma is a carrion fly species of forensic importance and associated with human activity (Lopes, 1973; d'Almeida, 1984; Dias et al., 1984; Tavares et al., 1988). Originally native to South America, it was first documented in the Galapagos Islands in the 1930s (Causton et al., 2006). In a study by Ferraz (1993), this species demonstrated strong competition against other carrion fly species, and Ramirez et al. (2024) showed competition and possible displacement of resident Diptera in the Galapagos, including an endemic species, *Sarothromyiops dasycnemis*. Concerns about possible parasitism of this fly by *C. annulifera* in the field, based solely on this chapter, might not be warranted, as the findings in Chapter 3 could help clarify the safety of the wasp. This research suggests that *P. chrysostoma*, along with other carrion fly species, exhibits subterranean pupation behavior, providing a spatial refuge from potential parasitism of *Conura annulifera.* These results are aligned with the broader study on host specificity and ecological factors influencing the safety of releasing biological control agents. More importantly, this biological control project mirrors the conclusions of the other biological control intervention done in Galapagos as the vedalia beetle was able to attack an endemic non-target host. However, due to the non-target subterranean life history, the vedalia beetle was deemed safe for release (Causton et al., 2004).

In my results, parasitism rates varied significantly in *P. downsi* between the two approaches used in this chapter. These notable differences could be attributed to the spatial separation between the wasp and the pupae. The distance between the parasitoid and the pupae may have influenced the parasitism rate, especially in the second approach (larger arena), where the wasp might have

stumbled upon the pupae less frequently. Many parasitoids depend on sensory cues to locate their preferred hosts, and the observed differences in parasitism rates suggest that olfactory cues could play a role in host finding for this particular species. This is unsurprising as several other parasitoids depend on olfactory cues to locate their host. Exploring and comprehending the host-finding behavior of *C. annulifera*, especially in relation to olfactory cues, could be beneficial for a more comprehensive understanding of its host-finding behavior.

Human-induced biological invasions are an ongoing threat to ecosystems, posing risks to vulnerable species. In tackling these challenges, especially when alternative control methods are not viable, classical biological control could be a powerful tool to avert extinctions based on such introductions. Consequently, prioritizing the evaluation of the safety of releasing potential biological control agents becomes paramount in such projects. While no-choice testing provides valuable insights, its limited in its ability to assess the behavior and performance of biological control agents under natural conditions. Therefore, adopting a more holistic approach to test the safety of potential biological control agents, such as field testing, is crucial for a thorough understanding of their effectiveness and impact in the pest.

Figure 4.1. A) Picture of the 'silver platter' approach arena. The *C. annulifera* female was placed with a single pupa of a fly species in no-choice trials. B) Picture of the second approach 'mass exposure' where five pupae of a single species were expose to *C. annulifera* inside the small mesh cage inside the larger plastic cage.

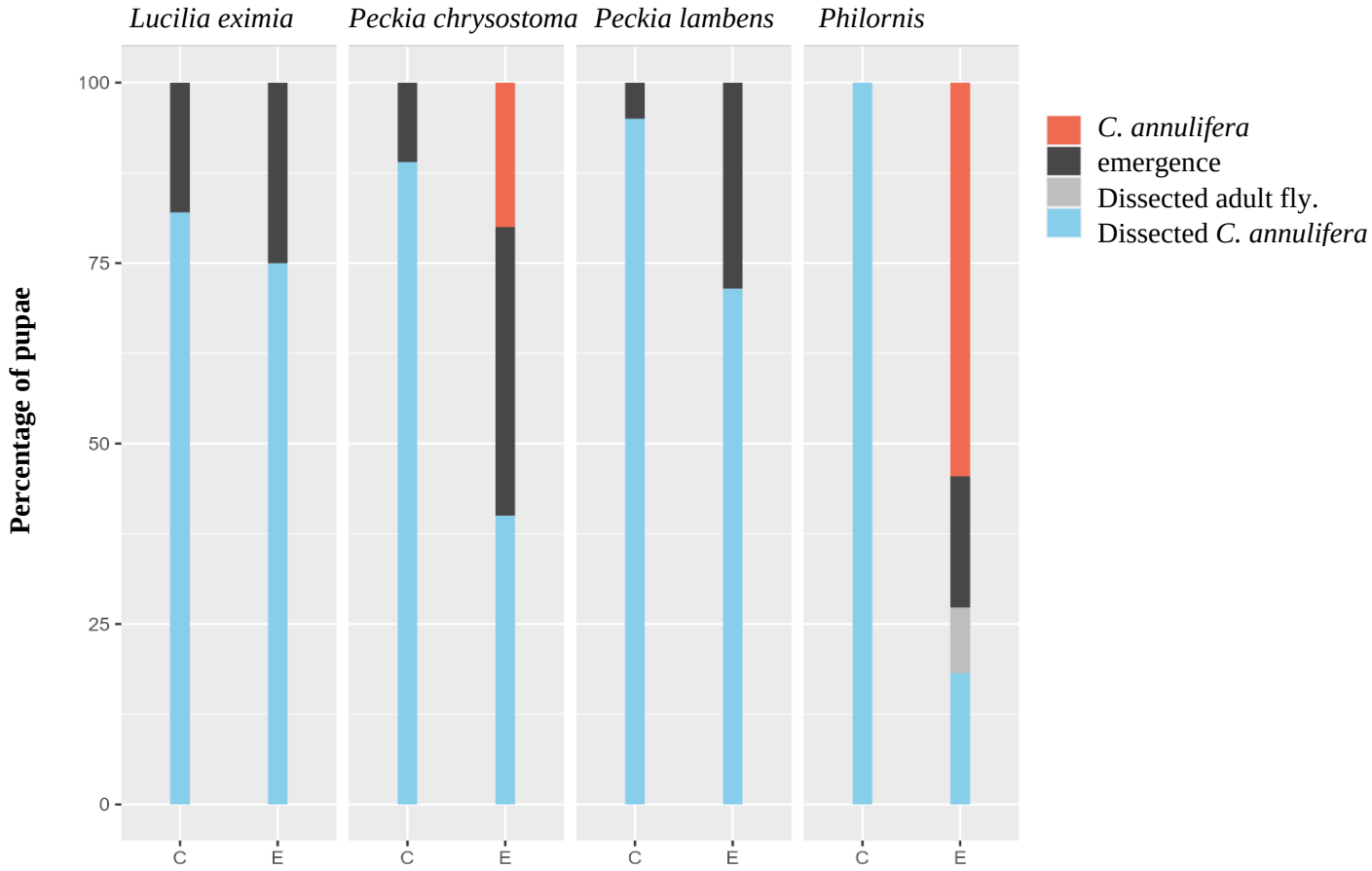

Figure 4.2. The percentage of fly pupae exposed to *C. annulifera* yielded parasitoid wasps, adult flies, dissected parasitoids, and dead puparia (dissected fly hosts) in both approaches. The x-axis represents the 'E' as experimental (exposed to *C. annulifera*) and 'C' as control (negative controls).

Bibliography

Abram, P. K., Haye, T., Clarke, P., Grove, E., Thiessen, J., & Gariepy, T. D. (2023). Partial refuges from biological control due to intraspecific variation in protective host traits. *Ecological Applications*, **33**, e2796.

Abram, P. K., Labbe, R. M., & Mason, P. G. (2021). Ranking the host range of biological control agents with quantitative metrics of taxonomic specificity. *Biological Control*, **152**, 104427.

Allen, G. R., & Pape, T. (1996). Description of female and biology of *Blaesoxipha ragg* Pape (Diptera: Sarcophagidae), a parasitoid of *Sciarasaga quadrata* Rentz (Orthoptera: Tettigoniidae) in Western Australia. *Australian Journal of Entomology*, **2**, 147-151

Barton, P. S., Cunningham, S. A., Lindenmayer, D. B., & Manning, A. D. (2013) The role of carrion in maintaining biodiversity and ecological processes in terrestrial ecosystems. *Oecologia*, **171**, 761-772.

Benbow, M. E., Barton, P. S., Ulyshen, M. D., Beasley, J. C., DeVault, T. L., Strickland, M. S., & Pechal, J. L. (2019). Necrobiome framework for bridging decomposition ecology of autotrophically and heterotrophically derived organic matter. *Ecological Monographs*, **89**, e01331.

Benbow, M. E., Lewis, A. J., Tomberlin, J. K. & Pechal, J. L. (2013) Seasonal necrophagous insect community assembly during vertebrate carrion decomposition. *Journal of Medical Entomology*, **50**, 440-450.

Bodino, N., Ferracini, C., & Tavella, L. (2016). Is host selection influenced by natal and adult experience in the parasitoid *Necremnus tutae* (Hymenoptera: Eulophidae)?. *Animal Behaviour*, **112**, 221-228.

Bouček, Z. (1951) The first revision of the European species of the family Chalcididae (Hymenoptera). *Acta Entomologica Musei Nationalis Pragae*, **27**(Suppl. No. 1).

Boulton, R. A., & Heimpel, G. E. (2018). Mind the Gap: The evolution of oviposition site and specialization in the parasitoid superfamily Chalcidoidea. *Biological Journal of the Linnean Society*, **124**, 213–227.

Boulton, R. A., Bulgarella, M., Ramirez, I. E., Causton, C. E., & Heimpel, G. E. (2019). Management of an invasive avian parasitic fly in the Galapagos Islands: is biological control a viable option? *Island Invasives: Scaling up to Meet the Challenge*, **62**, 360 - 363.

Boulton, R. A. & Heimpel, G. E. (2017). Potential for biological control of a parasite of Darwin's finches. Pp. 23–28 in Van Driesche, R. G., and Reardon, R. C., eds. *Suppressing Over-Abundant Invasive Plants and Insects in Natural Areas by Use of their Specialized Natural Enemies*. Morgantown, WV, USA: FHTET, USDA Forest Service.

Bradley, S. W., & Sheppard, D. C. (1984). House fly oviposition inhibition by larvae of *Hermetia illucens*, the black soldier fly. *Journal of Chemical Ecology*, **10**, 853-859.

Brundage, A., Benbow, M. E., & Tomberlin, J. K. (2014). Priority effects on the life-history traits of two carrion blow fly (Diptera, Calliphoridae) species. *Ecological Entomology*, **39**, 539-547.

Briese, D. T., Zapater, M., Andorno, A., & Perez-Camargo, G. (2002). A two-phase open-field test to evaluate the host-specificity of candidate biological control agents for *Heliotropium amplexicaule*. *Biological Control*, **25**, 259-272.

Bulgarella, M., Quiroga, M. A., Vera, G. A. B., Dregni, J. S., Cunninghame, F., Muñoz, D. A. M., Heimpel, G. E. (2015). *Philornis downsi* (Diptera: Muscidae), an avian nest parasite invasive to the Galápagos Islands, in Mainland Ecuador. *Annals of the Entomological Society of America*, **108**, 242–250.

Bulgarella, M., Quiroga, M. A., Boulton, R. A., Ramírez, I. E., Moon, R. D., Causton, C. E., & Heimpel, G. E. (2017). Life cycle and host specificity of the parasitoid *Conura annulifera* (Hymenoptera: Chalcididae), a potential biological control agent of *Philornis downsi* (Diptera: Muscidae) in the Galápagos Islands. *Annals of the Entomological Society of America*, **110**, 317-328.

Bulgarella, M., Quiroga, M. A., & Heimpel, G. E. (2019). Additive negative effects of *Philornis* nest parasitism on small and declining Neotropical bird populations. *Bird Conservation International*, **29**, 339-360.

Burks, B. D. (1960). A revision of the genus *Brachymeria* Westwood in America north of Mexico (Hymenoptera: Chalcididae). *Transactions of the American Entomological Society (1890-)*, **86**, 225-273.

Carmo, R. F., Vasconcelos, S. D., Brundage, A. L., & Tomberlin, J. K. (2018). How do invasive species affect native species? Experimental evidence from a carrion blowfly (Diptera: Calliphoridae) system. *Ecological Entomology*, **43**, 483-493.

Carter, D. O., Yellowlees, D., & Tibbett, M. (2007). Cadaver decomposition in terrestrial ecosystems. *Naturwissenschaften*, **94**, 12-24.

Carvalheiro, L. G., Buckley, Y. M., Ventim, R., Fowler, S. V., & Memmott, J. (2008). Apparent competition can compromise the safety of highly specific biocontrol agents. *Ecology Letters*, **11**, 690-700.

Causton, C. E. (2009). Success in biological control: the scale and the ladybird. *Galapagos: Preserving Darwin's Legacy. Ed. T. De Roy David Bateman, Auckland, New Zealand*, 184-190.

Causton, C. E., Lincango, M. P., & Poulsom, T. G. (2004). Feeding range studies of *Rodolia cardinalis* (Mulsant), a candidate biological control agent of *Icerya purchasi* Maskell in the Galapagos islands. *Biological control*, **29**, 315-325.

Causton, C. E., Peck, S. B., Sinclair, B. J., Roque-Albelo, L., Hodgson, C. J., & Lancool, B. (2006). Alien insects: threats and implications for conservation of Galápagos Islands. *Annals of the Entomological Society of America*, **99**, 121-143.

Cock, C., Mason, P. G., Haye, T., & Cappuccino, N. (2021). Determining the host range of *Diadromus collaris* (Gravenhorst) (Hymenoptera: Ichneumonidae), a candidate biological control agent for diamondback moth *Plutella xylostella* linnaeus (Lepidoptera: Plutellidae) in Canada. *Biological Control*, **161**, 104705.

Couri, M. S., Tavares, M. T., & Stenzel, R. R. (2006). Parasitoidism of chalcidid wasps (Hymenoptera, Chalcididae) on *Philornis* sp. (Diptera, Muscidae). *Brazilian Journal of Biology*, **66**, 553-557.

Couri, M. S. (1999). Myiasis caused by obligatory parasites. Ia. *Philornis* Meinert (Muscidae). *Myiasis in man and animals in the Neotropical region. Editora Pleiade, Sao Paulo, Brazil*, 44-70.

David, P., Thebault, E., Anneville, O., Duyck, P. F., Chapuis, E., & Loeuille, N. (2017). Impacts of invasive species on food webs: a review of empirical data. *Advances in ecological research*, **56**, 1-60.

D'Almeida, J. M. (1984). Sinantropia de Sarcophagidae (Diptera) na região metropolitana do Estado do Rio de Janeiro. *Arquivos da Universidade Federal Rural do Rio de Janeiro*, **7**, 101-110.

Delvare, G. (1992). A reclassification of the Chalcidini with a checklist of the New World species. *Memoirs of the American entomological institute*, **53**, 119-441.

Delvare, G., & Huchet, J. B. (2017). *Brachymeria mochica*, a new Neotropical species of Chalcididae (Hymenoptera: Chalcidoidea) discovered on the archaeological site of Huacas de Moche, Peru with a review of related species. *Zootaxa*, **4290**, 43-60.

Desneux, N., Blahnik, R., Delebecque, C. J., & Heimpel, G. E. (2012). Host phylogeny and specialisation in parasitoids. *Ecology Letters*, **15**, 453-460.

Dias, E. S., Neves, D. P., & Lopes, H. (1984). Estudos sobre a fauna de Sarcophagidae (Diptera) de Belo Horizonte-Minas Gerais. I-Levantamento taxonómico e sinantrópico. *Memórias do Instituto Oswaldo Cruz*, **79**, 83-91.

Ferraz, M. V. (1993). Interespecific competition between *Peckia chrysostoma* and *Adiscochaeta ingens* (Diptera: Sarcophagidae) larvae reared in laboratory. *Memórias do Instituto Oswaldo Cruz*, **88**, 189-194.

Fessl, B., Heimpel, G. E., & Causton, C. E. (2018). Invasion of an avian nest parasite, *Philornis downsi*, to the Galapagos Islands: colonization history, adaptations to novel ecosystems, and conservation challenges. *In Disease ecology* (pp. 213-266). Springer, Cham.

Fessl, B., Kleindorfer, S., & Tebbich, S. (2006). An experimental study on the effects of an introduced parasite in Darwin's finches. *Biological Conservation*, **127**, 55–61.

Fessl, B., & Tebbich, S. (2002). *Philornis downsi*–a recently discovered parasite on the Galápagos archipelago–a threat for Darwin's finches? *Ibis*, **144**, 445-451.

Frederickx, C., Dekeirsschieter, J., Verheggen, F. J., & Haubruge, E. (2014). Depth and type of substrate influence the ability of *Nasonia vitripennis* to locate a host. *Journal of Insect Science*, **14**, 58.

Frost, C. M., Allen, W. J., Courchamp, F., Jeschke, J. M., Saul, W. C., & Wardle, D. A. (2019). Using network theory to understand and predict biological invasions. *Trends in Ecology & Evolution*, **34**, 831-843.

Frost, C. M., Peralta, G., Rand, T. A., Didham, R. K., Varsani, A., & Tylianakis, J. M. (2016). Apparent competition drives community-wide parasitism rates and changes in host abundance across ecosystem boundaries. *Nature Communications*, **7**, 1-12.

Futuyma, D.J. & Moreno, G. (1988). The evolution of ecological specialization. *Annual Review of Ecology and Systematics* **19,** 207–233.

Geden, C. J. (2002). Effect of habitat depth on host location by five species of parasitoids (Hymenoptera: *Pteromalidae*, Chalcididae) of house flies (Diptera: Muscidae) in three types of substrates. *Environmental Entomology*, **31**, 411-417.

Gessner, M. O., Swan, C. M., Dang, C. K., McKie, B. G., Bardgett, R. D., Wall, D. H., & Hättenschwiler, S. (2010). Diversity meets decomposition. *Trends in Ecology & Evolution*, **25**, 372-380.

Gippet, J. M., Liebhold, A. M., Fenn-Moltu, G., & Bertelsmeier, C. (2019). Human-mediated dispersal in insects. *Current opinion in insect science*, **35**, 96-102.

Godfray, H.C.J., Hassell, M.P. & Holt, R.D. (1994). The population dynamic consequences of phenological asynchrony between parasitoids and their hosts. *Journal of Animal Ecology*, **63**: 1–10.

Gomes, L., Godoy, W. A. C., & Von Zuben, C. J. (2006). A review of post-feeding larval dispersal in blowflies: implications for forensic entomology. *Naturwissenschaften*, **93**, 207-215.

Gomes, L., Gomes, G., & Von Zuben, C. J. (2009). The influence of temperature on the behavior of burrowing in larvae of the blowflies, *Chrysomya albiceps* and *Lucilia cuprina*, under controlled conditions. *Journal of Insect Science*, **1,** 1–6.

Grassberger, M., Friedrich, E., & Reiter, C. (2003). The blowfly *Chrysomya albiceps* (Wiedemann) (Diptera: Calliphoridae) as a new forensic indicator in Central Europe. *International journal of legal medicine*, **117**, 75-81.

Greenberg, B. (1990). Behavior of Postfeeding Larvae of Some Calliphoridae and a Muscid (Diptera). *Annals of the Entomological Society of America*, **6**, 1210-1214.

Guimarães, J. H., Prado, A. P. & Buralli, G. M. (1979). Dispersal and distribution of three newly introduced species of *Chrysomya* Robineau-Desvoidy in Brazil (Diptera, Calliphoridae). *Revista Brasileira de Entomologia*, **23**, 245–255.

Hasan, S. & Delfosse, E. S. (1995). Susceptibility of the Australian native, *Heliotropium crispatum*, to the rust fungus *Uromyces heliotropii* introduced to control common heliotrope, *Heliotropium europaeum*. *Biocontrol Science and Technology*, **5**, 165-174.

Hajek, A.E. & Butler, L. (2000). Predicting the host range of entomopathogenic fungi. In *Nontarget Effects of Biological Control* (Follett PA & Duan JJ, eds.) Dordrecht, The Netherlands, Kluwer, pp. 263-276.

Hickman, J. (1985). The Enchanted Islands: Galapagos Discovered. *Tanager Books*, Dover New Hampshire, USA.

Heimpel, G. E., Abram, P. K., & Brodeur, J. (2021). A phylogenetic perspective on parasitoid host ranges with implications for biological control. *Current Opinion in Insect Science*, **44**, 95-100.

Heimpel, G.E. & Cock, M.J. (2018) Shifting paradigms in the history of classical biological control. *BioControl*, **63,** 27-37.

Heimpel, G. E., Hillstrom, A., Freund, D., Knutie, S. A., & Clayton, D. H. (2017). Invasive parasites and the fate of Darwin's finches in the Galapagos Islands: The case of the Vegetarian Finch (*Platyspiza crassirostris*). *The Wilson Journal of Ornithology*, **129**, 345-349.

Heimpel, G. E., & Mills, N. J. (2017). *Biological control*. Cambridge University Press.

Henneman, M. L., & Memmott, J. (2001). Infiltration of a Hawaiian community by introduced biological control agents. *Science*, **293**, 1314-1316.

Hoddle, M.S., Ramírez, C.C., Hoddle, C.D., Loayza, J., Lincango, M.P., Van Driesche, R.G. and Causton, C.E. (2013). Post release evaluation of *Rodolia cardinalis* (Coleoptera: Coccinellidae) for control of *Icerya purchasi* (Hemiptera: Monophlebidae) in the Galápagos Islands. *Biological Control*. **67**: 262–274.

Hofkin, B. V., Hofinger, D. M., Koech, D. K., & Loker, E. S. (1992). Predation of *Biomphalaria* and non-target molluscs by the crayfish *Procambarus clarkii*: implications

for the biological control of schistosomiasis. *Annals of Tropical Medicine & Parasitology*, **86**, 663-670.

IUCN. (2021). Invasive Alien Species. IUCN. Retrieved from https://www.iucn.org/our-work/topic/invasive-alien-species

Johnson, M. T., Follett, P. A., Taylor, A. D., & Jones, V. P. (2005). Impacts of biological control and invasive species on a non-target native Hawaiian insect. *Oecologia*, **142**, 529-540.

Kimberling, D.N. (2004). Lessons from history: predicting successes and risks of intentional introductions for arthropod biological control. *Biological Invasions* **6**, 301-318.

Kleindorfer, S., & Dudaniec, R. Y. (2016). Host-parasite ecology, behavior and genetics: a review of the introduced fly parasite *Philornis downsi* and its Darwin's finch hosts. *BMC Zoology*, **1**, 1-19.

Komo, L., Hedouin, V., & Charabidze, D. (2021). Benefits of heterospecific aggregation on necromass: influence of temperature, group density, and composition on fitness-related traits. *Insect Science*, **28**, 144-152.

Koop, J. A. H., Kim, P. S., Knutie, S. A., Adler, F., & Clayton, D. H. (2016). An introduced parasitic fly may lead to local extinction of Darwin's finch populations. *Journal of Applied Ecology*, **53**, 511–518.

Koop, J. A. H., Causton, C. E., Bulgarella, M., Cooper, E., & Heimpel, G. E. (2021). Population structure of a nest parasite of Darwin's finches within its native and invasive ranges. *Conservation Genetics*, **22**, 11–22.

Kuhlmann, U., Schaffner, U., & Mason, P. G. (2006). Selection of non-target species for host specificity testing. In *Environmental impact of invertebrates for biological control of arthropods: methods and risk assessment* (pp. 15-37). Wallingford UK: CABI Publishing.

Kuusela, S., & Hanski, I. (1982). The structure of carrion fly communities: the size and the type of carrion. *Ecography*, **5**, 337-348.

Lahuatte, P. F., Lincango, M. P., Heimpel, G. E., & Causton, C. E. (2016). Rearing larvae of the avian nest parasite, *Philornis downsi* (Diptera: Muscidae), on chicken blood-based diets. *Journal of Insect Science*, **16**, 84.

Lasso, L., & Espinosa, J. (2017). Soils from The Galapagos Islands. In: Espinosa, J., Moreno, J., Bernal, G. (eds) *The Soils of Ecuador* (pp. 139-150). World Soils Book Series Cham: Springer International Publishing.

Lopes, H. D. S. (1973). Collecting and rearing sarcophagid flies (Diptera) in Brazil during forty years. *Anais da Academia Brasileira de Ciencias*, **45**, 279-291.

Lopes, H. D. S. (1978). Sarcophagidae (Diptera) of Galapagos Islands. *Revista Brasileira de Biología*, **38**, 595–611

Lopez-Nunez, F. A., Heleno, R. H., Ribeiro, S., Marchante, H., and Marchante, E. (2017). Four-trophic level food webs reveal the cascading impacts of an invasive plant targeted for biocontrol. *Ecology*, **98**, 782-793.

Malek, R., Kaser, J. M., Anfora, G., Ciolli, M., Khrimian, A., Weber, D. C., & Hoelmer, K. A. (2021). *Trissolcus japonicus* foraging behavior: Implications for host preference and classical biological control. *Biological Control*, **161**, 104700.

McNew, S. M., & Clayton, D. H. (2018). Alien invasion: biology of *Philornis* flies highlighting *Philornis downsi*, an introduced parasite of Galapagos birds. *Annual Review of Entomology*, **63**, 369–387.

Memmott, J. (2000). Food Webs as a Tool for Studying Nontarget Effects in Biological Control. In: Follett, P.A., Duan, J.J. (eds) *Nontarget Effects of Biological Control*. Springer, Boston, MA. Nixon, G. E. J. (1980). Diapriidae (Diapriinae). In *Royal Entomological Society of London*. London, United Kingdom.

Merritt, R. W., De Jong, G. D., Benbow, M. E., Tomberlin, J. K., & Tarone, A. M. (2015). Arthropod communities in terrestrial environments. In: Benbow, M. E., Tomberlin, J.K., Tarone, A. M. (eds) *Carrion Ecology, Evolution, and their Applications.* CRC Press, Boca Raton, FL, pp. 65-92.

Meurisse, N., Rassati, D., Hurley, B. P., Brockerhoff, E. G., & Haack, R. A. (2019). Common pathways by which non-native forest insects move internationally and domestically. *Journal of Pest Science*, **92**, 13-27.

Morehead, S. A., & Feener Jr, D. H. (2000). An experimental test of potential host range in the ant parasitoid *Apocephalus paraponerae*. *Ecological Entomology*, **25**, 332-340.

Moreno, J., Sevillano, G., Valverde, O., Loayza, V., Haro, R., Zambrano, J. (2018). Soil from The Coastal Plane. In: Espinosa, J., Moreno, J., Bernal, G. (eds). *The Soils of Ecuador* (pp 27–77). World Soils Book Series Cham: Springer International Publishing.

Novak, B. J., Phelan, R., & Weber, M. (2021). US conservation translocations: Over a century of intended consequences. *Conservation Science and Practice*, **3**, e394.

Ollivier, M., Lesieur, V., Raghu, S., & Martin, J. F. (2020). Characterizing ecological interaction networks to support risk assessment in classical biological control of weeds. *Current Opinion in Insect Science*, **38**, 40-47.

Omar, A. H. (1995). Studies on *Chrysomyia albiceps* (Wiedemann) one of the most important carrion flies in Egypt. *Journal of the Egyptian Society of Parasitology*, **25**, 607-624.

Ong, S., Ahmad, H., & Tan, E. H. (2018). Substrate Moisture Affects the Development of *Megaselia scalaris* (Diptera: Phoridae): An Implication of the Growth Circumstances of the Fly in Forensic Entomology. *Environmental Entomology*, **47**, 1582-1585.

Pacheco, H. M., S. L. Vanlaerhoven, M. A. M. Garcia, & D. W. Hunt. (2018). Food web associations and effect of trophic resources and environmental factors on parasitoids expanding their host range into non-native hosts. *Entomologia Experimentalis et Applicata*, **166**, 277-288.

Pape, T. (1994). The world *Blaesoxipha* Loew, 1861 (Diptera: Sarcophagidae). *Entomologica Scandinavica, Supplement*, **45**, 1-247.

Pemberton, R.W. (2000). Predictable risks to native plants in weed biological control. *Oecologia* **125,** 489-494.

Poisot, T., Canard, E., Mouquet, N., & Hochberg, M. E. (2012). A comparative study of ecological specialization estimators. *Methods in Ecology and Evolution*, **3**, 537–544.

Prinkkilá, M. L., & Hanski, I. (1995). Complex competitive interactions in four species of *Lucilia* blowflies. *Ecological Entomology*, **20**, 261-272.

RStudio Team. (2023). RStudio: Integrated Development for R. RStudio, PBC, Boston, MA. Retrieved from http://www.rstudio.com/

Ramirez, I. E., Causton, C. E., Gutierrez, G. A., Mosquera, D. A., Piedrahita, P., & Heimpel, G. E. (2022). Specificity within bird–parasite–parasitoid food webs: A novel

approach for evaluating potential biological control agents of the avian vampire fly. *Journal of Applied Ecology*, **59**, 2189-2198.

Ramirez, I. E, Yar, J., Sinclair, B.J., Torres A.K., Causton, C.E., & Heimpel, G.E. (In-Press). Competition among invasive and endemic carrion fly species in the Galapagos Islands with implications for biological control risk assessment. *Journal of Insect Conservation and Diversity*.

Reaser, J. K., Meyerson, L. A., Cronk, Q., De Poorter, M. A. J., Eldrege, L. G., Green, E., Kairo, M., Latasi, P., Mack, R. N. & Vaiutu, L. (2007). Ecological and socioeconomic impacts of invasive alien species in island ecosystems. *Environmental Conservation*, **34**, 98-111.

Ren L., Shang Y., Chen W., Meng F., Cai J., Zhu G., Chen L., Wang Y., Deng J., Guo Y. (2018). A brief review of forensically important flesh flies (Diptera: Sarcophagidae). *Forensic Sciences Research*, **3**, 16-26.

Roberts, R. A. (1933). Biology of *Brachymeria fonscolombei* (Dufour), a hymenopterous parasite of blowfly larvae. *United States Department of Agriculture Technical Bulletin*, 365, 1–21.

Román, A.C., Chicaiza-Herrera, J., Betancourt-Cargua, L. & Pinto, C.M. (2023). Scavenging insects found on a carcass of the endangered Galápagos sea lion *Zalophus wollebaeki*. *Therya Notes*, **4**, 199-203.

Russell, J. C., Meyer, J. Y., Holmes, N. D., & Pagad, S. (2017). Invasive alien species on islands: impacts, distribution, interactions and management. *Environmental Conservation*, **44**, 359-370.

Sanford, M. R., Tomberlin, J.K. & VanLaerhoven S.L. (2015). Behavioral Ecology and Forensic Entomology, In Mark E. Benbow (Eds.), *Forensic Entomology: International Dimensions and Frontiers*, 331-339. CRC Press LLC.

Seebens, H., Blackburn, T. M., Dyer, E. E., Genovesi, P., Hulme, P. E., Jeschke, J. M., ... & Essl, F. (2018). Global rise in emerging alien species results from increased accessibility of new source pools. *Proceedings of the National Academy of Sciences*, **10**, E2264-E2273.

Sheppard, A. W., Van Klinken, R. D., & Heard, T. A. (2005). Scientific advances in the analysis of direct risks of weed biological control agents to nontarget plants. *Biological control*, **35**, 215-226.

Simberloff, D. (2010). Invasive species. In: Sodhi, N.S., Ehrlich, P.R. (eds) *Conservation Biology for All*. Oxford University Press, Oxford, pp 131–152.

Simberloff, D. (2013). *Invasive species: what everyone needs to know*. Oxford University Press.

Sinclair, B. J. (2023) An annotated checklist of the Diptera of the Galápagos Archipelago (Ecuador). *Zootaxa*, **5283**, 001-102.

Singer, M. S. (2008). 3. Evolutionary ecology of polyphagy. In *specialization, speciation, and radiation: The evolutionary biology of herbivorous insects* (pp. 29-42)., Ed. K.J. Tilmon, University of California Press. Berkeley, CA, United States.

Singer, M.S. & Stireman, J.O. (2005) The tri-trophic niche concept and adaptive radiation of phytophagous insects. *Ecology Letters*, **8,** 1247-1255.

Skidmore, P. (1985). *The Biology of the Muscidae of the World* (Vol. 29). Springer Science & Business Media, Heidelberg, Netherlands.

Spatz, D. R., Zilliacus, K. M., Holmes, N. D., Butchart, S. H., Genovesi, P., Ceballos, G., ... & Croll, D. A. (2017). Globally threatened vertebrates on islands with invasive species. *Science Advances*, **3**, e1603080.

Spencer, E. E., Barton, P. S., Ripple, W. J., & Newsome, T. M. (2020). Invasive European wasps alter scavenging dynamics around carrion. *Food Webs*, **24**, e00144.

Stenoien, C. M. (2017). *Behavioral Ecology of Parasitoid Diet Breadth and Insect Defenses* (Doctoral dissertation, University of Minnesota).

Szpila, K., Mądra, A., Jarmusz, M., & Matuszewski, S. (2015). Flesh flies (Diptera: Sarcophagidae) colonising large carcasses in Central Europe. *Parasitology Research*, **114**, 2341-2348.

Tantawi, T.I., & Sinclair, B.J. (2013). An update of the blow flies (Diptera: Calliphoridae) of the Galápagos Islands, and first record of *Chrysomya rufifacies* (Macquart) from mainland Ecuador. *Zootaxa*, **3750** (3), 237–250.

Tavares, D. E. S., Porthun, J. R., Fernandes, E. A., & Oliveira, S. P. (1988). Estudo sobre comunidades de sarcofagídeos sinantrópicos (Diptera, Sarcophagidae) no Instituto Oswaldo Cruz, Manguinhos, RJ. *Resumos XV Congr. brasil. Zool., Curitiba-PR*, p. 233.

Todd, J. H., Pearce, B. M., & Barratt, B. I. (2021). Using qualitative food webs to predict species at risk of indirect effects from a proposed biological control agent. *BioControl*, **66**, 45-58.

Toral-Granda, M. V., Causton, C. E., Jäger, H., Trueman, M., Izurieta, J. C., Araujo, E., ... & Garnett, S. T. (2017). Alien species pathways to the Galapagos Islands, Ecuador. *PLoS One*, **12**, e0184379.

Trueman M., & d'Ozouville, N. (2010) Characterizing the Galapagos terrestrial climate in the face of global climate change. *Galapagos Research*, **67**, 26–37.

Tye, A., Francisco-Ortega, J., Bramwell, D., & Caujapé-Castells, J., 2011. Origins and evolution of Galapagos endemic vascular plants. *The Biology of Island Floras*, pp. 89-153.

Tylianakis, J. M., & Binzer, A. (2014). Effects of global environmental changes on parasitoid–host food webs and biological control. *Biological Control*, **75**, 77-86.

Van Driesche, R. G., Carruthers, R. I., Center, T., Hoddle, M. S., Hough-Goldstein, … van Klinken, R. D. (2010). Classical biological control for the protection of natural ecosystems. *Biological Control*, **54**, S2–S33.

Van Driesche, R. G., Nunn, C., Kreke, N., Goldstein, B., & Benson, J. (2003). Laboratory and field host preferences of introduced *Cotesia* spp. parasitoids (Hymenoptera: Braconidae) between native and invasive *Pieris* butterflies. *Biological Control*, **28**, 214-221.

Van Driesche, R.G. and Reardon, R.C. (2017). *Supressing Overabundant Invasive Plants and Insects in Natural Areas by Use of Their Specialized Natural Enemies*. USA: FHTET, USDA.

Van Driesche, R., Simberloff, D., Blossey, B., Causton, C., Hoddle, M., ... & Warner, K. D. (Eds.). (2016). *Integrating Biological Control into Conservation Practice*. John Wiley & Sons, Hoboken, NJ, United States

Vaughan, I. P., Gotelli, N. J., Memmott, J., Pearson, C. E., Woodward, G., & Symondson, W. O. (2018). econullnetr: An r package using null models to analyse the structure of

ecological networks and identify resource selection. *Methods in Ecology and Evolution*, **9**, 728-733.

Veldtman, R., Lado, T. F., Botes, A., Procheş, Ş., Timm, A. E., Geertsema, H., & Chown, S. L. (2011). Creating novel food webs on introduced Australian acacias: indirect effects of galling biological control agents. *Diversity and Distributions*, **17**, 958-967.

Verves, Y. G., & Protsenko, Y. V. (2019). Potter Wasps (Hymenoptera: Vespidae, Eumeninae) as Hosts of *Amobia* Robineau-Desvoidy, 1830 (Diptera: Sarcophagidae, Miltogramminae) in Ukraine. *Jordan Journal of Natural History*, *6*, 39-49.

Voss, S. C., Spafford, H., & Dadour, I. R. (2009). Hymenopteran parasitoids of forensic importance: host associations, seasonality, and prevalence of parasitoids of carrion flies in western Australia. *Journal of Medical Entomology*, **46**, 1210-1219.

Wapshere, A. J. (1974). A strategy for evaluating the safety of organisms for biological weed control. *Annals of applied biology*, **77**, 201-211.

Wapshere, A.J. (1989). A testing sequence for reducing rejection of potential biological control agents for weeds. *Annals of Applied Biology* **114**, 515-526.

Weatherbee, C. R., Pechal, J. L., & Eric Benbow, M. (2017). The dynamic maggot mass microbiome. *Annals of the Entomological Society of America*, **110**, 45-53.

Willis, A. J., and Memmott, J. (2005). The potential for indirect effects between a weed, one of its biocontrol agents and native herbivores: A food web approach. *Biological Control*, **35**, 299-306.

Wyckhuys, K. A., & Heimpel, G. E. (2007). Response of the soybean aphid parasitoid *Binodoxys communis* to olfactory cues from target and non-target host-plant complexes. *Entomologia Experimentalis et Applicata*, **123**, 149-158.

Wyckhuys, K. A., Koch, R. L., & Heimpel, G. E. (2007). Physical and ant-mediated refuges from parasitism: implications for non-target effects in biological control. *Biological Control*, **40**, 306-313.

Wyckhuys, K. A., Koch, R. L., Kula, R. R., & Heimpel, G. E. (2009). Potential exposure of a classical biological control agent of the soybean aphid, *Aphis glycines*, on non-target aphids in North America. *Biological Invasions*, **11**, 857-871.

Zavaleta, E. S., Hobbs R. J., and Mooney H. A. (2001). Viewing invasive species removal in a whole-ecosystem context. *Trends in Ecology & Evolution*, **16**, 454.