HOW DOES CLIMATE CHANGE INFLUENCE PARASITE PRESSURE ON BIRDS?

by

Kyle McKay Davis

A thesis submitted to the faculty of
The University of Utah
in partial fulfillment of the requirements for the degree of

Master of Science

in

Biology

School of Biological Sciences

The University of Utah

August 2023

The University of Utah Graduate School

STATEMENT OF THESIS APPROVAL

The thesis of **Kyle McKay Davis**

has been approved by the following supervisory committee members:

Sarah Elizabeth Bush	, Chair	**6/21/2023** Date Approved
Colin Dale	, Member	**6/22/2023** Date Approved
Kevin Johnson	, Member	**6/20/2023** Date Approved

and by **Frederick R. Adler**, Chair/Dean of

the Department/College/School of **Biological Sciences**

and by David B. Kieda, Dean of The Graduate School.

ABSTRACT

Climate change will impact ecosystems as abiotic factors such as temperature and humidity change. Changes in ecosystems will lead to changes in species composition as species either relocate, adapt to their new environment, or go extinct. It is only possible to make robust predictions about how climate change will influence the distribution of species if information about their natural history is known. There is, however, a gap in our knowledge about the natural history of many organisms, which makes it difficult to predict how these species will be influenced by changes in the environment. Organisms such as parasites, are understudied even though they represent a large percentage of eukaryotic biodiversity and can impact their hosts. With ectoparasites on doves and pigeons (*Columbiformes*), it has been found that humidity may influence their populations, with more ectoparasites being found in regions with higher humidity than in regions with lower humidity. Unfortunately, there is a sampling bias with more surveys of parasites being carried out in humid regions than arid regions.

To better understand how parasites will be influenced by climate change, I conducted surveys of ectoparasites found on birds in urban and montane Utah. The prevalence of chewing lice was 10% in the montane region and 5.6% in the urban region. Although many of the birds sampled are common to Utah, new louse host records were found in each survey, highlighting how understudied these ectoparasites are.

For Chapter 3, faunal surveys of birds and their ectoparasitic lice were used to investigate how likely changes in humidity will influence ectoparasite pressure on birds. Louse prevalence was significantly positively correlated with humidity, and louse abundance tended to increase with humidity. The results from this study imply that the number of lice will decrease as areas become arid and increase in areas becoming wetter. However, more surveys should be carried out in regions with lower humidity so that more robust predictions can be made on how climate change could influence ectoparasite populations.

TABLE OF CONTENTS

ABSTRACT iii

LIST OF TABLES vii

LIST OF FIGURES viii

Chapters

1. INTRODUCTION 1

    1.1 Literature Cited 5

2. POPULATION ECOLOGY OF ECTOPARASITES ON BIRDS IN AN ARID ENVIRONMENT 7

    2.1 Abstract 7
    2.2 Introduction 8
    2.3 Methods 9
    2.4 Results 13
    2.5 Discussion 15
    2.6 Acknowledgments 18
    2.7 Literature Cited 27

3. RELATIONSHIP BETWEEN HUMIDITY AND ECTOPARASITE PRESSURE: A STUDY OF FEATHER LICE ON BIRDS 29

    3.1 Abstract 29
    3.2 Introduction 30
    3.3 Methods 33
    3.4 Results 35
    3.5 Discussion 36
    3.6 Acknowledgments 41
    3.7 Literature Cited 47

Appendices

A. SCREENING WILD BIRDS IN UTAH FOR *SODALIS* 52

B. MICROINJECTION OF *SODALIS* INTO FEATHER LICE (*COLUMBICOLA COLUMBAE*) 63

LIST OF TABLES

2.1 List of birds examined in montane Utah 22

2.2 Ectoparasites recovered from birds in urban Utah 24

2.3 Summary of host-louse association in montane Utah 25

2.4 Summary of host-louse associations for urban Utah 26

3.1 Regions used to look at the influence of humidity on ectoparasites 46

A.1 List of wild birds sampled for *Sodalis* 60

LIST OF FIGURES

2.1 Locations in montane Utah where lice were surveyed 19

2.2 Examples of lice genera found in the montane survey 20

2.3 Lice genera that were most common from both surveys 21

3.1 Scatter plot of humidity to louse prevalence 42

3.2 Humidity map of locations found in the literature search 43

3.3 Scatter plot of humidity to log transformed plus one louse abundance 44

3.4 Scatter plot of humidity to louse prevalence 45

CHAPTER 1

INTRODUCTION

Climate change is posing problems for species across the globe. Climate change will influence species as abiotic factors such as temperature and humidity change (Trenberth 2011, Zurbenko and Luo 2012, and Pasqui and Giuseppe 2019). Changes in humidity and temperature will allow some species to expand or shift their range to coincide with the ecosystems they are adapted to (Thomas 2010, Hill et al. 2011, Ryan et al. 2019). Other species are predicted to go extinct because they are unable to adapt to changes in their environment or because they will be outcompeted by species better adapted to the changing planet (Foden et al. 2018, Román-Palacios and Wiens 2020).

It is possible to make robust predictions about how climate change will influence species, for which we have a lot of information about their natural history. There is, however, a gap in our knowledge about the natural history of many organisms, which makes it difficult to predict how these species will be influenced by changing environments. Many biodiversity studies have focused on charismatic megafauna, yet the diversity of parasites is understudied even though they represent a large portion of eukaryotic biodiversity (Price 1980, De Meeûs and Renaud 2002).

Although understudied, parasites can greatly impact their host's survival (Clayton 1990, Owen et al. 2010, Jenni and Winkler 2020). Parasites can also be host specific,

which has led to predictions of coextinction of parasites with their hosts (Roberts et al. 2001, Poulin and Morand 2004, Dunn et al. 2009, and Loker and Hofkin 2015). In some instances, it is predicted that parasites will go extinct quicker than their hosts since a minimum host population size threshold is needed to keep ectoparasite populations from crashing (Roberts et al. 2001 and Dunn et al. 2009). We also know that parasite populations can be influenced by abiotic factors such as humidity, with areas of higher humidity having more ectoparasites than areas of lower humidity (Moyer et al. 2002). Therefore, changes in abiotic factors caused by climate change may influence parasite pressures on animals. Yet, in most regions, we do not even have a baseline understanding of parasite diversity. Missing baseline data can make it hard to predict how changes in humidity will influence parasite populations, which could influence host populations.

In parasites, there is some bias in what species are surveyed. Parasites that cause infectious diseases in humans are often investigated to determine how climate change will influence their populations. In many instances, parasites of humans are predicted to expand their range, which will lead to a greater risk of outbreaks of infectious diseases (Sutherst 2004, Bouchard et al. 2019, Ryan et al. 2019, Casadevall 2020). An example of a group of parasites with gaps in the baseline data are the ectoparasites that can be found on birds. By studying the ectoparasites on birds, we may be able to understand how changes in the environment will influence parasite populations, which in return may influence their host populations.

Although it may not be possible to determine how populations of ectoparasites have already been influenced by climate change, predictions can be made about how it will influence ectoparasites in the future. We can make predictions if we know how

organisms respond to current ranges of abiotic factors. However, this cannot be done right now because we do not know how parasites respond to arid conditions because there isn't enough data. This thesis aims to investigate how humidity influences parasites and establish baseline data in areas where it is missing, such as low-humidity environments.

In the second chapter, birds were surveyed in montane and urban regions of Utah for ectoparasites from the orders Accipitriformes, Apodiformes, Columbiformes, Galliformes, Passeriformes, Piciformes, and Strigiformes. The birds in these surveys represented 44 genera and 56 species. The regions in Utah where ectoparasites were collected have an average humidity of 45% (worlddata.info), making it ideal for investigating what ectoparasite populations are like in an arid environment. The prevalence of chewing lice (Phthiraptera: Ischnocera) was 10% in the montane region and 5.6% in the urban region. While many of the birds sampled were common to Utah, 11 new louse host records were found in this survey, highlighting how understudied these ectoparasites are. The low prevalence of lice and flies combined with the absence of other ectoparasites suggest that ectoparasites populations are small or absent from arid environments.

The third chapter of my thesis used published surveys of birds and their ectoparasitic lice to investigate whether ambient humidity influences ectoparasite pressure on birds. It has been predicted that humidity will be influenced by climate change, and ectoparasites may be influenced by changes in humidity (Moyer et al. 2002, Trenberth 2011). This chapter includes data from birds from 26 regions across the globe. When comparing these regions, louse prevalence was significantly positively correlated with humidity, and louse abundance also tended to increase with humidity. The results

from this study imply that lice will decrease as areas become arid and increase in areas becoming wetter.

In summary, this thesis highlights the important role that humidity may play in ectoparasite diversity and population size. Few studies examine the population ecology of ectoparasites in arid environments. Chapter 2 carried out ectoparasite surveys on birds in Utah to help establish baseline data of ectoparasite diversity for arid regions. Chapter 2 helped determine how ectoparasite populations may change as climate change causes regions to become drier. Chapter 2 also found that ectoparasite prevalence, abundance, and intensity tend to be low in arid environments. It also found that genera such as *Brueelia* and *Ricinus* may be more common in arid environments. When focusing on prevalence and abundance, it seems as though ectoparasites will decrease as areas become arid and increase in areas becoming wetter (Chapter 3). More research should investigate ectoparasites in low-humidity environments. By studying low-humidity environments, we might be able to determine if there are arid-adapted genera of lice, which could suggest that they will become more common in environments that are drying out. Studying ectoparasites from both humid and arid environments can also help us establish baseline data that can be used to see how parasites change over time as their environment is impacted by climate change.

1.1 Literature Cited

Bouchard, C., A. Dibernardo, J. Koffi, H. Wood, P. A. Leighton, and L. R. Lindsay (2019). An increased risk of tick-borne diseases with climate and environmental changes. *Canada Communicable Disease Report* 45:83-89.

Casadevall, A. (2020). Climate change brings the specter of new infectious diseases. *The Journal of Clinical Investigation* 130:553-555.

Clayton, D. H. (1990). Mate choice in experimentally parasitized rock doves: Lousy males lose. *American Zoologist* 30:251-262.

De Meeûs, T., and F. Renaud (2002). Parasites within the new phylogeny of eukaryotes. *Trends in Parasitology* 18:247-251.

Dunn, R. R., N. C. Harris, R. K. Colwell, L. P. Koh, and N. S. Sodhi (2009). The sixth mass coextinction: Are most endangered species parasites and mutualists? *Proceedings of the Royal Society. B, Biological Sciences* 276:3037-3045.

Foden, W. B., B. E. Young, H. R. Akçakaya, R. A. Garcia, A. A. Hoffmann, B. A. Stein, C. D. Thomas, C. J. Wheatley, D. Bickford, J. A. Carr, D. G. Hole, T. G. Martin, M. Pacifici, J. W. Pearce-Higgins, P. J. Platts, P. Visconti, J. E. M. Watson, and B. Huntley (2019). Climate change vulnerability assessment of species. *Wiley Interdisciplinary Reviews* 10:e551.

Hill, J. K., H. M. Griffiths, and C. D. Thomas (2011). Climate change and evolutionary adaptations at species' range margins. *Annual Review of Entomology* 56:143-159.

Jenni, L., and R. Winkler (2020). *The Biology of Molt in Birds*. Bloomsbury Publishing Plc, London, United Kingdom.

Loker, E., and B. Hofkin (2015). *Parasitology: A Conceptual Approach*. Garland Science, Taylor and Francis Group LLC, New York, NY, USA.

Moyer, B. R., D. M. Drown, and D. H. Clayton (2002). Low humidity reduces ectoparasite pressure: Implications for host life history evolution. *Oikos* 97:223-228.

Owen, J. P., A. C. Nelson, and D. H. Clayton (2010). Ecological immunology of bird-ectoparasite systems. *Trends in Parasitology* 26:530-539.

Pasqui, M., and E. D. Giuseppe (2019). Climate change, future warming, and adaptation in Europe. *Animal Frontiers: The Review Magazine of Animal Agriculture* 9:6-11.

Poulin, R, and S. Morand (2004). *Parasite Biodiversity*. Smithsonian Institution Scholarly Press, Washington, D.C., USA.

Price, P. W. (1980). *Evolutionary Biology of Parasites*. Princeton University Press, Princeton, NJ, USA.

Roberts, M. G., P. J. Hudson A. Rizzoli, B. T. Grenfell, H. Heesterbeek, and A. P. Dobson (2001). *Parasite Community Ecology and Biodiversity*. Hudson. Oxford University Press, Oxford, MA, USA.

Román-Palacios, C., and J. J. Wiens (2020). Recent responses to climate change reveal the drivers of species extinction and survival. *Proceedings of the National Academy of Sciences* 117:4211-4217.

Ryan, S. J., C. J. Carlson, E. A., Mordecai, and L. R. Johnson (2019). Global expansion and redistribution of Aedes-borne virus transmission risk with climate change. *PLoS Neglected Tropical Diseases* 13:e0007213.

Sutherst, R. W. (2004). Global change and human vulnerability to vector-borne diseases. *Clinical Microbiology Reviews* 17:136-173.

Thomas, C. D. (2010). Climate change and range boundaries. *Diversity & Distributions* 16:488-495.

Trenberth, K. E. (2011). Changes in precipitation with climate change. *Climate Research* 47:123-138.

Zurbenko, I., and M. Luo (2012). Restoration of time-spatial scales in global temperature data. *American Journal of Climate Change* 1:22477.

CHAPTER 2

POPULATION ECOLOGY OF ECTOPARASITES ON BIRDS IN AN ARID ENVIRONMENT

2.1 Abstract

Most biodiversity studies focus on charismatic megafauna, leaving a gap in knowledge of the biodiversity of organisms such as parasites. Co-extinction of parasites with their hosts happens, and parasites may even go extinct before their hosts do. Yet, in most regions, we do not even have a baseline understanding of parasite diversity. For example, relatively few studies have examined parasite diversity in arid regions. In order to establish a baseline for ectoparasites in an arid region, we surveyed the parasites of birds in urban and montane Utah. We sampled birds from the orders: Accipitriformes, Apodiformes, Columbiformes, Galliformes, Passeriformes, Piciformes, and Strigiformes. The birds in this study represented 44 genera and 56 species. For our montane survey, we captured and collected birds at Big Canyon and Echo, Summit County, Utah. For the urban survey, birds were caught at the University of Utah, located in Salt Lake City, Utah. The prevalence of chewing lice (Phthiraptera: Ischnocera) was 10% in the montane region and 5.6% in the urban region. Along with lice, birds were also examined for feather mites (Acari), flies (Diptera: Hippoboscidae), fleas (Siphonaptera), and ticks (Acari). When comparing the lice found between the montane and arid regions, *Brueelia*

and *Ricinus* were commonly found, suggesting they may be arid-adapted. Although many of these birds are common to Utah, new louse host records were found in these surveys, highlighting how understudied these ectoparasites are.

2.2 Introduction

Parasites are usually host specific and are typically found on one or a few host species (Poulin and Morand 2005). Since parasites are highly specialized, co-extinction with their host should be common (Moir et al. 2010). Parasites may even go extinct before their hosts since a minimum host population is needed to keep the parasites population from collapsing (Roberts et al. 2001). Therefore, monitoring parasite diversity can be a way to determine how their host population is doing. To understand how parasite diversity is affected by changes in the environment, baseline data is needed for the parasites. Ectoparasites in arid regions are especially understudied. By carrying out surveys of ectoparasites in arid regions, insight can be gained into what parasite diversity may look like as climate change causes ecosystems to change (Overpeck and Udallb 2020).

To establish baseline data for arid regions, surveys of ectoparasites infesting birds were carried out in the southeastern USA, where the average humidity is 45% (worlddata.info). Utah's semi-arid foothills, mountain zones, and high plateaus are examples of areas with very few studies of ectoparasites. In order to get a baseline of parasite diversity for this region, ectoparasites were surveyed on birds found at Echo, Utah (41.032 °N, -111.317 °W) and Big Canyon, Utah (40.844 °N, -111.444 °W) from May until August 2021. Both locations are montane regions found close to the Wyoming

border in northeastern Utah. Our site in Echo was in semi-arid foothills and was at an elevation of 2100 meters. Birds were captured in Sagebrush, Grama Grass, Mountain Mahogany, and Gambel Oak habitat. Oak scrub and aspen forests were found throughout the area (Woods et al. 2001). Our site in Big Canyon was located between the Wasatch Mountains and high plateaus zones and was at an elevation of 2200 meters. Birds were captured in forests made up of Quaking Aspen and Douglas-fir. Some of our nets were also located in meadows found throughout the area (Figure 2.1).

After surveying ectoparasites in montane habitats another survey was carried out using dead, window-strike birds at the University of Utah (40.7649° N, 111.8421° W), located in Salt Lake City. The birds used in this survey were collected between 2018 and 2020, and the ectoparasites were collected from them in 2022. Any ectoparasites found on the birds were collected, which led to mites, chewing lice, and ticks being collected.

2.3 Methods

2.3.1 Ectoparasite collection and processing methods

Ectoparasites were collected from birds by post-mortem ruffling, dust ruffling, or body washing. All three methods are effective ways of collecting ectoparasites. All recovered ectoparasites were preserved in 95% ethanol and deposited in the Price Institute of Parasite Research at the University of Utah. Photo vouchers were taken of the ectoparasites by using an EOS 90D attached to an Olympus microscope.

2.3.2 Survey of montane ectoparasites

2.3.2.1 Capture of birds

For the survey of montane ectoparasites, birds were caught in mist nets or sacrificed. Birds sacrificed in this study were deposited as a voucher at the Biodiversity Institute and Natural History Museum at the University of Kansas after they were examined for ectoparasites. To capture live birds, we used mist nets. Mist nets are a type of net made of fine mesh that is not easily seen, making it more likely for the birds to fly into them. Mist nets were opened just before dawn and were checked every 30 minutes until 17:30. To prevent cross-contamination of ectoparasites, birds were placed into a paper bag after they were taken out of a net. Each paper bag was only used once. Once a bird was captured, the sex, mass, tarsus, bill length, width, and depth were measured and recorded along with the bill overhang. The bill overhang is the area of the upper mandible that sticks out past the lower mandible. The overhang was measured three times for each bird. From the three overhang measurements, an average overhang was found and recorded for each bird. A unique USGS aluminum band was also placed on the leg of each bird.

2.3.2.2 Post-mortem ruffling

Lice were collected from dead birds using the "post-mortem-ruffling" method described in Clayton and Drown (2001). In brief, this method is used on dead birds placed in a container containing cotton balls soaked in ethyl acetate for 20 min. The birds were then ruffled over a sheet of paper, and ectoparasites were collected. The inside of

the fumigation chamber was also checked for ectoparasites and wiped clean before it was used again.

2.3.2.3 Dust-ruffling

Ectoparasites were collected from live birds by "dust-ruffling" methods described in Clayton and Drown (2001). Dust-ruffling was used on live birds with "Happy Jack Flea and Tick Powder," which contains 2% pipreonyl butoxide and 0.5% permethrin. The flea powder was rubbed into the bird's back, tail, belly, and wings. The flea powder was dusted over the bird and rubbed into the feathers to ensure that it was evenly distributed throughout the plumage. Two minutes after the flea powder was applied, the bird was ruffled over a tray, and ectoparasites were collected. After a bird was dust-ruffled, it was released on site.

2.3.3 Survey of ectoparasites on urban birds

For the urban survey of ectoparasites, birds were collected during daily walks, which led to recently deceased birds being collected. Carcasses collected that showed signs of decay were used unless the bird was falling apart. Only three birds were not used because of how decomposed their bodies were. Ectoparasites, such as lice and mites, can be easily quantified on recently deceased birds since these parasites cannot easily move off their host. (Clayton et al. 2008**,** Pence 2008). Although ticks will move off a dead host over time, there is evidence that some ticks will remain on a body for a short amount of time, which is probably why only a few ticks were found (Tahir et al. 2020, Choi et al.

2022). Parasites such as flies, fleas, and dermal mites usually leave the host soon after it dies.

When a bird was found, it was placed individually into a plastic bag to avoid cross-contamination of specimens and stored in a freezer set to -22 °C. Tarsus measurements and bill length, width, and height were taken to 0.01 millimeters. Mass to the 0.1 grams was also recorded. After taking these measurements, ectoparasites were collected from the bird by using the "body washing" method described in Clayton and Drown (2001). Briefly, the body washing method was carried out by washing the bird carcasses in a 10 min wash and then 10 min rinse cycle. The water was then passed through filter paper, which captured the ectoparasites. Once washed, the bird was necropsied, and sex was determined by the presence of sexual organs (ovaries or testis). To determine if ectoparasites were present, filter paper was examined under a dissection microscope.

2.3.4 Comparing ectoparasites between urban and montane regions

Tentatively, parasite abundance, intensity, and prevalence can be compared between the regions. Comparing parasite abundance, intensity, and prevalence can be difficult since different parasite collecting methods were used for each site (Bush et al. 1997, Clayton and Drown 2001). The body washing method removes more ectoparasites than post-mortem ruffling or dust ruffling (Clayton and Drown 2001). Even though different ectoparasite collecting techniques were used between the regions, lice genera can be compared to see if they are different between the urban and rural regions.

2.4 Results

For the montane survey, birds in 6 orders: Accipitriformes, Apodiformes, Galliformes, Passeriformes, Piciformes, and Strigiformes were studied. For this region, 351 birds representing 37 genera and 45 species were examined (Table 2.1). For the montane region, 10.5% (37/351) of birds were infested with ectoparasites. The mean ectoparasite abundance for montane Utah was 13.3 (507/38), and the mean intensity of ectoparasites was 1.4 (507/351). For the urban survey of ectoparasites, data were collected from 89 window-strike birds representing 3 avian orders: Apodiformes, Columbiformes, and Passeriformes, which represent 22 genera and 27 species (Table 2.2). In total, 27.0% (24/89) of birds in urban Utah had ectoparasites (Table 2.2) Mean intensity of ectoparasites in urban Utah was 11.5 ectoparasites per bird (277/24), and the mean abundance of ectoparasites was 3.1 ectoparasites per bird (277/89).

2.4.1 Dust ruffling

The prevalence of lice found on birds that were dust ruffled was 6%. The mean louse abundance of dust-ruffled birds was 0.12 lice per bird. The mean louse intensity was 2.0 lice per bird.

2.4.2 Post-mortem ruffling data

The prevalence of ectoparasites on birds that were post-mortem ruffled was 24%. The mean louse abundance was 5.7 lice per bird, and the mean louse intensity was 23 lice per bird.

2.4.3 Lice

For the montane survey, 10% (36/351) of birds had lice. The mean louse abundance was 1.4 (506/351) lice per bird. The mean louse intensity was 13.7 (506/37) lice per bird. There were 11 genera of lice collected: *Chelopistes, Breelia, Myrsidea, Oxylipeurus, Penenirmus, Picicola*, *Strigiphilus*, *Menacanthus*, *Philopterus*, *Brueelia*, and *Ricinus* (Table 2.3, Figure 2.2, Figure 2.3). The montane survey had 10 new host records (Price et al. 2003). For the urban study, a total of 5.6% (5/89) of birds were infested with lice. The mean intensity of lice was 13.2 (66/5) lice per bird. The mean abundance of lice was 0.74 (66/89) lice per bird. Only two genera of lice were collected in the urban survey. The lice genera collected were *Brueelia* and *Ricinus* (Figure 2.3). From these genera, five species of feather lice were recovered (Table 2.4). All genera found are known to be associated with Passerines. The *Ricinus sp.* found on *Cardellina pusilla* is a new host record and possibly a new species (Price et al. 2003)

2.4.4 Feather mites

For the montane survey, no mites were found. In the urban survey, 21% (19/89) of birds were infested with mites. The mean abundance of mites was 2.3 (277/89) mites per bird. The mean intensity of mites was 11 mites per bird (209/19).

2.4.5 Ticks

No ticks were found in the montane survey. In the urban survey, ticks were found on 2.2% (2/89) of birds. The mean abundance of ticks was 0.02 (2/89) ticks per bird, and the mean intensity of ticks was 1 (2/2) tick per bird.

2.4.6 Other ectoparasites

No fleas were found in this survey, and a single hippoboscid fly was observed flying off an American Robin (*Turdus migratorius*) before it was dust ruffled in the montane survey.

2.5 Discussion

These surveys examine the diversity of ectoparasites in arid regions using three different methods for collecting ectoparasites. Ectoparasites collected in these surveys were lice, ticks, and feather mites. Although most aspects of birds are highly studied, 11 new louse host records were found with these surveys (Price et al. 2003) (Table 2.3 and Table 2.4). A new host record means that the genus of louse was found on a host that had not previously been recorded on that host. Some of the new host records are likely new species, but additional taxonomic work will need to be carried out with the lice to fully identify and describe these species. With most species only found on one or a few hosts (Galloway and Lamb 2021). The high number of new host records demonstrates that ectoparasites on birds are not normally studied.

Furthermore, only 2 genera of lice were found in the survey of ectoparasites on urban birds. They were *Brueelia and Ricinus,* with *Ricinus* having the most species collected. Finding only *Brueelia and Ricinus* in this survey gives support to the idea that they are arid-adapted. Especially since the most abundant genera of lice found in the montane survey were *Brueelia* and *Ricinus,* research should investigate whether these species are arid-adapted. By figuring out which species of lice are arid-adapted, we can start to predict how ectoparasite composition will change over time as climate change

impacts the humidity of regions. Perhaps in North America, where areas are getting dryer (Overpeck and Udallb 2020), we might see the genera of ectoparasites change so that those genera that are more arid-adapted become more prevalent.

Ectoparasites such as lice can be influenced by changes in humidity (Moyer et al. 2002). The low prevalence, abundance, and intensity of lice at our study sites could be due to the low humidity found in Utah (Moyer et al. 2002**)**. Utah has a mean humidity of 45% (worlddata.info). In areas with a higher humidity, the louse prevalence is usually 30% or higher (Moyer et al. 2002, Carrillo et al. 2007, Bush et al. 2018). In contrast, the prevalence at our sites was 10% in the montane region and 5.6% in the urban region. If we compare the prevalence of lice found between rural and urban Utah, we find a difference in prevalence between urban (5.6%) and rural (10%) regions. There is also a difference in the abundance of lice found between the regions, with 1.4 lice per bird being found in the rural region compared to the 0.74 lice per bird found in the urban region. It is important to remember that the collection method used can influence how many lice are collected from each bird. In the rural region, Post-mortem ruffling was used, which has a $r2$ of 0.92, along with dust ruffling, which has a $r2$ of 0.86. These methods are different from the washing method used in the urban region, which has a $r2$ of 0.99 (Clayton and Drown 2001). The montane region having a higher louse abundance and prevalence than the urban regions is probably not due to the way the lice were collection since the urban regions had the more robust collection method.

Since the parasite collection method is not contributing to a higher prevalence and abundance of lice in rural regions compared to urban regions, factors like bird body size could be influencing the results. It has been found that the number of lice found on birds

is correlated with the bird's body size, with more lice found on birds with a larger mass (Roza 1997). When comparing the regions, there were more large birds sampled in the rural region than the urban region (Table 2.1, Table 2.2). Therefore, a higher abundance and prevalence of lice in the rural region may be due to a greater number of larger birds in that region.

When comparing the mean intensity of lice per bird between the regions, it was found that birds in each region had a similar intensity, with 13.2 lice per bird in urban areas and 14 lice per bird in rural areas. As mentioned, the low humidity of Utah could be a factor leading to the low prevalence, abundance, and intensity of lice in Utah (Moyer et al. 2002).

There is a difference between the louse prevalence, abundance, and intensity results found by the two collection methods used in the mountain region. Post-mortem ruffling has a higher prevalence, abundance, and intensity of lice found when compared to dust ruffling. In part, the higher prevalence, abundance, and intensity of lice found by post-mortem ruffling could be due to it being a more robust method for removing ectoparasites than dust ruffling. Post-mortem ruffling has a $r2$ of 0.92, which is larger than the $r2$ of 0.86 for dust ruffling. The difference in $r2$ values could lead to more ectoparasites being collected off post-mortem ruffled birds than those dust ruffled (Clayton and Drown 2001). More ectoparasites being collected by post-mortem ruffling would lead to a higher prevalence, abundance, and intensity of lice than dust ruffling.

As mentioned, it has also been found that larger birds tend to have more ectoparasites (Clayton and Walther 2001, Rozsa 1997). When comparing the two different methods, post-mortem ruffling included birds that have a higher mass, such as

Strigiformes and Galliformes, which were not sampled by dust ruffling. The order with the largest birds that were dust ruffled was Accipitriformes. The fact that post-mortem ruffling had more birds from orders with a higher mass could also explain why the louse prevalence, abundance, and intensity were higher for it and lower for dust ruffling.

2.6 Acknowledgments

I would like to thank Mathew Waller, Aoife Galvin, Noelle Atkin, Sonora Clayton, Austin Clayton, and Mark Robbins for their help with fieldwork. I would also like to thank Sarah Bush and Dale Clayton for their help in editing this chapter and for their help with the fieldwork.

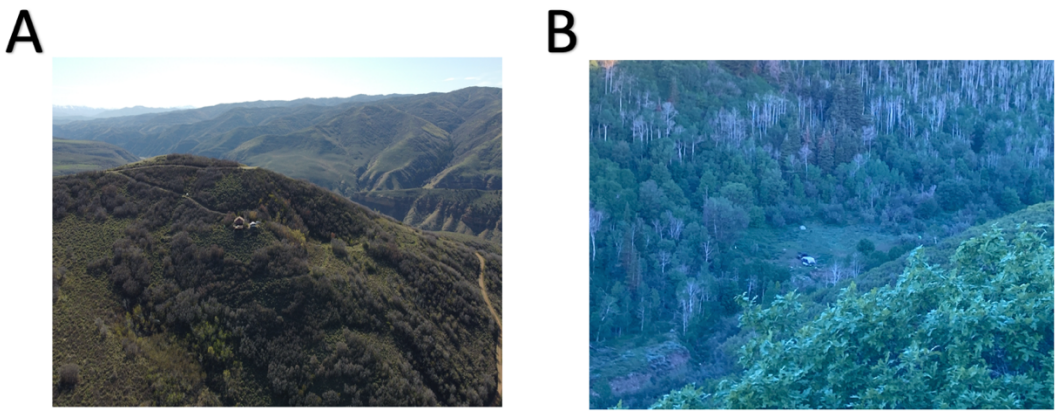


Figure 2.1: Locations in montane Utah where lice were surveyed. (A) Is the study site at Echo, Utah (41.032 °N, -111.317 °W). (B) Is the study site at Big Canyon, Utah (40.844 °N, -111.444 °W).

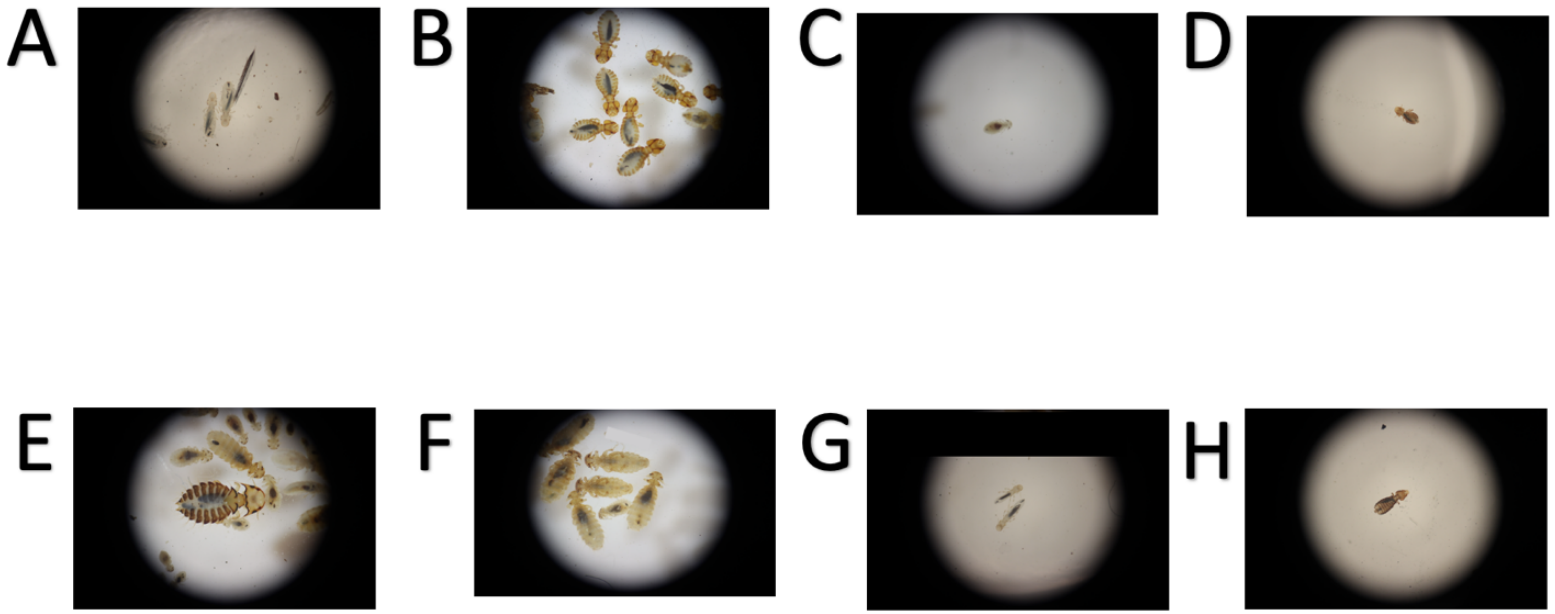


Figure 2.2: Examples of lice genera found in the montane survey. (A) *Picicola*, (B) *Strigiphilus*, (C) *Myrsidea*, (D) *Philopterus*, (E) Chelopistes, (F) *Menacanthus*, (G) *Oxylipeurus*, and (H) *Penenirmus.*

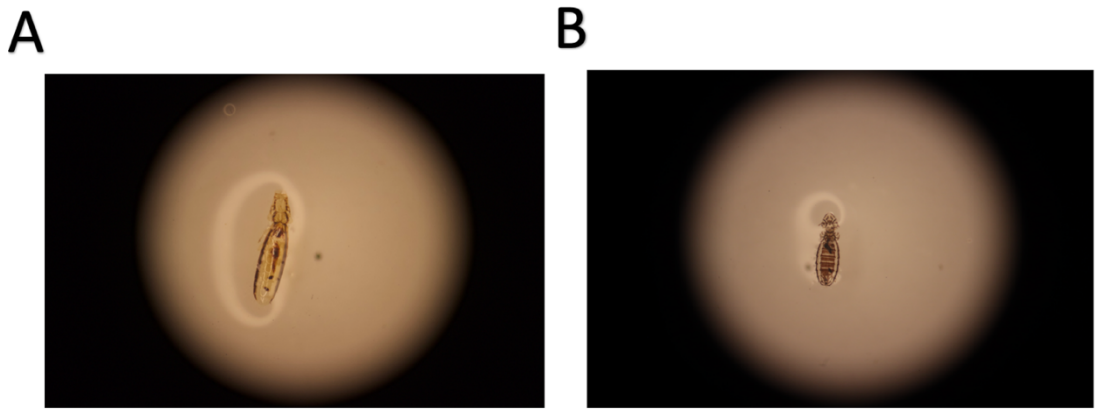


Figure 2.3: Lice genera that were most common from both surveys. (A) *Ricinus* and (B) *Brueelia*.

Table 2.1: List of birds examined in montane Utah.

orders	Species	Number of birds Post-mortem ruffled	Number of birds infested with lice PMR* (intensity***)	Number of birds Dust ruffled)	Number of birds infested with lice DR**(intensity***)	Total number of birds examined	Total number of birds infested with lice (intensity***)
Accipitriformes	*Accipiter striatus*	3	0	1	0	4	0
Apodiformes	*Selasphorus platycercus*	3	0	0	0	3	0
Galliformes	*Bonasa umbellus*	1	0	0	0	1	0
	Meleagris gallopavo	2	2 (45-125)	0	0	2	2(45-125)
Passeriformes	*Aphelocoma californica*	4	1 (2)	0	0	4	1(2)
	Carpodacus cassinii	3	1 (12)	0	0	3	1(12)
	Catharus guttatus	2	1 (2)	2	0	4	1(2)
	Contopus cooperi	0	0	1	0	1	0
	Cyanocitta stelleri	3	0	0	0	3	0
	Empidonax hammondii	0	0	1	1 (1)	1	1(1)
	Empidonax oberholseri	2	0	14	1 (1)	16	1(1)
	Empidonax occidentalis	2	0	1	0	3	0
	Geothlypis tolmiei	1	0	32	1 (5)	33	1(5)
	Junco hyemalis	9	5 (1-30)	1	0	10	5(1-30)
	Leiothlypis virginiae	2	0	20	1 (1)	22	1(1)
	Melospiza lincolnii	0	0	3	0	3	0
	Melospiza melodia	2	0	1	0	3	0
	Myadestes townsendi	2	1 (40)	0	0	2	1(40)
	Passerella iliaca	5	0	0	0	5	0
	Passerina amoena	1	0	2	0	3	0
	Pheucticus melanocephalus	1	0	9	0	10	0
	Pipilo chlorurus	1	1 (14)	25	3 (1)	26	4(1-14)
	Pipilo maculatus	0	0	47	3 (1-14)	47	3(1-14)
	Piranga ludoviciana	1	1 (7)	8	0	9	1(7)
	Poecile atricapillus	3	1 (4)	8	0	11	1(4)
	Poecile gambeli	2	0	0	0	2	0
	Polioptila caerulea	1	0	0	0	1	0
	Pooecetes gramineus	1	0	0	0	1	0
	*Regulus calendula**	1	0	1	0	2	0
	Setophaga coronata	2	0	1	0	3	0
	Setophaga petechia	1	0	1	0	2	0

Table 2.1 continued.

orders	Species	Number of birds Post-mortem ruffled	Number of birds infested with lice PMR* (intensity***)	Number of birds Dust ruffled)	Number of birds infested with lice DR**(intensity***)	Total number of birds examined	Total number of birds infested with lice (intensity***)
Passeriformes	*Sialia currucoides*	1	0	0	0	1	0
	Sitta canadensis	3	0	0	0	3	0
	Sphyrapicus nuchalis	3	2 (11-39)	0	0	3	2 (11-39)
	Spizella breweri	2	1 (2)	3	0	5	1(2)
	Spizella passerina	0	0	4	0	4	0
	Troglodytes aedon	2	0	1	0	3	0
	Turdus migratorius	0	0	14	0	14	0
	Vermivora celata	0	0	56	7 (1-3)	56	7(1-3)
	Vireo gilvus	1	0	6	0	7	0
	Vireo plumbeus	2	0	1	0	3	0
	Colaptes auratus	4	1 (75)	2	0	6	1(75)
Piciformes	*Picoides pubescens*	1	0	0	0	1	0
	Picoides villosus	2	0	0	0	2	0
Strigiformes	*Bubo virginiatus*	1	1 (61)	0	0	1	1(61)
Total		**86**	**20**	**267**	**16**	**353**	**36**

* PMR = Post-Mortem ruffled

** DR = Dust ruffled

*** Intensity is reported as the range in number of lice infesting individual birds.

Table 2.2: Ectoparasites recovered from birds in urban Utah.

Orders	Species	Number of birds examined	Number of birds infested with lice (Intensity*)	Number of birds infested with mites (Intensity*)	Number of birds infested with ticks (Intensity*)
Apodiformes	*Archilochus alexandri*	2	0	2(2)	0
	Selasphorus platycercus	2	0	2(2-3)	0
	Selasphorus sasin	1	0	1(1)	0
Columbiformes	*Zenaida macroura*	2	0	0	0
Passeriformes	*Bombycilla cedrorum*	45	0	4(3-29)	0
	Cardellina pusilla	3	1 (15)	0	0
	Certhia americana	2	0	0	0
	Geothlypis tolmiei	3	0	0	1 (1)
	Junco hyemalis	6	0	3(2-83)	0
	Melospiza lincolnii	2	0	2(7-9)	1(1)
	Molothrus ater	1	1(35)	1(14)	0
	Myadestes townsendi	1	0	0	0
	Passerina amoena	1	0	1(1)	0
	Passerina caerulea	1	1(1)	0	0
	Pipilo maculatus	1	0	0	0
	Pipilo chlorurus	1	0	0	0
	Piranga ludoviciana	1	0	0	0
	Poecile atricapillus	1	0	0	0
	Regulus calendula	2	0	0	0
	Setophaga coronata	2	1(14)	0	0
	Setophaga petechia	1	1(1)	0	0
	Setophaga townsendi	1	0	0	0
	Sphyrapicus nuchalis	1	0	0	0
	Spizella breweri	1	0	1(2)	0
	Spinus tristis	2	0	1(17)	0
	Turdus migratorius	1	0	0	0
	Vermivora celata	2	0	1(2)	0
	Total	**89**	**5**	**19**	**2**

*Intensity is reported as the range of parasites infesting individual birds.

Table 2.3: Summary of host-louse association in montane Utah.

Orders	*Species*	Lice	Total number of birds infested	Intensity (range)	Number of birds infested in Echo	Intensity range for echo	Number of birds infested in Big Canyon	Intensity range for Big Canyon
Galliformes	*Meleagris gallopavo*	*Chelopistes meleagridis*	2	(1-83)	0	0	2	(1-83)
		Menacanthus stramineus	2	(30-37)	0	0	2	(30-37)
		Oxylipeurus polytrapezius	2	(2-17)	0	0	2	(2-17)
Passeriformes	*Aphelocoma californica*	*Brueelia sp.*	1	1	0	0	1	1
	Carpodacus cassinii	*Breelia sp.**	1	12	0	0	1	12
	Catharus guttatus	*Myrsidea sp.*	1	2	0	0	1	2
	Empidonax hammondii	*Philopterus sp.**	1	1	1	1	0	0
	Empidonax oberholseri	*Philopterus sp. **	1	1	0	0	1	1
	Geothlypis tolmiei	*Ricinus sp.*	1	6	0	0	1	6
	Junco hyemalis	*Ricinus sp.*	3	1	0	0	3	1
		Brueelia vulgata	2	(1-30)	0	0	2	(1-30)
	Leiothlypis virginiae	*Ricinus sp.**	1	1	1	1	0	0
	Myadestes townsendi	*Philopterus sp.**	1	35	1	35	0	0
		Brueelia sensu lato, sp.	1	5	1	5	0	0
	Pipilo chlorurus	*Ricinus sp.**	4	(1-12)	3	1-12	1	1
	Pipilo maculatus	*Ricinus sp.**	3	(1-5)	2	(1-5)	1	1
	Piranga luiviciana	*Brueelia sensu lato, sp.**	1	7	1	7	0	0
	Poecile atricapillus	*Menacanthus sp.*	1	4	0	0	1	4
	Spizella breweri	*Brueelia sp**	1	2	1	2	0	0
	Vermivora celata	*Ricinus sp.*	7	(1-3)	7	(1-3)	0	0
Piciformes	*Colaptes auratus*	*Picicola sp.*	1	75	1	75	0	0
	Sphyrapicus nuchalis	*Penenirmus sp.**	2	(11-39)	2	(11-39)	0	0
Strigiformes	*Bubo virginianus*	*Strigiphilus*	1	61	1	61	0	0

*New host record

Table 2.4: Summary of host-louse associations for urban Utah.

Host	Lice	Number of birds infested	Intensity
Cardellina pusilla	*Ricinus sp**	1	15
Molothrus ater	*Brueelia ornatissima*	1	35
Passerina caerulea	*Ricinus australis*	1	1
Setophaga coronata	*Ricinus dendroicae*	1	14

*New host record

2.7 Literature Cited

Bush, S. E, D. R. Gustafsson, and D. H. Clayton (2018). New records of ectoparasites from passerine birds in the high tatras of Slovakia. *Oecologia Montana* 27:43-45.

Bush, A. O., K. D. Lafferty, J. M. Lotz, and A. W. Shostak (1997). Parasitology meets ecology on its own terms: Margolis et al. revisited. *Journal of Parasitology* 83:575-583.

Carrillo, C. M., F. Valera, A. Barbosa, and E. Moreno (2007). Thriving in an arid environment: High prevalence of avian lice in low humidity conditions. *Écoscience* 14:241-249.

Choi, C., H. Kim, T. A. Klein, H. Nam, and G. Bing (2022). Introduction of non-native ticks collected from fresh migratory bird carcasses on a stopover island in the Republic of Korea. *Korean Journal of Parasitology* 60:57-63.

Clayton, D. H., and D. M. Drown (2001). Critical evaluation of five methods for quantifying chewing lice (Insecta: Phthiraptera). *Journal of Parasitology* 87:1291-1300.

Clayton, D. H. and B. A. Walther (2001). Influence of host ecology and morphology on the diversity of neotropical bird lice. *Oikos* 94: 455-467.

Clayton, D. H., R. J. Adams, and S. E. Bush (2008). Phthiraptera, the chewing lice. In *Parasitic Diseases of Wild Birds* (C. T. Atkinson, N. J. Thomas, and B. Hunter, Editors). John Wiley and Sons, Inc, Hoboken, NJ, USA, 513-526.

Galloway, T. D., and R. J. Lamb (2021). Population dynamics of chewing lice (Phthiraptera) infesting birds (Aves). *Annual Review of Entomology* 66:209-224.

Moir, M. L., P. A Vesk, K. E. C Brennan, D. A Keith, L. Hughes, and M. A McCarthy (2010). Current constraints and future directions in estimating co-extinction. *Conservation Biology* 24:682-690.

Moyer, B. R., D. M. Drown, and D. H. Clayton (2002). Low humidity reduces ectoparasite pressure: Implications for host life history evolution. *Oikos* 97:223-228.

Overpeck, J. T., and B. Udall (2020). Climate change and the aridification of North America. *Proceedings of the National Academy of Science* 117:11856-11858.

Pence, D. B (2008). Acariasis. In *Parasitic Diseases of Wild Birds* (C. T. Atkinson, N. J. Thomas, and B. Hunter, Editors). John Wiley and Sons, Inc, Hoboken, NJ, USA, 527-536.

Poulin, R., and S. Morand (2005). *Parasite Biodiversity*. Smithsonian Institution Scholarly Press, Washington, D.C., USA.

Price, R. D., R. A. Hellenthal, R. L. Palma, K. P. Johnson, and D. H. Clayton (2003). *The Chewing Lice: World Checklist and Biological Overview.* Illinois Natural History Survey, Champaign, IL, USA.

Roberts, M. G., P. J. Hudson A. Rizzoli, B. T. Grenfell, H. Heesterbeek, and A. P. Dobson (2001). *Parasite Community Ecology and Biodiversity*. Oxford University Press, Oxford, MA, USA.

Rozsa, L. (1997). Patterns in the abundance of avian lice (Phthiraptera: Amblycera, Ischnocera). *Journal of Avian Biology* 28:249-254.

Tahir, D., L. Meyer, J. Fourie, F. Jongejan, T. Mather, V. Choumet, B. Blagburn, R. K. Straubinger, and M. Varloud (2020). Interrupted blood feeding in ticks: Causes and consequences. *Microorganisms* 8:910.

Woods, A. J., D. A. Lammers, S. A. Bryce, J. M. Omernik, R. L. Denton, M. Domeier, and J. A. Comstock (2001), Ecoregions of Utah. Geological Survey (map scale 1:1,175,000), Reston, VA, USA.

CHAPTER 3

RELATIONSHIP BETWEEN HUMIDITY AND ECTOPARASITE PRESSURE: A STUDY OF FEATHER LICE ON BIRDS

3.1 Abstract

Climate change will impact species composition as ecosystems change. Changes in abiotic factors in the environment could lead to changes in species distributions. One example of an abiotic factor that is predicted to change is the relative humidity, with some areas predicted to become more arid and others predicted to become wetter. There is evidence that lice gain water through the air, so changes in humidity caused by climate change should influence their populations. One previous study found very few ectoparasites on hosts in arid environments. Interestingly, another study found that Trumpeter finches (*Bucanetes githagineus*) living in a relatively arid region were still infested with feather lice. These two studies focus on just a few species, yet many bird species live in arid regions, and many more may experience arid conditions if the climate changes as predicted. Here, I use published surveys of birds and their ectoparasitic lice to investigate whether ambient humidity is likely to influence ectoparasite pressure on birds. This chapter includes data from birds from 27 regions across the globe. Louse prevalence was significantly positively correlated with humidity, and louse abundance also tended to increase with humidity. The results from this study imply that lice will decrease as areas

become arid and increase in areas becoming wetter. However, only a few studies focused on areas with low humidity. More studies should be carried out in low-humidity environments to establish a baseline for ectoparasite diversity and to make more robust predictions about how ectoparasite diversity will change in areas that become more arid over time.

3.2 Introduction

Climate change has and will continue to impact the environment. In most regions, the earth is predicted to get warmer (Trenberth 2011, Zurbenko and Luo 2012, and Pasqui and Giuseppe 2019). Models also show that some ecosystems are expected to become more arid, and others are predicted to become wetter (Trenberth 2011). With changes in the humidity and temperature of ecosystems, there may be changes in their fauna. For example, vectors of human disease and parasites that cause diseases are predicted to spread to new areas (Sutherst 2004). While some parasites will expand their range, others have been projected to go extinct (Roberts et al. 2001 and Dunn et al. 2009). It is unclear what will happen with some parasites since there are gaps in our baseline data for them. One group of parasites with gaps in baseline data are ectoparasites of birds, making it hard to determine how much climate change has already influenced their populations.

Although it may not be possible to determine how populations of ectoparasites have already been influenced by climate change, predictions can be made about how it will influence ectoparasites in the future. One way to estimate how climate change may influence ectoparasites is by surveying regions with varying humidities. Ectoparasites such as lice have been shown to glean water through specialized mouth parts that help

extract moisture from the air (Williams 1970, Rudolph 1983). Since lice gain water from the air, low humidity could cause lice to desiccate and may cause louse populations to decrease (Moyer et al. 2002, Harbison et al. 2008, Malenke et al. 2011). If louse populations are small in arid environments right now, predictions can be made that if an ecosystem starts to dry out, the future ectoparasite composition will resemble that of currently arid ecosystems. It can also be predicted that in ecosystems that are getting wetter, the ectoparasite composition will resemble the ectoparasite composition of regions that currently that have higher humidity.

However, it seems that there are contradictory results on how humidity influences the number of ectoparasites on birds. Some results suggest that lice on birds in areas with a low humidity will have fewer ectoparasites than those in a higher humidity (Moyer et al. 2002). Moyer et al. (2002) found that the prevalence and abundance of lice on Mourning doves (*Zenaida macroura*), Inca doves (*Columbina inca*), and Feral pigeons (*Columba livia*) was lower in less humid environments than in more humid environments (Figure 3.1). On the other hand, results also suggest that even in low-humidity areas, birds can have a high number of ectoparasites. For example, Carrillo et al. (2007) found a high prevalence of lice (40.9%) on Trumpeter finches in an environment with a low mean humidity of 51%. With this being said, Trumpeter finches are not in the same order as Mourning doves, Inca doves, and Feral pigeons. Mourning doves, Inca doves, and Feral pigeons are all in the order Columbiformes, whereas Trumpeter finches are Passeriformes, which suggest that the trend found by Moyer et al. (2002) only pertains to lice on Columbiformes. Passeriformes, represent about 60% of bird diversity (Oliveros et al. 2019), whereas Columbiformes represent about 3% of bird diversity. Understanding

how humidity influences lice on Passeriformes could give better insight into how a large percentage of birds will be influenced by climate change.

Changes in ectoparasite composition have been suggested to influence how hosts spend their time and energy, especially if the host's fitness is impacted (Clayton 1990, Lehmann 1993). For example, ectoparasites have been attributed to reducing the insulative capabilities of birds' feathers as they are eaten (Clayton 1990, Jenni and Winkler 2020). To conserve heat, birds will reduce activities that cause heat to escape, such as courtship displays (Clayton 1990). Ectoparasites can also activate the immune system if blood meals are taken (Owen et al. 2010). Causing an immune defense is costly and can use resources needed for other activates, such as self maintenance for adults and growth and development for juveniles (Owens et al. 2010). Therefore, it may not be surprising that animals increase behaviors that reduce the number of ectoparasites they have.

One behavior that reduces the number of ectoparasites is grooming (Clayton 1991, Goodman et al. 2020). Grooming is defined as a behavior used by an organism to maintain and clean the body's integument. Grooming is seen across multiple taxa, including birds, insects, and mammals (Cotgreave and Clayton 1994, Yanagawa et al. 2020, and Zhang et al. 2021). In birds, grooming can be split into preening and scratching. Preening is defined as a bird manipulating its feathers with its beak, and scratching is defined as a bird manipulating its feathers with its feet. Grooming is energetically costly; it can lead to a rise of 196% above the resting metabolic rate in birds (Croll and Mclaren 1993) or a range of 1.6 to 2.3 times the basal metabolic rate (Goldstein 1988). Grooming is also time-consuming, with birds spending about 8.5% of

their time grooming (Walther and Clayton 2005). It has been found that birds increase the amount they groom as the number of ectoparasites on them increases (Villa et al. 2016).

If ectoparasites increase in regions that are becoming more humid, the increase in time needed for grooming could stress birds that are already experiencing other pressures caused by climate change. On the other hand, in regions that are getting drier, there might be a reduction in the amount of grooming as ectoparasites pressure is reduced. The decrease in ectoparasites may free up time and energy for these birds.

Through a literature review, the presence of lice on bird populations located in regions throughout the world was carried out. By studying lice in various regions, this chapter aims to see if there is a relationship between humidity and prevalence and abundance of ectoparasites on birds. While previous studies compared the relationship between lice and humidity on one bird order from a few geographic regions (Moyer et al. 2002, Carrillo et al. 2007, Sychra et al. 2010). this chapter will investigate lice from hundreds of bird species surveyed around the world so that a variety of lice populations can be compared to various humidity.

3.3 Methods

To determine how changes in humidity will influence lice on birds, I used the Web of Science and Google Scholar to find papers published between 1901 and 2023. to find published papers containing surveys of lice on birds from any region in the world. The following search terms: “feather lice AND humidity,” “Louse Census,” Louse Diversity,” “Feather Louse Census,” “Lice on Birds,” and “ectoparasites and humidity”

were used to find publications for this chapter. With these search terms, 34 relevant studies were examined, representing 58 regions across the globe.

For each paper, the region the lice were collected in was documented. When birds were surveyed from multiple regions within the same paper, they were analyzed by the region they were found in. For each surveyed region, I obtained humidity data for that region using worlddata.info. Climate data from worlddata.info is collected from weather stations and displayed as an average for each month and representing 20 years' worth of data. Additionally, humidity data was only used for a region for the months that the louse surveys were carried out. Louse abundance, louse prevalence, how many birds were caught, how many bird species were examined, and louse collection methods were also recorded from each paper (Table 3.1). Louse abundance is the average number of lice found on each host across a population. Louse prevalence is the percentage of individuals within a population that are infested with lice (Bush et al. 1997).

Some studies found in the literature search were excluded from analyses based on the following criteria. Studies were excluded if fewer than 12 birds were captured. Parasites are typically overdistributed, with 20% of the hosts having 80% of the parasites (Atkinson et al. 2008, Loker and Hofkin 2015). What this means is that a large number of parasites could be missed if too few of the hosts are sampled. Bush et al. (2013) found that a sample of 12 hosts yielded a 90% probability of accurately sampling parasite species richness; thus, studies included in this review were restricted to surveys that included at least 12 individuals. Papers were also excluded if lice were only visually censused on the birds. There are a few reasons that visual censuses were excluded. It can be difficult to visually census lice when many or a few are on the bird. It can also be

difficult when the louse species move quickly among the feathers, when lice are the same color as the plumage (Galloway and Lamb 2021). Visual examination only finds between 9 and 14% of lice (Clayton and Drown 2001, Koop and Clayton 2013), so if there are only a few lice on the bird, they could be easily missed, which would especially influence the estimated abundance and prevalence of lice for some regions with low numbers of lice. With this being said, papers were kept if visual examination was used along with another parasite collection method. Ultimately, the meta-analysis included data from 27 regions for the louse prevalence to humidity comparison and 27 regions for the louse abundance to humidity comparison (Figure 3.2).

R-package 4.0.3 was used to generate the scatter plots and run this chapter's least squares linear regression analyses. Least squares linear regression models were used to compare louse abundance and prevalence to humidity. For least squares linear regression, the abundance data was log+1 transformed, and the prevalence data were log-transformed.

3.4 Results

Lice have been found on birds on every continent, including Antarctica (Vanstreels et al. 2020), which means they are subjected to a range of humidities. When comparing humidity to louse abundance, there was a lower abundance of lice in regions with lower humidity than regions with higher humidity (Figure 3.3). The lowest log+1 abundance of lice was 0.11 and the highest was 2.9. The result from comparing louse abundance to humidity is a weak, nonsignificant trend since the linear regression of the log+1 abundance data gave a p-value of 0.11. There was a positive correlation between

humidity and louse prevalence (Figure 3.4; log prevalence, linear regression p-value = 0.047). The lowest louse prevalence was 5.6% and the highest was 69.2%. The lowest humidity in both comparisons was 37% and the highest humidity was 85%

3.5 Discussion

There was a significant positive correlation between louse prevalence and humidity. Finding a significant positive correlation between humidity and ectoparasite prevalence is consistent with a pattern that birds in areas with higher humidity have more lice than birds in areas of lower humidity. Therefore, the results from this chapter are in line with the findings of Moyer et al. (2002) but broaden the scope to include birds other than Columbiformes. About 70% of the data used in this chapter came from surveys of lice found on Passerines, suggesting that lice's prevalence on Passerines follows a similar pattern to Columbiformes.

At first, the results of this chapter may seem to not be in line with the results of Carrillo et al. (2007), which found that Trumpeter finches have a high prevalence of lice (40.9%) in study areas that have a lower average minimum humidity (30%). However, when the results of Carrillo et al. (2007) are looked at, the number of lice between the 2 regions that Trumpeter finches were sampled from was not the same. More lice were collected in the region with the higher humidity than in the region with the lower humidity, which is in line with the results from this chapter. Perhaps the results from Carrillo et al. (2007) suggest that in some regions, humidity influences lice in ways other than the percentage of birds that have them. Instead of birds being free of lice in low-humidity environments, each bird might have fewer lice. It would be interesting to know

what the louse abundance was for the finches studied in Carrillo et al. (2007) so that it could be determined if a lot of birds had lice, but they had them in low numbers. One explanation for there being low number of lice is that some lice are better at extracting water from the air than others. The ones that can extract the moisture from the air survive, and those that cannot get enough moisture die. It could also be that lice do not lay as many eggs when there is a lower amount of humidity. Perhaps lice need to invest more resources in the eggs they lay so they do not desiccate in a lower-humidity environment. If they invested more resources in eggs that they lay, they may not be able to lay as many eggs, which would lead to fewer lice on the birds. Unfortunately, I could not calculate louse abundance from the data provided by Carrillo et al. (2007).

The high prevalence of lice in Carrillo et al. (2007) could also be due to the Trumpeter finches being in areas with arid-adapted lice genera. Two genera of lice were found on Trumpeter finches, *Brueelia* and *Philopterus*. *Brueelia* was found to have the highest prevalence, similar to what is seen in the previous chapters of this thesis. Finding a high prevalence of *Brueelia* on the Trumpeter finches and finding them in the previous chapters of this thesis could further support it being an arid-adapted genus.

The findings of Bush et al. (2009) support the hypothesis that *Brueelia* spp. may be arid-adapted. Bush et al. (2009) sampled lice found on Western scrub-jays (*Aphelocoma californic*) in regions across the Southwestern United States; they found that some Scrub-jays in areas with a mean relative humidity below 55% had *Brueelia* sp.. Another factor in support of *Brueelia* being an arid-adapted species comes from Rudolph (1982), which looked into how quickly different louse species lose and gain water. Out of the 15 genera of lice examined, only one genus lost water slower than *Brueelia. Brueelia*

also had the second-highest water uptake-to-loss ratio. The louse genus with the highest water uptake-to-loss ratio was *Bovicola,* which is normally found on cattle and, therefore, not found on any of the birds sampled in the surveys. The low amount of lost water and the high water uptake-to-loss ratio could be useful in surviving in an arid environment because water is lost slowly and can be taken in quickly when available. It would be interesting to know what the water uptake-to-loss ratio would be for *Ricinus* and *Philopterus* since they were genera found in arid regions (Chapter 2). Unfortunately, Rudolph (1983) did not examine *Ricinus* and *Philopterus*, so it cannot be determined if they show similar patterns to *Brueelia*.

When looking at abundance, there were more ectoparasites on individuals in humid environments than in arid environments, but this result is barely a trend (P=0.11). There may be a few reasons why the trend between abundance and humidity was not significant. Abundance looks at the mean number of ectoparasites on hosts in a given population. Since various bird orders were used when calculating louse abundance, there is a wide range of body sizes. It has been found that the body size of a bird influences the number of ectoparasites it has (Rozsa 1997). Therefore, the wide variety of bird body sizes in the literature review may have masked a biological relationship between humidity and louse abundance. Even in Passerines, there is a large difference in body size (Gosler et al. 1998). Unfortunate, the mass data for the birds captured in the studies used in this chapter were not recorded, so I could not account for body size in my analyses. Future studies could try to account for body size to determine if there is a relationship between louse abundance and humidity.

On the other hand, having a wide range of bird body sizes would not influence the results found between louse prevalence and humidity. Prevalence is the percentage of birds in a population with ectoparasites, larger birds with more ectoparasites should not skew the prevalence for a sampled population but could skew the abundance for a sampled population. Therefore, prevalence is not as influenced by body size as abundance. Birds with as many as 1000 ectoparasites have the same prevalence as those with only 1 ectoparasite, which could be why a significant positive correlation exists between prevalence and humidity and only a slight trend between abundance to humidity.

Another factor that might be worth considering in future studies is whether or not the birds sampled were migrating. If the birds recently migrated, the abundance and prevalence results may not accurately represent the number of lice on birds in the collected region. In Sychra et al. (2011), researchers examined how ectoparasite abundance, prevalence, and intensity changed on birds between the pre and post-breeding seasons. They also looked at differences in prevalence and abundance between birds that migrated and those that were resident. It was found that both the prevalence and abundance of lice were higher in resident birds than in those migrating. There might be a lower prevalence and abundance of lice on migrating birds because heavily parasitized birds may die on long migrations (Brown et al. 1995, Cork et al. 2001). The birds considered as migrating in Sychra et al. (2011) were birds that completed long migrations. They lumped birds with short migrations into their non-migratory group. With short migrations, parasites may not kill off the bird, which means that where the birds are migrating from might influence their ectoparasite loads. (Sychra et al. 2011). It may be useful to limit data collected on louse abundance and prevalence to non-

migratory birds to represent better what louse abundance and prevalence are like for the humidity in which the lice were collected. One way to control for migration could be to limit the studies observed to seasons when birds are not migrating. Another way to deal with bird migration influencing louse abundance and prevalence could be to include humidity data from both the breeding and wintering grounds of birds surveyed for lice.

This chapter looked at any genera of lice reported on birds sampled in the surveyed regions. In the future, limiting the lice to only Ischnocera lice might be helpful since other lice suborders may be able to supplement the water they intake from the air with blood meals (Burgess 2022**)**. Ischnocera lice, on the other hand, only eat feathers and feather debris, which means they are probably more susceptible to ambient humidity. More data from low-humidity environments could also be helpful. For this study, only a few papers had regions that represented lower humidity. More research in lower humidity areas could give a better representation of what types of lice are in these regions and how louse abundance is influenced. Perhaps there were fewer studies found with ectoparasite data for less humid regions because previous bird surveys in these areas did not find ectoparasites. If no ectoparasites were found in a survey of birds, it may not be reported because the scientific literature is biased against publishing what may be interpreted as negative results. For example, in chapter 3 of this thesis, 45 Cedar waxwings (*Bombycilla cedrorum*) were washed, and no lice were found on any of these birds. Few researchers or journals would publish such findings. In contrast, ectoparasites may be more noticeable in regions with higher humidity, so they are reported more often.

In summary, baseline data for ectoparasites is missing in a lot of regions with low humidity. In part, this missing data could be due to a publishing bias. Since there are

more ectoparasites in humid regions, they are more likely to be found and recorded, which may be why more data exists for ectoparasites in these regions. More surveys should be conducted in arid areas to better establish baseline data. By studying ectoparasites in arid environments, a better understanding of ectoparasite diversity can also be established, allowing for better predictions on how parasite species composition may change as climate change continues.

3.6 Acknowledgments

I would like to thank Sarah Bush for helping edit this chapter. I would also like to thank Mathew Waller for their help with statistics.

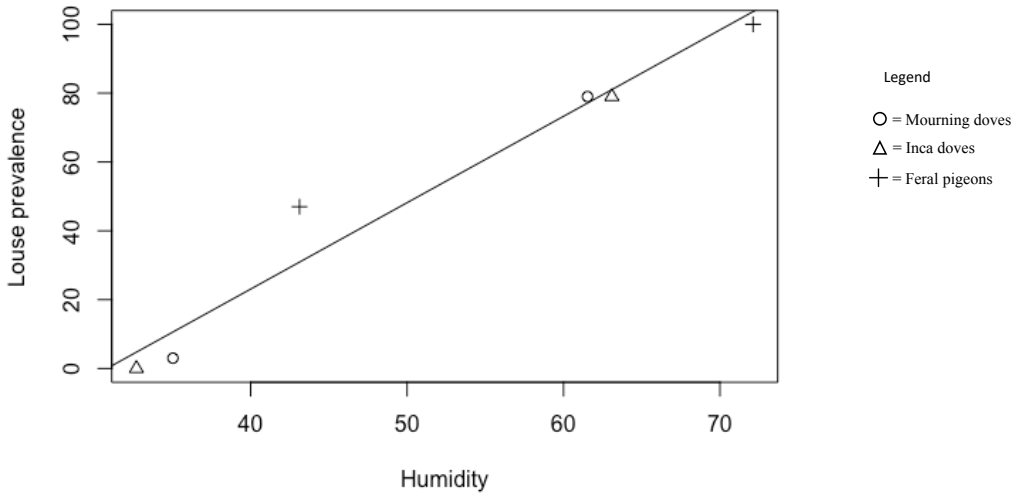
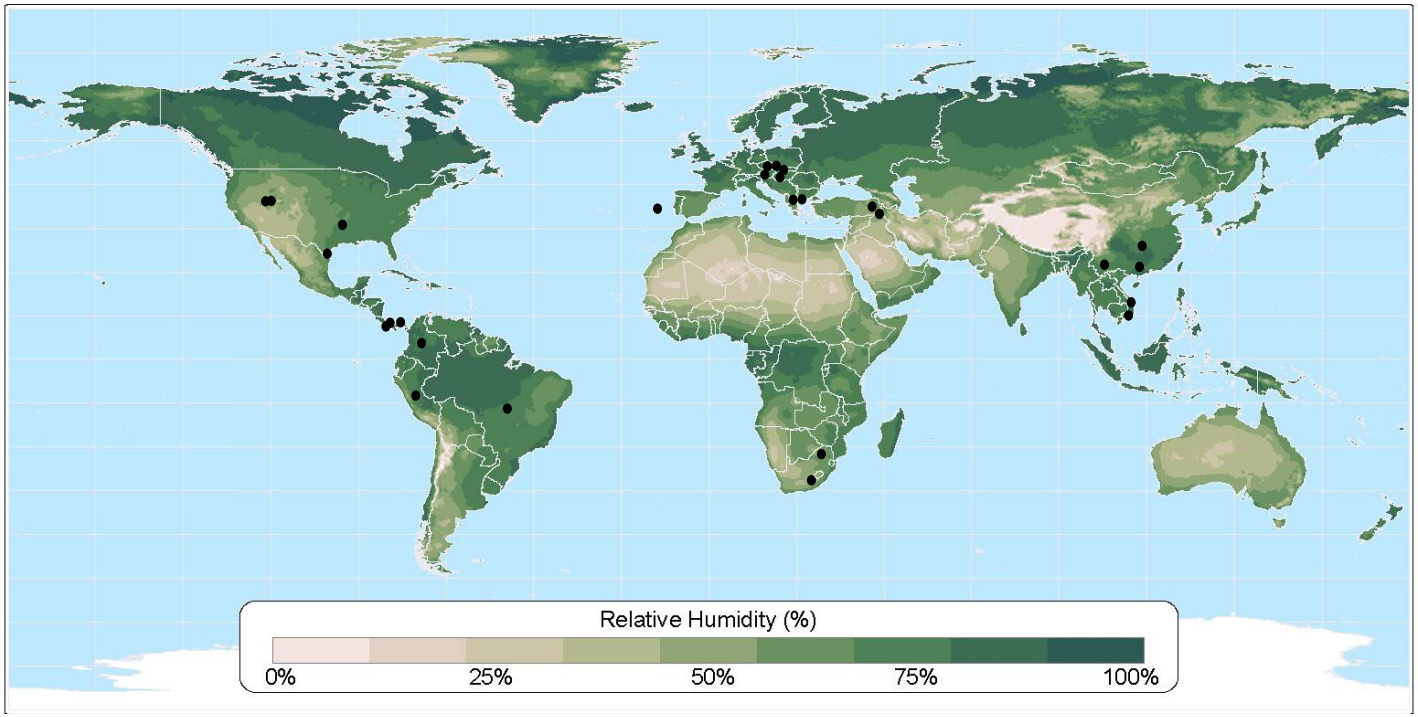


Figure 3.1: Scatter plot of humidity to louse prevalence. The scatter plot was made from data collected by Moyer et al. 2001. Each point is prevalence of lice found on species of Columbiformes.



From the Center for Sustainability and the Global Environment at The University of Wisconsin

Figure 3.2: Humidity map of locations found in the literature search. Each black dot represents a region that was studied.

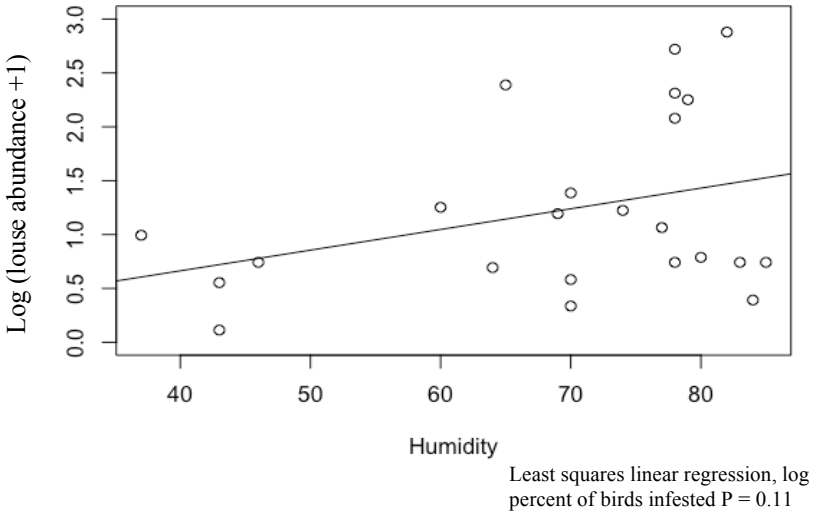


Figure 3.3: Scatter plot of humidity to log transformed plus one louse abundance. Each point is a separate location. The p-value from the least squares linear regression is 0.11.

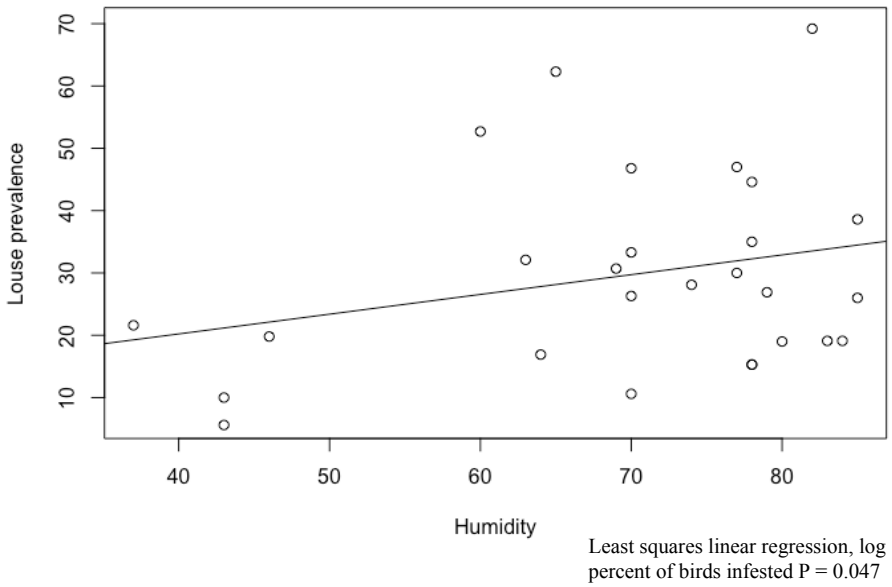


Figure 3.4: Scatter plot of humidity to louse prevalence. Each point is a separate location. The p-value from the least squares linear regression is 0.047. The linear regression used log transformed data for prevalence.

Table 3.1: Regions used to look at the influence of humidity on ectoparasites.

Citation	Location	Humidity (%)*	Prevalence	Mean abundance	Number of birds	Number of bird species	Collection method(s)**
Dik et al. 2011 a	Kars province, Turkey	37	21.6	1.7	51	22	DR
Chapter 2	Summit County, Utah, USA	43	10.0	0.12	351	45	PMR and DR
Chapter 3	Salt Lake City Utah, USA	43	5.6	0.74	89	27	Washing
Dik et al. 2011b	Aras River, Turkey	46	19.8	1.1	81	23	DR
Halajian et al. 2013	Limpopo, South Africa	60	52.7	2.5	91	18	FC and VE
Pistone et al. 2021	Texas, USA	63	32.1	Na	507	140	DR and PMR
Diakoua et al. 2017	Macedonia, Greece	64	16.9	1	543	65	FC and VE
Enout et al. 2012	Cerrado, Brazil	65	62.3	9.9	149	57	DR
Halajian et al. 2014	West Cape and KwaZulu-Natal, South Africa	69	30.7	2.3	13	6	FC and VE
Valim and Reiley 2015	Arkansas, USA	70	10.6	Na	66	1	DR
Diakoua et al. 2017	Porto Lagos, Greece	70	26.3	0.79	19	8	FC and VE
Sychra et al. 2014 a	Slovakia and Czech Republic	70	33.3	3	15	1	FC
Janiga 2018	Slovakia	70	46.8	0.4	149	2	FC
Xingzhi et al. 2019	East, Southwest, Central, and South China	74	28.1	2.4	603	215	FC and VE
Soto-Patiño et al. 2018	Colombia	77	30.0	Na	> 138	138	PMR, VE, and DR
Sychra et al 2009 b	Ba Be National Park, Vietnam	77	47.0	1.9	45	14	FC and VE
Sychra et al 2011	Moravskoslezský, Czech Republic	78	15.3	7	108	34	FC and VE
Sychra et al 2008	Moravskoslezský, Czech Republic	78	15.3	1.1	82	36	FC and VE
Bush et al. 2013	Central and South China	78	44.6	14.18	943	150	PMR
Oslejskova et al 2020	Azores shores, Portugal	78	35.0	9.1	266	8	FC and VE
Bush and Clayton 2018	Slovakia	79	26.9	8.5	52	19	FC and VE
Sychra et al. 2009 a	Cat Tien National Park, Vietnam	80	19.0	1.2	247	50	FC and VE
Sychra et al. 2014 a	Cascay, Peru	82	69.2	16.8	13	2	FC
Gustafsson et al. 2019	Yunnan, China	83	19.1	1.1	366	55	FC
Sychra et al. 2014 a	Limón, Costa Rica	85	19.1	0.48	21	3	FC
Lindell et al. 2002	Coto Brus Valley, Costa Rica	85	38.6	Na	36	2	DR
Sychra et al. 2010	Limón Costa Rica	85	26.0	1.1	170	5	FC and VE

*Humidity data from https://www.worlddata.info

**Collection methods are DR = dust ruffled, PMR = Post-mortem ruffled, FC = Fumigation chamber, and VE = Visual examination

3.7 Literature Cited

Atkinson, C. T., N. J. Thomas, and D. B. Hunter (2008). *Parasitic Diseases of Wild Birds*. John Wiley and Sons, Inc, Hoboken, NJ, USA.

Brown, C. R., M. B. Brown, and B. Rannala (1995). Ectoparasites reduce long-term survival of their avian host. *Proceedings of the Royal Society of London Series B-Biological Sciences* 262:313-319.

Burgess, I. F (2022). Sucking lice and spiracular transpiration: Turning a liability into a benefit and a necessity. *Journal of Experimental Biology* 225:7.

Bush, S. E., M. Reed, and S. Maher (2013). Impact of forest size on parasite biodiversity: Implications for conservation of hosts and parasites. *Biodiversity and Conservation* 22:1391-1404.

Bush, S. E., C. W. Harbison, D. L. Slager, A. T. Peterson, R. D. Price, and D. H. Clayton (2009) Geographic variation in the community structure of lice on western scrub-jays. *Journal of Parasitology* 95:10-13.

Bush, A. O., Lafferty, K. D., Lotz, J. M., and A. W. Shostak (1997). Parasitology meets ecology on its own terms: Margolis et al. revisited. *Journal of Parasitology* 83:575-583.

Bush, S. E., D. R. Gustafsson, and D. H. Clayton (2018). New records of ectoparasites from passerine birds in the High Tatras of Slovakia. *Oecologia Montana* 27:43-45.

Carrillo, C. M., F. Valera, A. Barbosa, and E. Moreno (2007). Thriving in an arid environment: High prevalence of avian lice in low humidity conditions. *Écoscience* 14:241-249.

Chu, X., B. Dik, D. R. Gustafsson, X. Che, Q. Zhang, and F. Zou (2019). The influence of host body size and food guild on prevalence and mean intensity of chewing lice (Phthiraptera) on birds in southern China. *Journal of Parasitology* 105:334-344.

Clayton, D. H. (1990). Mate choice in experimentally parasitized rock doves: Lousy males lose. *American Zoologist* 30:251-262.

Clayton, D. H. (1991). The influence of parasites on host sexual selection. *Parasitology Today* 7:329-334.

Clayton, D. H., and D. M. Drown (2001). Critical evaluation of five methods for quantifying chewing lice (Insecta: Phthiraptera). *Journal of Parasitology* 87:1291-1300.

Cork, S. C., T. Csörgö, S. Scebba, and G. Lövei (2001). The prevalence of nematodes parasites in transcontinental songbirds. *Research in Veterinary Science* 70:20.

Cotgreave, P. and D. H. Clayton (1994). Comparative analysis of time spent grooming by birds in relation to parasite load. *Behaviour* 131:171-187.

Croll, D. A., and E. McLaren. 1993. Diving metabolism and thermoregulation in common and thick-billed murres. *Journal of Comparative Physiology. B, Biochemical, Systemic, and Environmental Physiology* 163:160-166.

Diakou, A., J. B. Pedroso Couto Soares, H. Alivizatos, M. Panagiotopoulou, S. Kazantzidis, I. Literák, and O. Sychra (2017). Chewing lice from wild birds in northern Greece. *Parasitology International* 66:699-706.

Dik, B., M. A. Kirpik, C. Sekercioğlu, and Y. Saşmaz (2011a). Chewing lice (Phthiraptera) found on songbirds (Passeriformes) in Turkey. *Türkiye Parazitolojii Dergisi* 35:34-39.

Dik, B., C. H. Sekercioglu, and M. A. Kirpik (2011b). Chewing lice (Phthiraptera) species found on birds along the Aras River, Igdir, eastern Turkey. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi* 17:567-573.

Dunn, R. R., N. C. Harris, R. K. Colwell, L. P. Koh, and N. S. Sodhi (2009). The sixth mass coextinction: Are most endangered species parasites and mutualists? *Proceedings of the Royal Society. B, Biological Sciences* 276:3037-3045.

Enout, A. M. J., D. N. C. Lobato, F. C. Diniz, and Y. Antonini (2012). Chewing lice (Insecta, Phthiraptera) and feather mites (Acari, Astigmata) associated with birds of the Cerrado in central Brazil. *Parasitology Research* 111:1731-1742.

Galloway, T. D., and R. J. Lamb (2021). Population dynamics of chewing lice (Phthiraptera) infesting birds (Aves). *Annual Review of Entomology* 66:209-224.

Goldstein, D. L. (1988). Estimates of daily energy expenditure in birds: The time-energy budget as an integrator of laboratory and field studies. *American Zoologist* 28:829-844.

Goodman, G. B., M. C. Klingensmith, S. E. Bush, and D. H. Clayton (2020). The role of scratching in the control of ectoparasites on birds. *Auk* 137:1-9.

Gosler A. G., J. J. D. Greenwood, J. K. Baker, and N. C. Davidson (1998). The field determination of body size and condition in passerines: A report to the British Ringing Committee. *Bird Study* 45:92-103.

Gustafsson, D. R., L. Lei, K. Luo, X. Chu, X. Zhao, Q. Zhang, and F. Zou (2019). Chewing lice from high-altitude and migrating birds in Yunnan, China, with

descriptions of two new species of *Guimaraesiella*. *Medical and Veterinary Entomology* 33:407-419.

Halajian, A., O. Sychra, W. Luus-Powell, D. Engelbrecht, and I. Papousek (2014). An annotated checklist of Amblyceran chewing lice (Phthiraptera: Amblycera) from wild passerine birds (Passeriformes) in South Africa. *African Entomology* 22:762-778.

Harbison, C. W., S. E. Bush, J. R. Malenke, and D. H. Clayton (2008). Comparative transmission dynamics of competing parasite species. *Ecology* 89:3186-3194.

Janiga, M. (2018). Different coevolutionary breeding strategies of Ischnoceran lice on *Prunella collaris* and *P. modularis* in high mountains. *Polish Journal of Ecology* 66:182-193.

Jenni, L., and R. Winkler (2020). *The Biology of Molt in Birds*. Bloomsbury Publishing Plc, London, United Kingdom.

Koop, J. A. H., and D. H. Clayton (2013). Evaluation of two methods for quantifying passeriform lice. *Journal of Field Ornithology* 84:210-215.

Lehmann, T. (1993). Ectoparasites: Direct impact on host fitness. *Parasitology Today* 9:8-13.

Lindell, C. A., T. A. Gavin, R. D. Price, and A. L. Sanders (2002). Chewing louse distributions on two neotropical thrush species. *Comparative Parasitology* 69:212-217.

Loker, E., and B. Hofkin (2015). *Parasitology: A Conceptual Approach*. Garland Science, Taylor and Francis Group LLC, New York, NY, USA.

Malenke, J. R., N. Newbold, and D. H. Clayton (2011). Condition-specific competition governs the geographic distribution and diversity of ectoparasites. *American Naturalist* 177:522-534.

Moyer, B. R., D. M. Drown, and D. H. Clayton (2002). Low humidity reduces ectoparasite pressure: Implications for host life history evolution. *Oikos* 97:223-228.

Najer, T., O. Sychra, N. M. Hung, M. Capek, P. Padzemny, and I. Literak (2012). Chewing lice (Phthiraptera: Amblycera, Ischnocera) from wild passerines (Aves: Passeriformes) in northern Vietnam, with descriptions of three new species. *Zootaxa* 3530:59-73.

Najer, T., O. Sychra, F. Kounek, I. Papousek, and N. M. Hung (2014). Chewing lice (Phthiraptera: Amblycera and Ischnocera) from wild birds in southern Vietnam, with descriptions of two new species. *Zootaxa* 3755:419-433.

Oliveros, C. H., D. J. Field, D. T. Ksepka, F. K. Barker, A. Aleixo, M. J. Andersen, P. Alström, B. W. Benz, E. L. Braun, M. J. Braun, G. A. Bravo, R. T. Brumfield, R. T. Chesser, S. Claramunt, J. Cracraft, A. M. Cuervo, E. P. Derryberry, T. C.

Glenn, M. G. Harvey, P. A. Hosner, L. Joseph, R. T. Kimball, A. L. Mack, C. M. Miskelly, A. T. Peterson, M. B. Robbins, F. H. Sheldon, L. F. Silveira, B. T. Smith, N. D. White, R. G. Moyle, and B. C. Faircloth (2019). Earth history and the passerine superradiation. *Proceedings of the National Academy of Sciences* 116:7916-7925.

Oslejskova, L., S. Kounkova, D. R. Gustafsson, R. Resendes, P. Rodrigues, I. Literak, and O. Sychra (2020). Insect ectoparasites from wild passerine birds in the Azores Islands. *Parasite* 27:64.

Owen, J. P., A. C. Nelson, and D. H. Clayton (2010). Ecological immunology of bird-ectoparasite systems. *Trends in Parasitology* 26:530-539.

Pasqui, M., and E. Di Giuseppe (2019). Climate change, future warming, and adaptation in Europe. *Animal Frontiers: Review Magazine of Animal Agriculture* 9:6-11.

Pistone, J. P., J. E. Light, T. A. Campbell, T. A. Catanach, and G. Voelker (2021). Restricted geographic sampling yields low parasitism rates but surprisingly diverse host associations in avian lice (Insecta: Phthiraptera) from south Texas. *Diversity* 3:430.

Roberts, M. G., P. J. Hudson A. Rizzoli, B. T. Grenfell, H. Heesterbeek, and A. P. Dobson (2001). *Parasite Community Ecology and Biodiversity.* Oxford University Press, Oxford, MA, USA.

Rozsa, L. (1997). Patterns in the abundance of Avian lice (Phthiraptera: Amblycera, Ischnocera). *Journal of Avian Biology* 28:249-254.

Rudolph, D. (1983). The water-vapour uptake system of the Phthiraptera. *Journal of Insect Physiology* 29:15-25.

Soto-Patiño, J., G. A. Londoño, K. P. Johnson, J. D. Weckstein, J. E. Avendaño, T. A. Catanach, A. D. Sweet, A. T. Cook, J. E. Jankowski, and J. Allen (2018). Composition and distribution of lice (Insecta: Phthiraptera) on Colombian and Peruvian birds: New data on louse-host association in the neotropics. *Biodiversity Data Journal* 6:e21635.

Sutherst, R. W (2004). Global change and human vulnerability to vector-borne diseases. *Clinical Microbiology Reviews* 17:136-173.

Sychra, O., I. Literák, P. Podzemný, and V. Benedikt (2008). Insect ectoparasites from wild passerine birds in the Czech Republic. *Parasite* 15:599-604.

Sychra, O., I. Literák, P. Podzemný, P. Harmat, and R. Hrabák (2011). Insect ectoparasites on wild birds in the Czech Republic during the pre-breeding period. *Parasite* 18:13-19.

Sychra, O., F. Kounek, I. Papousek, M. Capek, J. Cardenas -Callirgos, S. Franco, and I. Literak (2014). Chewing lice (Phthiraptera: Amblycera et Ischnocera) from wrens (Passeriformes: Troglodytidae), with description of a new species of *Myrsidea*. *Acta Entomologica Musei Nationalis Pragae* 54:1-28.

Sychra, O., T. Najer, F. Kounek, M. Capek, and I. Literak (2010). Chewing lice (Phthiraptera) on manakins (Passeriformes: Pipridae) from Costa Rica, with description of a new species of the genus *Tyranniphilopterus* (Phthiraptera: Philopteridae). *Parasitology Research* 106:925-931.

Sychra, O., I. Literák, N. M. Hung, and P. Podzemný (2009). Chewing lice from wild passerines (Aves, Passeriformes) from Vietnam, with description of a new species of the genus *Brueelia* (*Phthiraptera, Ischnocera, Philopteridae*). *Acta Parasitologica* 54:154-157.

Trenberth, K. E. (2011). Changes in precipitation with climate change. *Climate Research* 47:123-138.

Valim, M. P., and B. M. Reiley (2015). The chewing lice (Insecta, Phthiraptera) fauna of the Swainson's warbler, *Limnothlypis swainsonii* (Aves, Parulidae). *Journal of Medical Entomology* 52:850-857.

Vanstreels, R. E. T., R. L. Palma, and S. V. Mironov (2020). Arthropod parasites of Antarctic and subantarctic birds and pinnipeds: A review of host-parasite associations. *International Journal for Parasitology. Parasites and Wildlife* 12:275-290.

Villa, S. M., H. E. Campbell, S. E. Bush, and D. H. Clayton (2016). Does antiparasite behavior improve with experience? An experimental test of the priming hypothesis. *Behavioral Ecology* 27:1167-1171.

Walther, B. A., and D. H. Clayton (2005). Elaborate ornaments are costly to maintain: Evidence for high maintenance handicaps. *Behavioral Ecology* 16:89-95.

Williams, R. T (1970). In vitro studies on the environmental biology of *Goniodes colchici* (Denny) (Mallophaga: Ischnocera). *Australian Journal of Zoology* 18: 391-398.

Yanagawa, A., W. Huang, A. Yamamoto, A. Wada-Katsumata, C. Schal, and T. F. C. Mackay (2020). Genetic basis of natural variation in spontaneous grooming in *Drosophila melanogaster*. *G3* 10:3453-3460.

Zhang, Y., L. V. Cifuentes, K. N. Wright, J. P. Bhattarai, J. Mohrhardt, D. Fleck, E. Janke, C. Jiang, S. L. Cranfill, N. Goldstein, M. Schreck, A. H. Moberly, Y. Yu, B. R. Arenkiel, J. N. Betley, W. Luo, J. Stegmaier, D. W. Wesson, M. Spehr, M. V. Fuccillo, and M. Ma (2021). Ventral striatal islands of calleja neurons control grooming in mice. *Nature Neuroscience* 24:1699-1710.

Zurbenko, I., and M. Luo (2012). Restoration of time-spatial scales in global temperature data. *American Journal of Climate Change* 1:154-163.

APPENDIX A

SCREENING WILD BIRDS IN UTAH FOR *SODALIS*

A.1 Introduction

Symbiotic relationships can form between organisms that interact in the environment. A symbiotic relationship involves interaction between two different organisms living in close association with each other. These interactions can positively or negatively influence the organism's fitness in the relationship. At times the nature of these relationships can change. For example, some bacteria may start as pathogens but form mutualistic relationships with their hosts over time (McCutcheon et al. 2019). Eventually, these bacteria may become obligate endosymbiotic organisms that rely on their host to survive. While endosymbiotic bacteria can be found in multiple taxa, insects, in particular, have a large number of obligate endosymbiotic bacteria, with 10% of species estimated to have endosymbiotic bacteria (Stork 2018). Endosymbiotic organisms can help their host in a variety of ways, including predator defense (Oliver et al. 2003), protection against toxins (Blanton and Peterson 2020), and nutrient supplement (Su et al. 2022). By supplementing their hosts' nutrients, endosymbiotic microorganisms have been able to help insects spread into nutrient poor niches, leading to insect species' radiation (Cornwallis et al. 2021). While many insects form associations with bacterial

endosymbionts, there has not been much success in finding out where hosts pick up their endosymbiotic organisms in the environment.

An example of an association between a host and an endosymbiotic organism is the association between *Columbicola columbae* and a genus of bacteria called *Sodalis*. *Columbicola columbae* are obligate ectoparasites found on the feathers of Rock pigeons (*Columba livia*). They complete their whole life cycle on their hosts and only eat feathers and feather debris found on the host (Clayton et al. 2008). *Sodalis* is a genus of bacteria that is the primary endosymbiont for *Columbicola columbae*. Due to *Columbicola columbae* only eating feathers and feather debris, it is thought that *Sodalis* helps compensate for nutrients absent in its nutrient poor diet (Fukatsu et al. 2007**)**.

The association between *Columbicola columbae* and its *Sodalis* is useful to study for multiple reasons. One reason is that there are strains of *Sodalis* that can be cultured in the lab and then injected into insects (Su et al. 2022**).** There is also a free-living strain of *Sodalis* called *Sodalis praecaptivus*. *Sodalis praecaptivus* was isolated from a puncture wound caused by a branch (Chari et al. 2015). Since a branch caused the puncture wound, it could give insight into where *Sodalis* is typically found in the environment. With *Sodalis,* it would seem that it can be found on plants.

Since a lot is known about the association between *Columbicola columbae* and *Sodalis,* it is a good system for figuring out how an endosymbiotic association forms. One puzzle in this association is how *Columbicola columbae* came into contact with *Sodalis.* As mentioned, *Columbicola columbae* spend their whole life cycle on their host, meaning there are few opportunities for the lice to come into contact with *Sodalis*, especially if *Sodalis* is normally found on vegetation. One possible way that *Columbicola*

columbae could have come into contact with *Sodalis* is if the *Sodalis* ended up on the pigeons that the lice were infesting. Pigeons could pick up *Sodalis* when they come in contact with vegetation in their environment. If the Pigeons came into contact with vegetation, *Sodalis* could end up on the pigeon's plumage or feet. In such a scenario there is a chance of *Columbicola columbae* coming in contact with it, which may be how *Sodalis* was acquired from the environment by *Columbicola columbae*.

In this Appendix, I am investigating if *Sodalis* can be found on the plumage and the feet of birds caught in Utah. By sampling the plumage and feet of birds for *Sodalis*, there can be a better understanding of how *Columbicola columbae,* and by extension, other ectoparasites, gain their endosymbiotic organisms from the environment.

A.2 Methods

A.2.1 Screening for *Sodalis* on wild birds

A total of 266 birds were sampled in Echo and Big Canyon, Utah, from May through August 2021. Birds used in this study were caught in mist nets, and feathers samples were collected from the outer tail feathers of the birds captured. In total, 2 tail feathers were taken from each bird and stored in whirl-pak bags. Whirl-pak bags are sterile, so the feathers would not get contaminated while transported from the field site to our lab at the University of Utah. To prevent the feathers from getting contaminated, they were collected once the bird was extracted from the net. Before extracting the bird, hand sanitizer was used to prevent bacteria from spreading from the researcher's hands to the bird. Once the hand sanitizer had dried, the bird was taken out of the net. Feather samples were kept in a cooler in the field to try and slow down bacterial growth.

Feather samples were transported back to the University of Utah and kept in a fridge set to - 20 °C until they were processed. To grow bacteria off the feathers, 1cm of the feather was cut off the collected tail feather with scissors that had been sterilized in 75% ethanol. Before cutting the feather, the excess ethanol was burned off. Once cut, feathers were transferred to a sterile test tube that contained 5 ml of sterile saline. The feather was then briefly vortex mixed, and 1 ml of the saline solution was pipetted onto the LB agar mentioned below.

A.2.2 Screening for *Sodalis* on Rock pigeons

Pigeons were sampled for *Sodalis* in downtown Salt Lake City Utah. 40 Pigeons were captured in walk-in traps baited with pigeon mix. Hand sanitizer was used to prevent bacteria from spreading from the researcher to the pigeons. Pigeons were then extracted from the traps, and their feet were placed on the LB agar mentioned below. After the pigeon's feet were pressed into the plate, the pigeon was released. The petri plates were then transported back to the University of Utah, where they were incubated.

A.2.3 Media, controls, and incubation methods used to screen for *Sodalis*

LB agar was used to grow *Sodalis* that contained IPTG, X-gal, and polymyxin B. Once inoculated, the plates were incubated at room temperature (20 °C) for up to a week inside a sterile fume hood. After a week, the plates were checked for growth. *Sodalis* was identified by blue colonies on the LB agar. The *Sodalis* colonies appear blue on the LB agar since they utilize lactose (Maas 1999). For every 10 plates, there was a positive

and negative control for *Sodalis* growth. The positive controls were a known strain of *Sodalis* inoculated onto the LB agar that was made to sample for *Sodalis*. The negative controls were LB agar that were not inoculated with bacteria.

A.3 Results

A.3.1 Screening for *Sodalis* on wild birds

A total of 263 Passeriformes and 3 Piciformes representing 21 genera and 28 species of birds were examined for *Sodalis* (Table A.1). No *Sodalis* was isolated from any of the birds sampled. No bacteria grew on any of the negative controls, and *Sodalis* did grow on the positive controls.

A.3.2 Screening for *Sodalis* on rock pigeons

In total, 40 pigeons were sampled for *Sodalis*. No *Sodalis* was grown from the sampled pigeon feet or the negative controls. There was growth of *Sodalis* from the positive controls.

A.4 Discussion

This research aimed to find *Sodalis* on birds to help determine if feather lice come into contact with *Sodalis* in their hosts' feathers or feet. In this appendix, no *Sodalis* was found on the sampled birds. It is unlikely that the media was improperly made since the positive controls were able to grow *Sodalis*. Since the positive controls grew, it is also unlikely that the environment in which the plates were incubated caused the *Sodalis* not

to grow. There were other colonies that grew on the plates of the experimental plates, but they were probably not *Sodalis* since what grew was not blue.

Plants were not sampled in this project to see if *Sodalis* was on them, so we do not know if *Sodalis* was even in the area we were sampling. It would seem unlikely that *Sodalis* doesn't occur on or in plants considering the person that was infected with *Sodalis* was impaled with a branch. That said, maybe the *Sodalis* was on their skin and then infected them after the branch pierced their skin. In the future, it may be useful to sample the vegetation for *Sodalis* in the area where the birds are being sampled to help determine if it can even be found on the surface of the plants in the study area. Sampling plants in the study area could also help determine if *Sodalis* is on the surface of plants, or located inside plants.

Although research has found that birds' feathers can hold a wide range of microorganisms (Javůrková et al. 2019), no *Sodalis* was cultured from the feathers or the feet of the studied birds. Even though the plumage can hold a large range of microbes, it can still be hard for microbes to inhabit due to defenses against bacteria like uropygial oil, which can have antimicrobial properties (Shawkey et al. 2003). Perhaps *Sodalis* cannot defend itself well against uropygial oil and dies shortly after coming in contact with the uropygial oil on the bird's plumage. Birds have been shown to spread uropygial oil onto their feet, so even if the *Sodalis* ended up on the bird's feet, it would still encounter it and possible kill it (Simmons 1961).

Perhaps *Sodalis* is not often found in Utah, or it may not be able to exist outside of a host for a long time due to the arid environment of Utah. Some bacteria cannot handle low-humidity environments well and will desiccate if not enough water is

available (Fredrickson et al. 2008). *Sodalis* that end up on bird feathers in Utah might desiccate rather quickly due to the low humidity of Utah. Some behaviors, such as dusting and sunning, are carried out by birds and may also help fight bacteria by drying out their plumage and feet (Clayton 1999).

The areas where the wild birds were sampled for *Sodalis* had various plant species, but no *Sodalis* was found on their plumage. Perhaps no *Sodalis* was found on the wild birds because the *Sodalis* sampled from them died before it was inoculated onto the LB agar. Bacteria will die if there are not enough nutrients, some bacteria can survive in low nutrient environments by going into spore form (Burtt and Ichida 1999), but *Sodalis* cannot form endospores. Since *Sodalis* does not form endospores, it might only remain on the plumage for a short amount of time and die after the nutrients on the feather run out. If this is true, feather samples may need to be processed faster to find *Sodalis.* There may also not be many nutrients on the feet of the pigeons, which would also lead to *Sodalis* dying before being sampled.

Although *Sodalis* was not found on the sampled birds, future studies should continue investigating where it can be found in the environment. Perhaps the first step should be to sample plants to determine if *Sodalis* is associated with them. If it is found that *Sodalis* is associated with plants, knowing if it is in or on the plant's surface would help determine how often birds come in contact with it. If it is found on the plant's surface, it is more likely that birds will come into contact with it since they are likely to pick it up when they interact with vegetation. If it is found inside the plant, birds may be less likely to come into contact with it since they do not interact with the inside of vegetation as often.

A.5 Acknowledgments

I would like to thank Mathew Waller, Aoife Galvin, Noelle Atkin, Sonora Clayton, Austin Clayton, and Mark Robbins for their help with fieldwork. I thank Sarah Bush and Dale Clayton for their help editing this chapter and for their help with the fieldwork. I also thank Colin Dale, Liszhen Teh, and Crystal Su for their help with media prep and troubleshooting.

Table A.1: List of wild birds sampled for *Sodalis*.

Orders	Species	Number of birds examined	Number of birds infested with *Sodalis*
Passeriformes	*Catharus guttatus*	2	0
	Contopus cooperi	1	0
	Empidonax hammondii	1	0
	Empidonax oberholseri	14	0
	Empidonax occidentalis	1	0
	Geothlypis tolmiei	32	0
	Junco hyemalis	1	0
	Leiothlypis virginiae	20	0
	Melospiza lincolnii	3	0
	Melospiza melodia	1	0
	Passerina amoena	2	0
	melanocephalus	9	0
	Pipilo chlorurus	25	0
	Pipilo maculatus	47	0
	Piranga ludoviciana	8	0
	Poecile atricapillus	8	0
	Regulus calendula	1	0
	Setophaga coronata	1	0
	Setophaga petechia	1	0
	Spizella breweri	3	0
	Spizella passerina	4	0
	Troglodytes aedon	1	0
	Turdus migratorius	14	0
	Vermivora celata	56	0
	Vireo gilvus	6	0
	Vireo plumbeus	1	0
Piciformes	*Colaptes auratus*	2	0
	Sphyrapicus nuchalis	1	0
Total		**266**	**0**

A.6 Literature Cited

Blanton, A. G., and B. F. Peterson (2020). Symbiont-mediated insecticide detoxification as an emerging problem in insect pests. *Frontiers in Microbiology*. 11:547108.

Burtt, E. H., and J. M. Ichida (1999). Occurrence of feather-degrading *Bacilli* in the plumage of birds. *The Auk* 116:364-372.

Chari, A., K. F. Oakeson, S. Enomoto, D. G. Jackson, M. A. Fisher, and C. Dale (2015). Phenotypic characterization of *Sodalis praecaptivus* sp. nov., a close non-insect-associated member of the *Sodalis*-allied lineage of insect endosymbionts. *International Journal of Systematic and Evolutionary Microbiology* 65:1400-1405.

Clayton, D. H. (1999). Feather-busting bacteria. *The Auk* 116:302-304.

Clayton, D. H., R. J. Adams, and S. E. Bush (2008). Phthiraptera, the chewing lice. In *Parasitic Diseases of Wild Birds* (C. T. Atkinson, N. J. Thomas, and B. Hunter, Editors). John Wiley and Sons, Inc, Hoboken, NJ, USA, 513-526.

Cornwallis, C., A. Padje, J. Ellers, M. Klein, R. Jackson, T. Kiers, S. West, and L. Henry (2021). Symbiont-driven niche expansion shaped the adaptive radiation of insects. Preprint at Research Square.

Fredrickson, J. K., S. W. Li, E. K. Gaidamakova, V. Y. Matrosova, M. Zhai, H. M. Sulloway, J. C. Scholten, M. G. Brown, D. L. Balkwill, and M. J. Daly (2008). Protein oxidation: Key to bacterial desiccation resistance? *Multidisciplinary Journal of Microbial Ecology* 2:393-403.

Fukatsu, T. R. Koga, W. A. Smith, K. Tanaka, N. Nikoh, K. Sasaki-Fukatsu, K. Yoshizawa, C. Cale, and D. H Clayton (2007). Bacterial endosymbionts of the slender pigeon louse, *Columbicola columbae*, allied to the endosymbiont of grain weevils and tsetse flies. *Applied and Environmental Microbiology*. 73:6660–6668.

Javůrková, V. G., J. Kreisinger, P. Procházka, M. Požgayová, K. Ševčíková, V. Brlík, P. Adamík, P. Heneberg, and J. Porkert (2019). Unveiled feather microcosm: Feather microbiota of passerine birds is closely associated with host species identity and bacteriocin-producing bacteria. *Multidisciplinary Journal of Microbial Ecology* 13:2363-2376.

Maas, S. (1999). Efficient and rapid procedure for blue-white screening of recombinant bacterial clones. *BioTechniques* 27:1126-1128.

McCutcheon, J. P., B. M. Boyd, and C. Dale (2019). The life of an insect endosymbiont from the cradle to the grave. *Current Biology* 29:485-495.

Oliver, K. M., J. A. Russell, N. A. Moran, and M. S. Hunter (2003). Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. *Proceedings of the National Academy of Sciences* 100:1803–1807.

Simmons, K. E. L. (1961). Problems of head-scratching in birds. *Ibis* 103:37-49.

Shawkey, M. D., S. R. Pillai, and G. E. Hill (2003). Chemical warfare? Effects of uropygial oil on feather-degrading bacteria. *Journal of Avian Biology* 34:345-349.

Stork, N. E. (2018). How many species of insects and other terrestrial arthropods are there on earth? *Annual Review of Entomology* 63:31-45.

Su, Y., H. Lin, L. S. Teh, F. Chevance, I. James, C. Mayfield, K. G. Golic, J. A. Gagnon, O. Rog, and C. Dale (2022). Rational engineering of a synthetic insect-bacterial mutualism. *Current Biology* 32:3925-3938.

APPENDIX B

MICROINJECTION OF *SODALIS* INTO FEATHER LICE (*COLUMBICOLA COLUMBAE*)

B.1 Introduction

Many insects form associations with bacterial endosymbionts, but we have not had much success replicating how these relationships form, and fully understand how the symbiosis benefits the host. It is particularly hard to study these symbioses since the bacteria are hard to cultivate outside their hosts. It is also difficult to determine what they are doing for the host because most hosts contain many endosymbiotic organisms. Moreover, even if the endosymbiont is isolated and its contributions to the symbiosis are determined, it can be difficult and costly to keep live animals in the lab to carry out long-term endosymbiotic research.

One host-endosymbiont system that can be maintained under laboratory conditions is *Columbicola columbae*, a species of feather louse that commonly infests Rock pigeons (*Columba livia*), and its *Sodalis* endosymbiont ally. I worked with *Columbicola columbae* for multiple reasons. One reason is that there are strains of *Sodalis* that can be cultured in the lab and then injected into insects (Su et al. 2022**).** *Columbicola columbae* only has one endosymbiotic bacterium, and the nutrients the

bacteria provide probably compensate for nutrients absent in its feather-rich diet (Fukatsu et al. 2007**)**.

Most importantly, interesting ecological and evolutionary questions can be explored with the *Columbicola* system. For example, feather lice in the genus *Columbicola* all occupy a similar niche. They are obligate ectoparasites found on the feathers of pigeons and doves (Columbifomes). They do not travel off the feathers, and their diet is made up of feathers. Different strains of *Sodalis* show different evolutionary paths, with different genes being retained or lost as different lineages transition to an endosymbiotic lifestyle. Experimental simulation of the evolution of endosymbiosis could conceivably be carried out through the experimental injection of a free-living lineage of *Sodalis* into feather lice. While injections have been carried out with other insects (Su et al. 2022**)**, they have not been accomplished with feather lice. I attempted to successfully inject *Columbicola* lice with *Sodalis* bacteria.

B.2 Methods

B.2.1 Prepping the bacteria

For injections, I used a strain of mCherry *Sodalis praecaptivus* that was inoculated into LB medium overnight in a 30°C shaking incubator. After a day, the concentration of bacteria was checked with a mass spectrometer. Once the concentration was checked, a 200 microliter pipet was used to load the bacteria into a needle made from a capillary tube. Once the needle was loaded with bacteria, it was inspected under a light microscope, and the tip of the needle was clipped with forceps to allow the bacteria to flow through it. The needle was then placed in an injecting apparatus, a drop of

halocarbon oil was added to a slide, and the needle was tested to see if a steady stream of bacteria would come out of the opening. The force that caused the bacteria to flow out of the needle came from a hand syringe pump that was hooked up to the needle. For a control, I made a separate needle and loaded it with a 10% saline solution.

B.2.2 Finding eggs on feathers

Columbicola columbae typically lays its eggs on the ventral surface of the first row of under coverts of wing feathers on Rock pigeons. I maintained a small population of pigeons that are only infested with *Columbicola columbae* in the animal facility at the University of Utah. I used these as a source of *C. columbae* eggs. Louse eggs are glued to feathers with a strong glandular cement, which makes it difficult to remove lice eggs from feathers that are still attached to the pigeon without damaging them. To remove eggs, I removed feathers that had *C. columbae* eggs attached to them. Feathers were removed by plucking them off the bird (rather than cutting them off), which ensures that the feathers will grow back. After collecting feathers with lice eggs attached, I would prep the eggs for injection with the bacterial solution as described below.

B.2.3 Housing lice off pigeons

To maintain lice cultures off pigeons, I set up an incubator that was kept at 37°C and 75% humidity, which are optimal conditions for these lice (Nelson and Murray 1971). Lice are negatively phototactic and prefer a dark environment, thus I kept the light off in the incubator. I housed the lice in 5dram *Drosophila* vials. The vials had detachable plastic caps that I drilled holes into to allow for gas exchange. Each vial had four covert

feathers (on which lice lay eggs) and the rest of the space was filled with downy feathers taken from the rump of pigeons in order to provide food for the lice. I would take birds that had *C. columbae* and put them in a fumigation chamber filled with CO_2 as described in Clayton and Drown (2001), which is a way to remove lice from live birds. I placed 15 lice in each prepared vial, then placed the vials in a test tube rack lying flat. The lice would not move throughout the vial if they were kept standing up. The vials needed to be cleaned weekly, or the lice would die. After 2 to 3 weeks, the lice in the vial would start laying eggs, which could be used for injections. To clean the vials, each feather within the vial was blown off with a circulating fan, which removed louse frass, but would prevent the lice from escaping. I would move the lice every three weeks to a new vial with fresh feathers.

B.2.4 Avoiding the desiccation of eggs during transport

I found that eggs desiccate quickly, e.g. during the walk from the animal rooms up to the lab where injections would take place. Desiccated eggs can be difficult to inject and lead to death of the embryo in the egg. To prevent the eggs from desiccating, I took a plastic sandwich container and placed two squares of paper towel in it that were soaked in warm water. Excess water was wrung out before placing the paper towel in the container. I found that if there was enough water to submerge the eggs, they would not hatch. I would then close the sandwich container and make sure it was sealed.

B.2.5 Removing eggs from the feathers

Once in the injection room, I placed a single feather with louse eggs in a Petri dish set under a light microscope. I examined the feathers under the microscope to find eggs that had not hatched and could be injected. While looking for injectable eggs, I kept feathers I was not examining in the humid container, which would prevent the eggs from drying out. While looking for eggs under the microscope, I noticed that the eggs on the feather I examined could dry out. To prevent the eggs from drying out, I partially filled the Petri plate with a 10% saline solution that was occasionally refilled as the water evaporated. I would put enough saline solution into the Petri dish that the feather would float on it but not enough to fill up the whole plate. It is important to ensure that feathers remain buoyant and not submerged because too much saline solution on the eggs prevents them from hatching. It can also be difficult to remove the eggs when there is too much saline solution since the saline solution could cause the eggs to stick to the feather. After adding saline solution, the feather would move when I tried to extract eggs. To prevent the feather from moving, I would place my thumb over the rachis at the end of the feather so that no eggs were smashed and the feather did not move while eggs were extracted. I tried a variety of tools to try to remove eggs from the feathers, as described below.

B.2.6 Cutting feathers to remove and inject eggs

I also tried cutting the feather barbs that the eggs were attached to and then attaching those barbs to a microscope slide. I found that this did not work well because viable eggs are usually surrounded by eggs that are not viable. If you cut the feather

section with the viable egg out, it is hard to position the eggs on the slide to allow for injection of a particular egg. Louse eggs would not stick well to the slide if they were still attached to the feather, making it difficult to inject them. Also, this procedure made it difficult to access the posterior end of the egg (see below).

B.2.7 Review of egg removal tools and strategies

Forceps: At first, I tried using forceps to remove the eggs from the feathers with mixed success. Most forceps are too large to remove lice eggs from the feathers easily. Fine-tipped forceps could be used to remove eggs occasionally by sliding the forceps along the barb to which the egg is attached. It was difficult to remove eggs from the forceps when the forceps got wet. Moreover, it didn't take much force to accidentally break louse eggs with the forceps, which would dent the egg, preventing it from hatching, or sometimes cause the egg to break open.

Dental pick: I also used a dental pick to try and remove eggs with little to no success. The pick was bulky and could not easily remove the eggs from the feathers. Since the eggs were attached to the barbules, there was not much resistance to the dentistry pick, and the barbules would move, making the dentistry pick ineffective at removing eggs. The pick also caused a high puncture rate, resulting in eggs breaking. The pick could be useful for removing the eggs from the forceps or the other instruments if the water caused the egg to stick to their surface. In this case, the pick was used to push the egg to the edge of the forceps or the dentistry spatula, where it could be submerged in water, which would dislodge the egg. The pick could also move a stuck egg from the instrument to the sticky side of an injection slide.

Dental spatula: The spatula was the best tool for removing lice eggs from feathers. It is small enough to target individual eggs and blunt enough that it would not puncture them. The spatula was also useful in identifying viable eggs that could be injected. To identify viable eggs, I would run the spatula through the saline solution and then move it over the mass of eggs on the feather. I would do this while looking at the eggs under a microscope. By running the spatula over the eggs on the feather, I could see if they deflated, meaning that the lice had already hatched from the egg. I also found that if the eggs were discolored, with more of a yellowish color than glistening white, they would not hatch. In addition, these old eggs were not as firm as newer eggs. I would gently push on the eggs with the spatula, and eggs that were likely to hatch would retain their shape. Eggs that were less likely to hatch would dent and remain dented. It is important to check throughout the mass of eggs because viable eggs do not seem to be laid in any particular place on the feather. Once an egg was identified that might be viable, I would get a small amount of saline solution on the tip of the spatula and run it along the bottom of the egg. I would flick my wrist while running the spatula under the egg to give a bit of force to the area where the egg was cemented to the feather. The saline solution underneath the feather in the Petri dish helped to soften the cement so that the egg would come off easier. The saline solution on the spatula would also make the egg stick to the spatula, making it easy to transfer. Occasionally the eggs would get stuck to the spatula. If the egg ended up getting stuck, I would use the dental pick to move it into a position that would help remove it from the spatula.

B.2.8 Keeping eggs hydrated after removing them from the feather

Originally, I would keep eggs moist by putting them in a Petri dish with a couple of drops of 10% saline solution. However, I found that if eggs remained in the solution for too long, they would not hatch. It was also difficult to remove eggs from the solution. I therefore started attaching eggs directly to the injection slide (see below) after I removed them from the feather. I would keep the slides in a humid container, which prevented them from desiccating and becoming waterlogged.

B.2.9 Attaching the lice to the slide and injecting lice eggs

I attached the louse eggs to a microscope slide to inject them. I first attached louse eggs to the slide with heptane glue. I found that the glue was not strong enough to hold the eggs in place while I was injecting the eggs. Some eggs would remain in place, but most would slide through the glue when the needle was pushed into them. I then tried using double-sided tape, which prevented the lice eggs from moving during injections. One problem that I had with the double-sided tape was that the lice would get stuck on it once they hatched. To prevent the emerging lice from getting stuck, I would cut some paper and cover the exposed parts of the tape with the paper. I would place ten lice eggs on a single slide. After attaching the eggs, I would cover them in 2 ml of gas-permeable halocarbon oil 700, which prevents the eggs from being infected by other microorganisms.

To help orient myself under the microscope, I would take a sharpie and mark the slide, which I could use to focus the microscope. Once focused, I would inject the eggs from the posterior end.

It is best to inject eggs from their posterior end. When injecting from the anterior end the operculum will sometimes detach leading to the lice being ejected out of the egg prematurely. The procedure was most successful when I removed eggs from the feather with the dental spatula, it was less successful when I did not remove lice from the feathers prior to injection.

After injecting the eggs, I would place them back into the humid container and transport them to an incubator kept at 37°C and 75% humidity, where they were kept until the lice hatched or three weeks had passed. Every few days, I would check the eggs under a compound light microscope to see if any had hatched. For every egg that was injected with *Sodalis*, I had a control egg that was not injected and an egg that was sham injected with saline.

B.2.10 Problems with the halocarbon oil

Lice did not hatch or, if they did hatch, they did not develop correctly if the egg had been submerged in the halocarbon oil. However, if no oil was used, I could get lice to hatch from the eggs. I tried various techniques to enable the oil (or other substances) to coat the egg and protect against secondary infection and yet still have the lice hatch (see below).

B.2.11 Using glue instead of halocarbon oil

First, I tried using Elmer's glue to cover up the hole after the egg had been injected. Unfortunately, none of the eggs that I glued went on to hatch. I also tried using superglue to block the injection hole. I put one drop of glue on the hole after the egg was

injected with bacteria. But none of the eggs that were superglued hatched. It seemed like the glue would partially crush the egg once it hardened. To try to prevent the eggs from being crushed, I used a fine-tipped paintbrush to apply just enough super glue to cover the posterior end of the egg. While performing these tests, I had a separate group of control eggs with no glue. These would be kept in the same condition as the eggs with glue. Although the control eggs hatched, eggs with Elmer's or super glue did not hatch.

B.2.12 Trying to prevent the halocarbon oil from covering the louse egg

I tried to use less halocarbon oil to test whether this would help eggs hatch. I only put enough oil on the egg to cover its posterior end. When I did this, the oil would spread around the egg when it was put into the incubator. I tried to reduce the amount of oil even more by using a fine-tipped brush so that the oil would only lightly cover the posterior end of the egg. Even when I did this, the eggs would not hatch. I tried to fold the tape so the oil would not move up around the egg while it was in the incubator. First, I folded the tape in half, so there was a slight incline to the egg. I was hoping the incline would be enough to keep the oil from spreading along the eggs in the incubator. After the slight incline did not work, I folded the tape so that the egg was at a steeper incline. I also put the egg at the seam of the fold so that the oil was more likely to drain along the seam and not up along the egg. I also used the fine-tipped brush with these eggs so that less oil would be on the egg. The eggs still did not hatch. With the folding techniques and the fine-tipped brush, I would have controls that went through the same process but did not have oil. The control eggs hatched. I then started wiping the excess oil off the eggs after they had been in the incubator for a few days. I still folded the tape so that the oil would

run off the egg, and I also used the fine-tipped brush on the egg so that only the posterior side would be covered in oil. After wiping the egg off, one of 300 eggs hatched. I tried to replicate this result but was never able to get another louse to hatch. I never saw lice emerge or partially emerge from the egg on their own. The oil clearly prevents the lice from hatching. The oil may be suffocating the lice, either in the egg, or when it tries to emerge from the egg.

B.2.13 Attempts to facilitate lice hatching

I noticed that some of the lice in the eggs covered in halocarbon oil would develop in the egg but would not emerge from the egg. I decided to try and help the lice hatch. I did this by taking the eggs out of the incubator and checking them under a compound light microscope. By checking them under the microscope, I could tell how developed the lice were. Once the lice were fully developed, I would remove the egg cap (operculum), which would cause the lice to emerge. I removed the egg cap with an injection needle, which would allow the louse to emerge. Unfortunately, lice in eggs covered in oil never seemed to develop as much as lice in control eggs without oil. They seemed to die in the egg before getting to full size. When I opened the egg, The lice inside were either already dead or could not walk properly and died soon after hatching. Even if I transferred the newly hatched lice to the tubes with feathers, they would still die. In conclusion, a method needs to be developed in which the lice are injected with bacteria without the use of injection oil. The injection oil clearly hampers the development of the lice and prevents them from hatching properly.

B.2.14 Injecting adult lice

Most of my injections were carried out on louse eggs, but I also tried to inject adult lice. First, I restrained the lice with a coverslip. I had difficulty finding an area to inject the lice without the injection needle breaking. Once I identified where they were not protected, the lice would break the needle as they tried to get out from under the coverslip. To prevent the lice from moving, I would try to inject them shortly after getting them off the pigeons while they were still incapacitated by the CO_2. However, the lice would usually begin to move before I could inject them. To try to slow down movements of the lice, I would place them in the refrigerator for ten minutes. I would then also place a small amount of dry ice next to them, which kept them from moving. However, I still had trouble with the needle breaking during injections. Some of the adults I used during the trials ended up surviving, and I could keep them alive in the glass vials I set up in the incubator.

B.2.15 Future directions

Future trials might build on what I tried with the adult injections. It would be good to look at the adults and eggs under a fluorescent microscope to determine if bacteria were successfully introduced by injection. If the lice were periodically checked under the fluorescent microscope, one could determine how long the bacteria remain viable in the adult lice. It might also be helpful to try injecting nymphal lice in the future.

B.3 Acknowledgments

I also thank Colin Dale, Liszhen Teh, and Crystal Su for their help with injections and troubleshooting. I would also like to thank Sarah Bush, Kevin Johnson, and Dale Clayton for their help with figuring out solutions to problems that arose with injections.

B.4 Literature Cited

Clayton, D. H., and D. M. Drown (2001). Critical evaluation of five methods for quantifying chewing lice (*Insecta*: *Phthiraptera*). *Journal of Parasitology* 87:1291-1300.

Fukatsu, T., R. Koga, W. A. Smith, K. Tanaka, N. Nikoh, K. Sasaki-Fukatsu, K. Yoshizawa, C. Dale, and D. H. Clayton (2007). Bacterial endosymbiont of the slender pigeon louse, *Columbicola columbae*, allied to endosymbionts of grain weevils and tsetse flies. *Applied and Environmental Microbiology* 73:6660-6668.

Nelson, B. C. and M. D. Murray (1971). The distribution of Mallophaga on the domestic pigeon (*Columba livia*). *International Journal for Parasitology* 1:21-29.

Su, Y., H. Lin, L. S. Teh, F. Chevance, I. James, C. Mayfield, K. G. Golic, J. A. Gagnon, O. Rog, and C. Dale (2022). Rational engineering of a synthetic insect-bacterial mutualism. *Current Biology* 32:3925-3938.