**The conservation and food-safety impacts of birds in working landscapes**

***Numbers of animals involved in study***

* Food safety (864)
  1. 500 birds surveyed to build pathogen prevalence database
  2. 264 for the two focal bird species pathogen survival experiments
  3. 80 from 10 species for species variation
* Nestbox
  1. 640 individuals
* Total 1484

***Brief summary of procedures***

Our project will evaluate the impact of climate change on birds in agricultural landscapes, alongside the potential food-safety risks birds might post to agriculture (i.e., by carrying foodborne diseases).

***Objectives***

The fate of biodiversity in the Anthropocene will largely depend on the ability of species to survive alongside us in landscape mosaics of farms and patches of natural habitat. While recent work suggests that large concentrations of wildlife are often found in farming landscapes, at least two major barriers exist to their continued persistence. First, because farms often lack tree canopies that shade the understory, increasingly common temperature spikes associated with climate change may make many agricultural systems inhospitable in the future. Second, fear that wildlife carry foodborne diseases (*e.g.,* pathogenic *E. coli*) has created great pressure on farmers to discourage wildlife from visiting their farm fields. We propose using wild birds in the California Central Valley as a model system to (1) quantify and compare the impacts on temperature spikes on bird health and reproduction between farms, grasslands, and forests and (2) develop a holistic assessment of the potential food-safety risks of wild birds.

***Significance***

Co-managing agricultural landscapes for people and nature requires understanding how farming practices could be altered to both facilitate species persistence and mitigate any potential conflicts with producing safe and sufficient food. Our first objective seeks to unravel the mechanisms through which climate change may affect the ability of birds to survive in human-dominated habitats. To achieve this objective, we will leverage a large network of ~200 songbird nest boxes in California’s Central Valley, maintained by the UC Davis Museum of Wildlife and Fish Biology (MWFB) since the year 2000. The network encompasses boxes in riparian forests, oak savannas/grasslands, orchards, and row-crop agriculture. Across this network, we will monitor nest temperatures with thermal sensors, food provisioning with remote cameras, and nestling growth, health, and survival with morphometric measurements and blood sampling.

Results from this work will help begin to identify the key processes that dictate how climate change and habitat conversion to agriculture will interact to affect biodiversity. Doing so may provide concrete avenues through which working landscapes could be modified to better accommodate cavity-nesting birds. For example, if we find that temperature spikes directly affect bird health, then nest boxes could be modified to increase nesting success (*e.g.,* by adding white paint or solar shields to roofs, or by installing boxes in more shaded areas). If, in contrast, we find that temperature spikes primarily affect reproduction via declines in food provisioning, then maintaining patches of non-crop habitats in working landscapes to support food resources for birds (*i.e.,* insects) may be more effective.

Farmers, however, will be reluctant to take these actions if they result in heightened food-safety risks. Birds are indeed a cause for concern for many farmers as they are known to carry multiple pathogens, are difficult to exclude from farms, and regularly defecate on crops. However, the little food-safety-related work that has been done on wild birds has focused on only a few species (and those species form a minority of farmland bird communities). Moreover, existing studies stop at examining pathogen prevalence and do not holistically assess food-safety risk. For a species to pose a significant risk, it must carry pathogens, visit fields, and produce feces that support pathogen survival. We will first identify species that carry pathogenic E. coli, Salmonella, and Campylobacter by coupling existing studies with assays of field-collected feces. We will then experimentally compare *E. coli* survival between feces placed on different substrates (crops, organic/conventional soils, plastic mulch) and between feces from different species to determine whether bird feces could result in crop contamination.

Improving our understanding of the risks associated with birds on produce farms could allow for more informed management, helping farmers avoid food-safety risks while taking advantage of some of the benefits that birds provide. For example, by consuming insect pests, birds can enhance farm yields and profits. Knowing when farmers could benefit from birds without incurring significant food-safety risk may improve food safety, bird conservation, and crop production outcomes. On the other hand, it is equally vital to know when food-safety risks preclude ‘win-win-win’ scenarios; for example, if species known to be effective reservoir hosts are often intruding into farms and defecating on produce. To help farmers identify and manage birds, we will produce a photographic guide that describes the food-safety risk associated with >50wild bird species.

***Refinement***

Birds will need to be captured to (1) quantify nestling growth rates, (2) obtain blood samples for assessing nestling health and physiology, and (3) obtain fecal samples for assaying pathogen prevalence and conducting pathogen survival experiments. Hand-capturing birds from artificial nest boxes provokes the least amount of stress of any trapping procedure and will thus be employed for all studies of birds in nest boxes. However, there are only a few species that use nest boxes and understanding the food-safety risks of wild birds requires studying many more farmland species. Our literature search did not reveal any viable alternatives to our proposed protocol of using mist nets to capture birds and collect their fecal samples. First, fecal samples could be collected passively (through scouring areas for bird feces and collecting them opportunistically); however, these samples may be contaminated and we would not know which species produced them. Second, some species can be captured through other methods (*e.g.,* potter traps, which we may occasionally use); however, no other method will effectively capture enough of the farmland bird community to holistically assess food-safety risks. As such, we decided to capture birds with mist nets, as they represent a standard method for surveying and capturing passerine birds. Finally, taking small blood samples will be necessary for quantifying avian health and stress levels. Total blood samples extracted will represent <1% of the individual’s body mass (our intent is to draw ~50 microliters of blood).

***Replacement (species rationale)***

Wild birds are ideal model organisms to explore the impacts of climate change in working landscapes and the potential food-safety risks of conserving wildlife on farms. In bird species with altricial young, nestlings are ectothermic; thus, both low and high temperatures divert energy from growth to thermoregulation. While the lethal effects of cold are well-documented, only recently have biologists begun documenting effects of temperature spikes on birds. Recent studies suggest that temperature spikes will have severe impacts on avian fitness and nestling health, especially in very hot regions like the California Central Valley. For example, in the Putah Creek ecosystem near Davis, CA, we found that warm temperatures during nesting are associated with lower nestling growth (and survival in some species). These thermal impacts can have severe repercussions: recent research in the Mojave Desert has shown that climate-induced drying and warming severely increased ‘cooling costs’ for birds (*i.e.,* the amount of water needed to maintain their body temperatures), leading to a collapse of the bird community. Finally, our preliminary analyses of a large citizen science database (N= 152,863 nesting attempts across 58 species) show that, across North America, temperature spikes lower nesting success in agriculture and urban environments but do not affect them in forests. The mechanisms through which temperature spikes affect nestlings in different habitat types, however, is unknown (motivating our current research).

At the same time, birds are also a primary focus of food-safety concerns among farmers, as they carry enteric pathogens and move long distances, including between livestock operations and fresh produce fields. As a result, growers often implement economically and ecologically costly measures to prevent bird intrusion (e.g., bird netting and habitat removal). However, despite widespread concern, our knowledge of the risks posed by different species by farming context is still in its infancy. Few species have been studied, with disproportionate focus on species that frequent feedlots, refuse sites, or water bodies rather than crop fields. Moreover, no studies have assessed the entire pathogen spillover cycle; that is, the combined likelihood that bird species carry pathogens, enter farms, defecate on crops, and produce feces that enable pathogen survival.

**Reduction**

The numbers of birds that will be captured and safely released was carefully chosen to balance animal welfare and necessary sample sizes. To quantify effects of temperature spikes on birds in different habitat types, we plan to systematically monitor 20 active nests per year for two years within nest boxes placed in four habitat types: riparian forests, open grasslands, row-crop agriculture, and orchards (total= 160 boxes monitored). Assuming an average of 4 nestlings per box, we will obtain blood samples and growth measurements from a total of 640 individuals (study group 1).

To assess pathogen prevalence in farmland birds, we will collect fecal samples from birds captured in mist nets and birds hand-captured from nest boxes. We have compiled the most comprehensive database of enteric pathogen tests in wild birds to date, consisting of 3023 Campylobacter spp. tests (n= 80 species), 4093 Salmonella spp. tests (n= 93 species), and 4693 STEC tests (n= 94 species). Despite the database’s size, most samples came from few species, with many common farmland birds under-sampled.

We will strategically supplement this database, acquiring fecal samples for species that are under-sampled and abundant on farms. To produce holistic risk assessments for the more common birds found in farmland, our ideal goal would be that our final database will include >20 tests of each pathogen for >45 species (i.e., 15 additional species). Thus, we intend to collect at least fecal samples from 300 individuals (20 tests \* 15 species). It is impossible, however, to only capture target species in mist-nets and we anticipate capturing many more individuals that have moderate sample coverage in our database. As these birds will have already been captured and obtaining a fecal sample will cause minimal additional distress, we anticipate assaying 200 additional individuals for foodborne diseases, bringing the total number of individuals captured and assessed to 500 (study group 2). The number of samples necessary was determined from consulting with scientific literature about relative detection frequencies of pathogens/pests in bird fecal materials. For example, we expect that *Campylobacter* will be detected in ~5% of the samples of suitable disease vectors. Given this expectation, a sample size of at least 20 should allow for at least 1 *Campylobacter* detection.

Finally, we will capture birds to acquire fecal samples that will be used in pathogen survival experiments (methods detailed below). These experiments will help us determine how long foodborne pathogens can survive in the feces of different farmland bird species, and on different substrates found in the farm field environment. Feces will be acquired from wild birds through three approaches, depending on the target species: (1) hand captures from nest boxes (for cavity-nesting birds), (2) following larger birds (*e.g.,* Canada Goose and Wild Turkey) and waiting for them defecate, and (3) mist nets (for all other species). Feces will be inoculated with either pathogenic *E. coli* strains (in a BSL-2 lab at a USDA facility) or with nonpathogenic strains (placed at the UC Davis student farm). They will then be placed on four different common farm substrates: lettuce leaves, organically-managed agricultural soil, conventionally-managed agricultural soil, and plastic. Fecal samples will be collected at multiple time point to quantify pathogen half-lives. To do so, we will collect feces from 240 birds (2 bird species, 2 pathogen strains, 4 substrates, 3 replicates per substrate, and 5 time points to quantify). We will also acquire 24 additional samples to compare survival between the pathogenic and nonpathogenic strain in the lab. Finally, we will acquire 80 additional samples to compare among a broader array of species (10 bird species, 1 strain, 8 replicates, 1 time point). Thus, in total, 344 birds will contribute fecal samples to this study (study group 3). These sample sizes were carefully chosen to minimize the number of fecal samples needed (and thus birds that need to be captured) while still having enough statistical power to quantify pathogen survival rates.

**Procedures**

*Study group 1:* Nestlings will be hand-captured from nest boxes at weekly intervals after hatching, until 1-2 weeks before fledging. We will record the mass, tarsus length, bill length, and wing chord of each individual. During the last measurement, nestlings will be banded with a metal leg band and a small blood sample will be acquired via puncture of the medial metatarsal vein with a sterile needle and collection into a sterile hematocrit tube (approximately 50 microliters and/or <1% of the individual’s body mass).

*Study group 2:* Adult birds will be captured to obtain fecal samples for pathogen testing either via hand captures from nest boxes (for cavity-nesting birds) or mist nets (for all other species). After extraction from mist-nets (see methods below), birds will be placed in sterile cotton bags where they defecate >75% of the time. Birds will then be identified, banded, and measured (*i.e.,* mass, tarsus length, bill length, and wing chord) before being released.

*Study group 3:* Birds will be captured to obtain fecal samples for the pathogen survival experiment either via hand captures from nest boxes (for cavity-nesting birds), following larger birds and waiting for them to defecate (*e.g.,* Wild Turkeys and Canada Goose), or mist nets (for all other species). After extraction from mist-nets (see methods below), birds will be placed in sterile cotton bags where they defecate >75% of the time. Birds will then be identified and released.

**Procedure Details**

*Objective 1—* Our study leverages a large network of songbird nest boxes in California’s Central Valley, established by the UC Davis Museum of Wildlife and Fish Biology (MWFB) in 2000. Our focus is on the two most common nest box species: Tree Swallow and Western Bluebird. We plan to systematically monitor 20 active nests per year for two years within nest boxes placed in four habitat types: riparian forests, open grasslands, row-crop agriculture, and orchards (total= 160 boxes monitored). Birds in this area experience some of the most severe temperatures while nesting that have been documented in the U.S., with temperatures regularly soaring over 40°C. The system is thus ideal to study whether closed canopies buffer nesting birds from temperature spikes. We will quantify microclimates, monitor box occupancy, and assess nestling growth, physiology, and survival across the four habitat types detailed above.

Specifically, from April-July for two years, boxes will be monitored weekly to quantify box occupancy. From the occupied boxes, we will select at least 20 active nests per habitat type per year for monitoring (N= 160 boxes; 20 boxes/habitat/year, 4 habitats, 2 years). We will then use temperature/relative humidity loggers to record microclimates in each nest box every 5 min from egg-laying to fledging. We will also place remotely triggered cameras outside nest boxes to monitor food provisioning. To track nestling growth and survival, we will visit active nests each week, hand-capture nestlings, and collect morphometric growth data (nestling weight, wing chord, tarsus length, and bill length). One to two weeks prior to fledging, we will affix a small metal leg band to the nestling’s leg. PI Karp is a federally licensed Master Bander; personnel on this IACUC request have been sub-permitted on his license. To quantify nestling stress physiology, we will also collect a small blood sample via puncture of the medial metatarsal vein with a sterile needle and collection into a sterile hematocrit tube (approximately 50 microliters and/or <1% of the individual’s body mass). Samples will be kept on ice (max. 4h) and then centrifuged to separate plasma from red blood cells. Plasma will be aspirated, frozen (-20 ºC), and then corticosterone concentrations will be quantified via an immunosorbent assay.

We will compare microclimates between land-use types, expecting to observe higher average and maximum temperatures, and lower relative humidity, in open habitats. We will also assess inter-habitat differences in nest box occupancy, nestling stress physiology, nestling growth, and nestling survival. We predict that temperature spikes in open habitats, especially anthropogenic types, will reduce nest box occupancy and cause stress hormones to spike, leading to lower nestling growth and survival.

*Objective 2—* We will determine pathogen prevalence in birds and pathogen survival in bird feces through collecting feces from wild birds near UC Davis. First, we will strategically supplement our existing pathogen prevalence database, acquiring fecal samples for species that are under-sampled and abundant on farms. Specifically, we will collect fecal samples from birds captured in mist nets and birds hand-captured from nest boxes. Mist nets will be placed around the Student Farm, the Russell Ranch Sustainable Agriculture Facility, and/or adjacent non-crop habitats near UC Davis. Nets will be operated under standard protocols (Ralph et al. 1993), beginning at sunrise and continuing for 5 hours. Dangers to birds during mist netting include: exposure to severe weather, predation, or asphyxiation from entanglement. These risks can be properly mitigated through practicing responsible banding protocols. First, visiting sites in early morning hours maximizes capture probability (due to bird activity at that time), while minimizing heat exposure. Severe heat can lead to heat stroke and death; as such, we will cease all banding operations by 11am (when fields become intolerably hot). We will also reframe from capturing birds during extreme weather (i.e., during periods of severe heat and/or when it is raining). Second, checking nets regularly and frequently can mitigate risks of from predation, severe entanglement, and/or exposure to bad weather. As such, we will check nets every 20-30min, a time period considered standard responsible procedure. At this point, birds will be extracted from nets, placed in sterilized, breathable cotton bags and transported to a nearby banding station. Bags will be chosen to be large enough to allow birds to move comfortably and without restraint.

Past experience suggests that most birds (~75%) will defecate during transport. At the banding station, feces will be removed from bags and placed in vials. Samples will be placed in vials for pathogen screening. Prior to collecting each fecal sample, however, we will identify and band each bird. We will follow standard banding procedure, ensuring that bands are properly placed on birds such that they will cause no future discomfort (i.e., they are correctly sized and applied). Finally, we will collect morphometric measurements (*i.e.,* mass, tarsus length, bill length, and wing chord) before release. No bird will kept for more than 2 hours (and most will be released within 1 hour of capture).

If capture rates are too low to achieve our sampling goals, then we will also capture birds with Potter Traps (small wire cages, baited with food, that have pressure-triggered closing doors). These traps do not harm birds, but are much less likely to capture sufficient numbers for our purposes. As such, we will only use Potter Traps if mist nets completely fail. In either case, birds will be placed in bags, allowed to defecate, identified, and banded before release.

We will assay feces for pathogens utilizing the same methods as in (Smith et al. 2020). Briefly, DNA will be extracted from each sample using the QIAamp DNA Mini Stool Kit (Qiagen) and the manufacturer’s protocol. We will than test for Campylobacter spp. by using a multiplex PCR to test for C. jejuni (hipO gene), C. coli (glyA gene), C. fetus (sapB2 gene), and the 23S rRNA gene from Campylobacter spp., as in (Wang et al. 2002). We will test samples for Salmonella spp. using PCR as in (Park et al. 2011). Finally, we will assay samples for E. coli virulence genes using a multiplex PCR to test for stx1, stx2, eaeA, hlyA, and saa genes, as in (Paton and Paton 2002).

Finally, we will combine lab and field experiments to quantify E. coli survival in wild bird feces. For our first experiment, we will parameterize E. coli survival curves in feces from two species: a large waterbird often detected on farms (Canada Goose) and a small songbird common in farmland (Western Bluebird). Samples will be collected using the same practices as above for Western Bluebird (*i.e.,* using the same mist-netting procedures and hand capture from nest boxes). For Canada Goose, we will follow individuals and collect feces after they defecate.

Samples collected for survival experiments will be placed in Eppendorf tubes held on ice until inoculation (<12 hours from collection). They will then be inoculated with a non-pathogenic E. coli strain collected from a lettuce field in Yuma, Arizona (E. coli O145:H11 strain RM14721). Half of the samples will be placed in the Student Farm at UC Davis and the other half will be placed in a BSL 2 growth chamber in co-PI McGarvey’s lab. Inoculated feces will be placed on four substrates: lettuce leaves, organic soil, conventional soil, or plastic. Organic and conventional soil will be collected from a 27-year soil management study at Russell Ranch, UC Davis. Lettuce will be grown from seed in a pot (for lab experiments) or in the field (at the Student Farm). Plastic will either be petri dishes (lab experiments) or plastic mulch (field experiments). Lab samples will be regularly watered at rates consistent with field irrigation.

We will quantify E. coli concentrations after 1, 3, 7, 14, and 21 days. At each time interval, we will collect samples and place them in blender jars containing 100 ml of sterile phosphate buffered saline and blend them on high speed for one minute. The resulting mixture will be serially diluted in phosphate buffered saline and plated onto McConkey sorbitol agar plates supplemented with cycloheximide, rifampicin and nalidixic acid and counted after 16 hours. In total, we will analyze 240 samples (2 bird species, 2 strains, 4 substrates, 3 replicates per substrate, 5 time points). We will model half-lives of each strain and then compare survival between substrates and species.

Next, we will compare survival of the non-pathogenic strain to a pathogenic isolate E. coli O157:H7 strain RM4403. Unlike the above experiments, experiments will only take place in the biosafety level 2 growth chamber (and not in the field). Moreover, to reduce pathogen waste, we will only assess survival at one time point, determined from decay curves in experiment 1 (N= 24 additional samples).

Finally, we will compare E. coli O157:H7 survival, in the BSL-2 lab, among a broader array of species. Feces will be collected from birds using hand-capture from nest boxes, mist-nets, and/or following birds (*i.e.,* using the same methods as above). We will note the mass of each fecal sample to determine if variation among species is due to fecal mass or other species-level characteristics. We will follow the same procedures as above; however, we will only evaluate one substrate type (lettuce leaves), one time point, and only the pathogenic strain. In total, 80 samples will be analyzed (10 bird species, 1 strain, 8 replicates, 1 time point). Across all experiments, we expect that survival of pathogenic and non-pathogenic strains will be similar and always lowest in organic soils, plastic mulch, and in small songbird feces.

**Adverse effects**

***Describe all significant adverse effects that may be encountered during the study.***

As noted above, capturing birds in mist nets will expose birds to several risks including exposure to extreme weather, severe entanglement, and predation. By checking nets frequently and only operating nets at appropriate times, these risks can be mitigated. Indeed, our prior experience suggests a mortality rate of less than 0.5%. Finally, obtaining blood samples is necessarily invasive; however, we will only obtain samples from individuals in good body condition, with a total amount <1% of the individual’s body mass.

***Describe criteria for monitoring the wellbeing of animals on the study and criteria for terminating/modifying the procedure(s) if adverse effects are observed.***

We will check nets every 20-30min. If more than 1 bird dies at any given net, the net will be closed immediately. We will then determine the cause of death and whether birds in other nets will likely be affected (e.g., if weather related, then all nets will be closed). If one bird is found dead, then we will determine the cause of death and look for signs of stress in other captured individuals (e.g., panting etc.). Our observations will inform whether we should close nets and abandon sampling for the day.

***How will the signs listed above be ameliorated or alleviated?***

If a bird is stressed or exposed to heat exhaustion or hypothermia, we will use portable coolers, heat packs, and towels to help return the bird to an adequate temperature. Once the bird exhibits normal behavior, it will be immediately released. If a bird is severely injured (e.g., broken wing/leg, severe laceration etc), then we will humanely euthanize the bird using cervical dislocation (a standard method for small songbirds).

***Criteria for euthanasia:***

Birds will be euthanized when recovery is viewed as very unlikely and/or impossible. This can occur if severe entangle and/or predation while in the net causes: severe wounds, lacerations, and/or broken bones. In this case, birds will be humanely euthanized via cervical dislocation. In our experience, it is incredibly rare that birds need to be euthanized (<0.1% of birds captured).

***Disposition***

Animals will be released within 2 hours of capture.

**Qualifications:**

*Daniel Karp*

I was trained in mist net operation by the Klamath Bird Observatory (KBO) in 2006. For the first two weeks of the 10 week program, I was constantly supervised by a Master Bander and was trained in all standard mist-netting protocols including: net setup, extraction, banding, aging and sexing, feather sampling, net closure, data management, and net repairs. I learned how to minimize bird mortalities (e.g., through closing nets on cold, rainy, or especially busy days) and how to humanely euthanize injured birds. By the end of the field season, I had banded >1000 individuals across 71 species, and received a bander’s merit badge, demonstrating banding skill. My next major banding project began in earnest in 2011. At that time, I selected and set up a mist netting operation at 6 study sites in Costa Rica to collect bird fecal samples. Each site was visited for 6, 56 hour periods, beginning at sunrise. 20 nets were operated at each site. In 2012, I returned to the sites to continue monitoring bird and bat abundance and collect additional fecal samples. Over both field seasons, I personally banded ~1250 birds across ~90 species and ~100 bats across 25 species.

In 2017, I joined the faculty at the University of California Davis and was awarded a Master Bander license. After acquiring CDFW permits and IACUC approval, I then led a study quantifying the benefits and potential harms associated with birds in strawberry fields. Our objectives were to (1) analyze DNA content in bird fecal samples to identify pest, disease vector, and beneficial species and (2) quantify how farming practices affect birds’ net economic impact on yields. We were also interested in analyzing the effects of farmland diversification on avian health (via physiological analyses of blood samples). Thus, I was trained alongside other lab members in safe blood sampling techniques, capturing birds near UC Davis with Potter traps and practicing venipuncture alongside Dr Jesse Krause (a postdoctoral fellow who had taken blood samples from >2000 birds). Following our training, our team collected >1000 fecal, blood, and feather samples across ~60 species; conducted a bird exclusion experiment across 15 farms; and surveyed birds, nest density, strawberry damage, and fecal contamination across 20 farms. Our fecal analyses identified species that consume pests, consume pest predators, consume strawberries, and/or vector foodborne diseases.

*Katherine Lauck*

I was employed as a field assistant for the Golondrinas de las Americas project, an international effort to understand the evolutionary biology and migration ecology of swallows, with a focus on Tree Swallows. I worked for two summers as a nestling and adult bander in which my primary responsibilities were to measure, band, and sample blood from nestlings (~200 birds), and to capture, measure, band, and sample blood from adults (~70 birds). I also used mist-nets, wigwag, and flap traps (as well as hand-captured birds from nest boxes). After my first year as a field assistant, I enrolled as a student in a banding course taught by master bander David Bonter at Cornell University. During this course, I practiced extracting birds from mist nets and improved my ageing and sexing skills. I also learned how to use pressure plate traps (*i.e.,* Potter Traps) and funnel cage traps. Before and after this banding course I traveled to Malaysia as part of an expedition to gather natural history data on Old World suboscines. As part of this expedition, I set up and managed mist nets in primary tropical forest. I extracted and banded about a third of the birds that we captured (N= ~200 birds), honed my bleeding skills, learned how to sample feathers, and handed an eclectic variety of lowland tropical passerines, Old World suboscines (including pittas), woodpeckers, and barbets. Finally, this past summer I banded and measured Tree Swallow and Western Bluebird nestlings as part of my ongoing dissertation research (~300 nestlings).