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

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***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.

***Methods summary***

*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.

***Methods 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 refrain 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.

***South Fork Preserve’s role***

I request permission to capture birds using mist nests and monitor nest boxes and associated nestlings in the South Fork Preserve weekly as described above in Methods from mid-March through August 2022.