CENTER for PRODUCE SAFETY

CPS 2021 RFP
FINAL PROJECT REPORT

Project Title
Towards a holistic assessment of the food-safety risks imposed by wild birds

Project Period
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Objectives

1. *Combine research team's pathogen database with new Campylobacter, Salmonella, and STEC tests from field-collected feces—focus on under-sampled species that frequent farms, especially species that can be actively managed (e.g., birds that use nest boxes).*
2. *Quantify how proximity to rangeland vs. natural habitats affects bird community composition, crop contact, and fecal densities on farms—census birds, collect feces, and use DNA barcoding to identify species defecating on crops at 15–20 produce farms.*
3. *In the field and in the lab, parameterize and compare E. coli survival curves in feces from a large waterbird (Canada Goose, Branta canadensis)* and a small songbird (Western Bluebird, Sialia mexicana) placed on four substrates: lettuce plants, conventional soils,* organic soils, and plastic mulch—on lettuce plants only, compare E. coli survival in feces from 8 more species that carry pathogens and/or are common on farms.*
4. *Combine data on pathogen prevalence, fecal densities, and pathogen survival to develop holistic risk assessments for multiple farmland birds; produce a photo guide to aid farmers in identifying species and farming contexts that present the gravest risks.*

*Objective changes: Wild turkey (*Meleagris gallopavo*) replaced Canada goose, and conventional soils were removed (sample size for the other three substrates was increased).

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Abstract

Members of the fresh produce industry often express concerns about wild birds because they can carry foodborne pathogens, are difficult to exclude from farms, and regularly contaminate fresh produce with feces. Nonetheless, very few studies have quantified the combined likelihood that different bird species carry pathogens, visit farm fields, and defecate feces on crops that are conducive to pathogen survival. To address these gaps, this research project sought to (1) augment prior datasets with pathogen assays of under-studied species, (2) quantify spatiotemporal patterns in bird intrusion and fecal contamination on California lettuce farms, and (3) assess *E. coli* survival in feces across diverse bird species and substrate types. Pathogen prevalences in wild birds were very rare. Combining >500 new pathogen assays with a prior pathogen database yielded new prevalence estimates of 0.2% of 5,247 birds for STEC, 0.4% of 4,647 birds for *Salmonella*, and 5.8% of 3,577 birds for *Campylobacter* spp. Across 29 lettuce farms in California's Central Coast, bird communities exhibited strong seasonal dynamics, with more individuals, flocks, and feces observed in fall than winter or spring. Molecular identification of bird feces in fields suggested that species observed more often in fields also caused more fecal contamination, with flocking blackbirds, crows, ravens, sparrows, and finches depositing the highest number of feces. Across field and greenhouse experiments, fecal size strongly predicted *E. coli* persistence. *E. coli* rapidly died off in feces deposited by small songbirds but persisted in large feces deposited by geese or turkeys, especially on lettuce. Finally, this work reaffirmed that bird feces are ubiquitous in the farm environment and that implementing no-harvest buffers around all feces would be cost-prohibitive. Fortuitously, combining results across objectives suggested that most birds on farms and most bird feces in fields present low food-safety risks: pathogens are rare in birds, and most birds defecate small feces that are unlikely to facilitate pathogen survival. Growers could thus consider implementing no-harvest buffers only around large feces deposited on lettuce, thereby decreasing affected acreage. More broadly, results from this study suggest a path forward for co-managing produce farms for food safety, crop production, and conservation, as promoting small, insect-eating songbirds on farms would likely incur low food-safety risks while potentially providing ancillary pest-control benefits.

Background

Despite evolving food-safety standards and fundamental changes in farming practices, foodborne disease outbreaks continue to plague the fresh produce industry [1–3]. Foodborne pathogens can enter and contaminate farm fields via multiple pathways, including but not limited to agricultural water, soil amendments, farm equipment, farm workers, runoff, flood events, and domestic or wild animals. Farm management strategies have been identified to address many of these contamination routes, some of which are now enforced via mandatory or voluntary food-safety regulations [4,5]. For example, after wildlife were implicated in several high-profile foodborne disease outbreaks [6–8], many growers began fencing their fields, deploying traps, and/or implementing 1 m no-harvest buffers around animal feces encountered in their fields [9].

Though many such practices at least partially address large mammal (e.g., deer and pigs) and rodent intrusion, identifying and addressing risks associated with wild birds has proven more complicated [10]. Wild birds are known to at least occasionally carry foodborne pathogens, including Shiga-toxin producing *E. coli* (hereafter, STEC), *Salmonella*, and *Campylobacter* spp. [10–14]. Additionally, birds often move long distances, potentially carrying pathogens between high-risk areas (e.g., livestock operations) and fresh produce fields [15]. Birds are also difficult to exclude from farms. Indeed, birds rapidly habituate to auditory or visually deterrents, and

other exclusion methods, while effective, are also quite costly (e.g., falconry and netting) [16,17]. As a result, birds commonly contact produce and defecate in fresh produce fields, raising concerns among growers and the broader fresh produce industry that birds could introduce significant food-safety risks to the farm environment [18].

Despite these concerns, whether or not wild birds a represent significant food-safety risk remains unclear [10,19]. To date, wild birds have only been implicated in one foodborne disease outbreak: Sandhill Cranes (*Grus canadensis*) caused a *Campylobacter* outbreak linked to contaminated Alaskan pea fields [6]. Additionally, much of the historic pathogen surveillance work in wild birds has focused a narrow range of species (e.g., pigeons, starlings, gulls, and waterfowl), neglecting many species commonly found in the farm field environment [7,10]. A recent study compiled a comprehensive database of pathogen assays of wild birds in produce fields, reporting very low prevalences of 0.22% for STEC and 0.46% for *Salmonella* (STEC: 4,693 tests from 94 species; *Salmonella*: 4,093 tests from 93 species) [13]. *Campylobacter* spp. were more common (8% of 3,023 tests for 80 species); however, whether the strains present in wild birds also cause campylobacteriosis in humans is unclear [10,13,20,21]. Regardless, though this database majorly advanced understanding of pathogen prevalences in wild birds, representation among species was still highly skewed, with >75% of 130 species represented by fewer than 20 individuals [13]. Under-sampled species included many species commonly found in produce fields, especially wintering or migratory species (given that prior studies have largely occurred in summer [11,14,22]). Birds that occupy nest boxes were also poorly represented, complicating efforts to assess the food-safety risks associated with the common practice of constructing nest boxes to attract insectivorous birds that consume crop pests [23,24].

Comprehensively assessing the food-safety risks associated with different bird species requires not only understanding which species carry pathogens, but also which are likely to enter farms and defecate on crops [10]. However, a prior meta-analysis found that only 3.3% of studies reported data across the entire pathogen spillover cycle [10]. Different bird species vary dramatically in their inclination to enter fresh produce farms, with some species never venturing into agriculture, others primarily occupying diversified fields adjacent to natural areas, and still others only using highly-itensified fields in crop monoculture landscapes [13,25–27]. While many studies have identified which species enter various types of agricultural fields, few have also explored whether bird presence in farm fields correlates to contamination risk. It may be that some species are often found in crop fields but rarely defecate on crops, while others exhibit the reverse trend [22]. Identifying which species not only carry pathogens but also defecate on produce (and in which spatial contexts) is thus a major priority.

Finally, our understanding of pathogen survival in wild birds is quite limited. A few studies have shown that *Salmonella* and STEC can at least occasionally survive for long periods in Canada Goose (*Branta canadensis*) and European Starling (*Sturnis vulgaris*) feces, respectively [28,29]. Desiccation risk may be key to survival, with pathogens surviving longer in humid conditions [30] and after exposure to irrigation water [31] but rapidly dying off in the hot summer [32]. What is not known, however, is whether pathogen survival depends on the substrate upon which feces are defecated as well as the species that defecated the feces. For example, pathogen survival may decline on substrates prone to more rapid desiccation (e.g., plastic mulch and soil versus crops). Survival may also decline in small songbird feces that desiccate rapidly as opposed to large feces that may better retain moisture.

This project was designed to address the aforementioned knowledge gaps and, in doing so, comprehensively evaluate the food-safety risks across a wide range of common farmland bird species. Three research objectives guided the work plan. The first objective was to collect feces from species under-represented in current pathogen prevalence databases and then assay them for the presence of *Campylobacter* spp*., Salmonella*, and STEC. The second objective

was to quantify bird intrusion and defecation rates on lettuce farms located across landscape gradients in the Central Coast, thereby identifying the spatial contexts and species that regularly venture into farm fields and defecate on fresh produce. Given that prior work has largely been restricted to spring and summer, surveys were to occur throughout the year to quantify seasonal variation in food-safety risks. The third objective was to implement a series of field and greenhouse experiments to quantify *E. coli* survival in wild bird feces and, in doing so, assess how different substrate types and species identities influence survival trajectories. Finally, the project included an objective focused on synthesizing results across objectives to develop holistic risk assessments for wild birds and distribute this information to the produce industry.

Research Methods

Objective 1— To quantify pathogen prevalences across a broader array of wild bird species, fecal samples were opportunistically captured from wild birds throughout the project period and then assayed for foodborne pathogens. All bird handling techniques were permitted and approved by relevant institutional authorities (IACUC Protocol #22562; CDFW Specific Use Permit S-212650004-21266-001-01; US Federal Bird Banding Permit #24033).

Feces were obtained from birds via five methods. First, wild birds were captured in 12 m, 38 mm mesh mist nets, placed on or adjacent to farming areas in the California Central Valley near Davis, California. Nets were erected at dawn and kept open following standard protocols (*i.e.*, nets were closed during extreme heat, cold, or inclement weather) [33]. Birds were extracted from nets every 20 minutes and then placed in sterilized cotton bags. Birds usually defecated in bags within 15 minutes and were held for no more than 1 hour before processing. During processing, birds were identified to species and marked with a unique leg band before being released. Feces were collected from cotton bags with sterilized tweezers, placed in vials, and then transferred to a cooler. Feces were then placed in a -80ºC freezer until subsequent molecular analysis. Across 2022 and 2023, five sites near Davis, California, were visited a total of 28 mornings (e.g., the UC Davis Student Farm, Putah Creek Riparian Reserve, and Russell Ranch research facility), comprising approximately 1008 total net-hours of effort (*i.e.*, total numbers of hours that each net was open). In total, 596 individuals of 36 species were captured.

Second, feces were obtained from birds nesting in ~200 artificial nest boxes located at 12 sites in row crops, orchards, and adjacent riparian and grassland areas along Putah Creek in California's Central Valley. Flaptraps were placed at the entrance of nest boxes, and, after being triggered, adult birds were hand captured from boxes. Birds were placed in sterilized cotton bags and feces were obtained as described above. In total, feces were collected in this manner from individuals of the following species: Western Bluebird (*Sialia mexicana*; 19 individuals), Tree Swallow (*Tachycineta bicolor*; 30 individuals), and Ash-throated Flycatcher (*Myiarchus cinerascens*; 2 individuals). Third, Wild Turkey (*Meleagris gallopavo*; 23 individuals), Canada Goose (*Branta canadensis*; 20 individuals), California Scrub-jay (*Aphelocoma californica*; 1 individual), and Hermit Thrush (*Catharus guttatus*; 1 individual) were opportunistically followed, fresh fecal samples were collected upon defecation with tweezers, and feces were placed into sterile vials. Fourth, in the late evening, sterilized plastic tarps were placed beneath known roosting sites of the following species: American Crow (*Corvus brachyrhynchos*; two sampling occasions, resulting in 22 fecal samples) and Cliff Swallow (four sampling occasions, resulting in 9 fecal samples). Only roosts composed of single species were targeted. The following morning, sterilized tweezers were used to acquire bird feces and place them in vials.

Finally, in addition to collecting feces from known species in the manner described above, feces from unknown birds were collected from lettuce farms throughout California's Central Coast.

Molecular techniques were then used to identify the originating bird species (methods for sample collection and molecular analysis are detailed below).

After fecal sample collection, 1,352 fecal samples were selected for pathogen testing. This included 1075 fecal samples from lettuce farms in the Central Coast, of which 277 could be attributed to bird species via molecular techniques (four additional samples were identified as domestic chickens and one additional sample could be identified as a sparrow, but not to a specific species). In addition, 277 samples obtained from mist net captures, nest boxes, following birds, and/or plastic tarps were selected for pathogen testing from the 34 species that were relatively under-represented in existing pathogen databases.

Each fecal sample was tested for *Campylobacter* spp*., Salmonella*, and *E. coli* virulence genes via PCR as in [14,22]. DNA was extracted from fecal samples using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany) as directed by the manufacturer. DNA from *Campylobacter jejuni* RM1615, *C. coli* RM1875, *C. lari* RM2808, *C. fetus* RM1265, *Salmonella enterica* RM3363, and *Escherichia coli* O157:H7 RM2323 were extracted from 1 ml of an 18h culture using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI), as directed by the manufacturer, for use as positive controls. First, a generic assay for *Campylobacter* spp. was implemented, targeting the 23S rRNA gene as in [34]. PCR positive samples were followed up with more specific tests for *C. jejuni* (hipO), *C. lari* (glyA), *C. fetus* (sapB2), and *C. coli* (glyA) genes. PCR for all the *Campylobacter* genes was performed in 25 µl reactions containing 12.5 µl Taq 2X Master Mix (New England Biolabs, Ipswich, MA), 0.4 µM for each primer, 50 ng DNA, and water to volume. PCR conditions were: 1 cycle of 95°C for 6 min; 30 cycles of 95°C for 30s, 59°C for 30s and 72 for 30s followed by one cycle of 72°C for 7 min. Second, samples were assayed for the *E. coli* virulence genes stx1, stx2, eaeA, hlyA, and saa using multiplex PCR as in [35,36]. 50 µl reactions contained 25 µl Taq 2X Master Mix, 50 ng of sample DNA, 0.4 µM each primer, and water to volume. PCR conditions were: 10 cycles of 95°C for 1 min, 65°C for 2 min, 72°C for 1.5 min followed by 5 cycles of 95°C for 1 min, 60°C for 2 min, 72°C for 1.5 min followed 10 cycles of 95°C for 1 min, 60°C for 2 min, and 72°C for 2.5 min. Third, samples were tested for *Salmonella*, targeting the invasion gene InvA using methods from [37]. 50 µl reactions contained 12.5 µl of Taq 2X Master Mix, 0.8 µM of each primer, 50 ng of sample DNA, and water to volume. PCR conditions were one cycle of 94°C for 2 min followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s, followed by a final extension at 72°C for 5 min. All PCR products were electrophoresed in 2% agarose gels at 100 V for 25 min. The gels were stained with ethidium bromide (10 mg/ml) and visualized on a UV transilluminator.

Objective 2— To quantify and assess bird intrusion and fecal contamination in the farm environment, avian censuses and fecal contamination surveys were conducted across 29 organic vegetable farms growing leafy greens in the California Central Coast. Farms were selected to span a gradient in landscape composition, from farms completely surrounded by agriculture to those immediately adjacent to seminatural or grazed areas. To quantify seasonal dynamics in bird abundances and fecal deposition, surveys occurred across three distinct periods: summer (5/26/2022–7/23/2022), fall (9/14/2022–11/18/2022), and winter (2/15/2023–4/20/2023). Notably, the winter surveys represented additional effort (beyond what was originally proposed), but the repeated occurrence of heavy rains and flooding limited sampling effort. In total, 18, 18, and 11 farms were visited in summer, fall, and winter, respectively.

At each farm, point-count sites were established in fields growing lettuce, with 3–8 sites per farm, depending on farm size (mean 5.8 sites). Within farms, point-count sites were established a median of 220 m apart from one another (inter-quartile range: 120 m to 447 m). In total, 274 sites were surveyed across all three seasons. Each site was usually visited three times within a one-week period, with several exceptions. In summer, 8 of 101 sites were only visited twice due to the presence of heavy irrigation during sampling. In fall, 15 of 106 were visited 4 times, as

entire farms were resurveyed if any point could not initially be surveyed due to irrigation. In winter, 3 of 67 sites were surveyed 4 times (for the same reason as in fall). Additionally, to increase replication (given rain and flood events), 10 sites were visited 6 times, with two rounds of counts at the beginning and end of the season.

Within seasons, all points counts were conducted by the same expert observers (technician Rose Albert in summer and technician Max Leibowitz in fall/winter). In summer and fall, surveys were timed to occur near harvest (*i.e.,* the highest risk period for fecal contamination). However, given crop phenology, most surveys occurred at the seedling stage in winter. Surveys usually began at sunrise, with 1–2 farms surveyed per day. After arriving at each count site, observers waited 5 minutes before beginning the count. Then, during each 10-minute point count, observers recorded all birds heard or seen within the 50 m survey radius. Fly overs and birds outside the radius were recorded and noted as such. For visually detected birds within the count radius, observers recorded each bird's microhabitat; most importantly, whether or not the bird was physically interacting with crops. For each bird, observers also noted the time of detection, the method of detection (auditory or visual), and, when possible, the bird's behaviors (e.g., foraging, aggression, flying, perching, *etc.*).

Covariates that may influence bird detection were also recorded, including: the temperature and wind speed (using a hand-held thermometer and anemometer), the presence of loud noises, the number of people visible within and outside the 50 m count radius, and weather conditions (sun, partly cloudy, overcast, fog, and rain; though surveys always occurred in the absence of heavy rain and fog). Anti-bird practices were also noted, including bird traps (not detected), artificial raptor calls (9 of 862 surveys), scarecrows (not detected), sound canons and whistlers (5 surveys), streamers (35 surveys), and fake owls (2 surveys). Finally, observers visually characterized the 50m point count, noting the presence of nest boxes, bird seed, fencing, hedgerows, wind blocks, tractors, trees, powerlines, sprinklers, pesticide tanks, weeds, flower strips, grass strips, forest cover, paved surfaces, residential areas, water sources, *etc.* The presence and phenological state of all crops growing within the count radius was also noted.

To quantify and assess fecal contamination, observers established three 25 m transects on each farm. Transects were located at a field edge, in a field interior (*i.e.,* up to 500 m into a crop field), and halfway in between. Transects were visited once per season, for a total of 141 transects surveyed (3 transects per farm; 18, 18, and 11 farms surveyed in summer, fall, and winter, respectively). Along each transect, observers used a $1m^2$ square to divide the transect into 25, $1m^2$ adjacent quadrats. The number and location (*i.e.,* on crop or soil) of bird feces was noted for each quadrat. Up to 10 feces were then collected from each transect with sterile tweezers, placed in vials, and then transferred to a cooler. If 10 feces could not be located from each transect, then observers scoured adjacent areas (sometimes a few additional samples were collected if encountered). Samples were placed in a -20ºC freezer until being transported to either UC Davis or the USDA Western Regional Research Center for DNA extraction. In total, 1075 samples were collected across the summer, fall, and winter field seasons.

Feces collected in lettuce fields were assayed for foodborne pathogens (*i.e.,* STEC, *Salmonella,* and *Campylobacter* spp.) using the methods described above in Objective 1. In addition, to identify the bird species that deposited each fecal sample, the bird cytochrome c oxidase I (COI) gene was PCR amplified and sequenced as in [38]. Briefly, DNA was extracted from bird feces using the QIAamp Fast DNA Stool Mini Kit as described above. PCR were performed in 50 µl that contained 25 µl of Taq 2X Master Mix, 0.4 µM of both primers (K_Bird_F1 and K_Bird_R1), 50 ng of sample DNA, 20 µg of bovine serum albumin, and water to volume. The PCR conditions were one cycle of 95°C for 2 min followed by 5 cycles of 95°C for 1 min, 46°C for 1 min, and 72°C for 30 s, followed by 45 cycles of 95°C for 1 min, 53°C for 1 min, and 72°C for 30 s, and a final extension at 72°C for 5 min. PCR products were purified via QIAquick PCR

Purification Kit (Qiagen, Hilden, Germany). Purified PCR products were sequenced at the University of California DNA Sequencing Facility.

Finally, to quantify the landscape composition around each farm, 2016 NAIP imagery was manually digitized within a 1 km radius of all sampling sites in ArcMap (ESRI, Redlands, CA, USA). Specifically, observers denoted the presence of forest, shrubland, bare ground, water features, herbaceous vegetation, pasture, greenhouses, annual crops, orchards/vineyards, and urban, exurban, and suburban areas. A map of grazeable land—or land that is suitable for grazing landscape—was acquired from the Farmland Mapping and Monitoring Program [39] and overlaid with the hand-classified land-cover map to identified grazed areas.

Generalized linear mixed models (GLMMs) were implemented to assess spatiotemporal variation in bird communities and fecal densities. Specifically, the total number of birds detected per count and the number observed contacting crops were modeled as a function of season (summer, fall, or winter) as well as other covariates that may influence bird detectability, including time of day, number of people observed within the count radius, the presence of loud noises during the count, average wind speed during the count, and the average temperature recorded during the count. Both response variables were modeled with negative binomial distributions to address issues related to zero inflation. In contrast, binomial distributions were implemented to model the probability of observing at least one bird flock during a point count, defined as 10 or more individuals of the same species. Due to convergence issues, only noise, time of day, and temperature could be included alongside season in this model. For all models, point-count location, nested within farm identity were included as random effects to address spatial autocorrelation and account for repeated visits to the same count locations. Finally, negative binomial models were used to assess the effects of season and transect position (edge, interior, or half-way in between) on fecal densities (*i.e.,* the number of bird feces observed within each 25m by 1m transect). Again, farm identity was included to account for spatial autocorrelation. All models were implemented in R [40].

Objective 3— To quantify *Escherichia coli* persistence on different substrate types and in feces from different bird species, a field experiment was implemented on an organic vegetable farm in Davis, California. The experiment focused on two bird species that are frequently observed on farms but vastly differ in size: Wild Turkey (5,791 g; *Meleagris gallopavo*) and Western Bluebird (26 g, *Sialis mexicanus*) [41]. Wild Turkeys are resident across much of the United States, while Western Bluebirds contain migratory and resident populations along the west coast of North America to Central Mexico. Throughout the spring and summer of 2022, fecal samples were collected from each species near Davis, California. Turkey feces were obtained by following individuals on the UC Davis campus and collecting feces immediately after defecation with sterile forceps. In contrast, bluebirds were captured from nest boxes as described above, and then feces were collected with sterile forceps.

After collection, feces were immediately placed on ice in a cooler, returned to the UC Davis campus, aggregated into one composite sample by species, and then inoculated with a non-pathogenic *E. coli* strain collected from a lettuce field in Yuma, Arizona; specifically, *E. coli* 0145:H11 strain RM14721NR, a spontaneous nalidixic acid and rifampicin-resistant mutant of *E. coli* 0145:H11 strain RM14721 [42]. An overnight inoculation of *E. coli* was created by placing one colony forming unit (CFU) into 5 ml of tryptic soy broth (TSB). The inoculated TSB was placed in an incubator at 37°C for 18–24 hours prior to fecal sample collection. The following day, Wild Turkey and Western Bluebird samples were inoculated at a projected concentration of 0.1 mL *E. coli* inoculate per 2.0 g of fecal matter, approximating natural *E. coli* shedding concentrations found in wild birds [10]. After inoculation, Western Bluebird aggregated fecal matter was subsampled into two size classes (0.03 g and 0.06 g) and Wild Turkey aggregated fecal matter was subsampled into four size classes (0.03 g, 0.06 g, 2 g, and 4.75 g). Masses

were chosen to roughly correspond to the average fecal mass of (1) a small songbird (e.g., Yellow-rumped Warbler, *Setophaga coronata*), (2) a Western Bluebird, (3) a juvenile Wild Turkey, and (4) an adult Wild Turkey, based on fecal samples obtained from 30 warblers, 15 bluebirds, and 27 turkeys. To calculate exact *E. coli* concentrations within the inoculated TSB, the TSB was serially diluted in PBS to create a concentration of $1x10^{-7}$ of the original. This solution was plated in petri dishes on agar gel and placed in an incubator at 37°C for 24 hours. The plate was collected and the number of CFUs were recorded the following day. The initial concentration of *E. coli* that was used to inoculate samples was then calculated by multiplying the sample concentration (CFUs/mL) by the mass of the fecal sample. In general, fecal samples were inoculated with equal concentrations of *E. coli* per gram of sample, but some variation existed depending on *E. coli* growth within the initial TSB inoculation.

After inoculation, feces were taken to the UC Davis Student Farm—an organic, experimental, and teaching farm on the UC Davis campus—and placed on one of three substrates: lettuce (*Lactuca sativa*; butterhead variety), soil, or black plastic mulch, within 4 hours of original collection. To measure abiotic conditions, three temperature and humidity loggers were placed on the lettuce, soil, and black plastic mulch. Samples were revisited after 1–30 days (64 samples for 1–2 days; 63 for 3–5 days; 66 for 6–8 days; 32 for 14–15 days; and 52 for 21–30 days; total = 277 samples). If the fecal sample had not completely disintegrated, the sample and surrounding substrate was collected with sterile tweezers, placed into unique plastic bags, and transported on ice to the USDA Western Regional Research Center in Albany, CA.

To quantify the amount of *E. coli* remaining in the samples, approximately 10 g of feces and substrate (lettuce leaves, plastic, or soil) were added to 90 ml of PBS in a sterilized blender jar and blended with an Osterizer Beehive blender (Oster, Neosho, MO) on high speed for 60 seconds. The resulting solution was 10-fold serially diluted in PBS, plated onto MacConkey agar plates containing 50 mg/l nalidixic acid and 100 mg/l rifampicin, incubated at 30°C for 24 h, and then counted. Samples that showed no growth were enriched by adding 1 ml of blended sample into 9 ml tryptic soy broth containing 50 mg/l nalidixic acid and 100 mg/l rifampicin. They were then incubated at 30°C for 24 h (shaking 200 rpm), plated onto MacConkey agar containing 50 mg/l nalidixic acid and 100 mg/l rifampicin, and examined for growth after 24 h. If the sample showed growth after enrichment, then they were assigned value of $1x10^{3}$ CFUs, as this is the conservative limit of detection for growth on MacConkey agar plates. If no growth was observed after the secondary enrichment, samples were designated as *E. coli* free.

Next, a greenhouse experiment was implemented at the USDA Western Regional Research Center to further quantify differences in *E. coli* survival among 10 bird species. A greenhouse experiment was necessary as fecal samples for different species were available at different times of year and abiotic conditions had to be standardized as much as possible. Specifically, from early December 2022 through late May 2023, feces were collected from Wild Turkey (N=17), Western Bluebird (N=16), Canada Goose (*Branta canadensis,* N=16), Barn Owl (*Tyto alba*, N=16), American Crow (*Corvus brachyrhynchos*, N=16), Rock Pigeon (*Columba livia*, N=16), American Kestrel (*Falco sparverius*, N=19), Cliff Swallow (*Petrochelidon pyrrhonota*, N=28), White-crowned Sparrow (*Zonotrichia leucophrys*, N=12, and Yellow-rumped Warbler (*Setophaga coronate*, N=16). The variation in sample size was due to the unpredictable nature of sample collection (particularly from feces collected via the tarp method). If additional samples were available, then they were used to increase sample size (*i.e.*, Cliff Swallow). Together, these species have wide ranges, represent six taxonomic orders, vary greatly in body size and thus fecal deposit size, are regularly found in farming environments, and exhibit a variety of migratory patterns, from resident to long-distance migration to South America.

As described in Objective 1, White-crowned Sparrow and Yellow-rumped Warbler samples were collected after capturing individuals in mist-nets; Western Bluebird samples were obtained after

capturing adult birds from nest boxes; Wild Turkey and Canada Goose samples were collected by following birds and waiting for them to defecate; and American Crow, Rock Pigeon, and Cliff Swallow samples were collected by placing tarps under known roosting sites. Finally, samples from American Kestrel and Barn Owl were obtained by placing sterile plastic tarps under perches of captive individuals at the California Raptor Center (https://crc.vetmed.ucdavis.edu/). The average fecal mass produced naturally by each species was determined by placing feces in airtight containers (sterile vials or plastic bags) within 30 minutes of defecation and weighing feces within 5 hours of collection. Captured birds were also weighed at the time of capture to relate fecal mass to bird mass. For uncaptured species (e.g., Wild Turkeys), an estimate of average mass for the species was obtained from the literature [41].

Immediately after collection, samples were taken to the USDA Western Regional Research Center. As above, samples were aggregated by species and then aggregates were inoculated with the same *E. coli* strain at same concentration as the field experiment, utilizing the same methods described above. To disentangle whether fecal mass or species identity *per se* influenced *E. coli* survival, feces were subsampled into one of two sizes: the average natural size for the species (0.03–9.8 g depending on the species) or a standardized mass (0.08 g; or the average mass of a common songbird: the White-crowned Sparrow). Within 6 hours of collection, samples were then placed on lettuce from the same seed stock in a greenhouse, alongside a temperature/humidity logger. After three days, samples were collected and the amount of *E. coli* remaining was quantified using the same methods described above.

GLMMs were used to analyze *E. coli* survival and persistence for all experiments. For the field experiment, response variables included *E. coli* survival (presence or absence of *E. coli*; binomial distribution) and the percent of original *E. coli* remaining (final divided by initial *E. coli* concentrations; Gaussian distribution with a log-link to address right-skewed data). Both response variables were modeled as a function of days in the field, mass class (0.03 g, 0.06 g, 2.00 g, or 4.75 g), substrate (lettuce, soil, or plastic), and species (Western Bluebird or Wild Turkey). Temperature and humidity were also modeled as a function of substrate. As substrate was highly correlated with temperature and humidity, *E. coli* survival and persistence models only included substrate. Interactions between mass and substrate as well as mass and days in the field were also tested, but then omitted as interactions were not significant. In addition, models were parameterized to assess whether the initial concentration of *E. coli* affected survival (*i.e.*, if *E. coli* was more likely to survive in larger fecal samples simply due to the fact that larger samples began the experiment with more *E. coli*). The initial concentration was not significant and was omitted to improve model simplicity.

For the laboratory experiment, the percent of original *E. coli* remaining was modeled as a function of fecal mass, (fecal mass)^2, species identity, temperature (°C), and relative humidity, using a Gaussian distribution with a log-link as above. The addition of the power function was to account for non-normal residuals. To test for the effect of explanatory power of species identity versus fecal mass, two additional models were created: one modeling the percent of original *E. coli* remaining as a function of fecal mass, temperature, and relative humidity only, and another as a function of species, temperature, and relative humidity.

All models were implemented using JAGS in R [40]. For each model, three chains of 50,000 iterations were thinned by 10 with a burn-in of 10,000, resulting in a posterior sample of 12,000. Parameter convergence was confirmed via visual inspection of traceplots as well as requiring a Gelman–Rubin statistic <1.1 [43]. Parameters were considered strong predictors (*i.e.*, significant) if the 95% Bayesian Credible Intervals (BCI) did not overlap zero.

Objective 4— Data were synthesized across objectives to (1) understand how no-harvest buffers could be strategically implemented to balance food-safety risks and crop losses and (2) identify species associated with relatively higher versus lower food-safety risks.

Results from pathogen survival experiments (Objective 3) provided estimates of *E. coli* survival according to fecal deposit size and substrate type. Results from bird and fecal transect surveys (Objective 2) provided information on the species of birds found on farms, the species that defecate on crops most often, and densities of feces encountered on various substrates (*i.e.,* lettuce and soil). Combining these data required estimating fecal deposit sizes across many species. To do so, fecal masses were correlated with bird masses (either obtained by weighing captured birds or by using published weights for uncaptured birds [41]; e.g.*,* Wild Turkey and Canada Goose). Bird weights were then used to predict fecal masses for all birds encountered in field surveys [41]. Finally, numbers of birds observed in counts, birds observed contacting produce, and birds defecating on soil and lettuce were divided into fecal weight categories to estimate the relative abundance of high versus low-risk bird species and fecal deposits on California Central Coast lettuce farms (solely defined by *E. coli* survival probability).

In addition, results were combined across objectives to generate holistic risk scores for wild bird species. Specifically, the pathogen prevalence dataset was merged with data on bird fecal sizes (estimated from bird body mass) as well as data on bird fecal contamination frequencies in farm fields. To quantify fecal contamination frequency, data from Objective 2 were combined with another dataset of 463 bird fecal samples attributed to bird species via molecular techniques and collected in *Brassica* fields or food washing stations on farms throughout the western U.S. [22]. Finally, a holistic risk score was computed for species that had been adequately sampled for all three pathogens (*i.e.,* 15 or more individuals tested for *Campylobacter* spp*., Salmonella*, and STEC). Species that (1) carried pathogens, (2) defecated in farm fields, and (3) produced large feces more conducive to *E. coli* survival received the highest scores.

Results

Objective 1— Pathogen prevalence in wild birds was very low (**Fig. 1**). Of the 554 identified birds tested, 12 (2.2%) from six species tested positive for the 23S general *Campylobacter* test (one American Crow- *Corvus brachyrhynchos*, two Brewer's Blackbirds- *Euphagus cyanocephalus*, one Common Raven- *Corvus corvax*, one House Finch- *Haemorhous mexicanus*, six House Sparrows- *Passer domesticus*, and one Yellow-rumped Warbler- *Setophaga coronata*). Including both identified birds and fecal species that could not be attributed to any species (N=1352), 20 samples (1.5%) tested positive for *Campylobacter* spp. Subsequent tests for more specific *Campylobacter* strains were all negative. Including the 554 individuals identified to species in the broader pathogen dataset reduced the original *Campylobacter* spp. prevalence estimate of 8% (of 3,023 birds) to 5.8% (of 3577 birds).

Only one identified individual (0.2% of birds) tested positive for *Salmonella*: an Orange-crowned Warbler (*Vermivora celata*). One additional unidentified fecal sample also tested positive (0.15% of 1,352 birds). Including the 554 tests of identified birds in the pathogen database caused estimates of *Salmonella* prevalence in birds to lower slightly from 0.5% (of 4,093 birds) to 0.4% (of 4,647 birds).

Finally, one identified individual (0.2% of birds) tested positive for *E. coli* eae, saa, stx2, and hly genes: a Common Raven. Four additional unidentified feces tested positive for the *E. coli* eae gene (but no other genes). Including samples from identified birds in the pathogen database did not change the estimate of STEC prevalence in wild birds, remaining at 0.2% for both the original dataset of 4,693 birds and the updated dataset of 5,247 birds.

Notably, pathogen prevalences among birds nesting in artificial nest boxes were particularly low. Not a single sample from the 73 individuals of five species that occupy nest boxes and were collected in this study was positive for any pathogen (Western Bluebird- *Sialia mexicana*, Tree Swallow- *Tachycineta bicolor*, White-breasted Nuthatch- *Sitta carolinensis*, Ash-throated Flycatcher- *Myiarchus cinerascens*, House Wren- *Troglodytes aedon*). Across the broader pathogen dataset and including more nest-box species (Violet-green Swallow- *Tachycineta thalassina*, Black-capped Chickadee- *Poecile atricapillus*, Chestnut-backed Chickadee- *Poecile rufescens*, and Oak Titmouse- *Baeolophus inornatus*), pathogen prevalences were 2.7% of 183 birds for *Campylobacter* spp., 0.47% of 213 birds for *Salmonella*, and 0% of 260 birds for STEC. Further, no pathogens were ever detected in the three species most likely to occupy nest boxes in Western U.S. agricultural settings (*i.e.*, Western Bluebird, Tree Swallow, and House Wren). Importantly, however, the two non-native species that sometimes usurp nest boxes exhibited much higher prevalences of *Campylobacter* spp. at 20% of individuals tested for House Sparrow (*Passer domesticus*) and 15% for European Starling (*Sturnis vulagris*). Though neither species tested positive for *Salmonella* or STEC, other studies have documented STEC and *Salmonella* in European Starlings outside the fresh produce environment [44–46].

Objective 2— A total of 34,794 individuals of 115 bird species were detected across the 862 point counts conducted in summer 2022, fall 2022, and winter 2023. Of those, 9,560 birds of 90 species were detected within the 50 m point count radius and 1,015 individuals of 25 species were observed contacting leafy greens. As mentioned, 277 of the 1,075 fecal samples collected from farms in summer, fall, and winter could be successfully attributed to originating bird species, with one additional sample identified as an unknown sparrow species and four more samples identified as chickens. Of the 277 identified to species, 227 samples were collected inside crop fields (the rest were collected along field margins, roads, or near farm infrastructure).

Species that were observed more often in 50 m point counts, species that were observed more often interacting with lettuce, and species that were more often observed in flocks of 10 or more individuals were also likely to produce more feces in fields, as determined from molecular analyses (**Fig. 2**; birds in point count: Pearson's r= 0.58, P< 0.001; birds interacting with lettuce: r= 0.60, P< 0.001; birds in flocks: r= 0.60, P< 0.001). For example, Brewer's Blackbird, House Finch, Common Raven, and White-crowned Sparrows were often detected in counts, regularly seen interacting with lettuce, exhibited flocking behavior, and produced many feces in fields. There were, nonetheless, exceptions suggesting that species occurrence in agricultural settings is not a perfect surrogate for contamination risk. For example, House Sparrows were not often detected in counts, never seen interacting with crops or in flocks, but were regularly identified as producing feces in fields. That said, House Sparrows do form large flocks (they were just relatively rare in counts, so flocks were not observed). On the other hand, some species were regularly often seen in crop fields but rarely produced feces in fields (e.g., Red-winged Blackbird and Yellow-rumped Warbler). Importantly, none of these results changed if all fecal samples were analyzed, including those from field margins.

Bird communities exhibited strong seasonal dynamics, translating into temporal variation in potential food-safety risks (**Fig. 3**). Models estimated the average number of birds observed per count in fall to be more than double the amount in summer or winter (summer: 5.4 birds, fall: 13.4 birds, winter: 4.9 birds). Similarly, the likelihood of observing a bird flock—defined as 10 or more individuals of the same species—was ~3 times as high in fall than in summer or winter (summer: 11%, fall: 32%, winter: 8%). The number of birds observed actually contacting crops exhibited slightly different trends, with models predicting extremely few birds interacting with produce in winter (0.04 per count) but significantly higher numbers in summer (0.47 per count) and fall (1.38 per count). Correspondingly, the density of feces encountered in fecal transects

was significantly higher in fall (0.11 feces/m^2) than in winter (0.05 feces/m^2), with intermediate numbers observed in summer (0.07 feces/m^2).

Initial evidence also suggested spatial variation in potential food-safety risks. Within fields, fecal densities were highest along transects at field edges (0.11 feces/m^2) compared to field interiors (0.07 feces/m^2) and transects halfway in between (0.06 feces/m^2) (**Fig. 4**). With respect to bird communities, models that included information on where surveys were located explained much more variation in total birds observed per count compared to models that did not take farm identity into account (ΔAIC= 98, P< 0.001). Though landscape analyses are not complete, these initial results suggest that ongoing analyses will yield insights into how rangeland, seminatural areas, and other land covers influence bird communities and fecal densities on farms.

Objective 3— In the field experiment, substrate and day of year affected microclimates. Specifically, temperature was higher on soil than on lettuce (95% BCI: 0.98, 2.19; **Fig. 5A**), and temperature was higher on plastic than soil (95% BCI: 2.99, 4.30; **Fig. 5A**). On the other hand, relative humidity was highest on lettuce, followed by soil (95% BCI: -15.41, -9.82; **Fig. 5B**), and plastic (95% BCI: -21.30, -15.49; **Fig. 5B**). Microclimatic differences were likely responsible for differences in *E. coli* survival (presence or absence of *E. coli*) and decay rates (fraction of original *E. coli* remaining) among substrates. Specifically, *E. coli* survival was predicted to be higher on lettuce than soil (95% BCI: -1.16, -0.07; **Fig. 5C**) or plastic mulch (95% BCI: -2.22, -0.59; **Fig. 5C**). Higher percentages of original *E. coli* concentrations were also found in lettuce than on soil (95% BCI: -2.71, -0.90; **Fig. 5D**) or plastic mulch (95% BCI: -4.62, -2.44; **Fig. 5D**).

After accounting for differences among substrates, fecal mass was the strongest predictor of *E. coli* survival and decay rates (95% BCI: 0.63, 1.14; **Fig. 6**). The largest fecal samples (4.75 g) had significantly higher survival and percent of original *E. coli* remaining than 2.00 g samples, which, in turn, were higher than both 0.03 g and 0.06 g samples. Specifically, a 4.75-g fecal sample, corresponding to a Wild Turkey, was predicted to have an 84.6% chance of *E. coli* detection on lettuce, 74.9% on soil, and 58.2% on plastic after 30 days. However, a 0.06-g fecal sample, corresponding to a Western Bluebird, was predicted to have an 8.6% chance of detection on lettuce, 4.9% on soil, and 2.3% on plastic over the same time period. These results suggest that, while the substrate a fecal sample is on is important, the effect of fecal mass is also a critical predictor of *E. coli* survival and decay. Critically, the model found no difference in *E. coli* survival or decay between feces from Wild Turkeys and Western Bluebirds after fecal size was taken into account (95% BCI: -0.93, 0.36).

The laboratory experiment arrived at similar conclusions as the field experiment. Among naturally sized fecal samples ranging from 0.03–9.8 g, mass had a strong positive relationship with the percent of *E. coli* remaining in the sample after 3 days (**Fig. 7A**; 95% BCI: 1.40, 2.17), even after controlling for temperature and humidity. However, when fecal samples were standardized across species to 0.08 g, the effect of species became marginal. While one species exhibited significantly higher *E. coli* persistence after 3 days compared to other species (Wild Turkey), all species had less than 10% of the original amount of *E. coli* remaining on average (**Fig. 7B**). This was confirmed in model comparisons between the full model with fecal mass and species versus the reduced models with no fecal mass or no species parameters. The full model had the most explanatory power (DIC: 730.1), however, the model with fecal mass but no species had much higher explanatory power (ΔDIC: 7.8) than the model with species and no fecal mass (ΔDIC: 111.7).

In conclusion, differences in fecal mass were the primary reason why *E. coli* survival varied among species, both field and laboratory settings. As a result, models could be used to predict how fecal mass affected *E. coli* survival in the field (95% BCI: 0.62, 1.14; **Fig. 8A, B**) as well as the percent remaining of *E. coli* in field (95% BCI: 0.87, 1.36; **Fig. 8C, D**) and in the greenhouse

(fecal mass: 95%CI: 1.83, 2.58; fecal mass^2: 95% BCI: 0.38, 0.68; **Fig. 8E**). Interestingly, models predicted higher overall *E. coli* persistence in the greenhouse than in the field, likely due to more benign conditions in the greenhouse (*i.e.*, lower temperatures, lower UV radiation). Nonetheless, greenhouse models still suggested that *E. coli* populations would exhibit declines after 3 days if fecal mass were smaller than 1.61 g (**Fig. 8E**).

Objective 4— Combining data across objectives gave key insights into no-harvest buffer implementation. As noted, pathogen survival models could quantify how *E. coli* survival changes as a function of fecal mass and substrate types. Specifically, models predicted that the fraction of *E. coli* remaining after 2 weeks in the field for a 0.15 g fecal sample would be $9.4x10^{-9}$ % on lettuce and $1.5x10^{-9}$ % on soil. Additionally, models predicted that the probability of detecting any *E. coli* after secondary enrichment would be only 35.2% on lettuce and 22.7% on soil after 2 weeks. If this were considered a tolerable risk threshold, then growers might consider ignoring feces <0.15 g, especially when located on soil.

What fraction of birds in farmland defecate feces below that size threshold? Answering this question required estimating fecal masses for birds encountered in field surveys. Fortuitously, bird body mass was a strong predictor of fecal mass (R^2= 0.82; P< 2e-16; **Fig. 9**). Bird fecal masses were thus derived from bird body mass data [41]. Doing so suggested that 90.2% of species encountered in point-count surveys and 89.6% of birds observed contacting crops defecated feces smaller than the 0.15 g threshold (equating to a 100g body size; **Fig. 10A, B**). Further, 81% of feces encountered in transects were attributed to birds that defecate feces <0.15 g (**Fig. 10C**). Bird feces were found in 10.3% of the 2700 1 m^2 quadrats assessed over summer and fall, meaning growers could lose a large fraction of their harvest if they were to implement no-harvest buffers around all bird feces. Importantly, however, only 0.9% of quadrats contained feces on lettuce vs. 9.6% on soil. If growers ignored small feces (<0.15 g) on soil, then they could reduce acreage affected by no-harvest buffers to only 2.7%. This is because 0.9% of fields would contain feces on lettuce and 1.8% would contain large feces on soil (*i.e.,* 9.4% of quadrats have feces on soil but not lettuce, 19% of which would likely be large feces).

Combining data across objectives on pathogen prevalences, fecal incidences in farm fields, and fecal sizes also helped categorize species according to food-safety risks. Specifically, species received higher food-risk scores according to the following formula:

- +1 if *Campylobacter* spp. prevalence was estimated at >5% or *Salmonella* or STEC were ever detected in the species.
- +1 if *Campylobacter* spp., *Salmonella*, or STEC prevalences were estimated to be greater than 10%, 0.5%, and 0.5%, respectively.
- +1 if more than 5 feces collected from produce fields could be attributed to the species.
- +1 if more than 10 feces collected from produce fields could be attributed to the species.
- +1 if the species was estimated to defecate feces >0.1 g in mass.
- +1 if the species was estimated to defecate feces >0.15 g in mass.

Of the 47 species adequately sampled for all three pathogens (**Table 1**), only one satisfied all six conditions (Common Raven). Two more satisfied five conditions (American Robin and European Starling). Eleven additional species satisfied three or four conditions (Brewer's Blackbird, California Quail, House Sparrow, Savannah Sparrow, Song Sparrow, American Crow, Barn Swallow, California Scrub-Jay, House Finch, Red-winged Blackbird, and White-crowned Sparrow). Twenty satisfied one or two conditions and the rest (thirteen) satisfied none. Importantly, all three native species that commonly use nest boxes in California farmland were in the absolute lowest risk category (Western Bluebird, House Wren, Tree Swallow).

Outcomes and Accomplishments

Objective 1— The first objective of this project was to collect and assay bird feces for foodborne pathogens, focusing on under-sampled species, defined as having <20 individuals represented in a recent avian foodborne pathogen database [13]. Species that use nest boxes and thus can be actively managed on farms were to be prioritized.

To address this objective, 554 feces were collected via diverse methods and then assayed for *Campylobacter* spp., *Salmonella,* and STEC (as discussed above). As a result, the number of species with at least 20 individuals represented in the database increased from 39 to 48 for STEC, 37 to 45 for *Salmonella*, and 30 to 41 for *Campylobacter* spp. Moreover, 47 species are now represented by more than 15 individuals for all three pathogens. Of these, 73 samples were from nest-box utilizing birds, including the two most common nest-box associated species (Western Bluebird and Tree Swallow), both of which are now adequately sampled.

As noted above, very few pathogens were detected in pathogen assays, especially in nest-box associated species. Overall, pathogen prevalences for birds in farming landscapes are now estimated to be 5.8%, 0.4%, and 0.2% for *Campylobacter* spp., *Salmonella,* and STEC, respectively. Results from this project reaffirm that pathogens are rare in wild birds and that nest-box associated species present particularly low food-safety risks. However, pathogens were more common in some non-native species that occasionally usurp nest boxes, suggesting boxes should be actively managed according to established best practices.

Objective 2— The second objective of this project was to assess spatiotemporal variation in food-safety risks from birds on lettuce farms in the California Central Coast, leveraging bird censuses, fecal transects, and molecular identification of field-collected feces. Particular emphasis was placed on understanding risks from adjacent rangeland areas and on identifying which species are most likely to defecate on crops.

Birds were surveyed and feces were collected from 29 lettuce farms along landscape gradients in rangeland and semi-natural habitat proximity. Farms were surveyed not only in summer and fall (as outlined in the original proposal) but also in winter. Across the 862 point-count surveys conducted, ~35,000 birds of 115 species were identified. In addition, 141 fecal transects were conducted, from which 1,075 fecal samples were collected for subsequent molecular analyses. Therefore, data collection efforts exceeded those outlined in the original proposal.

Species detected more often in bird surveys were also more likely to defecate in farm fields, suggesting that the species growers regularly encounter on farms are also most likely to be the ones causing fecal contamination. In particular, fecal contamination was often caused by flocking species, including crows, ravens, blackbirds, finches, and sparrows.

Bird abundances on farms and fecal contamination exhibited seasonal variation. In particular, fall represented a time of heightened fecal contamination risk. Regarding spatial risk factors, results suggest fecal contamination from birds is more common at farm edges. Because landscape analyses took longer than originally anticipated, work on landscape risk-factors is ongoing and will continue past the official project end date.

Objective 3— The third objective of this project was to assess *E. coli* survival in wild bird feces. This consisted of three sub-objectives, including (1) a field experiment comparing *E. coli* survival across two bird species and multiple substrate types, (2) a greenhouse experiment comparing *E. coli* survival across 10 bird species, and (3) a lab experiment comparing survival between non-pathogenic and pathogenic *E. coli* strains.

Unfortunately, the project experienced supply-chain issues and long delays associated with the installation of a BSL-2 growth chamber at the USDA Western Regional Research Center. The

lab experiment comparing survival of pathogenic vs. non-pathogenic *E. coli* strains in bird feces thus could not be conducted. Instead, sample sizes were increased in the other experiments.

The field experiment involved inoculating 277 fecal samples from Wild Turkeys and Western Bluebirds with a non-pathogenic strain of *E. coli* and then quantifying survival 1 to 30 days later. Results suggested that *E. coli* die-off is often rapid in bird feces; however, populations can grow under certain conditions. For example, *E. coli* survival was lower on plastic mulch and soil than on lettuce, likely because plastic mulch and soil are much hotter and drier than lettuce. Fecal size also proved critical, with *E. coli* exhibiting higher survival in larger feces.

For the greenhouse experiment, 172 fecal samples were collected from 10 bird species, inoculated with a non-pathogenic strain of *E. coli*, placed on lettuce in a greenhouse, and then collected 3 days later for survival analysis. The experiment confirmed that fecal size *per se*, rather than bird species identity, dictated *E. coli* survival. Specifically, when fecal deposit size was standardized, *E. coli* survival exhibited minimal variation across 10 bird species. However, *E. coli* survival varied by orders of magnitude across species when natural fecal deposit sizes were studied. Together, results from the field and greenhouse experiment suggest that fecal size is a key factor influencing *E. coli* survival.

Objective 4— Finally, the project's fourth objective involved integrating data on pathogen prevalences, bird surveys, and pathogen survival to holistically assess the food-safety risks associated with different farmland bird species. Disseminating information to the fresh produce industry was also a key priority; for example, a photo guide was proposed that would help growers identify birds and farming contexts associated with higher and lower food-safety risks.

First, data from fecal transect surveys, bird surveys, and pathogen survival experiments yielded novel strategies for implementing no-harvest buffers that balance crop production and food-safety risks. Specifically, field and greenhouse experiments suggested that *E. coli* die-off was very rapid in small feces deposited on soil, which, fortuitously, comprised the vast majority of feces encountered in lettuce farms. Ignoring small feces (<0.15 g) deposited on lettuce would thus reduce the area estimated to be subjected to no-harvest buffers from 10.3% to 2.7%.

Likewise, integrating data across all three objectives helped holistically categorize bird species according to their food-safety risks. Three species that more regularly carry foodborne pathogens, commonly defecate in farm fields, and produce larger feces were identified as having relatively higher food-safety risks: Common Raven, American Robin, and European Starling. On the other hand, many small, insect-eating species, including those that regularly occupy nest-boxes, were associated with the lower risks.

Finally, while the photo guide has been delayed (pending finalization of the landscape analyses in Objective 2), results dissemination to key stakeholders has remained a major priority throughout the project. Project members have shared project results via 17 webinars, conference presentations, virtual courses, academic seminars, field demonstrations, and podcasts to 800–1000 growers, industry officials, students, conservationists, academics, and bird enthusiasts. Project members are also working to integrate the bird pathogen database into an online platform in development by Western Growers.

Summary of Findings and Recommendations

Results from this project answered at least three questions of relevance to the produce industry that could be used to guide future risk assessments and farming practices for food-safety.

First, which, if any, bird species carry significant food-safety risks? Especially when combined with prior datasets [13], data from this project suggest that foodborne pathogens are very rare in

wild birds within farming landscapes. Across 5,247 and 4,647 individuals, STEC and *Salmonella* have been detected in only 0.2% and 0.4% of identified birds. While *Campylobacter* spp. were more prevalent (5.8% of 3,577 birds), it remains unclear whether the strains found in birds cause human illnesses [10,13,20,21]. Still, some bird species present higher risks than others. Holistic risk analyses singled out three species as potentially problematic: Common Raven, American Robin, and European Starling. More generally, data from this project and prior studies [13] suggest that some level of concern may be warranted if livestock-associated species, species that form large flocks, and larger species that defecate large feces are regularly seen in vulnerable produce fields [13]. On the other hand, results suggest that small, insect-eating birds carry low food-safety risks, including species that occupy artificial nest boxes in the Western United States. Erecting nest boxes to attract pest-eating birds is thus unlikely to compromise food safety. That said, several non-native species occasionally usurp nest boxes and carry relatively higher risks (*i.e.,* House Sparrows and European Starlings). Following established guidance for deterring nesting and/or removing nests from these species may thus be prudent, from both a food-safety and a conservation perspective (https://nestwatch.org/learn/all-about-birdhouses/managing-house-sparrows-and-european-starlings/).

Second, when and where might food-safety risks from birds be greatest? Though most prior studies of birds and food safety focused on spring and summer [14,47,48] (but see [11,49]), bird abundance and fecal contamination risk were highest in fall. Large flocks were also prevalent in fall, suggesting more vigilance may be needed during late-season harvests. Within farms, fecal contamination appears to be more likely at field edges. Across farms, landscape analyses are ongoing. Still, prior work suggests food-safety risks from birds may be higher near livestock-grazed areas and in crop monoculture landscapes compared to diversified farms surrounded by semi-natural habitats [12–14,47,48]. This is likely because large flocks of livestock-associated species are often found in monoculture landscapes, whereas diverse communities of species unlikely to carry pathogens are often found in more complex landscapes [12–14,47,48]. These findings align with a broader literature that has coalesced around the idea that removing non-crop vegetation near farms does not help achieve food-safety goals [1,50–52].

Third, and finally, what should growers do when they encounter bird feces in their fields? Because bird feces are so ubiquitous in farm fields, implementing no-harvest buffers around every fecal deposit in the field would be cost prohibitive. Pathogen survival experiments suggest that *E. coli* persistence in bird feces is very low, especially in small feces deposited on soil. Because pathogens are rare in wild birds, growers could thus choose to implement no-harvest buffers around large bird feces deposited on crops and ignore small feces on soil without incurring major food-safety risks. Fortuitously, small birds that defecate small feces dominate farmland bird communities and most feces are deposited on soil. Thus, ignoring small feces on soils would dramatically decrease crop losses associated with no-harvest buffers.

In summary, results from this project suggest that produce farms can be co-managed for food safety, crop production, and bird conservation. Birds are ubiquitous across nearly all farming contexts, and their presence is not necessarily a problem. Indeed, foodborne pathogens are rare in birds, and, in most cases, pathogens do not persist in bird feces for long. Therefore, instead of a deterring all birds from farm fields, exclusion efforts might instead focus on specific contexts; for example, if large flocks of large-bodied birds are visiting produce fields near a potential pathogen source (e.g., livestock operations). Doing so could help growers avoid unnecessary losses associated with no-harvest buffers, contribute to bird conservation, and even enhance pest control or other recognized benefits associated with birds on farms.

APPENDICES

Publications and Presentations

Publications
Three publications will result from this work, all of which are at various stages of preparation.

1. For objective 1, a manuscript presenting the pathogen prevalence dataset is being prepared for publication in the *Proceedings of the Vertebrate Conference*. The short abstract for the conference has been accepted and, after the conference occurs in mid-March 2024, the corresponding paper will be submitted to the 31st annual conference proceedings.
2. For objective 2, a manuscript is being prepared that will focus on spatiotemporal dynamics of bird communities and fecal contamination across fresh produce farms. While all temporal analyses are complete, landscape analyses are ongoing. As such, the manuscript will likely be submitted in the middle to end of 2024.
3. For objective 3, a manuscript is in draft focused on pathogen survival bird feces across species and substrate types. The manuscript will be submitted for publication in early 2024.

Presentations
- Virtual course entitled “Role of Birds on Farms - Designing a Farm to be Bird Friendly” organized by The Wild Farm Alliance
    - Title: *Comanaging produce farms for bird conservation, pest control, and food safety in California’s Central Coast*
    - Date: June 2022, Presenter: PI Karp, Audience: ~50 attendees from produce industry
- Poster at the Center for Produce Safety Research Symposium
    - Title: *Towards a holistic assessment of the food-safety risks imposed by wild birds*
    - Date: June 2022, Presenter: PI Karp
- Presentation at the Western Bird Banding Conference
    - Title: *Putah Creek’s role in understanding the connection between birds and food safety*
    - Date: October 2022, Presenter: Postdoc Spence, Growers: ~60 academics and students
- Presentation at the Mt. Diablo Audubon Society
    - Title: *Harmonizing biodiversity conservation and agricultural production across working landscapes*
    - Date: December 2022, Presenter: PI Karp, Attendees: ~50 bird enthusiasts
- Presentation at the California Plant and Soil Conference
    - Title: *Comanaging produce farms for bird conservation, pest control, and food safety*
    - Date: February 2023, Presenter: PI Karp, Audience: ~150 attendees from academia, government, and the produce industry
- Webinar for the International Association for Food Protection
    - Title: *Towards a holistic assessment of the food-safety risks imposed by wild birds*
    - Date: March 2023, Presenter: PI Karp, Audience: ~150 attendees from the produce industry
- Departmental seminar at University of California, Santa Cruz
    - Title: *Harmonizing biodiversity conservation and agricultural production across working landscapes*
    - Date: April 2023, Presenter: PI Karp, Attendees: ~50 academic scientists
- Presentation at the Fresno Audubon Society
    - Title: *Harmonizing biodiversity conservation and agricultural production across working landscapes*
    - Date: April 2023, Presenter: PI Karp, Attendees: ~25 bird enthusiasts

- Field day at Paicines Ranch organized by the Wild Farm Alliance
    - Mist netting demonstration showing field methods for bird capture
    - Date: June 2023, Presenter: Postdoc Spence, Audience: 30 growers, academics, and students
- Poster and 2-min Presentation at the Center for Produce Safety Research Symposium
    - Title: *Towards a holistic assessment of the food-safety risks imposed by wild birds*
    - Date: June 2023, Presenter: PI Karp
- Webinar for Food Safety and Quality Community of Practice Monthly Meeting
    - Title: *Towards a holistic assessment of the food-safety risks imposed by wild birds*
    - Date: August 2023, Presenter: PI Karp, Audience: ~15 attendees from the produce industry
- Presentation at the Ecology Society of America Conference
    - Title: *Towards a holistic assessment of the food-safety risks imposed by wild birds*
    - Date: August 2023, Presenter: Postdoc Spence, Audience: ~50 ecologists
- Field day at Braga Fresh organized by the Wild Farm Alliance
    - Title: *Comanaging produce farms for bird conservation, pest control, and food safety*
    - Date: November 2023, Presenter: PI Karp, Audience: ~150 attendees from the produce industry
- Departmental seminar at Oregon State University
    - Title: *Harmonizing biodiversity conservation and agricultural production across working landscapes*
    - Date: October 2023, Presenter: PI Karp, Attendees: ~100 academic scientists
- Podcast hosted by Dr. Ed Zaworski, Iowa State University
    - Title: *I see dead plants* podcast (discussed birds and food-safety risks)
    - Date: January 2024, Presenter: PI Karp
- Departmental seminar at University of California, Davis
    - Title: *Pathogen survival in avian fecal samples on farms is directly related to size, not species*
    - Date: January 2024, Presenter: Postdoc Spence, Audience: 25 students and academics
- Co-management symposium at EcoFarm Conference
    - Title: *Comanaging produce farms for bird conservation, pest control, and food safety in California's Central Coast*
    - Date: January 2024, Presenter: PI Karp, Audience: 50 growers, academics, and industry representatives.

Budget Summary

After several re-budget requests were granted by the Center for Produce Safety, the final spending allotment for this grant included: $170,535 in salaries, $28,935 in benefits, $20,452 in travel expenses, $12,726 in supplies, $115,110 for a subaward to co-PI McGarvey at the USDA-Agricultural Research Service, $6,924 in other direct costs, and $15,439 in indirect costs. The grand total of $370,123 was adequate to cover all project related expenses. That said, the project benefited from funds contributed by the USDA Agricultural Research Service, allowing the project to be expanded beyond what was originally proposed (e.g., adding an additional field season in Winter 2023). Below, spending in each cost category is briefly discussed.

Personnel (salary and benefits): This grant supported one postdoctoral scholar (postdoc Spence) throughout the duration of the grant. Spence was responsible for organizing and executing all project objectives. Spence hired undergraduate researchers (*i.e.,* Frank Fabbro,

Meirun Zhang, and Wentao Yang) as needed and on an hourly basis throughout the project to aid in fecal sample collection, other field work, lab preparation, and data entry. One graduate student researcher (Katie Lauck) was hired for one quarter to collect fecal samples from nest boxes in service of Objectives 1 and 3. Two field technicians were also hired to conduct bird censuses and fecal transects in the California Central Coast. Specifically, Rose Albert was hired to conduct surveys in Summer 2022 and Max Leibowitz was hired for two field seasons in Fall 2022 and Winter 2023. Importantly, funds for the Winter 2023 field season came from the USDA Agricultural Research Service. Similarly, funds from the USDA Agricultural Research Service were used to hire another laboratory technician (Kenzie Pollard) in Spring 2023 to extract DNA from bird fecal samples and use PCR to identify the originating bird species.

Travel: Mileage reimbursement was requested by Postdoc Spence throughout the project period to transport fecal samples to the USDA Agricultural Research Service in Albany, California for laboratory analyses. Spence and Karp also traveled to the California Central Coast regions multiple times throughout the project period to select study sites, train technicians Albert and Leibowitz in field methods, meet with industry partners, and disseminate project results to the produce industry. Funds were also used to rent a car from UC Davis fleet services to conduct fieldwork on produce farms in Summer 2022 and Fall 2022 (funds for the Winter 2023 field season were contributed by the USDA Agricultural Research Service). Finally, funds were used for Karp to travel to the Center for Produce Safety Research Symposium in June 2022 and June 2023. Spence also traveled to the Symposium in June 2023. Unspent funds currently remain to facilitate travel to the 2024 CPS Research Symposium.

Supplies: Field and laboratory supplies were acquired in service of diverse research goals. Field supplies included (1) mist-nets and potter traps for bird capture, (2) electronic balance and batteries for weighing birds, (3) GPS devices for logging locations, (4) vials, labels, sample boxes, tweezers, and sharpies for collecting fecal samples, (5) banding pliers, calipers, and rulers for measuring birds, (6) blowtorch for sterilization, (7) temperature and humidity loggers for pathogen survival experiments, (8) a rangefinder and binoculars for bird surveys, (9) paper and pens for data collection, (10) a cooler for sample transport, (11) speakers for attracting birds to nets, and other field-related expenses. Laboratory expenses included (1) DNA extraction kits, (2) reagents including various buffers, master mixes, primers, Taq polymerase, EDTA, nalidixic acid, rifampicin, cycloheximide, phosphate buffered saline, and others, (3) media including MacConkey agar and others, (4) consumables including 96 well plates, pipette tips, gloves, vials, pipetting reservoirs, tubes, and other lab-related expenses.

Subaward: A subaward to co-PI McGarvey's lab was used to fund a laboratory technician (SangIn Lee) throughout the project to assay bird feces for foodborne pathogens, identify species which defecated on farms via molecular analyses, and quantify pathogen survival in feces after field and laboratory experiments. The subaward was also used to procure laboratory supplies, many of which are detailed above. Finally, unspent funds remain to facilitate travel to the 2024 CPS Research Symposium.

Other direct costs: Other costs incurred during the project period included: (1) DNA sequencing costs to identify birds that defecate on produce farms, (2) tuition and fees associated with graduate student researcher Lauck, (3) registration fees for the CPS Research Symposium, and (4) costs to reimburse the UC Davis Student Farm for labor and materials associated with preparing the lettuce field trial experiment in Spring 2022.

Indirect costs: Finally, indirect costs were charged at a rate of 8% of the personnel costs.

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Figures 1–10 and Table 1 (see below)

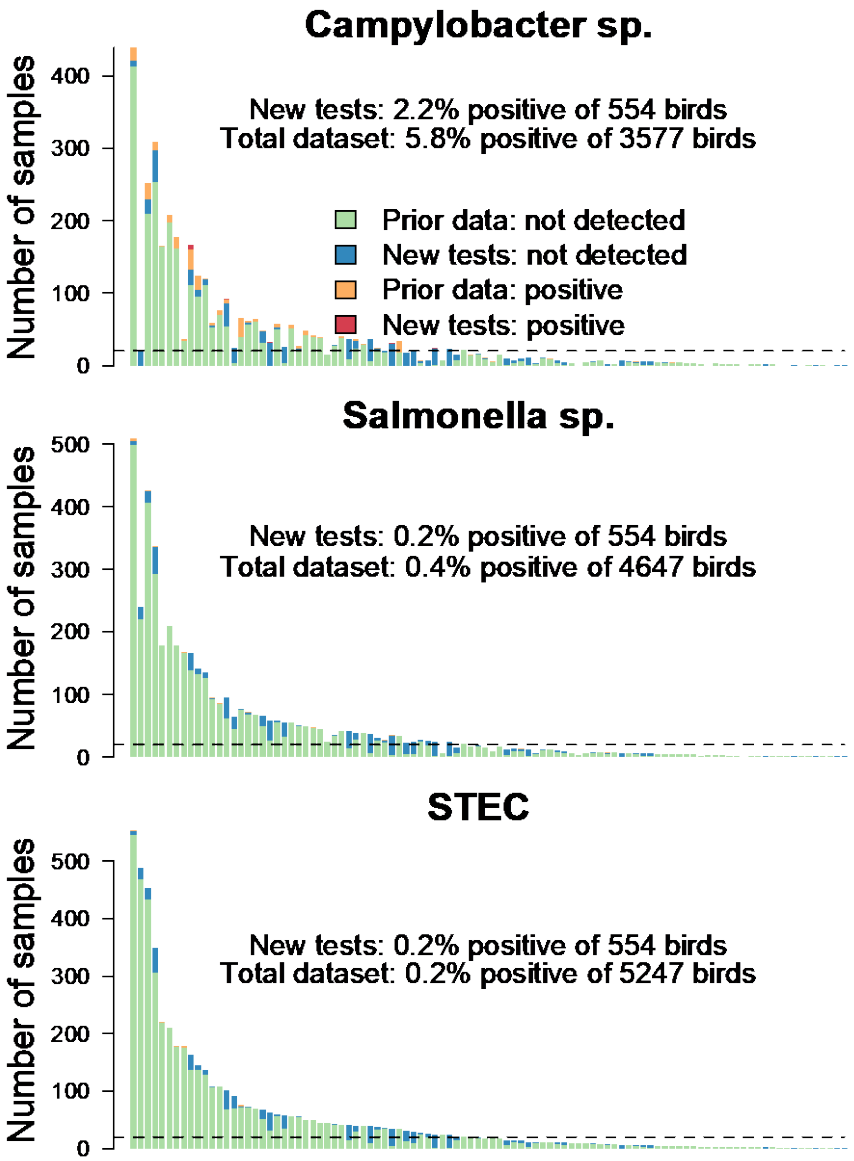

Figure 1: Prevalence of Campylobacter spp. (top), Salmonella (middle), and STEC (bottom) in wild birds. Each bar corresponds to a unique bird species, showing the number of individuals that tested positive (red) and negative (blue) in the 554 samples from identified birds analyzed here. Bars also depict the numbers testing positive (yellow) and negative (green) in the avian pathogen prevalence dataset from [13]. A horizontal dashed line is included to show those species for which more than 20 individuals have now been tested for each pathogen.

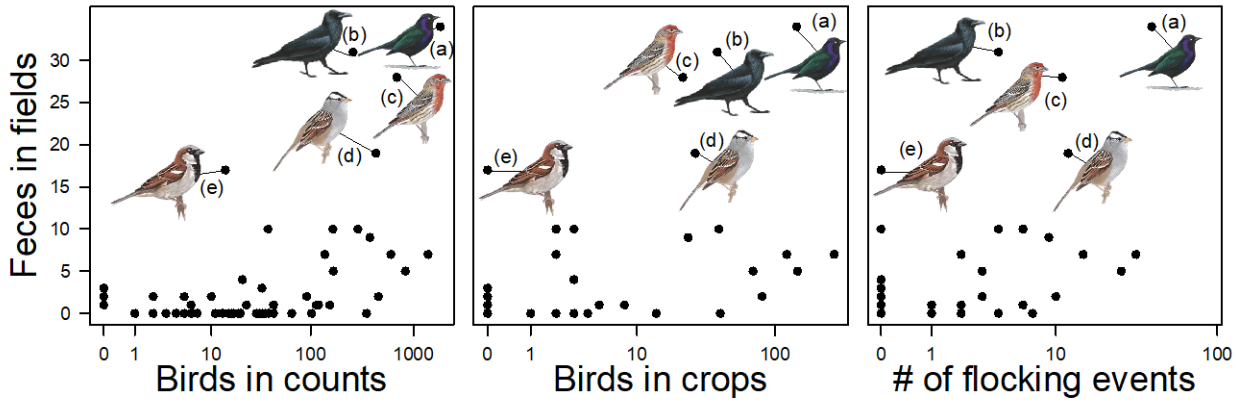

Figure 2: Correlation between bird abundance in fields, bird interactions with crops, number of observed flocking events, and fecal contamination. Black dots depict bird species. The top 5 species that produced the highest number of feces are depicted: (a) Brewer's Blackbird, (b) Common Raven, (c) House Finch, (d) White-crowned Sparrow, and (e) House Sparrow. In general, species that were more often observed in plots (left panel), species that were observed interacting with crops more often (middle panel), and species that were observed more often in flocks of 10 or more individuals (right panel) also tended to defecate more often in fields. However, there were exceptions; for example, House Sparrows produced a lot of feces in fields but occurred rarely in counts and were never observed interacting with crops or in flocks.

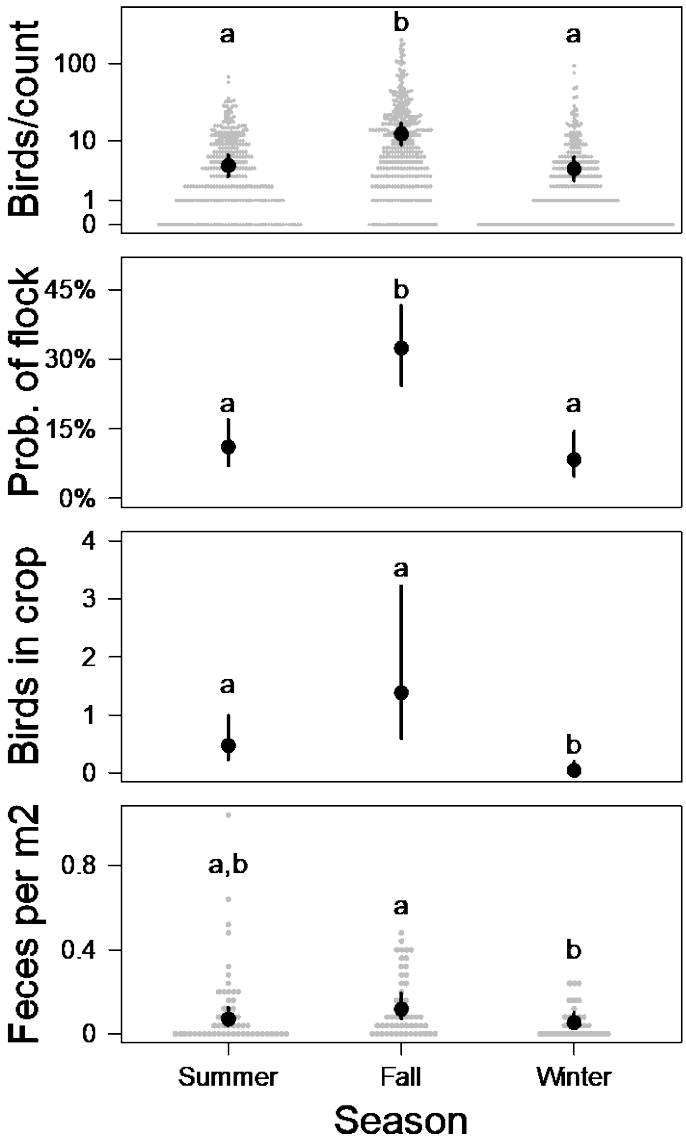

Figure 3: Seasonal dynamics in potential food-safety risks from birds. The number of birds observed per count (top panel) as well as the likelihood of observing a bird flock (2nd panel) were significantly higher in fall than summer or winter. Very few birds were observed actually contacting produce in winter (3rd panel) and, as a result, fecal densities in leafy-greens fields were significantly lower in winter than fall (bottom panel). Black lines are model predictions; lines are 95% confidence intervals from GLMMs. Gray dots indicate observations of bird numbers (top panel) and fecal densities (bottom panel). No observations are depicted for binomial data (*i.e.*, whether flocks were present or not) as well as for the numbers of birds contacting produce (due to the very low number of observations detected).

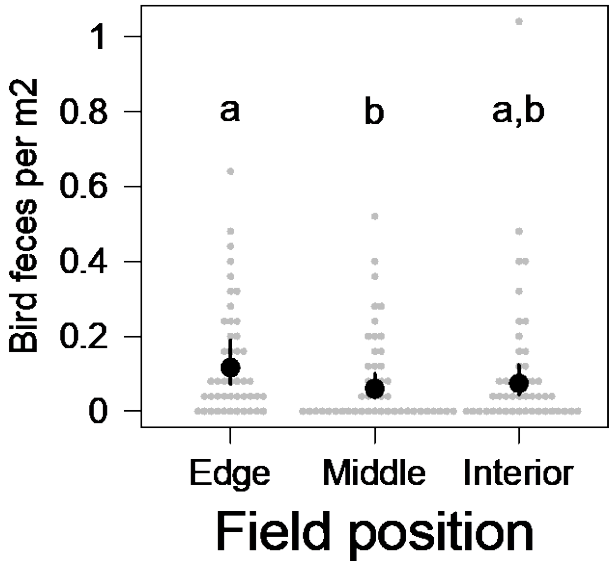

Figure 4: Spatial variation in fecal densities within farm fields. Models suggest fecal contamination in fields peaks at field edges. Black points and lines indicate model predictions and confidence intervals, respectively. Gray points indicate observations from fecal transects. Letters denote significance.

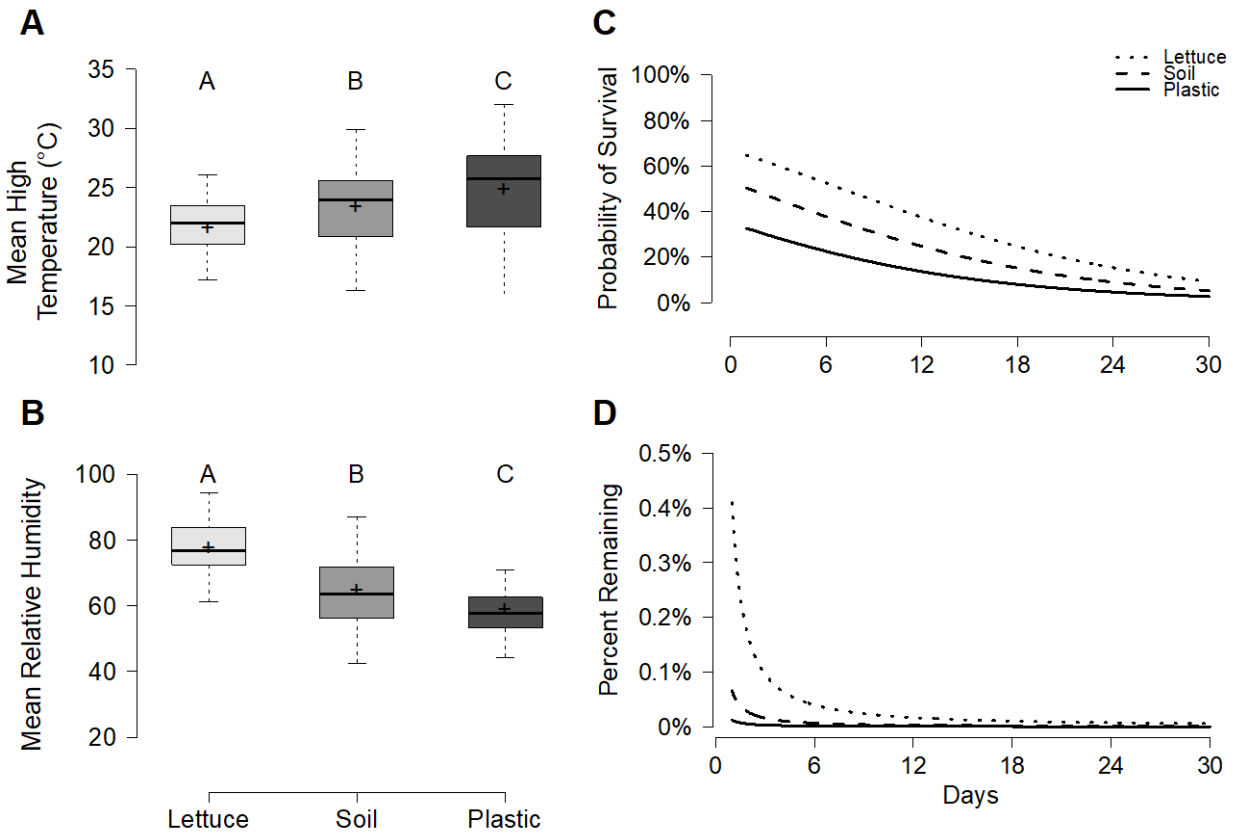

Figure 5: Variation in microclimate and E. coli *survival and persistence across farm substrates.* Temperature increases (panel A) and humidity decreases (panel B) from plastic mulch to organic soils to lettuce. In boxplots, bars represent the median, "+" represent the mean, error bars denote 95% confidence intervals, dots represent outliers, and letters denote significant differences. The likelihood of detecting any *E. coli* (*i.e.,* the probability of survival) declines over time and is lowest when feces are deposited on plastic, intermediate on soils, and highest on lettuce (panel C). Examining the percent of *E. coli* remaining resulted in the same trends as for the probability of survival (panel D). Lines depict model predictions for a 0.15-gram fecal sample on lettuce (dotted lines), soils (dashed lines), and plastic (solid lines).

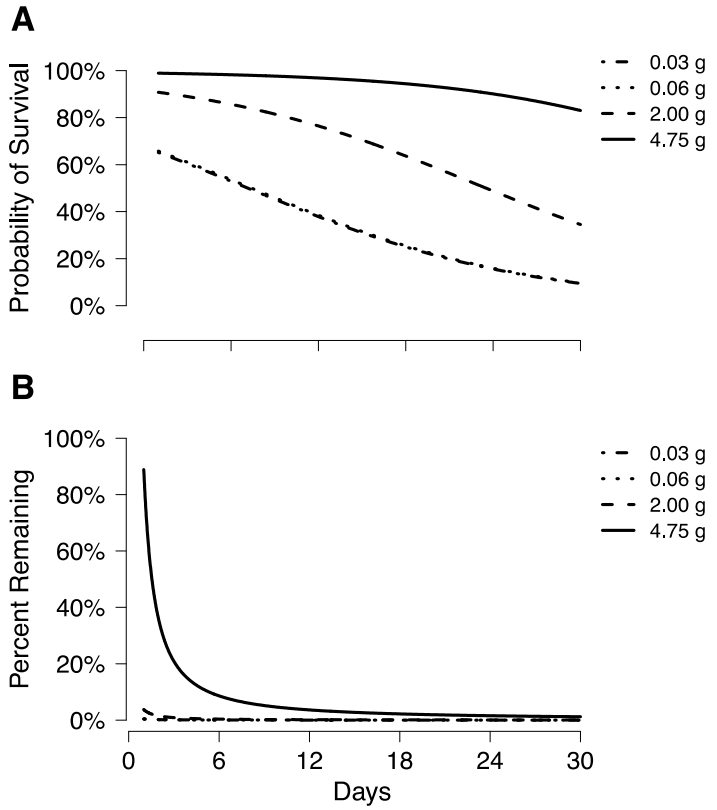

Figure 6: E. coli *survival and decay on lettuce as a function of fecal deposit size. E. coli* survival (*i.e.,* probability of detecting any *E. coli*; panel A) and *E. coli* decay (*i.e.,* percent of *E. coli* remaining; panel B) declined over time and exhibited significant differences according to fecal mass. Specifically, models predicted *E. coli* was significantly more likely to survive and persist in 4.75 g than 2.00 g fecal samples, which, in turn, were significantly better at hosting *E. coli* than 0.03 g and 0.06 g fecal samples (which did not differ from each other). Lines depict model predictions of survival or decay on lettuce for fecal samples of 4.75 g (solid lines; adult Wild Turkey Size), 2.00 g (dashed lines; juvenile Wild Turkey Size), 0.06 g (dotted lines; Western Bluebird size), and 0.03 g (dot/dashed lines; small songbird size, e.g., Yellow-rumped Warbler).

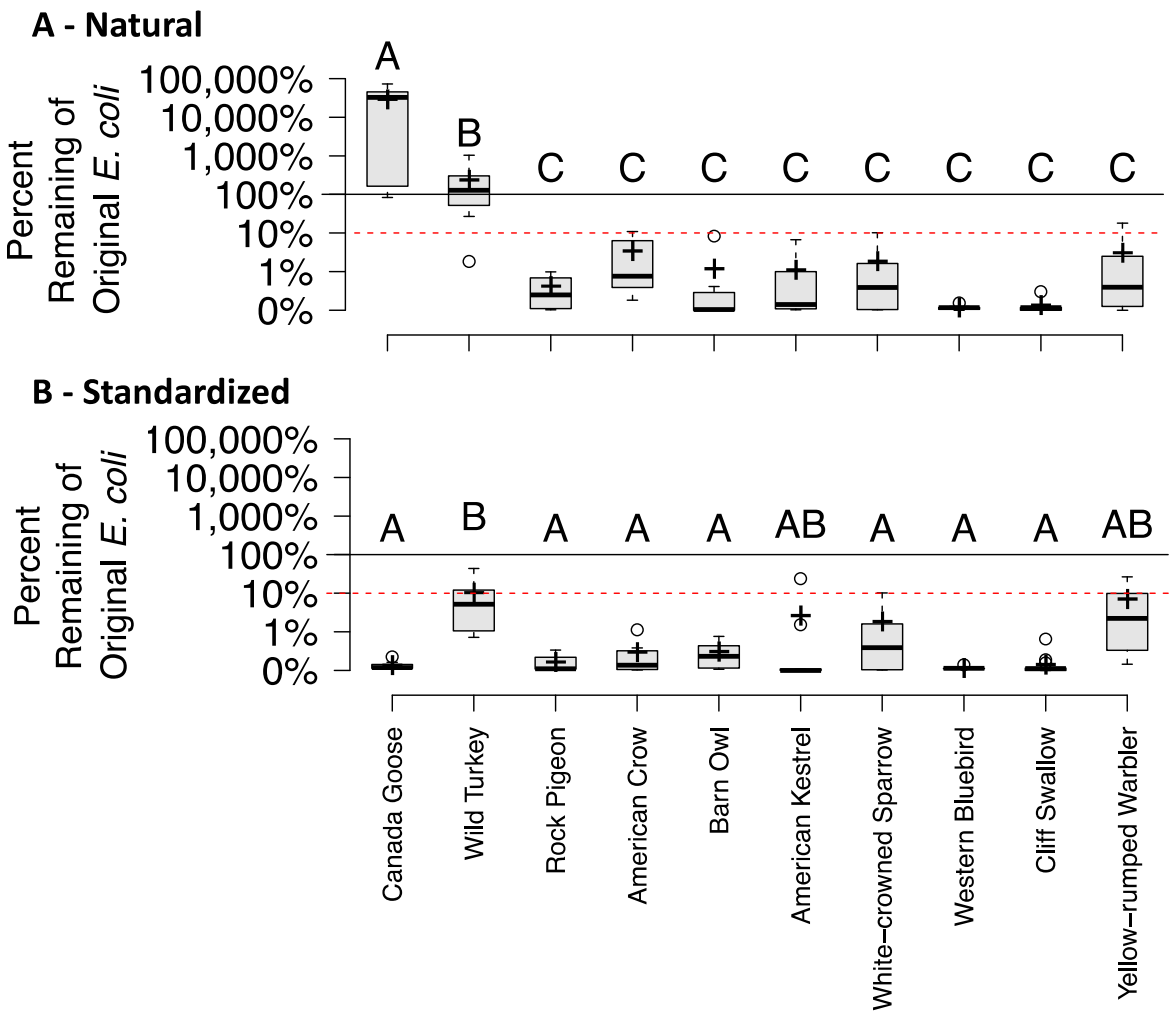

Figure 7: E. coli *decay in feces from different species, across natural and standardized sizes.* Panels depict the percent of original *E. coli* remaining after 3 days in bird feces placed on lettuce in a greenhouse, both for natural-sized feces (0.03 g – 9.8 g; panel A) and fecal masses standardized to 0.08 g (*i.e.,* the size of a large songbird; panel B). Natural fecal deposit sizes, arranged by size, were: 9.80 g (Canada Goose), 4.75 g (Wild Turkey), 0.62 g (Rock Pigeon), 0.43 g (American Crow), 0.39 g (Barn Owl), 0.28 g (American Kestrel), 0.08 g (White-crowned Sparrow), 0.06 g (Western Bluebird), 0.05 g (Cliff Swallow), and 0.03 g (Yellow-rumped Warbler). Models suggest that, compared to species identity, fecal deposit size accounts for much more variation in the fraction of *E. coli* remaining. Data below the black horizontal line represents *E. coli* decline, whereas data above represents growth. The red dotted line represents 10% of original *E. coli* remaining. Within boxplots, bars represent the median, "+" represent the mean, error bars denote 95% confidence intervals, and dots represent outliers. Lettuce denote significance.

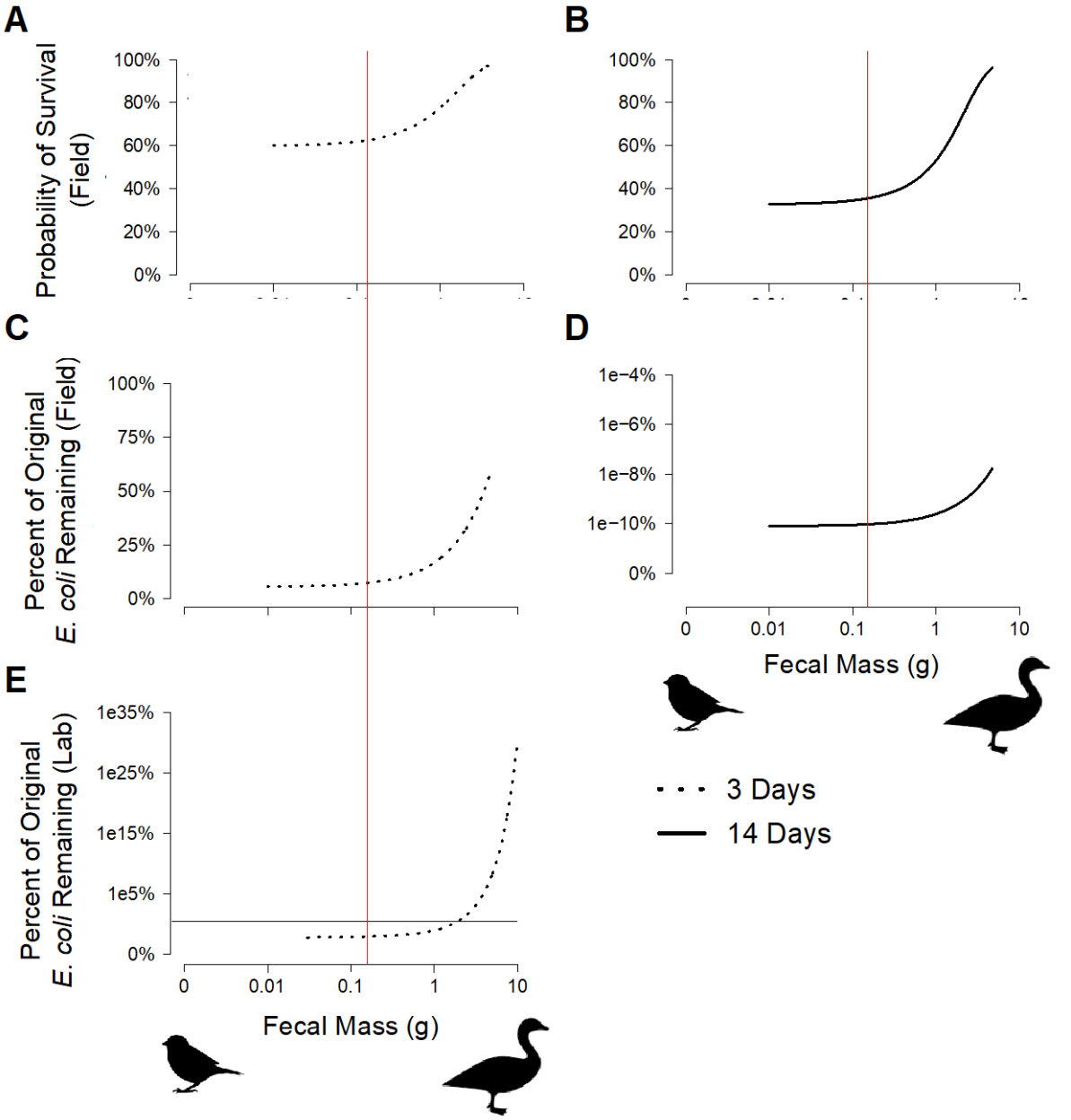

Figure 8: E. coli *survival and persistence as a function of bird fecal mass. E. coli* survival on lettuce showed a strong positive relationship with fecal mass in field settings over 3 days (panel A) and 14 days (panel B). Similarly, the percent of original *E. coli* remaining showed a strong positive relationship with fecal mass on lettuce after 3 days in the field (panel C), 14 days in the field (panel D), and 3 days in the greenhouse (panel E). Predicted values below the black horizontal line represent *E. coli* decline (above represents growth). The model predicts *E. coli* decline for fecal samples less than or equal to 1.61 g in the greenhouse. Vertical red lines depict the 0.15 g fecal size threshold, which would correspond to 63.1% and 35.2% probabilities of detecting *E. coli* after 3 and 14 days on lettuce in the field; 9.3×10^{-3} % and 9.4×10^{-9} % fractions of *E. coli* remaining after 3 and 14 days on lettuce in the field; and 1.0% fraction of *E. coli* remaining after 3 days on lettuce in the greenhouse.

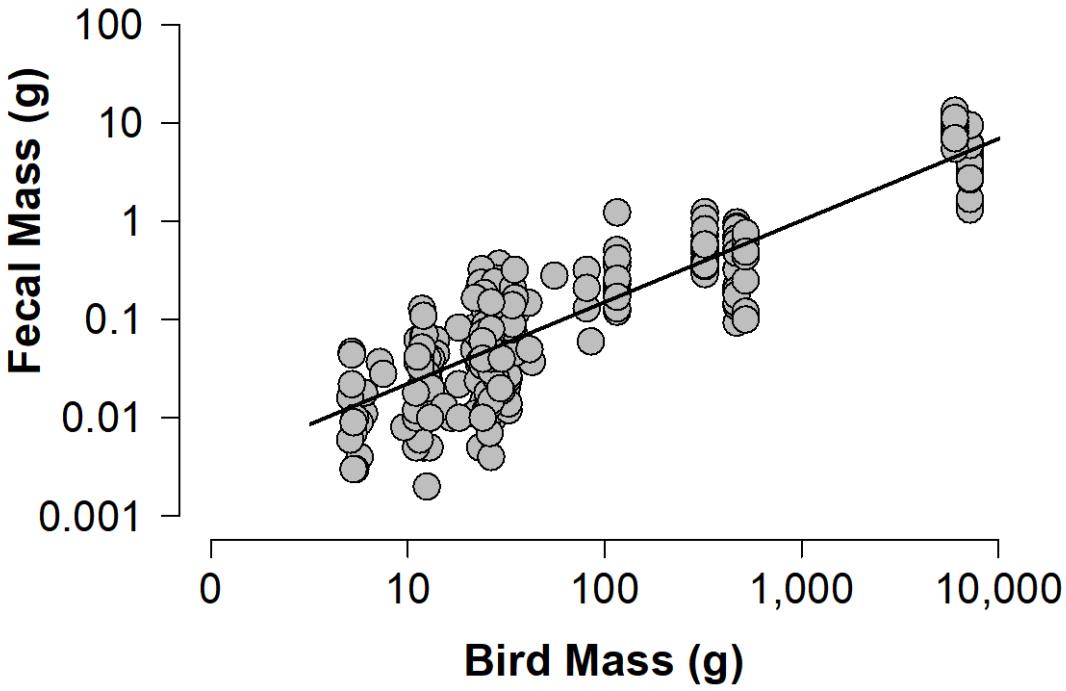

Figure 9: The relationship between avian body mass and fecal mass. Avian body mass was strongly predictive of avian fecal mass across 33 species ($R^2 = 0.82$; $p < 0.001$). Points depict individual birds; line depicts model predictions.

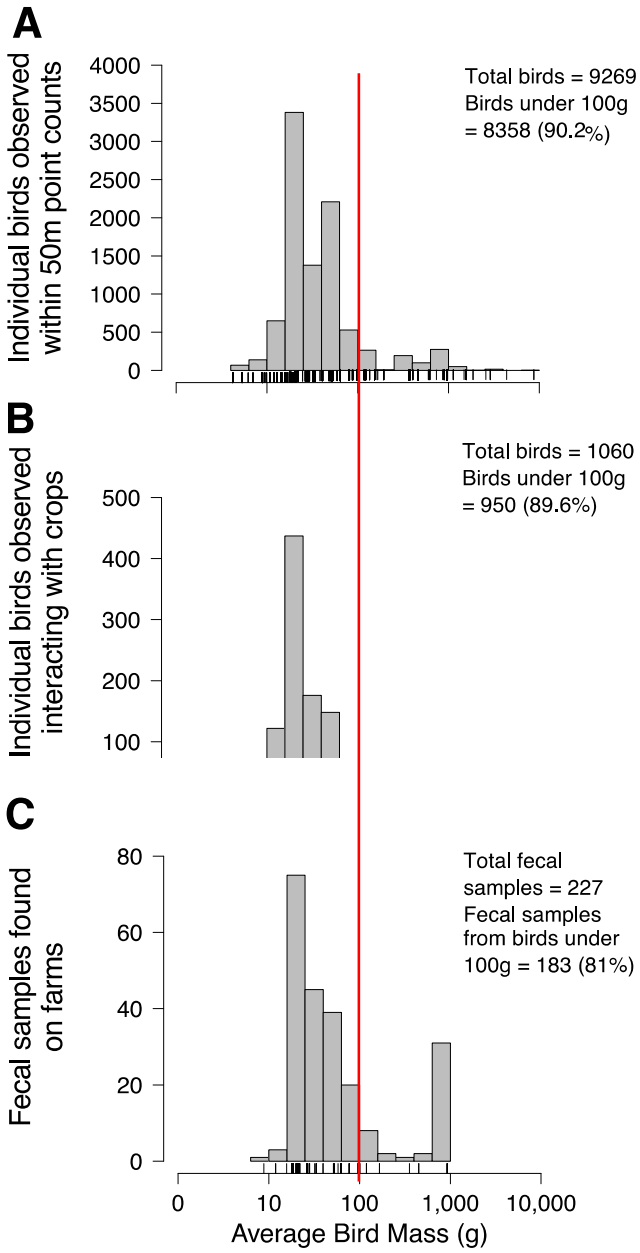

Figure 10: Variation in body masses of species observed in point counts and species identified through molecular analyses of field-collected feces. Histograms depict the number of individuals detected, arranged by bird body mass, for species observed within 50m point counts on leafy-green farms (panel A), species observed interacting with crops during point counts (panel B), and species identified from sequencing fecal samples found on farms (panel C). The red line indicates birds of 100 g, which would be predicted to defecate a 0.15g fecal sample.

Table 1: Holistic food-safety risk scores by species. Risk scores range from 0 to 6 (see Results above) and vary according to prevalences of *Campylobacter* spp., *Salmonella,* and STEC (% detected, with sample sizes in parentheses), numbers of feces in fields attributed to each species, and predicted fecal masses (in grams).

Common Name	Scientific Name	Campylobacter	Salmonella	STEC	Feces in Fields	Fecal Mass	Risk Score
common raven	Corvus corax	3.2% (31)	2.9% (35)	2.9% (35)	31	0.95g	6
american robin	Turdus migratorius	9% (178)	0% (178)	0.6% (178)	68	0.12g	5
european starling	Sturnus vulgaris	15.3% (124)	0% (142)	0% (146)	28	0.12g	5
brewer's blackbird	Euphagus cyanocephalus	7.5% (93)	0% (96)	0% (102)	65	0.1g	4
california quail	Callipepla californica	17.9% (28)	0% (51)	0% (56)	4	0.23g	4
house sparrow	Passer domesticus	20.4% (167)	0% (167)	0% (164)	86	0.05g	4
savannah sparrow	Passerculus sandwichensis	1.7% (121)	0.7% (136)	0% (136)	19	0.04g	4
song sparrow	Melospiza melodia	4.3% (440)	0.6% (509)	0.2% (554)	74	0.04g	4
american crow	Corvus brachyrhynchos	4% (25)	0% (25)	0% (25)	7	0.52g	3
barn swallow	Hirundo rustica	8.1% (37)	0% (39)	0% (40)	26	0.04g	3
california scrub-jay	Aphelocoma californica	0% (21)	7.1% (28)	0% (37)	0	0.13g	3
house finch	Haemorhous mexicanus	4.2% (310)	0.3% (337)	0% (350)	42	0.04g	3
red-winged blackbird	Agelaius phoeniceus	5.6% (36)	1.2% (168)	1.7% (178)	10	0.09g	3
white-crowned sparrow	Zonotrichia leucophrys	9.1% (252)	0.5% (427)	0.2% (454)	109	0.05g	3
black phoebe	Sayornis nigricans	4.1% (49)	0% (66)	0% (68)	11	0.04g	2
black-headed grosbeak	Pheucticus melanocephalus	14.3% (49)	0% (50)	0% (50)	1	0.08g	2
brown-headed cowbird	Molothrus ater	39.4% (66)	0% (77)	3.9% (76)	0	0.07g	2
bushtit	Psaltriparus minimus	4.8% (42)	2.1% (48)	0% (50)	0	0.01g	2
california towhee	Melozone crissalis	6.8% (59)	1.1% (95)	0% (109)	2	0.09g	2
canada goose	Branta canadensis	0% (20)	0% (240)	0% (489)	0	2.39g	2
cedar waxwing	Bombycilla cedrorum	47.1% (34)	0% (34)	0% (34)	1	0.06g	2
dark-eyed junco	Junco hyemalis	1.2% (166)	0% (178)	0.5% (220)	6	0.04g	2
lesser goldfinch	Spinus psaltria	4.8% (62)	1.4% (72)	0% (74)	3	0.02g	2
spotted towhee	Pipilo maculatus	7.9% (76)	1.2% (86)	0% (108)	2	0.07g	2
wild turkey	Meleagris gallopavo	4.2% (24)	0% (24)	0% (24)	0	4.35g	2
american goldfinch	Spinus tristis	4.8% (208)	0% (210)	0% (210)	9	0.03g	1
black-capped chickadee	Poecile atricapillus	7.1% (56)	0% (56)	0% (56)	0	0.02g	1
chipping sparrow	Spizella passerina	7.3% (41)	0% (42)	0% (42)	3	0.03g	1
cliff swallow	Petrochelidon pyrrhonota	0% (26)	0% (56)	0% (58)	10	0.04g	1
northern mockingbird	Mimus polyglottos	5% (40)	0% (45)	0% (45)	3	0.08g	1
oak titmouse	Baeolophus inornatus	6.2% (16)	0% (25)	0% (44)	0	0.03g	1
purple finch	Haemorhous purpureus	6.7% (15)	0% (17)	0% (22)	0	0.04g	1
swainson's thrush	Catharus ustulatus	8.6% (58)	0% (58)	0% (59)	1	0.06g	1
willow flycatcher	Empidonax traillii	6.2% (16)	0% (17)	0% (17)	0	0.03g	1
bewick's wren	Thryomanes bewickii	4% (25)	0% (31)	0% (38)	1	0.02g	0
bullock's oriole	Icterus bullockii	3.3% (30)	0% (39)	0% (40)	3	0.07g	0
common yellowthroat	Geothlypis trichas	4.7% (64)	0% (68)	0% (70)	0	0.02g	0
golden-crowned sparrow	Zonotrichia atricapilla	0% (25)	0% (65)	0% (91)	1	0.06g	0
hermit thrush	Catharus guttatus	0% (18)	0% (23)	0% (31)	0	0.06g	0
house wren	Troglodytes aedon	0% (15)	0% (15)	0% (22)	2	0.02g	0
lazuli bunting	Passerina amoena	0% (22)	0% (22)	0% (22)	1	0.03g	0
pacific-slope flycatcher	Empidonax difficilis	0% (29)	0% (35)	0% (43)	1	0.02g	0
ruby-crowned kinglet	Regulus calendula	0% (20)	0% (25)	0% (29)	0	0.01g	0
tree swallow	Tachycineta bicolor	0% (36)	0% (37)	0% (40)	1	0.04g	0
western bluebird	Sialia mexicana	0% (36)	0% (41)	0% (41)	0	0.05g	0
wilson's warbler	Cardellina pusilla	0% (17)	0% (19)	0% (19)	0	0.02g	0
yellow-rumped warbler	Setophaga coronata	3.1% (32)	0% (59)	0% (64)	4	0.03g	0