

In less than a human lifetime, North America has lost more than a quarter of its birds, equating to 2.9 billion breeding birds¹¹. Among the species that showed some of the steepest declines were migratory shorebirds, one of which is the Lesser Yellowlegs (LEYE, *Tringa flavipes*). LEYE, a once common bird that breeds in the boreal forest, has declined by 80% range-wide since 1966^{1,3} and is estimated to lose an additional 50% of its global population within 11 years⁹. As a result, LEYE has been designated as federally threatened in Canada and a species of high conservation concern in the U.S.⁹ This species likely encounters multiple threats during its 8000-mile migration journey³, but agricultural practices in one of their most critical stopover regions, the Prairie Pothole Region (PPR) have the potential to impact much of the breeding population³. Reductions in survival due to exposure to agricultural insecticides in the PPR is one novel hypothesis that has been proposed to explain why many shorebirds in North America have declined, including LEYE. However, **this hypothesis has not been thoroughly explored**. Investigating population-level threats to rapidly declining species like LEYE is a critical conservation priority.

The migratory period may be the most critical to annual survival due to the high energetic demands that if not fulfilled can lead to reduced survival and reproductive success¹⁰. However, to ensure a timely arrival at breeding and wintering sites, migratory birds must balance their time spent refueling at stopover sites with their migration speed¹⁰. The **optimal bird migration theory** predicts that migrants constrained by time should adjust their stopover duration to their refueling rate, and thus minimize time spent on migration to maximize their fitness². Research has shown that individuals with low refueling rates depart later from their stopover sites relative to individuals with higher refueling rates, indicating that birds wait until they reach a threshold of fuel stores before departing¹⁰. This suggests that the quality of stopover habitat affects the decision of when to leave a stopover site, which is of critical importance for migration success.

During migration, shorebirds are exposed to neonicotinoids⁶, the most widely used class of insecticides in the world, which pose significant risks to birds and other wildlife^{2,4}. Neonicotinoids cause impaired immune function, rapid reduction in food consumption, and lower reproductive success, which can result in greater energetic demand, reduced fat stores, delayed migration and low survival^{1,4}. Because migration delays can carry over to affect survival and reproduction¹, **neonicotinoids have the potential to impose population-level impacts**. Although their adverse impacts have been established in songbirds^{1,4}, we have little information regarding their effect on shorebirds, highlighting a **critical information gap**. In a recent study, GPS transmitters were deployed on over 100 LEYE in Alaska and Canada³. Of the birds that bred west of James Bay, Ontario, **90% stopped in the PPR to refuel** during their migration to South America, with stopover duration times varying from a few days to over a month, indicating the **importance of this region during migration**. High presence of neonicotinoids has been reported in these prairie wetlands and agricultural fields⁵, which are important foraging habitats for migrating shorebirds.

Proposed Research: Using the optimal bird migration theory as a framework, I will investigate the threat of neonicotinoids on the fitness and migration of fourteen shorebird species of high conservation concern⁹ that heavily rely on the PPR. This study will investigate a potential contributor to the observed population declines of shorebirds and will help guide on-the-ground management decisions for agricultural solutions.

Hypothesis: Migrating shorebirds with high plasma concentrations of neonicotinoids will be physiologically and behaviorally impaired relative to birds with low concentrations. Similar to what has been observed in studies of captive birds¹, I predict that wild shorebirds with high neonicotinoid concentrations will exhibit: A) lower plasma triglyceride and higher uric acid levels, indicating lower fueling rates and fat deposition, B) poorer body condition (measured by body mass and fat scores), C) reduced foraging behavior, D) prolonged migration stopovers, and E) later migration departure dates.

Research Plan: To establish an environmental gradient in pesticide contamination, I will pre-screen wetlands by measuring neonicotinoid concentrations in water samples. At sites with low and high concentrations of this pesticide, I will capture LEYE and thirteen other shorebird species, collect blood samples, and measure body mass and fat over two fall and two spring migration seasons. I will measure the concentrations of neonicotinoids in bird plasma as well as key metabolites in blood using **cutting-edge LC-MS/MS techniques**⁸. *Prediction A:* I will measure plasma concentrations of triglycerides and uric acid and correlate them to plasma neonicotinoid concentrations⁷, thereby testing for a link between pesticides and fuel deposition rates. *Prediction B:* I will compare body mass and fat scores of birds with

high, moderate, and low neonicotinoid concentrations to better understand how neonicotinoids affect body condition. *Prediction C*: I will conduct behavioral surveys on shorebirds at high and low contamination wetlands to determine if there is a relationship between neonicotinoid exposure and foraging behavior. After randomly selecting an individual, I will record the length of time spent in different behavior categories (foraging, resting, etc.) for a duration of 5 minutes. This will be repeated for 10 individuals per wetland. To account for time and weather, I will conduct surveys in the morning and will record temperature, wind, and cloud cover. *Predictions D & E*: To understand if neonicotinoids are impairing migratory ability and causing migration delays, I will deploy Lotek PinPoint GPS transmitters that will allow me to track the migration, departure dates, and stopover durations of birds with varying levels of neonicotinoid exposure. The results of this study will provide critical information on how environmental contaminants interfere with optimal migration. To **minimize confounding factors**, I will only capture adults, and will stratify results by sex, species, and migration season.

Facilities & Mentorship: I have two mentors: Dr. Christy Morrissey at the University of Saskatchewan and Dr. Courtney Conway at the University of Idaho (UI) where I will matriculate. Dr. Morrissey is a global leader in avian ecotoxicology and has developed novel and extremely sensitive methods for neonicotinoid analysis⁸. Dr. Conway is a renowned expert in ecology and migration of birds.

Intellectual Merit: Regional efforts to study neonicotinoids in songbirds¹ and wetlands⁶ in the PPR are ongoing and **our project expands on this** by investigating the effects of neonicotinoids on shorebird health, **a novel yet timely research topic**. This project would **build upon an existing and growing partnership** among 8 state agencies, federal agencies, South American agencies, and universities in both the U.S. and Canada, as well as farmers and landowners in both countries. My findings will advance the fields of migration ecology and ecotoxicology and will be highly applicable to developing conservation strategies for shorebirds in the PPR because it will improve our understanding of the effects of agricultural insecticides. This project aligns with the 3-Billion Birds Campaign¹¹ to reverse population declines and is **part of an international effort to understand threats** impacting LEYE throughout their annual cycle. This study fills a **critical information gap** by investigating a major threat during migration that may have carry over effects to survival and reproduction and will inform managers and farming communities about the effects of agricultural insecticides on birds.

Broader Impacts: To **increase participation of underrepresented minorities in STEM**, I will develop an internship opportunity through the Doris Duke Conservation Scholars Program at UI that will engage students from diverse backgrounds to participate in my research and develop their own independent projects. To **improve STEM education and outreach**, I will **create a program called Backyard Bird Banding for underrepresented students** from rural schools and tribal communities to watch how we capture and band shorebirds and participate when deemed appropriate. I will enhance the experience with engaging kid-friendly games and shorebird ID cards for teachers and students to use while out in the field. This event will be recorded and made **publicly available world-wide** on social media. I plan to develop this program with the following rural ND schools: Glenburn, Kenmare, and Turtle Mountain Community High School. In addition to hands-on field activities, I will use real-time shorebird migration data to link schools through social media platforms in ND and Alaska, and through the Outreach International Environmental program in South America. Students will be able to track the migrations of birds tagged in or passing through their neighborhoods via Movebank, an animal tracking database. Our outreach goal is to engage with at least 200 students in our programs. To **increase public engagement**, I will **develop high-impact outreach and educational materials** about shorebird friendly agricultural practices and alternative biological pesticides in the PPR. I will work closely with the Lesser Yellowlegs working group, the Coalition for Conservation & Environmental Education, farmers, and landowners in the PPR to find practical, long-term solutions that will benefit bird populations and farming communities.

¹Eng et al. 2019. *Sci.* 365:1177; ²Alerstam et al. 1990. *Bird Mig.* 331-351 ; ³McDuffie et al. 2021. *Pro.* 1-134; ⁴Gibbons et al. 2015. *Env. Sci. Poll. Res.* 22:103; ⁵Main et al. 2014. *PLOS* 9:1; ⁶Malaj et al. 2020. *Sci. Tot. Env.* 1-10. ⁷Li et al. 2020. *Nat. Sus.* ⁸Bianchini et al. 2018. *Env. Sci. & Tech.* 52:13562; ⁹U.S. Shore. Cons. Plan. 2016. ¹⁰Zhao et al. 2017. *Move. Eco.* 5-23; ¹¹Rosenberg et al. 2019. *Sci.* 120-124.