

THE SAP PROJECT POLLEN SAMPLING PROTOCOL - 2023 SEASON



The Songbirds as Pollinators (SaP) project welcomes pollen samples from any songbird species, with an emphasis on those listed below. When a sterile swab is swirled over the head of a bird, the tiny fibers pick up grains of pollen, which remain in the fibers of the swab head. The grains of pollen are removed from the swab and processed at Colorado State University. The pollen DNA can tell us what flowers the songbird has foraged in, giving us an idea of plants possibly pollinated by songbirds, and subsequently plants of interest for protecting declining bird species.

To prioritize your efforts, we are seeking *30 samples per species from your station*, as feasible, from the list below. We are actively seeking samples during all stages of the annual cycle with a *greater emphasis on spring (including migration)* and early summer banding:

Orange-crowned Warbler (OCWA)	Black-throated Gray Warbler (BTYW)
Yellow Warbler (YEWA)	Townsend's Warbler (TOWA)
MacGillivray's Warbler (MGWA	Hermit Warbler (HEWA)
Nashville Warbler (NAWA)	Wilson's Warbler (WIWA)
Yellow-rumped Warbler (YRWA)	Western Tanager (WETA)
Bullock's Oriole (BUOR)	Anna's Hummingbird (ANHU)
Rufous Hummingbird (RUHU)	Black-chinned Hummingbird (BCHU)
Allen's Hummingbird (ALHU)	Bushtit (BUSH)
Warbling Vireo (WAVI)	Ruby-crowned Kinglet (RCKI)

After obtaining a minimum of 30 samples/species, please continue to collect up to 5 additional samples each week for each species, as feasible. If you capture a bird with visible pollen on it, please swab the areas where you see the pollen, even if you have already collected the number of samples for that species outlined above.

If you are interested in helping to collect air samples to compare against bird pollen samples, please contact Carolyn Coyle (<u>carolyn.coyle@colostate.edu</u>).

POLLEN COLLECTION

When collecting pollen, we ask that pollen sampling be done early in the banding process to minimize contamination. If possible, please sanitize your hands between each bird you are sampling. We also ask that you do not sample from birds that are in bags that have been used since they were last cleaned. Pollen collection is performed using sterile swabs. We strongly encourage collaborators to watch the detailed sampling instruction video available on the SaP project website: https://carolyncoyle.wixsite.com/sapproject.

If you are banding and/or sampling independently, the bird can be held in the bander's grip in one hand and sampled with the other. However, before removing the bird from the bag for processing, it is helpful to get the swab ready for use by opening its individual wrapping. If banding with at least one other person, one could hold the bird in the photographer's grip while securing the wings, while the other bander swabs. If the swab-head touches a hand or surface while sampling the bird, please discard the swab. If you notice **clumped feathers around the bill**, and are already permitted to collect feathers, we ask that you clip those feathers into the same tube.



When swabbing the bird, you may sample region 1-4 in any order. The swab is unwrapped, then swirled (spun) as you gently brush the facial feathers of the bird. While not mandatory, the bill is best to sample last so that you do not disperse pollen from the bill back onto the bird's feathers. The swirling motion should be done with the grain of the feathers, with care taken to get between feather tracts in case of trapped pollen.



After you have swabbed the bird, the swab head is snapped off into a centrifuge tube (~1 in. tall) and closed up with the attached lid. That tube is then placed into a coin envelope where metadata can be recorded: all you need to do is fill in the blanks!





Metadata

Place the feathers from each bird into one of the SaP provided pre-labeled envelopes and fill out metadata. Using your own envelopes is fine, but please use coin-sized envelopes and ensure that each envelope contains the required information listed below.

- Species name
- Band number
- Tube ID
- Date (Please use letters for the months instead of numbers, e.g., APR 25, 2023)
- Time Captured
- Station and location codes, or nearest town and city
- Age, sex, and breeding condition (as determined by brood patch or cloacal protuberance)
- Visible Pollen (was there yellow, white, or orange powder noticeable on the bird?)
- SaP ID# can be left blank; this will be assigned after we receive the samples
- Any notes about the sample, including if the sample is from a same-day recapture

On a separate paper or in an email, please also provide the following location details:

- Nearest town, state/province, country
- GPS coordinates in decimal format

PLEASE NOTE: If possible, please email an electronic version of metadata to Carolyn Coyle (carolyn.coyle@colostate.edu). The preferred format is available below. We will also be sending all collaborators a blank excel template of this data sheet.

Species	Band #	Tube ID	Date Collected	Time Captured	Sex	Age	Country	State	Near Town	Lat	Long	Visible pollen?	Sample
Orange-crowned	XXXX-	0003	dd/mm/yyyy	0850	M, F, or U	If	USA	MD	Silver	39.01	77.02	Y or N	Feather and
Warbler	XXXXX					known			Spring				pollen
Black-throated	XXXX-												pollen
Gray Warbler	XXXXX												

STORAGE OF POLLEN SAMPLES

You can store the coin-envelope samples in ziploc bags or any other easy-to-ship storage solutions. Samples are preferably stored in 4°C refrigerators but can also be stored at room temperature away from direct sunlight. Please store samples until the end of the field season to minimize the number of shipments (see shipping instructions below).

SHIPPING

When you're ready to mail your samples, please email Carolyn Coyle

(carolyn.coyle@colostate.edu) with the following information to request a prepaid FedEx or UPS label:

- Approximate dimensions and weight of package
- Preference for UPS or FedEx label
- Return address
- Contact number + email

If using your own label, please mail the samples to the following address: ATTN: Carolyn Coyle Colorado State University 1474 Campus Delivery 200 W Lake ST Fort Collins CO 80521

Copies of the following Permits must be included in the shipment
For domestic shipments of samples collected within the USA, please include:
1. A copy of your (the collector's) federal bird banding permit
2. A copy of your (the collector's) state scientific collecting permit
3. A copy of the importer's USDA domestic transport permit #138961*

*The SaP project will provide this form

Across the United States and beyond, songbirds are experiencing extensive population declines. Understanding the potential role of songbirds as pollinators is critical for sustaining this ecological process as well as informing bird conservation practice and policy. Thank you very much for your participation in the Songbirds as Pollinators (SaP) Project.

<u>Please visit our website to learn more and feel free to reach out to Carolyn Coyle</u> (carolyn.coyle@colostate.edu) with any questions!

